INSTITUTE OF TROPICAL MEDICINE ANTWERP

Lecture Notes on Tropical Medicine

Part I

2025

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Tropical Medicine
2025

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Opening remarks

In memoriam

These Lecture Notes are dedicated to the memory of Dr. Erwin Van den Enden, who passed away unexpectedly on May 5, 2013. The Illustrated Lecture Notes are Dr. Van den Enden's magnum opus. They form an invaluable body of reference for both students and practitioners of tropical medicine. Dr. Van den Enden spent decades on this project, expanding on the knowledge of his predecessors at the Institute of Tropical Medicine. His colleague and mentor, Dr. Jef Van den Ende, explains how Erwin enriched his life:

On May 5, 2013, our colleague Erwin Vanden Enden was unexpectedly taken from us. Dr. Vanden Enden was a cornerstone and buttress of the Medical Services of the Institute of Tropical Medicine, Antwerp.

Dr. Van den Enden preparing a vaccine vial at Marymount Mission Hospital, Zimbabwe, 1981

First and foremost, he was a homo doctus. He could engage in more than knowledgeable conversation about the Higgs particle, butterflies, crystals or even the Hubble telescope and distant galaxies. He pointed out to me one day that a medical doctor should read the 'Scientific American' too, to be truly deserving of the title "Doctor". I subscribed the same day.

As homo universalis he was a bicyclist and invested an enormous amount of time in raising his two sons and being a caring husband. He went on missions to Africa under dreadful conditions and took me by surprise once by - out of the blue - playing two magnificent pieces of jazz on my piano.

Above all we will remember him as a clinician and professor. He could thoroughly analyze clinical cases, study the publications and lead the team on a journey through biochemistry, physiopathology, pathology and the history of medicine, all based on one case.

As professor (he unfortunately never obtained the title because of a shift in focus from education to research at the end of the 20th century in most universities), he was unparalleled. He was a guest teacher in Amsterdam, at John Hopkins, in Lima and elsewhere, and could completely enthrall students with his lectures about tropical spiders and snakes – even those suffering from a serious hangover from the previous night.

"They don't make these kinds of people anymore," a student once said about him. Duly noted.

Jef Van den Ende, ITM

A tropical disease tour

Tribute to Dr Erwin Van Den Enden

We would like to give Dr. Van den Enden the word for the Introduction (written in 2013), which remains an extraordinarily accurate and modern diagnosis of the current global situation.

“We are taking a panoramic tour through a number of medical subjects. An attempt has been made to provide a picture of a number of problems. Hopefully this has whetted the reader’s appetite and he/she will be motivated to continue to learn and contribute to the ever-increasing knowledge and use this for commendable purposes. We hope this course will be highly interactive, and not lead to "death by power-point" in the class room.

Many rural areas in the tropics seem to be frozen in time (although cell phone use spreads fast), while the flow of medical and scientific information has become a deluge. Getting information resembles trying to drink from the water of a fire hose. We are living in the middle of several revolutions, from explosive expansion of genetic and biomedical data, to fundamental changes in information/ communication strategies. Rapid advances in robotic surgery, rapid diagnostic tests, the promise of stem cell therapy, the approval of the first gene therapy in 2012 (Glyvera), brainbow cell staining, fast internet access, attosecond lasers, quick gene cloning, optogenetics, metagenomics and transgenic organisms suggest that we enter a world with surprising new possibilities and risks. There is so much new information available that we are in danger of becoming overwhelmed by it, so choosing what to study becomes more and more important. Maybe a part of the style of learning is changing right under our noses as well. With lots of information coming in small "Mc"-bite size portions, there is a danger of losing an overview or a proper reference frame. With electronic data overload, certain students might have a diminishing ability to stay focused on a single item for a prolonged and reasonable time. We have to get a firm broad and constant education, based on factual knowledge and mastering applications, together with empathy as well as fluency in divergent and convergent thinking. I think it is a good idea
that a physician can talk with a marine biologist, a geochemist and an entomologist and of course the patient. Drawing hard dividing lines between academic disciplines makes it more difficult for researchers to communicate and cooperate.

Other items on a very different level -to name just a few- are the looming freshwater crisis in certain geographical areas, the dramatic increase of the world population, the "population greying" in several countries and loss of habitat, biodiversity and the near-exhaustion of unique natural non-renewable resources such as geological phosphate deposits.

The unrelenting spread of multiresistant pathogens is becoming a global emergency and will become one of the most serious infectious disease problems facing the world in the near future. The spectrum ranges from multidrug-resistant malaria, Staphylococcus aureus, tuberculosis, ceftriaxone-resistant gonorrhea, carbapenem-resistant Enterobacteriaceae to triclabendazole-resistant Fasciola hepatica. Treatment of bacterial infections with bacteriophages is still experimental at this moment.

Another threat is the possibility of a new pandemic of a highly contagious and lethal disease, be it a new influenza, a SARS-like pathogen or something nobody expected.

In this course, the emphasis is on tropical medicine. Let's try to get the outlines right, getting a good grip on basic principles as currently understood, before diving deep. It is true that a jack-of-all-trades is usually a master of none. So be it, but it eases communication between disciplines. A study of the details comes afterwards (since we'll all be life-long learners) and often shows how rich nature is if we only want to see. Admiration of nature goes best together with understanding of nature. Understanding the structure of a flower does not diminish the beauty of a rose. Hence the following by William Blake".

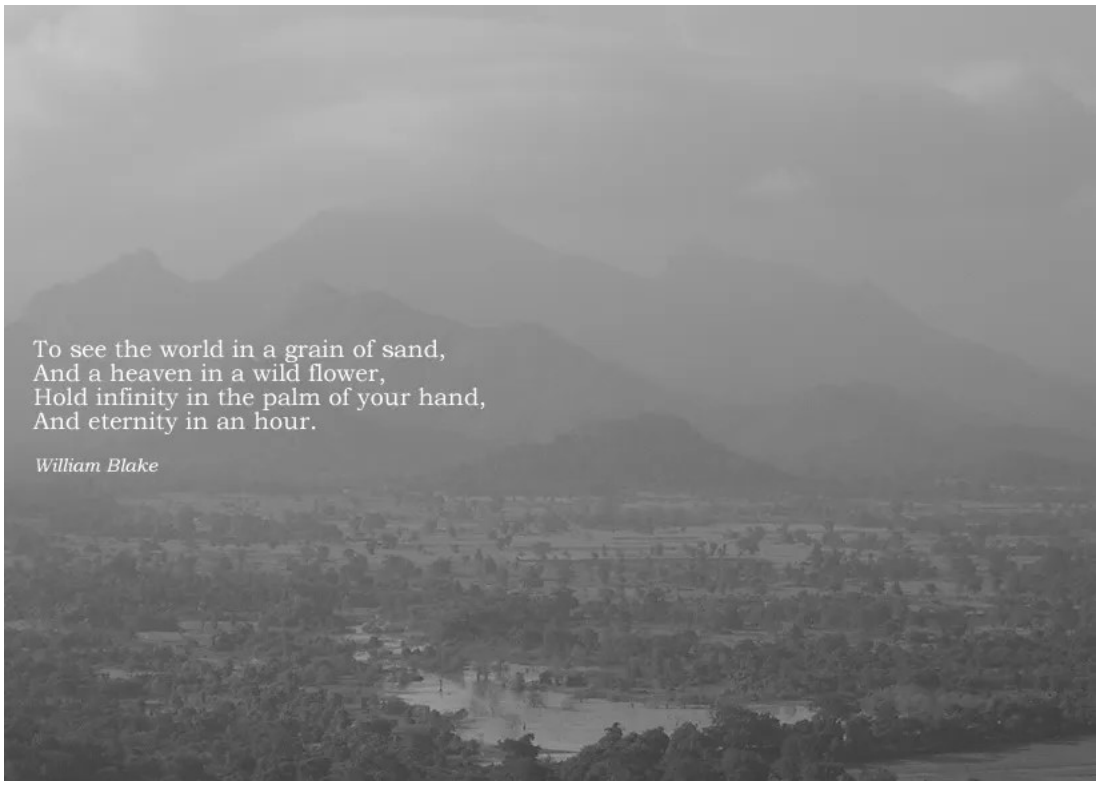

Photo courtesy of Mr Jan Van den Enden

Van den Enden Erwin, MD
Antwerp, Belgium
April 2013

Navigation guide

"To wrest from nature the secrets which have perplexed philosophers in all ages, to track to their sources the causes of disease, to correlate the vast stores of knowledge, that they may be quickly available for the prevention and cure of disease - these are our ambitions".
Sir William Osler.

The great majority of diseases in tropical regions are cosmopolitan which means they are found throughout the world: pneumonia, burns, fractures, diarrhoea, asthma, diabetes, hypertension and schizophrenia. Some disorders were also previously found in Europe, but here they have largely disappeared: leprosy, vivax malaria, plague. Only a few diseases occur exclusively in tropical regions, e.g. African sleeping sickness. A number of diseases have disappeared in the West as a result of the improvement of living conditions. The classic, predominantly parasitic tropical diseases are for the most part not the main cause of disease in developing regions, except in certain localized areas where there is a high prevalence. The main medical problems in Third World countries at present continue to be respiratory tract infections, diarrhoea, tuberculosis, malaria, AIDS, measles, accidents, anaemia and pregnancy-related problems. Hepatitis B and C, pelvic inflammatory disease and epidemic meningococcal meningitis are also frequent problems.

As economies develop, other diseases previously first seen in Western countries will become more common, such as cancer, dental caries, cardiovascular diseases and multiresistant micro-organisms. Problems typical of large cities will become more important in the near future as urbanization increases in less developed countries. The poor neighbourhoods and slums of conurbations such as Cairo, Lagos and Kinshasa in Africa, Sao Paulo, Rio, Lima and Bogota in South America, Dhaka, Calcutta, Bombay, Delhi, Karachi and Manila in Asia pose their own problems, but also offer opportunities for improvement.

Some concepts recur constantly in these lecture notes and are explained below.

Parasite: a parasite is an organism that lives in or on another organism and draws its nourishment from it (from the Greek "para-sitos": beside food). Strictly speaking, it has no connotation of harmfulness or otherwise. Usually, however, the meaning is taken in a narrower sense and the term is used to refer to various worms, protista and arthropods which have another organism as their habitat. Parasites often have a complicated life-cycle with well-defined hosts and a specific mode of transmission.

Protista: unicellular organisms that contain a cell nucleus surrounded by a nuclear membrane: eukaryotes (as opposed to prokaryotes - bacteria). There are specific treatments for each disease.
e.g. Sleeping sickness, malaria, amoebiasis, leishmaniasis, giardiasis, toxoplasmosis.

Metazoa: multicellular eukaryotic organisms, diverging considerably in size and taxonomic relationship.
E.g. whip worms, schistosomiasis, scabies, lung flukes.

Paratenic host: a host in which a parasite lives and survives, but does not develop further.

Vector: an intermediate host, which transports a parasite from the previous host to the subsequent one. E.g.: the tsetse fly is the vector of African sleeping sickness.

Arthropod: invertebrate animal with articulated legs. In medical practice the main arthropods belong to the group of insects and arachnids (including ticks and mites). Copepods are also arthropods and are vectors for a number of organisms.

Epidemic: infection which fairly suddenly affects a large number of people at the same time. E.g. the plague epidemics in the Middle Ages in Europe, the meningitis epidemics in the Sahel.

Pandemic: epidemic which spreads around the whole world. E.g.: Flu (influenza), AIDS pandemic

Endemic: a disease is endemic if it is chronically present in a particular region. E.g.: in Africa there are foci of endemic malaria.

Transmission: transport of an organism can occur in various ways.

- **Mechanical transmission,** comparable to sharing a dirty needle. This can occur in rapid repetitive blood meals of mobile insects on different hosts, e.g. the host reacts to the pain caused by the bite and interrupts the insect's feeding. The hungry insect will soon try to bite asecond host and infect him via the blood of the first host which is still sticking to its mouthparts. This sort of transmission, however, is rare, e.g. tularemia spread by tabanid flies.

- **Biological transmission,** in which the pathogenic organism either (1) reproduces in the vector (e.g. plague, arboviruses), (2) undergoes maturation before it becomes infectious (e.g. river blindness), (3) both reproduces and undergoes maturation (e.g. malaria, sleeping sickness).

Taxonomy, nomenclature and the concept of species

In 1758 Linnaeus published the tenth edition of "Systema naturae", in which he used the binomial system consistently, also for animals. This work represented a turning point in zoological terminology. It is due to his work that we have a naming system with two parts: first the genus and then the species. E.g. *Schistosoma mansoni, Escherichia coli, Aedes aegypti*. If there are subspecies (races), a third word is added, e.g. *Trypanosoma brucei gambiense*. Thus, living organisms are divided into hierarchical groups according to the similarities in their structure. The successive groups are: Kingdom, Phylum, Class, Order, Family, Genus and Species. A mnemonic sentence to help to remember the sequence is "**K**ing **P**hillip **C**ame **O**ver **F**or **G**ood **S**paghetti".

Sometimes a subgenus is given and is written between brackets, e.g. *Aedes (Stegomyia) aegypti*. When there are species complexes, as in *Simulium damnosum*, reference is often made to *S. damnosum s.l.* (sensu lato - in the broad sense, i.e. the species complex) or *S. damnosum s.s.* (sensu stricto - in the narrow sense). Different groups within a complex may

exhibit very different patterns of behaviour. Thus, *Anopheles gambiae* sensu strictu is highly anthrophilic, while the sister species *Anopheles quadriannulatus* is totally zoophilic and has no medical significance. The presence of the latter in an environment, however, can cause confusion in a control programme.

Example: Order: Diptera

Suborder: Nematocera

Infra-order: Culicomorpha

Superfamily: Chironomoidea

Family: Simuliidae

Subfamily: Simuliinae

Tribe: Simuliini

Genus: *Simulium*

Subgenus: *Erwardsellum*

Species: Simulium (Edwardsellum) damnosum

According to the "International Code of Zoological Nomenclature", the genus name is always written with a capital letter and the species name always with a lower case letter (e.g. *Glossina tachynoides)*. This applies even if the name is derived from a proper name, e.g. *Culicoides grahamii*. In scientific publications, genus and species name are italicised or underlined. Names also never contain an accent, apostrophe or umlaut (thus no *Aëdes aegypti* or *Tipula o'neili*). Two words are sufficient, e.g. *Mycena luxaeterna* for a luminescent mushroom species (instead of *Mycena lux aeterna*). The name of the genus can be abbreviated, e.g. *Anopheles funestus* becomes *A. funestus* if this does not lead to confusion or potential mistakes in the text. Sometimes the generic name is abbreviated to two letters to prevent confusion. Suppose a text contains the mention of *Culiseta* and *Culex*. If both are abbreviated to *C.* then it is no longer possible to know to which this refers. If *Culex* is identified by *Cx.* then clarity is restored.

Sometimes the name or the initials of the discoverer of the species are included (not italicised), possibly with the year of description: e.g. *Enterobiusvermicularis* (Linnaeus, 1758). This mention of the name, however, is optional and does not form any part of the actual scientific name. In view of the fact that knowledge and opinion are constantly changing, taxonomic classifications (certainly the "middle groups") sometimes differ from author to author and according to the time of publication. There is no such thing as "The One Final Correct Classification".

Conventionally, the species is defined as a population which can reproduce among itself and which is reproductively isolated from other populations. This appears clear when we talk for

example of humans, horses, wild ducks or rattlesnakes, but with other organisms it is much less obvious. What is the situation with the taxonomy of extinct species? What about symbiotic organisms, from lichen to protista, which cannot live without their symbiont? Some organisms have no sexual reproduction (for example amoebae). If there are sterile hybrids (e.g. horse x donkey-> mule), then this is an answer. Sometimes however there are fertile hybrids (some animals, many plants). The problem of species definition is central in biology at present. This has practical implications for example for the better understanding of the variability of diseases such as amoebiasis, leishmaniasis or Chagas' disease. Better insights into vector populations also depend on good definitions (some morphologically identical mosquitoes appear genetically to consist of various complexes with, for example, differing biting or reproductive behaviour).

Diagnosis

There are various ways of reaching a diagnosis. The saying: "One recognizes only what one knows" is of great importance. Knowledge of diseases and pattern recognition are the basis. Recognition of clinical presentations and reaching a diagnosis is the outcome. In the case of infectious diseases, an attempt can be made to detect the pathogenic organisms directly by microscopy (for example thick smear for malaria, Ziehl-Neelsen staining of sputum for pulmonary tuberculosis, fecal specimen for amoebae, bone marrow aspirate for visceral leishmaniasis, etc.). Cultures and serological tests are usually difficult or impossible in rural areas. Radiology and ultrasound are mostly of limited availability.

A patient will have certain complaints: symptoms, examples of which are neck pain, cough or loss of strength in the legs. There will also usually be objective signs identified by the physician treating the patient. Examples are neck stiffness, crepitations and hyperreflexia. One and the same disease may take different forms in different people. There is a spectrum of manifestations: there is individual variability (for example immunological resistance) and furthermore the symptoms and signs depend on the stage of the disease. Sometimes the degree of infection (for example worm load) is important. Whether a particular symptom, for example blood in the urine (hematuria), is highly indicative of a specific diagnosis depends on the local frequency (prevalence) of the disorder (for example, bilharziasis is frequent in Africa but not in India). A symptom may be specific to a greater or lesser extent, for example fever can be caused by numerous diseases. Fever is thus fairly unspecific on its own. On the other hand, muscle spasms triggered by sudden noise are strongly indicative of tetanus. This sign rarely occurs in other diseases and is thus relatively specific for tetanus.

Often, a definite diagnosis is not possible and a probable diagnosis must be established: the disease that is most likely in the differential diagnosis. The differential diagnosis is the list of those diseases that might explain the patient's clinical picture. It is not advisable to make a long list by including all sorts of rare possibilities. By definition, rare diseases are always rare. It is however important to think of a rare disease if it is severe and treatable in the given circumstances.

Treatment

Priorities play a major role. For example: with a limited budget, a renal dialysis unit will not be built at the expense of everything else. "What is the importance for this patient?" must be

asked, but: "What is the importance for public health?" should also be considered. In the choice of medications, cost price and availability are also of importance. The WHO has compiled a list of essential medicines.

Since the discovery of penicillin in 1928 and it's production in 1938 and Prontosil and related sulfa drugs after 1932, antibiotic resistance has been on the rise. This is currently threatening many gains in infectious diseases which have been made over all those decades. The first penicillin-resistant bacteria where already identified before penicillin came on the market in the 1940s, methicillin-resistance was documented in the 1980s and vancomycin-resistance in the 1990s. It is estimated that more than 50% of the world production of antibiotics is for use in the agricultural sector, not to cure sick animals by veterinarians, but for food additives. New threats include multi-resistance in a multitude of pathogens including *Plasmodium falciparum, Mycobacterium tuberculosis*, methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant enterococci and the appearance of *Klebsiella pneumoniae carbapenemase* (KPC) and New Delhi metallo-beta-lactamase (NDM-1) in several Gram-negatives in the first decade of the 21st Century. Since (1) Gram-negative bacteria easily share resistance genes across species, (2) fewer new antibiotics are discovered due to several reasons, (3) and the increase in world travel -both numbers of people and speed of travel-, the conditions for a perfect storm ("total resistance") are in the making, threatening to bring us back to conditions similar to where we were during World War One. The bottom line in pharmaceutical industries is financial gain. The development of new classes of drugs to treat multi-resistant bacteria is rather challenging. Even if successful, the drugs will probably be used for a short period of time before resistance arrives, and therefore are not considered to be worth the great expense of research and development time. Better incentives (e.g. longer patent protection) are needed. Maybe formal agreements such as were used during polio vaccine development are needed to protect companies against financial disaster. Basic as well as applied research needs to be boosted. Let's hope that we won't need a Manhattan-type of project if things seriously get out of hand. In our direct workplaces as doctors and nurses, meticulous disinfection of hands and surfaces will need to be instituted to limit spread and outbreaks.

Prevention

Prevention is better than cure. Sometimes prevention is the only feasible measure (for example HIV).

Prevention is based on:

- Vaccination: for example measles, polio, diphtheria, tetanus, whooping cough, yellow fever
- Chemoprophylaxis: for example the regular intake of antimalaria tablets
- Interruption of transmission. A good knowledge of the biological cycle if the pathogen is necessary for this. For example, control of the tsetse fly for sleeping sickness. Interruption of epidemic typhus transmission by delousing.
- Information, health education and encouragement of personal hygiene e.g. via school.
- Genetic counselling has its place in a number of hereditary diseases.
- Clean drinking water and food, use of good sanitary facilities. Quality control of food and drinking water is essential if it can be coupled to action to improve the situation.

- Food supplements: e.g. vitamin A, iodine deficiency
- Rapid isolation and treatment of infectious diseases (e.g. Ebola fever, open pulmonary tuberculosis, plague).
- Epidemiological surveillance (regional, national, international) is important.
- Combating poverty is the best prevention

Disclaimer

This book is not intended to be a comprehensive review of Tropical Medicine. Instead, it aims to provide an overview, highlighting the diverse and intriguing diseases predominantly found in tropical regions. We hope to spark further interest on this topic that we love.

To note, we purposely did not include Tuberculosis and HIV chapters, due to the fast changing nature of them and availability of high quality quidelines.

Useful manuals: Tropical medicine

- Medical practice in developing countries by Krawinkel (ISBN 3-8243-1276-X).
- Médecine tropicale by Gentilini & Duflo.
- Manson's Tropical Diseases by Cook and Zumla (published by Saunders, 24th edition 2023).
- Tropical Medicine and Parasitology by Goldsmith (published by Prentice-Hall International).
- Care of the critically ill patient in the tropics and subtropics by Watters (published by McMillan).
- 100 Clinical Problems in Tropical Medicine by Harries (published by Baillière Tindall).
- Common Medical Problems in the Tropics. Ed: Schull. MacMillan Publishers. ISBN 0.333.67.999.7
- Lecture notes on Tropical medicine by Bell (published by Blackwell Science).
- Online clinical cases Gorgas course (Peru): http://www.uab.edu/medicine/gorgas/cases-blog
- Online tropical radiology: http://www.isradiology.org/tropical_deseases/tmcr/main.htm
- Online mycology: http://www.mycology.adelaide.edu.au/Mycoses/
- Online medical algorithms (requires free log-in): http://www.medal.org/Visitor/login.aspx
- Kabisa: Interactive training program for clinical practice in tropical and subtropical countries available on: www.wikitropica.org

Reflections

Medicine is far from static. The work facing clinicians in the tropics mainly consists of cosmopolitan problems. The classic tropical diseases will probably become less important, while obesity, hypertension, heart disease are increasing in low-resource settings and a massive diabetes epidemic is unfolding. A tsunami of new data threatens to overwhelm scientists, although creative use of networked silicon chips helps.

In medicine, we would like not only to form highly qualified professionals, but also balanced caring people with a gentle touch and a spark in their eyes. The illustrations and case studies discussed during the actual teaching course will help to identify the important points. Sometimes, after all the studying, it is nice to let your mind drift and reflect on the big picture and dream your dream(s). The following has nothing to do with tropical medicine, but I would like to include here one of my favorite poems, just because I think it is beautiful. It was written by Max Ehrmann in 1927.

"Go placidly amid the noise and the haste, and remember
what peace there may be in silence.

As far as possible, without surrender, be on good terms with all persons.
Speak your truth quietly and clearly; and listen to the dull and ignorant;
they too have their story.

Avoid loud and aggressive persons; they are vexations to the spirit.
If you compare yourself with others, you may become vain or bitter,
For always there will be greater and lesser persons than yourself.

Enjoy your achievements as well as your plans.
Keep interested in your career, however humble;
it is a real possession in the changing fortunes of time.

Exercise caution in your business affairs, for the world is full of trickery.
But let this not blind you to what virtue there is;
many persons strive for high ideals and everywhere life is full of heroism.

Be yourself. Especially do not feign affection.
Neither be cynical about love; for in the face of all aridity and disenchantment,
it is as perennial as the grass.

Take kindly the counsel of the years, gracefully surrendering the things of youth.
Nurture strength of spirit to shield you in sudden misfortune.
But do not distress yourself with imaginings.
Many fears are born of fatigue and loneliness.

Beyond a wholesome discipline be gentle to yourself.
You are a child of the universe,
no less than the trees and the stars and you have a right to be here.

And whether or not it is clear to you, no doubt the universe is unfolding as it should.

Therefore, be at peace with God, whatever you conceive Him to be.
And whatever your labours and aspirations,
in the noisy confusion of life, keep peace with your soul.

With all its sham, drudgery and broken dreams, it is still a beautiful world.
Be cheerful. Strive to be happy."

Max Ehrmann, "Desiderata", 1927

Authors and collaborators

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This book is an ongoing work in progress. In case of suggestions or questions about these notes, please contact Steven Van Den Broucke (svandenbroucke@itg.be) or Sami Alcedo (salcedo@itg.be).

Protista

Malaria

Summary

- Malaria is very common; a very important cause of mortality and morbidity in the tropics
- Five parasites: *Plasmodium falciparum, P. vivax, P. ovale, P. malariae*, and since 2007; *P. knowlesi* (in Southeast Asia)
- Transmission via female Anopheles mosquitoes which bite at night
- Symptoms: fever and body-ache; sometimes atypical or "chronic" (anaemia, splenomegaly)
- Risk of complicated presentations, mainly with P. falciparum (severe anaemia, kidney failure, cerebral malaria)
- Infections often asymptomatic in semi-immune people (generally low parasitaemia)
- Clinical diagnosis not reliable.
- Often clinical over-diagnosis of malaria and under-diagnosis of other disorders in endemic areas
- Diagnosis via thick smear, thin smear, rapid antigen-detection, DNA-based methods
- Treatment of *P. malariae*: chloroquine
- Treatment of P. vivax and *P. ovale*: chloroquine or if possible with primaquine (hypnozoites, G6PD).
- Resistance of P. vivax to chloroquine is rising in several areas
- Increasing multidrug resistance of *P. falciparum*, including resistance to artemisinin derivatives
- Combination treatment of P. falciparum infection is strongly advised:
    - ACT: artemisinin combination treatment (e.g. artemether + lumefantrine; = Co-Artem, Riamet).
    - Quinine + (doxycycline or clindamycin)
    - Atovaquone + proguanil (Malarone)
- Individual prevention via pyrethroid-impregnated bed net ± chemoprophylaxis; stand-by emergency treatment (self-medication) for certain travelers?
- Population protection via vector control, but increasing resistance of mosquitoes to various insecticides
- First malaria vaccine undergoing phase 4 studies in children of endemic countries

Malaria in humans

Malaria is the common name for diseases caused by infection with single-celled parasites of the genus Plasmodium. Among the parasites of the genus Plasmodium five species have been identified which regularly cause disease in humans:

- *Plasmodium falciparum*
- *Plasmodium vivax*
- *Plasmodium ovale*
- *Plasmodium malariae*

- *Plasmodium knowlesi*

However, in 2017 several malaria outbreaks in Brazil were caused by *P. simium*, a malaria species closely related to P. vivax that was previously considered to be a monkey-specific malaria parasite.

Human malaria parasites have a restricted host-specificity. They don't develop disease in rabbits, rats or mice but need to be maintained either in human volunteers or in primates. A common used less than-optimal substitute is to perform experiments on primate, rodent or avian malaria parasites in their natural host. Most animal models are inadequate and, while they can help the researcher in answering specific questions, any extrapolation to human disease has to be considered with extreme caution. For example dexamethasone was considered to be useful in severe malaria caused by *P. knowlesi* in rhesus monkeys, but was found to be harmful in humans.

Historical note

Discovery of the parasite

Malaria has been with humanity since millennia. The most famous historical case of falciparum malaria is probably King Tutankhamen, the boy pharaoh from Old Egypt, in whose 3,000-year-old mummy the parasite was demonstrated. Although usually associated with the tropics malaria was endemic in North America and large parts of Europe until the middle of the 20th century. Malaria transmission occurred in Belgium, the Netherlands, Sweden, Finland and the United Kingdom. It was a significant impediment for the European nations during the colonial period. In Northern Europe, only *P. vivax* and *P. malariae* occurred. In Southern Europe, malaria was due to infection with P. falciparum, P. vivax and *P. malariae.*

In 1880 the French army doctor Charles Louis Alphonse Laveran discovered malaria parasites in fresh blood from malaria patients in the coastal town of Bone (Annaba), Algeria.

Transmission

The transmission of malaria had for long been a mystery. One of the researchers was the Briton Sir Ronald Ross. He left for India with a personal mission to prove transmission via insects. In 1897, after three years of hard work, he demonstrated parasites in mosquitoes which had bitten patients. Later he also demonstrated transmission of avian malaria via mosquitoes. He was able to describe the complete development of the parasite in the mosquito and also demonstrated that transmission took place via the bite of the mosquito (and not via the presence of dead mosquitoes in drinking water, as his mentor Patrick Manson had initially thought).

Life Cycle

After the cause and transmission of malaria became known, it was logical to assume that the parasites inoculated via a mosquito bite would directly penetrate red blood cells. This wrong idea was proposed in 1903 by Fritz Schaudinn, a distinguished German microscopist. It was based on faulty observation and due to his authority, it entered some textbooks. It was known that when blood from a patient with active malaria was inoculated into a healthy volunteer, the volunteer would develop malaria and would become infectious

nearly instantaneously. However, when a volunteer was inoculated via a mosquito bite, the blood was not infective for 6 days (in case of *P. falciparum*) to 9 days *(P. vivax).*

Why? This was a vexing problem which took decades to answer. It was by very careful animal experiments with *P. cynomolgi*, a primate malaria species, that the puzzle was solved. *Shortt and Garnham* collected a large number of infected mosquitoes, mashed them to pulp and injected the lot (including sporozoites) into monkeys. After waiting a period, they killed the animals and searched the various organs and tissues. The parasites (with a different shape) were found in the liver. They had to support their hypothesis of the existence of a pre-erythrocyte stage with a species of human malaria. They used *P. vivax* and a human volunteer. This man was inoculated IV with sporozoites isolated from 200 mosquito salivary glands. A week later, the volunteer was operated on and a piece of liver tissue was obtained. The parasites were present in the liver. A year later they obtained a strain of *P. falciparum*, infected 770 mosquitoes and inoculated another human volunteer. About 6 days later a liver biopsy was taken and again the parasite was found.

Pyrotherapy
In 1927 Julius Wagner-Jauregg won the Nobel prize for his discovery of malaria pyrotherapy for treatment of late stage neurosyphilis. To induce repeated spikes of high fever in patients with progressive paralysis, he inoculated them with blood from patients who were suffering from tertian malaria (*Plasmodium vivax*). Although not without risk, this treatment proved to be very successful.

Life cycle

When a mosquito lands on the skin, it attempts to pierce a small blood vessel with its proboscis in order to suck blood. To prevent the blood from coagulating the mosquito first injects some saliva. Besides vasodilating agents this saliva contains anticoagulants. However, the saliva may also contain microorganisms. When a human is bitten by an Anopheles infected with malaria, parasites (sporozoites) [Gr. sporos = seed] are introduced into the human body. On average 10-20 sporozoites are injected per bite, although this number can be higher, e.g. 100.

A certain protein of the parasite, (the circumsporozoite protein, CSP), plays an important role in the penetration of the sporozoite into a liver cell (cf. Mosquirix vaccine). Sporozoites reproduce asexually in liver cells, by schizogony [Gr. schizo = split, divided]. This is called exo-erythrocytic or pre-erythrocytic reproduction. The form of the parasite produced in this way is called a liver schizont. The multinuclear schizont splits into many thousands of small offspring (merozoites) [Gr. meros = part]. Every successful sporozoite can produce some 20,000 merozoites.

After some time the infected liver cells burst and the merozoites enter the blood stream. While the parasites are reproducing in the liver, there are no symptoms. Neither the sporozoites, nor the liver forms are sensitive to most of the drugs used in malaria prophylaxis (atovaquone/proguanil is an exception). The minimal required time from infection to the appearance of the first merozoites, is the prepatent period. The incubation period is somewhat long because signs and symptoms do not appear until the parasitaemia is

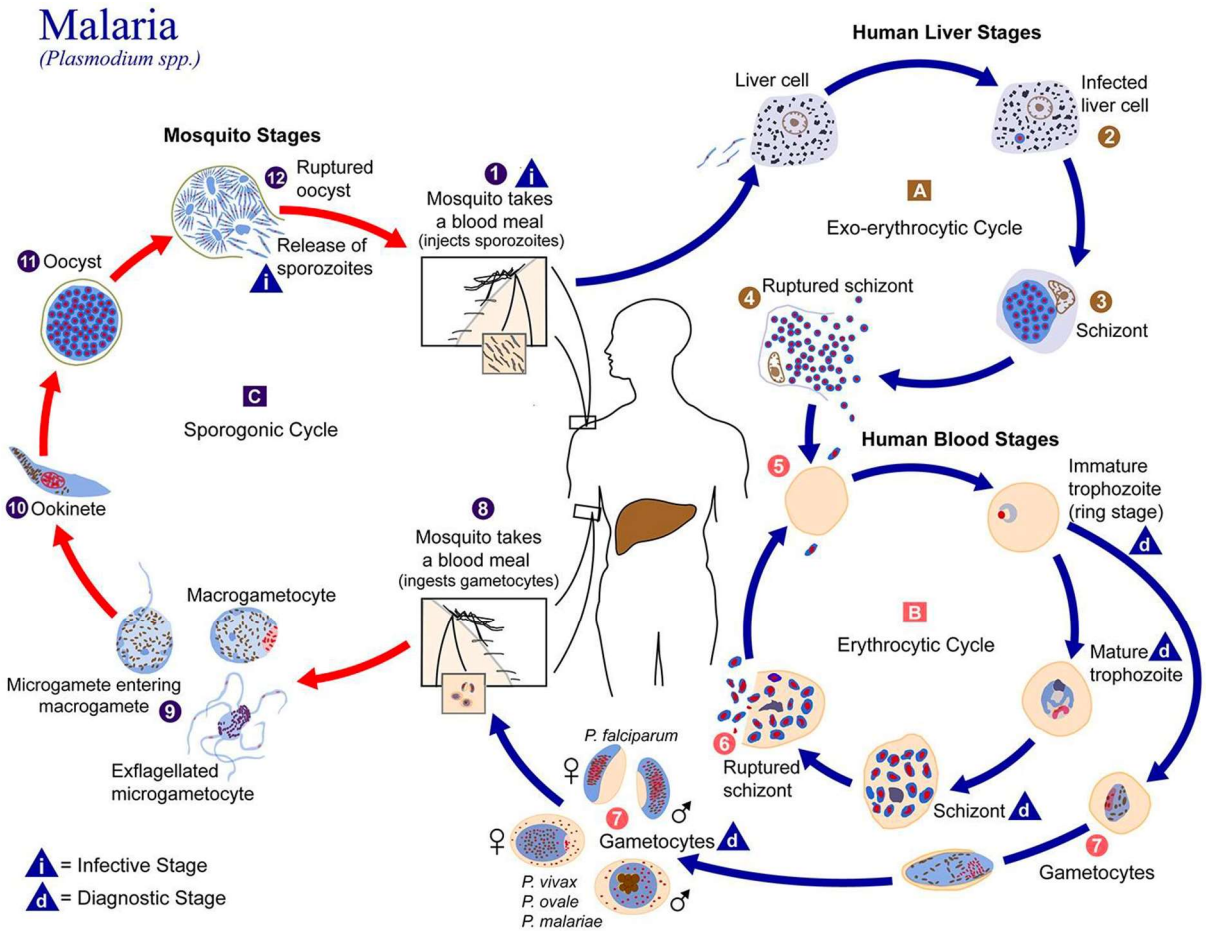
sufficiently advanced. Of note, merozoites in blood are usually too small to be seen by microscopy.

In the case of *P. vivax* and *P. ovale* only some of the infected liver cells burst. The parasites in the liver cells which do not burst (hypnozoites) [Gr. hypnos = sleep] may remain viable for years and are responsible for new attacks of the disease if reactivated. The trigger which reactivates the hypnozoites is not known. The existence of hypnozoites in P. vivax was only formally demonstrated in 1985 via fluorescence microscopy. Reactivation of these "sleeping" forms explains delayed exacerbations of the disease after treatment with chloroquine and other antimalarial drugs. They kill the blood forms, but not the liver forms. Hypnozoites are not present in *P. falciparum* and probably not in *P. malariae* (although this is controversial). This is important for treatment because hypnozoites are not sensitive to chloroquine, quinine, mefloquine or artemisinin. Accidental inoculation with infected blood (blood containing trophozoites) may lead to infection, e.g. transfusion malaria or malaria via shared contaminated syringes by drug users. Since the infection in these cases is not transmitted by sporozoites, there are no liver forms. Liver forms are also absent in congenital malaria. This is important for treatment (no primaquine for congenital malaria with P. vivax or P. ovale). The chronic nature of infections with P. malariae is traditionally explained by assuming that the parasite can induce a very low parasitaemia (or hidden erythocytic schizontes) for many years, which is below the detection threshold of normal diagnostic methods.

In the red blood cell the parasite feeds on haemoglobin. The form of the parasite when present in the red blood cell is now known as a trophozoite (Gr. trophe = nutrition). The young parasite possesses a digestive vacuole with lysosomal enzymes. This vacuole contains proteinases (plasmepsin and falcipain). The vacuole can be clearly seen in a blood smear and explains the ring shape of the young parasite. The breakdown of haemoglobin results in an iron-containing pigment: hemozoin. The vacuole disappears as the parasite becomes older. The trophozoites will once more reproduce asexually and lead to the formation of a multinuclear parasite (schizont). The latter divides to form merozoites. Each schizont produces 8 to 24 merozoites, depending on the species, within a time span of 48 hours (*P. falciparum, P. vivax, P. ovale*), 72 hours (*P. malariae*) or 24 hours (*P. knowlesi*). The infected red blood cells burst after a while so that once more merozoites appear in the blood from where they will penetrate new erythrocytes within a few seconds. This bursting (lysis) of the red blood cells is accompanied by a bout of fever. If the development is synchronous (all parasites being at the same stage of development) the fever will follow a typical pattern (see below). This is, however, unusual: asynchrony is more common than synchrony, especially early in infections. The development from merozoite to schizont takes place in the peripheral blood and all stages can be observed. In *P. falciparum* usually only very young forms (ring forms) can be observed in the peripheral blood because older parasites (and schizonts) adhere to the endothelium of blood vessels in deep organs (e.g. the brain).

After a few days some of the merozoites transform into male or female gametocytes. These are necessary for sexual reproduction of the parasite (which only occurs in the mosquito). Gametocytes are responsible for transmitting the disease but do not themselves cause symptoms. Adult *P. falciparum* gametocytes are not sensitive to chloroquine and quinine, while those of *P. vivax, P. ovale* and *P. malariae* are sensitive. This means that following adequate treatment of *P. falciparum* there may still be gametocytes in the blood, and this may

continue for several weeks. This does not mean that the treatment has failed. One interesting hypothesis is that chloroquine might significantly increase the gametocytemia of chloroquine-resistant *P. falciparum*, resulting in an increased infectivity for *Anopheles*. This could, therefore, contribute to the rapid spread of chloroquine resistance.



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Glucose metabolism and LDH

The trophozoite has no carbohydrate reserves and needs to consume glucose continually. The glucose metabolism in infected red blood cells is 50-100 times higher than that in non-infected cells. This probably contributes to the hypoglycaemia which is often seen in severe infections.

The parasite have mitochondria, but these play a minor role in the provision of energy (the last word on this has not yet been said). Glucose is converted by anaerobic glycolysis to pyruvate and then to lactate. This latter step, as in humans, is catalyzed by the enzyme lactate dehydrogenase (LDH). The parasite's LDH is clearly different from that of humans and forms the basis of a diagnostic test (see below).

Geographical distribution

Many lay people regard malaria as a purely tropical disease. However, the distribution of malaria used to be world-wide. Today, it still occurs in some 100 countries. The situation varies

from region to region. Until 1938 there was still *P. vivax* malaria ("polderkoorts") in Belgium, and in the Netherlands as late as 1958, although there was an unexplained (possibly autochthonous) case of *P. malariae* infection in a child in Zeeland in 1969. The WHO declared the Netherlands officially malaria-free only in 1970. It is chiefly the pollution of surface waters which makes reproduction of *Anopheles* mosquitoes difficult.

Yet some *Anopheles* persist and can transmit malaria. *Anopheles atroparvus* is able to transmit *Plasmodium vivax* malaria but cannot transmit *Plasmodium falciparum. Anopheles plumbeus* can transmit tropical falciparum malaria. In the last century there were important changes in the lifestyle of humans, resulting in less human/mosquito contact. Effective therapy was available. All these factors mean that malaria has disappeared in Northwest Europe. Cases in Western countries are generally dealt with swiftly and satisfactorily and one person with malaria very rarely leads to the infection of others. Chronic large scale reintroduction of the disease in Europe is thus improbable, although with the combination of the current economic crisis with its plummeting health budgets, the massive influx of tropical migrants refugees and global climate change, makes this possibility more real at present than in the last decades of the 20th Century. To maintain an infectious disease, it is necessary for one infectious case to lead to one other infectious case, otherwise the disease will die out in the area. One would need sufficient gametocyte carriers and vectors to ensure the continuation of the disease.

Malaria is a very important public health problem in most tropical countries although the incidence rate of malaria declined globally between 2010 and 2018 from 71 to 57 cases per 1000 population at risk. In 2018, an estimated 228 million cases of malaria occurred worldwide, compared with 251 million cases in 2010. In that same year 405.000 people died of malaria mainly young children in Africa. Most lethal infections are due to *Plasmodium falciparum*. Six countries cause more than half of all malaria cases worldwide: Nigeria (25%), DRC (12%), Ivory Coast, Mozambique and Niger (4% each). Of note, the decrease in incidence seems to stagnate these very last few years in sub-Saharan Africa.

P. falciparum is the most common form in sub-Saharan Africa (99,7% of malaria cases in this region), tropical South America and Southeast Asia. The parasite occurred previously in the Mediterranean basin.

P. vivax has the widest distribution area (previously as far as London, Norway, Denmark, New York, southern Canada and even Siberia). In 1922 the number of cases in Texas was estimated at 500,000. It is the most common form in certain regions (e.g. Maghreb, Middle East countries, parts of China, Argentina). P. vivax preferentially penetrates young red blood cells (reticulocytes). In 1976 Miller discovered that *P. vivax* uses the Duffy blood group antigens (Fya and Fyb) as receptors to penetrate red blood cells. People who do not have this protein on their red blood cells cannot be infected with P. vivax. These antigens do not occur in the majority of humans in West Africa [phenotype Fy (a-b-)]. As a result, *P. vivax* does not occur in West Africa, or occurs in low numbers (and could be systematically missed). Duffy blood group negative erythrocytes are, in vitro, also resistant to infection with *P. knowlesi* (monkey malaria).

P. ovale is found chiefly West Africa, less elsewhere in Africa and sporadically in the Far East.

P. malariae is not very common but can be found anywhere. Often confused with *P. knowlesi.*

P. knowlesi is known from Malaysia (including Borneo), The Philippines, Vietnam, Thailand and recently Myanmar. The vector is present in India (Kerala) and Sri Lanka, but in these areas there is no known zoonotic reservoir. *P. knowlesi* infections are often confused with P*. malariae.*

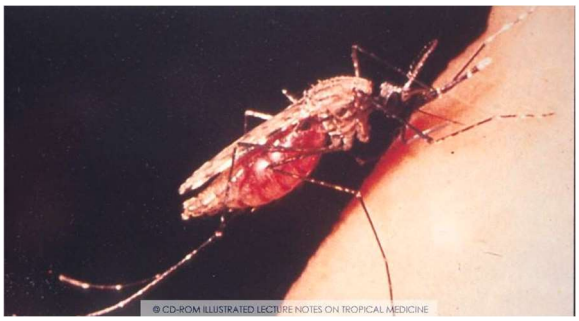
Malaria can only persist naturally when climatic conditions are suitable for the vector(s) and for the development of the parasites in the vector. Increased rainfall and higher temperatures may make larger areas favourable for malaria transmission in the future. Tropical *P. falciparum* requires a minimum temperature of 18°C, while tropical *P. vivax* strains require a minimum of 16°C. The European strain of *P. vivax* which persisted in the high North was uniquely adapted, with summer temperatures being sufficiently high for *P. vivax* development in the mosquito. Infection of patients occurred in autumn (September / October) when mosquitoes started to enter homes looking for shelter. The *P. vivax* parasites in humans had a very long incubation period of 6 to 9 months. A patient infected with these northern strains of *P. vivax* would remain asymptomatic during winter until the following spring. This clearly differs from tropical *P. vivax* dynamics. In Southern Europe *P. falciparum* used to be common in Portugal, Spain, Italy and Greece. It is likely that the strain of this parasite was genetically different from tropical strains.

However for malaria to become re-established, a sizable parasite reservoir (gametocyt carriers) must be present. This has not happened in other circumstances, such as after World War II, when great numbers of people (patients and gametocyt carriers) returned from tropical areas. In the current health system and socioeconomic situation in Europe, it is likely that patients will be treated early, diminishing the reservoir and lessening the threat of new epidemics. Small outbreaks and some local transmission might occur from time to time, but large reinvasion of the North European landmass is unlikely. South Europe would have a somewhat larger risk, as reflected by the outbreak of autochthonous *P. vivax* cases in Greece in 2012.

Epidemiological classification - stable versus unstable malaria

There is no completely satisfactory epidemiological classification of malaria. Stable malaria means that the clinical disease is characterized by preferentially affecting children and achieving a protective "immunity" in adults. Stability does not mean that there can be no variation in transmission. In some regions seasonal malaria occurs. In other areas there is unstable malaria: transmission differs greatly from year to year and occasionally epidemics occur. Then the disease also occurs in older persons. This is important in many respects; as irregular control of malaria may lead to changes in the immune status of the population. Sometimes malaria may appear again in a region after a long absence. For example: in 1972 the disease was eradicated in South Korea following an intensive eradication campaign with case detection and vector control. In 1993 one case of P. vivax was observed. There then followed 22 cases in 1994, in 1995 there were 107 cases, 356 in 1996 and more than 1600 in 1997. In 1995 all cases were still limited to the border area with North Korea, but in 1996 there was also transmission outside the demilitarized zone. After entomological surveys had shown that *Anopheles sinensis* was the chief vector, measures were taken to control the disease.

Vector, *Anopheles* mosquitoes



Malaria is transmitted via the bite of infected female *Anopheles mosquitoes*

Malaria is transmitted by *Anopheles* mosquitoes. This applies to the malaria of all mammals. Avian malaria is chiefly transmitted by Culicinae. There are some 400 *Anopheles* species, 40 of which are good vectors while 28 are poor vectors. Anopheles mosquitoes are active at night. They do not buzz much and are not easily noticed. The world's most important vector is *Anopheles gambiae*, an anthropophagic and endophilic freshwater mosquito which flourishes preferentially in moist regions. It typically breeds in exposed sunlit and often transient aquatic habitats such as pools, puddles, and irrigation channels. *Anopheles* mosquitoes are good flyers: they can cover several kilometres in one night. This is of course of great importance for their control. Endophagic (bite inside the house) mosquitoes will often rest on walls after a blood meal. Residual insecticides which are applied there will kill the vector.

How do mosquitoes find their prey?

Mosquitoes are attracted by an increasing CO2 gradient. The warmth of the skin, lactic acid and moisture (breath) play a part over short distances. Every animal produces several volatile substances in its skin, breath, faeces and urine. A number of the substances (kairomones) are used by the mosquito to find its prey. The details are complex. Anopheles gambiae prefers to land on the feet, while A. atroparvus prefers to bite the face.

Vector control

Malaria vector control is primarily based on the use of insecticides. Appropriate monitoring of vector resistance to insecticides is an integral component of planning and evaluation of insecticide uses in malaria control programmes. Pyrethroids and DDT, two important insecticides used for vector control, block the nerve-impulse conduction by preventing a sodium channel from closing after an action potential. An important mechanism that confers resistance to pyrethroids and DDT, known as knockdown resistance or kdr, was first described in the housefly Musca domestica. It has been reported that a single mutation in the sodium channel sequence is the molecular basis of kdr in Musca domestica. The gene has also been characterized for *Anopheles gambiae*. PCR tests have been developed for the detection of the kdr-mutation in *A. gambiae*.

Physiopathology

The incubation period may be short (minimum 7-9 days for *P. falciparum*) to very long (several years for *P. ovale*). In falciparum malaria the parasitaemia can be very high: up to 80% of erythrocytes may contain parasites, but even 5% is enough to result in severe disease. These situations may be lifethreatening. The other malaria parasites produce much lower parasitaemia (especially *P. ovale*). They do cause severe illness but are rarely life-threatening. *P. knowlesi* infections mimics severe *P. malariae* infections.

The rupture of the red blood cells (haemolysis) is accompanied by fever, muscle pain and general malaise. Massive haemolysis may cause kidney failure. Parasitized red blood cells are removed by the spleen. Splenomegaly will result. Anaemia occurs due to the destruction of erythrocytes, suppression of the bone marrow and excess activity of the enlarged spleen (hypersplenism). In severe falciparum malaria, there is activation of blood coagulation system along with thrombocytopenia, even before widespread DIC and coagulation failure occur. In falciparum malaria there will often be a drop in glycaemia that can be corrected by administration of glucose.

The details of how cerebral malaria happens, are not clear at present, and various researchers have different opinions. More than 100 years ago, the Italian pathologists Bignami and Marchiafava reported on the sequestration of parasitized red blood cells in the brains of people who died of cerebral malaria. Erythrocytes which contain schizonts of P. falciparum, develop small knobs on their cell membranes. These consist, among other things, of a histidine-rich protein, *P. falciparum* erythrocyte membrane protein 1 and rifins. Rifins are clonally variant proteins encoded by rif genes (“repetitive interspersed family") and are expressed at late ring or early trophozoite stage on the infected red cell surface. Their high copy number, sequence variability, and red cell surface location indicate an important role in hostparasite interaction. The knobs have an overall negative charge, allowing non-specific attraction to positive endothelial ligands, but specific molecular adhesion also play a part. With these knobs the infected cells cling to the walls of the capillaries and to the vascular endothelium of the post-capillary venules in the brain. The low local O2 pressure and high CO2 pressure are optimal for further maturation of the parasite. Infected red blood cells are less easily distorted and more rigid than normal erythrocytes. This impedes the blood flow, which can lead to cerebral malaria. Other organs may also be affected for example the placenta and the intestines (resulting in abdominal pain and diarrhoea). Red blood cells which contain schizonts of *P. malariae*, also develop knobs on their membranes, but these cells do not adhere to the vascular endothelium. When post mortem cerebral sequestration was compared with the peripheral parasitaemia, there were about 26 times more infected red blood cells in the brain microvasculature than in the peripheral blood if there were free-mixing. More blood vessels in the cortex and cerebellum than in the brain stem are affected. Some researchers found more sequestration in white matter than in cortex. Coma requires sequestration, but sequestration itself is not enough to provoke cerebral malaria. The rapid reversible nature of cerebral malaria led to the hypothesis that soluble neuroactive mediators might play a role in the pathogenesis possibly involving reversible disturbances of the blood brain barrier and biochemical disruption of normal metabolism.

There are two groups of parasites in *P. falciparum* infections: (1) the young forms in the peripheral blood which can easily be observed in a thin blood smear and (2) the mature group

which is attached to small blood vessels and which cannot be seen. Falciparum schizonts are rarely found in peripheral blood but these are important for the development of cerebral malaria. The whole mechanism of cerebral malaria has not to date been fully explained. As well as the attachment of parasitized red blood cells to the vessel walls (cytoadherence) other mechanisms possibly also play a part. Normal red blood cells sometimes attach to parasitized cells, which impairs the microcirculation. All kinds of released chemical substances (cytokines, oxygen radicals, etc.) may also play a part. Cytokines such as tumour necrosis factor (TNF-a) increase the expression of receptor molecules on the endothelium and will contribute to the cytoadherence and flow obstruction which characterize falciparum malaria. This mechanism is similar to the release of TNF-a by endotoxins in Gram-negative septicaemia.

Increased brain volume was seen in children who died from cerebral malaria but was uncommon in those who did not die from the disease; this suggests that raised intracranial pressure may contribute to a fatal outcome.

Carriers of the sickle cell anaemia gene (heterozygotes for haemoglobin S) have relative protection against severe infection with P. falciparum and thus have a survival advantage (in homozygous patients, malaria may be fatal and the disease itself tends to kill patients before the reproductive age). The same advantage probably applies to persons deficient in G6PD. This may explain why these two conditions are so common in Africa. In Papua New Guinea ovalocytosis is common. These red blood cells have an oval shape and cannot be penetrated by *P. falciparum* parasites. Heterozygotes are thus protected against *P. falciparum* (homozygosity is not compatible with life).

In West Africa, haemoglobin C is rather frequent. People with haemoglobin AC or CC can be infected with *Plasmodium falciparum* and can develop substantial parasitaemia. The presence of Hb C therefore does not protect against infection itself. Haemoglobin C might protect against the lethal effects of *P. falciparum* malaria by reducing cytoadherence of parasitized erythrocytes.

Haemoglobin E (chiefly Southeast Asia) does not protect against *P. falciparum* infections itself.

While circulating in human blood P. falciparum exhibits antigenic variation. On the surface of the infected red blood cell a certain protein is expressed: the P. falciparum erythrocyte membrane protein 1 (PfEMP-1). The parasite can make many variants of this protein. By interchanging which variant of PfEMP-1 is present, the parasite can evade the immune response to these immune dominant antigens.
These proteins are thought to be the major virulence factor found on the surface of infected red blood cells, directly contributing to the pathogenic nature of the infection and placing these genes at the centre of a disease responsible for several million deaths in developing countries. Although there are many var gene copies, only a single var gene is expressed at any given moment (i.e. there is mutually exclusive expression). Over the course of an infection, expression switches from one var gene to another, resulting in antigenic variation of the parasite population and a persistent infection which is difficult to clear by the human immune system.

Antigenic variation has important implications for the development of vaccines. The repertoire of proteins which are expressed in the *Anopheles* mosquito is far less pronounced probably because the vector has no adaptive immune system.

A large case-control study of malaria in West African children showed that a human leukocyte class I antigen (HLA-Bw53) and an HLA class II haplotype (DRB1*1302-DQB1*0501), common in West Africans but rare in other racial groups, are independently associated with protection from severe malaria. In this population they account for as great a reduction in disease incidence as sickle-cell trait. These data support the hypothesis that the extraordinary polymorphism of major histocompatibility complex genes as well as other genes has evolved primarily through natural selection by infectious pathogens.

Malaria is very often accompanied by thrombocytopenia, the causes of which seem to be multiple and not completely known. The severity of the thrombocytopenia correlates with the parasitaemia and the clinical severity of infection.

Clinical aspects

Classic acute uncomplicated attack

Most clinical episodes of malaria are characterized by fever with unspecific symptoms. Certainly, in children the presentation can be very misleading. Any fever should bring the possibility of malaria to mind. There is a danger, however, that every fever episode may be regarded as malaria and other important diagnoses are then likely to be missed.

P. falciparum: typical incubation time: 7 to 30 days. If a person is taking preventive antimalarials and if the parasite is partially resistant, there may be temporary suppression of a malaria attack. The fever is generally irregular. If the attack is not treated, after a few weeks a regular fever pattern will develop with peaks every 2 days (tertian malaria, so called because the fever reappears on the third day, reckoning the day of the paroxysm as the first. This is rare in everyday clinical practice). At the beginning of the attack the symptoms are similar to influenza: general malaise, tiredness, muscle pain, headache but in general without respiratory tract problems or running nose. These symptoms are nonspecific. After a while the muscle pain and headache become worse. Sometimes there is also abdominal pain and diarrhoea. Rarely there is a classic attack: this lasts for approximately 12 hours and occur every 48 hours. At first cold shivers with high fever occur, followed by an intense feeling of heat and fever, leading to a sweating stage with a drop in fever. Most falciparum attacks do not follow this classic pattern. Therefore what is referred to as a classic attack is paradoxically not the general rule.

P. vivax and P. ovale: the incubation time is a few weeks to years. The awakening of dormant parasites in the liver (hypnozoites) explains the potential for late relapses. The fever is sometimes regular (every 48 hours) especially in cases of recrudescence (tertian malaria). In 1922 *P. vivax* was introduced for the treatment of neurosyphilis. It was thought that the bacterium which causes syphilis had little resistance to heat, so the high fever would kill the bacteria (*Treponema pallidum*).

P. malariae: the incubation time is 3 weeks to many years. The very late attacks are probably not due to awakened hypnozoites (to date these have never been detected) but due to the

activation of blood parasites which are present at a very low concentration. Fever peaks may occur every 72 hours (quartan malaria).

P. knowlesi is a monkey parasite which can be misidentified as *P. falciparum* in the early ring stage and as P. malariae in the older stages. It has the shortest asexual life cycle of all i.e. 24h. The prepatent period is 9-12 days. At present, no hypnozoites have been found. PCR is needed to firmly identify this species.

Mixed infections: mixed infections do occur, but for reasons which are unclear they are much less common than would be expected based on the prevalence of the individual species. Underreporting may play a part, but this is probably a real phenomenon (partial cross-immunity to heterologous species? biological interference?).

Natural course of malaria in the autochthonous population

Children are very susceptible to infection. The highest mortality is found in children below the age of 5 years. Gradually, after repeated infections, a partial immunity develops in those who survive. There is a high degree of tolerance to the infection in adults, provided they live in a stable malaria region. This semi-immunity (or "premunition") is maintained by repeated infections and mild latent infections. It disappears after approximately 6 to 24 months if there is no further infection (e.g. a stay in a nonmalaria region).

This partial immunity is reduced during pregnancy. A pregnant woman is at increased risk of hypoglycaemia and cerebral malaria. Malaria is an important cause of severe (sometimes spectacular) anaemia in the mother, low birth weight, premature birth, abortion and increased perinatal death. Chondroitin sulphate and hyaluronic acid, both present in abundance around the syncytiotrophoblasts of the placenta, are mucopolysaccharides (glycosamine glycanes) which act as receptors for red blood cells infected with P. falciparum. Probably there are also other receptor molecules. Infected cells accumulate in the placenta resulting in reduced placental function.

The placental barrier is very seldom passed. Congenital malaria is not common and occurs chiefly in neonates of non-immune women. Neonates of semi-immune women receive transplacental antiplasmodium antibodies. Due to this passive resistance in the first 3-6 months they are at a lower risk of malaria.

Several observations of humans infected with both malaria and helminths suggest that co-infection provides a benefit to either parasite. The evidence indicates that malaria patients co-infected with helminths are protected from severe malaria possibly through skewering of the immune response towards T helper (Th)2 immunity.

Malaria and HIV interact in several, rather complex ways. The CD4+ lymphocytes play a central role in the defence against malaria and their characteristic decrease during the course of HIV infection explains why severely immunosuppressed HIV-positive individuals are so susceptible to severe malaria. In malaria-endemic regions, severe malaria may be considered as an "opportunistic infection" and any diagnosis of complicated malaria in adults should trigger HIV testing.

Acute severe malaria

Acute severe falciparum malaria is a medical emergency. This encompasses:

- Coma: repeated generalized convulsions
- Hypoglycaemia: reduced consciousness, aggressive behaviour
- Severe anaemia: weakness, tachypnoea, pale mucosae
- Tendency to spontaneous bleeding (pronounced thrombocytopenia)
- Circulatory collapse (shock); cfr. below “algid malaria”
- Pulmonary oedema (dyspnoea and bilateral crackles) leading to acute respiratory distress syndrome (ARDS)
- Haemoglobinuria (dark urine)
- Kidney failure: urinary output should be monitored (but attempts to force urine production may cause circulatory overload!)
- Acidosis (chiefly due to lactic acid): tachypnoea. If too many salicylates are given this may exacerbate acidosis (not unusual in febrile patients).
- Other important signs are: marked jaundice, confusion without coma, extreme generalized weakness or prostration (child cannot remain sit and don’t want to eat/drink).

The priorities are cerebral involvement, severe anaemia, hypoglycaemia, and the presence of hyperparasitaemia. The degree of parasitaemia correlates with the severity of the symptoms: the higher the parasitaemia, the greater the risk of severe symptoms. It should be borne in mind that the parasitaemia (the percentage of parasitized cells that are found in a smear preparation) changes by the hour. This is because the red blood cells with mature P. falciparum parasites (schizonts) attach themselves to the small capillaries of deep organs therefore are not found in a thin blood smear. A parasitaemia of 0.5% is already severe, 2% is pronounced, and patients with a parasitaemia of more than 10% have a relatively poor prognosis. Over 25% is often fatal. Another consideration is that a parasitaemia of 3% in someone who still has a normal red blood cell count, is different from a parasitaemia of 3% in an anaemic patient.

Hypoglycaemia may quickly lead to general deterioration and coma. It is common in children (up to 25% of cases) and pregnant women. Glucose may be life-saving. If the glycogen store in the liver is low (i.e due to malnutrition) the risk of hypoglycaemia increases [glycogen is converted to glucose]. The conversion of glycogen to glucose is also inhibited by certain cytokines which are released during infection with *P. falciparum* [Hypoglycaemic effects of TNF-a and possibly interleukin-1 and TNF-ß]. The parasites themselves also use glucose for their metabolism and contribute to the hypoglycaemia if they are present in large numbers. Quinine can stimulate the secretion of insulin from the pancreas and in this way can also contribute to hypoglycaemia.

The term "**algid malaria**" (L. "algidus" = cold) is obsolete. The condition is characterized by hypotension with progression to shock. The patient is clammy and often feels cold. There is no fever. Often there is concurrent septicaemia with Gram-negative bacteria. Mortality is high. Therapy with artesunate or quinine, treatment with antibiotics and (cautious, see ARDS) IV fluid administration is of great importance. Shock seldom occurs in malaria if there is no septicaemia. However splenic rupture can also cause hypovolemic shock.

Splenic rupture. This may occur spontaneously or after an unobserved trauma. This complication can occur in *P. falciparum*, *P. vivax, P. ovale* or *P. malariae*. The presence of intraperitoneal fluid is suggestive in this context. In these cases ultrasound can often detect a splenic hematoma, splenic rupture or intraperitoneal fluid. A diagnostic peritoneal lavage may be indicated.

Cerebral malaria is the main cause of death (80 %) in *falciparum malaria*. This complication occurs chiefly in non-immune persons (children, travellers). Cerebral signs include confused behaviour, psychosis, convulsions, stupor, coma, paralysis. Unlike meningitis, there is no real neck stiffness (pain) or photophobia (intolerance to light) but neck retraction and opisthotonos (neck muscle spam) may occur. Sometimes the difference between neck stiffness and neck retraction is not clinically clear. It is typical of the coma that it develops swiftly in 75% of cases and quickly disappears. If a child survives cerebral malaria it has approximately a 10% chance of significant long term sequelae. Children with cerebral malaria and with a normal eye fundus have a good prognosis, while papilledema and retinal bleeding suggest a guarded prognosis. Malarial retinopathy is increasingly considered as a specific diagnostic criteria of cerebral malaria, but sensitivity of this abnormality is rather poor (meaning that its absence does not exclude cerebral involvement). Repeated generalized convulsions should not be regarded as "normal" febrile convulsions. Severe convulsions with contraction of the abdominal muscles and compression of the stomach, may cause reflux of gastric acid and food into the pharynx. Aspiration of gastric contents into the lungs is a real danger as this may result in Mendelson's syndrome (chemical pneumonitis) or aspiration pneumonia. If there are convulsions, these are stopped by administering diazepam (Valium®) IV. A CT scan or MRI scan of the brain of patients with cerebral malaria shows few abnormalities except an occasional increase in cerebral volume. Herniation of the brain stem is a rare event.

If confronted by a febrile coma or confusion with fever in the tropics, glucose must be administered (preferably IV), artemisinin (or quinine if artemisinin unavailable) therapy should be instituted and a lumbar puncture carried out without hesitation (to rule out meningitis). Of the persons who die in hospital due to cerebral malaria, 50% of the fatalities occur within the first 12 hours after admission. At autopsy countless petechiae can be seen in the brain. Small ring-shaped haemorrhages also occur around cerebral blood vessels.

Febrile convulsion

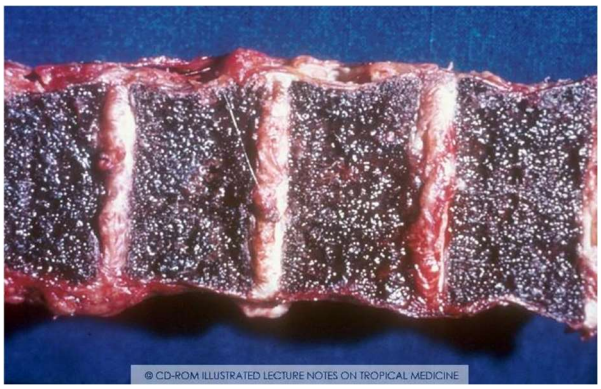
Febrile convulsions are generalized tonic-clonic convulsions. They only occur in children between the age of 6 months and 5 years and will not be repeated during the same fever episode. They occur during the phase in which the fever is rising fast. They always last less than 15 minutes, but post-ictal coma can take up to 1 hour. There is never postictal hemiparesis. It is important to differentiate between febrile convulsions and convulsions during fever (e.g. cerebral malaria, meningitis, cerebral abscess). Approximately 2% of children have a tendency (possibly genetic) for febrile convulsions. The risk that epilepsy will develop in this group of patients is no greater than in children without febrile convulsions. Brief and sporadic attacks have a good prognosis. No maintenance therapy with anti-epileptic agents is required.

Severe anaemia occurs due to haemolysis (of both parasitized and non-parasitized red blood cells – the latter via immune-mediated mechanisms), due to excessive action of the spleen i.e. hypersplenism (until weeks after the infection), due to possible haemorrhages (low blood platelets, splenic rupture) and due to disturbed production of new blood cells in the bone marrow (dyserythropoiesis) due to TNFalpha.

Hyperpyrexia (very high fever above 40°C) should be treated by cooling the patient and administering paracetamol. It is assumed that malaria fever is caused when lysis of the red blood cells releases malaria pigment (hemozoin) as well as GPI-anchors ("malaria toxin") which are absorbed by the reticuloendothelial system. This in turn releases endogenous pyrogens (cytokine network). The concentration of tumour necrosis factor in the peripheral blood correlates with the severity of the malaria. In cases of repeated malaria attacks the liver, spleen and bone marrow are stained black by the enormous amounts of hemozoin. Hyperpyrexia is no longer considered as a criteria of severity (WHO classification of 2000).

Black water fever

Black water fever is a severe life-threatening complication. Acute massive haemolysis occurs through immuno-allergic mechanisms which are not fully understood. It has been observed after taking halofantrine, artemisinin-derivatives and after irregular use of quinine. The parasitaemia is generally very low. There is high fever, jaundice, back pain, shock and very dark urine. Renal insufficiency occurs: the urine production is very low (oliguria) or zero (anuria). Mortality is very high. When quinine was no longer used prophylactically, black water fever became very rare. Differential diagnosis should be made with severe malaria itself, leptospirosis and viral haemorrhagic fever.



Black pigmentation of the bone marrow in the spine, due to accumulaiton of malaria pigment (repeated malaria). Photo Dr Gigaes. Copright ITM

Acute renal failure may also be caused by shock, hypovolemia with reduced renal circulation, disseminated intravascular coagulation (DIC), obstruction of the renal glomeruli by parasitized red blood cells and by the precipitation of released haemoglobin in the kidney (pigment nephropathy). The combination of these factors can result in acute tubular necrosis. Glomerulonephritis may occur in chronic *P. malariae* malaria (cfr. infra), but this complication plays no part in acute renal problems.

Pulmonary oedema is a common complication of severe malaria. The dividing line between

overhydration and dehydration is narrow. Adults easily develop non-cardiogenic pulmonary oedema if there is excessive fluid overload, but on the other hand dehydration and hypovolemia may lead to hypotension, shock and renal failure. Pneumonia is observed quite often if coma lasts for longer than 3 days. ARDS (acute respiratory distress syndrome) may occur. This is caused by diffuse damage to the vascular endothelium and the alveolar epithelium. There is a rapid progression towards dyspnoea, arterial hypoxia, bilateral patchy pulmonary infiltrates due to pulmonary oedema with a protein-rich fluid. The treatment is both etiological and symptomatic: mechanical ventilation, with or without intubation or an endotracheal cannula, possibly with NO (nitrogen monoxide), high-dosed oxygen and positive end-expiratory pressure (PEEP).

Chronic falciparum malaria

Where *P. falciparum* is partially resistant to the therapeutic drug locally used (e.g. chloroquine), the parasite may be suppressed, but will remain present (not completely cleared). This may lead to a whole range of clinical pictures, from asymptomatic parasitaemia through to mild unspecific symptoms, to significant chronic malaise, anaemia and fatigue. Curative therapy with atovaquone/proguanil or artemisinin-based combination therapy (ACT, see below), for example, produces rapid improvement.

Hyperreactive malaria splenomegaly (HMS)

Some adults have a very strong immunological reaction to P. falciparum antigens. The level of IgM in the blood is very high. Due to the polyclonal immune stimulation, all kinds of autoantibodies can appear. Immune complexes are formed, and are removed by the reticulo-endothelial system, which leads to splenomegaly and sometimes hepatomegaly. In these individuals the swollen spleen swells also breaks down normal, non-parasitized red blood cells. The number of parasites is very low, but very high concentrations of anti-P. falciparum antibodies can be detected. The splenomegaly disappears after curative therapy with, e.g. ACT followed by months or even years of adequate malaria chemoprophylaxis if persistent exposure in a malaria region), but recovery is very slow. In rare cases splenectomy is necessary. Steroids have no place in the treatment.

HMS and splenic lymphoma

HMS may be very similar to a certain indolent splenic lymphoma (e.g. splenic lymphoma with villous lymphocytes). The latter disorder is related to B-cell chronic lymphocytic leukaemia and occurs chiefly in elderly persons. The disease is often accompanied by significant cytogenetic abnormalities and monoclonal "villous" B-lymphocytes in the peripheral blood. It is likely that in HMS, excessive stimulation of the B-lymphocytes by malaria antigens increases the risk that oncogenic mutation may occur, followed by clonal growth of these cells.

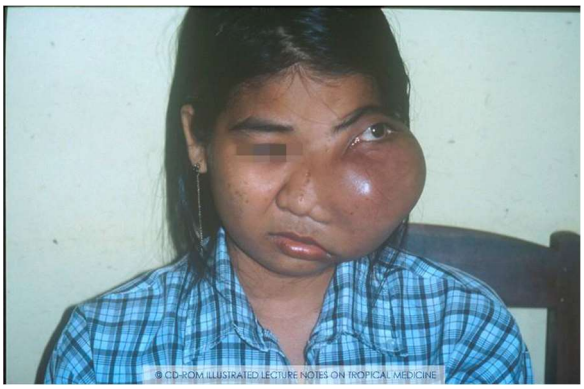
Burkitt's lymphoma

This malignant tumour originates from B-lymphocytes. It is very aggressive with a volume doubling time of about 3 days. The endemic form occurs in sub-Saharan Africa and is also found in Papua New Guinea. In these areas, it accounts for up to 50% of childhood tumours.

One hypothesis states that repeated malaria attacks may have a mitogenic effect on infected Blymphocytes (polyclonal B-cell stimulation) increasing the risk of mistakes during chromosomal replication which subsequently would lead to neoplastic behaviour.

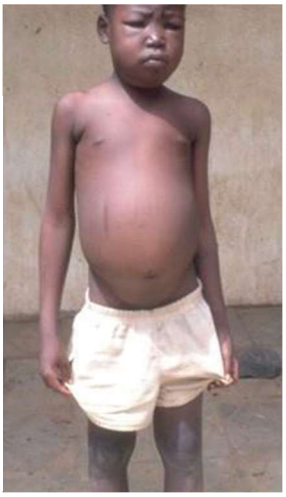
Burkitt's ymphoma generally presents with swelling of the jaw and mouth ulcerations (75%, especially maxilla tumours), abdominal swelling with ascites (60%) and central nervous system involvement (30%, including cranial nerve palsies, malignant pleocytosis or paraplegia). Infection with the Epstein-Barr virus (cf. mononucleosis) plays an important part in the endemic form of Burkitt's lymphoma, probably by causing genetic instability. Epstein-Barr viral DNA is found in about 90% of African Burkitt's lymphomas.

The tumour responds well to cytostatic drugs. The alkylating agent cyclophosphamide (Endoxan®) is first choice (the target dose 1-1.5 gram/m2 IV every 3-4 weeks with 2 doses in remission), but more complex chemotherapies (methotrexate, vincristine, CHOP-R, hyper-CVAD,…) are difficult to evaluate in low-resource settings. About 80% of patients can achieve complete tumour regression and 10% have a partial response. About 50% will relapse.



Burkitt's lymphoma in a Cambodian woman, aspect before chemotherapy. Phoro Dr Lut Lynen, Copyright ITM

Nephotic syndrome secondary to chronic Infection with *Plasmodium malariae.* Notice the swollen face ans ascites. Photo Prof. Gigase. Copyright ITM



Nephrotic syndrome in P. malariae

Chronic infection with P. malariae may, via immunological mechanisms (chronic immune complex glomerulonephritis) cause a nephrotic syndrome, characterized by oedema and proteinuria (more than 3.5 gram per 24 hours). There is often significant hyperlipidaemia and lipid bodies are sometimes found in the urine.

If a kidney biopsy is carried out, it should be borne in mind that severe bleeding will occur in 1% of cases. The treatment of nephrotic syndrome is difficult. Curative malaria treatment is of course indicated but will not produce improvement of the kidney function. Salt restriction and diuretics are indicated (both thiazide and loop diuretics). Treatment with an ACE-inhibitor [angiotensin-converting enzymeinhibitor such as enalapril] should be ideally initiated in settings where it is available. Steroids and immunosuppressive agents are of little benefit in this disorder. An important challenge is to distinguish the entity from minimal change glomerulonephritis (electron microscopy needed to confirm "minimal change" on biopsy specimen).

Diagnosis

General

When can one assert that someone has the disease "malaria"? There are several problems and the question has still not been fully resolved. The demonstration of malaria parasites in the blood is essential but insufficient in itself. Most cases are accompanied by thrombocytopenia and normal white count. Many people will develop an acquired immunity after several years of exposure and may harbour parasites without exhibiting symptoms. The degree of parasitaemia may help, but there is no absolute criterion (the higher the parasitaemia, the more chance that malaria is in fact the diagnosis). There are patients with malaria for whom the thick smear is negative (luckily this is rare in a good laboratory). There are no pathognomonic clinical signs. An accurate diagnosis is becoming more and more important, in view of the increasing resistance of *P. falciparum* and the higher price of modern combination treatments.

Clinical aspects

No single clinical sign allows the diagnosis of malaria. Most cases are accompanied by thrombocytopenia, a normal white count and a positive parasitaemia. Yet malaria must always be considered in cases of fever in the tropics. Since the symptoms can be quite diverse, a clinical diagnosis is unreliable and the diagnosis should be based on identification of the parasite. Microscopic confirmation of the diagnosis is often not possible in many regions and situations. It is of the greatest importance that other important diagnoses are ruled out before instituting a blind anti-malaria therapy. All too often fever is considered as malaria without considering alternative diagnoses. This tendency is reflected in the quote: "if you only have a hammer, you tend to see every problem as a nail" (Abraham Maslow).

The presence of parasites does not rule out an additional diagnosis: e.g. someone with fever may well have some malaria parasites in a thick smear, but this does not rule out meningitis or pyelonephritis. Chronic carriers are people who, although they have malaria parasites in their blood, have no symptoms of this. When such people develop another infection their symptoms are often attributed to the malaria parasites in their blood, although these are not responsible. The absence of parasites in a single preparation does not rule out malaria but does make the diagnosis of P. falciparum highly improbable (if the microscopist searched carefully). Where there is any clinical suspicion it is best to repeat the test 12h later.

Microscopy

A **thick smear** concentrates the parasites 10 to 25 times. It is rather more difficult to interpret than a thin smear preparation and often does not permit species identification. A thick smear contains no intact red blood cells (haemolysis due to the distilled water used in the staining). If a thick smear is positive, a thin smear should be examined.

> *Parasitaemia*
> The parasitic density can also be roughly determined in a thick smear, by counting the number of parasites per 200 leukocytes and multiplying this by 30. It is assumed that on average there are 6000 leukocytes per µl blood and that there is one leukocyte per 500 red blood cells. For example: 5 parasites per leukocyte (1000 parasites for every 200 leukocytes) corresponds to a density of 30,000 parasites per µl. Roughly 30,000 parasites per µl corresponds to a parasitaemia of 1% (5 parasites per 500 RBC's): a moderately anaemic person.

If the thick smear is found to be negative in a reliable laboratory and if there is strong suspicion of malaria, the test is repeated every 12 hours for 48 hours. One great disadvantage of the thick smear method is that reliable technical expertise is needed which should be monitored (e.g. quality control). The argument that a lab technician has carried out the test for years and thus has plenty of experience is absolutely no guarantee of quality or reliability. The test also requires plenty of time if the parasitaemia is low, or before a negative result can be concluded.

A **thin blood film** has many advantages:

- it demonstrates the species present
- detection of mixed infections is possible
- distinguish asexual stages from gametocytes
- assesses parasitaemia (in % of infected red blood cells)
- can detect a new or unexpected parasite
- gives information on red cell morphology
- allows a white cell differential count
- inexpensive

Other points include: Sensitivity and specificity is operator dependent. In a good average lab, the sensitivity is good but limited to about 50 parasites per µL, this is somewhat better in a reference lab. Most routine laboratories cannot detect parasitaemia below 100 to 500 parasites per µL. DNA amplification techniques have better sensitivity and can give information when species is in doubt but this technique remains limited to reference laboratories (even in high resource settings).

If the parasite cannot be identified it is regarded as a P. falciparum as a safety precaution. Mixed infections do occur.

Antigen detection

Malaria rapid diagnostic tests (RDTs) based on lateral-flow immunochromatography are increasingly used in endemic and non-endemic settings. They are easy to use, provide results rapidly and require no specific training and equipment. Reported sensitivities vary between

different RDT products but are generally good for Plasmodium falciparum, with rapid tests based on the recognition of *P. falciparum* antigen **histidine-rich protein-2** (PfHRP2) scoring slightly better than those which recognize *P. falciparum*-**lactate dehydrogenase** (LDH). Sensitivity is lower for *Plasmodium vivax* (66 – 88%) and usually poor for *Plasmodium ovale* (55 – 85%) and *Plasmodium malariae* (21 – 45%). Rapid diagnostic tests have some limitations. The test strips are susceptible to heat and humidity. A positive result can be obtained after correct treatment, when there are no more parasites visible in the thick blood smear.
This is due to persistence of the PfHRP2 antigen (up to several weeks) after successful treatment. The pLDH based tests have the advantage of turning negative sooner after parasite clearance (several days). Occasionally there is cross-reactivity of P. falciparum with the non-falciparum test line and vice versa and rare false-positive reactions due to other infectious agents or immunological factors. False negative results occur in the case of low parasite densities, prozone effect (saturation of binding sites due hyperparasitaemia) or pfhrp2 gene deletions as observed in Pf strains from South America, but also in Mali, DRC and India. The latter two reasons for false negativity are only observed with HRP2-based RDTs. Finally when instructions are not followed (delayed reading, incorrect sample and buffer volumes, not recognizing invalid test results, disregarding faint test lines) errors in interpretation can occur. **Rapid diagnostic tests do not give information about parasite density.**

Depolarized light scatter

Automated cell counters, such as certain Cell-Dyn instruments, use 90° depolarized light scatter to distinguish eosinophils from other leukocytes. Eosinophils are normally the only leukocytes that depolarize light. Some automated haematology analyzers display an alert for possible malaria based on the presence of activated monocytes (Coulter Counter), hemozoin containing white blood cells (Cell-Dyn series) and an additional peak in the reticulocyte fraction (Cell-Dyn series). During malaria infection, the parasites consume haemoglobin and produce malariapigment, a form of polymerized haeme. This pigment, also known as hemozoin, is birefringent. When peripheral blood is analyzed by automated flow cytometry, the pigment will cause atypical depolarization of the laser beam that can be recognized in a scatterplot. Although diagnostic accuracy of these features is too low to exclusively rely on these flags for malaria diagnosis, such an alert is especially useful in situations where the initial clinical suspicion of malaria is low (non-endemic setting).

PCR

At present, in case of doubt, mixed infections, low parasitaemia, forensic questions, suspicion of zoonotic malaria, etc… PCR technology (e.g. multiplex real-time PCR) can give answers to several questions, but is in general slower than the traditional methods since such tests are not performed everyday even in larger centres. However, point-of-care PCR based techniques are being developed and their importance might grow in the future in countries contemplating malaria elimination, especially if this technique can combine detection of multiple infectious agents (multiplex-PCR). The future will learn whether they will have a place in diagnosis even in low-resource settings.

Serology

Serology can only be carried out in reference hospitals and is of no importance for the individual diagnosis in acute fever. The antibodies are positive from the tenth day therefore at the beginning of the attack they will be negative. The presence of antibodies only shows that

there has been contact with the parasite. This does not mean that there is immunity. There will be high titers of antibodies in the tropical hyperreactive malaria splenomegaly. Malaria type IgG antibodies penetrate the placenta and will give the neonate temporary and partial protection against malaria during the first months of life. Antibodies after infection remain positive for a longer time.

Indirect aspects

Signs of haemolysis include yellow serum, dark urine while faeces have a normal colour, elevated indirect bilirubinaemia and low haptoglobin. Often there is thrombocytopenia. Sometimes there is malaria pigment in white blood cells (sign of severity).

Test therapy

In endemic regions fever, muscle pain or even generally feeling unwell are often attributed to "malaria". An anti-malaria treatment is then instituted, without obtaining confirmation of the diagnosis or often even without considering alternative diseases. The argument given is that such a treatment can do no harm, that the diagnosis of malaria is always probable because the disease is common and that this is a good strategy for first-line care. Each of these arguments can be defended to a certain extent, but in this way often useless and sometimes expensive treatments with potential side effects are administered. In addition, not recognizing and treating other diseases (borreliosis, rickettsiosis, kidney infections, amoebic liver abscess, pneumonia, sepsis and so on) is a daily reality in many tropical regions. The over-diagnosis of malaria often leads to under-diagnosis of other treatable disorders. It is sometimes stated that fever which does not disappear after three days of adequate therapy, is not malaria. This may however not be completely true, in case of drug-resistant malaria (resistance R3, with no decrease in the parasite load during treatment) or co-infection with another pathogen (commonly sepsis).

In face of the increasing resistance to P. falciparum parasite and the need of more complex and expensive treatment (ACT), WHO recommends since 2010 the diagnosis of malaria being parasitebased as often as possible either by microscopy or antigen-based RDTs. Ideally no malaria treatment should be provided without confirmation of the diagnosis.

Treatment

Specific anti-malaria drugs

General

Most people are not very interested in the history of a particular medicine. Quinine, however, is rather different and occupies a special place. For 300 years this was the only specific treatment for malaria. The story of its discovery, the important part which quinine has played in the colonization of the tropics, its role in both World Wars and during the Vietnam war, and the present come-back of this product all make it unique. At present quinine and related products are used in the treatment of P. falciparum malaria, as an antiarrhythmic, as a muscle relaxant and as a flavouring (Schweppes!)[1]. There are also some minor applications such as the treatment of babesiosis. Quinine is obtained from the bark of Cinchona trees.

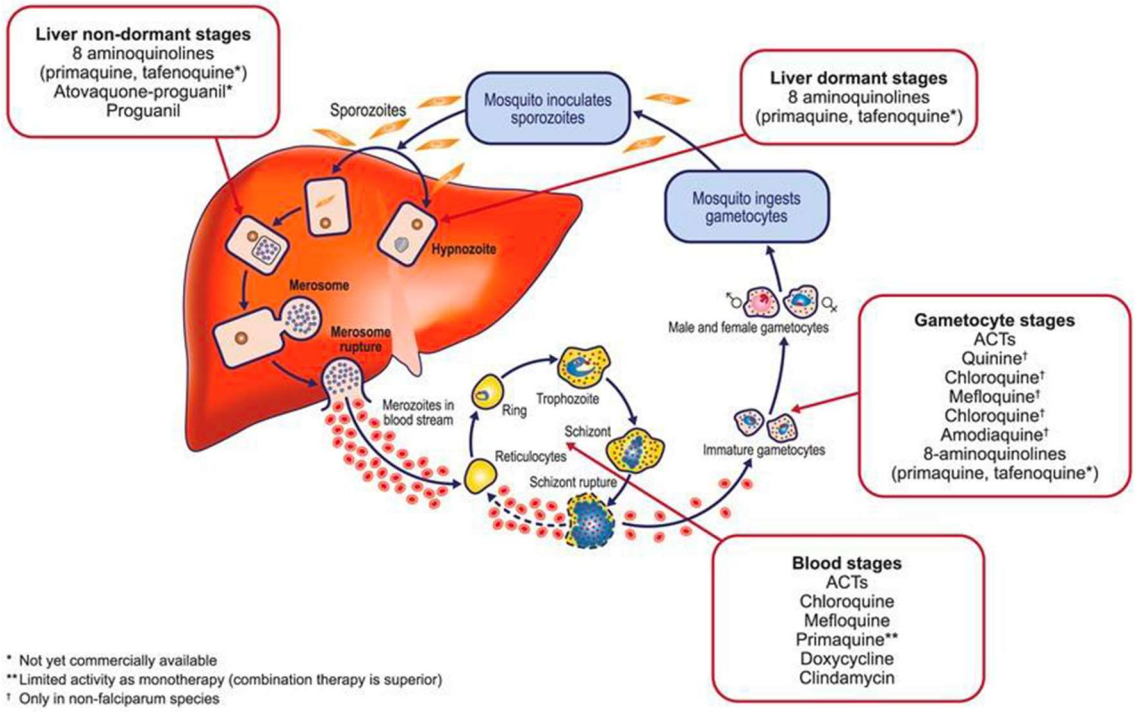
[1] It is remarkable that also qinghaosu, wormwood, the base for the artemisinin derivates is used in an Italian aperitif called Cinzano (which is a phonetic equivalent of quinguasu).

In 1934 resoquine was discovered by the German H. Andersag. Only after the allies took North Africa there was renewed interest in the product. It was renamed chloroquine. Preparation in the laboratory was also economically viable. It quickly became the first choice agent and quinine was pushed into the background. In 1950 in Brazil, Mario Pinotti introduced the strategy of adding chloroquine to cooking salt (as was also done with iodine).

The synthetic preparation of primaquine was perfected after the war. The British war programme led to the development of proguanil, which itself served as a model for the development of pyrimethamine. Pyrimethamine in combination with sulphadoxine was introduced in 1970 under the name Fansidar®. After World War II it was hoped that malaria would be definitively eradicated. The use of chloroquine and the world-wide campaign to eradicate malaria by the World Health Organization, led initially to a considerable reduction in malaria infections all over the world. After the anti-malaria campaign vanished due to various circumstances, the resistance of *Anopheles* to various insecticides and the development of chloroquine-resistant and multi-resistant *P. falciparum*, malaria once more became one of the major problems.

Whereas World War II led to the discovery of some new anti-malaria agents, the Vietnam war stimulated a huge programme for the discovery of new drugs. The Walter Reed Army Institute of Research of the United States army investigated thousands of constituents. This research resulted in mefloquine (Lariam®) and halofantrine (Halfan®). Research in China produced artemisinin, pyronaridine and benflumetol.

Treatment overview



P. vivax life cycle and site of action for different antimalarials.
Source: Quique Bassat, PLoS Neglected Tropical Diseases 5(12):e1325, dec 2011

Broadly speaking, anti-malaria drugs can be divided into four major classes

- Blood schizonticides
- Antifolates
- Antimitochondrials
- Redox process-based agents

Blood schizonticides

When the malaria parasite leaves the liver and penetrates an erythrocyte, it can begin a haemoglobin diet. Chloroquine, quinine, mefloquine and halofantrine interfere with the detoxification of haemin in the digestive vacuole of the parasite, so that haemin can generate free radicals and parasitic membrane damage follows. It is therefore logical that the drugs are not active against the parasitic stages which precede the blood forms (sporozoites, liver forms) and which do not consume haemoglobin.

Antifolates

Folic acid is an important metabolic factor. Humans obtain this vitamin from the food they eat. The malaria parasite must produce it for itself. Para-aminobenzoic acid (PABA) is used at an early stage of the biosynthesis of folic acid by the enzyme dihydropteroate synthetase. This step is inhibited by structural analogues of PABA, such as sulphonamides and sulphones, e.g. sulphanilamide, sulphadoxine and dapsone.

The next synthesis step is catalysed by dihydrofolate reductase. This step is prevented by pyrimethamine, trimethoprim and cycloguanil (prodrug = proguanil), to such an extent that tetrahydrofolate - the end product - is not formed. The combination of these two sequential inhibitors forms the basis of Fansidar® (similar to cotrimoxazole). Resistance to both antifolates easily develops.nA specific point mutation in each gene (dhps and dhfr) is sufficient.

Antimitochondrial products

Although artemisinin derivatives and 8-aminoquinolines (primaquine and tafenoquine) cause mitochondrial swelling, this organelle is not their chief target. Some antibiotics such as tetracycline and clindamycin prevent protein synthesis by mitochondrial ribosomes (these are similar to the ribosomes found in bacteria). They are slow-acting.

Atovaquone is a naphthoquinone which specifically destroys the electron transport chains of Apicomplexa. The molecule is similar to ubiquinone (coenzyme Q) which plays a role in the energy transfer between cytochrome B and C1. The enzymes of Plasmodium falciparum are 1000 times more sensitive to atovaquone than the corresponding enzymes in humans. Resistance can easily develop if used in monotherapy.

Redox reactions

Primaquine and tafenoquine exercise their action via redox-active quinone metabolites. They are selectively toxic for the pre-erythrocytic stages and are the only medicaments which kill hypnozoites. Tafenoquine has in addition a pronounced blood schizonticidal action.

Current treatment of malaria

A summary of the WHO recommendations in 2020 is provided first in these notes for clarity. For detailed dosages and special groups, see additional information in "Guidelines for the treatment of malaria: WHO; third edition, 2015.

All drugs used currently or in the recent past are described in detail below the summary.

- Complicated malaria (whatever the species, and also in all risk groups)
    - First choice: Artesunate IV (2.4 mg/kg in adults and children > 20 kg; 3 mg/kg in children < 20 kg)
    - Second choice (only if artesunate not available): quinine IV (see dosage below)

- Uncomplicated malaria (whatever the species)
    - Artemisinin-based combination treatment (ACT); five ACTs are currently accepted; all are in fixeddose combination (FDC) nowadays and consist of 3-day regimen:

        1. Artemether-lumefantrine
        2. Artesunate-amodiaquine
        3. Artesunate-mefloquine
        4. Artenimol (dihydroartemisinin)-piperaquine
        5. Artesunate + sulfadoxine-pyrimethamine (SP)

NB1: In low-endemic countries, a single dose of primaquine (0.25 mg/kg) should be added at the end of the ACT to decrease transmission (no need of G6PD determination)

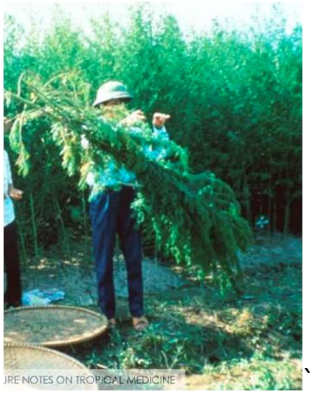
NB2 : Chloroquine (see dosage below) is a good alternative for uncomplicated P. vivax, P. ovale, P. malariae and P. knowlesi in areas where no resistance is reported

NB3 : Primaquine (30 mg/day for 14 days) should be administered in case of P. vivax and P. ovale infections, after determination of the G6PD activity (alternative regimens available in case of low activity)

Anti-malaria drugs

Qinghaosu and Artemisinin derivatives

Artemisia annua in Vietnam. This plant is harvested to extract artemisinin from the leaves. Copyright Charles Lugt (with special thanks to prof Kager).



Artemisia annua in Vietnam. This plant is harvested to extract artemisinin, used for malaria treatment Copyright Charles Lugt (with special thanks to prof Kager).

Artemisinin and its derivatives have become essential components of antimalarial treatment. ACTs are now recommended by WHO as the first-line treatment for all falciparum malaria in malaria endemic countries. These plant-derived peroxides are unique among antimalarial drugs in killing the young intra-erythrocytic malaria parasites, thereby preventing the more pathogenic mature stages. Huang hua hao or qinghaosu ("essence of qinghao") originates from a Chinese plant, Artemisia annua (sweet wormwood). The antimalarial properties of the traditional Chinese medicine qinghaosu were discovered and developed by Chinese scientists in 1971 (secret "project 523"). This research effort was prompted by the requests of Ho Chi Minh to Zhou En Lai for antimalarial drugs for the Vietnamese troops (cfr the efforts of the American forces to develop halofantrin and mefloquin).

Artemisinin has the derivatives artesunate (the hemisuccinate; -CO(CH2)2COOH), arteether (the ethyl ether; -OCH2CH3), artemether (the methyl ether; -OCH3) and the reduced substance artenimol, syn. for dihydroartemisinin. Their plasma half-life is very short: 1 hour, both in healthy volunteers and in patients with active malaria.

Artemisinins are not active upon liver stages, but upon both the immature sexual and the all asexual blood stages. Their broad stage specificity (as opposed to quinine) has several therapeutic consequences. Killing young circulating ring-shaped trophozoites results in a more rapid reduction in parasitaemia as compared to other antimalarials and reduces the number of parasites that mature and sequester in the post-capillary venules. Quinine does not stop sequestration since it acts on the mature parasite stages, which have already adhered to the vascular endothelium. Since artemisinin reduces the number of gametocyte carriers, it helps to prevent malaria transmission, although artemisinin does not kill mature gametocytes of P. falciparum. In low-transmission areas, where symptomatic infection constitutes the main source of transmission, ACTs reduce gametocyte carrier rate, and if widely employed is expected to reduce the incidence of malaria. Artemether, artesunate and dihydroartemisinin reduce the number of parasites by a factor of approximately 10,000 for each asexual cycle. After two cycles (3-day treatment) there is a 108-fold reduction of the parasitaemia. The longer acting partner drug will then eliminate the remaining low numbers of parasites.

The medication is best avoided during the first trimester of pregnancy, if a good alternative is available (quinine + clindamycin). Recent large studies (PREGACT) have demonstrated the safety of ACT administered during the second and third trimesters on pregnancy and infant outcome. In observational studies of pregnant women treated with artemisinin derivatives during the first trimester, no differences were noticed in the risk of miscarriage, stillbirth or congenital anomalies when compared to quinine treatment. Although data are limited, the use of ACTs is probably safe throughout gestation, especially if alternatives are not available.

Artemether (Paluther®, Arteminth®, Cotexcin®, Artenam®) is an oil-soluble derivative that can be used for IM administration.

Artesunate (Artenam®, Artesunate®, Arsumax®, Artemax®, Arinate®, Plasmotrim®) is the fastestacting artemisinin derivative. It can be administered parenterally (IV, IM), rectally or orally. For IV use the dose is 2.4 mg/kg as start dose. This dose is repeated at least after 12 hours and 24 hours. The side-effects are mild and are difficult to distinguish from the effects of malaria itself. However, delayed onset haemolytic anaemia has been observed in about 20%

of travellers who receive artesunate after about 2 weeks. This post-artesunate delayed haemolysis is also described in endemic countries. Haemoglobin monitoring 1 and 2 weeks after artesunate administration is strongly recommended for this reason, particularly after an episode of severe malaria.

There is now strong pharmacological and clinical evidence that artesunate is superior over quinine for treating severe malaria (35% reduction of fatalities in Asian adults and 22% reduction of fatalities in African children). If patients with severe malaria cannot be treated orally and transport to a hospital for IV therapy will take more than 6 hours, a single inexpensive artesunate suppository at the time of referral substantially reduces the risk of death or permanent disability. A single dose of artesunate, given rectally (by e.g. parent), can provide parasiticidal blood concentrations within 10–20 min and can already halve parasitaemia numbers within 6–12 h.

Artenimol (more commonly named dihydroartemisinin) is obtained by reduction (hydrogen addition) of artemisinin. Together with piperaquine it is available as a fixed drug combination known as Eurartesim®. Artenimol has a short half-life, as opposed to piperaquine which has a long half-life.

After a decade of use in monotherapy in Southeast Asian countries, it has become clear that monotherapy would quickly lead to resistance to artemisinin derivatives (5-10% recrudescence after 7 days of monotherapy). Since 2005, to protect this “last-line” drug, WHO has strongly recommended to systematically combine artemisinin with another, partner drug with a longer half-life to treat all falciparum malaria in endemic countries. “Accepted” partner drugs are amodiaquine, pyrimethamine/sulphadoxine, lumefantrine, piperaquine or mefloquine (see other drugs). New ACT compound are emerging.

Artemisinins also have some activity against other parasites, for example they kill the young stages of trematodes such as schistosomes and Fasciola. They are studied also in animal models of clonorchiasis.

Lumefantrine or Benflumetol

Lumefantrine (= benflumetol) was registered in China in 1987 for the treatment of P. falciparum malaria. The half-life in the blood is approximately 4 days. The product is not active on the liver stages or gametocytes. Lumefantrine, like chloroquine, probably destroys heme polymerization (a detoxifying pathway for the parasite). It is synergistic with artemether. The combination artemether-lumefantrine is known as co-artemether (AL; Riamet®, Coartem®: artemether 20 mg/lumefantrine 120 mg, adul dose 2x4 tablets/d for 3 days). The combination artemether-lumefantrin is probably the most used ACT worldwide.

The possibility of drug-interaction and QTc-prolongation needs to be studied further, especially if this product would be used as stand-by medication in travellers to the tropics who also might take certain quinolones, azoles, macrolides or prokinetics (domperidone).

Absorption in the intestine is highly variable from person to person and is greatly increased (up to 16fold) by fatty food. Since people who are ill generally do not eat much, this has important consequences. Early in the treatment very little lumefantrine is absorbed. In

combinations, such as Coartem, the artemether is responsible for the initial important reduction in the number of parasites and the low residual numbers of parasites is then cleared up by lumefantrine.

In HIV-infected children, lopinavir-ritonavir-based ART (Kaletra) was associated with a decreased incidence of recurrent malaria (reinfection) as compared to an NNRTI-based regimen, largely because of an interactions that increases drug levels of lumefantrine.

Piperaquine

Piperaquine is a Chinese synthetic drug belonging to the bisquinolines. Half-life of piperaquine is 9 days. Piperaquine is a highly lipid-soluble drug. The combination dihydroartemisinin (artenimol) 40 mg with piperaquine 320 mg per tablet (Artekin®, Eurartesim®, Duo-cotecxin®, adult dose: 1x4 tablets/day for 3 days) is increasingly used in first-line in many endemic countries.

In 2006 Papua New Guinea became the first country to implement dihydroartemisinin-piperaquine treatment for P. falciparum and P. vivax infection in pregnant women during the second and third trimesters as well as its first-line therapy for any case of malaria. Because of the slow elimination of piperaquine, this treatment provides up to 6 weeks posttreatment prophylaxis against new infections and relapsing P. vivax infection (better than all other ACTs). It is recommended in travel medicine to check first an ECG to exclude an underlying QTc prolongation in people with serious liver, kidney or heart diseases or in people taking other QTc prolongating medication (macrolides, fluoroquinolone, domperidone, …). It is contra-indicated if > 500 msec and to be used withcaution if QTc > 450 msec.

Amodiaquine

Amodiaquine is closely related to chloroquine. Long-term use causes grey skin pigmentation in white people. Sometimes there are severe side effects (agranulocytosis in approximately 1/2000, liver toxicity in approximately 1/15,000). Amodiaquine (Camoquine®, Flavoquine®, Malarid®) had been rarely used in monotherapy. There is therefore less resistance to amodiaquine than to chloroquine. Since the product is eliminated slowly, a single dose of 600 mg was (and is) sufficient.

Amodiaquine is nowadays the partner drug of artesunate in one of the 5 recommended ACTs. This therapy exists now in fixed-drug combination (Coarsucam®, ASAQ: 100 mg artesunate/270 mg amodiaquine, adult dose: 1x2 tablets/d on 3 consecutive days) and because of its low price, has become the first-line ACT for P. falciparum in many African countries.

Mefloquine

Mefloquine (Lariam®) is a long-acting product. After 2 to 3 weeks half of the dose is still present in the body. Mefloquine has a rather slow onset of action. For curative use, mefloquine is always combined with other antimalarials, and its use in monotherapy for treatment is now strongly discouraged (major side effects, while effective alternatives exist). The combination mefloquine + pyrimethamine + sulphadoxine is known as Fansimef®. Now, mefloquine is used with artesunate in a fixed-drug combination and is one of the first-line

therapies of Pf malaria in many countries: artesunate 100 mg/mefloquine 220 mg (ASMQ), 1x2 tables/d for 3 consecutive days (adult dose).

Mefloquine plays an important (although decreasing) role in prophylaxis: cfr. infra.

Quinine

Quinine has long been a first line anti-malarial drug and was for a long time one of the only parenteral treatment options. More recent studies however, showed clinical benefit of parenteral Artesunate and oral artemisinin combination treatment over quinine, together with less side effects. Quinine is still a powerful product, which acts upon the schizonts of the parasites in the blood (it is a schizonticide). It thus acts chiefly in the second half of the maturation cycle: on the parasites which are sequestered in the small blood vessels (not on the young ring forms in the peripheral circulation). Quinine also possesses gametocytocidal activity against P. vivax, P. malariae and P. ovale (but not against gametocytes of P. falciparum). As for chloroquine, quinine causes an inhibition of hemozoin biocrystallization in the heme detoxification pathway, which facilitates the aggregation of cytotoxic heme. Free cytotoxic heme accumulates in the parasites causing their deaths.

Quinine sulphate is administered orally. It is absorbed well in the intestines. Quinine bihydrochloride is injected, preferably by slow IV (infusion with glucose because of the risk of hypoglycaemia). IM injections may lead to sterile abscesses but can be used where necessary if there are no alternatives available. For IM injection, it is best to use a diluted solution (60 to 100 mg/ml) instead of the concentrated solution (300 mg/ml). Quinine administered via IM injection is absorbed well even in severe malaria. Treatment with quinine is unpleasant (bitter taste, cinchonism) and poor compliance after the acute phase is common.

Treatment regimens

The basic regimen is 10 mg salt/kg, every 8 hours, orally or slow IV. Currently, a loading dose of 20 mg/kg IV over 4 to 8 hours is universally recommended for the first administration (followed by 10 mg/kg every 8 hours). This should be continued for at least 4 days, preferably 7 to 10 days (if used in monotherapy). This is an unpleasant treatment. Because there is still a risk of relapse if quinine is used in monotherapy even for > 7 days, another product is generally combined with it, e.g. tetracycline or clindamycin. This allows also to shorten the quinine administration to 4-5 days. Sometimes treatment with Fansidar® is given after a few days, which shortens the treatment period, but only in regions where this drug is still sufficiently effective. If a patient vomits within an hour after swallowing the medication, the whole dose should be repeated. If vomiting occurs longer than one hour after ingestion, no new dose is necessary. In case of repeated vomiting IV administration is required.

Side effects of quinine

Quinine is a substance with highly irritating properties (also for the gastric mucosa: nausea is not uncommon). Capsules are therefore best taken after a meal. Quinine increases the secretion of insulin from the pancreas, increasing the risk of hypoglycaemia. Quinine allergy is not common. What is common is a range of side effects such as tinnitus, temporary deafness for high frequencies, headache, nausea and palpitations. These toxic phenomena are known as cinchonism: quinine was first isolated from the bark of the cinchona tree. This reduces the patient's compliance.

Quinine increases irritability of the pregnant uterus. In case of need one must not hesitate to use quinine in a pregnant woman with malaria (malaria itself can lead to abortion, preterm labour or death in utero). To prevent an impending premature labour, a tocolytic agent can be given such as the beta 2mimetic ritodrine, fenoterol or salbutamol. The calcium antagonist nifedipine is as effective a tocolyticum as the beta-mimetics. Prolongation of the PR, QRS and QT intervals may occur during the use of quinine (as with quinidine). If the patient has atrial fibrillation, conversion to sinus rhythm may occur with possibly arterial embolic complications. Atrial fibrillation which has already been present for more than 48 hours is a contra-indication for quinine. Congenital long QT syndrome and Brugada syndrome are equally formal contra-indications for using quinine. ECG monitoring to detect QTcprolongation is recommended during quinine therapy, especially in case of kidney failure.

Overdose of quinine may lead to very severe situations such as deafness, delirium, bradycardia, hypotension, respiratory arrest or death (lethal dose approximately 8 gram). Overdose may also lead to blindness via a direct toxic effect on the retina and possibly also due to spasms of the retinal blood vessels and subsequent retinal ischemia.

Quinine and Gin Tonic

Unlike the majority of other bitter products which occur naturally, the bitter taste of quinine is short-acting with no annoying after-taste. It is therefore used as a flavouring to produce tonic water. The British colonialists in India often drank gin and tonic. The present-day tonic water contains approximately 15 mg per litre, however, only enough to give a bitter taste. Copious drinking of gin and tonic in order to prevent malaria, is thus only an excuse for drinking gin.

Why is quinine resistance still rare?

The product has been used for more than 360 years. This is in stark contrast to the resistance to other malaria drugs or antibiotic resistance in bacteria where the "useful life" of a product is measured in years or a few decades. The concept of a standard dose was only developed in the twentieth century. Earlier the duration of treatment and the dosage were left to the discretion of the doctor. This together with the fact that the concentrations of alkaloidsvaried greatly from plant to plant and that quinine was never pure, meant that malaria was treated with a therapy which must have produced the most varied blood levels. Yet no wide spread quinine resistance has been reported. The answer to the question why there is virtually no quinine resistance, could be very important. Is the target molecule of quinine so special that mutation is not possible? It would then be very helpful to know this target. It could also be that there is quinine resistance, but that it was not, and has not been recognized. However, this is doubtful. Is it that the present recommended dose is much higher than that which was formerly necessary? Is it the fact that "quinine" is actually a mixture of various active products, which prevents resistance developing? Resistance to combined therapy requires multiple, simultaneous mutations which is less readily achieved than that to single products. It is possible that quinine has not previously been used at levels which create sufficient evolutionary pressure. The majority of malaria cases in Europe and America were P. vivax infections. Even in British India, P. vivax represented the lion's share

of infections. In P. falciparum endemic regions, only a few fortunate people were able to take quinine and then only when they had to (because of unpleasant side effects). Few used quinine as a prophylactic agent (especially among the indigenous population). What is more, quinine has a short half-life, so that the parasite was only exposed to subtherapeutic concentrations for a short time. Probably its limited use is the reason for the absence of resistance, but if used on a larger scale, quinine resistance may yet become a reality in years to come.

Chloroquine

Despite the presence of this resistance, chloroquine still has a place in treatment. It is still active against non-falciparum plasmodium species almost everywhere and could theoretically still be used against chloroquine-sensitive *P. falciparum* in very limited areas: Central America and the Caribbean. Elsewhere, chloroquine is not recommended any more against P. falciparum even in immune patients, who do not usually appear very ill.

The trophozoite in the red blood cell breaks down haemoglobin using lysosomal enzymes. In this digestive process ferriprotoporphyrin IX (haemin) is formed, which is toxic to the parasite and is usually polymerized to non-toxic malaria pigment. Chloroquine binds to ferriprotoporphyrin IX and prevents detoxification.

Since the liver parasite do not feed on haemoglobin this drug is not active at the pre-erythrocytic stages of Plasmodium sp.

Chloroquine is available in tablet form as chloroquine sulphate (Nivaquine®) and as chloroquine diphosphate (Resochine®). Hydroxychloroquine sulphate (Plaquenil®) is different and is used in e.g. rheumatoid arthritis, lupus erythematosus and Q-fever. The injectable form is chloroquine dihydrochloride. Nivaquine® tablets contain 100 mg chloroquine, but availability of this drug has decreased over the last years.

Chloroquine is a powerful schizonticide. It has strong affinity for various tissues and organs. It is fastacting and remains in the blood for many days. A brief treatment (3 days) is therefore possible.

Chloroquine may be given orally, SC, IM or SLOW IV (infusion). Never inject an ampoule of chloroquine IV rapidly as a bolus or rapid infusion (fatal arrhythmia). The injections are not painful.

There are several different treatment regimens. Most of the time it is given orally, 25 mg/kg spread over three days. Parenteral administration should be discontinued as soon as oral administration is possible.

Chloroquine is cheap and not very toxic in normal use.

- Some people are allergic (pruritus, rash) or suffer nausea.
- People with psoriasis are more at risk of side effects.
- A reversible precipitation of chloroquine in the cornea may occur, resulting in small opacities.

- This may result in seeing haloes around objects, blurred vision or photophobia. This form ofnkeratopathy may become manifest quite rapidly (a few weeks after beginning treatment). After discontinuing the medication it is completely reversible.
- Chloroquine accumulates in melanin-containing tissues. Chronic use may lead to abnormalities of the choroid and retina (chorioretinitis). This toxic retinopathy is not reversible. The abnormalities are always bilateral and symmetrical. Often there is maculopathy (bull's eye lesion) with central and paracentral scotomata, but constriction of the peripheral field of vision may also occur. The total cumulative dose before such problems occur is generally 100 gram chloroquine or more.
- Chloroquine has a narrow safety margin (just 30 mg/kg may be fatal). In case of overdosage myocardial depression, hypotension and severe arrhythmias may occur. ST-segment abnormalities and T-wave inversion occur. Broadening of the QRS complex (>0.12") and ventricular arrhythmias have a poor prognosis. The patient may become comatose, vomit and aspirate stomach contents. In acute intoxication diazepam is given (Valium® 1 mg/kg) and adrenalin (= epinephrine) or dopamine if these are available.

Pyrimethamine + / - Sulphonamides

Fansidar® is a combination product of pyrimethamine 25 mg and sulphadoxine 500 mg per tablet (Mekalfin® is another commercial name). The curative treatment for an adult is 3 tablets taken as a single dose. Sulphadoxine is a long-acting sulphonamide (t½ = 8 days) which in case of allergy may cause severe skin lesions (erythema multiforme and Stevens-Johnson syndrome). Plasmodium falciparum has rapidly developed resistance to this product in many parts of the world. It is not used any more as monotherapy but may be combined to artesunate (at least in regions where no resistance has been observed): Sulfamon®, Artecospe adult® (AS+SP) = artesunate 100 mg + sulphadoxine/pyrimethamine 500/25 mg: 1x2 tablets AS/d for 3 days + 3 tablets SP single dose. This combined treatment is available in co-blister packs (this is not the same as coformulated tablets!).
Fansidar is also widely used as intermittent preventive treatment for pregnant woman in Africa (either they present with blood parasite or not, once or twice during pregnancy) and still provide substantial benefit in preventing maternal and infant anaemia and low-weight birth (even in areas with increasing resistance). Though recent studies comparing dihydroartemisine-piperaquine (and other artemisininbased combination therapies [ACTs]) vs pyrimethamine-sulphadoxine as intermittent preventive treatment during pregnancy (IPTp), showed that ACTs are usually superior in decreasing the malaria burden during pregnancy. Use of ACT in IPTp is however not yet a WHO recommendation, pending results on the long-term risk of developing resistance.

Halofantrine (Halfan®)

This is fast-acting, effective and has few but potentially lethal side effects. Given a series of casualties, it is no longer used and production has been abandoned. It has been replaced by a similar but non-toxic product: lumefantrin. Halofantrin was very dangerous in people with a long QT interval: reportedly lumefantrin does not present the same toxicity, but this deadly experience with halofantrin makes clinicians very cautious, ordering always an ECG before treatment whenever possible and almost always in high income settings.

Primaquine

Primaquine is an 8-aminoquinoline. It is inactive upon asexual blood forms. It does have an important though only partial causal prophylactic effect (on both P. falciparum and P. vivax) but only if it is taken 24-48 hours (max. 96 hours) after inoculation with sporozoites. It acts upon the exo-erythrocytic stages of the parasites (liver schizonts). The half-life is relatively short (4 hours). For causal prophylactic use a daily dose of 15-30 mg may be taken. These regimens are not very popular and there has been little experience of them. Chemoprophylaxis with primaquine can be stopped 3 days after leaving a malarious area.

In cases of P. vivax or P. ovale malaria, hypnozoites remain in the liver after therapy with ACT or chloroquine/quinine. These may be destroyed by primaquine. In the past, 15 mg base per day was used for 14 days [26 mg primaquine biphosphate = 15 mg primaquine base], but current medical opinion favours 30 mg per day for 2 weeks (increasing tolerance of some P. vivax strains). This drug is contraindicated in pregnant women and in people with a significant deficiency of G6PD (glucose-6-phosphate dehydrogenase), an enzyme in the red blood cells (risk of haemolysis in patient and/or fetus).

Primaquine also acts on P. falciparum gametocytes. Therefore, in some circumstances (e.g. refugee camps) it may be given to reduce transmission (single dose of 45 mg). It is nowadays thoroughly investigated (in low dosage) as a potential strategy to decrease/suppress transmission in low-endemic areas contemplating elimination. Detection of underlying G6PD-deficiency is however a major hurdle for its use on a larger scale. Reliable point-of-care tests to detect G6PD deficiency would remediate this problem. Several low-endemic countries have already adopted the systematic administration of primaquine (0.25 mg/kg) at the end of the course of antimalarials/ACT administered to treat a clinical malaria episode. Mild methaemoglobinemia is often observed with primaquine but rarely with clinical consequences.

Tafenoquine or Etaquine

Etaquine or tafenoquine is a new 8-aminoquinoline, derived from primaquine. It has a half-life of two weeks, which is much longer than the half-life of primaquine. It may be taken orally and has low toxicity. It is active against P. falciparum and P. vivax. It is an effective schizonticide and is also active on the preerythrocytic stages, including the hypnozoites of P. vivax. Screening for G6PD deficiency is also required and this is always a limiting factor in low-resource settings. Tafenoquine has been approved in 2018 for the radical (relapse-preventing) treatment of P. vivax and P. ovale malaria (single dose of 300 mg just after the treatment of the clinical episode) and for malaria chemoprophylaxis (200 mg weekly). The experience of this new drug in not yet that large in clinical practice, but it is expected that replace primaquine soon, due to its much shorter/easier administration.

Proguanil and Chlorproguanil

Proguanil (Paludrine®) and chlorproguanil (Lapudrine®) are biguanides which are converted in the body to the active product cycloguanil.

The combination of chlorproguanil with dapsone is also known as Lapdap®. It is used as a cheap, shorthalf-life antifolate. It may be combined with artesunate (combination known as "CDA or Chlorproguanil-Dapsone-Artesunate"). In Malarone®, proguanil is combined with

atovaquone and both drugs have a synergetic effect explaining its increased efficacy (despite the use of two drugs with moderate activity).

Atovaquone

Atovaquone (Wellvone®, Mepron®) is a lipophilic hydroxynaphthoquinone. Atovaquone is a powerful schizonticide for P. falciparum and P. vivax. On monotherapy recrudescence occurs very quickly. To avoid this problem, it is combined with proguanil (brand name of the atovaquone + proguanil combination = Malarone®). Atovaquone/proguanil is both used in curative and prophylactic regimen. It cannot be used in renal failure because the blood levels of proguanil/cycloguanil are much higher.

Simultaneous use of atovaquone/proguanil and rifampicin is not recommended (blood levels 50% lower). Most recent data state that it's probably safe in pregnancy, even during the first trimester. The curative dose is 4 tablets of atovaquone/proguanil 250/100 mg for 3 consecutive days.

The product is also being studied in toxoplasmosis, babesiosis, leishmaniasis, microsporidiosis and in Pneumocystis jirovecii pneumonia. In the treatment of babesiosis it proved more active in some animal studies than the combination of clindamycin/quinine.

In general atovaquone/proguanil is very well tolerated. Nausea, diarrhoea and headache are the most frequent side-effects. Stevens Johnson syndrome has also been described. Resistance to atovaquone/proguanil has been rarely described even though a single mutation is enough to substantially decrease its activity. The limited use due to its high price might explain in part the lack of resistance.

Miscellaneous products

Tetracycline, minocycline and doxycycline are antibiotics which are active against malaria parasites but are very slow-acting. For this reason, they are never given as monotherapy, but in combination with quinine. They very much reduce the risk of relapse. Doxycycline has the advantage that it only needs to be administered once daily. Doxycycline is is sometimes used for malaria prophylaxis (cfr. infra). Clindamycin (Dalacin®) is also active against plasmodia but is a second choice drug (risk of pseudomembranous colitis due to Clostridioides difficile). It is given together with quinine for Pf attack during pregnancy.

Drug resistance

In chloroquine-sensitive *P. falciparum* the drug is concentrated in the parasite. There is slow outflow (t½ = 50 minutes) of chloroquine from the sensitive parasite. In resistant parasites t½ for outflow = 1 to 2 minutes. Resistance is thus not due to inactivation, breakdown or neutralization of chloroquine. The parasite quickly pumps the product away to the blood, so that the concentration of chloroquine within the parasite is low. At present this cannot be counteracted in humans (in vitro reversible with verapamil).

PCR technology [polymerase chain reaction] is required to differentiate a recrudescence (or relapse) in an endemic region from a re-infection with the same species. Several polymorphic

loci are analyzed. Every combination of alleles that is tested, is rare and permits differentiation between strains.

The first signs of chloroquine-resistant P*. falciparum* infections occurred in the '60s, more or less simultaneously in Colombia and Thailand (countries which mixed chloroquine in commercial table salt). This resistance spread progressively and is now a significant problem in most continents. There are three grades of chloroquine resistance (RI, RII, RIII). In RI the parasitaemia after therapy is so low that it falls below the detection threshold, to rise above it again within 28 days. In RII the parasitaemia is reduced by at least 75 %, but the parasites remain detectable in the peripheral blood throughout the treatment. In RIII chloroquine has no effect on the parasitaemia.

In 1991 chloroquine-resistant *P. vivax* strains were discovered in Papua New Guinea. In 2006, already 65% of the Papuan *P. vivax* strains were chloroquine resistant. *P. vivax* chloroquine resistance in other areas, such as Indonesia, India, Brazil, Guyana is spreading. Chloroquine is not the first-choice treatment any more for *P. vivax* in Indonesia and neighbouring islands. To date resistance to chloroquine in *P.ovale* is very rare. The first chloroquine-resistant P. malariae has been reported (Malaysia, 2002).

Resistance also developed against other drugs, including Fansidar®. The situation is evolving rapidly and is a serious threat to the future use of the artemisinins. The highest resistance against anti-malarial drugs is found in some regions in Southeast Asia, including Cambodia, the Thailand-Cambodia border and the Thailand-Myanmar border. It is only a question of time before these resistant strains spread further geographically. There are several reasons for this increasing resistance. Important factors include inadequate individual patient compliance, treatments that are often discontinued prematurely, frequent underdosing, earlier mass-treatment campaigns reaching only part of the population and therapy being sometimes only partly administered, as well as the use of chloroquinated salt [Cambodia, Brazil (the Pinotti method)]. Among the causes of the swift increase in geographical spread are the large-scale migrations of today, and the ability to move rapidly from place to place. Some products are eliminated slowly from the body (e.g. mefloquine t1/2 = 2 weeks) so that for some weeks a subtherapeutic level of the product is present in the body (as what happened with the addition of choloquine to the salt). When malaria parasites are exposed to such low concentrations, partially resistant strains have a selective advantage. The occurrence of subclinical cases (premunition) functions as a source and reservoir for transmission of parasites with reduced sensitivity. Since the cost price of alternative drugs is generally higher than that of traditional treatments, under-dosing with new drugs will become even more important in future.

On the other hand, reversion of chloroquine resistance has been described in areas where chloroquine was not routinely used for several years. In Malawi, treatment of uncomplicated malaria with chloroquine was 99% effective 12 years after withdrawal due to resistance. This opens possibilities for the use of chloroquine as a partner drug in combination treatments. Stricter control on "fake" drugs (counterfeited medication), some of which contain small amounts of active material will be an essential component in health programs. One idea to combat counterfeit drugs and piracy is to tag individual genuine medication boxes with an authentication number (itemunique code) under a scratch-off label on the wrapping. When

revealed just before purchase, this number can be sent toll-free by telephone text message to an independent certifying company (e.g. Sproxil) which then instantly and automatically replies.

In 2008 resistance to artemisinin has been documented for the first time in Cambodia (borders with Thailand and Vietnam) and in the years after it was described in Thailand, Vietnam, Laos and Myanmar. Treatment failures rates after artesunate-mefloquine and artemether-lumefantrine often exceed 10% in these areas, which is worrying, and higher than anywhere else at present. Full resistance seems to be adequately predicted by a failure to clear the parasites after 3 days of combined treatment. This delay in parasite clearance is the best early marker of resistance so far and is therefore scrutinized in all these regions. Resistance to artemisinin is associated with mutations in the kelch protein gene on chromosome 13 (kelch13). It is worrisome that in some regions in Southeast Asia decreased sensitivity to the artemisinin partner drug was found in combination with the kelch13 mutation. High MIC's (minimal inhibitory concentration) to piperaquine in Cambodia lead to recrudescence in dihydroartemisinin-piperaquine treated patients. Treatment in this region should therefor consist of mefloquine plus artesunate. In 2019, a 15% treatment failure was noted among patients treated with artesunate-sulfadoxine-pyrimethamine due to a mutations in the kelch13 gene combined with resistance to sulfadoxine-pyrimethamine.
In general, six-day courses of ACT still appear to be efficacious if three-day treatments are failing.

Outside of Southeast Asia, only a few cases of artemisinin resistance have been described in Guyana. In Africa, kelch13 mutations where found but it is not clear whether these mutations confer artemisinin resistance. Clinical failure of ACT has not been described in Africa. Ongoing worldwide molecular surveillance and assessment of the efficacy of ACT regimens are warranted to detect resistance and its spreading early.

Prevention

External agents

Anopheles mosquitoes only bite in the evening and at night. It is possible to protect oneself by wearing protective clothing and using an undamaged mosquito net. Effectiveness is increased by treating the net with pyrethroids (insecticides) such as permethrin (Permas®, Peripel®), lambda-cyhalothrin (Danger®, Demand CS®, Matador®) or deltamethrin (K-Otrine®). This will increase further in importance in the future. In most instances, permethrin will be augmented by piperonyl butoxide. Piperonyl butoxide is the most widely used synthetic pyrethrin synergist and there are no reports available on toxic effects on humans resulting from the exposure to it. Piperonyl butoxide is not an insecticide itself but a cytochrome P450 inhibitor which allows pyrethroids such as permetrin to be much more active (10x). Inhibition of the detoxification pathway allows higher unchanged systemic concentrations of the active insecticide to remain within the target animal for a longer period.

Long-lasting insectide treated nets

Mass produced long lasting insecticide treated nets (LLINs) are replacing older style bed nets. Olyset net was the first LLIN which became commercially available. Sumitomo's Olyset® technology incorporates permetrine insecticide directly into polyethylene filaments

which can be woven into sturdy bed nets to provide long-lasting protection from night-time biting mosquitoes. Olyset Plus, which received WHO approval in July 2012, retains the controlled release technology and durability, and contains permethrin and of the synergist piperonyl butoxide (PBO). The fibres have been designed to release the two ingredients at a constant ratio of 2:1. The 'bleed rate' at which permethrin and PBO migrate from the internal reservoir in the fibres to the surface of the net has been adjusted in order to make the net active again within 1-2 days of washing. For this work, Sumitomo Chemical became the co-winner of the 2012 'Application of Core Competence' category Global Business Coalition Health Award. A major production plant has been set up in Tanzania.

Fine-mesh gauze can be applied to windows and ventilation shafts. One good argument for using a mosquito net is the fact that it also protects from nuisance insects such as *Culex* mosquitoes and bedbugs. In regions where there are few *Culex*, people are not so ready to use a net: after all they cannot see or hear any mosquitoes (anopheline mosquitoes fly with little noise).

Insecticides based on pyrethrum can be dispersed by means of spraying (spray gun), evaporation (heated electric plate) or burning (mosquito coil, e.g. with esbiotrin). Insecticides can also be applied to the walls or to the curtains by the windows.

There are also various insect repellents. DEET (N,N-diethyl-m-toluamide, now called N,N-diethyl-3methylbenzamide) is moderately active and can be applied as an alcoholic solution to the skin. This produces a sticky effect when the alcohol evaporates. The effectiveness is only moderate. DEET is absorbed through the skin and is eliminated quickly via the urine. There is no accumulation in the body. The higher the concentration, the longer the duration of action: DEET 20-30% gives 4-6 hours protection, DEET 50% offers 8 hours protection. Concentration higher than 50% don't give significant longer protection.

Alternative repellents are (p)icaridine(Care-Plus®Repel-it; Parazeet®) and IR3535 (Cinq sur Cinq®, Moustidose®).

Intermittent preventive treatment (IPT) and seasonal malaria chemoprophylaxis (SMC)

In highly endemic countries (sub-Saharan Africa), several "preventive" strategies have been promoted and adopted for special risk groups or for some periods of higher transmission. They consist of administering some drugs with antimalarial activity at regular intervals to a group of population with no previous diagnostic testing for malaria. The main aim is to control the malaria morbidity and important reductions of clinical and severe malaria or malaria-related complications (on fetus/newborns for example) have been repeatedly demonstrated.
At this moment, IPT use is recommended by WHO

- in pregnancy (ITPp) as part of antenatal care: sulfadoxine-pyrimethamine (SP) starting from the second trimester with at least three administrations at one-month intervals minimum
- in infants (< 12 years) during the immunization program: SP (where still effective) at the second and third rounds of vaccination against tetanus/diphtheria/pertussis and at vaccination against measles

- in children (< 6 years) in the sub-Sahel region during the rainy season: SP + amodiaquine once a month during each transmission season (strategy called SMC)

On an important prospective note, ACTs are also increasingly explored as IPT in various populations for preventive purposes. ITP with ACT is currently investigated in pregnant women, infants, children < 6 years, school-age children, whole population where malaria is about to be eliminated. This field and the related WHO recommendations are expected to evolve deeply in the coming years.

Chemoprophylaxis for travelers

Chemoprophylaxis is in the first instance intended as prevention of P. falciparum malaria. No single drug which is taken preventively is 100% active against sporozoites and no single drug prevents the formation of liver forms (except primaquine). While taking prevention no vivax or ovale malaria will occur but after they have been discontinued an attack with these plasmodia is possible in the following months or years.

In view of the extensive resistance of P. falciparum, at present no 100% satisfactory protection against this latter parasite is possible. Advice as to whether or not to take medication and which kind of drug to take, will depend on the region and differ from person to person (short journeys, resident, local population, pregnancy, young children, allergy, chronic diseases, use of other drugs and so on).

Recommendations vary from country to country and evolve in time.

- In regions with only P. vivax and/or sensitive P.falciparum (WHO type A) chloroquine 300 mg/week will suffice.
- In zone C with resistant/multidrug resistant P. falciparum, 3 different regimens are currently recommended:
    - Atovaquone/proguanil 250/100 mg 1 tablet per day beginning 1 day before departure until 7 days after return
    - Doxycycline 100 mg/day during the stay and up to 4 weeks after return
    - Mefloquine 250 mg 1 tablet per week, to start two-three weeks before departure, and to continue up to 4 weeks after return

The decision should be individualized, since it depends on several aspects (side effect profile, type of trip, budget). Given the lower cost of generic drugs of atovaquone/proguanil and its good tolerance, atovaquone/proguanil is often chosen as the prophylactic treatment, especially for shorter journeys.

Doxycycline is an alternative in case of atovaquone/proguanil intolerance., Prolonged ingestion of doxycycline can lead to phototoxicity, including photo-onycholysis. Sunscreens do not block ultraviolet A well enough to prevent phototoxic reactions to doxycycline.

Today, the use of mefloquine as preventive treatment has decreased. The plasma half-life of mefloquine is 2 to 3 weeks. Ingestion of 1 tablet per week produces stable blood levels after 7 weeks. Traditionally it is said that mefloquine prophylaxis should be started before

departure. This guideline is based on the consideration that intolerance to the drug can be monitored in this way. It is safe to begin the medication 15 days before departure so that 3 tablets are taken before leaving. In this way 75% of the side effects can be detected. At the prophylactic dosage (adults one 250 mg tablet per week) side effects occur in 2 to 3% of people, which require that the prophylaxis be discontinued. Rarely (1 in 12,000 to 15,000) preventive dosages may trigger epilepsy or psychosis may occur. Epilepsy and arrhythmias (including the use of beta-blockers, calcium antagonists and digitalis) are contraindications for the use of this product. Latest data indicate that it is proven safe during pregnancy. There are sufficient data that it is safe if taken for longer periods. The first case of mefloquine resistance was described in Thailand in 1982. There is already mefloquine resistance on a small scale in many countries, but this can be significant locally: e.g. the cure rate in East Thailand was only 41% in 1993. P. falciparum malaria can thus sometimes occur in spite of correct prophylactic use of mefloquine. Mefloquine does not kill sporozoites and liver parasites (therefore P. vivax and P.ovale malaria are still possible after leaving an endemic zone and after discontinuing mefloquine chemoprophylaxis).

For longer stays we recommend after a period of adequate chemoprophylaxis (a few weeks) at arrival; to travel with stand-by emergency treatment (SBET) of quality, to use in case of malaria, either breakthrough under chemoprophylaxis or attack occurring later. It is of utmost importance to remain alert in case of fever even after several years of tropical stay. Malaria is always possible, even in regions of lower transmission and malaria should be investigated appropriately and treated accordingly.
Emergency treatment for travellers in 2016 includes Malarone®, Riamet® or Eurartesim®.

The local population should not take chronic chemoprophylaxis and most people develop semiimmunity. There are however some high-risk groups: e.g. pregnancy, children less than 5 years and HIV patients. During pregnancy particularly in the second and third trimesters and also immediately postpartum, the immunological resistance to malaria falls. Intermittent preventive therapy in pregnancy ("IPTp") protects against maternal anaemia and low birth weight, and its use in areas in medium to high transmission is recommended by WHO (in most African programs Fansidar is used). The efficacy of IPTp is reduced in HIV-positive women.

Vaccination

Research into a malaria vaccine is based on a number of possibilities. An immune response can be triggered against sporozoites and liver forms (pre-erythrocytic vaccines), erythrocytic forms (bloodstage vaccines) and/or gametocytes (transmission blocking vaccines). However the immune response does not necessarily have a protective effect. A 100% effective malaria vaccine is not likely to be developed in the foreseeable future but a vaccine which leads to partial protection is being evaluated in different fields.

RTS,S/AS01

In the early 1980s antibodies against sporozoites were used to identify the main antigen, circumsporozoite protein (CSP). The CSP is expressed on the surface of the parasite during the infective sporozoite stage.

In 1996 the first favourable results became known. A randomized and controlled study in the Gambia on 306 volunteers showed RTS,S/AS01 to provide significant protection against

natural *P. falciparum* infection. The RTS,S/AS01 is a recombinant vaccine against the pre-erythrocytic stage of the parasite in which regions of P falciparum CSP are fused to hepatitis B surface antigen. It was developed by a public-private partnership with support from the Bill and Melinda Gates Foundation. The results of the large phase III trial that enrolled 15,459 infants was carried out at 11 clinical trial centers in seven countries (Burkina Faso, Gabon, Malawi, Mozambique, Ghana, Tanzania, Kenya) were published in 2012. In this trial 3 vaccines were given with a 1-month interval and some received a booster 20 months after the first vaccine to assess if higher immunity is maintained with a booster vaccine. Initial results demonstrated a vaccine efficacy of about 31% for both clinical and severe malaria in African children and a 26% vaccine efficacy against severe malaria However, a follow-up study over 7 years showed that these results were offset by rebound in later years in areas with high exposure to malaria parasites. In year five to seven after vaccination, the vaccinated group even had a higher risk of febrile convulsions than the control group with a possible higher risk for cerebral malaria and meningitis in areas with high exposure. Nevertheless, pilot implementation studies are currently being initiated in Kenya, Malawi and Ghana and will *learn whether large-scale use of the RTS,S/AS01 vaccine may enter future malaria preventive* programs.

PfSPZ

PfSPZ is a newly developed vaccine, eliciting an immune response against Plasmodium falciparum. It is made of non-replicating irradiated whole sporozoites (SPZ), the parasite stage that infected mosquitoes inject during a bite. The vaccine is unique in using whole parasites as its ingredient. In healthy volunteers a strong protection was noted in lab studies with development of CD8+ T-cells producing IFNy. These T cells play a key role in the immune response to fight malaria in the liver. The difficulty with this vaccine however is that PfSPZ must be injected intravenously, that poses challenges for mass vaccination campaigns. On top of this, it must be stored in liquid nitrogen at – 195 °C or colder. Sanaria, the developing company, is developing a robot that can dissect salivary glands of mosquitos. This step should make preparation and further development of the vaccine faster and easier.

A pilot trial that will enrol 2.100 people aged 2-50 years on the west African island of Bioko is being planned. If the first results are promising, the plan is to vaccinate another 10.000 people and ultimately all 280.000 habitants of the island. PfSPZ's efficacy in the field will inevitable be lower than in lab studies because people might have weaker responses to the vaccine due to pre-existing exposure to malaria or local strains of the malaria parasite might differ from the one used in the vaccine. But combined with conventional measures such as indoor insecticide spraying and insecticide treated bed nets, there is the hope to be able to completely eradicate malaria on the island.

Trypanosomiasis

Human African trypanosomiasis

Summary
• Difference between Gambian (western) and Rhodesian (eastern) trypanosomiasis
• Restricted to well defined regions in Africa, determined by tsetse fly vectors
• Early/first stage: transient sore, fever, oedema, lymphadenopathy, splenomegaly
• Late/second stage: central nervous system symptoms with abnormal CSF (elevated cells and
protein, Mott cells, trypanosomes)
• Diagnosis: always try to detect the parasite
• Repeated thick smears, Buffy coat, Woo technique, mAECT, lymph node aspiration
• When parasite found in blood or lymph node, always lumbar puncture to determine the stage
• Indirect: serology (CATT for West African form), clinical evidence
• Difficult treatment depending on species and stage: pentamidine, suramin, melarsoprol, eflornitin and nifurtimox
• Currently nifurtimox-eflornithine combination therapy (NECT) in first-line against second-stage T. b. gambiense trypanosomiasis. Fexinidazole oral short course.
• Importance of early diagnosis and follow-up as well as integration of control in primary care.

General

Human African trypanosomiasis (HAT) is caused by infection with a unicellular parasite. There are two subspecies of these parasites: the West African or Trypanosoma brucei gambiense and the East African or *T. brucei rhodesiense*. They cannot be differentiated from each other on morphological grounds. *T. brucei gambiense* has two subtypes, *T. brucei gambiense* type 1 and 2. The main difference resides in their ability to avoid the uptake or (*T. b gambiense type 1*) or to neutralize/compensate (*T. b. gambiense type 2*) the trypanosome lytic factor, a human serum component. *T. b. gambiense type 2* resembles T. b. brucei, an animal infecting trypanosomeand causes a more acute disease than type 1 *T. b. gambiense*.

Transmission takes place through the bite of an infected tsetse fly (Diptera, genus *Glossina*). Since the parasites are transmitted via tsetse saliva, they are also known as "salivaria", as opposed to *Trypanosoma cruzi*, which belongs to the "stercoraria" because of its transmission via the feces of the kissing bug. In exceptional cases, mechanical transmission takes place via other biting flies (tabanids). Congenital infections are rare. Sexual transmission seems to be extremely rare.

Epidemiology

African trypanosomiasis occurs exclusively in sub-Saharan Africa, with its distribution being defined by the tsetse fly occurrence Because of its clinical presentation, the West African form is also called sleeping sickness. The area of distribution lies between 14° north of the Equator and 29° south of the Equator. The areas of distribution of West African and East African trypanosomiasis show little overlap. Most of the endemic countries have only one form of the

disease: the Western form, or the Eastern form. This facilitates national therapeutic guidelines. However, both West and East African trypanosomiasis exist in Uganda. Both forms have their own foci, but these are now converging in Uganda. They did not overlap in 2015, but are separated now by only a narrow corridor of about 100 km. If the transmission areas meet (as feared), it would considerably complicate diagnosis and guidelines for management of clinical cases in Uganda.

There have been several large epidemics in Africa in the last 120 years. One from 1896 till 1906 mostly in Uganda and the Congo Basin. Numbers were skyrocketing in many African countries in 1920 but by the mid-1960s, the disease was under control with less than 5000 cases reported in the whole continent, thanks to mobile teams which carried out the screening of millions of people at risk. After this success, surveillance was relaxed, and the disease reappeared, reaching epidemic proportions in several regions by 1970. In 1998, almost 40,000 cases were reported, but estimates were that 300,000 cases were undiagnosed and therefore untreated. In the last decades of the 20th century, prevalence reached 50% in several villages in Angola, the Democratic Republic of the Congo, and South Sudan. Sleeping sickness was the first or second greatest cause of mortality in those communities, even ahead of HIV/AIDS.

The efforts of WHO, national control programmes, bilateral cooperation and nongovernmental organizations (NGOs) during the 1990s and early 21st century reversed the curve. In 2009, after continued control efforts, the number of cases reported dropped below 10,000 (9 878) for the first time in 50 years. This decline in number of cases has continued with 997 new cases reported in 2018, the lowest level since the start of systematic global data-collection 80 years ago. The estimated population at risk today is 65 million people. The area reporting ≥ 1 case/10,000 inhabitants/year in the five-year period (2012–2016) has shrunk by 61% from the baseline period (2000–2004). Since the number of new human African trypanosomiasis (HAT) cases reported between 2000 and 2018 dropped by 95%, the WHO neglected tropical diseases road map targeted its elimination as a public health problem (< 1 case/10,000 inhabitants/year) by 2020 and interruption of transmission (zero cases) for 2030.

To achieve complete elimination of HAT, the main challenge is to set up a cost-effective, adapted and sustained HAT control and surveillance strategies. Integration of the vertical HAT control activities in the general health system will be needed, which is often particularly difficult in those peripheral rural areas where the disease is more entrenched and the health system is weak. Sustained commitment of donors will be crucial. The role human asymptomatic carriers, of parasites in the skin, and by the possible animal reservoirs in gambiense HAT epidemiology, will be essential.

Countries reporting cases (year 2019):
T. b. gambiense: Guinea, Equatorial Guinea, Nigeria, Cameroon, Gabon, Chad, Central African Republic, Congo, DR Congo, Angola, South Sudan, Uganda. Countries with historical T*. b. gambiense* HAT with surveillance activities not reporting any cases are Benin, Ivory Coast, Mali, Niger, Senegal, Sierra Leone and Togo. This anthroponotic subspecies affects mainly humans but is sometimes isolated in pigs, dogs, … The role of animals in transmission is unknown but probably very limited.

Between 1999 and 2019, the reported number of new cases of the chronic form of human West African trypanosomiasis (*T. b. gambiense*) fell by 97%, from 27 862 to 864. Importantly, the number of health facilities providing gambiense HAT diagnosis and treatment keeps increasing. Therefore, it can be considered that the observed trends are very likely to reflect a real abatement in disease transmission, despite the challenges always posed by under-detection. Notwithstanding the encouraging indicators, surveillance has weakened in South Sudan and the Central African Republic due to security constraints. So, the risk of deceleration is real and can have serious consequences as was already painfully experienced in the history of HAT.

T. b. rhodesiense: Uganda, Tanzania, Zambia, Malawi, Zimbabwe. No more cases reported from Burundi, Ethiopia, Kenya, Mozambique and Rwanda. This subspecies is a zoonosis affecting both wild animals and domestic cattle. Humans are sporadically infected "by accident". Contrary to West African HAT, the zoonotic nature of rhodesiense HAT does not presently allow to envisage complete interruption of its transmission.
From 1999 till 2009 , the number of newly reported cases of the acute form of human East African trypanosomiasis (T. b. rhodesiense) fell by 81% from 619 to 116. Of note, in 2018 only 24 cases of East African HAT where reported. In Malawi the reported cases rose from 15 in 2018 to 91 in 2019. In contrast with the West African HAT, surveillance has weakened in countries as Tanzania, Uganda, Zambia and Zimbabwe. The replacement of microscopic examination for malaria diagnosis by rapid serological tests now prevents the accidental diagnosis of rhodesiense HAT when testing for malaria. This is exacerbated by a concomitant decrease in HAT-skilled staff who could maintain knowledge and awareness of the disease. Opposed to gambiense HAT, very few innovative tools have been developed for rhodesiense HAT screening, diagnosis and treatment. These factors in combination with the acute clinical progression of rhodesiense HAT usually prevalent in remote rural areas, are likely to result in non-negligible under-detection. An indirect indication of this under-detection is the fact that 8 cases (6% of the total rhodesiense HAT caseload) were diagnosed in non-endemic countries among returning tourists in 2015–2016.

Trypanosomiasis does occur in South America, but Chagas' disease which is caused by *Trypanosoma cruzi* is clinically very different from African sleeping sickness. There are rare human infections with trypanosomes in India and Malaysia. They were due to accidental zoonotic infections with Trypanosoma lewisi, a rat and other rodents parasite transmitted by fleas, or *T. evansi*, a parasite mechanically transmitted by hematophagous biting flies and infecting mainly horses and camels but also buffalo and cattle. A number of human infections with *T. vivax* and *T. congolense* have also been reported. Such infections are very exceptional.

Surra

Trypanosomaevansi causes disease ("surra") in certain animals, such as camels, llamas, horses, buffalo, cattle, dogs, sheep and goats. There is considerable variation in the pathogenicity of different strains and the susceptibility of different host species. The disease ranges from inducing a subclinical infection, mild disease, chronic to overt forms (months to years) and rapid fatal infections (esp. in horses and camels). Deer, capybara and coati can become infected and ill and may also constitute a reservoir. Animals subjected to stress such as malnutrition, pregnancy, work, are more susceptible to disease. Suramin is the most

frequently used drug for treatment of surra in horses. Successful treatment by a single dose of diminazene diaceturate has been reported in dogs.

Trypanosomacongolense is the main trypanosome infecting cattle, causing animal African trypanosomiasis (AAT) Every year AAT is responsible for more than 3 million deaths in cattle with estimated annual agriculture economic losses of more than US$ 4.5 billion dollars, making AAT one of the major constrains for sustainable livestock production in Africa. A few indigenous African cow breeds, such as the N'dama breed, tolerate the parasite's presence remarkably well. However, these trypanotolerant animals are not popular with farmers because they grow slowly and are small. Many farmers prefer Boran cattle, which are more beefy with high resistance to heat and ticks but susceptible to AAT.

Trypanosomaequiperdum causes a chronic sexual transmitted disease (“dourine”) in horses, mules and donkeys. Infections are endemic in Eastern and Southern Africa, South America, Mongolia, Russia and Kyrgyzstan. T. equiperdum is the only trypanosome that is not transmitted by an insect vector.

Parasite

In general, among trypanosomes, one can distinguish several morphological forms based on the relative position of the kinetoplast to the nucleus. Extracellular African trypanosomes have two main morphologies:

1. Epimastigote: fusiform 20-40 µm long with an anterior placed kinetoplast, in front of the nucleus i.e. on the same side as the flagella is pointing. This stage occurs in the tsetse fly.

2. Trypomastigote: the kinetoplast is located behind the nucleus. The parasites are pleomorphic in human blood. Some are elongated and slender ("slender trypomastigotes") and others are shorter and stumpy. Reproduction in man occurs via longitudinal binary cleavage every 7 hours.

In intracellular trypanosomes, like *T. cruzi* (see Chagas' disease) the amastigote stage is present inside the cell. This multiplication stage is characterised by a spherical form without flagella.

Parasite information

The parasite has only one nucleus, is elongated, contains a giant mitochondrion and has a single flagellum. At the base of the flagellum is the basal body. This lies adjacent to the kinetoplast. The latter is a compact DNA (deoxyribonucleic acid) structure, located in the very long mitochondrion. This mitochondrion is almost as long as the entire trypanosome. The name of the Order to which the parasite belongs - Kinetoplastida - refers to this organelle. Between the basal body and the flagellum there is an undulating membrane which is required for motility. In the form of the parasite such as it occurs in man (trypomastigote), the kinetoplast lies in a posterior position and the flagellum points towards the front, rather like a bowsprit on a large sailing vessel. The parasite occurs in the salivary glands of the tsetse fly as an epimastigote (kinetoplast located just in front of the nucleus).

The genome of T. brucei was sequenced and published in Science in July 2005. The DNA in the kinetoplast (kDNA) stains like that of the nucleus (recognizable on a smear). The structure of the DNA in this kinetoplast is very complex. There are numerous (about 40) large DNA loops ("maxicircles") and even more (some 5,000-10,000) small DNA loops ("minicircles").

While in the human host, the parasites are diploid. The parasites replicate in humans by asexual mitosis. Diploid and polyploid forms can be found in tsetse flies. Experimental arguments for meiosis and a possible sexual reproduction in T. brucei were first proposed in 1986. In the laboratory tsetse flies were infected with 2 different clones after which hybrid parasites were isolated, which indicates exchange of genetic material. This could be important for a better understanding of the natural parasite populations, e.g. via the various iso-enzyme patterns that occur in nature. Even if these laboratory data were confirmed, it remains an open question how important this is in nature.

The notion of **antigenic variation** in African trypanosomes has been around for a long time. Early investigators would isolate trypanosomes and serum from an animal early in the course of an infection and then again later during the same infection. Early antiserum would kill the initial strain of trypanosome, but did not affect the trypanosome strain isolated later in the infection. It was apparent that the trypanosome population changed over time. When the parasite is present in an individual it is covered with a thick monotonous layer of a single type of glycoprotein, VSG (Variant Surfac Glycoprotein). The VSG coat is approximately 20% of total cell protein and includes more than 10 million molecules thus has a vast repertoire of surface antigens. The T. brucei genome has around 2000 distinct VSG genes but only one single VSG is expressed at a time. The entire VSG surface of a trypanosome is recycled every seven minutes by a process of VSG endocytosis and exocytosis. Whenthe parasite is transferred to the tsetse fly, the VSG coating disappears within 4 hours and is replaced by an invariant glycoprotein ("procycline" or PARP (procyclic acidic repetitive protein)). After the parasite has completed its cycle in the fly, colonizes the salivary glands and transforms into the metacyclic infectious stage the VSG coating reappears. The metacyclic VSG coat is different from the bloodstream VSG coat having only 12 to 20 VSG types. The metacyclic VSG coat is supposed to limit the first immune response and thus facilitating the parasite establishment and proliferation in the vertebrate host, making the VSG coating of vital importance for the parasite. This explains why only metacyclic trypanosomes (the mature forms in the salivary glands of the insect) are infectious. When an antigenically homogeneous population of parasites is in the human body, antibodies against the VSG of this population are produced. The immune system lyses the parasites which is accompanied by fever. Infections with trypanosomes would be cured quickly, if the parasite population could not constantly change its surface antigens. The switch of VSGs happens about once every 100 cell divisions.

Most of these VSG genes are located on specialized telomeric region defined as the expression site with around 80% of these telomeres residing on minichromosomes in the nucleus of the parasite. The parasite also has about twenty chromosomes of "normal" size. These do not condense during mitosis.
At any one time, only one VSG gene per parasite is active. After destruction of the first dominant population by the immune system, the heterologous parasites increase in number

until the variant VSG has induced antibodies and a new cycle of destruction begins. A third population of minority variants then emerges. Antigenic variation is a very important factor in the development of the disease and explains various symptoms (including its chronic course, fluctuating parasitemia and fever episodes).

Vector

Tsetse flies (*Glossina sp.)* are blood-sucking insects that occur only in sub-Saharan Africa and the Gisan oasis in Saudi Arabia. Four different species of fossil flies were discovered in 20-million-year-old mudrock in Colorado, USA, indicating that the insects once existed in North America. The name tsetse descends from the Tswana language. This name was also used by the Matabele and Zulus and refers to the sound that the insects make. An English reporter in Southern Africa at the end of the 19th century adopted this name when he wrote about a fly which attacked horses and cows.

The insects have prominent elongated mouthparts (proboscis), which explains their scientific generic name ("glossus": tongue). Tsetse flies have typical wing veins, with a "hatchet cell" in the middle. When resting they fold their wings over their back like a closed pair of scissors. Other flies hold their wings more to the side. There are 31 species and subspecies, but less than half are vectors of human trypanosomiasis. The genus *Glossina* is now divided into three subgenera:

1. The fusca-group (subgenus Austenina): not important in human pathology.

2. The palpalis-group (subgenus Nemorhina): these flies prefer dense vegetation in humid areas (e.g. on riverbanks, gallery forests). Their habitat should have exactly the right conditions of humidity, warmth and light intensity, and there should be a blood supply (nearby animals or humans). Humans are frequently bitten when working/standing close to the water's edge. The flies can also be found in cocoa, coffee, mango and banana plantations; this group is the vector of human West African trypanosomiasis and nagana (= animal trypanosomiasis).

3. The morsitans-group (subgenus Glossina) are distributed over the East African savanna and are zoophilic. They are the vector of East African trypanosomiasis.

Tsetse flies and their bloody bites

As obligate blood feeders, both male and female tsetse flies feed with blood every 3 to4 days. After landing on a host, the fly will lower its proboscis to a vertical position and stab with a rocking motion of the body. The rough dentate part of the proboscis saws through the tissues. The proboscis penetrates the skin while the teeth lacerate the capillaries walls and saliva is injected forming a small pool under the skin. The blood is actively pumped up and stored in the crop for a short time and is then passed to the midgut. Tsetse blood feeding implies manipulation of the host haemostasis, possible by producing and injecting a potent saliva anticoagulant cocktail at the biting site. Until now, two key molecules have been identified to facilitate tsetse blood feeding: the tsetse thrombin inhibitor with anticoagulant activities and the 5'Nuc apyrase with a dual role in platelet activation and aggregation. Bites of forest flies are less painful than those of savanna species. During a

bite, tsetse can injects with the saliva infectious metacyclic trypanosomes. Feeding time ranges from 20 to 25 seconds. In a single meal 5-80 mg (max 155 mg) of blood in taken up. A hungry fly can take up a bloodmeal greater than its fasting weight. When satiated, the fly heads to a roosting site to digest at leisure.

Tsetse flies live a few months. If parasites are taken up by a bloodmeal, 99% of the parasites die in the insect's stomach (midgut), but some transform in the procyclic (midgut) and later into the metacyclic trypomastigotes (salivary glands) The tsetse fly becomes infective 2 to 3 weeks after an infective bloodmeal.

Adult tsetse flies are airborne for short periods and rest for the remaining time. On average, they cover 200-300 meters in the dry season. In savanna areas they only take flight at times of the day when temperature is suitable. At the hottest time of the day (above 35°C) and during the night they rest.
Farmers take advantage of this trait by driving their herds through infested areas after dusk. In forested areas where temperature swings are less marked they fly more often. There are several different species of tsetse flies, each with its own ecological preference. In an endemic area usually less than 1% of the flies are infected.

Congenital transmission is possible and there are case reports of laboratory accidents, blood transfusion and organ transplantation as transmission route, but they are extremely rare.

Clinical aspects

Infection by T.b. gambiense

Any bite from a tsetse fly, whether infected or not produces a local reaction. When the bite is infected a small local wound can appear after 1 or more weeks, but in general after 5-15 days (trypanosomal chancre or sore or trypanoma). This often remains unnoticed in the local population, though it can sometimes reach quite substantial dimensions (2-5 cm). In infected Europeans it is described at a frequency of 25-40%. It involves a central blister or ulcer surrounded by red infiltrated skin. The lesion tends to be minimal painful. When it has healed after 1-3 weeks a depigmented scar can remain. The infection develops slowly if there is no medical intervention. The patient's condition gradually deteriorates, ultimately leading to his/her death in sometimes as short as a few months, sometimes much later. There are two quite artificially separated stages: a preliminary hemato-lymphatic stage and a second stage with symptoms of meningo-encephalitis. The boundary between these two stages is determined by the findings in the cerebrospinal fluid. The distinction is important for treatment. Asymptomatic human carriers (and spontaneous cure) are described but is rare.

Hematolymphatic stage (first or early stage)

The hematolymphatic stage lasts 6 to 12 months, but sometimes much longer. It is characterized by intermittent unpredictable bouts of fever separated by irregular intervals of days to a month or even more, headache and general malaise. The lymph nodes swell, especially those in the neck (Winterbottom sign). These glands are soft, mobile and not painful. In early West African trypanosomiasis, swollen posterior cervical lymph nodes are found in 50-85% of early stage patients and in fewer than 25% in the late stage. Oedema sometimes occurs (face), as well as pruritus (itching) and transient red spots or a circinate rash

(trypanides). This rash can be seen without difficulty on a white skin (reported in 50%) but is difficult to see on a dark skin. The liver and certainly the spleen can be enlarged. There is moderate to severe anemia. Neurological disorders (personality changes), increased sensitivity to pain, especially deep hyperesthesia ("Kerandel sign") can already be present in the first stage. This condition gradually evolves into increasing neurological collapse, characteristic of the meningo-encephalitic stage.

The condition is characterized by a chronic course with flare-ups and quieter periods. These flare-ups are to be interpreted as destruction of the trypanosomes, followed by the development of a new population of parasites carrying a different surface antigen. Lysis of the parasites releases large quantities of antigen into the bloodstream. These form immune complexes with circulating antibodies which then precipitate resulting in perivascular inflammatory symptoms (including vasodilation with increased vascular permeability and oedema). Successive generations of parasites each have a different glycoprotein on the outer membrane. It is to this outer membrane that the antibodies attach themselves. Whenever a new glycoprotein emerges, the immune system always has to start again from scratch, with the production of new antibodies. This explains the pronounced increase in the immune globulins (especially IgM) in blood and cerebrospinal fluid. The high IgM serum concentration thus results from chronic polyclonal B cell stimulation. A specific cross-reacting and auto-antibodies can also be produced, making serological diagnosis of other diseases more difficult. Meanwhile time goes on and the infection worsens.

Meningo-encephalitic stage (second or late stage)

If left untreated, the meningo-encephalitic stage will progress to death in 6 months to 2 years after neurologic symptoms arise. Personality changes increase and the patient usually loses interest in their surroundings. Psychosis sometimes occurs. The patient develops tremor, paresthesia, increased sensitivity to pain, gait disorders, speech- difficulties and reversal of the diurnal wake/sleep rhythm. Ataxic dyskinesia is present in most patients. Basal ganglia involvement can produce clinical features which overlap with those of Parkinson's disease. Weight loss and endocrine abnormalities with e.g. impotence are common. Damage to the hypothalamus (paraventricular and supraoptic nuclei) may lead to disturbance of the normal sleep pattern. The patient progressively deteriorates and develops stupor (sleeping sickness!). The patient can still be woken up but will quickly go "back to sleep" again. Daytime sleeping, insomnia and behaviour change are reported in 40%, 55% and 30% of cases respectively. This is finally followed by coma and the patient dies of malnutrition, concomitant infections, accidents and destruction of the central nervous system. This disease is not to be confused with neurosyphilis, tuberculosis, AIDS with cerebral toxoplasmosis or cryptococcal meningitis, alcoholism or schizophrenia.

Histopathological changes include leukoencephalitis with demyelination and accentuation of the periventricular areas. There is a characteristic infiltration of lymphocytes and plasma cells around cerebral blood vessels (perivascular cuffing).

Infection by T.b. rhodesiense

Infection with T. b. rhodesiense evolves much faster than West African trypanosomiasis. The incubation phase is shorter (1 to 3 weeks). An inoculation chancre often occurs (in traveller this is almost always present), and appears some days before the onset of pyrexia. There is

high fever and most patients have signs of multiorgan failure. Hepatitis leads to jaundice, elevated liver enzymes and coagulation disturbances. Myocarditis is common and often gives diffuse T-wave inversions. Heart failure can occur. ARDS can be detected on chest X-ray. Encephalitis leads to neurological symptoms, such as confusion and stupor. Daytime sleeping, insomnia and behaviour change are reported in 75%, 65% and 20% of cases respectively. There is usually no obvious lymph node swelling, but splenomegaly is frequent. The disease evolves to a fatal outcome within a few weeks or months.

Diagnosis

Detection of parasites

In the peripheral blood there is usually a normal white blood cell count (no leukocytosis or leukopenia), a normal platelet count and a slight normocytic anemia. The erythrocyte sedimentation rate is quite high, in part explained by the high immunoglobulins. The diagnosis is best made by detection of the parasite. The sensitivity of conventional parasitological techniques is however quite low. The parasite can be found in fluid from the inoculation chancre, blood (direct examination, thin smear, thick smear, buffy coat), lymph node fluid (needle aspiration) or cerebrospinal fluid (lumbar puncture). In a wet blood smear, the motility of the parasites attracts the eye but the sensitivity of the technique is too low. A Giemsa-stained thick blood smear is more sensitive, but parasites are frequently deformed in this preparation and are therefore easily missed. Lymph node aspiration is done with a dry needle. After puncture the needle is left in place for a while and the node is massaged. A syringe is then fitted to the needle and after aspiration the fluid is put on a microscope slide for direct examination (the motile trypanosomes can then be observed). Several samples will often be needed, as the parasites are not present in large numbers and appear in the blood in intermittent waves. Concentration techniques facilitate the diagnosis: Woo technique, buffy coat from a centrifuged microhematocrit tube or quantitative buffy coat test (QBC). In well-equipped laboratories a miniature anion exchange centrifugation technique (mAECT) is used (Lanham or Lumsden method). Such a column contains diethylaminoethyl-cellulose (DEAE-52). The separation of blood cells from trypanosomes depends on a difference in surface charge of the blood cells and the parasites. This charge is pH-dependent (importance of iso-electric point). Blood is mixed with a particular buffer (PSG = Phosphate SalineGlucose) and gently layered on top of the column. The blood will penetrate the gel on top of the column and red and white blood cells adhere to the DEAE gel particles. In this buffer, the trypanosomes are at their isoelectric point (=neither positive nor negative charge) so flow through the column. The eluate containing the parasites is collected and centrifuged. The sediment is examined microscopically to determine if parasites are present. The type of buffer and the temperature at which the test is carried out are of very great importance. The more the disease advances the less frequently are trypanosomes found in the blood, though they are then found more often in the cerebrospinal fluid. The parasites can be cultured in vitro in a specific medium (KIVI; Kit for in Vitro Isolation). In theory as few as 1 trypanosome can be detected in 5 ml, though in 50% of the tested cases the culture remains sterile.

Comparison of detection thresholds

Fresh blood preparation (10 µl)	6000 trypanosomes/ml
Thick drop (10 µl)	2000 trypanosomes/ml
Buffy coat (70 µl)	600 trypanosomes/ml
QBC (Quantitative Buffy Coat Test)	16 trypanosomes/ml

MAECT (500 µl)	usually 100/ml required
PCR (Polymerase Chain Reaction)	10 trypanosomes/ml
KIVI (Kit for In Vitro Isolation)	1 trypanosome per 5 ml.

Serology

Antibodies can be detected serologically. Several techniques (immunofluorescence etc.) have been developed. There are also methods for use in primitive rural conditions. A cheap and practical method is a direct agglutination reaction of trypanosomes on a plastic card, with macroscopic read-off (**CATT= Card Agglutination Test for Trypanosomiasis**), which was developed by the Institute of Tropical Medicine, Antwerp. This is a good screening method for *T. b. gambiense* in most areas. The sensitivity of the CATT test in areas (e.g. Cameroon) with T. gambiense strains which do not carry the variable surface antigen LiTat 1.3 is lower. A drop of blood (finger prick) and a drop of reagent that contains blue-colored parasites of a known serotype are mixed on a white plastic card. The card is mechanically shaken for 5 minutes and then immediately read. When the test is positive (presence of antibodies) the trypanosomes agglutinate and form a blue clot. The CATT has no place in the diagnosis of *T. rhodesiense* infections, except in a chronic form of T. rhodesiense which exist in Malawi. CATT must not be confused with the CIATT (Card Indirect Agglutination Test for Trypanosomiasis, an antigendetection test). Another method is to take a blood drop on very small filter papers (confetti) and examine this later in a laboratory. The patient should be called back later if the result is positive. Antigen-detection methods (ELISA) have also been developed, but are not yet in routine use. A problem arises in persons who have a positive serology, but who are asymptomatic and in whom no parasites are found (wait and see with follow-up or treatment with suramin or pentamidine?). After successful treatment the antibodies remain for years. Antibody detection therefore cannot be used for detecting relapse or reinfection. It is hoped that in the future we shall be able to prove a cure by monitoring reductions in the levels of circulating antigens.

The CATT is designed for mass screening and still requires agitator rotator, electricity, and refrigeration, there is a need for simple and individual point-of-care tests. In 2013, two lateral-flow **rapid diagnostic antibody-detection tests** were developed for *T. brucei gambiense*: the HAT Sero-KSeT test (Coris, Belgium) and the immunochromatographic HAT-RDT (Standard Diagnostics, Korea), designed for testing on whole blood, with results provided within 15 minutes. The tests contain variant surface glycoproteins (LiTat 1.3 and LiTat 1.5). Clinical field evaluation showed sensitivity and specificity similar to those obtained with the CATT, but with simpler use (no need of electricity and cold chain). In large multicenter prospective studies, sensitivity of both SD HAT-RDTs was found lower than expected (71%–89%) whereas specificity was very high (98%). However, combining any of these RDTs together or with CATT achieved a very high sensitivity. It appears therefore that both these RDTs achieve a diagnostic accuracy equivalent to that of CATT and may be used instead for both mass screening and clinical care, wherever local conditions do not favour the use of CATT.

In *Trypanosoma b. r.* HAT, the parasite load in blood is usually very high at symptom onset, so trypanosomes are relatively easily detected whenever a blood smear is performed, In travellers, diagnosis of *T brucei rhodesiense* HAT has been almost always made by thick and thin blood film examination, often as a surprise finding. It could, however, be missed when in

such circumstances malaria diagnosis is limited to RDT. Although antibody based assays exist for *T brucei rhodesiense* HAT, none have been developed in RDT format.

Genomic tests

The first PCR was developed in 1983 by Kary Mullis (Nobel Prize Chemistry 1993). Since then several PCR variants have been developed. Most techniques consist of an amplification step followed by amplicon electrophoresis in agarose gel, but there are other approaches. The sensitivity and specificity largely depend on the DNA sequence targeted by the primers. Preferred genomic sequences are those which are conserved and unique for the parasite and that occur as multiple copies in the genome. Tests based on extra-nuclear minicircle kinetoplast DNA have failed to live up to expectations. With PCR, formal molecular differentiation between T. brucei gambiense and T. brucei rhodesiense is possible. T. brucei gambiense-specific glycoprotein is only present in T. brucei gambiense, while the gene encoding the serum-resistance-associated protein (SRA) is only present in T. brucei rhodesiense. Both however are single copy genes. The most interesting next-generation diagnostic for active infection by trypanosomatids is the spliced leader RNA (SL-RNA) detected by PCR. The splice leader is a conserved species specific sequence capping the mature mRNAs.

Clinical diagnosis

A correct diagnosis can sometimes be reached even though parasites cannot be detected. These "clinical cases" are patients from an endemic area, with clinical symptoms of late stage trypanosomiasis. and lymphocytes in the cerebrospinal fluid. Such "clinical cases" may amount to no more than 5% of the total number of trypanosomiasis patients.

Diagnosis: IgM in cerebrospinal fluid

Antibodies should if possible be detected in the cerebrospinal fluid. Determining the IgM content in the cerebrospinal fluid can be very difficult or even impossible to carry out in endemic areas and under field conditions. An experimental latex agglutination test for detection of IgM was developed at the Institute of Tropical Medicine, Antwerp, Belgium. Blood-CSF barrier dysfunction is usually absent or mild and occurs in very advanced late-stage disease. It is possible to calculate and plot diagrams of the quotients CSF/serum concentration for IgG, IgA and IgM (demonstration of intrathecal production of antibodies). Especially intrathecal IgM production will be present in late-stage sleeping disease (occurs in 98% of people with leukocyte counts higher than 20/µl). Similar patterns do occasionally occur in Lyme neuroborreliosis, neurosyphilis, mumps meningoencephalitis and in non-Hodgkin lymphoma involving the central nervous system.

Usefulness of the lumbar puncture

A lumbar puncture is important:

1. sometimes in order to make a diagnosis
2. in order to determine the stage (main purpose)
3. in order to monitor therapy

In the 2nd stage the cerebrospinal fluid is characterized by:

- white blood cell count (WBC) > 5 per mm3, (normally <3)

- protein > 45 mg% (normally 15-45 mg%)
- IgM increase (difficult to carry out; Latex IgM)
- sometimes trypanosomes and/or Mott cells (= degenerated plasma cells: multiple varied size spherical inclusions/ Russell bodies within a plasma cell having an eccentrically placed clock face nucleus; also called morula cells of Mott (in Latin 'morus' means mulberry).

Treatment

There are several different treatment schemes that are determined by the vertical control program that is (or was) in place in many areas. The specific therapy is not simple. Drugs that do not penetrate into the cerebrospinal fluid and the brain are useful in the early stage only (prior to invasion of the central nervous system). Drugs that do penetrate the blood-brain barrier must be used in the late stage. Although recent progress has been made, there is an urgent need for less toxic, easy to administrate and cheaper drugs.

Here under are summarized the current guidelines for HAT treatment, according to the causal species and disease stage. Thereafter, each trypanocidal drug is described one by one for information.

Treatment summary

***T. b. gambiense* early stage:**
Pentamidine 4 mg /kg/day IM or IV for 7 days (preferred)
Or suramin test dose 5 mg/kg, then 20 mg/kg/day (max. 1 g) IV on days 3, 10, 17, 24, 31 (alternative)

***T. b. gambiense* late stage:**
NECT: eflornithine 200 mg/kg IV 2 times per day for 7 days + nifurtimox 5 mg/kg/day TID orally for 10 days
Or elflornithine 100 mg/kg IV 4 times per day for 14 days

NB: Melarsoprolol is not used any more for T.b. gambiense except in the very rare situations of treatment failure with NECT, and after expert advice. The regimen is then similar to the treatment of rhodesiense HAT late stage (see below: 2.2 mg/kg/day for 10 days with prednisolone throughout the whole period)

***T. b. rhodesiense* early stage:**
Suramin test dose of 100 mg (check urine for protein and cylinders), then 20 mg/kg/day (max 1 g) on day 1, 3, 7, 14 and 21 (alternative 20 mg/kg weekly for 5 weeks)

***T. b. rhodesiense* late stage:**
Melarsoprol 2.2 mg/kg/day for 10 days under cover of prednisolone (see above); the previous cumbersome alternative (3-4 series of 3.6 mg/kg/day IV for 3 days weekly) is being abandoned
NB: The first all-oral short course treatment fexinidazole has been approved in 2020 by WHO as a valid alternative treatment for T.*b. gambiense* early stage AND late stage (but only in children 6 years oor mre, if drug administration with food is directly observed T, and for the late stage if there is no advanced neurological disease nor presence of more tha 100 WBC/µL

in CSF examination). Details about the rationale are provided below in the paragraph on fexinidazole.

Trypanocidal drugs

Suramin (Germanine®). The compound was developed in 1920. It is best administered by slow intravenous infusion, as intramuscular administration (10% solution in distilled water) is very painful.

The drug is active against both T.b. gambiense and rhodesiense, but its toxicity restricts its use to the latter pathogen, since a better tolerated alternative (pentamidine) exists for the former. Suramin is excreted extremely slowly by the body. This is important if exfoliative dermatitis develops as a side effect. It can cause substantial proteinuria and a nephrotic syndrome. When a test dose of 100 mg is tolerated well, the daily dose 20 mg/kg (max. 1 g) can be given on days 1, 3, 7, 14 and 21. A urine strip should be performed before each administration, to look for occurrence of proteinuria. In the past 20 mg/kg (max 1g) weekly was given for 5 weeks. Fever sometimes initially occurs due to lysis of trypanosomes. Suramin also *kills Onchocerca volvulus* filaria. Patients with active onchocerciasis can exhibit severe side effects to suramin (cfr. Mazzotti reaction with DEC).

Pentamidine was developed in 1941. It is less active than suramin and not active against T. b. rhodesiense. pentamidine exists as an isethionate salt (Pentacarinat®, Pentam®) and must be administered parenterally. Intramuscular injections are painful and therefor slow IV administration is preferred. Rapid IV injection causes acute hypotension. Hypoglycemia can sometimes occur due to release of insulin from the pancreas. Other adverse events include pancreatitis, ventricular arrhythmias, hepatotoxicity and kidney failure. This medicine is also used in pneumocystosis in AIDS patients and the treatment of cutaneous Leishmania guyanensis. The recommended field treatment is 4 mg kg/day for one week.

Eflornithine (DFMO, Ornidyl®). Di-fluoro-methyl-ornithine or DFMO was first used for trypanosomiasis in 1985. It is very water soluble. This substance penetrates quite well into the cerebrospinal fluid. A cumbersome IV treatment is however required, divided in 4 administrations per day for 2 weeks. Eflornithine is rather well tolerated, although hematotoxicity is possible (and bacterial infection of IV lines in the tropics) as well as seizures. Unfortunately it is active only against T. b. gambiense. In monotherapy, the dosage regimen is 100 mg/kg/6 hours IV x 2 weeks via physiological fluid infusion. Concentrations in cerebrospinal fluid in children seem to be lower than in adults. Children require a higher dose (150 mg/kg 4 times a day). If used in combination with nifurtimox, the dose is 200 mg/kg 2 times a day (over 1 hour) for 7 days.

Nifurtimox (Lampit®): Cf. Chagas' disease. After thorough pharmacokinetic studies, a multicenter trial has evaluated the safety and efficacy of the combination of nifurtimox (oral, for 10 days) with eflornithine (IV, 2 administrations of 200/kg per day for 7 days), compared to eflornithine in monotherapy (100 mg/kg 4 times a day) for 14 days. The nifurtimox-eflornithine combination treatment (NECT) was not inferior to eflornithine (> 95% cure rate) but much easier to administer and cheaper (14 infusions of eflornithine instead of 56!). Further field phase 4 studies confirmed these excellent results. This combination has also the theoretical advantage to prevent emergence of resistance. The NECT has been endorsed by

WHO in 2012 and has become the first-line therapy in all endemic countries. The drugs are provided for free by WHO to the national HAT programs.

Melarsoprol (Arsobal®) was developed in 1949. Because of the demonstrated efficacy of eflornithine for second stage T. b. gambiense trypanosomiasis (with limited side effects), the use of melarsoprol is nowadays limited to the T. b. rhodesiense form (second stage). This trivalent arsenic compound is insoluble in water or alcohol. It is therefore dissolved in propyleneglycol. This solvent is highly irritant to tissues. It causes phlebitis and chemical cellulitis when administered paravenously. Melarsoprol may only be given by very slow IV infusion. It has a significant trypanocidal activity (as can be measured via bioassay) in plasma and cerebrospinal fluid for up to several days after administration, although melarsoprol can then no longer be detected with HPLC (high performance liquid chromatography). The molecule is transformed into biologically active metabolites such as melarsene oxide that irreversibly binds to pyruvate kinase, which disrupts energy production in the parasite. The same effects happen in host cells, rendering the drug highly toxic. Resistance to melarsoprol is described. Toxicity results in polyneuropathy and reactive encephalitis. Encephalopathy tends to manifests itself as a sudden violent neurological deterioration at the end of the first series or during the second series of injections. At present, there seems to be no way to predict which patient will develop encephalopathy.
Corticosteroids seem to diminish the risk and severity of the encephalopathy (controversial). It is therefore imperative to administer prednisolone before using melarsoprol. In general, one can expect lethal reactive encephalopathy after administration of melarsoprol in about 3-5% of cases. Clinically, there are three syndromes of reactive arsenic encephalopathy:

- convulsive status associated with acute cerebral oedema, due to diffuse lesions with hemorrhagic encephalitis.
- rapid progressive coma without convulsions
- acute nonlethal mental disturbances without neurological signs (e.g. psychosis)

Another toxic effect of melarsoprol is polyneuropathy (analogous to heavy metal intoxication). This results in diminished sensitivity and/or paresthesia's in hands and feet, as well as motor signs. In this case melarsoprol should if possible be stopped and vitamin B (e.g. thiamine) administered.

In the past, treatment regimens consisted of 12 injections over a 30-day period, but current regimens with daily administration of 2.2 mg/kg for 10 consecutive days have proven a similar efficacy towards *T.b. rhodesiense* and *gambiense* without increase in toxicity, avoiding very long hospitalizations.

Experimental medications

Fexinidazole is a product related to megazol, tinidazole and metrinidazole. After demonstrations of in vivo trypanicidal activity in 1983 the drug languished in obscurity for more than 25 years. Fexinidazole was "rediscovered" as an oral drug which might be used in early and late stage sleeping disease. In African trypanosomes, the mode of action of nitro drugs involves reductive activation via a NADH
(reduced form of nicotinamide adenine dinucleotide)-dependent bacterial-like nitro-reductase. In a randomized controlled trial including 394 patients, treatment success at 18

months was slightly lower with fexinidazole (1800 mg once a day, days 1 to 4; 1200 mg/day, days 5 to 10) than with NECT therapy (91% vs 98%) but this difference was within the predetermined acceptability margin of 13%. The death rates (none directly attributable to treatment) and severe adverse events were similar between the two groups. In a subgroup of patients with > 100 >WBC/µl in the CSF, fexinidazole was inferior to NECT (87% vs 99% cure). Based on these new key findings, the WHO has revised its recommendations in 2020, and considers nowadays fexinidazole as a valid treatment for BOTH stage 1 and stage 2 gambiense HAT, PROVIDED that the patient does not present with advanced neurological disease (and/or > 100 WBC/µL in CSF examination) and that treatment administration can be directly observed (lower efficacy if no concomitant food intake). Implementation of this new strategy is just starting.

In sharp contrast with the gambiense HAT, rhodesiense HAT treatment did not progress at all. Studies to examine the efficacy of fexinidazole against T. b. rhodesiense are ongoing. If successful, fexinidazole has the potential to become a safe, efficacious, affordable, oral short-course for both stages of *T. b. gambiense* and *rhodesiense*.

The Drug for Neglected Diseases Initiative (DNDI) has recently developed the compound SCYX-7158, the first oxaborole-based agent (the boron atom being essential for the trypanocidal activity even if the mechanism of action is unknown). Phase 1 studies have been completed. Phase 2/3 study results are expected in 2021. The drug has a very long half-life (17 days in healthy volunteers), making it very promising as a single dose oral treatment for both HAT studies.

Treatment follow-up

After treatment the patient should have a regular follow-up for 2 to 3 years for possible relapse *(T. b. gambiense:* check every 6 months). The first sign of relapse is often an increase in the cell count in the cerebrospinal fluid, followed by a rise in its protein content. Recurring fever, drowsiness and chronic headache are also signs of relapse. Unfortunately, obtaining a CATT negative result after treatment for HAT cannot be relied upon to confirm successful treatment. Further works needs to be done to address this question.

Recent studies have however demonstrated that it was possible to simplify and shorten the period of follow up after treatment for second stage *T. b. gambiense* infection according the following rules:

- At 6 months presence of trypanosomes or WBC > 50 in the CSF are considered as failures; WBC count < 5 in the CSF is considered as a cure (no further lumbar puncture). The remaining patients need to be re-evaluated again at 12 months by lumbar puncture.

- At 12 months: cure if no trypanosomes and CSF WBC < 20; failure if > 20 WBC in CSF or trypanosome.

American trypanosomiasis (Chagas disease)

Summary
• Trypanosomacruzi, only in the New World
• Transmission via bugs, blood transfusion, congenitally and orally (bug feces in food/drink)
• Importance of poverty (housing) in transmission
• Acute (especially children): chancre, Romaña's sign, fever, lymphadenophathy, myocarditis,
hepatosplenomegaly
• Chronic: cardiac arrhythmias, heart failure, emboli, apical aneurysms
• Chronic: dysphagia, constipation (mega-syndrome)
• Diagnosis: clinical + thick smear/buffy coat (early), serology, xenodiagnosis, ECG, X-ray (late), PCR
• Treatment in the early phase still reasonably successful with medication; in the late phase difficult and probably useless
• Nifurtimox badly tolerated as a 2 to 4-month treatment; benznidazole: problems with bone marrow toxicity, hypersensitivity, peripheral neuropathy.
• Prevention: much progress in recent years via vector control and control of blood banks.

Introduction

Historical note

In 1907 the physician Carlos Chagas (1879-1934) was working in Lassance, a small poverty-stricken town on the Sao Francisco river in the state of Minas Gerais, Brazil. The town had been built along the railway from Rio de Janeiro to Belem. Chagas treated the workmen for injuries, syphilis, malaria etc. He noticed that cardiac arrhythmias occurred frequently. One day an engineer brought him an insect of the type which was known to often suck the blood of humans at night. Chagas wondered if this creature could also transmit malaria like the Anopheles mosquitoes. In the bug, he discovered a unicellular parasite. In April 1908 he found the same parasite in a sick cat. Two weeks later, in the same house, the parasite was found in the blood of a 3-year-old child (Rita), who was ill with fever. Her face, liver, spleen and lymph nodes were swollen and the child died shortly afterwards. In the house there were countless bugs which tested positive for the parasite. He sent bugs to Rio, to Oswaldo Cruz, his former teacher (Brazilian physician 1872-1917). In the laboratory the parasite caused an infection in marmoset monkeys (Callithrix sp.), rodents and puppies. The disease caused by this parasite, American trypanosomiasis, was named after Chagas. The parasite was given the name Trypanosoma cruzi. The parasite did not always trigger disease, however. In 1908 Chagas also discovered the parasite in another person (Bernice). This woman died in 1989, still infected, but without signs of organ involvement.

The infection apparently already existed before contact with the West. In 1985, 22 mummies were found in the Andes mountains. These were 1500 years old. In approximately half of them the heart, colon and/or oesophagus were clearly enlarged (lesions typical for Chagas' disease). Trypanosoma cruzi DNA was found in 1999 in a 4000 year-old mummy in Northern Chile. In one of his books Charles Darwin describes how in 1835 in South America he was bitten by the bugs. It is possible that he incurred infection and later developed a chronic form of the disease.

Distribution

The infection only occurs in America in endemic regions. It is a disease associated directly with poverty. The severity varies from region to region. In the South of Texas there are very few occur in Central America sporadically. Although the disease is endemic in large areas of South America (in particular in the “Gran Chaco” region), the majority of those infected have no symptoms. Until recently it was thought that approximately 16 million persons were infected, but these figures are under review (see Prevention). The disease is transmitted via the faeces of an infected bug.

Reservoir

The parasite, *Trypanosoma cruzi,* occurs in more than 100 species of mammal (opossums, guinea pigs, goats, dogs, cats, rats, mice, and so on). There are several known (and probably also some unknown) subtypes each of which has its own distribution and probably also its own pathogenic features. In view of the extent of the animal reservoir eradication of the parasite will not be possible. This does not mean that the disease and the transmission cannot themselves be controlled. At present the strains are divided into two groups*. Trypanosoma cruzi* I has an extensive sylvatic reservoir, of which opossums appear the most important. This group is not very common in the "Southern Cone" countries (Argentina, Brazil, Chile, Paraguay, Uruguay), but it is virtually the only form which occurs north of the
Amazon region. *T. cruzi II* seems to be chiefly associated with rodents and is common in the Southern Cone.

Transmission

Transmission occurs chiefly **via infected bugs**. These large insects like to bite sleeping humans at night (a mosquito net gives protection). They have a sharp proboscis which at rest is folded below the head like a jack-knife. When biting they inject anticoagulants and an anesthetic substance into the wound. Since this makes their bite quite painless (kissing bugs), people seldom wake up and several bites may take place unnoticed in the course of one night. The parasite is not inoculated directly by the bite, as Chagas initially thought. In 1913 Brumpt showed that the parasite is found in the faeces of the insect. While the animals suck blood, they defecate. By scratching, a bitten person can bring the faeces into the bite wound or rub them into the conjunctiva. The parasites multiply in humans and then appear in the blood. The cycle is completed when a subsequent bug drinks infected blood. In the bug the parasite undergoes further changes and after 2 to 3 weeks is excreted with the faeces during a subsequent bite. It is estimated that the risk per bite by an infected *Triatoma* is one in a thousand. The existence of **oral transmission** has been suspected for quite a while. It was demonstrated in animals and has now been confirmed in some human cases. How frequent oral transmission happens is not clear yet. Food or drink contaminated with the liquid faeces of infected bugs or containing (crushed) dead bugs may lead to infection not only in experimental animals but also in humans. Small outbreaks of acute Chagas are regularly reported from Northern Brazil in the last years. The parasite could withstand short periods of freezing, but not decontamination with sodium hypochlorite or heating to 80°C. **Congenital infection** (1 to 2 % risk) and transmission via **blood transfusion** also occur (poor people often sell their blood). To give an idea of the scale, this implies for example that several thousands

of babies are born with congenital Chagas each year in the USA, and a lesser number in Europe (from immigrant mothers from endemic areas). Transmission via transfusion is particularly important in urban zones and has been reported outside endemic countries. The risk of infection after a contaminated blood transfusion is estimated at one in five. There are sporadic cases of **accidental contamination** of laboratory staff (finger prick, aerosol) and after **organ transplantation** (including in non-endemic countries).

Vectors

The bugs are also known locally as "vinchucas" or "barbeiros". Of the approximately 120 vector species only about 7 are important. Each species has its own region of distribution:

- Central America and northern South America: *Triatoma dimidiata* and *Rhodnius prolixus*
- South America (south of 5° S*): T. infestans, T. braziliensis, T. sordida, Panstrongylus megistus*

The bugs mentioned here are the main vectors. Other bugs also play a part in different regions. The bugs each have their own preferred biotopes. *T. dimidiata,* for example, is often found inside houses on the floor or the lower 150 cm of the walls or immediately outside in dung heaps, hollow trees, etc. In contrast, *R. prolixus* prefers to live in palm leaves either in the roof of the house or in the tree itself. In and around the house the bugs can feed on animals (e.g. dogs are important because they sleep at night when the bugs are active). The vectors often live in chicken runs but the chickens themselves are not infected (they do eat bugs). During the day the insects hide in all kinds of cracks and crannies (importance of earthen or adobe walls) and in the roofing (straw, wood, etc). It can be seen immediately that the key word in Chagas' disease is "poverty". These are insects which reproduce slowly and whose geographical spread is slow. Migration of bugs, by migrating birds for example still needs to be studied. In view of these characteristics and the fact that the important vectors live around houses they can easily be reached by eradication campaigns.

A fertilized female lays several hundred eggs in her lifetime. From the egg comes a nymph which always needs a blood meal for its subsequent development stages (both sexes suck blood). The last instar will develop into an adult insect. During a blood meal they suck more than their own weight in blood. This takes 10-25 minutes. The insects may live for up to 2 years (5 years for *T. barberi*). *Rhodnius prolixus* has a relatively short generation time (3-5 months), while for *T. dimidiata* this time is quite long (1 year or longer). Long generation times make the development of resistance to insecticides difficult.

Parasite

In stained blood preparations the parasites are C- or S-shaped with a prominent kinetoplast towards the rear (trypomastigotes). The nucleus is elongated and the undulating membrane is usually not clearly visible. After infection multiplication of the parasite in the human is solely intracellular. They form microscopic pseudocysts in the tissues (similar to toxoplasmosis and sarcocsytosis). This occurs mainly in the heart, muscle cells, some nerve cells and the lymphatic system. In the cell the parasite is small and rounded with no flagellum (amastigote). When the infected cell ruptures, parasites are released into the blood circulation where they

become elongated and develop a flagellum. These forms can then infect other cells or be ingested by a bug.

Clinical aspects

Infection and incubation

Incubation period after exposure to vector-borne T. cruzi is 1 to 2 weeks, although longer incubation times are sometimes reported. If the parasites penetrate via the conjunctiva, there is unilateral redness and oedema of the upper and lower eyelids after 4 to 12 days. This is “Romaña's sign”, named after the Argentinean physician Cecilio Romaña, who described the oedema in 1935. This swelling may last for weeks. Sometimes there is also swelling of the ipsilateral lymph nodes (including the preauricular lymph nodes). Trypanosomes may be found in the tears at this stage. If inoculation is in the skin there is local oedema and redness (“chagoma”) in 75% of cases. This remains for 1 to 4 months.
From these sites the infections spreads.

Acute stage

The incubation period is followed by the acute phase which lasts 4 to 8 weeks. Many infections are initially asymptomatic. Acute symptoms occur more frequently in children than in adults. Dissemination of the parasite from the inoculation site may go unnoticed but may also give rise to acute illness with muscle pain, local or generalized oedema, swollen liver, spleen and lymph nodes. Moderate fever is almost always present in symptomatic cases and may persist for a long time, two or even four months. Sometimes there is also acute inflammation of the heart (myocarditis) with arrhythmias, decreased blood pressure, and heart failure. As with other forms of myocarditis the electrocardiogram is frequently abnormal. There is low QRS-voltage, prolonged PR- and/or QTinterval, T-wave abnormalities. Rarely there are ventricular extrasystoles or atrial fibrillation (the prognosis is poor if this occurs). Acute inflammation of the brain and meninges (meningo-encephalitis) occurs, chiefly in young children. Inflammation of the heart and brain may be fatal. There is pronounced lymphocytosis and monocytosis. The acute-phase case fatality rate is nowadays estimated to be 0.25 to 0.50% with early treatment.

Latent period

If the patient survives the initial phase (which is usually the case), a latent period occurs of indeterminate duration. The patient is asymptomatic, seropositive and the parasitemia is very low.
Focal lesions are found in 60% of endomyocardial biopsies from patients in the latent phase. A positive xenodiagnosis can be obtained in 50% to 100% of these patients. For xenodiagnosis 10 to 40 noninfected bugs (e.g. Dipetalogaster maxima or Triatoma infestans) feed on blood from the patient. The faeces from these animals are investigated after 30, 60 and 90 days. In the event of immunosuppression there may be an acute flare-up, including meningo-encephalitis associated with AIDS or heart transplantation.

Chronic phase

Gradually the patient develops symptoms. These vary greatly from region to region. Lesions of the heart, oesophagus and colon are the most common.

Chronic heart problems

Chronic damage to the heart muscle cells and the cardiac conduction system (including that caused by auto-immune mechanisms) leads to heart failure. There is dyspnoea during exertion, orthopnoea and sometimes paroxysmal nightly dyspnoea, oedema of the feet and ankles, congestion of the neck veins, enlarged liver and crackles over the base of the lungs. Sometimes there is pulsus alternans: the peripheral arterial pulsations are alternately strong and weak. The precise pathophysiological mechanism is not fully known. The apex of the heart, which is normally situated on the mid-clavicular line, is displaced to the left. The heart sometimes becomes enormous, which may lead to clot formation in the heart. If blood clots break loose, there may be embolic complications: cerebrovascular accident (CVA), ischemia of limbs, renal infarction. Apical lesions in the left ventricle (wall thinning, intramural bleeding, aneurysms) are typical and occur in approximately 50% of patients. Unlike arteriosclerotic post-infarction aneurysms, in Chagas' disease the apical cardiac tissue does not consist of scar tissue, the wall is simply thinned. Right ventricular lesions occur in 10 to 20%. Cardiac arrhythmias may cause palpitations, dizziness, syncope and sudden death. On the electrocardiogram a right bundle branch block is often seen, together with a left anterior hemiblock, ventricular extrasystoles, abnormal Q-waves and/or AV-conduction disturbances. The coronary arteries are normal. A complete left bundle branch block is exceptional, unlike in idiopathic dilated cardiomyopathy. Sudden death is common in people with Chagas' disease. Probably this is due to ventricular tachycardia which changes suddenly into ventricular fibrillation.

In advanced heart failure, typical radiographic signs may be observed on a chest X-ray: cardiomegaly, prominent hili and distended pulmonary veins in the upper fields, pleural fluid, interstitial pulmonary oedema (fluid in the interlobular septa with Kerley B lines), peribronchial cuffing and finally alveolar pulmonary oedema ("butterfly oedema").

The degree of heart failure is often indicated using the New York Heart Association classification:

- Grade I: asymptomatic
- Grade II: symptoms only during the moderate to severe exertion
- Grade III: symptoms during mild exertion
- Grade IV: symptoms at rest. Patient generally confied to bed/chair.

Oesophagus and colon problems

Due to involvement of the small nerves in the oesophagus and colon, peristalsis is reduced and these organs are distended. This occurs in 5 to 10% of seropositive people south of the Amazon, and is virtually absent further north. *Trypanosoma cruzi I* and *II* are both associated with cardiac lesions, but intestinal lesions only occur in infection with *T. cruzi II* (the southern area).

Mega-oesophagus is characterized by difficulty in swallowing (dysphagia), choking, hiccups and nocturnal cough. This often leads to under-nourishment and loss of weight. Aspiration pneumonia is the most feared complication with substantial mortality. A clinical aid for detecting delayed oesophageal emptying is to measure the time between swallowing a mouthful of water, and observing the abdominal noises (stethoscope on the epigastrium).

Normally this is less than 10 seconds. A distended oesophagus may also be shown on X-ray. The parotid gland may hypertrophy and lead to socalled "cat's face".

Mega-colon can lead to pronounced constipation, meteorism (abdominal distension), abdominal pain and functional intestinal obstruction due to involvement of the myenteric (Auerbach) plexus and the submucosal (Meissner's) plexus). The abdomen is often distended. Fecaloma, volvulus and peritonitis are complications.

The nervous system

In no other infectious disease is the involvement of the autonomous nervous system as important as in Chagas' disease. Denervation of the parasympathetic nervous system is better documented and is much more pronounced than denervation of the sympathetic system. There can be sensorimotor polyneuritis. There is some hypoesthesia and paraesthesia, but chiefly a reduction or loss of tendon reflexes. The EMG is disturbed. In the central nervous system there is meningo-encephalitis in the acute phase, but the abnormalities in the chronic phase need to be better defined. In flare-up (e.g. AIDS) there may be intracranial hypertension, lesions of the cerebral nerves, paresis, plegia, stupor and convulsions. The cerebrospinal fluid exhibits a normal number of cells or pleocytosis with predominant lymphocytes and an elevated protein content. At times T. cruzi may even be detected in the cerebrospinal fluid. A CT scan of the brain shows one or more necrotizing lesions which may or may not be ring-shaped, with haemorrhages usually subcortical in the brain hemispheres and occasionally in the cerebellum or the brain stem. T. cruzi lesions rarely occur in the basal nuclei. These clinical pictures should be differentiated from cerebral toxoplasmosis, abscesses, mycoses, tuberculomata or other mycobacterial lesions, metastases or lymphoma.

Of all cerebral vascular accidents leading to stroke, about 20% are secondary to embolism from a blood clot secondary to atrial fibrillation. If patients do not take oral anticoagulants, an average of 5% CVA's per year can be expected, which roughly translates to 50% of patients with CVA within 10 years after onset of atrial fibrillation. However for several reasons (mostly haemorrhagic) 20-40% of patients cannot be treated with oral anticoagulants. Most of the clots (90%) originate when blood stagnates in the left atrial appendage, also known as the left atrial auriculum.

Congenital infection

About 1 to 2 % of babies born to seropositive mothers are infected. They may be asymptomatic (rarely) or may develop hepatosplenomegaly, neurological involvement, myocarditis, oedema and a bleeding tendency. The babies may be dysmature and/or premature. Fever is rare in these children. The mortality may be as high as 50% and they tend to die within a week. Those who survive will generally have permanent residual neurological damage.

Diagnosis

In the acute stage the parasite may be found in the blood via a thin blood smear, thick smear or buffy coat. As a concentration technique an anion-exchange minicolumn may be used (Woo's technique similar to Lanham's column, but with a different buffer, see African sleeping sickness). Strout's concentration technique includes the double centrifugation of serum (from

10-20 ml of blood), after which the motile trypanosomes can be detected in the sediment. PCR techniques for *T. cruzi* exist, but can only be carried out in better equipped laboratories. The serology is positive from the fourth week.
To know whether the neonate from a seropositive mother is infected, PCR is performed and IgM antibodies in its blood are determined. A positive serology (IgG) 6 months after birth also indicates infection. In-vitro and in-vivo culture is possible, but usually not available. Biopsies of lymph nodes, heart and muscles sometimes show parasitic pseudocysts (amastigotes in the cells). This is quite an aggressive technique and not very sensitive.

Dipetalogaster maximus is a blood sucking bug which can take up to 4 ml of blood in one meal. It is best known for its use in xenodiagnosis (cfr. supra, latent period) of Chagas' disease.

Following WHO recommendations in patient with latent infection (indeterminate), 2 or 3 different positive serological tests are required before ascertaining the diagnosis of Chagas disease.

Prognosis

In an endemic region an asymptomatic person with positive serology is probably a carrier (xenodiagnosis positive in 50 to 100 % of cases). The percentage of seropositive persons who develop symptoms is highly dependent on the geographical region (e.g. 10 to 30%). Some people have megaorgans but are asymptomatic.

Chagas' disease variables associated with adverse outcome

- 2 points: Male
- 2 points: Low QRS voltage on ECG
- 3 points: Non-sustained ventricular tachycardia on 24-h Holter monitoring (run of 3 or more consecutive VES, with a frequency >100).
- 3 points: Left ventricular systolic dysfunction: segmental or global wall-motion abnormality on echocardiography (quid apical aneurysms, intracavitary thrombus)
- 5 points: NYHA III or IV
- 5 points: Cardiomegaly present on CXR, defined as a cardiothoracic index > 0,5

Results:

- < 6: low risk 14% mortality rate in 10 years
- 7- 11: intermediate risk 44% mortality rate in 10 years
- 12-20: high risk 84% mortality rate in 10 years

Treatment

Acute phase

The acute phase lasts up to 60 days. All patients who are in this phase should be treated.

Congenital infection

All infected children should be treated. The earlier therapy is begun, the better the results.

Chronic phase

Etiological drug treatment is indicated for "recent" chronic infections (a few years). In practice all children younger than 10 years are treated. If mega-oesophagus is already present the dysphagia should be treated (the passage and absorption of oral medication may be severely impeded). Etiological treatment in these latter patients was not advised formerly but more recent data have brought this into question. In a study in Argentina, 131 patients with chronic Chagas' disease were treated with benznidazole. After an average follow up of 8 years, 4.2% exhibited ECG changes compared to 30% in the untreated group. There was also considerably less clinical deterioration in the treated group (2.1% compared to 17%).

The results of a large multicenter prospective study (the BENEFIT study) has however recently demonstrated that an etiologic treatment with benznidazole did not provide any clinical benefit when a patient with chronic infection had already developed a cardiomyopathy (no reversibility). Treating this group of chronic patients appear to be futile while exposing them to some drug toxicity. Whether this is also true for a patient with latent infection and no complication (yet) will require additional evaluation. Such studies are however very difficult to conduct due to the very long latency period to obtain robust clinical outcome data.

Accidental infection

This may occur for example in laboratory staff. A serum specimen should be frozen before beginning treatment and a second blood sample taken 4 weeks later. Serology is performed on these paired sera. Benznidazole 7-10 mg/kg/day x 10 days is the usual treatment regimen in this situation.

Transplant patients

There are two possible situations: transplantation of an infected organ into a non-infected patient and transplantation of a healthy organ into an infected patient. A donor may be infected so that the recipient becomes infected. Normally the donor is tested beforehand and positive donors are refused, but nevertheless these situations sometimes occur. Alternatively transplantation may be carried out on a patient who is a chronic carrier. The immune suppression that these patients undergo [steroids, azathioprine (Imuran®), tacrolimus (Prograft®) and cyclosporine (Sandimmun®)], may lead to reactivation of Chagas' disease. In both cases treatment with benznidazole 5 mg/kg/day x 60 days, is indicated.

HIV patients and Chagas

Infection with HIV may lead to significant flare-up of Chagas' disease. In endemic regions all HIV patients should be monitored for Chagas' disease. If positive, benznidazole is recommended. There is insufficient data concerning chemoprophylaxis. Since the initial step is often serology, one would normally first try to confirm the diagnosis with a second serological test (ELISA-based preferably) and by looking for circulating parasites by microscope (QBC or buffy coat), PCR and perhaps xenodiagnosis. If the diagnosis is confirmed, the patients deserve to be treated as their risk of severe complications (cardiac, digestive or CNS) is high. Benznidazole is preferred to nifurtimox, since nifurtimox is a treatment that is really badly tolerated in adults (notably a lot of nasty allergic reactions). Benznidazole 5mg/kg (max 300mg) daily for 60 days is not an easy treatment to administer neither (beware of skin toxicity!).

Pregnancy

Treatment during pregnancy is not recommended, although congenital Chagas has been well documented. It is clear that more understanding and better outcomes are sorely needed. Infants of infected mothers have to be carefully followed-up to early detect congenital Chagas.

Treatment: Drugs

There are several problems. The drugs have an unsatisfactory cure rate. The chronic lesions may be caused by auto-immune mechanisms and might not be improved by eradicating parasites (as suggested by the recent BENEFIT trial). Parasites play however some role since the disease worsens during immune suppression as in transplantation and in HIV. The drugs should be given long term (minimum 2 months). Results vary from country to country, possibly due to a difference in parasite susceptibility. Side effects occur more often in adults than in children. It is best to avoid steroids or other immunosuppressive drugs, since these may exacerbate the infection.

Nifurtimox (Lampit®) 5 mg/kg/day orally, slowly increased to 15 mg/kg/day (divided over 3 doses) for 2 to 4 months. There are regular problems for the sustainability of its production. Side effects: neurotoxicity (insomnia, tremor, polyneuritis), nausea, leukopaenia, thrombocytopaenia or hypersensitivity. May cause haemolysis in G6PD deficiency [glucose-6-phosphate dehydrogenase]. In the acute phase the parasites disappear from the blood in 80 % to almost 100 % of cases. The actual cure rate is 50-60%. In a prospective study conducted in Switzerland among Bolivian immigrants, more than 90% of the patients developed some side-effects, sometimes severe (angioneurotic oedema, Dressler syndrome) and half had to discontinue the drug before the end of the 2-month therapy.

Benznidazole (Radanil®, Ragonil®, Rochagan®) 5-10 mg/kg/day orally for 1 to 2 months. Administration (generally 100 mg tablets) is twice daily. The same side effects as nifurtimox, but less frequent and less pronounced, although skin rash occurs relatively frequently (up to 30-40% of patients, with probably some genetic predisposition) sometimes accompanied by swollen lymph nodes or angioedema. The pharmaceutical company Roche has donated all commercial rights and the technology to manufacture benznidazole to the Brazilian government. In all countries 2-month treatment is recommended, except in Argentina where experts recommend a one-month treatment only.

Other types of drugs for treatment

Posaconazole, an anti-fungal therapy, was found to be inferior (in terms of parasitological failure) to benznidazole in a randomized control trial in Spain, published in 2014.

Fexinidazole has a clear anti-*T. cruzi* in vitro activity, but no clinical study has taken place so far.

Ravuconazole is a new triazole with in vitro activity against species of Candida, Cryptococcus and Aspergillus, but also in vitro and in vivo (mice) activity against *Trypanosoma cruzi*. Ravuconazole has a long half-life in humans, which hopefully will facilitate compliance in patients. Clinical trials for its use in Chagas' disease are ongoing in Bolivia.

In the chronic phase the usefulness of these drugs could not be demonstrated (BENEFIT Trial) at least in patients having already (mostly heart) complications.

Symptomatic therapy is therefore indicated: oesophageal sphincter dilation, extramucosal cardiotomy (Heller's operation), colon surgery. An experimental treatment is the endoscopic injection of botulin toxin into the distal oesophageal sphincter (e.g. 20 U into each quadrant).

In heart failure diuretics, ACE-inhibitors and antiarrhythmic drugs may be beneficial. Beta-blockers are best avoided in view of the AV-conduction problems and brady-arrhythmias. Aspirin or anticoagulants are indicated for patients with atrial fibrillation, previous embolic phenomena and apical aneurysms. Amiodarone (Cordarone®) is effective in more than 50% of patients who develop ventricular extrasystoles or ventricular tachycardia. A bifascicular or trifascicular conduction block, also a second or third degree AV-block are contra-indications. A high incidence of "torsades de pointes" has been observed during use of disopyramide and other class I antiarrhythmic drugs.. Pacemakers, automatic defibrillators and cardiac surgery (including heart transplantation) are reserved in practice for those with financial means and these persons have an inherently low risk of infection. It is obvious that such costly procedures will not be within the financial means of the average Chagas' patient.

Prevention

The animal reservoir of *Trypanosoma cruzi* cannot be eradicated. There is no vaccine. Chagas' disease is typically a disease of poverty. Improvements in housing (brick or plaster walls, corrugated iron roofs, long-acting insecticides on house walls) diminish the insect population. A mosquito net has also proven usefulness here. Serological testing of the blood used for transfusion is very helpful. To date the various biological methods of eradication of the vectors (insecticide sprays, increasing natural enemies) which have been tested have not been effective because a new ecological balance is very quickly achieved but have brought substantial control in most regions.

In 1991-92 the "Southern Cone Initiative" project was launched by Argentina, Bolivia, Brazil, Chile, Paraguay and Uruguay with the objective of stopping the transmission of Chagas' disease. In 1997 Peru joined the project. After an initial phase for preparation (charting the foci, programming the activities, calculating the costs) there was an attack phase with insecticides, repeated after 3 to 6 months. Insecticide-containing paint is cheaper than the traditional insecticides which are applied by spraying.

Insecticides dispersed by fumigant canisters were also used. These are locally produced e.g. in Argentina, are cheap, effective and also active against *Aedes aegypti*, the important dengue vector. At present there are effective colourless long acting insecticides. The fact that people see the bugs, cockroaches, etc. lying dead after spraying is a bonus which makes it easier to accept the spraying procedure. In the Southern Cone Initiative, 1,800,000 houses were treated with pyrethroids (deltametrine, lambda-cyhalotrine, cyflutrine) by the year 2000.

Since then there has been further selective treatment of the houses which still exhibited infestation with triatomes. Simple "sensor boxes" of cardboard (traps for the bugs) were placed in the rooms and the occupants themselves could simply ascertain the presence of triatomes. The last phase is surveillance for the detection of residual foci. This is decentralized and involves the population. The effectiveness of the control program has been demonstrated by the very pronounced drop in seropositivity among young children. The surveillance phase has been reached in 6 countries of the Southern Cone. At present there are several South

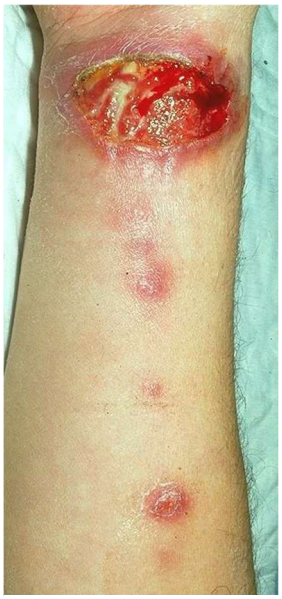
American countries (Colombia, Ecuador, Venezuela) which have a national control program. Similar programmes were begun in Central America in 1997: Costa Rica, El Salvador, Guatemala, Honduras, Nicaragua, Mexico and Panama. These programs can only be successful if there is participation of the population and if they can be continued for long enough. The latter is a political decision.

In July 2007 the WHO Global Network for Chagas Disease Elimination was launched in order to coordinate global efforts to eliminate this disease. It includes also many non-endemic countries (such as Spain or USA) where Chagas disease in Latin American immigrants have given rise to a substantial number of secondary transmission (by blood transfusion or transplantation), requiring locally adapted control efforts (screening).

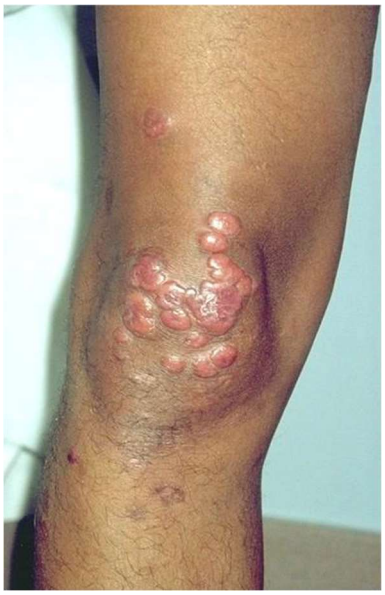
Leishmaniasis

Summary
•In humans obligate intracellular parasite with replication in macrophages
• Cutaneous form: chronic painless ulcers or nodules, amastigotes in smear
• Visceral form: chronic fever, hepatosplenomegaly, pancytopenia, persistent inflammatory state. Lethal if not treated
•Diagnosis of kala azar: amastigotes in bone marrow and other sites, serology, antigen detection
•Mucocutaneous: chronic destructive lesions in mouth/nose, frequent clinical diagnosis
•Transmission via about 30 species of sandflies
•Zoonotic transmission: animal reservoir (especially dogs and rodents)
•Anthroponotic transmission: human reservoir, e.g. Indian kala azar and in cutaneous L. tropica
•Treatment with antimony derivatives, amphotericin B, miltefosin, pentamidine. Combination
treatment increasingly in use.

General



Leishmania braziliensis ulcer on the wrist and spread via the lymphatics. Lesions occurred after a visit to rural Bolivia. Copyright ITM



Diffuse cutaneous leishmaniasis due to infection with *Leishmania aethiopica*. Copyright ITM

There are several species of *Leishmania* parasites and these can cause various clinical conditions. They can be responsible for chronic ulcers and skin nodules. Sometimes both skin and mucosae are affected (mucocutaneous leishmaniasis). When deep organs are affected, the condition is called visceral leishmaniasis. The *Leishmania* species that cause these various clinical conditions always have the same morphology under the microscope. However, there

are differences in parasite DNA, proteins, enzymes and mode of development in the insect vector, etc. *Leishmania* parasites can in turn be infected with a RNA virus (the "leishmania virus") though the significance of this is not yet known.

The classification, distribution and pathogenicity of the various *Leishmania* species is quite complicated. New data are regularly becoming available (for example, *L. tropica* was shown to be able to cause visceral leishmaniasis in rare cases). The whole taxonomy will probably change as more and more genetic information becomes available. A distinction is made between zymodemes (iso-enzyme patterns), schizodemes (kDNA analyses with restriction enzymes), serodemes (via reactions with monoclonal antibodies) and rapdemes (using PCR with random primers). Some 30 different *Leishmania* species have been described (10 in the Old World and 20 in the New World). Many of these can infect humans. The genus *Leishmania* is frequently subdivided into the subgenera *Leishmania* and *Viannia*.
There are substantial geographical genetic variations. Hence in the dry western part of *Peru L. peruviana* causes the disease "uta", an ulcerative form without mucocutaneous lesions. This organism contains less DNA in some of the chromosomes than the virulent L. braziliensis, the pathogen causing Espundia, a disease which occurs in the forests on the other side of the Andes in Eastern Peru. One of the differences is the number of copies of the leishmanolysin gene, which codes for an important surface antigen (gp63). This zinc protease has a role in adhesion to macrophages and survival in the phagolysosome. It is regarded as an important virulence factor. *L. braziliensis* contains more leishmanolysin genes than *L. peruviana*. The protein is being studied as, among other things, the basis for an experimental vaccine.

Classification

There is still no generally accepted internationally agreed definitive taxonomy. The following table can serve for orientation:

Leishmania species			
New World			
L. (Viannia) braziliensis	LCL, mucosal	zoonotic	Latin America
L. (Viannia) panamensis	LCL, mucosal	zoonotic	Northern South America and southern Central America
L. (Viannia) peruviana	LCL	zoonotic	Peru
L. (Viannia) guyanensis	LCL	zoonotic	South America
L. (Viannia) lainsoni	LCL	zoonotic	South America
L. (Viannia) columbiensis	LCL	zoonotic	Northern South America
L. (Leishmania) amazonensis	LCL, DCL	zoonotic	South America
L. (Leishmania) mexicana	LCL, DCL	zoonotic	Central America, Mexico
L. (Leishmania) pifanoi	LCL	zoonotic	South America
L. (Leishmania) venezuelensis	LCL	zoonotic	Northern South America
L. (Leishmania) garnhami	LCL	zoonotic	South America
Old World			
L. (Leishmania) aethiopica	LCL, DCL	zoonotic	Ethiopia, Kenya
L. (Leishmania) killicki	LCL	zoonotic	North Africa
L. (Leishmania) major	LCL	zoonotic	North and East Africa, Middle East, Central Asia
L. (Leishmania) tropica	LCL	anthroponotic	North Africa, Middle East, Central Asia
L. (Leishmania) donovani	LCL, visceral	anthroponotic	Central Asia, Africa
Old and New World			
L. (Leishmania) infantum	LCL, visceral	zoonotic	South Europe, North Africa, Central and South America

Legend: LCL : localised cutaneous leishmaniasis ; DCL : diffuse cutaneous leishmaniasis

Visceral leishmaniasis is mainly caused by the *Leishmania donovani* complex. There are several species in this complex:

1. *Leishmania donovani* (India, Pakistan, sub-Saharan Africa, East Africa)
2. *Leishmania infantum* (Mediterranean Basin, Middle East)
3. *Leishmania chagasi* (South America = *Leishmania infantum*)
4. *Leishmania archibaldi* (Africa) – of unclear importance

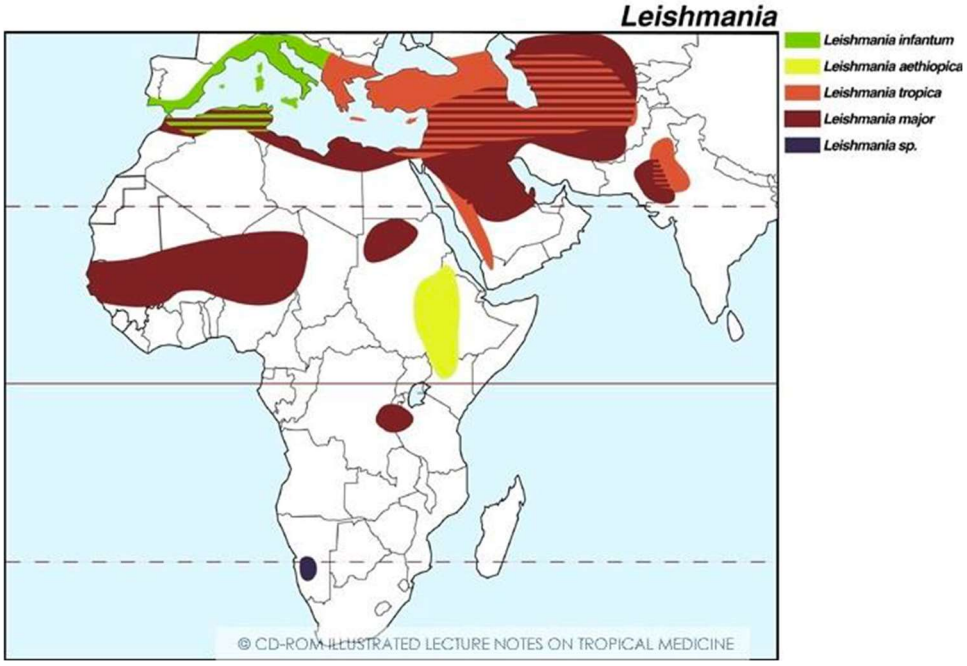
In the Old World skin lesions are mainly due to:
1. *L. tropica* (Mediterranean basin, Middle East). Frequently dry lesions
2. *L. major* (Middle East, sub-Saharan Africa). Frequently moist lesions
3. *L. aethiopica* (Ethiopia, Kenya). Sometimes also affects mucosa
4. *L. killicki* (North Africa) – of lesser importance
In addition, *L infantum* and *L donovani* (more exceptionally) can also cause skin lesions.

In (mainly South and Central) America skin lesions are caused by the *L. mexicana* and *L. braziliensis* complex. These complexes are subdivided into species:
1. *L. mexicana* complex: *L. mexicana, L. venezuelensis, L. amazonensis*
2. *L. braziliensis* complex: *L. braziliensis, L. panamensis, L. guyanensis, L. peruviana*

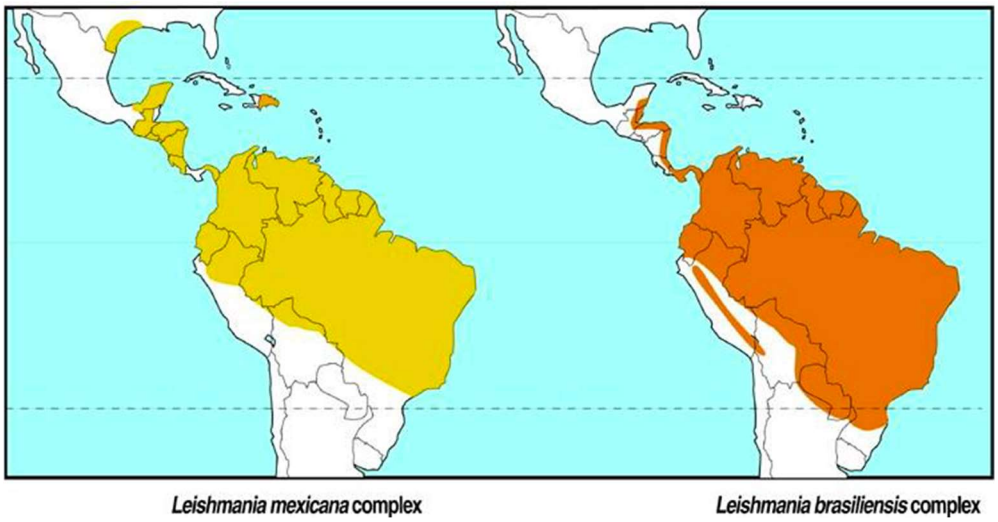
Mucosal lesions are common in infections with *L. braziliensis*. One should always keep in mind that the clinical lesions of leishmaniasis are a consequence of the parasite species on the one hand and of the immunological resistance and reaction of the patient on the other.

Infections occur very rarely with other *Leishmania* species: *L.(Viannia) naiff, L. (Viannia) shawi.*

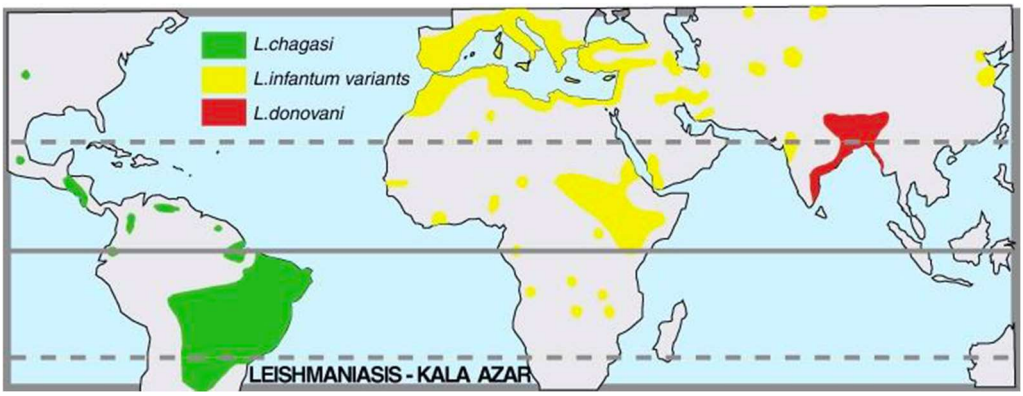
Distribution



Map Leishmania infantum, L. aethiopica, L. tropica, L. major. Adapted from Colour Atlas



Map *Leishmania mexicana* and *L.braziliensis*. Adapted from Colour Atlas.



Map of the areas endemic for *Leishmania chagasi, L. infantum, L. donovani,* pathogens leading to kala azar. Adapted from Colour Atlas

Mucocutaneous leishmaniasis occurs in Central and South America and occasionally in East Africa.

Visceral leishmaniasis occurs from western China to the Mediterranean Basin, East Africa and Central and South America. It is very rare in Africa south of the equator. The majority of cases occur in 6 countries: Bangladesh, Nepal, India, Ethiopia, Sudan and Brazil

The cutaneous form is seen from India to the Mediterranean Basin, the northern half of the African continent and in Central and South America.

Leishmaniasis does not occur in Northern Europe, Canada, Uruguay, Chile, South Africa, Australia and Oceania. While Southeast Asia was thought to be leishmania free, an increasing number of visceral leishmaniasis cases have been reported from Thailand more recently.

For additional information and geographical risk in Europe; see www.leishrisk.net

Vector

The parasite is transmitted by the bite of infected female sandflies: *Phlebotomus* in the Old World and Lutzomyia in Central and South America. These genera, together with the blood-

sucking genus *Sergentomyia* [little significance for man, as they suck blood from reptiles], belong to the Psychodidae family. Morphologically they very closely resemble each other. The name "sandfly" can be confusing as this name is sometimes used for other species as well. Sandflies are vectors of leishmaniasis, pappataci virus (an arbovirus) and *Bartonella* bacteria.

Sandfly. *Lutzomyia* and *Phlebotomus* species are vectors of leishmaniasis in the New, resp. Old World. Photo Cochabamba, Bolivia

Only some 10% of the approximately 600 known species of sandflies are vectors, and only 30 of these are important. A fly remains infected for life. In endemic areas, a minority of sandflies are infected usually below one per cent.

The female insects need blood in order to lay their eggs. Most species bite at night and at dusk. There are exceptions to this, such as Lutzomyia wellcomei, the main vector of L. braziliensis, which bites mainly during daytime. They can suck blood both from animals (cats, dogs, various rodents, cattle, birds and lizards, etc.) and man. They are small, soundlessly flying insects (approximately 2 mm in length). Because of these small dimensions they can get through standard mosquito nets. Impregnation with permetrine (cf. malaria) can help. Because of the very short mouthparts of the insects, they cannot bite through clothing. They are poor flyers. They will usually fly quite low and will remain in the vicinity of their breeding ground. They will also not fly when there is any wind. This knowledge can be exploited by having a fan or ventilator on at night in the bedroom to prevent sandflies from flying. They require high humidity and temperature for breeding, although they can be observed in dry regions provided there are sites with a favourable local microclimate (crevices, termite mounds, caves, hollows and holes in tree roots, etc) where 15 to 80 tiny eggs can be laid. The larvae cannot survive drying out. They will feed on organic waste and then pupate. Sandflies reproduce optimally at 23-28°C and at a relative humidity of 70-100%. Temperatures below 10°C or above 40°C are unfavourable for their survival. Measures used to control adult sandflies include the use of insecticides for residual spraying of dwellings and animal shelters, space-spraying, insecticide-treated nets, impregnated dog-collars and personal protection through application of repellents/insecticides to skin or fabrics. Bednets will be most useful in areas with peridomestic vectors (e.g. P*. argentipes* in India) whereas in areas where the vector bites in the field (e.g. *P. martini* in Kenya and Uganda) this can be expected to be less effective. Because the breeding-sites of sandflies are generally unknown, control measures that act specifically against immature are not feasible. Reports of insecticide-resistance refer to only three sandfly species (*P. papatasi, P. argentipes and S. shorttii*) against DDT in one country (India), although there are reports of DDT-tolerance in several countries.

Pathophysiology

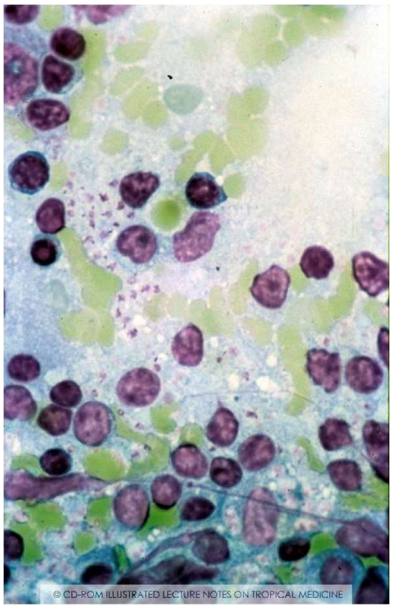
An important aspect of the immune system is the balance between two arms of the T-helper response. Broadly speaking, the T-helper1 (Th1) response is tailored to intracellular pathogens, such as viruses and some bacteria and parasites. Because these organisms live inside cells, they are not accessible to antibodies. The Th1 response therefore stimulates other defence mechanisms such as macrophages. The T-helper2 (Th2) system, by contrast, promotes a vigorous antibody response. The two arms are antagonistic, so a strong Th1 response means a weak Th2 response and vice versa. In leishmaniasis, where the parasites are intracellular, a strong Th1 response will kill the parasite and a strong Th2 response will lead to uncontrolled disease.

A gel produced by the *Leishmania* parasite in the gut of the sandfly prevents the insect from feeding properly. This causes more effort to feed, providing more chances for transmission of the parasite. The gel is injected into the human with the parasite and increases the severity of the infection. The crucial molecule in the gel, called filamentous proteophosphoglycan, interferes with the human immune system. The gel pushes the immune response to the non-protective T-h2 arm. The parasite thus manipulates the sandfly to make it feed more and then manipulates the host's immune system so that it can spread unchecked.

Sandfly saliva is important for the establishment of infection and disease pathogenesis. The sandfly saliva contains the vasodilator maxadilan. Saliva proteins seem to influence the immune response, resulting in a shift from Th1 to Th2 response. It is possible that the age-related decrease of susceptibility to leishmaniasis is due to anti-sandfly saliva antibodies.

Life cycle, *Leishmania* sp.

The parasite's life cycle is quite simple. When an infected sandfly bites, the parasite (as a promastigote) is injected directly into the skin. This unicellular parasite then penetrates the cells of the reticuloendothelial system (macrophages), where it multiplies in the form of amastigotes (the nonflagellate form) ("a" = without; "mastix" = whip). It is this form that can be seen in a skin biopsy or bone marrow aspirate. Multiplication results in bursting of the host cell, whereupon other cells become infected.



Leishmania amastigotes. This is the form present in human tissue.
Copyright ITM

Leishmania promastigotes. The parasite has this morphology when residing in the sandfly vector. Copyright ITM

When another sandfly later bites, these infected cells can be ingested. The parasite is then still located in infected macrophages. The blood meal in the stomach is completely surrounded by a peritrophic membrane. The parasite transforms into a different form (promastigote with flagellum) in the insect and then multiplies. After 2-3 days the peritrophic membrane is digested and the parasites are released into the lumen of the stomach and intestine. They then attach to the microvilli of the intestine by means of their flagellae. They produce an enzyme, chitinase which damages the chitin coating of oesophageal-gastric junction, so that the valve between stomach and oesophagus no longer functions adequately and leaks resulting in a backflow of parasites to the mouthparts. The parasites accumulate 7 to 10 days later in the insect's proboscis and can be injected when the insect bites its next victim. The insect is infectious 7-10 days after an infected meal and has to survive for this time in order to be transmitted. Haemoglobin degradation products inhibit the secretion of chitinase and/or inhibit the enzyme itself making backflow of parasites to the mouthparts more difficult. Certain plant sugars do not have this effect. The insects also feed on plant juices. A balance between plant and animal feeding is required for successful transmission. A botanical description of the vector's environment (biotope) can be important in scientific studies.

Kala azar can be transmitted in other ways, but these are exceptional, namely shared use of needles among intravenous drug users or infected blood transfusion. Very rare cases of congenital kala azar infection have been reported.

Historical note, discovery of the parasite

The search for the origin of kala azar initially proceeded with great difficulty. Many hypotheses were investigated: for example, hookworm infection (ancylostomiasis) or malaria were thought to be responsible for the clinical condition. In 1900 an Irish soldier developed kala azar, after a stay in Dum Dum, near Calcutta, India. He died in England. The Scottish physician

Dr. William Boog Leishman, later Director-General of the medical service of the British Army, carried out the autopsy. In spleen tissue he discovered small particles within the macrophages. He suspected that these were a sort of partly digested trypanosomes. A previously used name for visceral leishmaniasis was "Dum Dum fever" and refers to this historical event. The Irish physician Dr. Charles Donovan investigated splenic aspirates (needle biopsies of the spleen) from kala azar patients and confirmed Leishman's discovery. The tiny particles were called Leishman-Donovan bodies.

Visceral leishmaniasis - Kala Azar

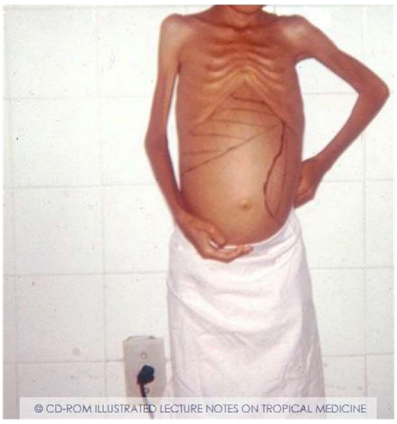
Distribution

Currently, 90% of all visceral leishmaniasis occurs in India, Bangladesh, Nepal, Ethiopia, Sudan and Brazil. Visceral leishmaniasis may be responsible for 500,000 new cases and > 50,000 deaths per year.

Clinical aspects

After an initial multiplication in the skin, causing a transient small lesion, the parasites can further multiply in bone marrow, liver and spleen, causing visceral leishmaniasis. The incubation period is usually 2 to 6 months. The pathogens are usually *Leishmania donovani* and *L. infantum*, but rarely *Leishmania tropica*. *L. chagasi* is now considered identical to *L. infantum* and was possibly introduced into the New World via infected dogs or rats at the time of the Spanish and Portuguese conquests, although there are doubts about this.

Visceral leishmaniasis in Southern Europe was initially considered a pediatric disease (hence the name L. infantum). However, it is clear that all age groups can be infected. The disease is characterized by a persistent inflammatory state with chronic fever, enlarged liver and spleen and a low blood count (pancytopenia = anemia + leukopenia + thrombocytopenia). This must be distinguished from an aplastic bone marrow. The patient becomes susceptible to other infections (pneumonia, tuberculosis, dysentery), which can sometimes prove fatal. Symptoms and signs of superimposed bacterial infections may confuse the clinical picture at the time of initial diagnosis. Low blood platelet counts result in a bleeding tendency (nosebleeds, bruising, etc.). Sometimes, there are other symptoms, such as swollen lymph nodes, which are more common in Sudan than in India. Weight loss and emaciation are frequent and substantial. The skin can turn a dark color: kala azar (Hindi) means “black fever” and refers to this hyperpigmentation. This was mainly described in Indian cases. The reason for this hyperpigmentation is not clear. The infection can proceed atypically in HIV patients (for example, without fever or splenomegaly or with negative serology). When immunosuppression is induced by chemotherapy, latent visceral leishmaniasis can become clinically apparent.



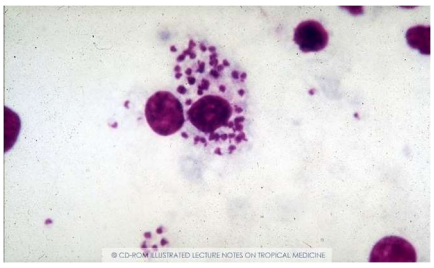
Visceral leishmaniasis (kala azar) with hepatosplenomegaly. Copyright ITM

Post- Kala-azar dermal Leishmaniasis (PKDL)

A skin condition called post-kala azar dermal leishmaniasis (PKDL) can occur after a patient has suffered from kala azar. PKDL rarely occurs without being preceded by kala-azar. PKDL occurs on average 4-8 months after kala azar (range 0-3 years), though there are strong regional variations (In India 2-3 years after the disease, in Sudan typically within six months). This disease occurs mainly in India (up to 20% of visceral leishmaniasis patients) and to a much lesser extent in the Middle East. In Sudan, the disease occurs regularly (56% of visceral leishmaniasis patients were diagnosed in one study). It is virtually unknown in the Mediterranean Basin or South and Central America. It involves discolored patches and painless nodules on the skin that usually contain high numbers of amastigotes. Most of the lesions occur on the face (98%) and to a lesser extent on the thorax (80%), arms (70%), legs (40%), tongue (40%) and genitals (6%). This disease has a chronic course (years) and is, therefore, important for transmission. Parasites do not affect internal organs in PKDL. There is sometimes a concomitant neuritis, which can further contribute to the clinical resemblance to leprosy. Contrary to India, in East Africa, this condition heals spontaneously in up to 80% of patients. Treatment with glucantime can be given for 2 months or longer (4 months in India, where resistance to antimony is higher). Amphotericin B is also effective. The therapeutic place of miltefosine for PKDL is not clear at present.

Diagnosis

In endemic areas, fever lasting more than 2 weeks and accompanied by splenomegaly not responding to antimalarial therapy strongly increases the suspicion of visceral leishmaniasis, but this clinical picture is insufficient to confirm the diagnosis.



Leishmania amastigotes. Copyright ITM

Diagnosis of visceral leishmaniasis is not easy, as none of the tests have 100% sensitivity and 100% specificity. Clinical syndromic diagnosis lacks specificity as malaria, hyperreactive

malaria splenomegaly, trypanosomiasis, typhoid fever, disseminated tuberculosis, brucellosis, hematological disorders, splenic abscess or splenomegaly due to portal hypertension all can be accompanied by an enlarged spleen, fever, wasting, anemia and/or lymphadenopathy. Because of the high cost and toxicity of current therapeutic options, empirical treatment is not advised. Therefore, confirmatory diagnostic tests must be used. The leishmanin skin test indicates past infection or cutaneous leishmaniasis and is not used to diagnose visceral leishmaniasis.

Direct diagnosis

Direct diagnosis is made by demonstrating the presence of amastigotes in a bone marrow, spleen or lymph node aspirate. The parasite is egg-shaped and measures 2-3 x 5 µm. With Giemsa staining, there is a pale blue cytoplasm, a well-defined nucleus and a smaller kinetoplast. Microscopy requires considerable expertise and training. Usually, bone marrow is obtained by sternum aspiration. The technique of spleen aspiration is more sensitive (in some studies approaching 100%, though in reality slightly lower) than bone marrow aspiration but can be risky (spleen rupture, hemorrhage). The platelet count should be above 40 x 109/litre. Active bleeding, severe anemia, jaundice, moribund state, pregnancy and lack of cooperation are contra-indications. Patients must lie in bed for several hours after the procedure. Vital signs must be checked frequently to allow early recognition of hemorrhage, and blood transfusion facilities must be available. To perform the procedure a 21-gauge needle and a 5 ml syringe is required. After penetration of the skin, the plunger is withdrawn, and the needle is quickly inserted into the spleen while maintaining suction and withdrawn immediately (i.e., less than 1 second). Lymph node aspiration and/or liver biopsy are sometimes necessary. The parasites can rarely be detected in peripheral blood monocytes.

Serology

Serology is positive in most cases of visceral leishmaniasis. Gel diffusions immunoelectrophoresis, complement fixation test, indirect hemagglutination, Western Blot and countercurrent immunoelectrophoresis have limited diagnostic accuracy and/or feasibility in the field. Indirect fluorescence tests (IFA) are an alternative but require a fluorescent microscope. The direct agglutination test (DAT) is often used as this has a high sensitivity and specificity. Both liquid and freeze-dried antigens can be used, although liquid antigen is associated with poor reproducibility in East Africa (most likely due to decay of liquid antigens during storage and transport). Note that freeze-dried antigens do not require refrigeration. The DAT is simpler than many other tests but requires equipment, such as microplates and micropipettes, training and regular quality control. A suggested cut-off value 1/3200 is often used but should be evaluated in each setting. An alternative is to consider titers < 1/1600 negative, borderline between 1/1600 – 1/12800, and positive > 1/12800. It can be defended that in a rural endemic area, a patient with more than two weeks of fever and splenomegaly with strongly positive DAT values and no response to antimalarials doesn't necessitate formal demonstration of parasites. With borderline serological values, tissue aspiration and a search for amastigotes will be needed. A possibility in a small regional clinic is to absorb a drop of blood from a patient suspected to have kala-azar on a small filter paper and then punch out a standard-size disk from the blood spot. This way, one obtains a well-defined, accurate aliquot of absorbed blood. This can be transported and used for DAT in a well-equipped laboratory. Serology remains positive after cure. The fast agglutination

screening test (FAST) is a simplified (single serum dilution) and more rapid version of the DAT (2-3 hours versus 18h). Because DAT is impractical in many field conditions, alternatives are being studied. ELISA is highly sensitive, but specificity depends on the antigen (amastigotes or promastigotes). Recombinant K39 antigen-based dipsticks using immunochromatography (ICT) have been an important step forward and have replaced DAT as a first-line test. K39 is a 39-amino acid repeat that is part of a kinesin-related protein of *L. chagasi.* This repeat is conserved within the *L. donovani* complex. The ICT tests are easy to perform, rapid and cheap. Twenty µl of serum is added to the dipstick, which is then placed vertically in a test tube. Two drops of chase buffer solution provided with the dipstick are then added. The results are read after 5 to 10 minutes. Even a weak band in the test region is considered positive. A control line has to be visible. It is the most promising tool for diagnosing visceral leishmaniasis in peripheral centers.

Formol-gel test

In kala azar there is a very high production of non-specific immunoglobulins (and a decrease In kala-azar, there is a very high production of non-specific immunoglobulins (and a decrease in albumin), especially in advanced disease (i.e., more than 3 months). This can be demonstrated by serum protein electrophoresis, which is usually unavailable in field conditions. The proteins can be precipitated as a gel by formalin. Twenty µl of 40% formaldehyde are added to 200 µl of serum in a glass tube. After twenty minutes, the gelification reaction is visually assessed as positive or negative. The test is simple and cheap. The test can also be positive in patients with hyperreactive malaria splenomegaly.

KAtex

A urinary antigen detection test using latex agglutination (KAtex) has been developed to circumvent the limitations of serological tests. It detects a heat-stable low molecular weight carbohydrate antigen. This will become negative upon successful treatment. It can, therefore, distinguish an active from a past infection. A very high specificity and moderate to high sensitivity were reported. The test requires the boiling of 1 ml of urine for 5 minutes. About 50 µl of the treated urine sample is added onto a reaction zone on a glass slide, and a drop of latex is added. The liquids are stirred to a completely homogenous mixture. Any agglutination reaction discerned when compared with a negative control is considered positive. The sensitivity varies with the parasite load.

Culture

Culture can be done from peripheral blood, buffy coat or tissue aspirates. The microculture method improves sensitivity and decreases incubation periods. Cultures are expensive, time-consuming and require expertise. A Leishmania parasite can survive for 3 days at 4° C but for only 1 day at room temperature in a Locke transport medium (a buffered glucose-salt solution with antibiotics).

Genome assays

Lack of standardization and quality control is a major concern of PCR and related assays. A multitude of gene targets, protocols and applications have been described. A PCR assay was developed to amplify the kinetoplast minicircle of Leishmania species (it can also be used in vector studies). The kinetoplast minicircle is an ideal target because it is present in 10,000 copies per cell, and its sequence is known for most Leishmania species. The very high

sensitivity of PCR-based assays may actually be a disadvantage by being a marker of infection (transient or permanent) instead of being a marker of disease, as it will also pick up asymptomatic carriers. Detailed genomic analysis of *L. donovani* showed that parasites can have two, three, four or even five sets of chromosomes in one organism. Further study of this ploidy-variation will investigate the possible clinical implications of this unexpected finding.

Montenegro test

Leishmanin is a compound obtained via in vitro culture of promastigotes. A skin test with leishmanin (Montenegro test) is negative during active visceral leishmaniasis but later becomes positive (after 6 to 12 months). The Montenegro test reflects the suppressed cellular immunity during infection. There is a specific anergy for *Leishmania* parasites during active disease. This test is mainly of epidemiological value and may support the diagnosis of cutaneous leishmaniasis. To perform the test, 0.1 ml is injected intradermally, and the local reaction is read after 48 hours (>5 mm induration = positive). A positive test eliminates the existence of active kala azar. Cutaneous leishmaniasis produces a positive Montenegro test.

Treatment of VL

Without treatment, the case fatality rate for fully manifest clinical visceral leishmaniasis (VL), or kala-azar, exceeds 90%. Mortality is often due to hemorrhagic or infectious complications.

Treatment primarily involves antileishmanial therapy, with cost and availability being the main constraints on drug selection. Additionally, local drug resistance must be considered, especially for VL cases originating in South Asia, as treatment responses can vary significantly by region. Supportive therapy is also crucial, addressing nutritional status, concomitant anemia, hemorrhagic complications, and secondary infections to optimize treatment outcomes.

Patients with VL should be screened for human immunodeficiency virus (HIV) coinfection, and if HIV is present, it should be treated aggressively. Without effective immune reconstitution, treatment response in HIV-VL coinfected patients is generally poor.
Species identification usually is not critical to treatment decisions for VL (in contrast with cutaneous leishmaniasis). However, sensitivity to specific drugs varies substantially by region, and first-line treatment recommendations in major visceral leishmaniasis-endemic areas have diverged.

Pentavalent antimonial compounds

One of the treatment options for visceral leishmaniasis is pentavalent antimony derivatives (antimony, chemical symbol Sb = Stibium). The derivative most frequently used is Glucantime® (meglumine antimonate, 85 mg Sb/ml) and rarely Pentostam® (sodium stibogluconate, 100 mg Sb/ml). One of their actions is to inhibit phosphofructokinase, the rate-limiting step in the parasites' glycolytic pathway. The standard dosing regimen consists of 20 mg/kg/day of antimony for 28 to 30 days. Monitoring for cardiotoxicity with ECG is advised: T-wave inversion and prolongation of the QT-time are indicative of threatening arrhythmia. The fever usually disappears after 1 week. The spleen begins to get smaller after 2 weeks but frequently requires 6 to 12 months to return to normal.

Alternative treatments:

Antimonials have been the mainstay for treating visceral leishmaniasis for decades, but the emergence of antimonial resistance in India and their toxicity caused a change in guidelines. Currently, the first-line drug in many settings is liposomal amphotericin B. Conventional amphotericin B, paromomycin, miltefosine and antimonials are alternatives. In HIV-infected patients, the combination of liposomal amphotericin B with a second drug is advised.

Amphotericin B is a polyene with a fairly complex structure and hydrophilic and lipophilic components. The recommended dose of amphotericin B [Fungizone®] is 0.5-1 mg/kg/day IV, given over 6 hours; total dose max. 1-3 g. This drug is mainly used for the treatment of deep mycoses, though it is also active against Leishmania. It is a rather toxic medication. Shivering, fever, nausea, vomiting, headache, anemia, phlebitis at the site of the infusion, cardiotoxicity, kidney failure, hypokalaemia and hypomagnesemia are frequent side effects. Side effects occurring shortly after administration can be reduced by cortisone IV or meperidine (pethidine), a morphine analog. Administration of 500-1,000 ml physiological isotonic saline solution before starting the IV drip reduces the risk of nephrotoxicity. The toxicity of the drug is reduced by pharmacological complexing with lipids before administration. The drugs are then concentrated in the reticuloendothelial system and not in the kidneys so that a higher daily dose per kg of body weight can be administered and treatment time shortened (e.g., to 5 days). There are good indications that single-dose treatment (high dose; 10 mg/kg of the liposomal formulation) is useful, at least in the Indian subcontinent (India, Nepal, Bangladesh). In 1990, AmBisome® was developed as a first-choice drug. Several lipid formulations of amphotericin B are now available. They differ from each other in the type of phospholipid and the ratio of lipid to amphotericin B. Good results have been obtained with these lipid formulations. The price of these medications (AmBisome®, Amphotec®, Abelcet®) has come down but is still high for the average rural farmer in a developing country.

Formulations of Amphotericin B

1. Fungizone®: Amphotericin B deoxycholate. Contains no lipids.
2. Emulsification of Fungizone® in Intralipid 20%: little reduction of toxicity
3. AmBisome®: L-AmB: incorporation in liposomes (vesicles).
4. Abelcet®: ABLC or Amphotericin B Lipid Complex. Microscopically small ribbon-like membranes formed by complexing with phospholipids.
5. Amphotec®: ABCD (= Amphocil®) Amphotericin B Colloidal Dispersion: AmB-cholesteryl sulfate forms disc-shaped structures.

Injectable aminosidine (paromomycin) is now a valid alternative. It is an aminoglycoside antibiotic. In 2007, the results of an Indian study showed that paromomycin IM, at a dose of 11 mg/kg/day x 21 days, was non-inferior to amphotericin B at a dose of 1 mg/kg IV every other day x 30 days. The combination with antimonials for 17 days was also effective in East Africa. Pain at the injection site, liver toxicity and ototoxicity were reported as side effects. Paromomycin for IM administration is licensed in India and, since 2012, also in Nepal. Combined with antimonials, it is the first-line regimen in East Africa.

Miltefosine (Impavido®, Miltex®) was approved for use in India in 1992. It became more widely became available in subsequent years. Miltefosine interferes with certain cellular signal cascades and membrane synthesis, though its precise mode of action is still

unknown. *Leishmania* contains many ether lipids in the cell membrane. The main advantage of the compound is that it can be given orally, in contrast to the injectable antimony derivatives and amphotericin B. It cannot be given IV as this would lead to hemolysis. The molecule is fairly easy to produce and should eventually bring down the price, which is quite high in the West. The daily dose for adults is 100-150 mg and for children, 2.5 mg/kg/day. It should be given for 4 weeks. The half-life is several weeks. The cure rate was high in studies in India, although lower efficacy was found in East Africa. Dose-dependent gastrointestinal discomfort often occurs, and reversible hepato- and nephrotoxicity sometimes occurs. It is teratogenic, and so cannot be given to pregnant women or women who want to conceive 6 months after treatment. How quickly resistance to miltefosine will develop when used as monotherapy in the field is not yet clear. It is relatively easy to induce resistance in vitro. In this regard, it is of concern that success rates have been declining over the last years in the Indian subcontinent, although it is not yet well defined whether this relates to true parasite resistance, underdosing or evolving parasite fitness are also considered as alternative explanations. This has led to using liposomal amphotericin B (AmBisome) as the first-line treatment in the Indian subcontinent.

Combination therapy is the suggested way forward to increase treatment efficacy, prevent the development of drug resistance, reduce treatment duration and possibly decrease cost. Pentavalent antimonials combined with paromomycin are now the first-line treatment in East Africa. Other combinations including liposomal amphotericin B, paromomycin and miltefosine were found effective in India in phase III trials.

Until now, the generic recommendation for treatment of a VL episode in an HIV co-infected patient was first to consider lipid formulations of amphotericin B, infused at a dose of 3–5 mg/kg daily or in 10 intermittent doses (on days 1–5, 10, 17, 24, 31 and 38) to a total dose of 40 mg/kg. Evidence from clinical trials in Ethiopia and India on the efficacy and safety of combination therapy (liposomal amphotericin B (L-AMB) plus miltefosine) to treat VL in HIV co-infected patients instead of monotherapy has offered new possibilities for case management. Some evidence has emerged for considering secondary prophylaxis after the first episode of VL, with pentamidine in Ethiopia and with amphotericin B or its lipid formulation in India.

Table: The main drugs currently used for the treatment of visceral leishmaniasis.

Drugs	**Regimen**	**Marketing** [a]	**Clinical efficacy**	**Resistance**
Pentavalent antimonials	20 mg/kg iv or im daily for 28-30 days	Albert David (SSG); GSK (Pentostam®) Sanofi Aventis (Glucantime®)	35-95% (depending on geographic area)	As high as 60% (Bihar, India)
Amphotericin B	0.75-1 mg/kg iv for 15-20 doses (daily or alternate days)	Bristol Meyers Squibb (Fungizone®) Generic companies	> 97% all regions	Not documented

Liposomal Amphotericin B	10-30 mg/kg total dose iv; usually 3-5 mg/kg/dose single dose (10 mg/kg) in India	Gilead (AmBisome®)	Europe and Asia: > 95%; Africa: not fully established (higher dose required?)	Not documented
Miltefosine	2-2.5 mg/kg/d orally daily over 28 days (India only)	Paladin (Impavido®)	Asia: 94% (India) Africa: single field study (93% in HIV(-)	Readily obtained in lab isolates
Paromomycin sulphate	15 mg/kg im daily for 21 days (India only)	IOWH/Gland Pharma	Asia: 95% (India) Africa: 15 mg/kg: 64% (Sudan <50%) 20 mg/kg: 80% (Sudan)	Readily obtained in lab isolates

[a] marketing authorization holder iv: intravenous; im: intramuscular; SSG: sodium stibugluconate

Table: The main drugs currently used to treat visceral leishmaniasis (continued).

Drugs	Toxicity	Cost/course	Issues
Pentavalent antimonials	Frequent, potentially severe; Cardiac toxicity, Pancreatitis, Nephro + hepatotoxicity	Generic ~ $53 Branded ~ $70	Quality control; Length of treatment; Painful injection; Toxicity; Resistance in India
Amphotericin B	Frequent Infusion-related reactions, Nephrotoxicity (in-patient care needed)	Generic price: ~ $21	Need for slow iv infusion; Dose-limiting; Nephrotoxicity; Heat stability
Liposomal Amphotericin B	Uncommon and mild; Nephrotoxicity (limited)	Preferential price: $280 (20mg/kg total dose) Commercial price: ~ 10x	Price; Need for slow iv infusion; Heat stability (stored <25° C)
Miltefosine	Common, usually mild and transient; gastro-intestinal (20-55%), Nephro + hepatotoxicity Possibly teratogenic	Preferential price: ~ $74 Commercial price: ~ $150	Price; Possibly teratogenic; Potential for resistance (half-life); Patient compliance

Paromomycin sulphate	Uncommon, Nephrotoxicity Ototoxicity Hepatotoxicity	~ $15	Efficacy variable between and within regions; Potential for resistance (?)

Response to treatment

The response to treatment of visceral leishmaniasis is generally assessed clinically. Key indicators include the resolution of fever within one to two weeks, a decrease in spleen size within a month of starting treatment, and weight gain. Additional parasitologic testing is usually not required, but patients should be monitored clinically for at least 12 months and instructed to return if symptoms recur. In immunocompetent patients, most relapses occur within 6 to 12 months after treatment completion, though relapses up to 18 months post-treatment have been described. Immunocompromised patients should be followed for a minimum of one year, ideally lifelong or until effective immune reconstitution, to monitor for post-treatment relapse symptoms.

For patients with an equivocal clinical response (e.g., no decrease in spleen size, continued fever) or those suspected of having a relapse, bone marrow or splenic aspirate should be performed to confirm relapse or to look for alternative diagnoses.

Cutaneous leishmaniasis

Distribution

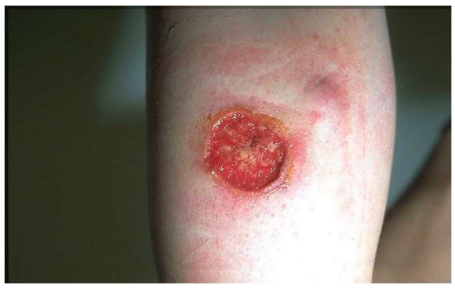
Approximately 90% of all cases of cutaneous leishmaniasis occur in Iran, Syria, Saudi Arabia, Afghanistan, Algeria, Peru and Brazil.

Clinical aspects

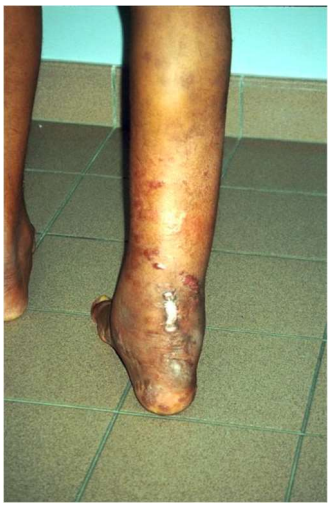
Various forms are clinically distinguished, the most important of which are:

1. Localized cutaneous leishmaniasis: skin ulcers that heal very slowly or nodular lesions, limited in extent and number. These chronic sores have regional names: clou de Biskra in Algeria and Aleppo boil in Syria.
2. Diffuse cutaneous leishmaniasis: cutaneous nodules and plaques that do not ulcerate but sometimes spread over the entire body.
3. Recurrent cutaneous leishmaniasis

"… After it is cicatrized, it leaves an ugly scar, which remains through life, and for many months has a livid color. When they are not irritated, they seldom cause much pain… It affects the natives when they are children and generally appears in the face, though they also have some on their extremities… In strangers, it commonly appears some months after their arrival. Very few escape having them, but they seldom affect the same person above more than once."



Skin ulcer due to cutaneous leishmaniasis. Copyright ITM



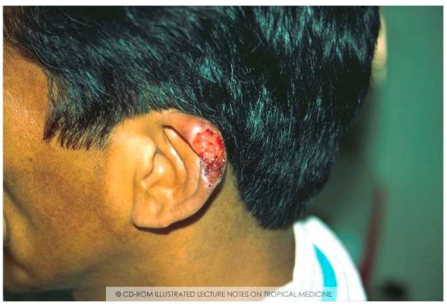
Diffuse cutaneous leishmaniasis.
Infection with *Leishmania aethiopica*.
Copyright ITM

Localized cutaneous leishmaniasis

After a bite by a sandfly infected with *L. tropica* (mainly urban infection), there is an incubation period of a few weeks or months, occasionally years. There is initially a small papule and usually only a single lesion, though sometimes there are several. This slowly spreads and can remain completely dry, become warty or nodular or develop into a painless, sharply delineated ulcer surrounded by a purplish raised border. Satellite lesions can occur. Spontaneous healing often occurs after 6 to 12 months, resulting in a depressed scar. Recurring cutaneous lesions – possibly with severe disfigurements – occasionally occur. There is usually immunity to any subsequent infection with the same organism. In infection with *L. major* (mainly rural infections, particularly from a rodent reservoir), the lesions are usually larger and develop more quickly, hence the name. There is a greater tendency to spread locally via the lymphatics, and this has to be distinguished from sporotrichosis. The lesions will eventually spontaneously heal with scar formation. Clinical cure starts when macrophages become activated and start killing amastigotes. This is mediated via a T-helper cell type 1 (Th1) response. This immune reaction also prevents recrudescence of latent chronic infection. The Th1 response is accompanied by the secretion of pro-inflammatory cytokines, such as interferon-gamma and interleukin 12. If the immune response would be towards the production of down-regulating cytokines (interleukin 4, 10, 13, TGF beta), macrophages will not be capable of eliminating the parasites, but tissue destruction will be limited.

In South America, the lesions often have their own local names and clinical expressions. Hence, in Peru, they are called “uta” (a solitary ulcer or a few restricted lesions brought about by L. peruviana, frequently on the face). In Guyana they are known as “bush yaws” or (French) “pian bois” (*L. guyanensis*) with raspberry-like lesions that resemble yaws. In Yucatan, Mexico an ulcer on the ear (usually caused by *L. mexicana*) is known as “chiclero” ulcer.

A “chiclero” is a man who collects chicle-latex (bubblegum) in the forest. During their work in the plantations the workers can get bitten by *Lutzomyia olmeca* and as such are exposed to a high risk of contracting leishmaniasis, hence the term “chiclero ulcer”.



Chiclero ulcer on an ear (leishmaniasis). Photo Cochabamba, Bolivia

Diffuse cutaneous leishmaniasis

Diffuse cutaneous leishmaniasis is a diffuse skin affliction with extensive non-ulcerative nodules and a chronic disease. It is sometimes followed by chronic lymphoedema of an affected body part. This disease is poorly understood but is probably caused by a diminished resistance to the parasite. This immunosuppression is possibly brought about by the parasite itself. One supposed mechanism of escape of *Leishmania* parasites is the downregulation of the expression of major histocompatibility complex (MHC) class II molecules on the macrophages they colonize. In East Africa, diffuse cutaneous leishmaniasis is often caused by *L. aethiopica* and in the New World, frequently by *L. mexicana*.
If there are generalized cutaneous lesions, the condition must be differentiated from lepromatous leprosy, keloids, neurofibromatosis and post kala azar dermal leishmaniasis (PKDL). Due to the patient’s weak immune response, numerous amastigotes are present, and most skin smears are positive. Treatment is difficult as the patient’s immune system itself is functioning poorly. DCL patients are anergic to leishmanial antigen. Patients with DCL have a predominantly Th2-type cytokine response. They have low concentrations of interferon-gamma and interleukin 12. There is no tendency to self-cure. Differentiation from PKDL is important, as the latter can still be treated reasonably well. In Sudan, 1 diffuse cutaneous leishmaniasis case is found for every 100 cases of localized cutaneous leishmaniasis. The incidence varies greatly from district to district. It occurs frequently in South America, but in contrast to this it does not occur in India (or very exceptionally –eg in HIV patients).

Recurring cutaneous leishmaniasis

Recurring cutaneous leishmaniasis seldom occurs (Iraq, Iran). This disease, also known as leishmaniasis recidivans, leads to significant tissue damage. Parasites are very difficult to detect in these very chronic lesions. Differentiation from cutaneous tuberculosis is important.

Diagnosis of cutaneous leishmaniasis

Attempts should be made to detect the parasite microscopically in a biopsy or smear from the edge of the wound. The biopsy should if possible, be divided up for pathology (seldom available, not very sensitive and is principally used more for exclusion of another cause) and cultures (bacteria, mycobacteria, fungi, *Leishmania*), and an impression preparation should also be made. Lesions on the face can be injected with 0.1 ml physiological saline and aspirated again while moving a small, thin needle back and forth in the skin. Serology is usually negative. Differential diagnoses include ulcers due to mycobacteria, cutaneous diphtheria, tertiary syphilis, yaws, cutaneous carcinoma and deep or subcutaneous mycosis. Field sore (cutaneous diphtheria) and tropical ulcers are painful, particularly in the early phase.

Differential diagnoses of disseminated nodular and ulcerated lesions include leishmaniasis, sporotrichosis, atypical mycobacteria and nocardiosis.

Treatment

The response to treatment varies according to the species. Drugs for systemic and topical treatment can be used. There is an urgent need for better and cheaper drugs.

Indications for local treatment

1. Lack of risk of developing mucosal lesions
2. Old World cutaneous leishmaniasis
3. Small, single lesion
4. Absence of spread to lymph nodes

Indications for systemic treatment

1. The presence of mucosal lesions or spread to lymph nodes
2. New World cutaneous leishmaniasis, except localized Leishmania mexicana infection
3. Lesions unresponsive to local treatment

Overview topical treatment of cutaneous leishmaniasis

1. Physical methods: cryotherapy (liquid nitrogen) for 15-20”, repeated 2-3 times with an interval of e.g. 3 weeks. Blistering will occur.
2. Applying local heat via a CO_2 laser or an infrared lamp (40°C to 42°C for 12 hours), but heat-induced skin bullae can occur.
3. Ointment with 15% paromomycin: twice daily application is advised for a duration of 20-30 days.
4. Skin infiltration with pentavalent antimony with a fine needle. Blanching of the lesions should be obtained. Treatment is repeated every 5-7 days, generally 2-5 times, sometimes more.

Overview systemic treatment of cutaneous leishmaniasis

1. Pentavalent antimonials (meglumine antimoniate [85 mg Sb/ml, IM] or sodium stibogluconate [100 mg/ml, IM or filtered IV] can be given parenterally for extensive skin lesions.

2. Pentamidine. First line against *L. guyanensis* (French Guyana). Several treatment schemes exist, and the cure rate is dose-dependent. Some short courses use 1200 mg as a total dose. In Guyana 3 mg/kg/day every other day is often used (4 injections).
3. Imidazoles, triazoles. Infections caused by *L. major* can be successfully treated with oral fluconazole 200 mg/day for 6 weeks (cure rate of 80%). Ketoconazole 600 mg per day x 28 days is moderately effective for *L. mexicana,* but much lower against *L. braziliensis*. Treatment with ketoconazole is sometimes complicated by hepatotoxicity, abdominal pain and nausea. Itraconazole (Sporanox®) gave good results in initial studies but this was not seen in the field.
4. Miltefosine. Not yet widely available, but allows oral therapy.
5. Amphotericin B and its liposomal formulations (IV).

Treatment of diffuse cutaneous leishmaniasis (*L. aethiopica*)
The treatment of diffuse cutaneous leishmaniasis caused by *L. aethiopica* is problematical, as this parasite is less sensitive to Glucantime®. Pentamidine can be used against *L. aethiopica*. A dose of 4 mg/kg/week has to be continued for at least 4 months after the disappearance of the parasites from the skin is an acceptable guideline here. Good results were obtained with amphotericin B.

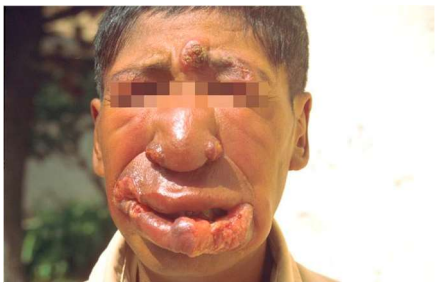
Mucocutaneous leishmaniasis

Distribution

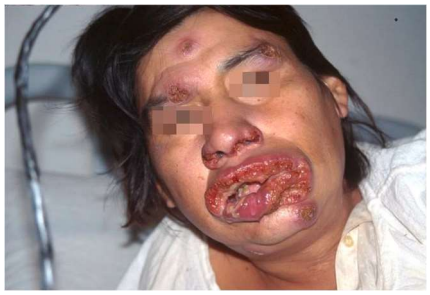
Currently, 90% of all mucocutaneous leishmaniasis occurs in Bolivia, Peru and Brazil. Illustrations of skin lesions and disfigurements suggestive of leishmaniasis are encountered on pre-Inca earthenware. These indicate that the disease existed in Peru and Ecuador in the 1st century AD. Texts dating from the 15-16th century Inca period and the Spanish conquest mention the risk of cutaneous ulcers in seasonal farmers. Espundia was also described as “white leprosy.”

Clinical aspects

When skin and mucosae are affected, the disease is known as mucocutaneous leishmaniasis. This is very rare in East Africa but frequent in South America, where it is known as “espundia”. After an initial skin lesion, that slowly but spontaneously heals, chronic ulcers appear after months or years on the skin, mouth and nose, with destruction of underlying tissue (nasal cartilage, for example). Tissue destruction with disfigurement can be very severe. Parasites are usually rare in the lesions. A substantial part of the disfigurement is possibly due to immunological mechanisms. One hypothesis is a relationship between the occurrence of mucocutaneous lesions and the presence of certain alleles of polymorphic tumor necrosis factor-a and b genes.



Espundia or mucocutaneous leishmaniasis often results from infection with *Leishmania brasiliensis.* Photo Cochabamba, Bolivia



Espundia or mucocutaneous leishmaniasis often results from infection with *Leishmania brasiliensis.* Photo Cochabamba, Bolivia

Diagnosis

The lesions often contain few parasites. Diagnosis is sometimes made solely on a clinical basis. Culturing the parasites is possible but is reserved for research purposes, and it is not feasible in primitive rural conditions. Serology in espundia can be positive or negative (the quality of the antigen is of crucial importance). A practical problem in South America is whether a certain skin lesion with *Leishmania* amastigotes is caused by *L. braziliensis* or not. The geographical origin of the lesion or PCR may give an answer here, though the latter is not available in rural areas.

Mucocutaneous leishmaniasis, differential diagnosis:

Differential diagnosis includes skin cancer, tertiary syphilis and yaws, leprosy, rhinoscleroma (a very chronic granulomatous infection with *Klebsiella rhinoscleromatis*), rhinosporidiosis, midline granuloma (a form of T-cell lymphoma), Wegener's granulomatosis, sarcoidosis, skin tuberculosis, infection with the free-living amoeba Balamuthia mandrillaris, chronic nasal cocaine abuse, noma, and fungal infections such cryptococcosis, histoplasmosis and South American blastomycosis (paracoccidioidomycosis). With this last disease, which is a very chronic infection, the lungs are frequently affected in a manner that can mimic tuberculosis. The yeast has typical oval cells with ectospores, which can be detected in sputum.

Overview: Differential diagnosis of nasal ulcers:

1. Mucocutaneous leishmaniasis (Espundia)
2. Fungal infections, such as paracoccidioidomycosis (syn. South American blastomycosis), histoplasmosis, cryptococcosis, coccidioidomycosis
3. Actinomycosis
4. Treponematoses (syphilis, yaws, bejel)
5. Leprosy
6. Tuberculosis
7. Rhinosporidiosis
8. Rhinoscleroma (chronic infection with *Klebsiella rhinoscleromatis*)
9. Balamuthiasis (infection with free-living amoeba)

Non-infectious

1. Granulomatosis with polyangiitis (formerly Wegener granulomatosis)

2. Midline granuloma (a form of T-cell lymphoma)
3. Other non-Hodgkin lymphoma
4. Squamous cell carcinoma
5. Sarcoidosis
6. Relapsing polychondritis
7. Cocaine abuse

Treatment

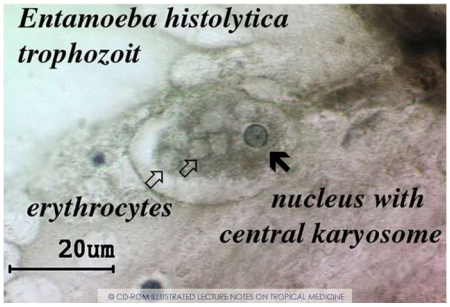
Mucocutaneous leishmaniasis is difficult to cure unless it is identified as mild. Treatment aims to prevent morbidity (e.g., disfigurement) and mortality (e.g., from aspiration pneumonia or respiratory obstruction). There are few randomized controlled trials to guide the treatment of mucocutaneous leishmaniasis, and a long duration of therapy is required, with close follow-up for relapse. Treatment options include parenteral pentavalent antimony drugs, amphotericin B including liposomal amphotericin, miltefosine and rarely pentamidine.

Amoebiasis

Summary
•Entamoeba histolytica: trophozoites and cysts; faeco-oral transmission
•Morphological identical but non-pathogenic *Entamoeba dispar*
•Intestinal infection: asymptomatic - colitis - fulminant dysentery - amoeboma.
•Amoebic colitis to be distinguished from bacillary dysentery, balantidiasis, colon cancer, Crohn's disease, ulcerative colitis
•Liver infection: Liver abscess with fever, liver pain, pus upon aspiration, leukocytosis, positive
serology, abnormal ultrasound.
•Liver amoebiasis to be distinguished from pyogenic abscess, cholangitis, cholecystitis
•Occasionally perianal skin ulcers and other extra-intestinal locations
•Treatment with nitro-imidazoles + contact amoebicides

General

Amoebiasis in our context means infection with *Entamoeba histolytica*. This is a unicellular cosmopolitan parasite. The first description of the parasite was in 1875 by Fedor Lösch in St Petersburg. This concerned an infection in a young Russian farmer in Arkhangelsk, 150 km from the Arctic circle. This illustrates the fact that the infection is not restricted to the tropics. Transmission depends on the level of sanitation and faecal hygiene in a country or region.



Entamoeba histolytica trophozoite in rectal mucosa.
Copyright ITM

Entamoeba histolytica cysts.
Cysts never contain red blood cells.
Copyright ITM

Pathogenicity of Entamoeba histolytica

There was considerable confusion concerning the nomenclature and pathogenic properties of Entamoeba histolytica. It is now recognized that there are morphologically identical amoebae, some of which are non-pathogenic and some of which are pathogenic. This concept was introduced in 1925 by the French parasitologist Emile Brumpt. The non-pathogenic amoebae
are called Entamoeba dispar. This should also not be confused with other completely non-pathogenic species, including *Entamoeba hartmanni* (previously sometimes called "small race" E. histolytica). In 1978 it was discovered in London that the two kinds of amoebae could be differentiated using isoenzymatic electrophoresis. Pathogenic amoebae always belong to one group and non-pathogenic amoebae always belong to the other group. In 1989 it was discovered that E. dispar always differs from *Entamoeba histolytica* by well-determined genetic (DNA) markers. Non-pathogenic Entamoeba dispar never changes into pathogenic Entamoeba histolytica. Earlier reports of this appear to be due to laboratory

errors: mixed cultures and/or contamination of cultures in the lab. In pathogenic Entamoeba histolytica isolates with low virulence and with high virulence can be seen (virulence is a measure of the severity of illness which certain strains can cause in certain circumstances). The degree of virulence is variable, because this is determined by several parameters, including the environment (in contrast to properties which are genetically determined). Isolates with low virulence are non-invasive, while isolates with a high degree of virulence are invasive.

Mobility of Entamoeba histolytica

E. histolytica trophozoites are highly motile. The fuel for this constant motion comes from the anaerobic conversion of glucose and pyruvate to ethanol. *E. histolytica* has no mitochondria (probably through secondary loss). Many of its metabolic enzymes seem to be of prokaryotic origin, possibly acquired from the lateral transfer of genes from bacteria.

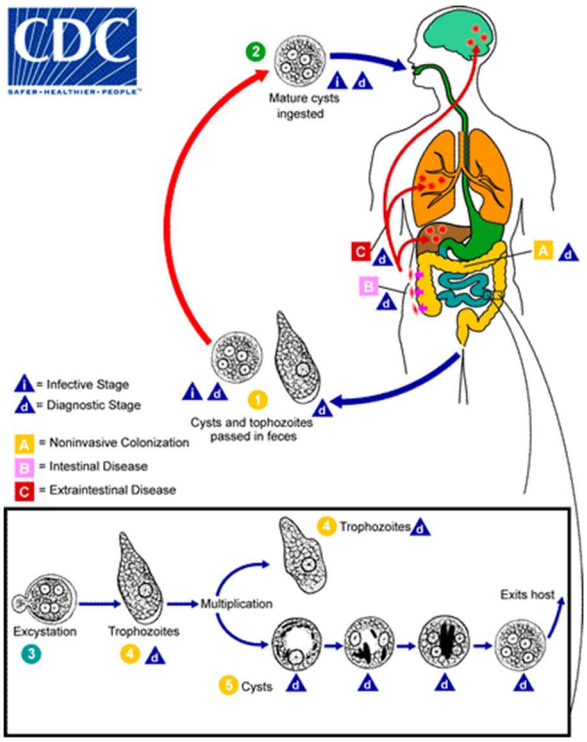
Life Cycle and transmission

Infection is caused by ingestion of *E. histolytica* cysts. One cyst develops in the small intestine into 8 motile trophozoites (one trophozoite with 4 nuclei divides 3 times and each nucleus divides once to produce 8 trophozoites from each cyst) which then find their way into the colon. The trophozoites multiply by asexual reproduction and in turn produce cysts, which are then excreted with the faeces. The cyst is quite resistant and can survive for a long time in the outside world. Excreted trophozoites die quickly and therefore are not responsible for transmission. Cysts of *E. histolytica* are never found in issues. The parasite is transmitted feco-orally as a cyst, usually from person to person. Transmission via water also occurs. Dogs, cats, rats, pigs and monkeys may become infected but do not form a significant animal reservoir (Note: kittens were used by E. Brumpt as a very susceptible animal model to test the pathogenicity of amoebae). Flies and cockroaches may carry cysts. Their role in transmission has not been properly investigated but is probably of minor importance. The main source of infection is humans. Amoebiasis is thus not a zoonosis. Infection via sexual intercourse is rare (via anal contact). The latter method of transmission may result in severe and mutilating lesions of the genitals.

Entamoeba histolytica is considered to be an asexual organism, but many mysteries persist. Some pieces of evidence don't fit with this asexual idea, such as the appearance of putative heterozygous populations after mixing homozygotic populations for certain isoenzym classes. Also, E. histolytica has the full complement of meoisis genes, which one would expect to have decayed over time if the organism abandoned the sexual life cycle.

Prevention

Amoebic cysts are resistant to normal chlorination of drinking water. Boiling and filtering drinking water eliminates the parasite. Large scale prevention depends mainly on improved sanitation and hygiene. No vaccine is available. Amoebiasis is not an opportunistic infection in HIV patients.



Entamoeba histolytica Life Cycle (courtesy of CDC)

Intestinal amoebiasis

Clinical aspects

We can differentiate 4 different situations in intestinal amoebiasis:

1. asymptomatic carriers
2. amoebic colitis
3. fulminant colitis
4. amoeboma

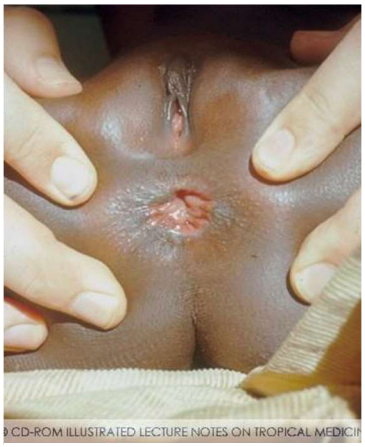
Asymptomatic carriers

Cysts can sometimes remain in the intestinal lumen for years without causing any damage: the patient is then an asymptomatic carrier. The majority (90%) of patients fall into this group. Asymptomatic carriers have by definition no symptoms of amoebiasis. These persons can be detected by faeces analyses. This may show cysts of non-pathogenic E. dispar or of potentially pathogenic *E. histolytica*, which for unknown reasons is not invasive. Differentiation with cysts of *Entamoeba coli* (which are larger and have 8 nuclei) is important. *Entamoeba coli* is not pathogenic.

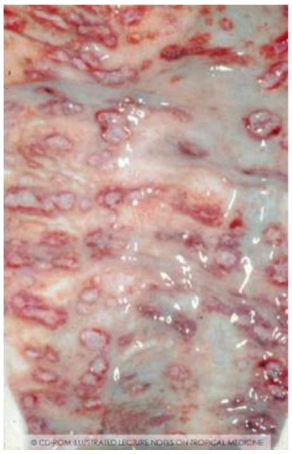
Amoebic colitis

The incubation period of amoebic colitis varies greatly. When *Entamoeba histolytica* penetrates the intestinal mucosa (becomes invasive) it produces ulcerations of the colonic mucosa [Gr. histo-lytica, i.e. referring to breaking down tissues]. The ulcerations are sharply defined and have eroded undermined edges. This is expressed clinically as abdominal pain, diarrhoea with blood in the faeces, and only moderate or no fever, with good general condition. When the rectum is affected there is tenesmus (painful cramps in the anus). Peri-

anal ulcers may occur via direct spread from rectal amoebiasis. The ulcers develop rapidly and are painful. After suffering from amoebic colitis there may be persistent intestinal problems, the aetiology of which is unclear.



Entamoeba histolytica rectitis, with spread to the perianal skin. Copyright prof Gigase, ITM



Entamoeba histolytica colitis. Notice the typical skipping lesions. Copyright ITM

Fulminant colitis

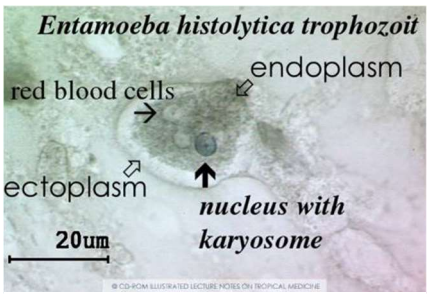
There is sometimes a fulminant course with high fever, a severely ill patient, intestinal bleeding or perforation of the colon. A slow seepage of intestinal content into the peritoneum is very likely in a severely ill patient whose condition deteriorates progressively, together with the formation of ileus (intestinal paralysis) and a distended abdomen. A fulminant course may occur if patients are treated with steroids or other immunosuppressive drugs (e.g. if amoebic colitis is wrongly thought to be Crohn's disease or haemorrhagic ulcerative colitis) and sometimes in very young children and elderly.

Amoeboma

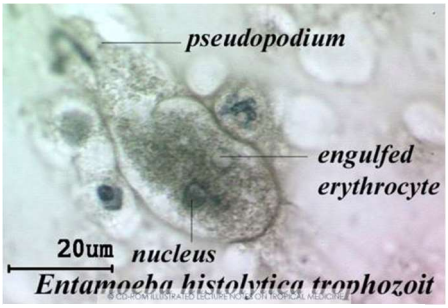
In 1% of patients an inflammatory thickening of the intestinal wall occurs. A mass may then be palpated (amoeboma). The diagnosis may be made via biopsy. The inflammatory mass may mimic colon carcinoma. Countless trophozoites are found in the tissues (never cysts). Correct therapy produces a pronounced reduction in the volume in approximately 3 days.

Diagnosis

When amoebic dysentery is suspected, a fresh faecal sample or a swab from a rectal ulcer should be examined under a microscope. If examined quickly (a fresh stool, still warm) the colourless motile trophozoites can be seen. Motility disappears when cooled, and the parasites are then difficult to recognize. They should be differentiated from actively motile macrophages. The trophozoite (motile form) has one nucleus. When colourless this nucleus is scarcely if at all visible. Once stained the nucleus is moderately visible. Lugol staining kills the parasite almost immediately (motility disappears). Stained Entamoeba histolytica trophozoites have a transparent outer border (ectoplasm) and an opaque inner border (endoplasm). The trophozoite measures 20 to 40 µm and may contain red blood cells (unlike other amoebae). The last detail is probably pathognomonic for pathogenic Entamoeba histolytica, but is not always present and this statement is contested by some.



Entamoeba histolytica trophozoite. Morphologically, it is only possible to differentiate Entamoeba dispar from E. histolytica if the trophozoite contains engulfed red blood cells.
Only *E. histolytica* is haematophagous, although this statement is contested. Copyright ITM



Entamoeba histolytica trophozoite. Copyright ITM

The cysts have 1, 2 or 4 nuclei and measure 8 to 15-20 µm. The nuclei are best revealed by means of an iodine stain. They have a dark circumference and a dark central point (karyosome), these features are helpful in distinguishing with non-pathogenic species such as *Entamoeba coli.* Iodine staining can also detect glycogen (brown) in young cysts. Fresh cysts of *Entamoeba histolytica* also contain what are called chromatoid bodies. These are squat, oval inclusions which can easily be detected (black) with an iron-haematoxylin stain (not with iodine stain). They are not present in *Entamoeba coli* or *Endolimax nana* cysts. In active dysentery, often no cysts are found in the faeces, but if there is little diarrhoea, the parasites have time to encyst. Since excretion of the parasites is intermittent, it is best to carry out 3 different stool analyses before deciding upon a negative result.

Antigen detection is sensitive, specific, rapid, easy to perform and can distinguish between E. histolytica and *E. dispar.* Stool and serum antigen detection assays that use monoclonal antibodies to bind to epitopes present on pathogenic E. histolytica strains (but not on non-pathogenic E. dispar strains) are commercially available for diagnosis of E. histolytica infection. Detection of parasitic DNA or RNA in faeces via probes can also be used to diagnose amoebic infection and to differentiate between the different strains. PCR is about 100 times more sensitive than faecal antigen tests.

Intestinal amoebiasis: Differential diagnosis

The intestines may contain several species of harmless commensal amoeba. Differentiation with these other non-pathogenic amoebae is important; they include:

Iodamoeba butschlii : mononuclear cysts, big glycogen supply
Entamoeba hartmanni : small cysts with four nuclei
Endolimaxnana : smaller round or oval cysts with 2-4 nuclei (measuring 6-12 µm) and slow-moving trophozoites (L.: limax =slug)
Entamoeba coli : larger cysts containing 1, 2, 4 or 8 nuclei
Entamoeba dispar is a special case (see above)

In dysentery it is important to distinguish between bacillary and amoebic dysentery since their treatment is completely different. A diagnosis may be made clinically but it is best to confirm this by microscopy as there is partial clinical overlap of the two diseases.

Balantidium coli is a pathogenic ciliate which can cause severe colitis. This illness is very similar to intestinal amoebiasis and the diagnosis can only be made by faeces examination. Treatment is with tetracyclines or metronidazole.

Pseudomembranous colitis is caused by infection with toxicogenic *Clostridioides difficile.* These bacteria can be selected out and can proliferate after administration of certain antibiotics. Metronidazole is a good treatment in this case. Vancomycin is equally effective but will not be given in third world countries in view of its high cost. A related bacterium, *Clostridium perfringens*, can cause necrotizing colitis (necrotic enteritis, Pigbel syndrome). This disorder has an acute course and is very severe.
Sometimes gonococcal proctitis or lymphogranulomatosis venereum (due to C. trachomatis) can be confused with amoebiasis. There are then no proximal intestinal lesions and culture of the mucus or PCR methods provide a diagnosis. Crohn's disease and ulcerative colitis are rare in the tropics.
Radiology and biopsies are essential for their diagnosis.

Bacillary dysentry	Amoebic dysentry
Actute onset	Gradual onset
Poor general conditoins	General condition normal
High fever	Little fever (adult)
Severe tenesmus	Moderate tenesmus
Dehydration frequent	Little dehydration (adult)
Faeces: no trophozoites	Trophozoites present
Faeces culture positive	Faecal culturel negative

Treatment

Asymptomatic carriers

Since high percentages of the population may be cyst carriers (e.g. 10%) there is little point in treating cyst carriers found by chance in an endemic region. In any case, 90-95% of these people are infected with the non-pathogenic Entamoeba dispar. If this is nevertheless desired (e.g. in people who prepare food) paromomycin (Gabbroral®, Humatin®) is indicated. Diloxanide furoate (Furamide®) and iodoquinol (Intetrix®) can be used. In regions of low endemicity it may make sense to treat asymptomatic carriers to prevent transmission and to prevent possible development of later invasive amoebiasis (even if this risk is low). 5-Nitro-imidazoles are not effective against cysts.

Amoebic colitis

Parasites in the tissues (intestinal wall) can be treated with nitro-imidazoles, such as metronidazole, secnidazole, ornidazole or tinidazole. Secnidazole has the longest serum half-life (17h) compared with 12-13h for tinidazole, 11h for ornidazole and 8h for metronidazole. The dose of metronidazole (Flagyl®) is 500 mg q.i.d. for 5 or more consecutive days (adults). Tinidazole (Fasigyn®) is more expensive but has fewer side effects. Two grams per day x 3 days

is sufficient for amoebic colitis. Ornidazole 500 mg b.i.d is given for 5 days. Alcohol is forbidden during treatment due to disulfuram effect with severe nausea. These drugs are rapidly absorbed in the proximal intestine. For this reason, they are insufficiently active upon the parasites in the distal intestinal lumen.

The latter are treated with paromomycine (Gabbroral®, Humatin®) 10 mg/kg or 500 to 750 mg t.i.d. for 7 days. These drugs are not active against parasites in the tissues. The two drugs thus complement each other. An alternative contact amoebicide is diloxanide furoate (Furamide® =a contact amoebicide). Dose: Furamide® 500 mg t.i.d. for 10 days (adults). Children: 30 mg/kg/day. Nitazoxanide (Alinia® 500 mg tablets and 100mg/5 ml oral suspension) proved very effective as a tissue amoebicide and as a luminal amoebicide. However it is not readily available and is extremely costly.

Hepatic amoebiasis

General

If amoebae are transported with the venous blood from the intestinal wall to the liver an abscess in the liver may be formed: hepatic amoebiasis. If the abscess is located adjacent to the fibrous capsule of the liver adhesions are formed. A subphrenic abscess is less frequent than direct perforation of the diaphragm with empyema or fistula formation to the bronchi. Perforation to the peritoneum is rare. Perforations of the intestine, biliary ducts or navel with secondary phagedenic ulceration of the skin are more frequent than generalized peritonitis. Abscesses of the left hepatic lobe may perforate the pericardium in a life-threatening manner. [The term "abscess" is not actually correct here in the strictest sense as this is not a collection of pus cells (white blood cells). It is local cytolysis of live tissue.]

Clinical aspects

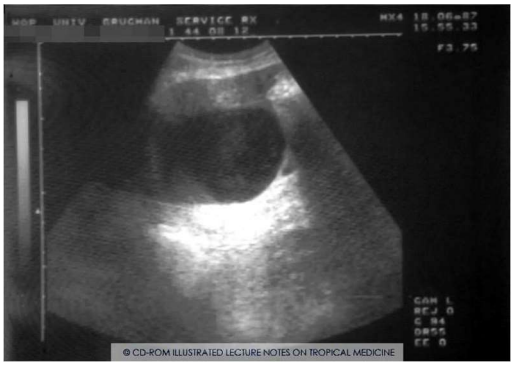
Upon physical examination, there is fever and pain in the liver region (pain upon palpation or percussion). The pain increases during deep inspiration or coughing. If the volume of the abscess is significant, the liver will be enlarged and the diaphragm will be elevated (percussion, auscultation, chest X-ray). The patient may develop pain in the right shoulder (referred pain). Dullness upon percussion of the base of the right lung may be due to the elevation of the diaphragm, to reactive pleural fluid or breakthrough to the pleura, or to atelectasis of the lung. Jaundice occurs in a minority (6-29%) of patients and tends to be a very late symptom. Jaundice can result from biliovascular fistula (with backflow of the bile into the hepatic veins) or from compression of bile ducts. The abscess continues to spread until it breaks through to the surroundings: the pleura (empyema), the lung, the pericardium or the skin. If fistulisation to the skin occurs, there may be swift progression of a painful skin ulcer. Untreated amoebic liver abscess is often fatal.

Liver amoebiasis with perforation of the abscess through the abdominal skin. Photo Prof. Gigase. Copyright ITM

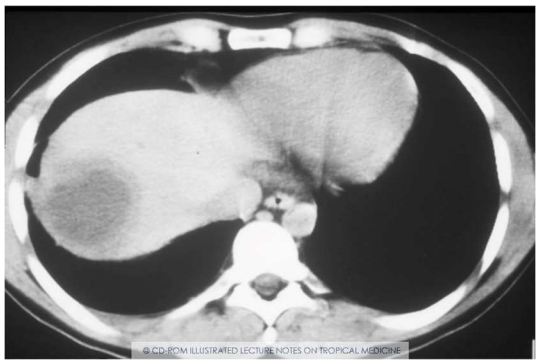
Liver amoebiasis with perforation of the abscess through the abdominal skin. Photo Prof. Gigase. Copyright ITM

Diagnosis

The diagnosis of a hepatic abscess may be suspected from clinical findings. Leukocytosis will be high (and there is no eosinophilia). Ultrasound and serology (ELISA, Latex agglutination) can confirm the diagnosis, but are often not available. Antibodies will remain present for a long time -often years- after infection. An amoebic abscess of the liver will contain necrotic liver tissue at its center. Upon aspiration, this often has a dark brownish red colour called "anchovy" or "chocolate" pus, but the pus may also be yellow, grey or greenish. The pus has no offensive odour, unlike most bacterial (anaerobic) abscesses which is an important difference. The wall of the abscess contains trophozoites, but the necrotic liver tissue itself does not. The presence of local oedema or bulging of the skin with or without fluctuation indicates the proximity of the abscess and the site where a puncture can be carried out. In case of doubt a trial therapy quickly produces a spectacular improvement. Fewer than 20 % of people with a hepatic abscess have Entamoeba histolytica in the faeces. The absence of amoebae in the stools is therefore does not rule out the diagnosis.



Ultrasound of liver showing an amoebic liver abscess. Copyright ITM



Liver abscess due to infection with *Entamoeba histolytica*. CT-scan of the liver shows a circular necrotic area. Copyright ITM

Hepatic amoebiasis: Differential diagnosis

1. Pyogenic/anaerobic hepatic abscess: stinking pus, poor general condition, often icterus, negative serology, sometimes portal-of-entry in the intestine (e.g. colon tumour, appendicitis).
2. Hydatid cyst: slow development, no fever, no toxaemia, serology positive for Echinococcus, sometimes calcifications on abdominal X-ray, no leukocytosis. Ultrasound may show daughter cysts.
3. Biliary cysts: ultrasound shows a thin wall and the content is anechoic, otherwise asymptomatic.
4. Haemangioma: hyperreflective on ultrasound, otherwise asymptomatic. On CT scan with dynamic sequences there is a centripetal staining with a delayed isodense appearance to the surrounding liver tissue. On MRI a haemangioma is extremely hyperreflective on T2-weighted images (T2 = "water images").
5. Metastases: ultrasound shows generally (but not necessarily) irregular and hyperreflective structure, central necrosis may occur. Frequently peripheral oedema.
6. Hepatoma: no fever or toxaemia, no response to trial therapy, elevated alpha-feto protein, negative serology, often related to HBV or HCV; biopsy is diagnostic.

Treatment

An amoebic abscess of the liver is treated with metronidazole for 10 days (often initially IV) or tinidazole 2 gr daily for 5 days, followed by paromomycine or diloxanide furoate for 10 days. The latter is to destroy any amoebae in the lumen of the intestines. If the diagnosis is known, aspiration is only carried out for very large abscesses or if there is a risk of breakthrough. Surgery is indicated if the abscess ruptures (e.g. into the peritoneum). If a relapse of the abscess occurs this usually happens within two months.

Amoebiasis of other organs

Amoebiasis of the lungs is generally the result of the spread of an amoebic abscess of the liver, which perforates through to the base of the lung. Breakthrough to a bronchus may occur. The prognosis is usually favourable. Amoebic pleuritis (empyema) is an unpleasant complication because of the need to drain the empyema. Other locations are rare and include:

Primary amoebiasis of the lung without prior hepatic amoebic abscess.

Abscesses in muscles, e.g. the thigh.

Ulceration of the skin of the lower limbs by amoebae, which could be the result of superinfections of skin wounds due to scratching with dirty nails.

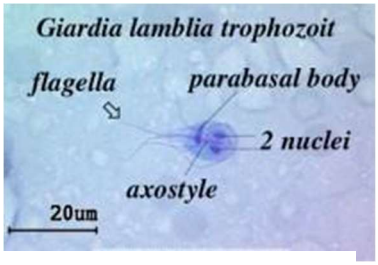
Urogenital forms, either due to fistula formation of intestinal lesions to the bladder or of peri-anal ulcers to the vagina and cervix of the uterus. Location on the penis if the partner has ulcers of the vagina/cervix or anal ulcers.

Parasites may appear elsewhere and lead to abscesses in other organs, e.g. the brain.

Giardiasis

Summary
- *Giardia lamblia* is an unicellular flagellate
- Faeco-oral transmission via cysts
- Sometimes asymptomatic infection
- Sometimes diarrhoea, atypical abdominal discomfort, bloated abdomen
- First-line treatment with nitroimidazoles, by preference tinidazole

General



Giardia lamblia trophozoite in faeces. Copyright ITM

Giardia lamblia cyst. Copyright ITM

Giardia lamblia (G. intestinalis, G. duodenalis) is a unicellular parasite (flagellate) which causes intestinal infections. The infections are often asymptomatic and *Giardia* was for a long time thought to be nonpathogenic. Since 1981 it has been regarded as potentially pathogenic and as the cause of diarrhoea and various forms of abdominal discomfort. In developing countries the infection occurs often in children but its frequency diminishes as they grow older. *G. lamblia* may infect various animals, including dogs, cats and beavers.

The taxonomy of this intriguing species is still to be clarified. At present, seven distinct genetic group based on protein and DNA polymorphisms can be distinguished in *Giardia*. Each group has its own host range, with group A and B able to infect humans.

Biological information

Giardia has no de-novo synthesis of lipids, which means that the parasite is dependent on exogenous lipids and bile salts (hence its location in the duodenum).

Giardia lamblia is possibly a complex of different species. Chemotaxonomy via determination of antigens using monoclonal antibodies shows that there is significant antigenic variation. DNA-analysis is promising, but Giardia has a complex genome. Using iso-enzymatic analysis, 13 zymodemes are known at present.

Giardia contains two functionally equivalent and apparently identical nuclei. The two nuclei remain physically distinct during mitosis in the trophozoit. Both nuclei are diploid and transcriptionally active. The two daughters of a single nucleus segregate to different trophozoites. It is still not clear yet at present if Giardia is asexual (as traditionally assumed), parasexual (diplomixis : nuclear fusion of the 2 nuclei during encystations, accompanied by

homologous recombination without meiosis) or sexual. Till present, Giardia has not been caught "in the act" however. If diplomixis occurs this would be unique to *Giardia*.

Life cycle of Giardia

Cysts are swallowed with water or food. In the duodenum excystation occurs which releases the trophozoite. This measures 12-18 µm. It attaches itself to the duodenal and jejunal intestinal villi by means of a kind of ventral sucking disk. The parasite reproduces only by asexual division. The trophozoites may multiply until the whole surface of the intestine is coated with parasites. Possibly this mechanical screening off the intestine contributes to malabsorption.

As trophozoites are carried to the more distal parts of the intestine, the parasite encapsulates. The cyst is resistant in the outside world but trophozoites perish. Cysts remain viable in a wet, cool outside environment. They are not very resistant to drying out. The cysts measure 10 x 7 µm. Transmission is via direct faeco-oral contact, food or via water. There is an animal reservoir and this is sometimes involved in human infection (giardiasis is known in Canada as "beaver fever"). In industrial countries dogs and cats are frequently found to be infected but almost always without symptoms.

Pathogenicity

In many cases infection is asymptomatic, but some patients develop symptoms. One hypothesis as to the pathogenicity is the mechanical covering of the intestinal epithelium (see above). This is not the only way in which the parasite gives rise to symptoms. *Giardia* is cytopathogenic on cell monolayers in vitro. Probably there is also in-vivo enterocytic damage with secondary disaccharidase (lactase) deficiency. Indeed, villous atrophy is found in patients. Another way in which *Giardia* may be pathogenic, is the destruction of conjugated bile salts with secondary steatorrhoea. Yet another unanswered question is whether the immune response contributes to the pathogenesis. In vivo *Giardi*a has frequent endosymbiotic bacteria up to 100 per trophozoite or so it was thought. This may possibly influence pathogenicity. The same question arises as regards any ectosymbionts. *Giardia* itself can be infected with an RNA virus of unknown clinical significance.

Clinical aspects

The disease is asymptomatic in approximately 80% of cases. The clinical spectrum ranges from silent carrier status to a malabsorption syndrome. The incubation time is 1 to 2 weeks. If symptomatic, an undifferentiated acute to subacute diarrhoea which lasts on average 1 to 6 weeks occurs. In some the diarrhoea is steatorrhoeic with malabsorption. This may be accompanied by mild fever, abdominal pain, ructus ("purple burps"), meteorism and anorexia, malaise and vomiting. The diarrhoea may be intermittent, chronic and recurrent, chiefly in patients with an IgA deficiency, hypogammaglobulinemia or agammaglobulinemia. This reflects the fact that secretory immunity in the intestinal lumen is more important for clearance than cell-mediated immunity within the intestinal lumen.

Diagnosis

Diagnosis is quite difficult due to the intermittent character of the presence of *Giardia* in the faeces. The diagnosis is mainly based on fresh or enriched faecal preparations. Sometimes several analyses of faecal specimens are needed. One specimen gives a detection rate of approximately 70% while 3 specimens increase this rate to approximately 85%. Generally cysts are found rarely trophozoites. Other techniques such as duodenal aspiration or the EnteroTest (the string test) are less practical. In rare cases infections have been recognised on jejunal biopsy material or mucus sampled during endoscopy. Recent techniques for detecting antigen in faeces have proved sensitive, specific and fast. PCR methods are increasingly available in high-resource settings. Microscopically a differentiation needs to be made between with other flagellatees such as the commensal *Chilomastic masnnilii, Enteromona hominis, Trichomonas hominis (= Pentatrichomonas hominis)* and *Retortamonas intestinalis*.

The histological intestinal lesions are not very pronounced: flattening of the intestinal villi, lymphocytic infiltration of the mucosa, no ulceration. Radiology of the small intestine is non-specific. If giardiasis is suspected, but cannot be proven a trial of therapy can sometimes be used.

Treatment

Giardia is an anaerobic protozoon, which possibly explains its sensitivity to nitro-imidazoles (e.g. metronidazole). The drug of first choice are nitro-imidazoles, especially tinidazole (Fasigyn®), of which 2 gramss is to be taken in one dose (adult patient). This gives a cure rate of 90 to 95%. Metronidazole may also be used but produces more side effects. Ornidazole (Tiberal®) 500 mg b.i.d. is an alternative but is best given for 5 days. Alcohol should be avoided since there may be an antabuse effect. Other nitro-imidazoles are also sometimes used: secnidazole (Flagentyl®), nimorazole (Naxogyn®).

Refractory giardiasis possibly related to lower susceptibility/resistance to nitro-imidazoles is increasing. Mepacrine (quinacrine, atebrine) is an old drug (3 x 100 mg/day orally for 5 days) which gives good results if tinidazole fails. It also kills cysts, as opposed to metronidazole. It is a yellow product and may cause a jaundice-like skin discoloration, which the patient should be warned about beforehand. It may also cause haemolysis if there is severe G6PD deficiency. Albendazole has also proved effective in vitro but produces varying results in vivo. Nevertheless it is a good second choice. Paromomycin (Humatin®, Gabbroral®) is an aminoglycoside which has very low absorption when taken orally and is thus active in the intestinal lumen. However there is quite a high relapse rate (25%). Nitazoxanide is an expensive alternative (500 mg BD x 3 days for an adult).

Metronidazole is often available in tropical countries when tinidazole is unavailable. Selective toxicity is achieved because the drug is only reduced in an anaerobic environment (reduction is prevented by oxygen). Its action is limited to anaerobic protista (Giardia, Entamoebahistolytica, Trichomonasvaginalis: all three lack mitochondria) and anaerobic bacteria. Side effects of metronidazole include a metallic taste in the mouth, gastrointestinal disturbances: vomiting, nausea, cramps, headache, and a disulfiram ("antabuse")-effect. Rarer are CNS toxicity, dizziness, drowsiness, lassitude, paraesthesia, pruritus and urticaria. In

therapy-resistant giardiasis the questions should be considered as to whether (1) compliance is failing, (2) is there a possibility of counterfeit medication, a growing problem in many countries, (3) if there may be re-infection (e.g. via an asymptomatic cyst carrier), or (4) immunodeficiency, including IgA-deficiency, (5) possibly the presence of a duodenal diverticulum (mechanical reason for relapse, as the concentration of the therapeutic drug might be rather low) or (6) whether this is a genuine problem of resistance. There is in-vitro cross-resistance between the different nitro-imidazoles. If symptoms persist, (7) long-term lactase deficiency or (8) bacterial overgrowth in the small intestine with possible inactivation of nitro-imidazoles by Gram-negative bacteria should be considered.

Prevention

Prophylaxis is difficult both individually and in the community. Giardiasis is much more common than amoebiasis. The importance of giardiasis is underestimated according to some and it is thought to be one of the ten most important parasitic diseases in humans and may be responsible in poor countries of impairment in child growth. Nevertheless there is little connection between the prevalence and the pathology attributed to the infection. In general the treatment of asymptomatic infections in endemic regions is considered unnecessary.

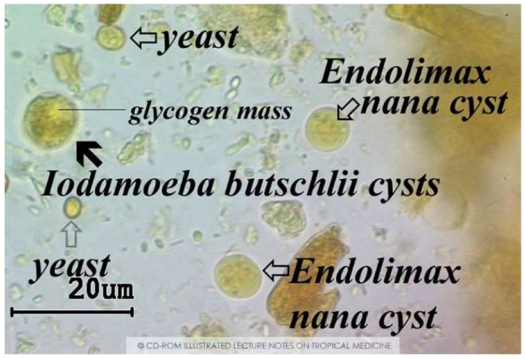
Treatment of large amounts of drinking water (flocculation, sedimentation, filtration and chlorination) is important. Chlorine compounds work best in water with a low pH and a high temperature when the water contains little organic debris.

Alternatives for chlorine and hypochlorite compounds includes chlorine dioxide, ozonation and ultraviolet irradiation. Boiling of large amounts of drinking water is too costly.

Infection with Various Protista

Non-E. histolytica intestinal amoebae

Entamoeba coli cyst in faeces. Cysts can obtain up to 8 nuclei. Copyright ITM



Iodamoeba butschlii in faeces. The glycogen mass will stain brown with an iodine stain. Copyright ITM

At least 10 different amoeba species are found in the intestinal lumen or mouth. Some consider all amoebae apart from E. histolytica as non-pathogenic commensals, but more investigation is needed to clarify some issues especially regarding *Blastocystis hominis* and *Dientamoeba fragilis*. Pathogenicity is probably due to strain differences that are increasingly
investigated. Genetic analysis indicates that *D. fragilis* is actually more closely related to Trichomonas than to amoebae.

1. Entamoeba histolytica
2. Entamoeba dispar
3. Entamoeba moshkovskii
4. Entamoeba hartmanni
5. Entamoeba coli
6. Entamoeba polecki
7. Entaoeba chattoni
8. Entamoeba gingivalis
9. Endolimax nana
10. Iodamoeba butschlii
11. Blastocystis hominis
12. Dientamoeba fragilis

E. dispar and ***E. moshkovskii*** are morphological identical with *E. histolytica.* In order to distinguish between *E. histolytica* and *E. dispar* molecular tools such as PCR technology are used. Most antigen-detection tests cannot distinguish the two organisms, although one test (Wampole *E. histolytica* test) uses reagents that differentiate between *E. histolytica* and E. *dispar*. If trophozoites in stool contain RBCs, they are pathogenic E. histolytica, but if the trophozoites do not contain RBCs no species identification can be reached. Limited research has been carried out on *E. moshkovskii*. At present there are no good practical tests to distinguish this organisms from the two other look-alikes. Its presence is suspected especially

in people who have E. *histolytica / E. dispar*-like cysts in the stools, but who test negative for *E. histolytica/E. dispar* antigen. *E. moshkovskii* is highly resistant to the current amoebicidal drugs.

The existence of these non-pathogenic look-alikes often results in clinical doubt and leads to overtreatment. Infections with non-pathogenic amoebae are much more frequent than infections with pathogenic *E. histolytica.*

E. hartmanni is a non-pathogenic intraluminal parasite which can only be distinguished from *E. histolytica* forms by its smaller dimensions.

Entamoeba coli is a non-pathogenic organism that is commonly mistaken for a pathogenic *E. histolytica.* Trophozoites move slowly and never conta
in red blood cells*. E. coli* cysts are larger
(10-30 µm) and may contain up to eight nuclei.
Endolimax nana is non-pathogenic. The trophozoites are small (up to 10 µm), move slowly with blunt hyaline pseudopods.

Iodamoeba butschliialso has small cysts, about 9 µm. These have only one nucleus and a glycogen mass which stains with iodine (Lugol), from which it gets its name.

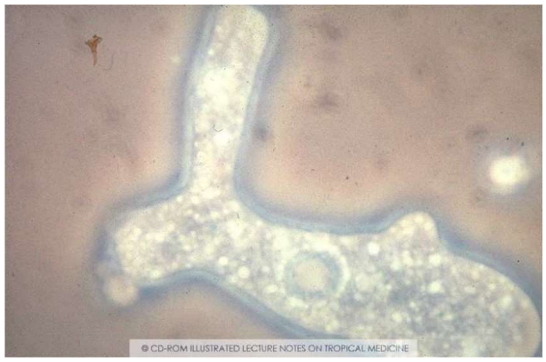
Dientamoeba fragilis is an amoeboflagellate. The fact that it can develop flagella puts it in a different taxonomic group from the above-mentioned amoebae. It is more closely related to *Trichomonas sp* than to *Entamoeba histolytica*. It is a non-invasive intestinal parasite. Many infections are asymptomatic, but it has been associated with non-specific diarrhoea. It is very difficult to demonstrate with the microscope because the vegetative form is easily damaged (fragilis = breakable). No cyst stage is known and it is unclear if transmission via trophozoites can take place. One hypothesis as to how transmission of such a fragile microorganism is possible is that *Enterobius vermicularis* (pinworms) could function as vectors but solid evidence is lacking. If the faeces cannot be brought quickly to the laboratory (ideally < 10 min), they should be fixed in PVA (polyvinyl alcohol) or SAF (sodium acetate formalin), otherwise the parasite will most likely not be detected. There seems to be wide genetic variability between isolates, e.g. as demonstrated by differences in DNA melting temperature or variability of certain DNA markers. As more information will become available in the future; it is possible we will encounter a scenario like the one with *Entamoeba histolytica* (being pathogenic) and *Entamoeba dispar* (non-pathogenic): i.e. a heterogeneous species with genetic variants that have similar morphologies but different pathogenicities. It is clear that more study is needed. *Dientamoeba fragilis* infections can be treated with a 5-day course of metronidazole, but a single 2-gram dose (adult patient) of ornidazole it is easier and gives less side-effects. Paromomycin and iodoquinol can also be used and actually give higher cure rates.

For ***Blastocystis hominis***, see below (separate chapter).

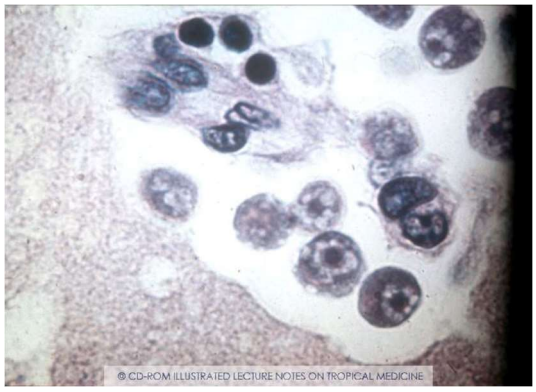
Free-living amoebae

Saprophytic amoebae from water, silt and wet soil which belong to the genera Naegleria, *Acanthamoeba, Balamuthia* and *Sappinia* are cosmopolitan and potentially pathogenic. They seldom cause infection although underreporting is probable. In industrialised countries with a moderate climate these amoebae prefer fresh water with a temperature higher than average, such as public swimming pools and warm waste water from factories or power stations. This suggests that these amoebae must be widely distributed in a tropical environment.

Naegleria fowleri



Naegleria fowleri trophozoite, one of the free- living amoebae.



Naegleria fowleri amoebae.
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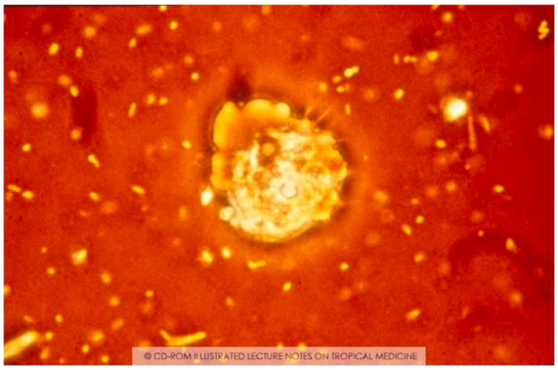

Acanthamoeba sp. Notice the typical thorn-like projections (acanthopoda).
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Historical note

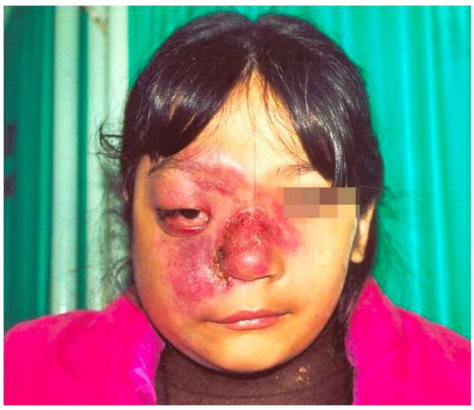
Culberston et al in 1958 were the first to launch the concept that free-living soil and water amoebae could cause disease in humans. In order to feed trophozoites form a kind of "cell-mouth", called an amoebostome. This is quite spectacular in electron microscopic pictures. In addition to phagocytosis via these food cups, contact-mediated cytolysis occurs. When flagella develop, e.g. after transfer from culture or from tissue to water, they sprout at the broad blunt end. This change to the flagellate form can take 2-20 hours. The flagellate form does not divide, but is motile. When the flagella are lost, the amoeboid form is regained and the parasite resumes asexual reproduction. Cysts measure 7-15 µm, but are absent from human tissue, in contrast with *Acanthamoeba* infections.

Infection with *Naegleria fowleri is* the consequence of bathing or swimming in contaminated freshwater ponds or lakes at quite high temperatures, such as fresh water lakes in the summer (e.g. southern USA) ponds, rivers and hot springs. Sampling of such warm water has indicated that *N. fowleri* is commonly present in such environments. The infection follows penetration of water into the nasal cavities. From there the lamina cribriforma of the ethmoid bone is penetrated, probably through phagocytosis of the olfactory epithelium. Via the first cranial nerve, the infection spreads to the lowermost part of the frontal cerebral lobes. Extensive tissue damage follows. The amoebae reproduce rapidly in the cerebrospinal fluid. There is virtually no inflammatory reaction. Haemorrhagic necrosis of the base of the brain, cerebral cortex and the olfactory lobes develops.

The incubation time is 2 to 15 days. Early in the infection, upper respiratory distress, severe headache, sore throat, runny or stuffy nose, altered smell and taste occur. Fever, vomiting and neck stiffness follow. Mental confusion and coma occur after 3 to 6 days. Most infections are lethal. A high index of suspicion is needed for diagnosis since CSF findings are very similar in acute bacterial meningitis. A history of bathing in surface water during the previous two weeks is significant. The disease closely resembles acute bacterial meningitis and is known as primary amoebic encephalitis (PAM).

In clinical practice, most cases will be diagnosed only at autopsy (immunofluorescense and immunoperoxidase techniques). Immediate chemotherapy is required for survival. N. fowleri is sensitive to amphotericin B. Combination treatment with ampho B, miltefosine, miconazole, rifampicin, azithromycin, chloramphenicol and/or ketoconazole has been used. Specialised advice is absolutely required.

Balamuthia mandrillaris



Balamuthia mandrillaris infection with important skin lesion. Copyright Alexander von Humboldt Institute, Peru

It is not clear how humans get infected, but transmission via swimming in contaminated surface water is one possibility. The pathogen has also been isolated from a potted plant in a home. Infection with this amoeba causes peri-orbital swelling and ulceration, followed by symptoms of granulomatous meningoencephalitis, in both immunocompetent and immunocompromised persons. Symptoms include headache, nausea, vomiting, fever, visual disturbances, dysphagia, seizures and hemiparesis. Both trophozoites and cysts are found in CNS tissues. Differentiation with *Acanthamoeba* is difficult when using only simple light microscopy. Electron microscopy, immunofluorescence testing and histochemistry are needed for definite species identification. In vitro studies show that B. mandrillaris is susceptible to pentamidine. Ketoconazole, propamidine, miltefosine, 5-flucytsosine, clotrimazole, sulfadiazine, fluconazole and clarithromycine have all been used in treatment of patients. Treatment is not standardised yet. Experience in Peru has shown that prolonged administration of itraconazole 400 mg/day (adults) can be useful.

Acanthamoeba sp.

Acanthamoeba sp are free-living protista which occur in numerous places (water, dust, waste). Several species have been described: *A. castellani, A. culbertsoni, A. polyphaga, A. healyi, A. astronyxis, A. hatchetti, A. rhysodes, A. griffini, A. quina, A. lugdunensis.*

Acanthamoeba species are responsable for several clinical problems: (1) granulomatous amoebic encephalitis, (2) keratitis, (3) disseminated lesions, including skin ulcers, but also lesions in adrenals, kidneys, liver, spleen, thyroid....

1. Granulomatous amoebic encephalitis (GAE): unlike with Naegleria, infection of the central nervous system progresses slowly and occurs where there is immunosuppression or in the course of a severe general illness. Infections are more common in AIDS patients with a low CD4-count. Generally it presents as a subacute meningo-encephalitis with signs of a brain abscess and develops in two to three weeks (range 7 days - 5 months). The cerebral hemispheres tend to be involved with an inflammatory exudate covering the cortex, granulomatous necrosis of the brain parenchyma and thrombosed blood vessels. Such infections can mimic malignancies, fungal infections or abscesses. In AIDS patients, the differential diagnosis with cerebral toxoplasmosis can be very difficult.

2. Keratitis. This is more common than cerebral inflammation. The amoebae may infect small wounds of the cornea and then trigger a dangerous ulcerative keratitis which may develop into painful uveitis with hypopyon, scleritis and panophthalmitis. Acanthamoeba keratitis should be considered in the differential diagnosis of uveitis in AIDS patients. Initially this diagnosis is often missed and the lesion is considered to be a herpetic or fungal keratitis. Infection can follow corneal trauma (e.g. corpus alienum). The number of cases has grown in recent years as the result of increased use of contact
lenses and the practice of rinsing these with tap water, as a result this is a cosmopolitan infection. It is likely that bacteria in the biofilm on dirty contact lenses constitute a good source of nutrition for the amoebae. The amoebae are often scarce in corneal smears. Culture is possible on nonnutrient agar plates with an overlay growth of Esch. coli or Pseudomonas aeruginosa bacteria on which the trophozoites feed. Sometimes the diagnosis is made purely on anatomopathological grounds, e.g. during a cornea transplantation.

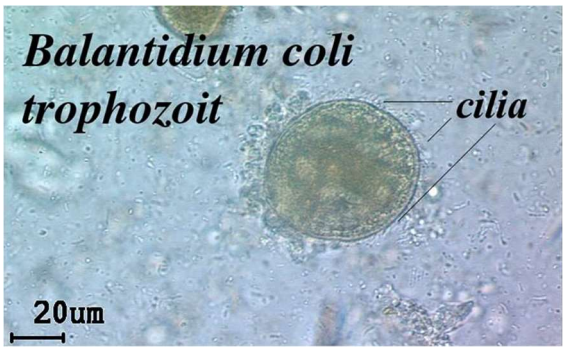
3. Other locations. Abscesses in other locations and granulomatous skin lesions in which histological investigations show amoebae, have also been observed. Skin lesions are more common in AIDS patients. Hard erythematous papulonodular lesions or non-healing indurated ulcers may be the first sign.

The optimal approach for GAE management is uncertain; therefore, combination regimens are preferred over single-drug regimens. An empiric treatment could be a combination of miltefosine, fluconazole, and pentamidine isethionate. Trimethoprim-sulfamethoxazole, metronidazole and a macrolide (azithromycin or clarithromycin) can be added to this regimen as well. Single cerebral lesions should be resected if possible.

Treatment of *Acanthamoeba* keratitis employs a combination of propamidine isethionate eye drops (Brolene®), topical neomycin, polyhexamethylene biguanide collyre (Lavasept®) and/or topical chlorhexidine (Hibitane®). Brolene® available in Great Britain is an antiseptic which is moderately toxic for the corneal epithelium. The use of topical steroids is controversial but probably beneficial. Oral itraconazole is probably also active. Topical miconazole is sometimes also used. Pentamidine (a diamidine related to propamidine) is being evaluated. Chronic refractory cases may require corneal transplantation. Unresponsive cases may require enucleation.

COMPARISON	
Naegleria fowleri	**Acanthamoeba sp**
Amoebic form with lobate pseudopodia; Flagellate form (two flagella)	No flagella, filiform sharp pseudopodia
Cystsnot present in tissue; they are small and smooth	Cysts can be found in tissues; large and wrinkled with a double wall
Culture requires living cells (bacteria or cell culture) No growth if NaCl concentration > 0.4%	May grow without bacteria; not affected by NaCl 0.85%
Smaller than Acanthamoeba; dense endoplasm; less distinct nuclear staining	Large round, less endoplasm; more distinct nucleus

Balantidium coli



Balantidium coli trophozoite. Notice the numerous cilia. Copyright ITM

Balantidium coli is a large protozoon. The trophozoite measures 30-200 µm x 40-60 µm. The whole surface of the trophozoite is dotted with countless cilia. These are very characteristic and because of this, it is classified as a ciliate (compare with Paramecium). Balantidium coli is the only ciliate pathogenic to humans. Transmission occurs from pigs to humans and from human to human in poor hygiene situations, also via water or food contaminated with cysts including poorly cooked pork sausages. As with amoebiasis the infection may be intraluminal and latent or invasive in the intestinal wall and symptomatic. In the invasive forms ulcerations of the intestinal wall are found which are quite similar to those of amoebiasis, with the same complications and the same clinical forms. Liver abscesses caused by B. coli have been observed but are extremely rare. Diagnosis is parasitological by direct stool microscopy or enrichment techniques. In a fresh preparation, B. coli can be very quickly recognised due to its swift manner of propulsion. Under the microscope, the creature is difficult to keep in the field of vision due to its relatively high speed. Treatment is not always simple. Tetracyclines (10 days) have been used as well as imidazoles in high doses.

Flagellum

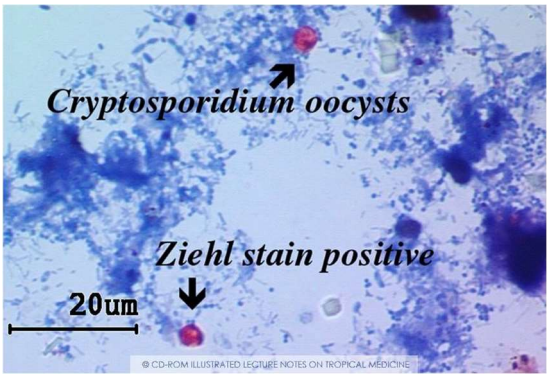
One important organelle to move in an aqueous environment is the flagellum. A certain group of micro-organisms (flagellates) take their name from the fact that they possess flagella. The term flagellum (L. flagellum = whip) is used, however for two totally different organelles. Some micro-organisms are dotted with myriads of these organelles which work in a coordinated way and which are then called "cilia".

Cryptosporidiosis

C. parvum is the most common parasite in this group in human infections, but *C. meleagridis, C. canis, C. muris* and *C. felis* are also found in immune-compromised persons with acute diarrhoea.

Biological classification

Cryptosporidia are coccidia and belong to the Apicomplexa phylum. Coccidia form an order of unicellular eukaryotic microorganisms, which includes the following human pathogens: *Toxoplasmagondii, Sarcocystis sp., Cryptosporidium parvum, Cyclospora cayetanensis, Isospora belli*. Microsporidia do not belong to the Coccidia and form a totally different taxonomic group. DNA analysis of Cryptosporidium suggests that there could be more than twenty different species.



Cryptosporidium parvum, Ziehl stain. Copyright ITM

Transmission to humans occurs from calves, dogs and cats. Transmission via drinking water or via insufficiently chlorinated water in swimming pools happens frequently. This species is resistant to standard chlorination. The parasite was first observed in humans in cases of persistent diarrhoea in patients with immunosuppression and since 1981 in cases of AIDS. Since 1983 the infection has frequently been recognized as a cause of benign and brief diarrhoea, both in children and adults, and it is one common aetiologies of travellers' diarrhoea.

Life cycle details

The complete cycle of the parasite, sporogony and schizogony, takes place in the same host. People become infected by swallowing thick-walled, resistant oocysts. Once in the intestine the parasites excyst and release sporozoites. They penetrate epithelial cells via the apical membrane. After maturation of the sporozoite there is asexual reproduction via schizogony with the formation of merozoites. These may either penetrate a new epithelial cell to repeat the cycle (type 1 merozoites) or undergo further intracellular changes (type 2 merozoites) to the sexual form of the parasite. The macrogamont is the female form, the microgamont the male form. The microgamont releases microgametes. After fertilisation and the formation of zygotes, thin-walled oocysts are produced, which after meiosis release sporozoites in their turn which amplifies the infection (auto-infection). Thick-walled oocysts are released into the lumen of the intestine, and are directly infectious via the faeco-oral route. *C. parvum* induces apoptosis in epithelial cells.

The parasites may be found throughout the entire digestive tract and even in the mucosa of the respiratory tract but are usually limited to the duodenum and jejunum. The incubation period is 4 to 12 days (usually 7-10 days) and is followed by moderately severe diarrhoea without fever usually and with little abdominal pain. Asymptomatic infections may occur. If there is no underlying immunosuppression, spontaneous recovery occurs within a few weeks. It is estimated that 4 to 10% of all cases of diarrhoea in children in tropical environments can be attributed to Cryptosporidium. In patients who have a deficiency in cellular immunity (such as in HIV infection), the diarrhoea is more pronounced can be chronic for several months and recurrent. Fulminant infection with cholera-like diarrhoea may occur in patients with fewer than 50 CD4 T-cells/mm3. Sometimes the protista enter the biliary tract, resulting in sclerosing cholangitis, strictures and papillary stenosis. Diagnosis is difficult and requires invasive procedures such as retrograde cholangiography (ERCP [endoscopic retrograde

cholangiography]). A biliary tract reservoir may contribute to the chronic course of infection. Diagnosis is based on looking for the parasite in the faeces on smears stained with modified Ziehl-Neelsen or Kinyoun staining. The small dimensions of the parasites and their similarity to yeast cells were responsible for the fact that infection in humans was only recognised in 1976. The parasites can easily be recognised on intestinal biopsy material obtained by endoscopy. There are other diagnostic techniques, such as immunofluorescence, antigen-capture ELISA and PCR, but these are not available in most tropical settings. Treatment is mainly symptomatic and can be quite difficult in AIDS patients. The best practical method in these patients is via HAART (highly active antiretroviral therapy). Paromomycin (Humatin®, Gabbroral®) is a non-absorbed aminoglycoside and is of limited use. At present the drug of choice is nitazoxanide (Alinia®, Cryptaz®, 500 mg tablets or syrup) but this drug is unaffordable. Cryptospodirium cysts are very resistant to chlorination (much more so than Giardia cysts, although even those have a certain resistance to standard concentrations in drinking water).

Cystoisosporosis

Isospora belli mature oocyst containing two sporocysts. Copyright ITM

Cystoisospora belli is a coccidian parasite of the duodenum and proximal small intestine (jejunum) in humans. It is cosmopolitan but more frequent in a tropical environment. The previous name was *Isospora belli.* No reservoir hosts other than man are known. The oocysts are very resistant to environmental conditions and may remain viable for months if kept cool and moist. The sexual and asexual cycles occur in the same host. The parasites are located intracytoplasmic, unlike Cryptosporidium. There is a prepatent period of about 9-10 days. Infection may be latent or lead to diarrhoea for one to two weeks occasionally with mild fever, headache, malaise and abdominal pain. The stools tend to be soft, watery or foamy, with an offensive smell, suggesting malabsorption. In immunosuppressed people the infection can become chronic. In such cases oocyst shedding can continue for years. Diagnosis is difficult and is based on stool examination, and biopsy of the duodenojejunal mucosa, in which the parasites are not very numerous. Charcot-Leydig crystals (derived from eosinophils) are occasionally found in stools samples of isosporiasis cases.

The infection can be severe and prolonged in case of immunosuppression (in particular in AIDS). It is like cryptosporidiosis a frequent cause of prolonged/recurrent cholangitis in AIDS patients through invasion of the biliary tract.

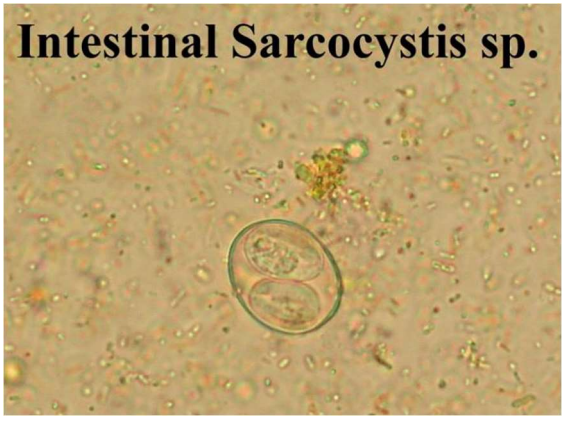
The condition can be treated with cotrimoxazole (e.g. Bactrim forte® 4 x 1 tablets/day for 10 days). If there is diminished sensitivity or resistance, either pyrimethamine (Daraprim®) 25 mg/day x 20 weeks or the combination ornidazole (e.g. 2 gram on day 1, 15, 30) with albendazole (400 mg BD x 30 days) is used. Ciprofloxacin is also moderately effective (70% cure rate).

Sarcocystosis

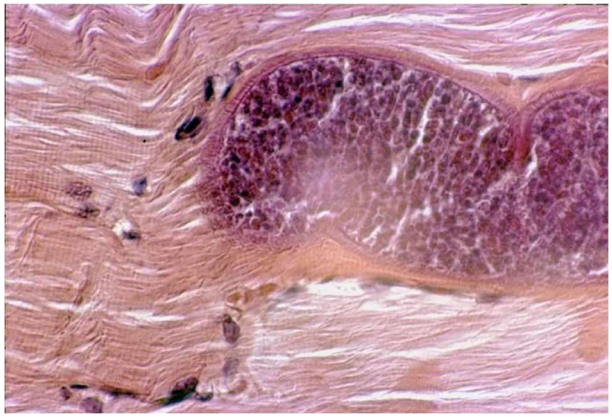
General

Sarcocystis species are parasites of mammals, birds and reptiles. Human sarcocystosis (syn. sarcosporidiosis) is rarely diagnosed. For some species humans are the definitive host i.e. the host in which sexual reproduction (gametogony followed by sporogony) is completed. In this case there is intestinal sarcocystosis. Humans may also act as accidental dead-end intermediate hosts – where asexual reproduction (schizogony) takes place - for several other species and in these cases there is muscular sarcocystosis.

Intestinal sarcocystosis



Intestinal Sarcocystis Copyright ITM



Sarcocystis, pseudocyst in muscle. Copyright ITM

Sarcocystis bovihominis and *Sarcocystis suihominis* are parasites of humans. Infection occurs due to eating raw or insufficiently cooked meat from cattle or pigs containing tissue cysts (intestinal infection cannot be triggered by the ingestion of sporocysts). The sexual cycle takes place within the cytoplasm in the cells of the human intestinal mucosa. The sporocysts which are released with the faeces are infectious for the intermediate host. These infections are cosmopolitan and generally asymptomatic. They can nevertheless trigger enteritis with peripheral hypereosinophilia. The diagnosis is based on faecal examination. Sometimes the parasites will be detected in surgical resected intestinal specimens.
Gastro-intestinal disease is often self-limiting and does not need treatment. There is no known effective treatment.

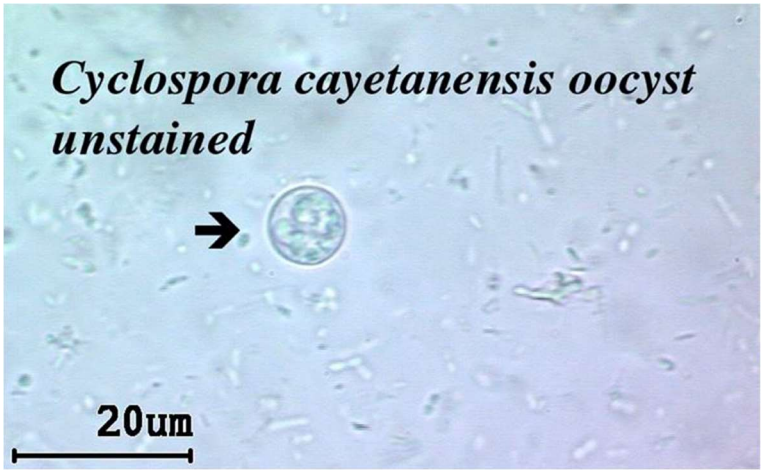
Muscular sarcocystosis

Muscle infection is caused after swallowing sporocysts (faeces of an infected predator). Each sporocyst releases 4 sporozoites. These penetrate the intestinal wall. Reproduction begins in the vascular endothelium. After dissemination of merozoites there is invasion of skeletal and cardiac muscle tissue and possibly the central nervous system (in animals). The merozoites

develop first to metrozoites and then to cystozoites. These tissue cysts remain dormant until the host is eaten by a predator after which the intestinal cycle begins. The tissue cysts gave the genus its name (Gr. sarx = flesh).

Most human infections are apparently asymptomatic. It is also possible that the diagnosis is systematically missed (data from investigation of routine autopsies). No cases of neurological involvement in human patients are known. Some patients with muscular sarcocystosis develop an eosinophilic myositis. The myositis is characterised by muscle pain, painful mild muscular swelling, mild fever, general weakness, bronchospasms and eosinophilia. This should be differentiated from trichinosis (*Trichinella spiralis*). Eosinophilic fasciitis, toxoplasmosis, polymyositis, dermatomyositis and polymyalgia rheumatica may lead to similar clinical pictures. Diagnosis is made via muscle biopsy. The intact cysts in the muscle generally do not trigger a local inflammatory reaction. Dead and ruptured cysts may cause inflammation. Muscular sarcocystosis can be treated with cotrimoxazole, although its efficacy is not proven. The use of corticosteroids is under discussion but often necessary to control the symptoms of myositis when they are prominent.

Cyclospora cayetanensis



Cyclospora cayetanensis in faeces, unstained. The parasite is about double the size of Cryptosporidium parvum. Copyright ITM

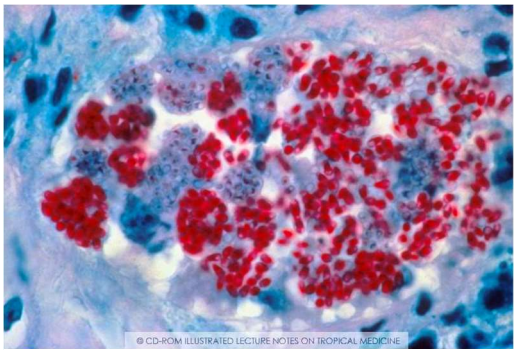
Cyclospora cayetanensis is a protozoon which belongs to the Coccidia. The name is derived from the morphology (the sporocysts are spherical) and from a Peruvian university (most of the epidemiological and taxonomic work has been carried out at the Universidad Peruana Cayetano Heredia, Lima, Peru). Distribution is probably cosmopolitan, but the species is only common in regions with poor hygiene. Protista can be detected in surface water with special techniques. No reservoir is known to date.

After swallowing mature (i.e. sporulated) oocysts, there is excystation after contact with bile salts. The released sporozoites penetrate the jejunal enterocytes. Infected persons eliminate non-sporulated oocysts in their faeces. Until they sporulate, which takes days or weeks these parasites cannot infect a new host. This delay makes direct human to human transmission unlikely.

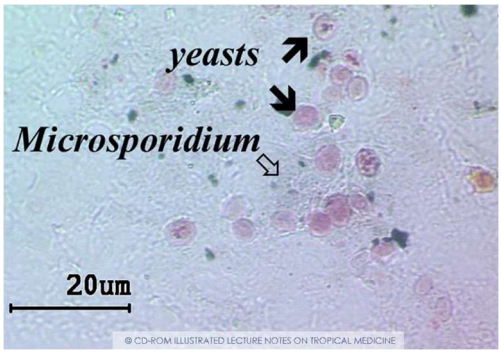
The protista are present in the duodenum and jejunum and cause persistent watery diarrhoea, often accompanied by significant abdominal discomfort, nausea, tiredness and anorexia and sometimes with mild fever. The symptoms may last several weeks. In particular, non-immune persons such as travellers or small children, will be symptomatic. Cotrimoxazole is used in treatment. This protozoon also causes persistent diarrhoea in HIV-positive persons. If patients cannot tolerate cotrimoxazole, the rather less effective ciprofloxacin may be used.

Microsporidiosis

General



Microsporidia in muscle of AIDS-patient. Ziehl-stain.



Microsporidium sp. in faeces. Species identification requires PCR or electron microscopy. Copyright ITM

Species belonging to the phylum Microspora are called microsporidia. At present more than 140 genera are recognized and 1200 species have been described. These obligate intracellular organisms appear to have separated very early from the eukaryotic family tree. They have true nuclei, but no mitochondria or peroxisomes. Their ribosomes are prokaryote-like (70S). Since the spore wall contains chitin some researchers regard them as aberrant fungi. They are obligate intracellular parasites and are recovered in countless widely varying host groups (insects, fish, rodents, and so on). Species which can parasitise humans are very small (1-2 µm).

DNA structure, ultrastructure and lifecycle

Encephalitozoon cuniculi holds the record at present for the smallest eukaryotic genome (<2.9 Mb). Other species known to infect humans are *Brachiola vesicularum, Encephalitozoon cuniculi, Encephalitozoon hellem, Encephalitozoon intestinalis (previously Septata intestinalis), Enterocytozoon bieneusi, Microsporidium africanum, Microsporidium ceylonensis, Nosema algerae, Nosema connori, Nosema ocularum, Pleistophora sp, Trachipleistophora hominis, Trachipleistophora anthropophthera* and *Vittaforma corneae (previously Nosema corneum)*. These organisms have mainly been described in immunodeficient persons.

The parasites have a very characteristic ultrastructure. The organism forms oval-shaped spores with an external exospore (glycoproteins) and an internal endospore (chitin). Within the spore is a coiled spiral tube (polar tube). After it is ingested, the spore is stimulated to protrude this polar tube which then penetrates a host cell. The sporoplasm is then injected via this tube into the cytoplasm of the host cell. Subsequently there is reproduction of the

parasite (merogony and sporogony). New spores may infect other neighbouring cells or be passed to the outside world to infect a new host.

Transmission

Transmission is chiefly via the faeco-oral route but much is still uncertain. Possibly transmission is via aerosol for those protista which cause corneal lesions. Transmission via infected water is being investigated.

Clinical aspects

Symptoms will be determined by the anatomical location of the parasites. Disseminated infections, corneal infections (keratitis), intestinal locations etc all occur, almost exclusively in immunosuppressed individuals. In HIV patients with low resistance (CD4 < 100/µL) there is often persistent diarrhoea, abdominal pain, loss of weight and sometimes sclerosing cholangiopathy.

Diagnosis

Diagnosis by light microscope (faeces, biopsies, corneal scraping) is often difficult due to the small dimensions of the parasites and the labour-intensive staining techniques. Experience is essential and the parasites must be properly differentiated from fungal spores and bacteria. Electron microscopy is a good technique for species identification, together with PCR but of course this can only be carried out in specialized centres. The organisms can be detected in routine formail-fixed and paraffin-embedded tissues.

Treatment

There is still too little known about treatment. Fumagillin is a product originating from *Aspergillus fumigatus* which is used in microsporidiosis of honey bees, has been used topically in keratitis with good results. Other drugs have been used with varying success. Albendazole is effective in infections with *Encephalitozoon intestinalis* and to a lesser extent in Enterocytozoon bieneusi. Nitazoxamide possibly has a place in treatment. Improving immunity in HIV patients, e.g. by combination antiretroviral therapy, often leads to remission of the infection. For symptomatic treatment (e.g. in persistent diarrhoea without knowing its cause), loperamide (Imodium®), opioids (laudanum) or even somatostatin analogues may be used. The latter is of course not easily available in developing
countries.

Blastocystosis

Blastocystis hominis is a rather common enteric unicellular protista. The parasite colonises chiefly the caecum and to a lesser extent the distal colon.

Blastocystis hominis. Copyright ITM

Biology

Very little is known of the basic biology of this organism, including the life cycle. Several morphological forms have been recognized: ameboid, vacuolar, avacuolar, multivacuolar, granular, cyst. Which of the forms is responsible for transmission is not known. The vacuolar stage divides, while the amoeboid stage might be invasive and is capable of budding. B. hominis forms pseudopods, and ingests bacteria and debris. It reproduces by binary fission or sporulation.

Transmission is faeco-oral through contaminated food or water. There seems to be a large animal reservoir. Its pathogenicity is controversial. Several studies using different methods and examining different patient groups have reported very variable results, from asymptomatic infection, acute symptomatic infection and chronic symptomatic infection; with abdominal pain, diarrhoea, constipation, irritable bowel, fatigue, skin rash, and other symptoms. The variation in results led to disagreements concerning a possible pathogenic role of *Blastocystis* in humans. Maybe *Blastocystis* has several variants which differ in their pathogenicity or virulence. The pathogenicity might depend on the parasitic load (more than 5 Blastocystis per 40x field, but different pathogenic properties of different strains will likely also play a role). Molecular typing has revealed extensive genetic diversity in morphological identical strains. According to current PCR-based genotype analysis there may be 12 different species which are lumped together under one name. It is possible that additional studies will show that what we call *Blastocytis hominis* will turn out to be a mixture of different microorganisms, a situation similar to the past confusion about the morphological identical Entamoeba histolytica, *E. moshkovskii* and *E. dispar.*

Classically Blastocystis is considered to be non-pathogenic and doesn't need treatment. However a treatment can be justified if symptoms are severe in the absence of other pathogens or in immunosuppressed patients (AIDS), or if the parasitic load is very high. If considered necessary, metronidazole/tinidazole or trimethoprim/sulfamethoxazole are used for treatment.

Rhinosporidiosis

Rhinosporidiosis is an infectious disease which occurs in the New World, Europe, Africa and Asia, but is most common in the tropics (India and Sri Lanka). The disease is characterised by slow-growing, painless polyps or tumour-like masses, which are usually found on the nasal mucosa, lachrymal sac, conjunctivae, palate, larynx or penis. Chronic rhinitis and/or epistaxis may occur. Treatment consists of surgical excision, but recurrence can be expected in approximately 10% of patients. No natural reservoir is known. It is also assumed that people become infected by swimming in fresh water lakes or rivers. It is likely that fish or other water creatures are the normal hosts.

Protothecosis

Protothecosis is a rare infection in humans. Infection is more common in cases of immunosuppression (AIDS, leukaemia). The disease is caused by Prototheca wickerhamii and P. zopfii (segbwema). These are aerobic unicellular round (P. wickerhamii) to oval (P. zophii) algae which activity belong to the Chlorococcales [Chlorophyta or green algae]. However they contain no chlorophyl and are colourless. The protista occur in still water, sewage sludge, mud and slime on trees.

Various animals may be infected (cattle, dogs, rabbits, mice, rats, pigs, deer). Humans are infected via traumatic inoculation of the germ into the skin or via infection of an open wound. Infection is usually limited to the skin, where local painles granulomatous hyperkeratotic dermatitis results. Bursitis and tenosynovitis have been described. Indolent olecranon bursitis can be tender. Sometimes there is systemic involvement including cholangitis, chronic meningitis and retinitis. There have been cases of peritonitis after peritoneal dialysis.

Diagnosis is made by biopsy. The pathogens are morphologically similar to mulberries. Confusion with yeasts is possible. For tissue sections a PAS [periodic acid-Schiff] or a Gomori methenamine silver stain are used.

Treatment is surgical with or without amphotericin B. Ketoconazole has frequently been used with success, but requires long-term administration. The possible therapeutic roles of itraconazole and fluconazole need to be better determined.

Babesiosis

General

Babesiosis is a zoonotic disease which is triggered by infection with a protozoon of the genus *Babesia.* The disease is also known as piroplasmosis. The order of Piroplasmida belongs to the Apicomplexa (cf. malaria). There are more than 110 species in the genus *Babesia*. Some infect fish, birds, reptiles or mammals. The rodent parasite Babesia microti (USA) and the bovine parasites *B. divergens* and *B. bovis* (Europe) cause most infections in humans. Occasionally other species may be responsible for human infections (e.g. the WA1 strain = *B. duncani*).

Transmission

Voles form the reservoir. Transmission is via the bite of hard ticks such as *Ixodes scapularis* and *Ixodes ricinus*. In the USA larval nymphs of *Ixodes scapularis* feed chiefly on Peromyscus maniculatus ("the white-footed deer mouse"). The adult ticks suck blood from deer (cf. Lyme disease). Strangely enough the deer are not infected with *B. microti*. In ticks trans-stadial transmission occurs. The parasite passes from larva to nymph to adult tick. There is no transovarian transmission. Infections in humans are accidental occurrences. After injection of saliva of the tick, the micro-organisms penetrate red blood cells and mature. *Babesia microti* trophozoites undergo asexual reproduction in human blood and divide into two or four merozoites. Infected red blood cells undergo haemolysis. This releases the protista which can then penetrate new red blood cells. Infections via blood transfusions have been described. Transplacental infection may occur.

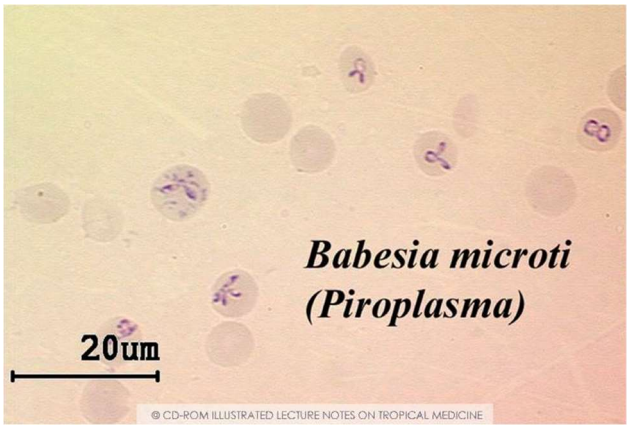
Geographical distribution

Endemic regions in the USA include Massachusetts and New York State with Nantucket Island, Long Island, the coast of Connecticut as well as foci in Georgia, California and Wisconsin. Cases have also been reported from various European countries such as Ireland, Scotland, Sweden, former Yugoslavia, France and Russia. There have been isolated case reports from Africa, Asia and Latin America.

Clinical aspects

Asymptomatic infection may persist for months or years. If symptomatic, the first symptoms occur after an incubation period of one to two weeks. Malaise, tiredness, fever, headache, nausea and abdominal pain, myalgia and joint pain are early but non-specific symptoms. The body temperature may rise to 40°C. Hepatosplenomegaly with haemolysis and jaundice, haemoglobinuria, mild neutropenia and thrombocytopenia follow. In severe cases ARDS [acute respiratory distress syndrome] with shock may develop. Infections may have a dramatic course in asplenic persons, chiefly in the European forms.

Diagnosis



Babesia microti. Copyright ITM

Diagnosis is made from a blood smear stained with Giemsa. The parasitaemia is generally 1 to 10%. Sometimes the mature parasite is in the form of a clover leaf: a so-called tetrad or Maltese cross. The intra-erythrocytic dimension of the merozoite is 1 to 2.5 µm. It is pear-shaped, oval or round. The circular appearance means that *Babesia* is often confused with

Plasmodium falciparum, but malaria pigment cannot be detected. There are also no gametocytes or malaria schizonts. In Babesia infections, large parasites may contain a central white vacuole, which is not present in malaria. Serological tests and DNA analysis may help in diagnosis.

Treatment

Quinine is the drug of choice, 650 mg TDS plus clindamycin 600 mg TDS or 1.2g BD IV for 7 to 10 days. Children receive 25 mg quinine/kg/day. Atovaquone (750 mg BD) and azithromycin (500 mg on day 1, then 250 mg daily) are also used and this combination is better tolerated. Exchange transfusion may be considered if there is life-threatening parasitaemia. A blood transfusion may be life-saving. Remember that ticks can be infected with more than one pathogen. In endemic regions co-infection with *Borrelia burgdorferi*, certain *Ricketssia, Anaplasma, Ehrlichia* or viral pathogen must be considered.

Prevention

Asplenic persons should avoid endemic regions and pay extra attention to tick prevention (proper clothes, repellent containing at least 30% DEET, permethrine and physical inspection after walking).

Viruses

Arboviral infections

Summary
•Arboviruses: Arthropod-borne viruses are viruses that can be transmitted to man by arthropod vectors (mosquitoes, ticks, flies)
• Over 500 virus species can be transmitted by arthropods, approximately 150 of those cause human disease
•Arboviruses belong to 5 different Families (Togaviridae, Flaviviridae, Bunyaviridae, Rhabdoviridae and Reoviridae)
• The main clinical syndromes are skin rash, arthralgia, neurological and/ or hemorrhagic manifestations.
• Clinical distinction between arboviral infections is difficult, because symptoms are often nonspecific. Clinical presentation is also similar to many non-arboviral infections.
•Geographical distribution of arboviral infections varies and is often related to outbreaks; knowledge of possible exposure is important for recognition of clinical cases and for choosing diagnostic tests.
• Laboratory diagnosis is required for confirmation of arboviral infections; the timeline of infection (date of exposure, date of symptom onset) is required to choose the appropriate diagnostic assays.
• Treatment is mainly supportive
• Preventive measures include personal protective measures like the use of protective clothing and insect repellents. Vaccination for selected arboviral infections is available.

General

Transmission

The WHO definition of arthropod-borne viruses (arboviruses) is as follows: “Viruses that are maintained in nature principally, or to an important extent, through biological transmission between susceptible vertebrate hosts by hematophagous arthropods or through trans-ovarian and possibly venereal transmission in arthropods.” Over 500 virus species can be transmitted by arthropods, and approximately 150 of those cause human disease. The viruses multiply in the vector, migrate towards the salivary glands and are transmitted via the saliva to the vertebrate host during a blood meal. There is therefore no simple mechanical transmission (e.g. the mosquito as a flying injection needle).
Different arthropod species often have different vector competence for a particular (strain of) arbovirus. Transovarial transmission in the vector has epidemiological significance, because it allows the arthropod to act both as a vector and as a reservoir.

Aetiologic agents of arboviral diseases are primarily zoonotic pathogens. Spillover from the enzootic cycle to humans occurs when humans enter areas of zoonotic transmission or when enzootic transmission is increased near humans. Examples include Eastern (EEEV) and Western equine encephalitis viruses (WEEV), as well as West Nile (WNV), St. Louis encephalitis (SLEV) and Yellow fever viruses. Spillover may involve direct transmission to humans by primary enzootic vectors (e.g. WNV, SLEV and WEEV) or by bridge vectors, i.e. vectors that take bloodmeals across species, including humans (e.g. EEEV). Some viruses, such as Rift Valley fever, Japanese encephalitis and Venezuelan equine encephalitis viruses (VEEV) infect

livestock animals, resulting in increased risk of infection in persons living in rural communities. Two of the most important human arboviral pathogens, Yellow fever and dengue viruses (DENV) have adapted to replication in humans only, allowing for urban transmission.

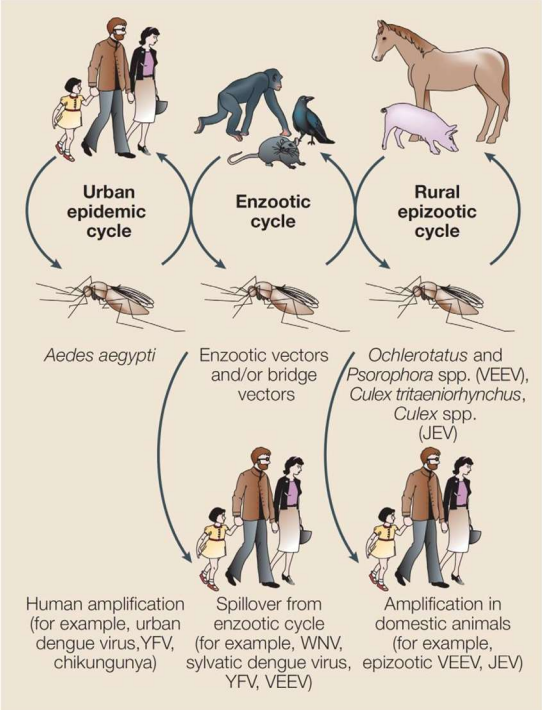


Figure: Typical mechanisms of arboviral emergence (Weaver et al., Nat Rev Microbiol.2004)

Virology

Thus, the acronym 'arbovirus' does not refer to a virological classification, but rather to the main mode of transmission. Taxonomy divides Arboviruses 4 different classes, that all have a single stranded RNA genome:

1. Togaviridae (genus Alphavirus, not Rubivirus); examples Chikungunya virus (CHIKV), Eastern/ Western/ Venezuelan equine encephalitis viruses (E,W,VEEV), Ross river virus (RRV), Mayaro virus
2. Flaviviridae (genera Flavivirus and Pestivirus and not Hepacivirus); examples Dengue virus (DENV), Japanese encephalitis (JEV), West Nile virus (WNV), Yellow fever virus (YFV), Zika virus (ZIKV), Tick-borne encephalitis (TBEV), Kyasanur forest disease virus
3. Bunyaviridae (genera Bunyavirus, Phlebovirus, and Nairovirus but not Hantavirus); examples Crimean-Congo hemorrhagic fever (CCHKV), Toscana virus (TOSV), Sandfly fever virus (SFV), Rift Valley fever virus (RVFV), Oropuche (OROV)
4. Rhabdoviridae; example Indiana vesiculovirus
5. Reoviridae; example Colorado tick fever virus

This Chapter will focus on the arboviral families most important in human medicine: Togaviridae, Flaviviridae and Bunyaviridae.

Vectors

Aedes mosquitoes are the most important vector species of arbovirus infections in Africa, America and Asia.

Aedes aegypti prefers peridomestic settings, where water containers are a typical example of preferred breeding sites. It also enters houses to feed. Aedes mosquitoes bite during the day, mainly in the late afternoon (unlike Anopheles). The adult mosquitoes buzz a little but do not keep people awake in their siesta (unlike night biting Culex mosquitoes).

Mosquito biology

Traditionally it was thought that Aedes aegypti had limited flying ability (100 m). This was called into question by more recent data. Studies with labelled mosquitoes revealed an area of ± 840 meters in diameter in which eggs were laid. In order to study the density of vectors in an area, entomological surveys are used. A frequently used index is the number of positive water containers per 100 houses ("Breteau index"). Dumps of old car tyres are favourite breeding sites.

Aedes albopictus is another dengue vector. The mosquito is recognisable because unlike A. aegypti, it has one longitudinal white stripe down its back. This vector breeds in all kinds of water reservoirs, from lucky bamboo stems to septic tanks, which is important for control purposes. This mosquito also called Asian Tiger mosquito, has been recognized among the world's most invasive species. Its territorial expansion has already been associated with dengue and other arboviral outbreaks in non-tropical countries, like France and Croatia in 2010 and on the Madeira islands of Portugal 2012 (over 2000 cases).

Culex species are the vector of Japanese Encephalitis and West Nile virus.

Vector control

If only the adult mosquitoes are to be controlled, for example with so-called "adulticides", very rapid reduction in the number of adult mosquitoes can be achieved. This reduction will however only be for a short time. The insecticides soon lose their effect, after which mosquitoes that have hatched occupy the ecological niche that has been vacated. It is therefore strongly advised that the breeding sites are controlled also using larvicides. Slow-release formulations of methoprene (Altocid®) can be used here for this purpose.

Aedes aegypti is a peridomestic mosquito and this means that the population can be controlled. The elimination of small water reservoirs (=breeding sites) near housing (cans, car tyres, vases, bottles, buckets, snail shells, coconut shells, bamboo stubble, hollows in plants, waste gullies, etc.) by clearing away rubbish and by having a "dry" day systematically once a week is important in controlling Aedes aegypti. On "dry days", all small water containers (buckets, vases) are emptied to interrupt the cycle of the mosquitoes. The larvae and pupae of the insects are destroyed before adult mosquitoes can emerge. Large reservoirs - drinking water for example - cannot of course be emptied quite so simply. Because large water containers have such a great epidemiological importance in some areas (Thailand for example) covering these with a fine-mesh net is effective in considerably reducing the Aedes mosquitoes (much better than a normal cover). Temephos pellets (Abate®, a larvicide) can be placed in water containers and is non-toxic for humans.

If Aedes albopictus plays an important role, appropriate measures are necessary for this (for example by expandable polystyrene beads that float on the water of septic tanks).

In epidemics the vector can also be controlled by using insecticides such as Bacillus thuringiensis H-14 or organophosphate larvicides (eg. Temephos pellets= Abate®).

Vector control for Culex mosquitoes consists of reducing contact with the vector by use of personal protective measures, such as protective clothing, mosquito repellents and impregnated mosquito nets.

Insecticide can also be sprayed indoors. In the case of large epidemics, outdoor vector control is also important (larvicides and adulticides). Today, several biological control methods can be used to diminish mosquito populations: the sterile insect technique (SIT) is a form of insect birth control where male mosquitoes are sterilized through irradiation. They are then released to mate with wild females that will lay non-viable eggs. RIDL (Release of Insects carrying Dominant Lethals) is a new tool to control *Aedes aegypti*. Genetically engineered mosquitos carry a lethal gene that is inherited by all offspring of RIDL mosquitoes. The lethal gene, which has an on and of switch, is switched on when the insects are released in the environment. The RIDL genes will then kill the larvae and pupae.
Incompatible Insect Technique (IIT) makes us of the Wolbachia gram-negative bacteria that competes with viruses like dengue, zika, chikungunya and yellow fever in Aedes aegypti. Wolbachia-carrying mosquitoes are bread and then released into areas affected by mosquito-borne diseases.

Ixodes ticks are the vector of Tick-borne Encephalitis viruses. The main prevention is vaccination. Vector control measures are not very effective. They include the use of tick repellents in combination with the wearing of appropriate clothing (for example, long trousers) and avoidance of the tick habitat if possible, although a recent study has shown that tick repellents are only moderately effective.

Hyalomma ticks are involved in the transmission of Crimean-Congo Haemorrhagic Fever (CCHF) virus, although sometimes other up to 31 tick species are involved (e.g. Rhipicephalus, Haemaphysalis, Amblyomma and Dermacentor sp). The virus can survive in a tick population because it is transmitted both by the transovarial and the transstadial route.

Geographical Distribution

Geographical distribution of arboviral infections varies and is often related to outbreaks; knowledge of possible exposure is important for recognition of clinical cases and for choosing diagnostic tests. When the distribution of arthropod vectors for pathogens overlaps, the distribution of the arboviruses can be similar (see also Figure). Co-infections (eg. two different serotypes of DENV, two different arboviruses and co-infections of malaria with arboviral infections) do occur. Online resources should be used to obtain up-to-date information concerning ongoing epidemics (eg. www.cdc.gov, www.who.int, http://ecdc.europa.eu/, http://www.promedmail.org/).

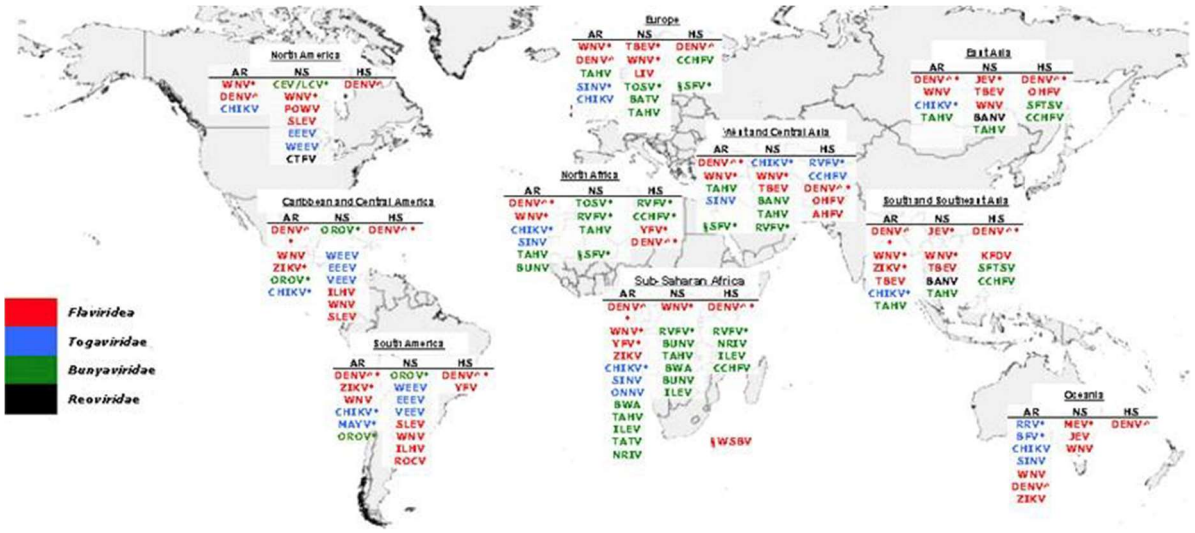


Figure: overlapping distribution of selected arboviruses (Cleton at al., PloS Negl Trop Dis)

Arbovirus include different families of viruses, as presented in this figure with colours. The Flaviviridea family, which is coloured in red, includes DENV, ZIKV and other species. The blue coloured viruses belong to the Togaviridea family, which include CHIKV among other species. The viruses belonging to the Bunyaviridea family are coloured in green and the viruses belonging to the Reoviridea family are coloured in black.

Clinical aspects

The clinical presentation of arbovirus infection varies from asymptomatic to critical illness with organ failure and death. It is not possible to distinguish between arboviral infections clinically, because symptoms are often non-specific. The clinical presentation is also similar to that of many non-arboviral infections.

However, a number of clinical syndromes may be distinguished. These are:

- Fever
- Skin rash
- Arthralgia
- Neurological manifestations
- Haemorrhagic manifestations

Skin rash

A non-pruritic skin rash tends to occur frequently. It can be maculopapular or morbilliform. Skin desquamation is uncommon. Skin vesicles can form in Sindbis virus infection.

Arboviral-induced arthritis

Arthralgia is a frequent finding in mosquito-borne arboviral disease, but some of them play a more prominent role than others. The six main mosquito-borne viruses associated with arthritis in humans belong to the Family of Togoviridae, genus Alphavirus. They are: Chikungunya, Sindbis, O'nyongnyong, Mayaro, Ross River and Barmah Forest virus.

All these viruses are transmitted via culicine mosquitoes, such as Aedes or Culex spp, except O'nyongnyong virus, which is transmitted via anopheline spp. Incubation is usually 2 to 10 days. The illness begins suddenly. The most common symptoms are fever, arthralgia and rash. Fever is usually low grade in O'nyong-nyong, Sindbis and Ross River virus infections, but high in Mayaro and Chikungunya infections.

Headache, photophobia, retro-orbital pain, myalgia and backache occur frequently. Anorexia, nausea and vomiting are also part of the clinical spectrum. Weakness can persist for several weeks, sometimes even months.

The severity of arthralgia can vary from vague stiffness to excruciating pain. Patients with Alphavirus infections (Chikungunya, Ross River virus) often have swollen tender joints; this does not occur in dengue or West Nile fever. Fingers, wrists, elbows, toes, ankles and knees are the most common affected. In most cases, the symptoms persist for several days and complete recovery follows.However, arthralgias may persist for several months and even for years. This results in prolonged disability. Intermittent attacks of joint pain and swelling can occur.

Incidence of arthralgia after Chikungunya virus infection varies greatly with factors such as genetic susceptibility of populations, cultural perceptions, and quality of study. In some cohorts, over 50% of patients develop chronic arthralgias and clinically detectable joint swelling at 3 years after their acute infection, so called post- Chikungunya rheumatic disorder. A 6-year retrospective study in La Réunion looked at patients referred to a rheumatologist due to rheumatic symptoms lasting more than 4 months following CHIKV infection. Out of 159 cases, they found that 59% met the criteria for de novo chronic inflammatory rheumatism (CIR) like rheumatoid arthritis, spondylarthropathy, and undifferentiated polyarthritis, and 31% had pre-existing rheumatic musculoskeletal disorders. Amongst those with de novo rheumatoid arthritis, 80% developed joint damage within 3–4 years. They found that some patients remained symptomatic for 6–8 years.

In those with persistent symptoms, there is little evidence on effective therapies. Several disease modifying drugs (DMARDs) have been studied with varying success. Chloroquine has some antiviral effect but has not been found to be more effective than other anti-inflammatories like meloxicam in acute and chronic CHIKV arthralgia. Methotrexate has been widely used, particularly in patients who present with a systemic polyarthritis. Up to 75% of patients may have a positive clinical response to this. Sulfasalazine has been shown to have good clinical efficacy, particularly when combined with methotrexate.

There are no vaccines against Togaviridae. Vector control and personal protection are the only effective preventive measures.

O'nyong-nyong virus

Poorly understood epidemiology. It was first isolated in East Africa in 1961. In this period, there was a massive epidemic involving millions of people. The virus is transmitted via anopheline mosquitoes, which is very unusual for an arbovirus.

Mayaro virus

This virus has been reported from Peru, Brazil, Colombia, Bolivia, Trinidad and Surinam. Most infections seem to occur in the forest. Forest-dwelling mosquitoes of the genus Haemagogus are believed to be the principal vector. Rodents or monkeys probably serve as reservoir.

Ross River virus

Human infection has been documented from Australia, New Guinea, the Solomon Islands, Fiji, Samoa and a number of South Pacific Islands. New Zealand seems to be spared. In Australia, infection with this virus is known as epidemic polyarthritis. The first recorded outbreak was described in 1928. A major epidemic occurred in 1979-80 on a number of South Pacific Islands.

The disease occurs in both an endemic and epidemic form. In Australia, the virus seems to be maintained in a wild vertebratemosquito cycle, with Culex annulirostris and Aedes vigilax serving as the principal vectors. In the Pacific the virus can be transmitted via Aedes polynesiensis.

Barmah Forest virus

This virus is so far only found in Australia. Barmah Forest virus was first isolated in 1974 from Culex annulirostris mosquitoes collected in the Barmah Forest of northern Victoria. It has also been isolated from numerous other mosquitoes including the coastal species Ochlerotatus vigilax and O. camptorhynchus, which have a salt marsh habitat, and from the midge Culicoides marksi in the Northern Territory. The virus was found to be pathogenic for man since 1988. Infections with this virus are less common than infections with Ross River virus. Wallabies and kangaroos are thought to form the reservoir.

Sindbis virus

Sindbis is the most widely geographically distributed of the six alphaviruses causing arthritis. It has been recovered from Europe, Africa, Asia, Australia and the Philippines. It has a broad host range. The basic life cycle involves Culex mosquitoes and wild birds. Because the vectors are mainly ornithophilic (“bird-loving”), human infection is uncommon.

Neurological symptoms

Although neurological symptoms may occur with many arboviral infections, the most important causes of neurological symptoms belong to the genus Flavivirus, of the family Flaviviridae. Important Flavivirus species which frequently cause neurological symptoms belong to the Japanese Encephalitis serogroup (Japanese encephalitis virus (JEV), West Nile virus (WNV), St Louis encephalitis virus (SLEV), Murray Valley encephalitis virus (MVEV)) and Tick-borne encephalitis virus (TBEV). Zika virus also has marked neurotropism.

Because of the clinical importance and vast distribution, these viruses are discussed in separate sections.

Laboratory diagnosis of arboviral infections

Laboratory diagnosis is required for confirmation of arboviral infections. As explained below,

information regarding the timeline of infection (date of exposure, date of symptom onset) is required to choose the appropriate diagnostic assays. This is illustrated for dengue virus in Figure 3.

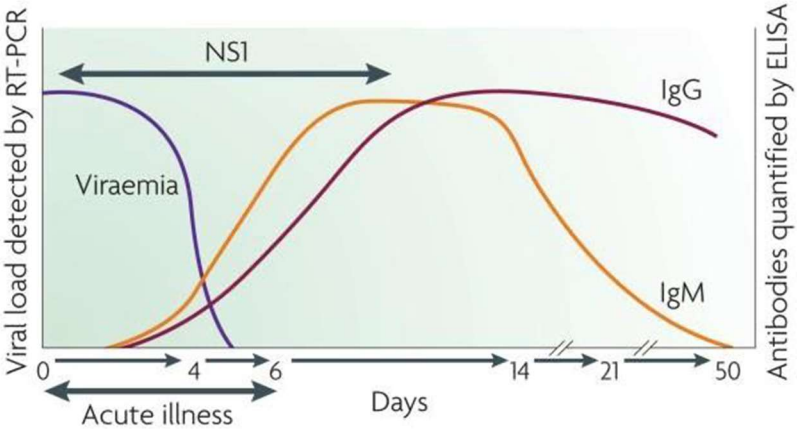


Figure 1: Typical timeline or arboviral infection (Dengue) (Guzman et al, Nat Rev Microbiol)

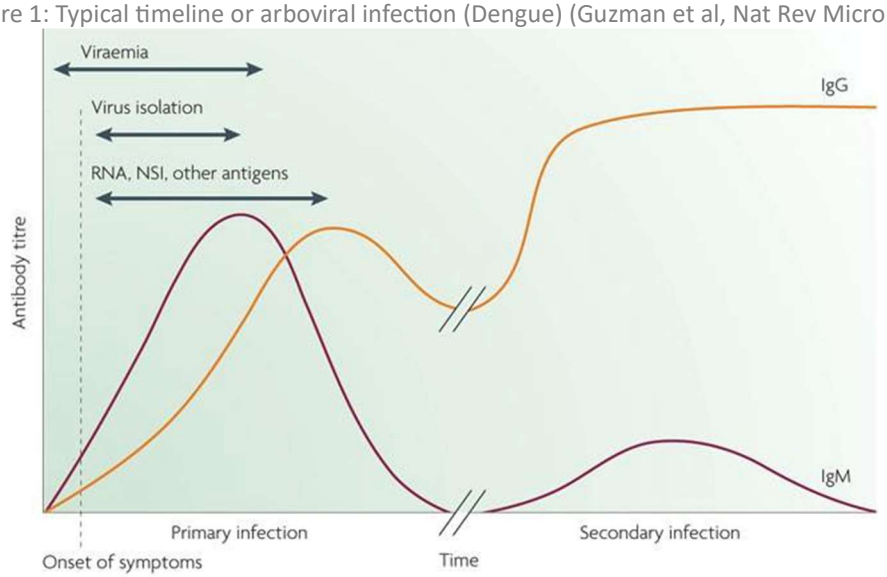


Figure: kinetics of anti-arbovirus antibodies (Dengue) (Peeling et al, Nat Rev Microbiol)

Direct tests

After the incubation period, the arbovirus is viraemic (ie. it circulates in human blood). In the acute phase of infection, the virus can be detected in serum or whole blood by molecular detection assays that target virus-specific sequences, such as real-time Reverse Transcriptase Polymerase Chain Reaction (RT-PCR). The viraemic phase is usually short-lasted (up to 7 days after symptom onset, depending on the arbovirus). RT-PCR may be used for detection of the arbovirus in other body fluids, thus possibly extending the diagnostic window. In addition, antigen detection tests have been developed. In the case of dengue virus infection, a rapid test targeting NS1, a glycoprotein that is essential for viral replication and viability has been introduced; this test can be used within 7 days after onset of dengue virus infection. Virus isolation from body fluids or tissues in cell lines is another means of confirming infection, but due to high costs and sophisticated technical requirements, its use is restricted to research settings.

Indirect tests

Antibody detection assays such as Enzyme Linked Immuno Sorbent Assay (ELISA) or Immune

Fluorescence Assays (IFA) are available for detection of arbovirus-specific antibodies. Only after developing a humoral immune response to an arbovirus, these tests can be used for detection of that virus. This generally limits their use in the acute phase of arboviral illness. Apart from limited sensitivity in the early course of the disease, serological assays that detect immunoglobulins present challenges to interpretation; specificity is frequently affected by cross-reactivity (particularly with other flavivirus infections or previous flavivirus vaccinations). It may also be difficult to discriminate subsequent infections because of persistence of IgG-class antibodies (see Figure 4).

A single indirect test can rarely confirm the diagnosis. To confirm a case by antibody detection assays, demonstration of seroconversion is required. Both seroconversion from negative to positive IgM antibody detection as well as a demonstration of a fourfold or greater increase in IgG antibody titers in paired sample analysis can be used to this end. Consecutive samples should ideally be taken at least 14 days apart.

To confirm the specificity of an antibody reaction to an arbovirus, Virus Neutralization Tests (VNT) can be used. Neutralization of a virus is defined as the loss of infectivity by binding to virus-specific antibody. Virus and serum are mixed and then inoculated into cell culture. Sera that contain antibodies that neutralise the virus will then prevent infection of the cells in culture. When little or no neutralizing antibody to the virus is present, the virus remains infectious. This can be observed microscopically by demonstrating a CytoPathogenic Effect (CPE) in the cell line, or by detecting higher viral loads using RT-PCR.

Table 1 Advantages and limitations of arboviral diagnostics tests (Peeling et al, Nat Rev Microbiol)

* virus neutralisation test is not included in this comparison

Diagnostic tests	**Advantages**	**Limitations**
Virus isolation and identification	Confirmed infection Specific Identifies serotypes	Requires acute sample Requires expertise and appropriate facilities Does not differentiate between primary and secondary infection Expensive
Viral RNA detection	Confirmed infection Sensitive and specific Identifies serotype and genotype Results within hours	Potential false-positives owing contamination Requires acute sample Doesn't differentiate between primary and secondary infection Expensive
Antigen detection		
	Confirmed infection Easy to perform Less expensive	Not as sensitive as virus isolation or RNA detection

Serological tests		
IgM or IgG seroconversion (paired samples)	Confirmed infection Least expensive Easy to perform Can differentiate between primary and secondary infection	IgM levels can be low in secondary infections Confirmation requires two or more serum samples
IgM detection (single sample)	Identifies probable cases Useful for surveillance, tracking outbreaks and monitoring effectiveness of interventions	IgM levels can be low in secondary infections

Medically important arboviruses

Table2 Medically Important Arboviruses

Family Genus	**Virus**	**Vector**	**Host**	**Transmission cycle**	**Incubation period**	**Clinical syndrome**
Bunyaviridea						
Nariovirus	Crimean-Congo hemorrhagic fever	Tick	Birds, small mammals	R; H2H	1–3 (1–9)	FD, HS, (NS)
Orthobunya virus	Bwamba virus	Mosquito	Unknown	R	1–14	FD, AR, (NS)
	Bunyamwera virus	Mosquito	? rodents	R	Unknown	FD, AR, (NS)
	Guaroa virus	Mosquito	Unknown	R	Unknown	FD, AR
	Ilesha virus	Mosquito	Unknown	R (U)	Unknown	FD, AR, (NS, HS)
	Ngari virus	Mosquito	Unknown	R	Unknown	FD, AR, HS
	La Cross virus	Mosquito	Small mammals	R	5–15	FD, NS
	Tahyna virus	Mosquito	Rodents, small mammals	U	3–7	FD, AR, (NS), conjunctivitis, pneumonia
	Oropouche virus	Midge	Humans, Sloths, ? primates/ birds	R, U	4–8	FD, AR, NS
	Tataguine virus	Mosquito	Unknown	R	Unknown	FD, AR

Family Genus	Virus	Vector	Host	Transmission cycle	Incubation period	Clinical syndrome
Phlebovirus	Toscana virus	Sandfly	Humans, bats	R	2–14	FD, NS, (AR)
	Sandfly fever Naples/Sicilian	Sandfly	Humans, rodents	R	2–14	FD
	Rift valley fever virus	Mosquito	Rodents, bats, cattle	R; H2H	1–7	FD, HS, NS, hepatitis
Flavivirus	Dengue virus	Mosquito	Primates, humans	R, U; H2H	4–7 (3–14)	FD, HS, AR
	Japanese encephalitis virus	Mosquito	Ardeid birds, pigs	R, U	5–14	FD, NS
	West Nile virus	Mosquito	Birds	R, U; H2H	3–5 (2–14)	FD, NS, (AR)
	St. Louis encephalitis virus	Mosquito	Birds	R, U	2–21	FD, NS
	Murray Valley virus	Mosquito	Ardeid birds	R	1–28	FD, NS
	Kyasanur Forest disease virus	Tick	Small mammals, humans	R	3–8	FD, HS, conjunctivitis, pneumonia
	Alkhurma hemorrhagic fever virus	Tick	Small mammals	R	3–12	FD, HS
	Tick-borne encephalitis virus	Tick	Small mammals, birds	R; H2H	7–14	FD, NS, (HS)
	Ilheus virus	Mosquito	Birds	R	Unknown	FD, NS
	Yellow fever virus	Mosquito	Primates, humans	R, U; H2H	3–6	FD, HS, hepatitis
	Zika virus	Mosquito	Primates, humans	R, U; H2H	3-12	FD, AR, NR, conjunctivitis, congenital syndrome
Reoviridea						
Coltivirus	Colorado tick fever virus	Tick	Small mammals	R; H2H	3–5 (0–20)	FD, NS, AR, HS

Family Genus	Virus	Vector	Host	Transmission cycle	Incubation period	Clinical syndrome
Seadronvirus	Banna virus	Mosquito	Unknown	R	Unknown	FD, AR, NS
Togaviridea						
Alphaviruses	Barmah Forest virus	Mosquito	Birds, marsupials	R, U	7–9 (5–2)	FD, AR
	Eastern equine encephalitis virus	Mosquito	Birds, small mammals, marsupials	R	3–10	FD, NS
	Chikungunya virus	Mosquito	Primates, humans	R, U	3–7 (1–12)	FD, AR, HS, NS, Conjunctivitis
	Mayaro virus	Mosquito	Primates	R, U	6–12 (3–12)	FD, AR, HS
	O'Nyong Nyong virus	Mosquito	Primates, humans	R, U	>8	FD, AR
	Ross river virus	Mosquito	Marsupials, mammals	R, U	7–9 (3–21)	FD, AR, HS
	Sindbis virus	Mosquito	Birds	R	1–7	FD, AR
	Western equine encephalitis virus	Mosquito	Birds, small mammals	R	2–10	FD, NS
	Venezuelan equine encephalitis virus	Mosquito	Small mammals	R	<1–5	FD, NS

A. Clinical syndromes: FD = febrile disease; AR = atralgia/ rash; HS = haemorrhagic syndrome; NS = neurological syndrome; () = less frequent.
B. Transmission cycle: R = rural, U = urban; H2H = human transmission reported
(adapted from Cleton[10])

Dengue

Summary
•Up to 350 million infections annually, 500 thousand cases of severe dengue, with an estimated 36,000 deaths
•Vector: mosquitoes, Aedes species
•Flavivirus, 4 main serotypes (DEN 1-4)
•Infection with one serotype produces lifelong immunity against this serotype, but only short-lasting cross-protection to other serotypes.
•Main clinical presentations: fever, artralgia/aash, haemorrhagic syndrome (FD, AR, HS)
•Plasma leakage is the hallmark of severe dengue
•WHO 2009 classification: Dengue and Severe Dengue (D/SD); Warning signs (WS) help clinicians identify cases in need of closer surveillance (dengue with warning signs [D +/- WS]).
•WHO 1997 classification: Dengue fever, dengue hemorrhagic fever and dengue shock syndrome (DF/DHF/DSS).
•No antiviral treatment is available at present, but mortality is greatly reduced by appropriate
supportive treatment
• 2 Dengue vaccines are licensed: CYD-TDV (Dengvaxia®) should only be administrated to seronegative people, TAK-003 (Qdenga®) may be used in seropositive and seronegative individuals. Future efficacy and safety monitoring is warranted

Virus

Dengue viruses belong to the Flaviviridae (yellow viruses),genus Flavivirus. The virus has a positive sense single-stranded RNA genome. It is translated into a large precursor protein, which is then cleaved by host-cell and viral proteases into three structural and seven non structural proteins (See Figure 5).

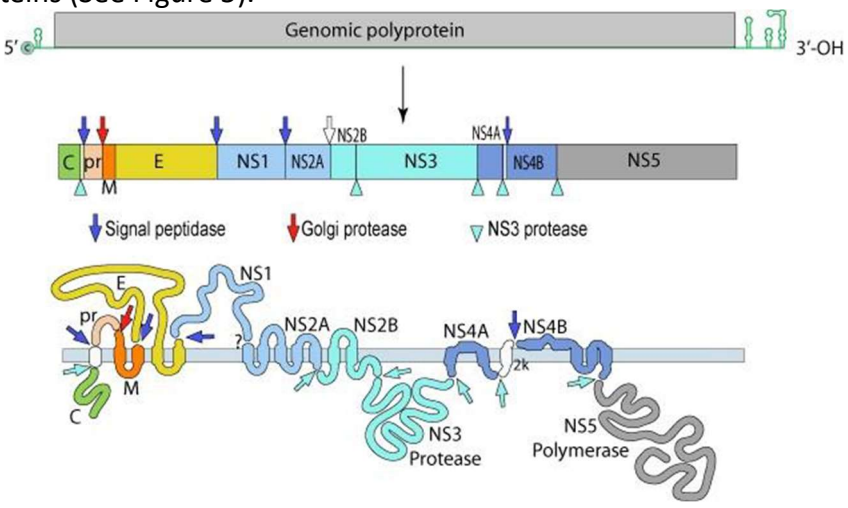


Figure 5: Dengue ssRNA genome and proteins (viralzone.expasy.org)

Dengue virus has 4 main serotypes. Infection with one serotype results in lifelong immunity to subsequent infection with that particular serotype (homologous immunity). There is no lasting crossprotection between the serotypes (heterologous immunity). In 2013, a 5th

serotype (DEN-5) was described, of which the clinical significance is not yet understood. Contrary to DEN 1-4, it has a sylvatic transmission cycle, which may hamper current dengue control efforts.

Epidemiology and transmission

Dengue prevalence increased over 15 fold over the last two decades attributable to three principal drivers: urbanization, globalization and lack of effective mosquito control. Dengue viruses have fully adapted to a human-Aedes aegypti-human transmission cycle in large, crowded urban centres in the tropics. In rapidly developing suburbs, running tap water is often lacking and people depend on fetching water in small reservoirs. Sewage systems are often open and are ideal breeding sites for mosquitoes. Increased mobilization with more car tires, together with a surge in the use of plastics, contributes to mosquito propagation since water containing mosquito larvae is co-transported.
International travel can transport the virus to new regions with little mosquito control. Transported rubber car tires and lucky bamboo plants have been shown to carry Aedes spp. larvae.

Dengue virus infects an estimated 300-530 million cases worldwide annually, of which almost 100 million manifest clinically. The estimated annual death rate of 36.000 deaths due to dengue virus is relatively low, but high numbers of less sick dengue patients can overburden health structures. Dengue occurs in 129 countries and 70 percent of the actual burden is in Asia. As with other arboviruses, the geographic distribution of dengue is determined by the distribution of its vectors (See Figure 6). The main reservoir of the dengue serotypes 1-4 is probably man.

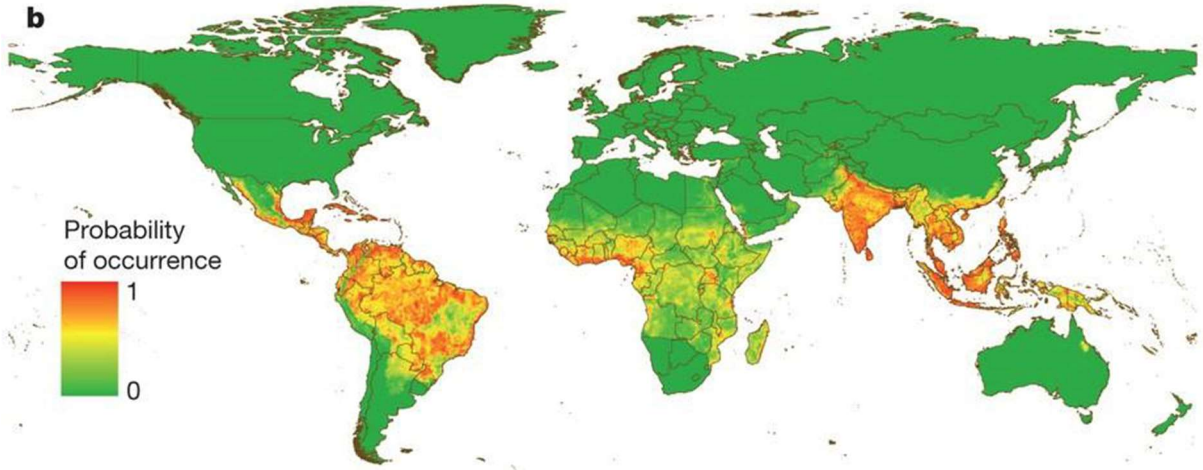


Figure 6: Probability of dengue occurrence (Bhatt et al, Nature)

The bite of infected female Aedes mosquitoes transmits dengue. The virus develops a life-long non-cytocidal infection in the mosquito. It may infect the mosquito ovaries and offspring (transovarial transmission). Aedes eggs can withstand dehydration for several months, and eggs of some Aedes species survive for several years. This cycle can be repeated for multiple generations and drive new outbreaks. It takes at least one week from the egg's hatching to the mosquito's adult stage. This is essential information for understanding the "dry day" principle (see below). Infection of humans occurs when dengue virus is introduced into the skin via the insect's saliva during a bite of female mosquitoes. Aedes albopictus is a less

competent vector for dengue virus but survives in a more temperate climate. Global warming might therefore increase the population at risk for dengue. Dengue transmission follows two patterns that are not mutually exclusive.

Dengue transmission follows two patterns that are not mutually exclusive. “Epidemic” dengue occurs when a single virus strain is introduced into a new region. Adults and children are affected, but dengue hemorrhagic fever is rare. In “hyperendemic” dengue, there is continuous circulation of multiple dengue serotypes. Seasonal variation is common and urban areas are particularly affected. Children are more at risk than adults, with a higher risk of dengue hemorrhagic fever.

Clinical aspects

Three-quarters of the estimated 390 million dengue virus (DENV) infections annually are clinically unapparent. These asymptomatic cases have the potential to contribute significantly more to virus transmission to mosquitoes than previously recognized, as high levels of viremia have been detected in infected people who do not have interruption to their daily routine and who continue to have exposure to the virus' vectors.

Symptomatic dengue infection begins with a sudden onset of a flu-like syndrome. Fever is common and lasts 2-7 days and is frequently biphasic (saddleback fever). Skin rash, headache and arthralgia are frequent symptoms. The rash may have a dengue-specific appearance of “white isles in a red sea” (Figure), but this finding has low sensitivity (up to 20%).

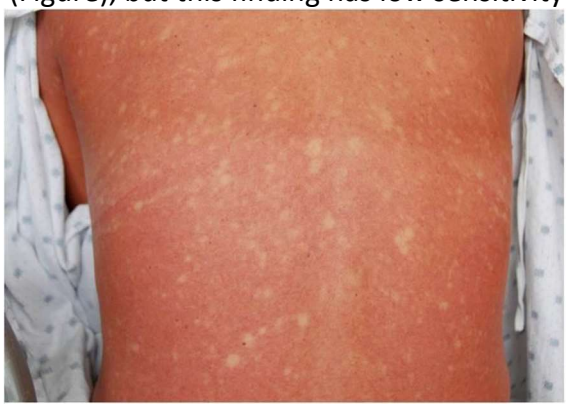


Figure: Dengue skin rash “white isles in a red sea” (Photograph Dr. R. Huits, ITM)

There may be marked muscle pain (breakbone fever), especially in the back and in the extraocular eye muscles (pain around and behind the eyes when looking sideways).

According to the 2009 WHO guidelines for diagnosis, treatment, prevention and control of dengue, a positive tourniquet test (aka. Rumpel-Leede or capillary fragility test) increases the probability of dengue in acute febrile illness. The sphygmomanometer is inflated around the upper arm to midsystolic blood pressure. After the cuff is left in place for 5 minutes, more than 20 petechiae in a 3 cm diameter circle in the crease of the elbow indicate a positive test. Recent literature suggests that the tourniquet test is more effective in detecting true negative than true positive cases and the test should not be used for diagnosing dengue.

Severe dengue

Severe dengue may rapidly be fatal and usually results from a second dengue infection more than 18 months after a resolved first infection. An estimated 500 000 people with severe dengue require hospitalization each year, a large proportion of whom are children.

Complications may develop after 3 to 5 days when the first fever subsides (defervescence), and endothelial dysfunction may lead to hemoconcentration. Patients may develop hemorrhage, ranging from petechiae, ecchymosis and purpura to overt bleeding from mucosal surfaces (epistaxis, melena), injection sites and cerebrovascular accidents. They may develop shock with plasma leakage; pleural or pericardial effusion or ascites can be observed by ultrasonography. Detection of an oedematous gallbladder wall by serial ultrasonography identifies patients at risk for developing severe dengue.

Prediction of severe dengue remains challenging, mainly because the determinants of a complicated course of dengue virus infection are poorly understood. Severe dengue was observed to occur more frequently in secondary dengue infections. In 1977, this led to the development of the concept of 'Antibody-Dependent Enhancement (ADE). Secondary dengue infections were found to be correlated with higher levels of viremia. A molecular model to support the ADE hypothesis was described by Dejnirattisai et al. Briefly: Dengue infection leads to the development of homologous neutralizing and protective antibodies. Upon subsequent infection with a different serotype, these antibodies may enhance the replication of even immature virus particles. This results in higher levels of viremia (replication of both mature and immature virions), thereby increasing the release of pro-inflammatory cytokines and, thus, the severity of the disease.

The prevailing dengue serotype may be a determinant of severe dengue. This should probably also be evaluated against existing population immunity to previous dengue serotypes. In a recent metaanalysis, Soo et al. compared the percentage of severe cases of both primary and secondary infections with different serotypes of dengue virus. They found that the presence of certain serotypes, including primary infection with DENV-3 from the SEA region and secondary infection with DENV-2, DENV-3, and DENV-4 also from the SEA region, as well as DENV-2 and DENV-3 from non-SEA regions, increased the risk of severe dengue infections.
Apart from the fever, rash, arthralgia, hemorrhage and symptoms related to the plasma leakage syndrome, additional manifestations of dengue infection are described:

•Liver failure, which is caused by hypoperfusion or hypoxia rather than direct viral liver damage
•Neurological symptoms such as encephalopathy and seizures

• Cardiac manifestations, including myocarditis, arrhythmias and heart failure
• Secondary hemophagocytic lymphohistiocytosis

There is no specific treatment for dengue or severe dengue, but early detection and access to proper medical care lowers fatality rates below 1%. To facilitate the clinical management of patients with dengue virus infections, a new classification system was introduced by the WHO in 2009.

WHO dengue classification

Recognizing Severe Dengue remains a challenge for the clinician. In 2009, WHO adopted a new classification of symptomatic dengue infections i.e.. dengue with or without warning signs (WS +/-). While the performance of the triage based on the presence of warning signs (WS) need further validation across different clinical settings, this practical classification helps clinicians identify those patients in need of closer surveillance and/or hospitalization. Dengue warning signs include spontaneous or provoked bleeding, severe abdominal pain, persistent vomiting, painful hepatomegaly, dyspnoea, lethargy and effusions (see Figure 8). Severe dengue is defined by the occurrence of plasma leakage and or fluid accumulation leading to shock or respiratory distress, and/or severe bleeding, and/or severe organ impairment.

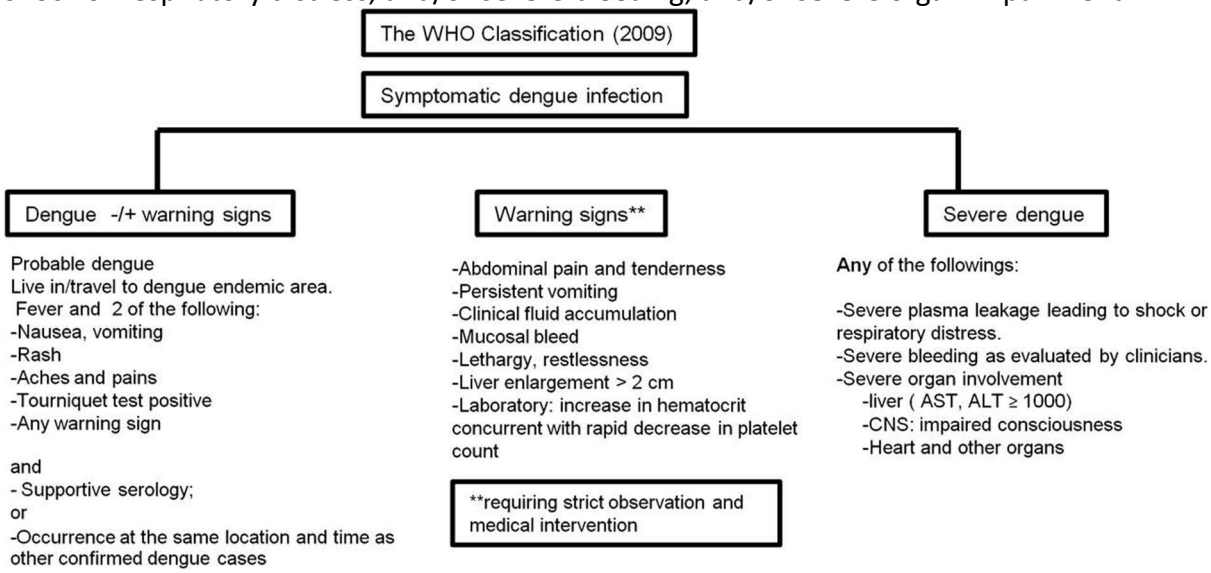


Figure: WHO dengue classification 2009

The former WHO classification (1975, revised in 1997) was derived from a paediatric population. It identified Dengue fever, dengue hemorrhagic fever and dengue shock syndrome (DF/ DHF/ DSS). It was used to classify disease severity for surveillance purposes. The main criticisms are summarized below:

1. poorly related to disease severity
2. misdirecting clinicians identifying severe disease
3. difficult to use (tests required are often not available/difficult to apply)
4. does not help for triage in outbreaks
5. leads to different reporting globally as a result of the difficulties in using the classification for reporting clinicians.

Further comparison of the usefulness of the 1997 and 2009 WHO Dengue Case Classifications can be found in recent publications.

Diagnosis

(see also the section: Laboratory diagnosis of arboviral infections).

Common hematological abnormalities include leukopenia and thrombocytopenia. Both are poor predictors of disease severity. Increased hematocrit (≥20% increase) indicates severe disease since it can point towards plasma leakage syndrome and evolution to shock syndrome.

Biochemical abnormalities correlate with disease severity and organ failure. Increased transaminase levels and hypoproteinemia are observed in severe dengue. Proteinuria, where proteins as large as albumin are lost, occurs and is consistent with disruption in the function of the endothelial glycocalyx layer. Hyperferritinemia in dengue-infected patients is associated with immune activation and coagulation disturbances and may reflect macrophage activation.

Patients with dengue or other febrile illness usually seek medical attention within several days of fever onset. Documenting the day of symptom onset (day 0) is essential to choose a single specimen diagnostic approach. DENV viremia occurs 3–5 days before fever onset and continues for approximately 5 days into the febrile illness. Viremia can be detected by molecular assays targeting DENV RNA (such as RT-PCR) or by immunoassays targeting DENV nonstructural protein 1 (NS1) antigen. An anti-DENV IgM response becomes detectable by IgM-capture immunoassays (Enzyme-Linked Immuno Sorbent Assay (ELISA) or Immune Fluorescence Assays (IFA)) 3–5 days after onset of fever. IgM levels peak 6–10 days after fever onset and may persist for up to 90 days. IgG antibodies can be detected from day 7 onwards and may persist for life. Anti-dengue IgG-antibodies may increase sooner in the event of secondary dengue infection. In view of these kinetics, laboratory diagnostic tests in a patient with suspected dengue infection should consider the day of symptom onset (Figure 9).

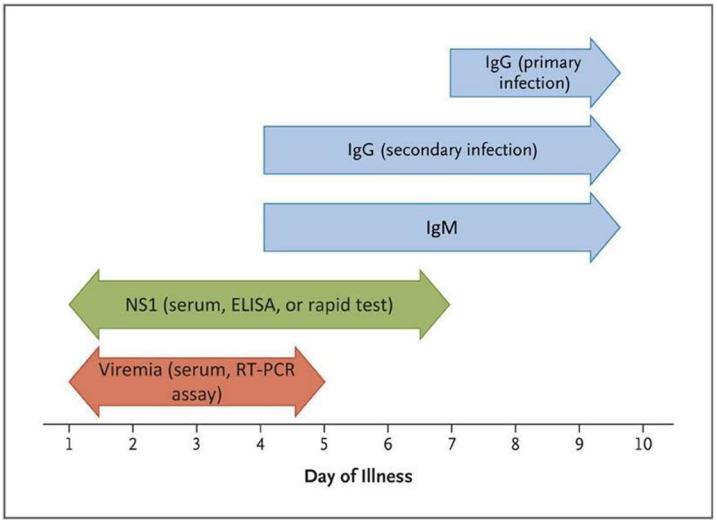


Figure 9: Laboratory Diagnostic Options in a Patient with Suspected Dengue Infection (Simmons et al, NJEM)

Flaviviruses share antigenic epitopes, which elicit cross-reacting antibodies. This cross-reactivity may result in false positive test results. To identify false positive test results or confirm true positives, virus neutralization tests can be performed. Because of the costs and technical expertise required, the use of these tests is mainly restricted to reference laboratories.

Treatment

No antiviral compounds are available for the treatment of dengue virus infections. Corticosteroids are not effective.

Most cases can be treated on an outpatient basis. Symptomatic treatment should avoid aspirin and NSAIDs (risk of bleeding), but paracetamol can be used. The patient or the parents of the sick child should be counseled on dengue complications. In-patient care is required if warning signs appear as these may predict severe dengue to occur on days 4-7 after symptom onset.

In the case of warning signs, isotonic crystalloid fluids such as Ringer's lactate should be used to restore circulating blood volume. Fluid resuscitation requires observation in intensive care units. When the endothelial function recovers, fluid overload may cause iatrogenic complications. In patients with severe dengue infection, adjuvant therapy, including vasopressor and inotropic therapies, renal-replacement therapy and further treatment of organ impairment may be necessary.

Blood transfusion and fresh frozen plasma are sometimes required to treat severe bleeding. In case of massive bleeding, platelet transfusion may be needed in addition to packet cell transfusion. Platelet transfusion is warranted in patients with a platelet count <10.000/µl and active bleeding, but there is no benefit in prophylactic platelet transfusion without active bleeding.

Prevention

Personal protection

Contact with Aedes mosquitoes can be reduced using insect repellents. Sleeping at night under a bed net does not give any protection against Aedes sp. that bite during the day but can be useful for e. g., children sleeping during the day.

Vaccination

Immunity to dengue virus infections is complex, as is the development of dengue vaccines. As discussed under the section 'Severe dengue', dengue infection with one serotype leads to the development of lasting homologous neutralizing and protective antibodies, but it induces only short-term immunity against other (heterologous) serotypes. Because of antibody-dependent enhancement (ADE), infection with a second serotype may lead to more severe illness. Hence there is concern over increasing the risk of severe dengue by vaccination. After infection with 2 different serotypes, broad immunity is observed.

Chimeric Yellow Fever-Dengue–Tetravalent Dengue Vaccine or CYD-TDV (Denvaxia®) is a tetravalent, live attenuated, chimeric vaccine and combines four chimeric yellow fever 17Ddengue vaccine viruses, where the premembrane and envelope proteins from each of the four DENV types replace the same proteins in a yellow fever 17D backbone virus. Three doses at months 0, 6 and 12 are administered. CYD-TDV is now used in about 20 countries in Latin America and Southeast Asia as part of their dengue control program after a study had shown an 80.3% efficacy against hospitalization and a 56,5 – 60,8% efficacy in contracting dengue disease in children. An additional analysis with retrospective determination of serostatus at the time of vaccination showed that children that were seronegative at the time of the first vaccination had a higher risk of developing severe dengue. Vaccination is therefore limited to people living in endemic areas ranging from 9-45 years of age who have had at least 1

documented dengue virus infection previously. This pre-vaccination screening for past dengue disease complicates the rollout of this vaccine in many low-resource settings.

TAK-003 (Qdenga®) is a tetravalent live attenuated DENV-2 virus with chimeras replacing the premembrane- and envelop genes of the DENV-2 with those from wild-type DENV-1, DENV-3 and DENV-4 strains. Two doses at months 0 and 3 are administered. The overall vaccine efficacy in children and adolescents 4 to 16 years of age was 80.9 % and 73,3 % at 12 and 18 months of follow-up, respectively. There was a 90.4 % efficacy against hospitalization for dengue. The vaccine efficacy was slightly higher among the baseline seropositive than baseline seronegative, without increased risk of severe dengue. Since DENV-2 was the backbone of TAK-003, efficacy was highest against DENV-2. There was no efficacy against DENV-3, and data were insufficient to evaluate efficacy against DENV-4.

Vector control

See general section.

Chikungunya

Summary
•Togavirus family, genus alphavirus
•Vector: mosquito, Aedes species
•Main clinical presentation: arthralgia/ rash, febrile disease (AR, FD), frequently post-Chikungunya rheumatic syndrome

Virus

Chikungunya virus (CHIKV) is a single-strand RNA virus of positive polarity; its genome encodes 4 nonstructural (nsP1-4) and three structural proteins (C, E1, E2). Phylogenetically, there are 3 distinct genotypes: the West African, the Asian and the Eastern-Central-South African genotype.

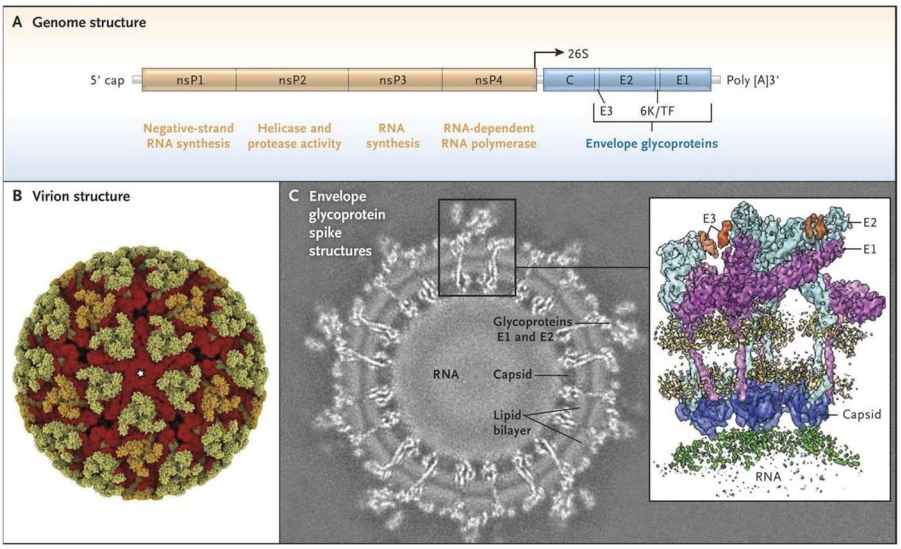


Figure: Structure of Chikungunya virus (Weaver et al, NEJM)

Transmission

Chikungunya virus was isolated during an epidemic in Tanzania in 1952 from both patients and mosquitoes. It has since been isolated frequently from humans and mosquitoes in tropical Africa, India and Southeast Asia, where large epidemics occur from time to time. Non-human reservoir species have not been identified unequivocally. Both Aedes aegypti and A. albopictus are vectors.

In 2004, there was an outbreak of Chikungunya fever in Kenya. The next year it reached the Comores. In 2005-6, outbreaks followed in Reunion (with 265,000 clinical cases out of a population of 770,000), Mauritius, Madagascar and other islands in the Indian Ocean. In Reunion, mortality was 237 deaths, about 1 per 1000 clinical cases. A single mutation (A236V) was identified in chikungunya virus strains in the 2005-2006 Reunion Island outbreak, that facilitated transmission by the Asian tiger mosquito (A. albopictus). CHIKV was capable of spreading via travellers, as was witnessed in July 2007, when about 160 people in Ravenna province, Italy fell ill. This was the first example of Chikungunya transmission via exotic mosquitoes (Aedes albopictus) outside the tropics.

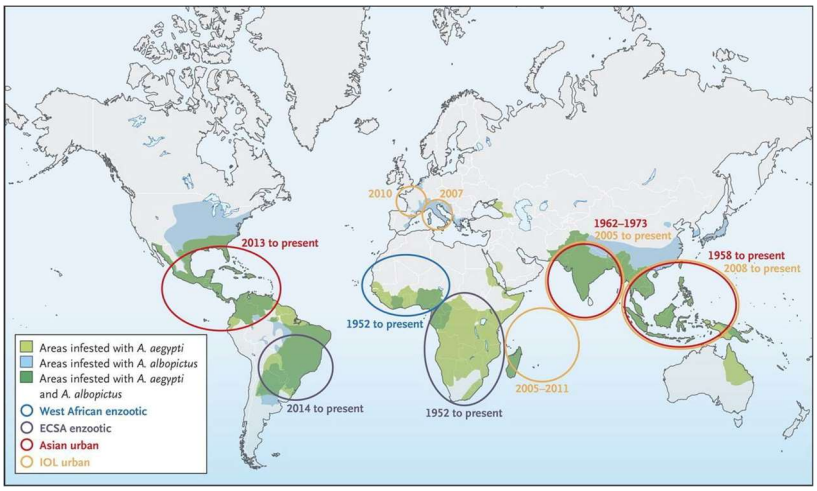


Figure: Distibution of Chikungunya (Weaver et al., NEJM)

Contrary to expectations and reports of introduction of so-called Indian Ocean Lineage of the ECSA genotype by travellers into the Americas, it was an Asian-lineage Chikungunya virus strain that caused a major epidemic in the Americas. The strain was introduced into the island of St. Martin in October 2013.

Clinical aspects

The clinical picture resembles that of classic dengue fever with which chikungunya fever is often confused. After a brief incubation period of 2 to 5 days, there is sudden onset of fever followed by crippling joint pains that may temporarily incapacitate the patient. In the Makonde language, "chikungunya" means "doubled up; that which bends up", referring to this important arthralgia. Conjunctivitis and skin rash are common. Arthralgias occur in around 70 percent of cases and can
persist for weeks to months. If no complications ensue, recovery takes 5 to 7 days. New severe clinical forms were reported in Reunion, including cases caused by peripartum mother-to-infant transmission. Rare complications include meningoencephalitis (also in newborns) and probably hepatic failure (possible role of high doses of acetaminophen). Common hematologic abnormalities in the acute phase include lymphopenia and thrombocytopenia that may lead to bleeding. Hepatic enzymes are commonly increased.

Chronic joint pains can be persistent or relapsing. These arthralgias are located mostly in the distal joints and may be associated with arthritis and may mimic rheumatoid arthritis (chronic inflammatory, erosive, and rarely deforming polyarthritis) in up to 50% of patients.

Diagnosis

Diagnosis in endemic areas is clinical, although it is very difficult to discriminate from co-circulating arboviral infections. A definitive diagnosis relies on virus detection through reverse-transcriptase– polymerase-chain-reaction (RT-PCR) testing during the viraemic phase (the first week). RT-PCR can be designed in a multiplex format to simultaneously detect several other arboviruses, such as dengue virus, which can be very useful for triage of patients. Chikungunya virus culture in a variety of cells permits further virologic characterization but has no added value over RT-PCR in clinical practice and is not performed routinely.

Sero-diagnosis is facilitated by the limited antigenic diversity of chikungunya virus and extensive cross reactivity of the antibodies induced by different strains. Serum IgM is detectable from day 5 (and even earlier) to several months after the onset of illness and is also considered diagnostic. Seroconversion can also be detected as a fourfold increase in IgG between acute-phase and convalescent-phase serum samples.

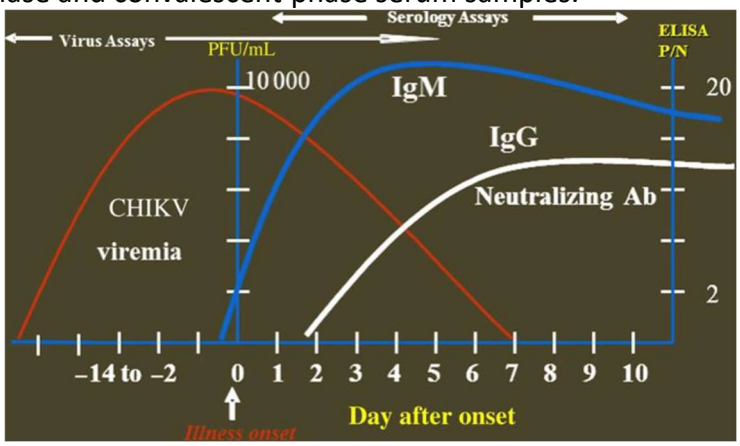


Figure: Chikungunya diagnostics in relation to kinetics of viremia and antibody response (Johnson et al, J Infect Dis)

Treatment

Treatment is symptomatic. Post-chikungunya rheumatism may require long-term treatment with nonsteroidal anti-inflammatory drugs or Disease Modifying Anti Rheumatic Drugs (DMARDs) such as methotrexate, although their safety and efficacy also have yet to be demonstrated in clinical trials.

Zika virus

Summary
• Flavivirus, belongs to Spondweni serogroup
•Vector: mosquito, Aedes species; human to human transmission occurs (sexually)
•Main clinical presentation: Artralgia/ rash, Febrile disease, neurological syndrome (AR, FD, NS), conjunctivitis, congenital syndrome
•WHO declared the Zika virus epidemic in the Americas a Public Health Emergency of International Concern (PHEIC), because of its association with microcephaly and other neurodevelopmental disorders

Virus

Zika virus (ZIKV) is a member of the virus family Flaviviridae, genus Flavivirus. It is a 40-nm virus and has icosahedral symmetry. ZIKV has a non-segmented, single-stranded, positive sense RNA genome.

Transmission

Prior to the 2007 outbreak in the Yap islands (Micronesia), no outbreaks and only 14 cases of human ZIKV disease had been recorded, although sero-surveillance studies in Africa had already indicated anti-ZIKV antibody presence of ca. 6% in some populations. The Yap outbreak indicated that the virus could now spread in human communities and establish a so-called urban transmission cycle. The biggest epidemic occurred in 2015-2017 in the Americas with spread to several countries in Asia. In 2016 the incidence peaked in the Americas and declined substantially throughout 2017 and 2018 probably due to herd immunity. In 2020, a total of 87 countries have had evidence of autochthonous transmission of Zika virus.

The reservoir of ZIKV are primates, both human and non-human. The virus is primarily transmitted by mosquitoes from the genus Aedes, most commonly Aedes aegypti. However sexual transmission of the virus (male to female, male to male, female to male) from symptomatic or asymptomatic persons I now well established. Uncertainty remains over the duration of infectivity of one person.

Table 4 A brief history of Zika virus infections

1947	ZIKV was first detected from rhesus monkey in Uganda.
1952	First human case has been identified in Uganda.
1968	ZIKV has been reported from Nigeria.
1951-1981	Incidences of this virus have been reported from various countries of Asia and Africa.
2007	The first outbreak was reported in Yap Islands, part of the Federated States of Micronesia. Prior to this event, no outbreaks and only 14 cases

	of human Zika virus disease had been documented worldwide. Zika virus infection is estimated to be asymptomatic in 80% of cases.
2012-2014	Cases have been reported from Thailand.
2013	The virus spread to French Polynesia with an estimated 28 000 cases. ZIKV rapidly spreads to the Cook Islands and Easter Island. An association of Zika virus with Guillain Barré Syndrome is observed.
2015	Zika virus infection was first diagnosed in Brazil. It was found to be associated with microcephaly in the infants of mothers with suspected ZIKV infection.
February 2016	WHO declares the Zika virus epidemic in the Americas as a Public Health International Concern (PHEIC) because of its association with microcephaly and other neurodevelopment disorders.
2015-2017	Epidemic in the Americas with 500.000 symptomatic cases reported at the peak of the pandemic in 2016

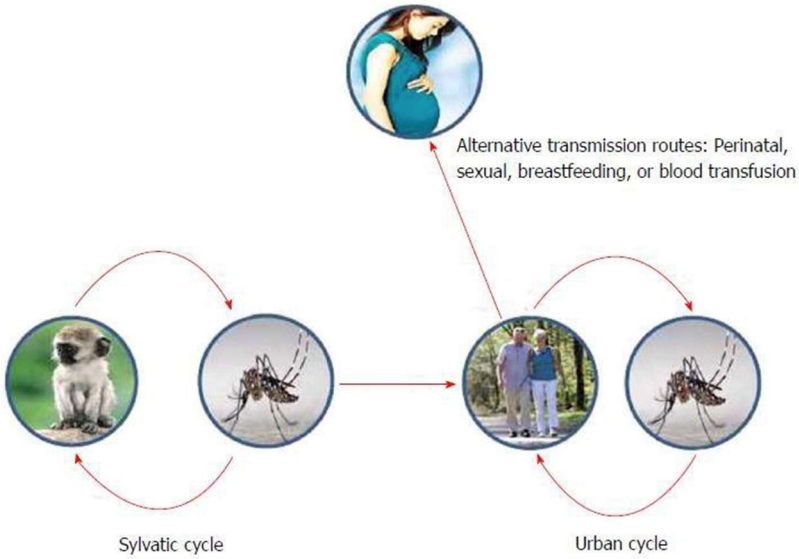


Figure: Important transmission routes of Zika Virus (Blázquez A B et al, World J Virol)

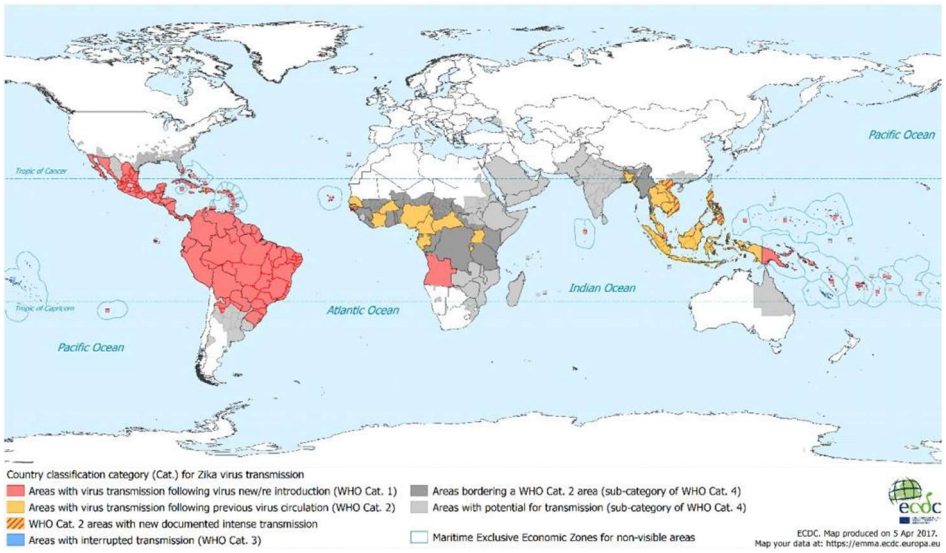


Figure: Global Transmission of Zika virus, ECDC April 2017

Clinical aspects

Symptomatic ZIKV infections

After a mosquito bite, the incubation period is 3-12 days, with a mean of 5.9 days (95% credible interval, CrI: 4.4–7.6), and 95% of people who developed symptoms doing so within 11.2 days (95% CrI: 7.6–18.0) after infection. Approximately 20% of patients are symptomatic. They can present with acute onset of low-grade fever with maculopapular rash, arthralgia or non-purulent conjunctivitis.

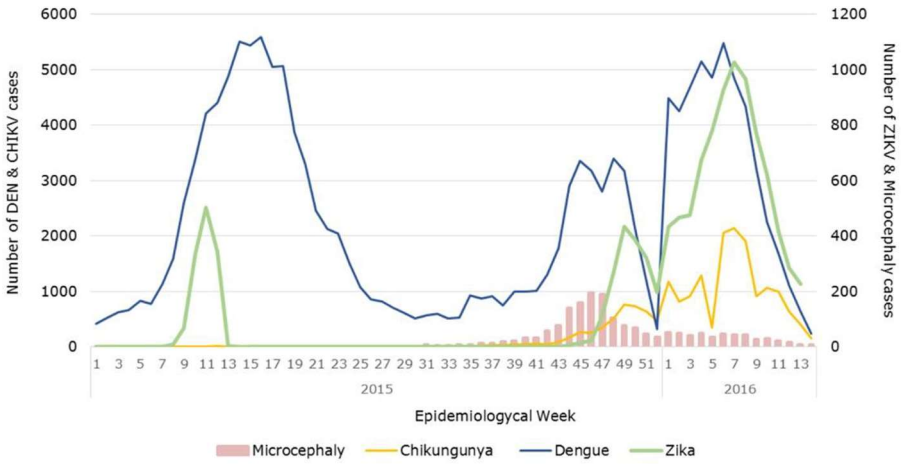
These symptoms feature in the (E)CDC case definition. Other commonly reported clinical manifestations are lymphadenopathy and ulcers on the mucous membrane are less common. Thrombocytopenia, palatal petechiae, and uveitis have been reported. In adults, ZIKV infection generally produces very mild disease. Infants and young children may present with irritability, walking with a limp, difficulty moving an extremity. There may be pain on palpation, or pain with active or passive movement of the affected joint.

Guillain Barré syndrome

Guillain-Barré Syndrome (GBS) is a post-infectious peripheral autoimmune neuropathy, characterized by progressive weakness of the limbs and absent or depressed deep tendon reflexes and cytoalbuminologic dissociation in cerebrospinal fluid (CSF) examination. Several electro-myographic (EMG) types exist. Up to 25% of those affected may require mechanical ventilation. Mortality is estimated at 3-5%. Global incidence of GBS varies from 0.8-1.9/ 100,000. The incidence of ZIKV-associated GBS is estimated to be 2 tot 3 cases per 10,000 ZIKV infections. The median time before onset of neurological symptoms was 6 days.

Neurodevelopmental disorders

The most disconcerting finding is the association of ZIKV infection with neurodevelopmental disorders. Health care personnel and authorities in Brazil observed a sharp increase in the number of neonates born with congenital microcephaly and found an epidemiological association with the ZIKV epidemic which hit Brazil early in 2015.



Source: Data published by the Pernambuco State Secretary of Health, Brazil.

Figure: Temporal association of microcephaly in newborns and Zika virus epidemic in Brazil (www.paho.org)

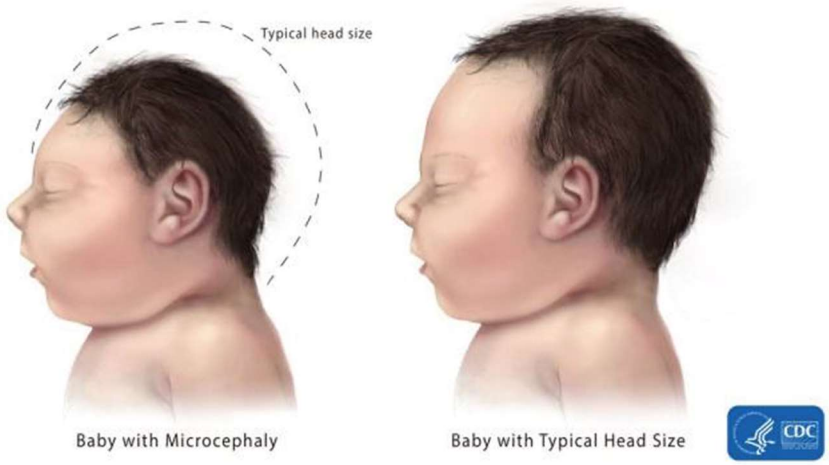


Figure: Microcephaly, from www.cdc.gov/ncbdd/birthdefects/microcephaly.html

Microcephaly is defined as Head Circumference (HC) at birth less than the 3rd percentile for gestational age and sex. Maternal-fetal ZIKV transmission can occur in all trimesters of pregnancy. There is no suggestion that pregnant women are more susceptible to ZIKV infection and there is no evidence of greater severity of this infection during pregnancy.
20-30% Of foetuses and neonates will become infected when mothers are infected during pregnancy. This will lead to foetal loss in 14%, to congenital Zika syndrome in 21% with microcephaly in about half the cases. 80-90% of all foetuses exposed to Zika (not necessarily vertically infected) will be asymptomatic during the first weeks of life. Follow-up is needed to know whether longer term sequalae (learning difficulties, …) in this last group will occur. Not just the brain that is affected in the congenital zika syndrome: infants from ZIKV infected mothers frequently show retinal defects, such as chorioretinal atrophy surrounded by a hyperpigmented halo and hyperpigmented mottling. Hence, the neurodevelopmental disorders observed in neonates and children after ZIKV infection of the mother can be referred to as Zika virus congenital syndrome.

Significance of asymptomatic ZIKV infections and sexual transmission

At present, approximately 80% of ZIKV infected patients are thought to have no clinical

manifestations of infection. In areas where suitable mosquito vectors are present, these patients will add to the reservoir and fuel the epidemic. It is estimated that 1% of ZIKV infections reported in Europe and the United States were acquired through sexual transmission. ZIKV RNA is detected up to 30 days after onset of symptoms, but shedding of infective virus is unlikely to occur after 30 days from the onset of illness.

Diagnosis

Laboratory diagnosis is needed to confirm the diagnosis of ZIKV infection.
Specific laboratory diagnosis is based on detection of viral RNA from clinical specimens by RT-PCR. The window of detection in blood samples is a period of 1–5 days after the start of symptoms. However, the sensitivity of RT- PCR is estimated to be 40 %. Because of the longer persistence of the virus in urine, RT-PCR on urine can be attempted up to the 15th day after the start of symptoms. Seroconversion (detection of anti-ZIKV IgM antibodies) is thought to occur from the 4th day after infection and IgG a little later. Seroconversion occurs on average at 9 days and 95% by 14 days

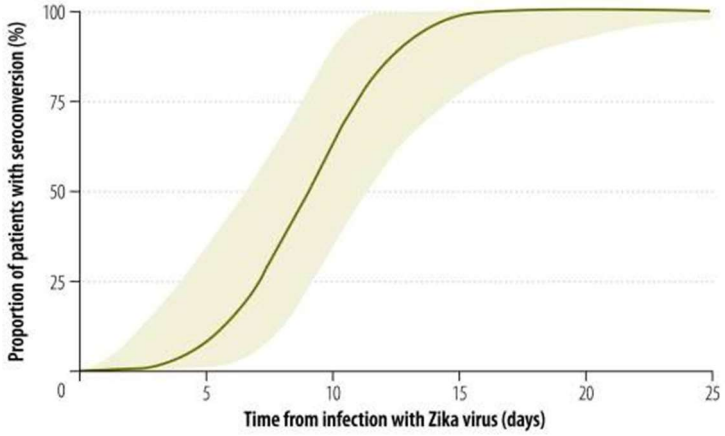


Figure: Time to seroconversion in Zika virus infection (Lessler et al, Bull WHO)

As with other serological tests for flavivirus infections, cross-reactivity of ZIKV antibody detection assays can yield false positive results; in endemic areas this may be a significant problem, because of possible simultaneous or previous circulation of other flaviviruses. Virus neutralization tests can be used to increase specificity.

Treatment

General

There is no specific antiviral treatment for treating ZIKV. Antipyretics or analgesics can be used for symptom relief. Non-Steroidal Anti-Inflammatory Drugs (NSAIDs) should not be used until dengue has been ruled out. NSAIDs should not be used in pregnant women beyond the 32nd week of gestation because of the risk of early closure of the arterial duct.

Management of pregnant women

Current CDC recommendations for the management of pregnant women with ZIKV infection include:
• Use of serial ultrasound examinations.
• In case of a confirmed diagnosis of fetal microcephaly, amniocentesis should be considered from the 15th week of pregnancy onwards.

Management of microcephaly/ ZIKV congenital syndrome

There is no specific treatment for microcephaly. Microcephaly may be accompanied by epilepsy, cerebral palsy, delayed cognitive, motor and speech development and hearing and eyesight problems. Since each child develops complications of different type and severity (eg. respiratory, neurological and motor problems), follow-up by specialists in different fields is warranted.

Guillain Barré Syndrome

Treatment of GBS in the acute phase consists of immunotherapy, such as plasmapheresis or application of human immunoglobulin (IVIG, dose: 400 mg/ kg of body weight per day, for a period of 5 days). IVIG is relatively simple to administer, however expensive and can be difficult to obtain. The bes results of IVIG or plasmapheresis are obtained when it is started within the first 2 weeks after the onset of neurological symptoms. Use of corticosteroids as a stand-alone treatment does not accelerate the recovery or alter the long-term result.

Prevention

A vaccine is not yet available.

Yellow fever

Summary
- Flavivirus, prototype
- Zoonosis
- Endemic and epidemics in Africa, South America.
- Vector: mosquito, Aedes species
- Main clinical presentations: Fever, haemorrhagic syndrome (FD, HS), hepatitis
- Effective vaccine available

Virus

The Yellow Fever virus (YFV) is the prototype virus of the family Flaviviridae, a group that also includes the epidemic arthropod-borne viruses causing dengue, Japanese encephalitis (JE), and Zika. It is an enveloped positive-sense, single-stranded RNA virus. The genome presents a single open reading frame encoding a polyprotein. Host proteases cut this polyprotein into 3 structural (C, prM, E) and 7 nonstructural proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B, NS5).

Transmission

Yellow fever is a zoonosis, caused by infection with Yellow Fever Virus (YFV). Yellow fever causes 200,000 infections and 30,000 deaths every year with nearly 90% of these occurring in Africa. It is endemic to large parts of Africa and South America. Its vectors are mosquitoes belonging to the Aedes genus. YFV maintains a sylvatic cycle mosquito-monkey-mosquito. In monkeys, viraemia lasts 2- 9 days. African monkeys do not die from the infection. Once infected African monkeys develop a lifelong immunity. In South American monkeys, the infection is often fatal. Sometimes large numbers of animals die.

Humans can be infected when they enter this biotope during the day, resulting in sporadic cases of yellow fever (sylvatic or 'jungle' transmission). Upon returning in their communities, infected persons may infect peridomestically living Aedes mosquitoes (notably Aedes aegypti). Subsequent transmission by peridomestic mosquitoes can take on epidemic proportions (epidemic or urban yellow fever).
Large outbreaks occurred Ethiopia (1960-62, 30,000 to 100,000 deaths), Senegal (1965, 2000 to 20,000 cases), Nigeria (1969, 1986 and 1988-1990), Uganda (2010), and Sudan (2003, 2005, 2012–2013) and Ethiopia (2012–2013). In 2016, Angola suffered an outbreak of yellow fever. Authorities have reported at least 3,867 suspected and confirmed cases nationally, including 369 deaths. There are frequently small outbreaks. The southern part and the east coast of Africa are relatively free of the disease. In South America, recent outbreaks affected southern Brazil, Paraguay and Argentina (2007– 2009).
Early 2016, sporadic yellow fever cases were introduced into China from Angola, where a large Chinese workforce is present. Since suitable vector species are also widespread in Asia, the prospect of sustained introduction of viraemic travellers raises the possibility of a yellow fever epidemic in Asia. Urban yellow fever transmission in an unimmunized population is a major public health concern. In order to prevent yellow fever from being imported, many countries where yellow fever does not occur require proof of vaccination following a recent visit to an endemic country.

Clinical features

Yellow fever begins after an incubation period of 3–6 days. It presents as a flu-like syndrome, with fever, chills, headache, backache, muscle aches, fatigue and vomiting. This phase lasts 3-4 days. A second febrile episode develops in 15% of infected persons (biphasic fever), characterised by mild jaundice (yellow or toxic phase). Liver (transaminases up to 15,000-40,000 IU/l) and kidney failure occurs. There is no splenomegaly. The patient's general condition then deteriorates dramatically, with haemorrhaging (skin, mucosa, uterus, intestines), hypotension and shock. Gastric bleeding ("Vomito Negro") is an indication of an extremely poor prognosis. There is considerable kidney involvement (proteinuria, oliguria). There is no real encephalitis but neurological signs such as convulsions can occur due to cerebral bleeding as well as hepatic encephalopathy.

Death occurs mainly between 7-10 days. If the patient survives after 12 days, complete recovery can be expected. Surviving the infection results in lifelong immunity and normally there is no permanent organ damage.

The toxic phase is fatal in 20 - 50 % of cases, resulting in an overall fatality rate for yellow fever of 3.0 to 7.5 %. Case fatality appears lower in Africa (20%) than in South America (40–60%); this suggests that genetic factors determine lethality of the infection.

The differential diagnosis of any case of fulminating hepatitis in an endemic area should include yellow fever, particularly if there is haemorrhaging and kidney involvement. If confirmed, the authorities must be made aware of it and the WHO notified.

Diagnosis

A presumptive diagnosis of yellow fever is often based on the patient's clinical features, places and dates of travel (if the patient is from a non-endemic country or area), activities, and epidemiologic history of the location where the presumed infection occurred. Laboratory diagnosis of YFV faces several challenges, such as a lack of commercial test kits, a lack of biosafety level 3 (BSL3) laboratories for virus isolation and the presence of serological cross-reactivity with other flavivirus infections. Current WHO recommendations for laboratory confirmation of YFV entail testing for specific IgM antibodies and/or a ≥4-fold increase in the specific serum IgG level when other flaviviruses are ruled out.

Antibody detection assays (IgM antibody capture by enzyme-linked immunosorbent assay (MACELISA), hemagglutination inhibition (HI), complement fixation (CF) and virus neutralization tests (VNT)) can be used for the diagnosis of YFV. However, anti-YFV IgM is detectable only from 5 days after the onset of symptoms, when the severity increases. There is cross-reactivity with other flaviviruses.

Yellow fever may be diagnosed on samples obtained during acute illness by the isolation of the virus in mosquito cell lines or by genome detection through PCR-based methods. A negative test result does not rule out infection.

Antigen detection: Antigen detection is only positive in serum during the first 3 days of illness. Monoclonal antibody-based antigen detection by ELISA are being developed, but they are currently not commercially available. Immunohistochemical detection of YFV antigen is performed on tissues in reference laboratories for post-hoc diagnosis.

Treatment

No anti-viral treatment is available for the treatment of YFV infection. Ribavirin reduced mortality and hepatocellular dysfunction in a hamster model, but was not effective in non-human primates. Supportive treatment reduces mortality. This requires hospitalization and close monitoring of vital functions and fluid balance. Hypotension, hypoxaemia and hypoglycaemia must be prevented or corrected. Kidney failure often has a combined aetiology here. Pre-renal failure can be corrected by giving fluid. Renal replacement therapy might be indicated for patients with acute tubular necrosis.

Prevention

There are 3 main strategies for preventing Yellow Fever virus infections.
1. Vaccination
2. Isolation of patients
3. Vector control

Vaccination

There is a very efficient vaccine. This consists of a live attenuated virus (17D strain). It is cultured on embryonated chicken eggs and is stored in freeze-dried form. After adding solvent the reconstituted vaccine is administered subcutaneously. A single vaccination offers lifelong protection in immunocompetent persons from 10 days after the injection. In rare cases post-vaccination encephalitis has been reported in babies (younger than 4 months) and the vaccine is therefore not recommended for children under 9 months of age. Other contra-indications to vaccination are pregnancy (except during a yellow fever outbreak); severe allergies to egg protein; and people wit severe immunodeficiency. Routine vaccination is part of the Extended Programme of Immunisation (EPI) of in number of endemic African countries. In the event of an epidemic vector control and a mass vaccination campaign is essential. The WHO keeps a special stock of yellow fever vaccine available to combat epidemics.
During an big Yellow Fever outbreak in 2016, millions of people were vaccinated and there was a threat for an international stock rupture. WHO authorized the us of fractional dose (one-fifth the usual dose) during the outbreak. A follow-up study showed that 98% of people had developed sufficient antibodies.

Isolation

During outbreaks; patients should be isolated in mosquito-free rooms. Medical staff should take personal protective measures: blood and body fluids of patients are infectious during the first few days. Staff and family members must be vaccinated. Suspected cases should be held in quarantine for the duration of the maximum incubation period, which is 6 days.

Vector control

Vector control efforts should target both peridomestic and sylvatic vectors: improving basic sanitation, improving the water supply and destroying breeding grounds. Sylvatic vectors have to be combated with appropriate agents.

Japanese encephalitis

Summary
•Flavivirus, belongs to JEV serogroup
•Vector: mosquito, Culex species
•Main clinical presentation: Febrile disease, neurological syndrome (FD, NS)
•Vaccine available.

Virus

JEV is the prototype virus of the JE serogroup Flaviviruses, which also includes several medically important etiological agents of encephalitis (see below). Taxonomically, JEV is closely related to other clinically important flaviviruses, including yellow fever virus (YFV), dengue virus, and tick-borne encephalitis virus. Like all flaviviruses, JEV is a small enveloped virus, with a single-stranded positivesense RNA genome. The genome encodes a single long open reading frame (ORF) flanked by 2 short non-coding regions (NCRs) at the 5′ and 3′ ends. The Japanese encephalitis serological group of flaviviruses counts 8 virus species and 2 subtype viruses with an extensive geographic distribution (Figure 11): Japanese encephalitis virus (JEV) in South-east Asia, Papua New Guinea and the Torres Strait of northern Australia. West Nile virus (WNV) in Africa, southern and central Europe, India, the Middle East and North America.
Kunjin virus (a subtype ofWNV) in Australia and Papua New Guinea.
Murray Valley encephalitis virus (MVEV) in Australia, Papua New Guinea and the western Indonesian archipelago
St. Louis encephalitis virus (SLEV) in North and South America.
Other minor members of the group are Usutu (USUV), Koutango and Yaounde viruses in Africa; Cacipacore virus in South America; and Alfuy, a subtype of MVEV, in Australia. Most members have avian vertebrate hosts and are vectored primarily by Culex spp. mosquitoes.

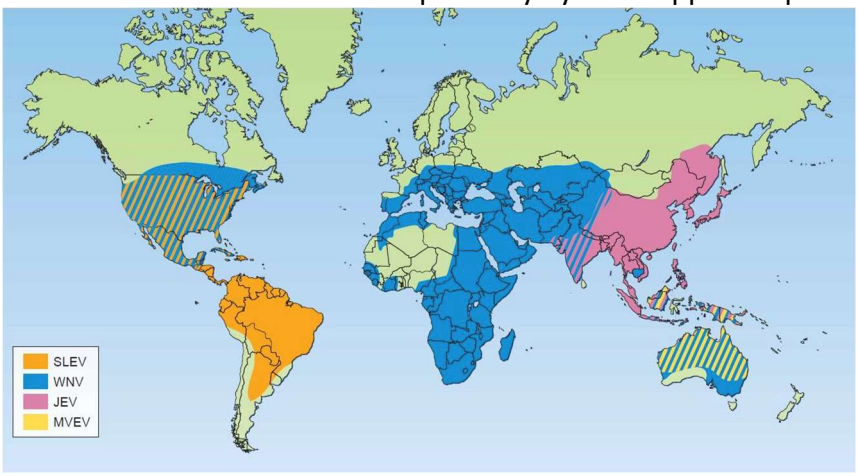


Figure 11 Global distrubition of Japanse Encephalitis serogroup flaviviruses (Mackenzie et al, Nat Med)

Transmission

JEV is the most important cause of viral encephalitis SEA, with 30,000–50,000 cases reported annually, although this may be a considerable underestimate because of inadequate surveillance and reporting. JEV is amplified in an enzootic cycle that involves mosquito vectors (mainly Culex species) and vertebrate hosts (primarily pigs and birds) (Figure 12). JEV is occasionally transmitted to dead-end hosts, such as humans and horses.

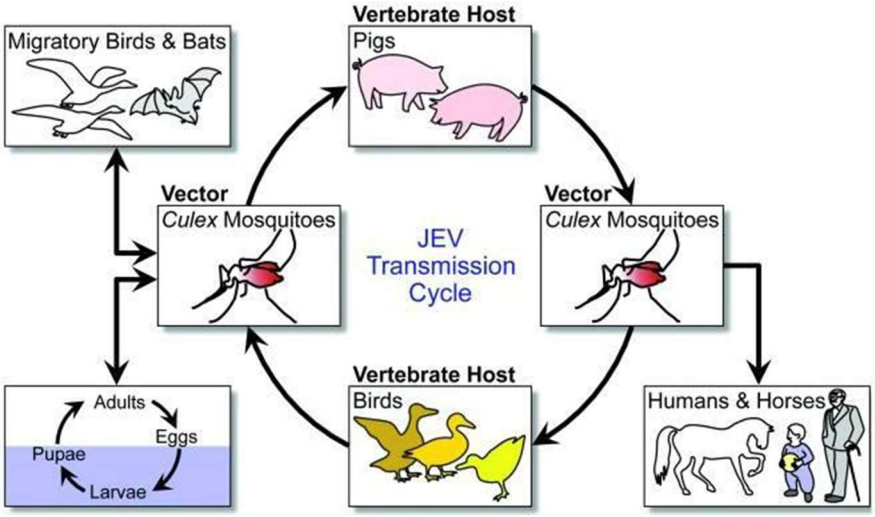


Figure 12: Japanese Encephalitis virus-Transmission cycle (Yn S I et al, Hum Vaccin Immunother)

Clinical features

The incubation period for JEV is 5-15 days. Most infections remain asymptomatic, with estimates of the ratio of symptomatic to asymptomatic infection from 1 in 25 or lower. Sero-surveys in JEV endemic areas have shown that the majority of adults have been exposed to JEV. As with other flaviviruses, the determinants of clinical disease manifestation are ill understood, but are likely to include endemicity, exposure to mosquitoes, pre-existing antibodies to flaviviruses and virus strain differences. Clinical disease often starts with unspecific febrile illness. In neuroinvasive JEV infections, patients usually seek consultation a couple of days after a prodromal syndrome, when meningeal irritation, headache, stupor, coma and convulsions occur. Classical description of Japanese encephalitis includes a Parkinsonian syndrome with a mask-like face, wide unblinking eyes, tremor, generalized hypertonia, cogwheel rigidity and other abnormalities of movement. Along with upper motor neuron signs, cerebellar signs and cranial nerve palsies may occur. Paralysis of the upper extremities is more common than that of the legs. Persistent motor deficits are common (30%), as are severe cognitive and language impairment (20%).

When performing lumbar puncture, CSF opening pressure is increased in about 50% of patients. High pressures (>250 mm) are associated with a poor outcome. Typically, there is a moderate CSF pleocytosis (10–100 cells/mm3), with predominant lymphocytes, mildly increased protein (50–200 mg%) and a normal glucose ratio. However, polymorphonuclear cells may predominate early in the disease, or there may be no CSF pleocytosis.

In about 50% of patients CT shows bilateral non-enhancing low-density areas in one or more of the thalamus, basal ganglia, midbrain, pons and medulla. Magnetic resonance imaging is more sensitive, typically demonstrating more extensive lesions, (typically high signal intensity on T2 weighted images) of the thalamus, cerebral hemispheres, and cerebellum. Thalamic lesions of mixed intensity may also be seen on T1 and T2 weighted scans suggesting haemorrhage.

Encephalitis has a high mortality rate (25-30%). Pregnant women are at risk of intra-uterine infection and death of the foetus during the first two trimesters.

Diagnosis

Anti-JEV immunoglobulin M (IgM) is produced soon after infection and is detectable in 90% of cases in cerebrospinal fluid (CSF) by 4 days and in serum by 7–9 days following the development of clinical illness. Anti-JEV IgM is less cross-reactive and therefore more specific than IgG. WHO recommends JEV-specific IgM antibody capture ELISA (MAC ELISA) as the first-line serological assay to diagnose acute JEV infection. However Serology MAC ELISA underestimates recent infection with Japanese encephalitis virus, in comparison to real time reverse transcriptase PCR50.

The diagnosis can be made by isolating the virus from the cerebrospinal fluid early in the disease or by serology, but it is not a sensitive method of laboratory diagnosis in clinical specimens because the lowlevel transient viremia is cleared soon after onset of illness.

Treatment

As with other flaviviruses, treatment for Japanese encephalitis is supportive. Convulsions and raised intracranial pressure should be treated when they occur. Randomized controlled trials failed to show benefit for the use of corticosteroids, interferon-alfa-2a or ribavirin. Intravenous Immunoglobulins (IVIG) produced in countries where flaviviruses are endemic contains high titres of specific neutralizing antibody, because most of the population have been exposed to the virus. A recent pilot study (2016) cleared the way for a phase III trial of treatment of JEV with IVIG in Nepal.

Prevention

Given its endozootic life cycle JEV cannot be eradicated. In absence of effective antiviral therapy, vaccination is the most important tool to control human JEV infections.

Vaccination

Four different vaccines are available, but all induce only short-term immunity. The vaccine IXIARO® (2 injections, on day 1 & 28) is approved in Europe for people aged 18 years and older. Indications for vaccination in travellers include people who travel at least 3-4 weeks in a rural endemic area or who intend to live in these areas for longer periods even in an urban environment. After a 2-dose primary immunization schedule (0-28 days), the seroprotection rate declines from 8% at 1 month to 48% at 24 months but reconversion is complete with a booster after 1 or 2 years. In older people the vaccine can be given safely, but a 3rd dose may be needed at primary immunization.

Vector control

In addition to vaccination, Japanese Encephalitis can be prevented by vector control measures, see also general section.

West Nile virus

Summary
•Flavivirus, belongs to JEV serogroup
•Vector: mosquito, Culex species
•Main clinical presentation: Febrile disease, neurological syndrome, (Arthralgia/ rash) (FD, NS, (AR))
•WNV neuroinvasive disease is an important clinical syndrome with up to 15% mortality and frequently accompanied by long-term sequelae

Virus

West Nile Virus (WNV) was originally discovered in 1937 in the West Nile district of the Northern Province of Uganda. It belongs to the Flaviviridae. It belongs to the Japanese Encephalitis group flaviviruses (see section on Japanese Encephalitis). Kunjin virus is regarded as a variant of West Nile Fever Virus. There is a considerable variation between different strains (isolates).

Transmission

WNV is transmitted by mosquitoes, mainly Culex species. Culex univittatus and C. pipiens are the main vectors in Africa and the Middle East. WNV has also been known to circulate Southern Europe (Romania, southern France, Spain), Israel, Asia, the Ukraine and Southern Russia. The main reservoir is probably formed by viraemic birds and a zoonotic mosquito-bird-mosquito cycle is assumed. Many bird varieties can be infected and can be viraemic for a long time (amplifying host). Since 1999 WNV has become endemic in the USA and Canada, where it demonstrates seasonality: 90% of infections occur in August and September.

One of the reasons why West Nile virus has spread so rapidly in the United States, is due to a hybrid mosquito species (Culex pipiens s.s. X Culex pipiens molestus), which bites both birds (ornithophilic) and man (anthropophilic). The infection usually takes a subclinical course in birds, but in an outbreak of West Nile Fever-like virus (afterwards confirmed as being West Nile Fever virus) in Queens in New York in the autumn of 1999 hundreds of birds in this city died from the infection (mainly crows, magpies and a few flamingos in the Bronx zoo). Prior to this the virus was unknown in the New World.

The infection is not transmitted directly from man-to-man, but it can be transmitted by blood transfusion, organ transplantation and breast feeding.

Kunjin virus occurs in Australia, Papua New Guinea (including Saibai island in the Torres Strait) and Borneo.

Geographical distribution

Past epidemics occurred in South Africa in 1974 (with more than 3000 clinical cases), the Camargue (France) and the Ebro delta (Spain). From 1999 through 2010, 3 million WNV infections are thought to have occurred in the USA, resulting in 780 000 clinical illnesses. From 1999-2012 the USA have recorded 16,196 patients with WNV neuroinvasive disease and 1549 deaths.

Clinical aspects

Incubation period varies from 3-15 days. Many infected patients experience a subclinical infection or a mild flu-like syndrome. Symptomatic patients present with headache, generalized weakness, morbilliform or maculopapular rash (often at time of defervescence), fever (often low grade, lasting 5 days on average), myalgia. Less commonly reported symptoms are joint pains, chills, painful eyes, vomiting or diarrhoea and lymphadenopathy.

Table: Symptoms experiences by WNV viraemic blood donors in 14 days preceding donation (Zou et al, J Infect Dis)

Symptom	No. (%) of donors with symptom
Headache[a]	125 (75)
Generalized weakness[a]	125 (75)
New rash[a]	97 (58)
Fever[a]	94 (56)
Severe muscle pain[a]	90 (54)
Joint pain[a]	81 (49)
Chills[a]	79 (47)
Painful eyes[a]	67 (40)
Vomiting or diarrhea	45 (27)
Swollen glands	36 (22)
Abdominal pain	31 (19)
New difficulty thinking	29 (17)
Bone pain	27 (16)
Tremor	4 (2)

Neuroinvasive disease occurs in less than 1% of those infected by a mosquito bite and appears more frequent in elderly persons. The risk may approach 1 in 50 among persons aged at least 65 years, a rate 16 times higher than that for persons aged 16 to 24 years. In addition, a history of cancer, diabetes, hypertension, alcohol abuse, or renal disease also increases the risk.

Other host factors associated with an increased risk of neuroinvasive disease are and chemokinereceptor CCR5 deficiency (which diminishes the risk for HIV infection) as well as male sex.

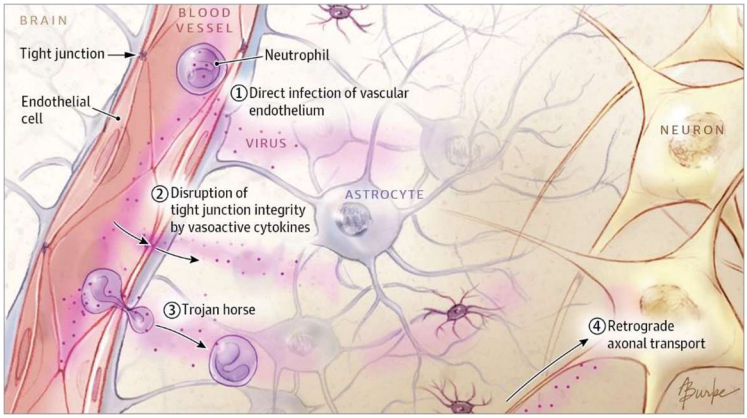


Figure: Potential mechanisms for neuroinvasion of West Nile virus (Petersen et al, JAMA)

Mechanism of neuro invasion

Neuroinvasive disease occurs in less than 1% of those infected by a mosquito bite and appears more frequent in elderly persons. Potential mechanisms for neuroinvasion of West Nile virus include (1) direct infection of the vascular endothelium and subsequent entry to the central nervous system, (2) viral passage through the vascular endothelium due to disruption of the blood-brain barrier integrity by vasoactive cytokines, (3) a Trojan horse mechanism through which infected monocytes are trafficked into the central nervous system, or (4) retrograde axonal transport to the central nervous system following infection of peripheral neurons.

Reported clinical syndromes of WNV neuro-invasive disease are:

• Meningitis, characterized by clinical signs of meningeal inflammation, including nuchal rigidity, Kernig or Brudzinski sign, or photo- or phonophobia.
• Encephalitis characterized by depressed or altered level of consciousness, lethargy or personality change lasting more than 24 hours.
• Acute flaccid paralysis, characterized by acute onset of limb weakness with marked progression over 48 hours, which is usually asymmetric, areflexic or hyporeflexic, and without sensory abnormalities. 80% of acute flaccid paralysis cases occur in conjunction with encephalitis or meningitis.

Examination of CSF of patients with neuroinvasive disease shows normal glucose, elevated protein (generally <150 mg/dL) and moderate pleocytosis (generally <500 cells/µL) usually with a predominance of lymphocytes; however, neutrophils may predominate in early infection.

Imaging studies are usually normal, but focal lesions in the pons, basal ganglia, thalamus and anterior horns, and enhancement of the leptomeninges, the periventricular areas or both are occasionally seen. These lesions may appear hyperintense on T2-weighted magnetic resonance and fluid attenuated inversion recovery images.

The duration of WNV neuroinvasive disease is weeks to months; long-term functional and cognitive difficulties are common in these patients, but the number of quality studies (with adequate control groups) is low. The mortality rate is 0% in the unspecific flu-like syndrome, 2% in meningitis and up to 15% in the case of encephalitis.

Diagnosis

West Nile virus is mostly diagnosed by detection of IgM antibody in serum or cerebrospinal fluid (CSF) by IgM antibody-capture ELISA (MAC-ELISA). Presence of anti-WNV IgM in CSF indicates CNS infection; it is found in 90% of patients with neuro-invasive disease within 8 days of symptom onset. However, anti-WNV IgM may not be detected in serum at clinical presentation. Demonstration of seroconversion in a convalescent sample will provide a definitive diagnosis. Testing for IgG antibodies has no utility in the acute clinical diagnostic setting. Cross-reactivity with other flaviviruses can be distinguished by performing a plaque-reduction neutralization test (PRNT), but the test is only available in reference laboratories.

Nucleic acid amplification testing (eg. RT-PCR) is used in blood donor screening in the United States and Canada has nearly eliminated the risk of West Nile virus transfusion transmission. It also has utility in the diagnosis of WNV in symptomatic patients as an adjunct to MAC-ELISA. In a study of 276 WNV cases, 191 were tested by both serology and NAAT. Of these, 86 (45.0%), 111 (58.1%), and 180 (94.2%) were detected by NAAT, serology, and combined NAAT and serology, respectively. RT-PCR may prove useful to diagnose WNV in immunocompromised patients when antibody development is delayed or absent.

Treatment

No antiviral treatment is available. Intravenous immunoglobulin (IVIG), West Nile virus–specific neutralizing monoclonal antibodies, corticosteroids, ribavirin, interferon α-2b, and antisense oligomers were not effective.

Prevention

Vaccination

In spite of 4 licensed equine vaccines and promising preliminary results from several phase 1 and 2 human vaccine candidates, phase 3 efficacy trials have not been attempted, probably because universal vaccination against WNV disease is unlikely to be cost-effective unless disease incidence increases substantially.

Surveillance

Potentially epidemic conditions due to increased virus transmission can be monitored by regularly testing the blood of birds for the presence of the virus or antibodies. So-called "sentinel birds" are used for this. Crows are very sensitive to infection. Analysis of samples of dead crows is useful in the New World.

Personal protection

The risk can be limited by reducing contact with mosquitoes. When there is an outbreak it is recommended that covering clothing is worn and that mosquito repellents are used. Insecticide can also be sprayed indoors. In the case of large epidemics, outdoor vector control is also important (larvicides and adulticides).

Rift Valley Fever

Summary
• Main clinical features: fever, haemorrhagic disease and neurological symptoms (FD, HS, NS), hepatitis
• Acute disease mainly of African domesticated ruminants, sometimes humans
•Transmission via mosquitoes and direct contact with infected animals
•Intermittent but severe epidemics

Virus

The virus which causes Rift Valley Fever (RVF) is a Phlebovirus and belongs to the Bunyaviridae family. There are several subtypes with each apparently having their own pathogenic capability. Zinga virus is currently regarded as a variant of the RVF virus. It is possibly identical.

Transmission

Between the epidemics it has never been possible to demonstrate a sylvatic vertebrate reservoir, but RVFV has been isolated from over 30 species of mosquitoes in six genera. The virus is passed from generation to generation of mosquito via the transovarial route. Mechanical transmission by arthropods is also documented.

The disease is primarily a zoonosis which affects sheep, goats, cattle and buffalo. Rodents are highly susceptible, although subclinical infections do occur. Birds, reptiles and amphibians are refractory. An epidemic in animals is called an epizootic. In animals the virus causes a severe infection with high mortality, mainly in newborn lambs. Adult pregnant animals often abort. A subclinical infection may occur in dogs, cats and camels (can abort). Horses and pigs are resistant.

The disease was first described in detail by Daubney, in Kenya in 1931 (epidemic in sheep on a farm near Lake Naivasha, one of the lakes in the Rift Valley). Until 1977 it was assumed that the illness only occurred in sub-Saharan Africa and Madagascar, but in 1977-78 there was a great epidemic in Egypt so that the area of distribution was found to be more extensive. Other important epidemics occurred in 1950-51 in South Africa (sheep: an estimated 100,000 dead and 500,000 abortions), in the river basin of the Senegal river in Senegal and southern Mauritania (1987) and in Kenya-Somalia (1997-1998). In 2000 numerous cases were reported from Saudi Arabia and the neighbouring Yemen. More than 200 people died. It was the first time the virus was detected outside Africa.

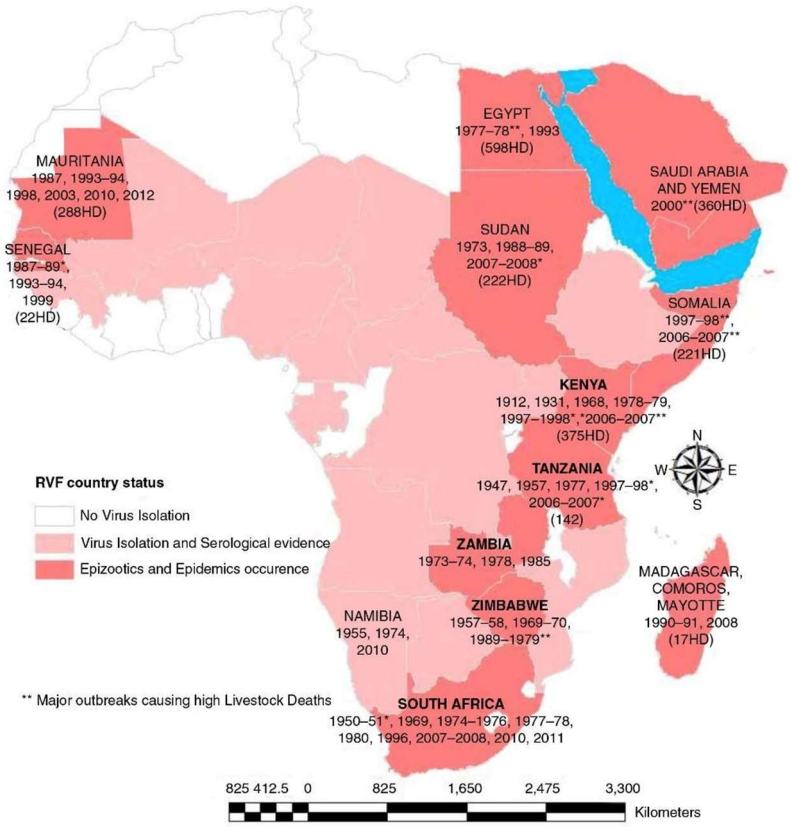


Figure: Rift Valley Fever epidemics (Nanyigi et al,Infect Ecol Epidemiol)

Rift Valley Fever occurs in intermittent epidemics with intervals of 10 to 15 years, mainly after periods of exceptionally heavy rainfall. It has been proposed that factors such as rainfall, ocean temperature and climate change all play roles in determining the likelihood of an epidemic.

Transmission of Rift Valley Fever to man can occur either via direct contact with the blood of a viraemic animal (e.g. in slaughterhouses, farmers, butchers, ranchers, veterinary surgeons, herdsmen, etc.), possibly via the milk of an infected animal or via a bite from an infected insect. There are numerous types of mosquitoes which can transmit the virus. Aedes sp. are usually the most important but Anopheles, Culex, Eratmopodites, Mansonia, Mansonoides and Coquillettidiae mosquitoes also play a role. The virus can be transmitted transovarially in Aedes mcintoshi (= Aedes lineatopennis sl.) and can survive for a long time (years) in a mosquito egg. In heavy rainfall, floods etc. numerous infected mosquito eggs will simultaneously hatch due to the rising water level and moistening of the eggs.

Rift Valley Fever virus can also be transmitted by mechanical vectors such as stomoxyds, phlebotomes, simulids and Culicoides sp. Infected insects can be carried over large distances by the prevailing winds such as the north and south trade winds. Transporting infected cattle to a non-epidemic area is an important factor in the epidemiology.

Clinical aspects

The incubation period of Rift Valley fever is 3 to 7 days. Clinically the disease can provoke a non-specific flu-like syndrome, sometimes with biphasic fever. Fever develops together with muscle and joint pain, anorexia, diarrhoea, vomiting, headache and sometimes photophobia and retro-orbital pain. Sometimes there is petechial rash. The acute phase of the disease lasts

4-7 days. Complications occur in fewer than 5% of cases. In case of a haemorrhagic form, diffuse intravascular coagulation, bleeding (epistaxis, melena, haematemesis, seeping of blood at infusion and needle prick sites) and jaundice predominate such that the disease resembles yellow fever. Pneumonitis, shock, hepatic failure and renal failure with proteinuria and shock can occur. Sometimes bilateral vision disturbances occur about a week after the start of the fever. These are the result of vasculitis of the retina with arteriolar thrombosis, retinitis, retinal ischaemia, bleeding and detachment of the retina. The macular and perimacular areas are affected preferentially. The lesions can result in permanent blindness or slowly improve over the course of the following weeks. Neurological complications also occur (< 1%): meningeal signs, dizziness, confusion, hallucinations, hypersalivation, grinding of teeth, chorea, convulsions and other signs of encephalitis. Coma, with or without decerebration, can occur in the terminal stage. In the complicated forms mortality is high.

Diagnosis

The disease may be suspected if large numbers of young lambs and goats die, with or without epidemic abortion among the animals and when at the same time multiple human cases with fever and haemorrhagic or neurological symptoms occur in an endemic area. In animals there is congestion in the liver, with small haemorrhagic areas and necrotic foci. The bile may be dark, almost black, and may contain blood. Tissue biopsies of animals can be used for anatomopathology, immunoperoxidase techniques for detecting the virus and of course virus isolation. Confirmation of the diagnosis in man is based on serology (IgM antibodies, including in the cerebrospinal fluid) and on virus isolation. Definitive identification is based on neutralisation tests with reference sera. Initially there is leukocytosis, then leukopenia and thrombocytopenia. Schistocytes may be found. With neurological symptoms lymphocytes predominate in the cerebrospinal fluid.

Treatment

There is no specific treatment. Symptomatic therapy is essential and occasionally requires intensive level care. There are insufficient data about the use of ribavirin and/or of convalescent plasma. Ribavirin inhibits virus replication in cell culture. Ribavirin is a ribonucleoside analogue that induces lethal mutagenesis of RNA viral genomes. The possible therapeutic place of interferon is not clear yet. Hepatotoxic medication as well as aspirin and NSAIDs should be avoided during the acute disease.

Prevention

Vaccination

A live attenuated strain (also known as the Smithburn strain) has shown to be potent in inducing protection from viral infection, and it is used as a vaccine for livestock. However, its ability to induce abortions and exhibit pathogenicity in European cattle has limited its use to areas threatened by an imminent outbreak. Studies on new vaccines are ongoing. These candidate vaccines can be classified into four groups: live attenuated, inactivated, viral-recombinant, and DNA vaccines. There is still no commercial vaccine available for humans.

Vector control

The transport of animals should be limited. In epidemics the transporting of cattle should be prohibited, or the animals must be quarantined. Contact with sick or dead animals must be avoided. Cattle can be vaccinated. If the epidemic has already started it is usually too late to employ with vaccination as a control strategy. Thus in sheep-farming areas it is advised that the animals be vaccinated regularly either with the live Smithburn vaccine (single dose, life-long protection), or vaccination with the formol-inactivated vaccine (boosters needed).

Because of the variety of vectors, it is difficult to control insects breeding sites. Sometimes in epidemics insecticides are used on a large scale. For personal protection covering clothing (long sleeves, long trousers), insect repellents (best with DEET) and impregnated mosquito nets are adequate in normal situations. Barrier-nursing is indicated in the care of patients.

Crimean Congo Haemorrhagic fever

Summary
•Main clinical presentation: Febrile disease, haemorrhagic symptoms, neurological syndrome (FD, HS (NS))
•Transmission via ticks (esp. Hyalomma marginatum marginatum) and direct contact with infected animals
•Human to human transmission occurs; CAVE nosocomial infection
•High mortality (up to 40%)

Transmission

Crimean-Congo Haemorrhagic Fever (CCHF) occurs throughout Africa, in Asia, in the former USSR and in Eastern Europe, the Balkans (Kosovo, Albania), the Middle East (including Oman and the United Emirates), Pakistan and the Maghreb, including Egypt. The virus was originally isolated in 1944-45 in the Crimean Peninsula in the north of the Black Sea, during an outbreak in Soviet military personnel. In 1956 it was found in Kinshasa, Congo, first in a patient and shortly afterwards in a scientist who acquired a subsequent laboratory infection. In 1967 it was shown by Chumakov and Casals that both viruses were virtually identical, so now it is referred to as Crimean-Congo Haemorrhagic Fever Virus.

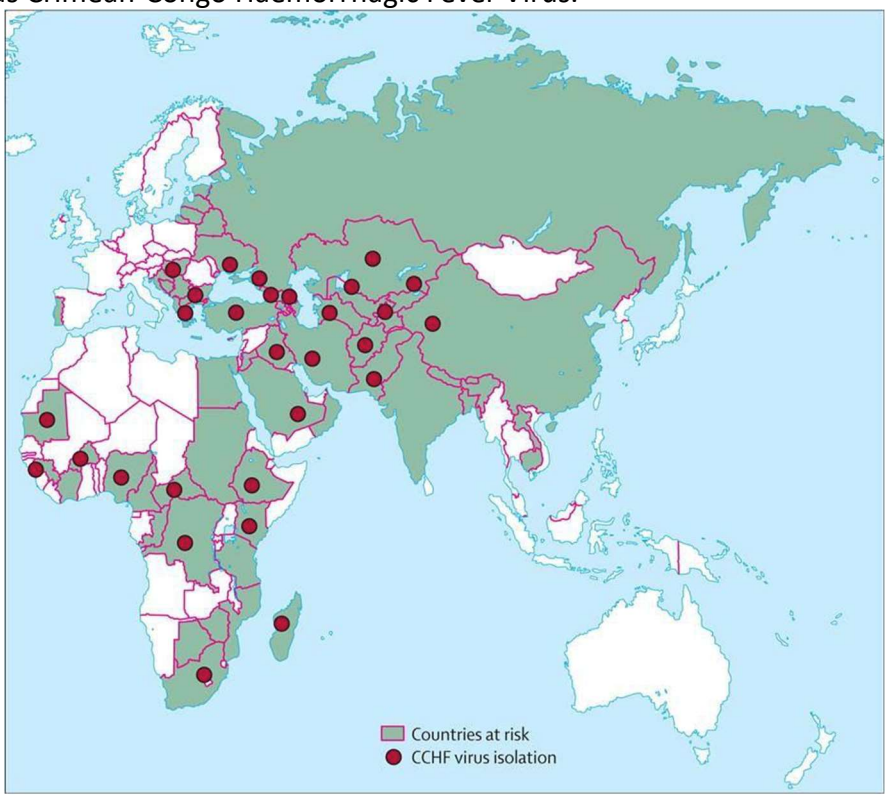


Figure: Distribution of Crimean-Congo Haemorrhagic Fever (Ergönül et al, Lancet Infect Dis)

Man can be infected by the bite of ticks, especially Hyalomma ticks, although sometimes many other tick species are involved. The virus can survive in a tick population because it is transmitted both by the transovarial and the transstadial route. The larvae and nymphs of the ticks become infected when they suck blood from viremic small mammals and birds. Adult ticks infect themselves through the blood of infected wild or domesticated ruminants. Man can be also infected by direct contact with infected animal tissue or blood (goats, cattle, sheep,

hares, ostriches) and during shearing of tick-infested sheep. In sheep and goats, the viraemia lasts a week. When these animals are slaughtered or die from their babesiosis/anaplasmosis, they can still be viraemic. The people who looks after the animal or deals with the carcass can therefore become infected. Herdsmen, farmers, veterinary surgeons and slaughterhouse workers have an increased risk of infection. Human to human transmission is well documented, and nosocomial transmission also occurs. A classic scenario is a patient with bleeding who requires surgery after which the virus spreads to medical staff and/or members of the family.

Virus

CCHF virus that causes Crimean-Congo Haemorrhagic Fever belongs to the family of the Bunyaviridae, genus Nairovirus. Other genera within the family include Orthobunyavirus, Hantavirus, Phlebovirus, and Tospovirus. CCHF is a tripartite, negative-sense, single-stranded RNA genome that comprises Large (L), Medium (M) and Small (S) segments. The three genome segments encode four structural proteins—the RNA-dependent RNA polymerase (L protein) is encoded by the large (L) segment, the glycoproteins (GN and GC) are encoded by the medium (M) segment, and the nucleocapsid protein (N) is encoded by the small (S) segment.

Clinical aspects

After an incubation period of 3 days after a tick bite and up to 6 days after contact with infected animal tissues, the disease starts with a sudden onset of fever. Clinical features commonly show a dramatic progression characterised by haemorrhage and myalgia, headache and vomiting. A discrete exanthema/enanthema can be seen, mainly on the palate. On the 4th day petechiae and extensive ecchymoses appear, followed by severe systemic bleeding including melaena, haematemesis, epistaxis and haematuria. There is no direct effect on the central nervous system, although confusion, lethargy and aggressive behaviour can occur.

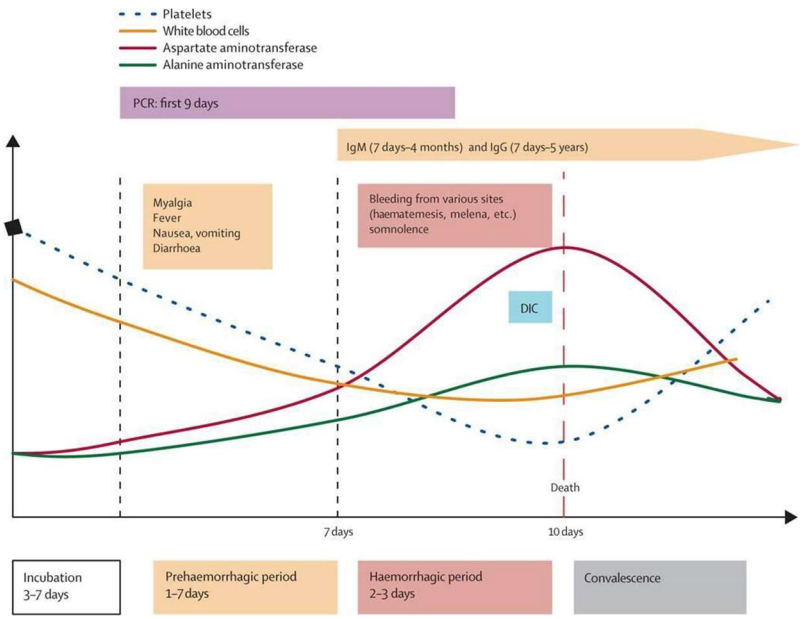


Figure: Clinical course of Crimean-Congo Haemorrhagic Fever (Ergönül et al, Lancet Infect Dis).
DIC: Disseminated Intravascular Coagulation

Haematology results frequently show leukopenia and thrombocytopenia. The levels of liver enzymes creatinine phosphokinase, and lactate dehydrogenase are raised and coagulation markers are prolonged. Infection of the endothelium has a major pathogenic role. Besides direct infection of the endothelium, indirect damage by viral factors or virus-mediated host-derived soluble factors that cause endothelial activations and dysfunction are thought to occur. Mortality is high (15-40%), especially during an epidemic but mild cases and spontaneous recovery also occurs.

Diagnosis

Early diagnosis is critical both for patient management and for the prevention of human to human transmission. The diagnosis is made by demonstrating the presence of the virus in viraemic phase plasma either by culturing or RT-PCR or by detecting a seroconversion. RT-PCR is highly sensitive and specific.

IgM and IgG antibodies are detectable by ELISA and immunofluorescence assays from about 7 days after the onset of disease. Specific IgM declines to undetectable levels by 4 months post-infection, but IgG remains detectable for at least 5 years.

Treatment

General supportive measures and symptomatic therapy. People who are infected should be treated in strict isolation since airborne transmission can occur. Barrier-Nursing should be in place for infection control.

Ribavirin (Virazole®) was used to treat CCHF. There is no evidence from randomised clinical trials for the use of ribavirin to treat human CCHF — its effectiveness has only been described in observational studies. Patients should be treated for 10 days (30 mg/kg as an initial loading dose, then 15 mg/kg every 6 hours for 4 days, and then 7·5 mg/kg every 8 hours for 6 days).

Another study suggested treatment using passive immunotherapy, transferring the plasma of convalescing survivors to infected patients However the study had no control groups and was limited to seven patients, therefore conclusions cannot be made.

Prevention

Vector control

In endemic areas ticks should be eliminated from animals two weeks before they are slaughtered (e.g. with a pyrethroid acaricide). The virus is sensitive to heat and is not resistant to an acid environment. This explains why transmission by eating infected meat is rrare.

Vaccination

There is no commercial vaccine.

Kyasanur Forest disease

Kyasanur forest disease is caused by Kyasanur forest disease virus, a flavivirus. It occurs principally in the Shimoga and Kanara district of Karnataka (formerly Mysore), India. The geographic distribution of this virus is not restricted to Karnataka, e.g. 22 percent of persons living in the Andaman and Nicobar Islands were found to be seropositive for KFD in 2002. Human infection by closely related viruses is known in Saudi Arabia (Alkhurma virus) and China (Nanjianyin virus).

The virus was identified in 1957 when it was isolated from a sick monkey from the Kyasanur forest in Karnataka state. This happened during a fatal epizootic among wild monkeys. The main hosts of this virus are small rodents, but shrews, bats, and monkeys may also carry the virus. Transmission is via the bite of an infected tick, mainly Haemaphysalis spinigera. Apart from tick bite, humans can also get infected by contact with an infected animal, such as a sick or recently dead monkey. Goats, cows, and sheep may become infected with KFD, but they do not have a role in the transmission of the disease. There is no evidence of the disease being transmitted via the unpasteurized milk of any of these animals.

The incubation period is not well known, some state 3-8 days, others 1-2 weeks. The patient develops sudden onset fever, severe headache, followed by back pain, muscle pain in the extremities, inflammation of the eyes, dehydration, gastrointestinal symptoms with or without gastrointestinal bleeding. Hypotension and pancytopenia can ensue. Some patients develop cough due to bronchopneumonia prior to coma and death. Some patients recover without complications after this first phase. However in most patients, the illness is biphasic, and the patient begins experiencing a 2nd wave of symptoms at the beginning of the 3rd week. These symptoms include fever and signs of encephalitis. The diagnosis is made by virus isolation from blood or by serologic testing using ELISA. There are approximately 400-500 symptomatic cases of KFD per year with a case fatality rate of 3-5 percent.

There was an important outbreak in May and June 2003. Forest workers are particularly at risk. There is a safe, effective formalin-inactivated vaccine available for control of Kyasanur Forest disease since 1990. More than 80,000 people were immunized in trials during 1990 to 1992, with no report of adverse effects. The vaccine is prepared from tissue culture and administered at a dose of 1.0 ml subcutaneously (0.5 ml below age 6), with a booster dose after 4 weeks.

Nipah virus

Nipah virus is at present not considered to be an arbovirus but is included in this chapter because of its close resemblance to Japanese Encephalitis.

From September '98 to March '99 a new paramyxovirus appeared in Malaysia. It was given the name Nipah virus and is related to Hendra virus which in 1994 caused fatal infections in horses and people in Australia. Nipah virus causes an encephalitis that clinically is indistinguishable from Japanese Encephalitis. An incubation period of 4-18 days is followed by 3-14 days of fever, headache, vomiting, reduced consciousness, meningism, myoclonus, convulsions, areflexia and hypotonia, tachycardia, abnormal pupils, nystagmus. There is often

a considerable effect on the brain stem, often resulting in an abnormal oculovestibular reflex (abnormal "doll's eye reflex"). Sometimes there is a non-productive cough. In man the mortality rate is high. During the first epidemic more adults than children were affected, mainly those who were working as pig-farmers. Pigs can be infected and develop a cough. In animals infection often results in death, unlike with Japanese Encephalitis. Flying foxes (large bats, including *Pteropus hypomelanus*) are thought to be the reservoir. The virus has been isolated from their urine and saliva.

There are multiple reasons why several zoonotic diseases originate in bats (rabies, Nipah virus, Hendra virus, SARS-CoV, Marburg, ...). About a quarter of all mammal species on the planet are bats. The genetic diversity among the more than 1000 species of bats creates numerous niches for viruses. Bats live from 5 to 50 years, which is much longer than most small mammals. This could be useful for viruses seeking stable reservoirs. Many species roost packed together in large clusters, making it easy for a virus to spread through a colony. Cave-sharing among different species also facilitates infection across species, which in turn increases the chances of viral recombination. Some bats can fly up to 20 km a day, foraging, and some species are migratory. Such animals have the capacity of widely transporting a pathogen over a relatively short period. Some bats seem to be able to carry and shed a virus for a long time without getting sick and without clearing the infection, but more study is required.

Venezuelan Equine Encephalitis

Summary
- New World arboviral infection with mainly neurological symptoms
- Transmission via mosquitoes
- No vaccine available for people

General

The virus only occurs in the New World. It belongs to the Alphaviridae. The virus is normally maintained enzootically in a cycle between small mammals and Culex mosquitoes, mainly those belonging to the subgenus Melanoconium. Rodents form the reservoir. This is an acute viral disease that is transferred from horses to man by various mosquitoes (Aedes and Culex sp.). In a minority of those infected this leads to a serious and sometimes fatal encephalitis. It is the main arbovirus (together with dengue) in (sub)tropical America. The infection occurs in Central America and in a sickle-shaped area in the north of South America. There are regular outbreaks and epidemics, such as in Mexico in '93 and '96. In 1995 there was an outbreak in Colombia with ± 75,000 cases (3,000 with neurological complications). Epidemics in man are always preceded by epidemics in horses. Encephalitis occurs in 90% of infected horses, 50% of which die.

Clinical aspects

Asymptomatic infections are rare in man. Usually there is a flu-like syndrome lasting for 3 days. Fever, myalgia, headache, vomiting and diarrhoea are frequent and for this reason the disease is often assumed to be dengue. In a minority of the symptomatic patients (4% in children) this develops into encephalitis with various neurological symptoms. Confusion, stupor and convulsions can follow. There is leukopenia as well as an increased level of proteins and an increased number of lymphocytes in the cerebrospinal fluid. Sequelae are more frequent in children than in adults. Abortion is frequent in infected pregnant women. Diagnosis is clinical, epidemiological and serological.

Prevention

In the case of an epidemic of VEE, horses should be vaccinated, and the vector should be controlled (insecticides). Most horses are not vaccinated because the vaccine is expensive. The vaccine is not in general use or readily available for man. For other encephalitis viruses such as Eastern Equine Encephalitis, Western Equine Encephalitis, St. Louis encephalitis, La Crosse Encephalitis, California Encephalitis, Jamestown Canyon and Cache Valley (now West Nile as well) surveillance is carried out in North America. This involves, among other things, using birds such as chickens or pheasants because the vectors preferably bite birds. Sera are taken from these sentinels every two weeks and tested for antibodies to VEE. Surveillance can also be carried out by catching mosquitoes. After the catch has been sorted, virus culture or PCR is then carried out on the insect collections. If the virus becomes too frequent, insecticides can be sprayed. If a sudden increase in mosquitoes is anticipated, such as after a severe rainstorm or hurricane, surveillance is increased.

Eastern and Western Equine Encephalitis

Alphaviruses that are related to VEE include the viruses that cause Eastern Equine Encephalitis (EEE) and Western Equine Encephalitis (WEE). EEE is a very serious, but quite rare arbovirosis in the east of the USA, but also occurs sporadically in Central and South America. An incubation period of 7-10 days, fever, meningism, severe encephalitis and a mortality rate that can be as high as 50% characterise the disease. The epidemic potential became evident in 1938. After a severe storm in Boston, Massachusetts there was a major outbreak with a high mortality rate. What was the connection between the storm and the disease? Birds form the reservoir. The virus is transmitted between birds by mosquitoes such as Culiseta melanura. This mosquito lays its eggs in dark underground hollows in an acid soil, such as root hollows in marsh cypresses or red maple trees. It is an unusual habitat for oviposition (egg-laying). The larvae are not in open water and are not easy to find. Such places easily become water-logged after heavy rainfall. In this way huge numbers of mosquitoes can appear simultaneously. Transmission between birds then increases. More than 75 different types of bird can be infected. When mosquitoes that bite both birds and man are infected (such as Aedes vexans, Coquilletidia perturbans), the infection can be transmitted to man. Culex tarsalis is also important in transmission. Horses and donkeys can be infected. In these animals the course of the infection is often dramatic and death among horses can precede an epidemic. Surveillance is carried out with sentinel birds. If there is a threat of an epidemic, insecticides are sprayed, e.g. by ULV (ultra low volume spraying).

In the west of the USA WEE occurs sporadically in man and animals. In other regions of the USA and South America WEE also occurs, but until now apparently only in animals. It is not known whether this is to do with the different antigenic types in North and South America. Most of the infections in adults are pauci- or asymptomatic. After an incubation period of 5-10 days there is a gradual onset of fever, malaise, headache, neck stiffness and dizziness. In serious cases this develops into stupor, coma, flaccid and spastic paralysis. There is pleocytosis in the cerebrospinal fluid as well as an increase in the protein content. Children often have permanent neurological sequelae. The mortality rate among symptomatic patients is about 10%.

Tick-borne encephalitis

Summary
- Flavivirus, 3 subtypes
- Vector: Ticks, Ixodes species
- Main clinical presentation: Febrile disease, neurological syndrome (FD, NS)
- Effective vaccine is available

Virus

Tick-borne encephalitis (TBE) is caused by 3 closely related flaviviruses. These as known at present are European, Siberian, and Far Eastern strains.

Transmission

Tick-borne encephalitis (TBE) is also called Frühsommer Meningo-Enzephalitis (Early Summer Meningo-Encephalitis). This name is a misnomer, since transmission lasts well into autumn (April till October). TBE refers to both Central European encephalitis (CEE, syn. FSME) and Russian spring summer encephalitis (RSSE). TBE is transmitted to humans usually by the bite of a tick (either Ixodes persulcatus or Ixodes ricinus). In contrast with Lyme disease, transmission of the TBE virus occurs immediately after the tick bite, hence tick removal will not prevent the disease. Occasionally, cases occur following consumption of infected unpasteurized milk.

All 3 subtypes co-circulate throughout most of the TBEV endemic areas. However, currently the Siberian subtype dominates in many endemic regions from Eastern Europe to Eastern Siberia. The geographical distribution of TBE is from eastern France, over South Germany, Switzerland, Austria, the previous East Block countries via Russia to northern Japan, and from Scandinavia (Sweden) and the Baltic states to Croatia and northern Italy. In Europe and Asia between 10000 and 15000 TBE cases are reported annually. The number is very likely underestimated because in many countries notification of the disease is not mandatory and only in a subset of the countries TBE case definition is in place. TBE is endemic in 27 European countries, and is a reportable disease in only 16 countries.

Vertical transmission in laboratory animals has been demonstrated to be widespread.

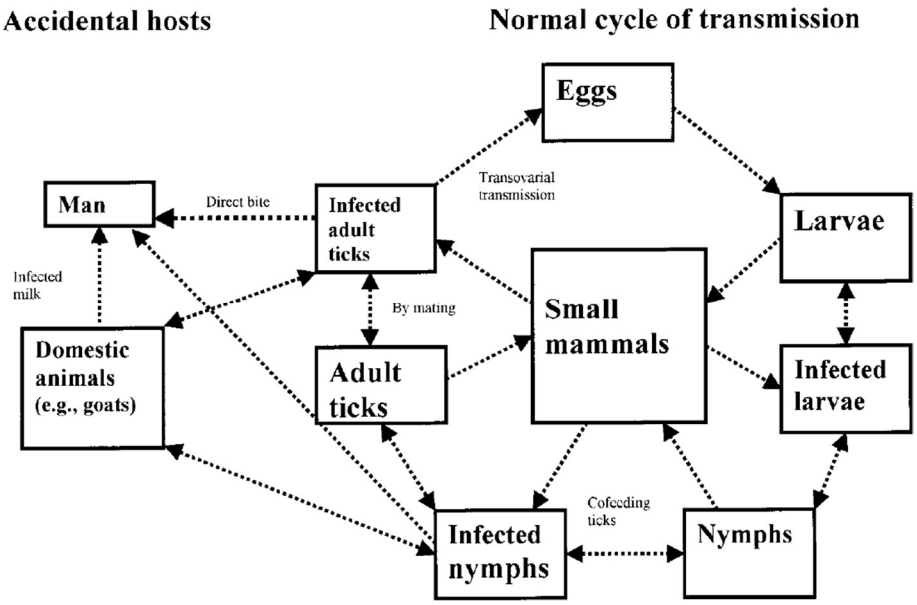


Figure: Transmission cycle of TBE (Dumpis et al, Clin Infect Dis)

Clinical aspects

The incubation period of TBE ranges from 2 to 28 days (7-14 days). After alimentary TBEV transmission the incubation period is generally 3 to 4 days. published data suggest that the ratio of asymptomatic infections is between 70% and 98%. However the proportion of asymptomatic cases is hard to ascertain because patients with mild clinical signs and symptoms may remain undiagnosed.

The initial phase correlates with viremia and like in other neurotropic flaviviruses, it presents with unspecific flulike symptoms (moderate fever, headache, body pain (myalgia and arthralgia), fatigue, general malaise, anorexia, nausea).

This phase lasts for 2 to 7 d and is followed by amelioration or even an asymptomatic interval that usually lasts for about 1 week (1-21 d). Then the second phase appears: in approximately 50% of adult patients it presents as meningitis, in about 40% as meningoencephalitis and in around 10% as meningoencephalomyelitis

The severity of TBE increases with age; in children and adolescents, meningitis is the predominant form of the disease. The long-term prognosis is unfavorable in about 40% to 50% of patients who sustain sequelae (paresis, ataxia, and other gait disturbances) for months to years, and severity of TBE-related sequalae also seems age-related.

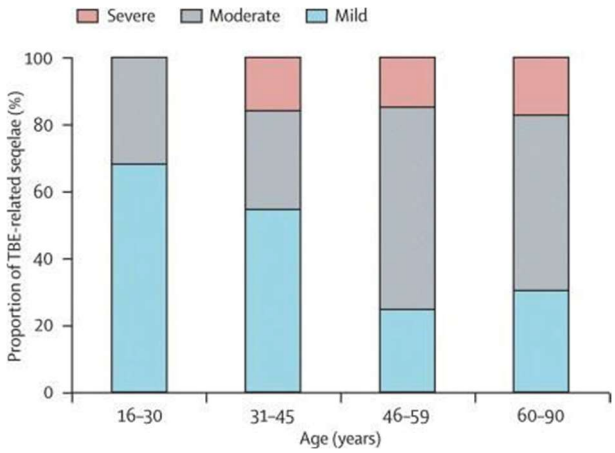


Figure: Relation TBE-related sequelae and age, (Lindquist et al, Lancet; after Mickiene et al, Clin infect Dis)

Classification of sequelae:

1. Mild- without any real impact on quality of life.
2. Moderate- residual symptoms or signs that affected quality of life but that did not require adjustments of daily activities.
3. Severe- symptoms or signs that led to an inability to continue previous activities or that required adjustments of daily activities.

In general the case fatality rate is approximately 1–2 % following European subtype infection but can be as high as 20–40 % following infection with a far-eastern subtype. Infection with the Siberian subtype produces a mortality rate of 2–3 %. However it is possible that the high mortality figures for the fareastern subtype may be due to the lack of detection of mild cases therefore skewing the mortality data.

Diagnosis

As a rule, anti-TBEV- IgM and usually TBEV-IgG antibodies are present in the first serum samples taken when CNS symptoms manifest in the second phase of the disease. In the first phase of illness, the virus can be isolated or detected by RT-PCR from blood, but only rarely is TBEV detected at the beginning of the second phase in CSF and occasionally in cases of progressive disease. Intrathecal IgM and IgG antibody response can be detectable in CSF, but several days later than in serum, and in all cases by day 10.
Enzyme immunoassays are usually used for specific serodiagnosis; these assays could be based on either purified virions or recombinant virus-like particles obtained by expression of prM and E proteins. ELISA for serum and/or CSF IgM antibodies to TBEV has been shown to be the most reliable serological test. Haemagglutination inhibition is also widely used but measures all antibody classes and needs a rise in antibody titre for definitive diagnosis. High cross-reactivity of the antigenic structure in the flavivirus may reduce specificity.

Treatment

There is no specific antiviral treatment for TBE. Patients as a rule need hospitalization and supportive care based on the severity of signs/symptoms, and usually encompasses administration of antipyretics, analgesics, antiemetics, maintenance of water and electrolyte balance and if necessary administration of anticonvulsive agents. In patients with neuromuscular paralysis leading to respiratory failure, intubation and ventilatory support are necessary.

Prevention

Personal protection

Personal protective measures help in prevention of tick bites (repellents like DEET being less effective than against mosquitoes) and protective clothing.

Vaccination

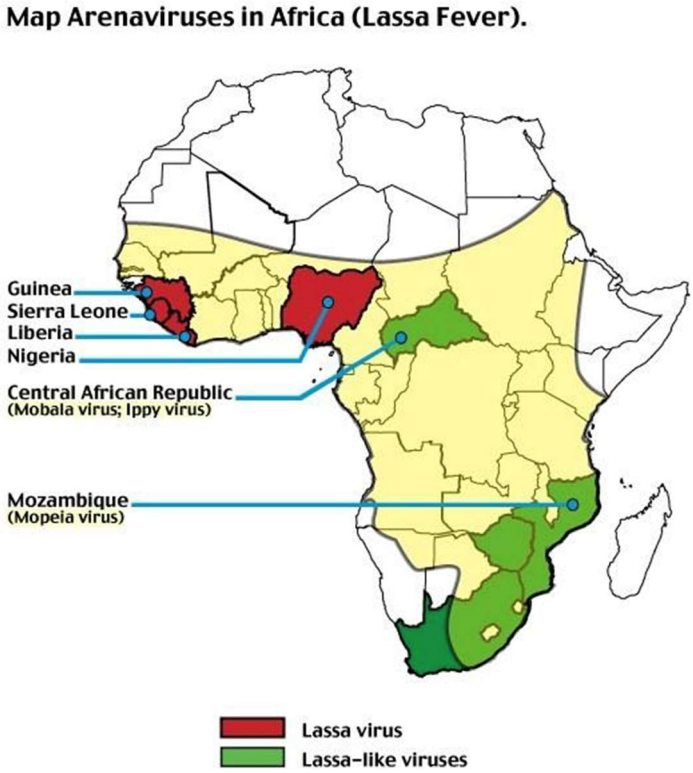
In Europe two vaccines are licensed: FSME immun® (from Baxter) and Encepur® (from Chiron Behring). 14 days after the second dose of basic vaccination protective antibodies develop in about 85% of the subjects, while after three doses more than 98% of persons with normal immunity are protected.

In some countries, such as Austria, vaccination coverage is very high. Other areas where the cost of vaccination is prohibitive lag behind.

Arenaviruses

Summary
•Arenaviruses: Zoonotic viruses transmitted via rodents mainly, but for some also via secondary person-to-person transmission and nosocomial infection
•Clinically atypical febrile, haemorrhagic, neurological or pulmonary syndrome.
•Ribavirin is used in Lassa fever

General



Map arenaviruses – Africa (in yellow: distrIbution of Mastomys mice)

The name of arenaviruses refers to their granular appearance under an electron microscope (L. arena = sand). This structure is created by the inclusion of electron dense host cell ribosomes in the viral envelope. They are RNA viruses, of which the genome consists of a short and a long RNA fragment. Some viruses from this group are pathogenic for humans. Our knowledge concerning these viruses is clearly incomplete. Most arenaviruses have a rodent reservoir. The rodent hosts are chronically infected with the virus, without causing them an obvious illness. Human infection occurs when a person comes into contact with excretions or other materials contaminated with excretions of the infected rodent via ingestion, via direct contact through broken skin/mucosa or via aerosol transmission. Taracibe virus was isolated from fruit-eating bats.

Known pathogenic arenaviruses:

1. Lymphocytic choriomeningitis virus
2. Lassa virus (with substrains Josiah, Nigeria, LP, AV)
3. Junin virus
4. Machupo virus

5. Lujo virus

Non-pathogenic arenaviruses and viruses with unknown pathogenicity:
1. Old World: Mopeia, Mobala, Ippy, Acar
2. New World : Tacaribe, Tamiami, Parana, Amapari, Flexal, Pichende, Latino, Oliveros

Incubation time

Nosocomial transmission and transmission via infected body fluids are known for Lassa fever, Ebola and Marburg virus as well as other non-arboviral haemorrhagic fevers. The Bunya-, Filo- and Flaviviruses are cytolytic. They destroy cells particularly endothelial cells. The incubation time is usually less than one week.

Arenaviruses are not cytolytic. They act indirectly by forming antigen-antibody complexes and activating complement. The incubation time tends to be longer than in the other groups.

New Arenaviruses

It is very likely that new viruses will be discovered in the future. An example is Lujo virus, a new member of the family Arenaviridae. This haemorrhagic fever virus was discovered in 2008, when it was responsible for an outbreak in South Africa (the index patient came from Zambia, 5 cases in total). Human disease is characterized by nosocomial transmission and a very high case fatality rate of 80 percent.

Lymphocytic choriomeningitis virus

The first arenavirus to be isolated was lymphocytic choriomeningitis virus (LCM). It was discovered in 1933 during an epidemic of St Louis encephalitis in the USA. The virus can infect mice. Neonatally infected mice become chronic carriers and excrete the virus for a long time in their urine. The course of the infection is determined by age, immunological resistance, the virus strain and the genetic makeup of the rodent. Both Mus musculus and Mus domesticus (the common house mouse) can be infected. Other rodents, such as hamsters, which are sometimes kept as pets, can also become infected and can be responsible for transmission.

Lymphocytic choriomeningitis virus can also be transmitted via organ transplantation. In humans it is mainly known for causing an "aseptic" meningitis, with or without fever about 10 days before the meningeal signs appear, though infection is more often without symptoms or a mild febrile illness. LCMV infection in immune compromised patients tends to be severe. Sometimes there is severe damage to the central nervous system. Transient hydrocephalus has been described. Chorioretinitis and congenital hydrocephalus may occur in foetal infection. The cerebrospinal fluid exhibits lymphocytic pleocytosis, an elevated protein content and in 25% of patients there is also reduced sugar. Rarely transverse myelitis, ascending myelitis or bulbar paralysis occur. Some cases of residual deafness have been described after LCM infection. At present, a significant fraction of cases of neonatal mental retardation and blindness remain unexplained. Congenital LCMV infection is an understudied potential cause of a portion of these cases.

There is no specific treatment. There is no vaccine. In general, mortality is less than 1%.

Lassa fever

Lassa virus

Lassa virus is an arenavirus. There are some subtypes, such as the Josiah, Nigeria, LP and AV strains. The disease "Lassa fever" takes its name from a small town in Nigeria. The disease occurs, endemically, in West Africa: Sierra Leone, Guinea, Liberia and Nigeria, but probably also outside these countries, based on case reports and serosurveys in humans and animals (Ghana, Ivory Coast, Burkina Faso, Senegal, Mali, Central African Republic). The total number of annual cases is estimated between 100.000 and 300.000 case with 5000 deaths.

Transmission is via ingestion of food infected with urine or faeces of infected peridomestic rats (Mastomys natalensis = Praomys natalensis). The rat itself exhibits no symptoms. There are many morphologically similar rodents, which differ in karyotype.

Transmission via aerosol has been demonstrated in the laboratory. Person-to-person transmission occurs, as does nosocomial transmission, including due to re-use of needles.

Transmission may also occur via sexual intercourse (Lassa virus has been isolated from semen up to 6 weeks after the acute stage).

Isolation and strict barrier nursing are sufficient to prevent transmission in the hospital.

Avoidance of contact with rodents is important (especially of food storage areas where these rodents are common). From time to time there are imported cases in Europe and North America.

Clinical aspects

In about 80% of patients, the disease has a mild course. After an incubation period of 7-18 days, infected persons gradually develop a sore throat with an inflammatory exudative pharyngitis, fever, malaise and myalgia, conjunctivitis and swollen eyelids, abdominal pain with or without nausea, vomiting and diarrhoea, cough, dyspnoea and tachypnoea, thoracic pain, pleural fluid and pain in the joints and loins. Oedema of the face may occur. Patients do not die with a clinical picture of DIC [diffuse intravascular coagulation], but with liver necrosis, haemorrhage, shock and pulmonary oedema. Icterus occurs rarely. Diffuse haemorrhages and swelling of the head and neck indicate increased vascular permeability and a poor prognosis. The cerebrospinal fluid is usually normal. After a few weeks pericarditis and/or cerebellar ataxia occur. There is moderate thrombocytopenia, but there is significant and pronounced blood platelet and endothelium dysfunction. Proteinuria is common. Death results from multiorgan failure in about 20% of those hospitalized. In those surviving there is often sensorineural deafness (25%). ARDS is a frequent cause of death in Lassa fever. Spontaneous abortion is a possible complication in pregnancy.

Diagnosis

Diagnosis is suggested via clinical symptoms in West Africa (thoracic pain, fever, haemorrhage, pharyngitis). In Lassa fever, the white cell count tends to be normal. In severe cases, lymphopenia with neutrophilia as well as haemoconcentration can occur. Mild

thrombocytopenia can be expected. Confirmation will be obtained via serology (ELISA IgM and/or seroconversion IgG), virus isolation or RT-PCR [reverse transcriptase polymerase chain reaction] for viral RNA in a high-containment laboratory (urine, blood, throat swab). IFA (indirect fluorescence assay) can be done on serum using a fluorescence microscope using anti-Lassa monoclonal antibodies. Immunoblotting with gel electrophoresis can detect Lassa proteins using specifically labelled antibodies.

Treatment

Patients should be isolated in an intensive care unit. Ribavirin (Virazole®, Rebetol® - caps. 200 mg), a guanosine analogue, administered during the first 6 days of the disease, is effective (30 mg/kg IV loading dose; then 16 mg/kg IV every 6 hours for 4 days, then 8 mg/kg IV every 8 hours for 6 days). Probably it is also beneficial as chemoprophylaxis (direct contacts PO 500 mg QID for 7 days). In practice ribavirin will often not be available. In the West this drug is used as an aerosol for the treatment of severe pulmonary infection with RSV (respiratory syncytial virus). In China it is used in hantavirus epidemics.

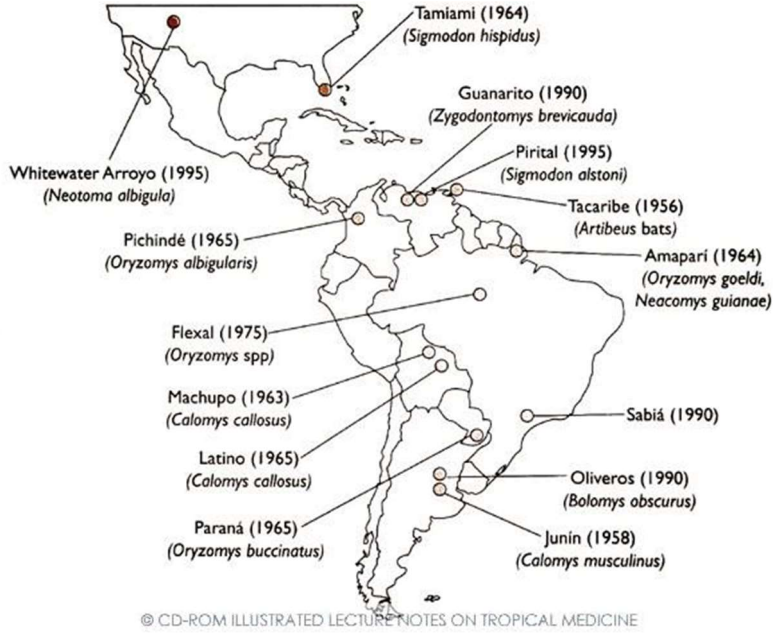
Prevention

Contact with rodents and their excreta (especially urine) should be limited as far as possible. Infected patients should be cared for and treated with the necessary caution (barrier nursing) to avoid nosocomial transmission. In experiments it has been possible to protect primates with a vaccinia virus-expressed Lassa virus vaccine. However, vaccines based upon vaccinia constructs might be dangerous in a population with a high seroprevalence of HIV infection. A recombinant vesicular stomatitis virus-based vaccine protected primates from lethal Lassa virus infection. There is no commercial vaccine for humans available.

New World arenaviruses

General

There are at least 16 arenaviruses in the New World, but most of these are not pathogenic for humans. Junin and Machupo virus occur in South America. The viruses were named after places in Argentina and Bolivia. Guanarito virus causes Venezuelan haemorrhagic fever. Sabia virus causes Brazilian haemorrhagic fever. In North America in 1970 the apathogenic Tamiami virus was found in cotton rats in Florida, but otherwise it was thought that arenaviruses did not occur in North America. In 1996 Whitewater Arroyo virus was identified in the USA. The name refers to a place in the state of New Mexico. It was not known at the time whether this virus was pathogenic or not. In 2000 several people became infected with this virus, with serious consequences. Bear Canyon virus is a third North American arenavirus, the pathogenic capacity of which is to date still unknown.



Map Arenavirusses - New World. Copyright ITM

Transmission

Transmission of Junin and Machupo virus is via rodents (Calomys musculinus and Calomys callosus respectively) which live in the fields (not peridomestic). Female rodents infected neonatally with Junin or Machupo virus are subfertile. Infection is via inhalation of swirling dust containing dried rodent urine (aerogenic transmission). Infection with Junin virus is seasonal and shows a peak during the harvest in autumn. Calomys musculinus has a preference for linear habitats, e.g. hedges and roadsides. Calomys callosus prefers to live in open fields. An outbreak of 1963-64 with 637 cases and 113 deaths was due to a proliferation of the rodents in a Bolivian town. Transmission was stopped by catching or killing the rodents. Many children all over the country gave their pet cats in an emotional gesture to help catch the rodents.

Clinical aspect

Machupo and Junin viruses cause similar clinical pictures. Initially there is a rather slow onset of unspecific malaise and fever, muscle pain, conjunctivitis, nausea, vomiting and sometimes photophobia. Unlike Lassa fever, pharyngitis is not pronounced. Enlarged lymph nodes and

pronounced erythema of the face, neck and thorax are common. Thrombocytopenia, leukopenia and albuminuria are generally present. Chest X-ray is usually normal. Machupo and Guanarito virus infections often cause neurological symptoms. Haemorrhage and shock herald a poor prognosis. Whitewater Arroyo virus causes high fever, liver problems, internal haemorrhage and possibly death. Only a few cases of Sabia virus infection have been documented.

Treatment

Physical protection of doctors and nurses is necessary (barrier nursing). Good results have been described with convalescent plasma from survivors, especially if this is administered early. Ribavirin is active in vitro against all arenaviruses. The penetration of ribavirin into the cerebrospinal fluid is very low. Salicylates and intramuscular injections should be avoided. Thrombocytes should be transfused in case of severe thrombocytopenia. In view of the heightened vascular permeability, caution is advised with IV fluid (risk of pulmonary oedema).

Prevention

Sometimes high-risk persons are given ribavirin preventively for two weeks (1.2 g daily PO).

Hantaviruses

Summary
•Hantaviruses are enzootic viruses transmitted to humans by rodents.
•Symptoms: fever, flu-like syndrome followed by nephropathy, haemorrhage, hyper acute pulmonary syndrome
•A febrile patient with acute respiratory distress due to pulmonary oedema who has a combination of bandemia (left shift), atypical lymphocytosis with possible lymphoblasts, hemoconcentration, thrombocytopenia is highly suspect for infection with Sin Nombre virus (North America) or Andes virus (South America) if he recently visited a transmission area.

General

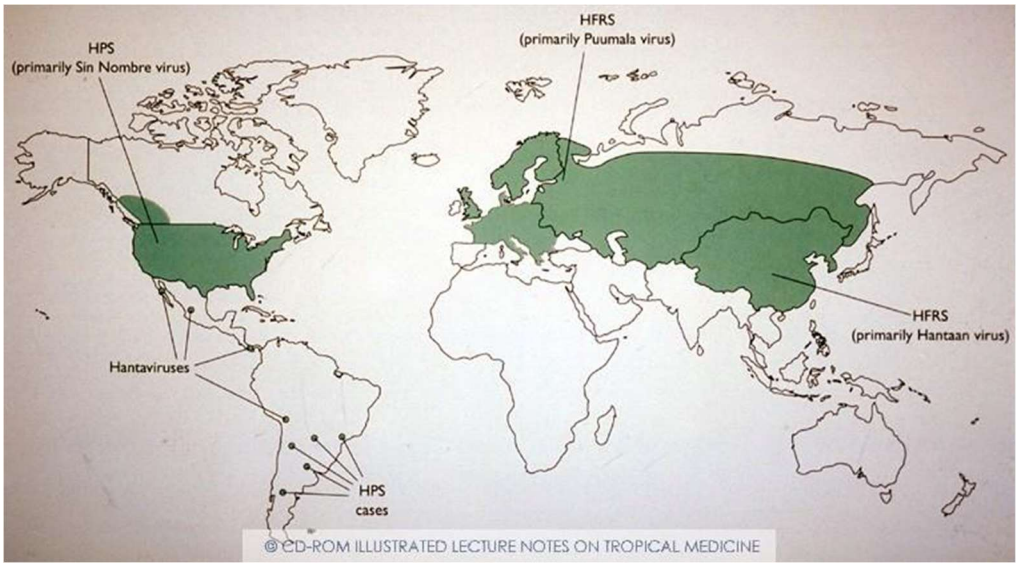
Hantaviruses belong to the Bunyaviridae family. They are spread by rodents and rarely by insectivores.

There are several viruses named for instance, Hantaan, Dobrava, Seoul, Puumala, Andes and Sin Nombre.

Transmission

The viruses are transmitted to man mainly via the inhalation of infected particles and more rarely via ingestion of food contaminated with urine, saliva or faeces of rodents. Once infected, these animals excrete the virus for a long time. In the case of some of the South American viruses, it is thought that occasionally they can be transmitted from human to human. There is a close connection between the specific virus and the rodent species that forms the reservoir.

Geographical distribution



Map Hantaviruses. Copyright ITM

Infections occur worldwide, however each serotype has its own geographical range. During the Korean war, approximately 3000 UN soldiers were infected with Hantaan virus which is

very virulent, producing Korean Hemorrhagic Fever. The virus derives its name from a river in Korea.

In 1993, a previously unknown serotype emerged in the USA. The virus responsible was initially called the Four Corners virus, then Muerto Canyon, and finally the name *Sin Nombre* ('no name' in Spanish) was adopted. Pulmonary Hantavirus syndrome is also seen in South America.

Hantavirus infection also occurs in Belgium (especially Wallonia) and the Netherlands, as so-called epidemic nephropathy. At first the disease was called "muroid virus nephropathy", assuming that rats or mice were involved but this nomenclature has now been abandoned.

A serious form with renal involvement, caused by the Dobrava serotype occurs in the Balkans (in Bosnia, among others).

A mild form, caused by the Puumala serotype, occurs in Scandinavia. A large outbreak of nephropathia epidemica occurred in North Sweden in 2007.

Infections with Seoul virus occur worldwide because the normal host (rat) is distributed worldwide.

Clinical aspects

Depending on the hantavirus serotype and the host, the course of the disease varies from benign to lethal. In humans the incubation period is approximately 1 to 6 weeks. Initially, there is an acute nonspecific flu-like syndrome with fever, headache, asthenia, muscle pain, abdominal pain, sometimes some discomfort in the eyes with blurred vision and red conjunctivae. The benign form (Puumala) has a low mortality rate (0-0.2%) and the serious form a high mortality rate (up to 40% in the case of Sin Nombre).

In Puumala and Dobrova virus infection lumbar pain and oliguria can be expected about 4-10 days after onset. The urine contains protein and blood and interstitial nephritis is present. The creatinine and urea levels increase. In severe forms, kidney failure can be fatal, but if the patient survives, after a polyuric phase, kidney function returns to normal within two to six weeks. In approximately three quarters of cases, thrombocytopenia is present. The leukocyte count is either normal or raised. Haemorrhages can occur.

The Sin Nombre virus often leads to hantavirus pulmonary syndrome with development of tachycardia, hypotension or shock and acute pulmonary oedema with tachypnoea. The fulminant pulmonary oedema is initially non-cardiogenic and is based on a capillary leakage syndrome. Most deaths are caused by myocardial dysfunction (cardiogenic shock) and hypoperfusion rather than hypoxia. This led to the use of the term "hantavirus cardiopulmonary syndrome" (HCPS) rather than the name "hantavirus pulmonary syndrome".

Note. Infection with Junin, Machupo, Sabia and Guanarito virus, which are New World arenaviruses transmitted through rodents, produce similar clinical syndromes with haemorrhagic tendency and sometimes neurological signs: absence of tendon reflexes, tremor, ataxia, confusion, delirium and convulsions can occur.

Diagnosis

The combination of thrombocytopenia, leucocytosis (often with left shift), elevated haematocrit, and presence of immunoblasts in peripheral blood smear is a sensitive and specific early clue to the diagnosis of pulmonary Hantavirus syndrome. These findings in a patient with rapid onset of respiratory insufficiency should suggest the diagnosis.

The diagnosis is confirmed via serology (seroconversion, IgM), RT-PCR and immunohistochemistry. The latter can be carried out on tissue biopsies, which are stored in formalin. Viral RNA can be detected via reverse transcriptase PCR. Due to the extreme sensitivity of this technique, laboratory
contamination is a considerable problem. Virus culture is possible but is rarely performed.

The differential diagnosis of pulmonary Hantavirus syndrome encompasses septic shock, leptospirosis, meningococcal septicaemia, plague, tularaemia, severe influenza, SARS, myocardial infarction and fulminant pneumonia due to other causes.

The diagnosis of the other hantaviral infections will be laboratory based. Patients with acute renal failure and interstitial nephritis with or without haemorrhagic symptoms will be tested.

Treatment

In the acute phase, it is necessary to treat severe cases in an intensive care unit. Ribavirin may have in vitro activity against some viral strains but showed no benefit against Sin Nombre virus in a clinical study. Symptomatic treatment and supportive measures are essential (haemodialysis, treatment of pulmonary oedema, extra-corporeal membrane oxygenation). A great deal of attention goes to proper oxygenation, fluid balance and blood pressure control. Mechanical ventilation, extra oxygen, IV fluid and inotropic drugs should be used when needed.

Isolation of the patient is not needed.

Prevention

There is still no vaccine for most Hantaviruses. Hantavax® is a vaccine which can be used in the Far East against Seoul and Hantaan virus. Booster injections are necessary. This vaccine is not available in Europe.

Contact with rodents and their excretion products must be avoided. Places where there have been rats are best decontaminated with bleach and ventilated (do not brush away the dry dust: airborne particles!). Attracting rodents must be avoided by careful monitoring of potential food sources and hiding places. Rodent control: see further.

Rodents

Medical significance

Most medically significant rodents belong to the Muridae and the Cricetidae. Rodents play a part in many diseases, such as plague, transmitted by the rat flea *Xenopsylla cheopis* and Weil's disease, a severe form of leptospirosis transmitted via infected rat urine. Rodents play a part in conditions such as echinococcosis *(E. multilocularis),* trichinellosis, Lyme borreliosis, recurrent fever *(Borrelia recurrentis),* salmonellosis, rat bite fever, tularemia, lymphocytic choriomeningitis, *Hymenolepis diminuta* and rickettsioses such as RMSF, scrub typhus and murine typhus. Haemorrhagic fevers that are transmitted by rodents ("rodent-borne") include Hantaviruses and Arenaviruses such as Junin, Machupo and Lassa fever. Infection with *Talaromyces marneffei* is essentially a disease of rodents but can occur in AIDS patients in Southeast Asia. In 2003 an imported and infected Gambian giant rat spread monkeypox virus in the USA, a country where there had been no cases until that moment.

Importance in research

Numerous laboratories use mice and rats as experimental animals, to gain knowledge which would otherwise be impossible or very difficult to obtain. Today, working with experimental animals is avoided as much as possible, but alternative in vitro experiments are not always available. Rodent strains have been bred to provide experimental models for, e.g. immune deficiency, increased likelihood of forming tumours or hypertension, etc. These strains are maintained by inbreeding.

Filoviruses

General

These viruses are filamentous in structure and are therefore known as filoviruses. Marburg virus and Ebola virus belong to this group. Infections with some of these viruses have a very high case-fatality ratio (e.g. Zaire ebolavirus), other are seemingly non-pathogenic (e.g. Reston ebolavirsus). Epidemics with human pathogenic filoviruses have become more common in the beginning of the 21st Century and the risk is not negligible that the infections become endemic, at least in Central Africa.

In the last years, several new filoviruses were detected in bat and fish species. Lloviu virus was discovered in 2010 in Schreiber's long fingered bats (Miniopterus schreibersii) found dead in a cave (the dead bat was already found in 2002), the so-called Cueva del Lloviu, Asturias, northern Spain. Later similar discoveries were made in caves in France,Portugal and Hungary. In 2018, Bombali virus sequences were discovers in bats from Sierra Leone, Guinea and Kenya and the virus is considered to be a new ebolavirus species. Měnglà dianlovirus (diān is the Chinese abbreviation for Yunnan) was found in Rousettus bat in Mengla County, Yannan province in China in January 2019.Fish-derived filoviruses constitute members of two new genera: striavirus and thamnovirus. At present it is uncertain if these new virusses are pathogenic for the concerned animal species. These new filoviruses have not been cultured yet, only their RNA genome has been sequenced. No human infections or human disease have been detected (yet) and since no isolates are available, their zoonotic or pathogenic potential cannot be tested.

Marburg virus

In 1967 there was an epidemic of Marburg virus infection among laboratory staff in Marburg, Germany. These people worked with African green monkeys (Cercopithecus aethiops), imported from Uganda. Some people in Frankfurt and Belgrade, Yugoslavia, who encountered the same batch of animals also fell ill. In all, 32 people were affected: 26 primary infections and 6 secondary infections. The mortality rate of the primary infections was 25%. In the next few years a few sporadic cases were seen in Zimbabwe ('75), Kenya ('80 and '87) and a laboratory infection in Russia ('87). In 1999 and 2000 multiple cases were diagnosed in the north east of Congo, in the area of Watsa and Durba. Infection occurred mainly in gold miners, working in very primitive conditions in old mines. There had probably already been a low level of transmission in this area for some considerable time (maybe even years). Social unrest and armed conflicts in the area hindered local research. The end of the epidemic coincided with the flooding of the mine.

Early 2005 there was a large epidemic in Uige, Northern Angola, with 374 cases (initial case fatality rate 92%). It was the largest Marburg epidemic to date (the initial estimate was above 400 cases). Two viral subtypes are responisble for all described outbreaks: MARV (Marburg virus), RAVV (Ravn virus)wich both diverg from the prototype Marburg virus variant Musoke (MARV/Mus) by < 10% at nucleotide level. There are very probably other subtypes as well. In 2007, it was found that certain fruit bats (Rousettus aegyptiacus) were carrying Marburg viral RNA as well as antibodies against the virus. It seems more and more likely that bats form the reservoir, although more research is needed. In 2009, the successful isolation of infectious Marburg virus was reported from caught healthy Egyptian rousettes (Rousettus aegyptiacus).This isolation strongly suggests that Old World fruit bats are involved in the natural maintenance of marburgviruses and makes bats the prime suspect as reservoir for Ebola virus, though the latter has never been cultured from bats. Further studies are necessary to establish whether Egyptian rousettes are the actual hosts of MARV and RAVV or whether they get infected via contact with another animal and therefore serve only as intermediate hosts.
Experimentally infected bats developed relatively low viremia lasting at least 5 days but remained healthy and didn't develop any notable gross pathology. The virus also replicated to high titers in major organs (liver and spleen) and organs that might possibly be involved in virus transmission.

In 2008 a Dutch tourist became infected after visiting a cave in Uganda. She became sick after her return home and subsequently died in the Netherlands. Also, in 2008 an American tourist developed chills and diarrhoea, severe leukopenia, massively elevated transaminases, coagulation problems, pancreatitis and renal failure after a similar voyage. The diagnosis of Marburg infection was obtained in retrospect, when she was informed of the death of the above-mentioned Dutch tourist. In October 2012, the disease flared-up in Uganda, short after an outbreak of Ebola virus. The clinical signs and symptoms are similar to Ebola (see further). There is no effective treatment.

At present, an experimental vaccine against Marburg has been developed. It is based on a live attenuated recombinant vesicular stomatitis virus, a well-known pathogen of horses, bovines and pigs. The gene coding for Marburg glycoprotein was inserted into the viral genome (similar work was performed with the Ebola Zaire virus). Experiments in monkeys showed a good

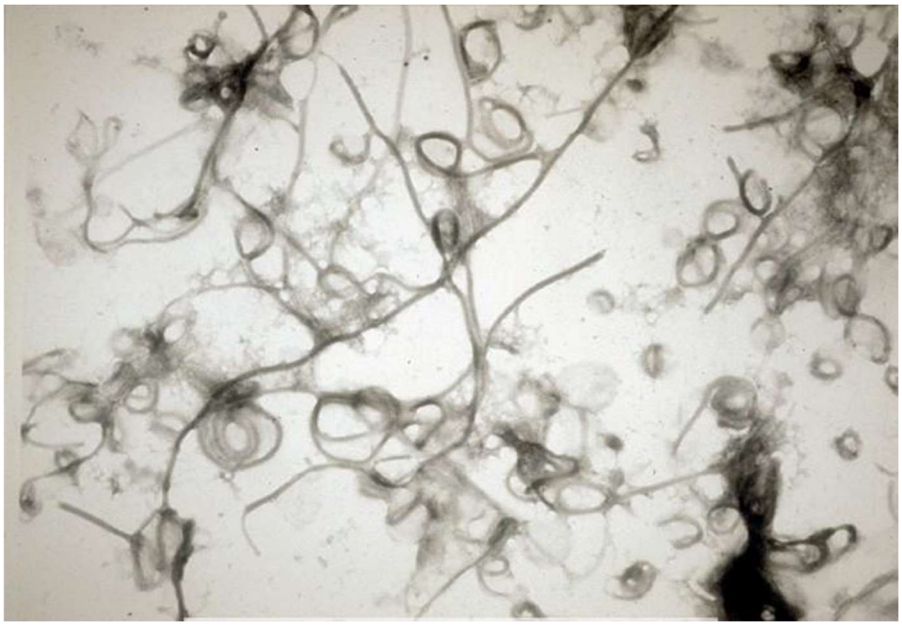
humoral and cellular immune response and protection against infection with wild type virus. There was no cross-protection against other filoviruses, such as Ebola virus. So far, the vaccine has shown no evidence of pathogenicity in four species of animals (mouse, guinea pig, goat, monkey).

In 2012 it was demonstrated that macaque monkeys could be protected from Marburg virus disease by post-exposure treatment with hyperimmune serum (Marburg virus-specific IgG). No clinical human trials have been performed to date. On the basis of efficacy in nonhuman primates and pharmacokinetic data in humans, AVI-7288 - a phosphorodiamidate morpholino oligomer with positive charges that targets the viral messenger RNA that encodes Marburg virus (MARV) nucleoprotein – has potential as postexposure prophylaxis for MARV infection in humans.

Ebola virus

General

Ebola virus is a member of the Filoviridae family (Mononegavirales order). It is a enveloped filamentous particle with a non-segmented, negative-sense RNA genome. The viral spike on the viral envelope is formed by the sole trimeric transmembrane glycoprotein and mediates viral entry; this spike is a target for the host immune response and for vaccine development. EBOV or Ebola virus refers to the Zaire ebolavirus in the genus ebolavirus. The other known species within the genus are Bundibugyo ebolavirus (Bundibugyo virus), Reston ebolavirus (Reston virus),Sudan ebolavirus (Sudan virus), Taï Forest ebolavirus (Taï Forest virus), and Bombali virus. Only Bundibugyo, Sudan, and Ebola viruses hav been associated with disease outbreaks in humans. Ebola virus disease (EVD) refers to a disease caused by four of five viruses of the genus Ebolavirus: BDBV, SUDV, TAFV and EBOV.



Ebola virus, Electron microscopy, copyright ITM, with special thanks to Guido Van der Groen

Ebola-Zaire (EBOV) and Ebola-Sudan (SUDV)

In 1976 there was a sudden large-scale epidemic of 2 different Ebola viruses in Maridi (South Sudan) and in Yambuku, on the Ebola river in North Congo. The mortality rate in Yambuku was very high (280 deaths out of 318 cases = 88%) and slightly lower in Sudan (53%). In 1977 there was one fatal case in Tandala, North Congo. New major outbreaks occurred in 1979 in Nzara (South Sudan), in 1995 in Kikwit, Congo and in 2003 in Kelle, Congo Brazzaville. The virus, which emerged in Kikwit, very closely resembled that in Yambuku (less than 1.6% difference in RNA). This is a sign of a genome, which is not under selection pressure, suggesting a stable ecological niche between epidemics. The Sudanese virus isolates of 1976 and 1979 were also almost identical.

From 1994 till 2012, several small epidemics occurred with the number of infections never exceeding 500, with the case fatality rate varying between 41 and 100% (100% was 4 times due to a single case) (see table below). During an outbreak in October 1996, an infected doctor was flown over to South Africa and there caused a fatal secondary case in a nurse. This

illustrates how easily pathogenic organisms can be spread in this age of long-distance transport. Early in 2003, a large-scale epidemic occurred in Mbomo and Kelle, a very remote and rural area of Congo Brazzaville, just south of Odzala National Park. It started by a large-scale die-off among the lowland gorillas in the park. The disease flared up again in the same area, in November the same year, but was contained before New Year 2004.

Ebola outbreak in West-Africa

On 14 March 2014, rumours of a 'mysterious disease' were reported by the Ministry of Health in Guinea. Several health staff taking care of the sick had died and mortality was very high. Suspicion of Lassa viral haemorrhagic fever rose, but what jumped out were the hiccups, a typical symptom associated with Ebola. 28 March 2014, the World Health Organization was notified of an outbreak of a communicable disease characterized by fever, severe diarrhoea, vomiting, and a high fatality rate in Guinea.

Virologic investigation identified Zaire ebolavirus (EBOV) as the causative agent. Full-length genome sequencing and phylogenetic analysis showed that EBOV from Guinea forms a separate clade in relationship to the known EBOV strains from the Democratic Republic of Congo and Gabon. the suspected first case of the outbreak was a 2-year-old child who died in Meliandou in Gu Gundou prefecture on December 6, 2013. A health care worker from Guom Gu with suspected disease, seems to have triggered the spread of the virus to Macenta, Nzcenta, and Kissidougou in February 2014. The initial case fatality rate was 86% (12/14 patients). What followed was an unprecedented outbreak going from bad to worse. Ebola had been stealthily spreading undetected for more than three months. It is not unusual for Ebola to go undiagnosed for a substantial period of time; the past eight Ebola outbreaks each took two months on average to be discovered and investigated. Ebola's symptoms are easily confused with other diseases, such as cholera and malaria, and experts trained to recognise it are rare. However, past outbreaks took place mostly in remote villages in central and eastern Africa, where they were more easily contained. In a twist of geographic fate, Ebola erupted at the junction of Guinea, Liberia and Sierra Leone, where people regularly move across the porous borders. Fear and suspicion of the unknown virus, unsafe burial practices, mistrust in politicians, the hiding of cases, and a weak public health system, which lacked the resources to recognise and efficiently respond to Ebola, all contributed to the virus surging through the region. For months, the epidemic spread faster than the international community's response. The Ebola virus was introduced into Nigeria on 20 July 2014 when an infected Liberian man arrived by airplane into Lagos, Africa's most populous city. The man, who died in hospital 5 days later, set off a chain of transmission that infected a total of 19 people, of whom 7 died.

On August 8 2014, the WHO declared the epidemic to be an emergency of international concern. In Mali 8 people were infected of whom 6 died and 1 case was detected in Senegal. On 6 October 2014, the World Health Organization (WHO) was informed of the first confirmed autochthonous case of Ebola virus disease in Spain. This case represents the first human-tohuman transmission of EVD outside Africa. The case is a female healthcare worker with no travel history to West Africa but who participated in the medical care of an EVD case in a Spanish citizen, who had been infected in Sierra Leone and evacuated to Madrid, Spain on 22 September 2014 and who died on 25 September 2014. She was in contact with the repatriated EVD case twice; on 24 and 25 September 2014. On both occasions she is

reported to have worn appropriate personal protection equipment (PPE). Following the Spanish national protocol for EVD cases, the healthcare worker was considered a low risk contact and monitored accordingly. The female case developed a fever on 29 September 2014 and was admitted into isolation on 6 October 2014 where she tested positive for Ebola.

In total 28.652 Ebola cases are recorded during the 2013-2016 epidemic with a death toll of 11.325. Ebola has destroyed lives and families, left deep scars, and ripped at the social and economic fabric of Guinea, Liberia and Sierra Leone. The virus cut a vast swathe through the three countries, in a cross-border geographical spread never seen before. Fear and panic set in, the sick and their families were desperate, and national health workers and MSF teams were overwhelmed and exhausted. Medical workers are not trained to deal with at least 50 percent of their patients dying from a disease for which no treatments exist. Nevertheless, the world at first ignored the calls for help and then belatedly decided to act. Meanwhile, months were wasted and lives were lost. No one knows the true number of deaths the epidemic will have ultimately caused. Across the three countries, local healthcare workers were tragically dying by the dozens. In Ebola outbreaks, health facilities without proper infection control often act as multiplying chambers for the virus, become dangerous places for both health workers and patients. This outbreak was no different, but it happened on a massive scale. The resulting collapse of health services means that untreated malaria, complicated deliveries and car crashes will have multiplied the direct Ebola deaths many times
over. Why was the world so slow to wake up to its severity and respond? Was it due to fear, lack of political will, lack of expertise, or a perfect storm of all three?
See also: https://www.who.int/features/ebola/storymap/en/

Country	Total Cases (Suspected, Probable, and Confirmed)	Laboratory-Confirmed Cases	Total Deaths
Guinea [2]	3814	3358	2544
Sierra Leone [3]	14124	8706	3956
Liberia[4]	10678	3163	4810
Total	**28616**	**15227**	**11310**

Ebola cases in 3 countries with widespread transmission during the 2013-2016 epidemic. Source: CDC

Country	Total Cases (Suspected, Probable, and Confirmed)	Laboratory-Confirmed Cases	Total Deaths
Nigeria	20	19	8
Senegal	1	1	0
Spain	1	1	0
United States	4	4	1
Mali	8	7	6
United Kingdom	1	1	0
Italy	1	1	0
Total	**36**	**34**	**15**

Countries with lower case load during the 2013-2016 epidemic. Source: CDC

During the West-African outbreak, 17 persons with EVD disease have been cared for outside Africa of which three persons have contracted Ebola outside Africa. In the United States eleven cases of EVD have been reported: nine of them contracted the disease outside the US and travelled into the country, either as regular airline passengers or as medical evacuees; of those nine, two died. Two nurses have contracted Ebola in the United States, both treating an Ebola patient; both have recovered. Of the eleven cases, four have been diagnosed within the US: the two above mentioned nurses and two travellers that became ill in the US.
Only 6 cases of Ebola have been diagnosed in Europe, all in connection with the Ebola outbreak in West Africa: one in Italy, one in Spain and three in the United Kingdom and one locally acquired in a health care worker in caring for an evacuated Ebola patient in Spain.

In August 2018, the Democratic Republic of Congo MOH tested 4 individuals positive for the Ebola virus in North Kivu. In this war-ravaged province in which there is mistrust of the government and mistrust of the Ebola response, the outbreak became the second largest ever recorded with a total of 3406 cases (3262 confirmed and 144 probable) and 2243 deaths, corresponding with a mortality rate of 65.9% which is significantly higher than in the West-African epidemic (39.5%).

Ebola Ivory Coast (Thaï Forest virus, TAFV)

In 1994 many chimpanzees died following an Ebola epidemic in the Tai nature reserve in Côte d'Ivoire on the border with Liberia. Here one person was infected during an autopsy on a chimpanzee that had died. She was evacuated to Switzerland where she was treated. The causative agent turned out to be a new genetic subtype of Ebola virus. In late 1995 another (unconfirmed) case occurred in the same area (Plibo) in a Liberian refugee.

Reston virus (RESTV)

In 1989 an epidemic of another Ebola virus occurred in a primate centre in the USA in Reston, a town near Washington D.C. A number of people were infected but without any illness both in Reston (4) and in the Philippines (12) where the monkeys came from. Unlike the case of Ebola-Zaire, there were arguments here for aerogenic transmission. Research was complicated by the fact that another haemorrhagic fever virus epidemic was taking place at

the same time among the monkeys (Simian Haemorrhagic Fever Virus). Late 2008 a Philippino farm worker was found infected by the Ebola-Reston virus that was discovered in pigs at 2 farms north of Manila. It was the 1st time Ebola-Reston was found outside monkeys. The infected man had not shown any symptoms and was healthy. Later 5 more persons were found to have been infected, all were asymptomatic. RESTV sequences have been found in Chines pigs, raising fear about food safety.

Bundibudyo virus (BDBV)

Bundibudyo was the region in Uganda were the 2007 Ebola epidemic was centred. The epidemic in Uganda was caused by a fifth viral species. The genome differs by about 32% of its nucleotides, compared with the other Ebola strains. This may complicate efforts to produce a universal vaccine.

Fifty-six cases of Bundibugyo Ebola virus infection were laboratory confirmed during the first epidemic. Signs and symptoms were largely nonspecific. The proportion of deaths among those infected was about 40%. A new outbreak occurred in August and September 2012, centred on Isiro and Viadana, Haut-Uele district.

Bombali virus (BOMV)

In 2018 a new ebolavirus – Bombali virus was detected in free-tailed bats in Sierra Leone: little freetailed (Chaerephon pumilus) and Angolan free-tailed (Mops condylurus) bats. The bats were found resting inside houses but it is not known whether human exposure has occurred or if BOMV is pathogenic in humans.

Summary of known human Ebola Disease cases

Year	Cases, deaths	Place
1972	1 non-fatal case (retrospective diagnosis)	Tandala, DRC (not confirmed)
1976	318 cases, 280 deaths	Yambuku , DRC (discovery of the virus)
1976	284 cases, 151 deaths	Nzara, Maridi, Tembura and Juba, Sudan
1977	1 fatal case	Tandala, DRC
1979	34 cases with 22 deaths	Nzara and Yambio, Sudan
1980	1 suspected case	Kenya (not confirmed)
1994	44 cases, 28 deaths	Minkouka, Gabon
1994	1 non-fatal case	Tai Park, Côte d'Ivoire
1995	315 cases, 255 deaths	Kikwit, DRC
1996	1 non-fotal case	Plibo, Liberia (not confirmed)
1996	37 cases with 21 deaths	Mayibout and Makokou, Gabon
1996	60 cases with 40 deaths	Booué, Gabon One exported case in South Africa with one fatal secondary case
2000	425 cases with 244 deaths	Gulu, Masini, Mbarara (Uganda)
2002	43 deaths in Congo, 53 deaths in Gabon	Gabon - DRC
2002	No reliable numbers available	Mbombo, DRC

2003	About 140 cases with about 130 deaths (Feb-Mar). Flare-up in Nov-Dec, with 35 cases (29 deaths)	Mbombo, DRC
2004	25 cases with 6 deaths	Mbombo and Mbandza, Congo Brazzaville
2005	About 10 cases	Etoumbi, DRC
2007	About 187 cases	Kampungu, Mweka, Luebo, DRC (Western Kasia)
2008	About > 90 cases	Western Uganda
2008-2009	New epidemic in Congo, lasting till early 2009. Number of cases unclear.	November 2009, outbreak in Mweka, DRC
2009	In March 2009, accidental needle stick injury in Hamburg (virologist)	Germany, the first time that vesicular stomatitis virus-based vaccine is used in a human (post-exposure)
2011	Isolated case	Uganda
2012		
2013-2016	28.652 cases with 11.325 deaths	Guinea, Sierra Leone, Liberia, Nigeria, Malia, Senegal, USA, Spain
2014	66 cases, 49 deaths	Équateur province, DRC
2017	8 cases, 4 deaths	Likati, DRC
2018	54 cases, 33 deaths	Bikoko, Mbandaka, DRC
2018-2020	3470 cases, 2287 deaths	North-Kivu and Ituri province, DRC

Epidemiologic and ecologic features

Ebola epidemic in Kikwit, Congo 1995. Small animal trapping and study, as a part of the search for the reservoir of this virus. Notice the protective gear of the researchers. Copyright ITM

Today, neither EBOV nor other filoviruses are endemic anywhere, but the discovery of persistent virus in humans after infection during the 2013-2016 epidemic, indicates that the virus can temporarily circulate in persons. The natural reservoir of these viruses remains unconfirmed, nevertheless bats are the prime suspects. To date the analysis of the numerous arthropods and living vertebrates has not produced a single positive viral isolate, although Ebola virus was demonstrated in several carcasses in the Central African rainforest, esp.

primates. Analysis of 98 animal carcasses in Gabon - Congo (study period 2001-2003) showed on 10 Ebola-positive gorillas out of a 50 gorilla carcasses, 3 positive chimpanzees out of 15, and 1 positive duiker (Cephalophus) out of 14. The monkey species, which have been studied thus far, all die from the infection and therefore cannot form the natural reservoir.

Filoviruses are considered regionally epizootic. Contact with infected monkeys plays a role in the beginning of an epidemic but how these animals are initially infected is not known. The epidemic, which started in November 2003 in Mbomo, Congo Brazzaville, was rumoured to have started after villagers found a dead wild pig in the forest and ate its meat. This would be the first case that such an animal would be implicated. Certain fructivorous and insectivorous bats can be experimentally infected and certain species are seropositive in nature. In 3 bat species (Epomops franqueti, Hypsignathus monstrosus and Myonycteris torquata) Ebola RNA sequences have been detected. These animals usually develop an asymptomatic infection.To date, ebolaviruses surprisingly were never isolated in a bat, which might be explained by low viral loads or inhibitors in bat tissue. Epidemics may start after spillover events from bats to humans and other mammals that serve as end-, intermediate- or amplifying hosts. These animals are often shot and eaten as "bush meat". The Zaire strain of Ebola virus can also replicate in pigs. Infected animals develop severe lung disease. They shed large numbers of virions in the respiratory tract. Shedding continues for up to 2 weeks after infection. Infected animals can transmit the infection to non-infected pigs and possibly to humans.

Pathophysiology

Transmission takes place through direct contact with infected body fluids (including sexual contact) and nosocomial through infected needles and contact with infected blood. Sexual transmission is described up to 6 months after survival. Aerogenic transmission of Ebola has been demonstrated in the laboratory in Rhesus monkeys, thought this is never described in humans. Viral particles land on mucous membranes or occasionally enter percutaneously. Filoviruses replicate in the cytoplasm of their target cells, which are initially dendritic cells and macrophages and potently shut down early innate immune responses by blocking interferon production. Later, dendritic cells migrate to lymphoid tissues and the virus is released in the circulation with spread to the liver, spleen and other tissues.

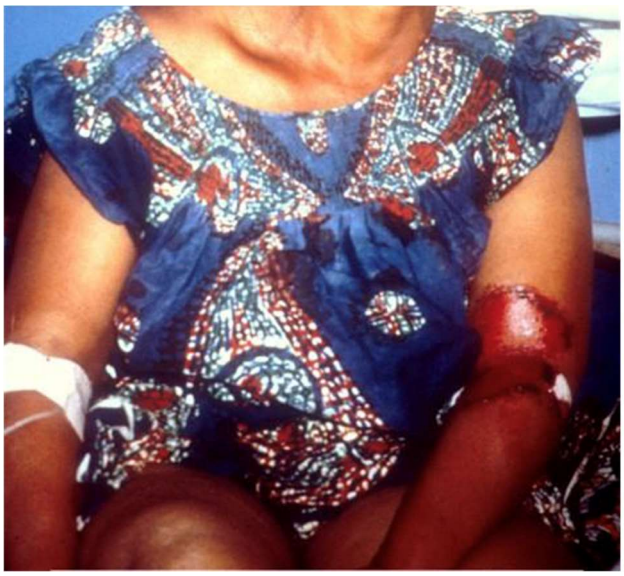
Disease is caused by the cytopathogenic effects of the virus itself leading to cell lysis, but also by an exaggerated host immune response inducing a cytokine storm causing a septic shock. Several cytokines (IL-1ß, IL-6 and TNF) and chemokines cause T-cell activation, which is rendered ineffective in severe or fatal cases due to T-cell exhaustion followed by an impaired adaptive immune response.

Endothelial-cell dysfunction is caused by inflammatory mediators triggering vascular permeability and fluid extravasation. Tissue factor is produced by infected macrophages and lead to fibrin deposition in the spleen, lymphoid tissues, glomeruli and renal proximal tubules. Diffuse intravascular coagulation arises due to consumption of clotting factors, endothelial dysfunction and platelet dysfunction with coagulopathy and bleeding as a consequence.

Multiple organ failure (MOF) with tissue hypoperfusion develops due to microvascular anomalies and hypovolemia due to gastro-intestinal fluid losses. Bacterial translocation can be a consequence of the disrupted gut mucosa triggering bacteraemia and bacterial septic

shock. Fatal cases are associated with defective immune responses and high viremia. Survivors have early and vigorous cellular as well as humoral immune responses. The immunological course early in the infection determines how quickly the Ebola virus replicates and whether the host will die or recover. Surviving an infection is linked to an early appearance of IgM and IgG, followed by the activation of cytotoxic cells.

Clinical aspects



Patient with Ebola haemorrhagic fever with bleeding at injection sites. Photo Dr Van den Enden, Copyright ITM

Ebola, 2003, Kelle, Congo. Patient presenting with bleeding gums, a sign of haemorrhagic diathesis. Photo Dr Erwin Van den Enden, Copyright ITM

The clinical disease is not called Ebola haemorrhagic fever anymore but Ebola virus disease (EVD) which downplays bleeding as a clinical hallmark and stresses the great variability in symptoms. After an incubation period of 2 to 21 days (average 7 days) infection often leads to multiple organ failure, with death occurring on average 6 to 9 days after the onset of symptoms. But asymptomatic infection with Ebola can occur. People infected with Ebola virus initially present with nonspecific febrile illness with malaise, fatigue and myalgia. In a second stage, gastro-intestinal symptoms with anorexia, nausea, abdominal pain, vomiting and diarrhoea develop. Patient can lose up to 10 liters per day and severe electrolyte disturbances rise: hypokalaemia, hyponatremia, hypomagnesemia, ... Dysphagia, headache, conjunctival injections, maculopapular rash and joint pain are other common symptoms. Hiccups can be caused by uncontrolled diaphragm contractions due to viral invasion of the CNS that controls

the diaphragm. Hiccups were a clue that led researchers to suspect that the West-African epidemic was not caused by Lassa virus but possibly by Ebola virus. One should not focus too much on bleeding as a presenting symptom as this is a late symptom and cases will be missed. Even in end-stage disease patients, bleeding abnormalities occur in less than half of them. Bleeding from gums, petechiae, persistent oozing from venepuncture sites, subconjunctival haemorrhage, haematemesis and bloody diarrhoea can be present. Hepatitis arises due to lysis of hepatocytes and liver hypoperfusion. Once kidney failure sets in, the fluid management becomes very difficult with the risk of fluid overload, pulmonary oedema and difficult to manage hypo-/hyperkalemia. The occurrence of renal failure almost universally leads to death if renal replacement therapy is not available.
Neurological complications have a multifactorial aetiology: hypoglycaemia, viral meningo-encephalitis, intracranial haemorrhage, hepatic encephalopathy, delirium, …

Healthcare providers should not minimize the psychological impact of receiving the diagnosis 'Ebola' on a patient, knowing that the mortality rate in some epidemics surpasses 60 percent. Anxiety and depression are common symptoms and psychological support to help patients cope with their fears is part of good patient care.

The post-Ebola syndrome refers to musculoskeletal pain, headache, encephalitis and ocular problems (uveitis) that were frequently noted in thousands of EVD survivors of the 2013-2016 EBOV epidemic. The mental health effects on survivors, their family and community are considerable.

Diagnosis

Diagnosis during an epidemic is based on clinical suspicion, with serum PCR as confirmation. The viral RNA can be detected via a quantitative reverse transcriptase PCR on a blood sample(qRT-PCR). Results are expressed in Cycle Threshold (Ct) levels: low (< 20) Ct levels indicate detection of the virus after a low number of cycles required for the fluorescent signal to cross the detection threshold, hence ahigh viral load translating in a poor prognosis. Diagnostic studies during the 2013-2016 outbreak have mainly relied on molecular diagnostic platforms. In general these tests are highly sensitive and specific.
Various assays are currently available & FDA approved including an Xpert-based machine (Xpert Ebola Assay), which is a fully automated and closed device now rolled out for tuberculosis diagnosis. If this assay is installed within an Ebola treatment unit, time between sample collection and result was 2.5-3 hours in a study by MSF. The assays itself runs over around 90 minutes and cartridges specific for the EBOV Zaire strain were developed to target highly conserved sequences in the nucleocapsid protein (NP) and glycoprotein (GP) genes. Results can come out positive or negative and a cycle threshold (Ct) for both gene targets is given. These molecular assays may be negative early in the disease course, warranting follow-up testing in patient with recent onset symptoms. If the initial PCR test is negative and the patient has symptoms that started less than 48 hours previously, a second sample must be taken at 72 hours of illness (after another 24-48 hours). Simple bedside antigen-based tests have become available, but their sensitivity and thus negative predictive value is lower than PCR. These tests can thus be used for quick confirmation, but not to exclude the infection. Virus can be cultured in a few BSL-4 laboratories (e.g. on Vero cells).

Serological testing has no place in the diagnosis of an acute ill patient, but can be used for epidemiological research. It is worth knowing that each of the various geographical isolates have their own antigenic structure and therefore problems can arise with serological testing. Other laboratory findings are elevated transaminases linked with hepatitis and creatinine kinase due to myositis. Consumption of clotting factors due to DIC leads to disturbed coagulation tests (PTT, Ddimers, fibrinogen). Thrombocytopenia is present in most patients and initially there is lymphocytopenia and later neutrophilia. Histologically there is focal necrosis in various organs (testes, kidneys, liver, etc.). Lower baseline viral load, creatinine and aminotransferase levels correlate with improved survival.

Patients can be safely discharged from Ebola treatment units when two sequential tests come back negative (Ct > 40) in a patient that has clinically improved.

Treatment

Early diagnosis and prompt initiation of care increase survival ratios. Paediatric patients and elderly are at higher risk of dying (however in the 2018-2020 epidemic in Eastern DRC extremes of age were not associated with poorer outcomes) as well as patients with a high viral load.

During epidemics, good patient care may lower the mortality. Care for EVD patients is based on three pillars: supportive care to restore normal physiology, management of discomfort or distress and presumptive treatment of concurrent infections. In all epidemics so far, treatment was mostly done in very basic field conditions and treatment in an intensive care unit was rarely possible. Throughout the experiences gained in the recent epidemics, Ebola treatment Centres (ETC) in the field have more and more evolved towards provision of individualized care, with advances in laboratory and technical support. Staffing ratios of 1 or more more clinicians for four patients, and assessments (evaluation of each patient) performed at least three times per 24 hours are recommended. A comprehensive guidance “Optimized Supportive Care for Ebola Virus Disease” has been published by the World Health Organisation (https://www.who.int/publications-detail-redirect/optimized-supportive-care-forebola-virus-disease).

Gastro-intestinal symptoms can be controlled with metoclopramide (Primperan®) or domperidone (Motilium®) against vomiting and loperamide against diarrhoea. Omprazole is given as stress ulcer prophylaxis. Prevention intravascular volume depletion and avoidance of organ hypoperfusion is critical. Fluid losses from vomiting, diarrhoea and vascular leakage may require more than five liters per day of crystalloid solution intravenously if the patient is unable to compensate the losses with oral rehydration. In the last epidemics, the use of point-of-care ultrasound has been a useful addition to estimate fluid status and has the potential to increase diagnostic capacity and individually tailored patient care. On-site biochemical testing was often available, permitting correction of electrolyte abnormalities (hyponatremia, hypo-/hyperkalaemia, hypomagnesemia and hypocalcaemia) and hypoglycaemia. Oral nutrition should be encouraged, ideally guided by a nutritionist. If necessary nasogastric tube can considered. High calorie liquid food is easier to swallow than solid food, since many patients suffer from severe throat pain. Antipyretic agents as paracetamol are given to manage pain and to decrease fever. Stronger pain killers (tramadol, morphine) might be needed, but NSAID’s should be avoided to minimize the risk of renal failure and to decrease the risk of

bleeding. Chlorpromazine and even haloperidol might be considered in case of agitation and confusion. Seizures are treated with diazepam.

Since coinfections are often difficult to diagnose in low resource settings with blood cultures rarely available, presumptive treatment with broad-spectrum antibiotics, in the form of a third generation cephalosporin, are usuallypart of the initial standard treatment. It is not unusual for EVD patients to develop new-onset fever that may be associated with leukocytosis in the second or third week of the hospital course, often despite initial improvement in the presenting symptoms and the viral load. In this setting, the development of ETU-acquired secondary infections while on broad spectrum antibiotics, and the development of resistant gram-negative bacteremia or Clostridium difficile infection, should be considered. Antibiotic management, including drug choice as well as doses, should be adjusted accordingly. In malaria endemic regions, anti-malarial treatment is sometimes added for all admitted patients, but robust data justifying this approach are lacking. A natural experiment due to a 12-day stock rupture of artesunate-lumefantrine in a Liberian MSF Ebola treatment center, noticed a lower risk of death in patients prescribed artesunate-amodiaquine compared with patients that received artesunate-lumefantrine. Amodiaquine is a compound with anti-Ebola activity in vitro. It is however not excluded that artemether-lumefantrine is associated with an increased risk of death due to torsades de points with fatal arrythmias in patients with a long QTc interval especially when combined with hypokalemia/hypomagnesemia and/or ciprofloxacine and/or metoclopramide. Another explanation is that artesunate-amodiaquine use was associated with unmeasured patient characteristics that altered the risk of death (e.g. effective malaria infection, higher viral loads, age, patients admitted during busier periods, patients in need of parenteral treatment with worse prognosis, …).

In the rare events when parenteral nutrition, renal-replacement therapy and mechanical ventilation where available, these treatments probably had a lifesaving impact.

Survivors of EVD need comprehensive follow-up care, including rheumatological, auditory, and ocular function with special attention to visual acuity deficits or raised intraocular pressure. Appropriate psychological and social support should be offered after mental health screening examinations.

Aside from good supportive care, several investigation treatments with anti-EBOV activity exist. The main categories are antibodies (plasma from convalescent patients, whole blood or monoclonal antibodies) and antivirals. A study with convalescent plasma in Guinea did not show sufficient mortality benefit, neither did a study with whole blood transfusion. A phase II study with the antiviral favipiravir only decreased case fatality rate in patients with a low viral load and seemed to increment mortality in patients with higher viral loads. At the end of the 2013-2016 outbreak in West-Africa, a randomized clinical trial (PREVAIL II) with ZMapp - a cocktail of three potent monoclonal antibodies - showed a fatality rate of 37% (13 of 35 patients) in those receiving the standard of care and a fatality rate of 22% (8 of 36 patients) in those receiving standard of care together with ZMapp. Although this result seems beneficial, the decline in the epidemic reduced participant enrolment hence results did not reach the statistical threshold for efficacy.

During the 2018-2020 outbreak in DRC, the Pamoja Tulinde Maisha (PALM "Together Save Lives" in Swahili) trial compared ZMapp with three newer agents: mAb114 (Ridgeback Biotherapeutics) which is a single monoclonal antibody derived from the memory B cells from a survivor of the Kikwit EVD epidemic, REGN-EB3 (Regeneron Pharmaceuticals) combining three triple monoclonal antibodies obtained by immunizing mice and the antiviral remdesivir (Gilead), a prodrug nucleotide analogue. mAb114 and REGN-EB3 have the advantage over ZMapp that they are given as a single dose whereas ZMapp is given in 3 doses, spaced 3 days apart. Remdesevir is given in daily doses for at least 10 days. An interim analysis showed superiority of Mab114 and REGN-EB3 to ZMapp and remdesivir with respect to mortality. In the mAb114 and REGN-EB3 group mortality was 35% and 33% as compared with 50% in the ZMapp and 53% in the remdesivir group. Shorter duration of symptoms before admission with earlier treatment initiation improved survival, which had not been the case in previous epidemics. Surprisingly, mortality with ZMapp in the PALM trial was 50% compared to 22% in the above mentioned PREVAIL II trial. The reasons remain unclear and subgroup analysis is ongoing to shed more light on potential differences among treatment groups. After the interim analysis patients were randomized to receive mAb114 or REGN-EB3, dropping the ZMapp and remdesivir study arm. Final results of this trial are still pending. Hundreds of patients in the recent outbreak in Eastern DRC that were not included in the PALM trial still received above mentioned investigations drugs under the Monitored Emergency Use of Unregistered and Investigational Interventions (MEURI) framework. Despite the lack of randomization, an analysis of patients receiving drugs under MEURI showed remarkably similar results for the same therapeutics that were provided in the PALM trial.

It is important to notice that patients developing EVD despite previous vaccination for EBOV had much better outcomes.

Future research might focus on combination therapy considering the possible synergistic effect of remdesivir – that has a delayed onset of action as compared with a antibodies – combined with antibodies. A next generation human antibodies (i.e. MBP134, FVM04 and CA45) have shown protection against EBOV, SUDV and BDBV, whereas ZMapp, REGN-EB3 and Mab114 only protect against EBOV. Nevertheless, these products will first have to prove their non-inferiority in a welldesigned future trial.

Table 1. Clinical Trials of Vaccines and Antiviral Therapies for Ebola Virus Infection in Humans.*

Treatment and Study Design (Country)	Filovirus Species (Strain)	Dose	Regimen	No. of Patients and Outcome	Study
Vaccine					
rVSV-ZEBOV; open-label, cluster, randomized trial of ring vaccination (Guinea)	Ebola (Makona)	2×10^7 PFU	Single injection (IM)	5837 vaccinated; estimated efficacy, 100% (95% CI, 79.3–100.0)	Henao-Restrepo et al.[51]
rVSV-ZEBOV; randomized, placebo-controlled phase 2–3 trial (Liberia)	Ebola (Makona)	2×10^7 PFU	Single injection (IM)	500 vaccinated (phase 3 eliminated because of decline of Ebola in Liberia)	Kennedy et al.[52]
rVSV-ZEBOV; open-label, cluster, randomized trial of ring vaccination (DRC)	Ebola (Kivu)	2×10^7 PFU	Single injection (IM)	93,965 vaccinated; efficacy, 97.5% (95% CI, 95.8–98.5)	World Health Organization[53]
ChAd3-EBO-Z; randomized, placebo-controlled phase 2–3 trial (Liberia)	Ebola (Makona)	2×10^{11} particle units	Single injection (IM)	500 vaccinated (phase 3 eliminated because of decline of Ebola in Liberia)	Kennedy et al.[52]
Antiviral Therapy					
Convalescent plasma; nonrandomized comparative study	Ebola (Makona)	Unknown	Two consecutive IV transfusions of 200–250 ml each	84 enrolled; no significant survival benefit	van Griensven et al.[44]
Convalescent blood; nonrandomized comparative study	Ebola (Makona)	Unknown	One IV transfusion of 450 ml given over a period of 1–4 hr	43 enrolled; no significant survival benefit	Sahr et al.[45]
ZMapp; phase 2–3 trial (Liberia, Sierra Leone, Guinea, United States)	Ebola (Makona)	50 mg/kg	One dose every 3 days (IV) for a total of three doses	36 enrolled, 28 survived (77.8% survival rate)	PREVAIL II Writing Group[46]
ZMapp; PALM trial (DRC)	Ebola (Kivu)	50 mg/kg	One dose every 3 days (IV) for a total of three doses	323 enrolled, 160 survived (49.5% survival rate)	Mulangu et al.[54]
MAb114; PALM trial (DRC)	Ebola (Kivu)	50 mg/kg	One dose (IV)	174 enrolled, 113 survived (64.9% survival rate)	Mulangu et al.[54]
REGN-EB3; PALM trial (DRC)	Ebola (Kivu)	150 mg/kg	One dose (IV)	155 enrolled, 103 survived (66.5% survival rate)	Mulangu et al.[54]
Remdesivir (GS-5734); double-blind, placebo-controlled, natural history trial (Liberia)	Ebola (Makona)	100 mg	Once daily for 5 days (IV)	Ongoing, with planned enrollment of 60 survivors to assess viral shedding in semen	Siegel et al.[55]
Remdesivir (GS-5734); PALM trial (DRC)	Ebola (Kivu)	200 mg loading dose; 100 mg thereafter	Once daily for 9–13 days (IV)	175 enrolled, 82 survived (46.9% survival rate)	Mulangu et al.[54]
Favipiravir (T-705); single-group trial with historical controls (Guinea)	Ebola (Makona)	6000 mg loading dose; 2400 mg thereafter	Two 1200-mg doses daily on days 1–9 (oral)	126 enrolled; no significant survival benefit	Sissoko et al.[48]
TKM-130803; single-group, phase 2 trial with historical controls (Sierra Leone)	Ebola (Makona)	0.3 mg/kg	Once daily for up to 7 days (IV)	12 enrolled; no significant survival benefit	Dunning et al.[47]

* CI denotes confidence interval, IM intramuscular, IV intravenous, PALM Pamoja Tulinde Maisha, and PFU plaque-forming units.

Source: NEJM, 2020; 382:1832-42, Feldmann et al.

Prevention

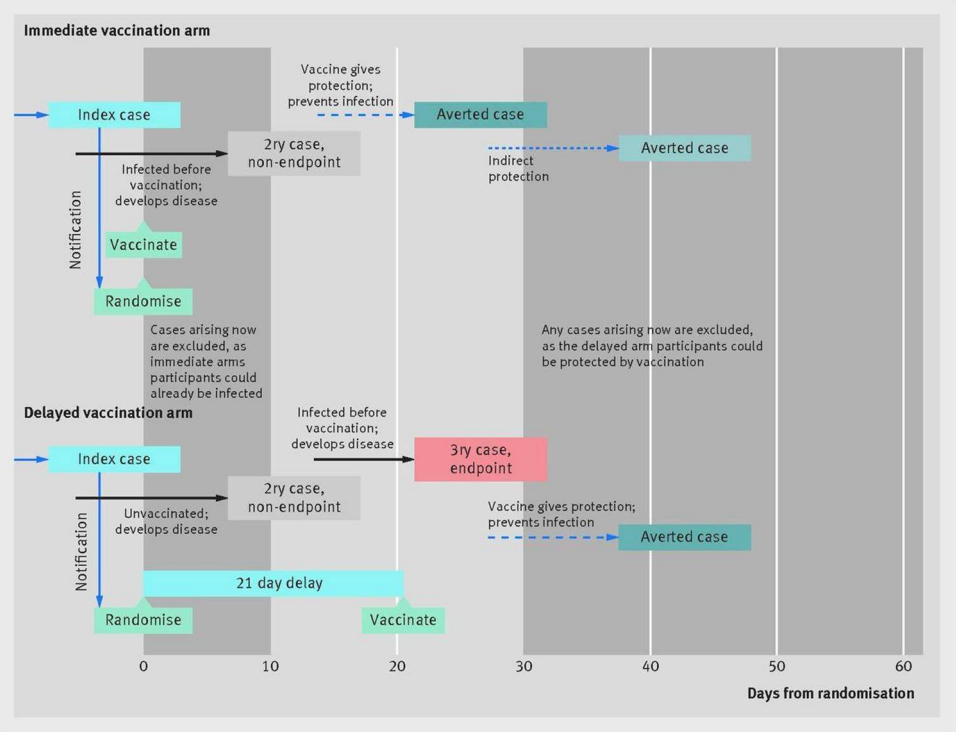
Prior to the 2013-2015 Ebola outbreak, no effective vaccine was commercially available nevertheless, such a vaccine was explored with several purposes, such as after lab-accidents, during epidemics, and probably in the stockpile for biowarfare defence.

Clinical development of several vaccines has advanced substantially during the 2013-2015 EBOV outbreak. Successful vaccination relies on the development of an immune response against the viral glycoprotein (GP), which is critically involved in cell attachment, fusion and cell entry. While assumed that the protection against EVD predominantly relies on the development of anti-GP antibodies, the role of the cellular immune response in vaccine protection remains to be defined. There is indeed not yet a well-defined immune marker (biomarker) that correlates with protection against EVD after vaccination. Currently, the level of IgG antibodies against the EBOV GP is the most commonly used measure of immunogenicity in vaccine trials.

The vesicular stomatitis virus (VSV)-based vaccine (rVSV-ZEBOV), a live (replication-competent) vaccine expressing the Zaire ebolavirus glycoprotein has shown limited reactogenicity ("toxicity") and good immunogenicity in phase I/II studies, although arthritis was documented in some. This vaccine was subsequently evaluated in a phase III trial in Guinea using a ring vaccination strategy, as previously successfully employed during the eradication of small pox. In principle, ring vaccination represents a strategy of targeted vaccination (in contrast with vaccination in the general population), targeting risk individuals at high risk of exposure to and development of EVD.

After the identification of a new EVD case ('index') case, his/her contacts, and the contacts of these contacts are eligible for vaccination. The trial compared clusters with immediate vaccination with clusters with delayed vaccination 21 days later. As cases occurring early after vaccination might have been infected before the vaccination, and since it takes some time for the vaccine to induce immunity, only cases occurring ten days or more after vaccination were taken into account. No EVD cases were seen after this ten day period in the immediate vaccination group, whereas cases continued to accrue with delayed vaccination. This yielded a vaccine efficacy of 100% although with a wide confidence interval (74.7%–100%). The vaccine effectiveness, taking into account all individuals that could/should have been vaccinated was 76.3% (95% CI –15.5% to 95.1%).

The rVSV-ZEBOV has also been used in front-line workers in Guinea and Sierra Leone. During the latest epidemic in eastern DRC, more than 265,000 people have received it as part of a ring vaccination strategy (with ring vaccination not only offered to 1st, but also to 2nd and 3th generation contacts) with an efficacy of 97.5% for vaccines with an onset of illness more than 10 days after vaccination, and 88.1% for all those with EVD regardless of the timing of illness onset. The rVSV-ZEBOV vaccine is now approved for use by WHO during epidemics and is approved for use in Europe and the United States, mainly to protect international healthcare workers that will work in Ebola treatment units.



Source: http://www.bmj.com/content/351/bmj.h3740

Since November 2019, a second vaccine Ad26.ZEBOV/MVA-BN was used to complement the ongoing ring vaccination with rVSV-ZEBOV. This vaccine is given in two doses, 2 months apart: the first dose consists of a recombinant human adenovirus 26 encoding the Zaire ebolavirus glycoprotein, while the second dose is a modified vaccinia Ankara virus (MVA) containing glycoproteins of Zaire and Sudan ebolavirus and Marburg Musoke virus as well as the nucleoprotein of the Tai Forest ebolavirus. The 2 dose prime-boost regimen is expected to give longer protection and therefor the vaccine is given to at-risk populations neighbouring an Ebola epidemic regions where there is no active transmission yet and to health care workers. It is not part of the ring vaccination strategy when rapid immunity is needed since
the single-shot rVSV-ZEBOV appears to induce a quicker immune response. Future work on vaccine efficacy, stability, storage, transport and administration as well as supply adequacy are needed.

Further clinical research

Clinical research initiatives started only late during this EVD outbreak. Only in August 2014, WHO declared the outbreak as a “public health emergency of international concern” and funding became available from the main funding organizations from September on, in part driven by EVD infections of health care workers from international healthcare workers with the threat of EVD spreading to the US and Europe. WHO developed an inventory of vaccines and therapeutics in the pipeline. However as many had often not undergone clinical evaluation, there was a lot of discussion whether it was ethical to use/evaluate these interventions during the 2014-2015 outbreak. In September 2014, the WHO Ethics Working Group released a statement recommending that “investigational drugs or vaccines that have shown promising results in the laboratory or in animal models be urgently tested in humans by scientifically sound, rigorous methods”. Since then, many clinical studies have

been launched in a relatively short time span, complemented with studies in non-human primates. A process that would otherwise take several years now had to be done in months.

Ebola outbreak management

Recognition

The very first step is to recognize possible clinical cases, which is why case definitions must be determined and widely distributed. The current case definition used by WHO for Ebola Virus Disease is:
a patient with any ONE of the following:

- Sudden onset of fever (≥38DC) AND contact with confirmed or probable Ebola case or dead or sick animal; OR
- Sudden onset of fever (≥38DC) AND ≥3 symptoms (Headache, vomiting, diarrhoea, anorexia/loss of appetite, lethargy, stomach pain, myalgia, dysphagia, breathing difficulties, or hiccups); OR
- Contact AND ≥3 symptoms; OR
- Unexplained bleeding or miscarriage; OR
- Sudden unexplained death.

This generic suspect case definition may be adapted to local circumstances (clinical presentation, mode of transmission). During outbreaks, expanding the suspect case definition to include patients with mild symptoms increases sensitivity, but increases the case load in triage centres. The performance of the case definition during outbreaks should be assessed.

Steps should be taken to identify and type the virus (send a blood sample safely to a well-equipped laboratory). In a laboratory which is protected and equipped to work with dangerous pathogens (biosafety level 4), an attempt will be made to detect viral antigen, antibodies and viral RNA (reverse transcriptase PCR) and carry out an analysis of the genome in order to establish which Ebola subtype is involved.

Central organization

If it is established that it really is Ebola, the government will be notified. Central control, registration and coordination is essential for combating an epidemic. WHO and CDC will be notified. Groups specifically responsible for a certain part of the campaign will be set up: clinical care, surveillance in the community, logistics, collecting the dead and safe burials, investigating rumours, informing the population, epidemiological study, research, reception center, etc. These days it is also useful to appoint someone who can handle the press correctly. Every day information will be exchanged between the various teams and the latest developments will be reported to the WHO in Geneva.

Vaccination

In the most recent and future epidemics, vaccination of health care workers, ring vaccination and vaccination of populations at risk play a much bigger role than in earlier epidemics (cfr. above). More info can be found in the Strategic Advisory Group of Experts (SAGE) on Immunization document by WHO:

https://www.who.int/immunization/policy/position_papers/interim_ebola_recommendations_may_2019.pdf?ua=1

Isolating patients

The patients' movements should be limited. They should be isolated (no direct physical contact with patients, blood, excreta etc.). Any newpatientmust be directed to a triage zone. Here, based on the history (contact with Ebola patients, fever, symptoms), patients must be divided into Ebola suspects and non-Ebola patients according to the predefined case definitions. Ebola suspects must be kept in isolation, awaiting results of their PCR. If fever came up less than 3 days ago and the PCR result is negative, the PCR test will be repeated after 2 days before a patient is considered definitely negative. Often contact with Ebola will not be reported due to superstition, fear of stigmatization or if there was sexual contact with a person who subsequently developed Ebola infection. The absence of a lab facilities on-site can be a practical problem for the clinicians working in the field.

Barrier nursing

During an outbreak, there is a crucial need to protect health care workers. The small inoculum and the high mortality rates despite (investigational) treatments impose a zero-tolerance practice. Personal protection (masks, goggles, aprons, boots, disinfection supplies) for medical staff and for people who care for the sick person in case of refusal to admission to an ETC (often family) is necessary.

Demonstration of how to use the protective equipment and proper explanation are imperative. Donning and doffing is done through standard operational procedures and under direct supervision of a team member. Personal protective equipment has many inconveniences, but none greater than heat stress, limiting the time that can be spent for caring patients under tropical conditions.

Reusable equipment should be disinfected rigorously with, for example, bleach (hypochlorite solution). Objects that cannot be sterilized must be burnt under supervision. People who are suspected of being infected with the Ebola virus should be cared for by people who understand and use personal protection. Basic needs (drink, food, pain-relief, hygiene, etc.) have to be met. Vomitus, sputum, faeces and urine must be collected in a plastic bucket and mixed with strong bleach before disposal.

Centers with a poor medical infrastructure and with a high risk of nosocomial transmission must be closed down temporarily. This applies both to large hospitals and small one-person clinics with only a few needles and syringes. Strict guidelines have to be issued to centers which continue functioning, particularly with regards to triaging of suspect cases, disinfection, the use of needles and syringes, vaccinations and surgical procedures. In many places non-qualified private individuals have only a few (non-sterile) needles and syringes, which they use for all injections.Ebola, field treatment.

Cfr. section on treatment above.

Surveillance and contact tracing

The goals of Ebola virus disease (EVD) surveillance are to promptly detect new, suspected EVD cases and deaths so as to trigger an appropriate response. Communities and local authorities should always report all deaths. In past epidemics a system of alerts was put in place. An alert is a condition that meets a very broad (sensitive) definition that aims to identify all signals that could potentially be an EVD case or death. Alerts can be generated by the community, at health facilities, or picked-up in the media.
Often checkpoints are put up at so called points of entry and exit. Alerts are reported to those in charge of surveillance through various means, e.g. a telephone hotline. If an alert is validated and a new case identified, it is primordial to establish the chain of transmission.

People who have recently had contact with Ebola patients but do not display symptoms have to be placed under supervision (surveillance) for 3 weeks, the maximum incubation period. A contact is defined as: any person who has been exposed to a suspected, probable, or confirmed case of EVD in at least one of the following ways:

- has slept in the same household as a case
- has had direct physical contact with the case (alive or dead) during the illness
- has had direct physical contact with the (deceased) case at a funeral or during burial preparation rituals
- has touched the blood or body fluids (including urine, faeces, vomit, tears, or sweat) of a case during their illness
- has touched the clothes or linens of a case
- a baby who has been breastfed by the case

Note: This should include health workers (including those involved in cleaning, waste management, laboratory technicians, nursing, etc.)

If symptoms arise, immediate investigations should be carried out. Each diagnosed patient has on average 10 to 15 contacts which are to be monitored daily for 21 days. In large epidemics, contact tracing and follow-up can be a vastly resource-intensive activity.

Convalescent patients

Ebola virus might persist up to several months in selected immunologically privileged body sites of survivors. Sexual transmission is possible up to 6 months or longer after clinical recovery. Male survivors and their partners should be counselled on safe sex practices for 6 months or until their seminal fluid is free of viral RNA.
Convalescent serum can be stored if necessary, even though studies with convalescent serum so far failed to prove survival benefit This serum has the possibility to produce monoclonal antibodies as was the case in mAb114 development.

Information

Nothing may be as important as community engagement and public perception. A part from education about the disease and control measures, populations should be encouraged to quickly alert authorities about febrile cases and unexplained deaths. Transmission of the disease will only stop when the community is no longer caring for the sick in unprotected settings and burying the dead in an unsafe manner. Trust is not always a given in an epidemic,

which was clearly shown in the 2018-2020 DRC outbreak, with several structures of the outbreak response attacked. A general large-scale information campaign with adequate and practical information for the population should be started. If this results in many questions and tips, a permanent center can be set up where information about possible new cases can be examined. In view of the extreme virulence, the incomplete knowledge about these pathogens and memories of the impact of the earlier plague and yellow fever epidemics, these pathogens can capture the imagination of the general public. Superstition and belief in witchcraft can lead to misunderstandings and violence. In an environment of mistrust towards the national or local government and towards international organizations, experimental countermeasures as vaccination experimental drugs can fuel rumours of unsavoury experimentation. Crystal clear communication about the ongoing interventions with transparent answers to questions are a prerequisite for the interventions to be successful.

Burials

The deceased should not be washed, and the bodies have to be isolated and buried as quickly as possible and reasonable. This sometimes causes problems with the family and acquaintances of the deceased because of the disruption of traditional rituals. The government has a role to play here in law enforcement avoiding the disrespect of cultural values as much as possible.

Social impact

Caring for orphans in the community should be organized if this does not take place through the traditional system of the extended family. The latter sometimes does not work because of fear, prejudice and practical problems.

Logistics

Logistics play a very important role and include, among other things, infection control, equipment and materials, administrative support, accommodation, money and wages, communication, transport, fuel, safety and stock management. Good management is essential and has to be entrusted to reliable people. The NGO Médecins Sans Frontières and Alima (Alliance for International Medical Action), an international non-profit medical organization have a lot of experience in handling the logistics of such operations. Specific "Ebola kits" of different sizes have been prepared and are kept in stock, ready to be used in emergency situations.

Personnel

Experts in various areas cannot, in most cases, make themselves available quickly for a long time and a rotation system should be organized. It is best if (international) staff do not change too frequently in order to achieve a minimal continuity locally. Realistic guidelines for cases in which medical personnel are infected accidentally must be drawn up. According to the scale of the outbreak, the need for formation of national and/or international staff should be assessed.

Epidemiology

Epidemiological research should attempt to identify transmission routes and secondary cases. Risk factors for infection should be identified: unsafe burials, screening systems in health

facilities, fear/mistrust in the community. An attempt will also be made to trace the first case in order to understand how the chain of infection started. However this person may well have died. Several people, such as customers, work colleagues, neighbours, family and friends may be able to provide useful information. A reminder of the terminology: the index case is the patient in whom the disease first indicated the existence of an outbreak. The index patient always remains the same person irrespective of whether earlier cases are discovered later. The very first case is called the primary case, not the index case. Later secondary, tertiary, etc. cases can follow. The first case might change over time with incoming retrospective data, whilst the index case of an epidemic will never change in the future.

Reservoir

Because an animal reservoir is assumed to exist where the virus "hides" between epidemics, extensive attempts have been and are being made to identify this. An "ecological" team should be exclusively involved in this and will study different animals in the vicinity. An investigation should also be carried out into whether the virus is "exported" from the isolation units in the hospital to the environment. In addition to the fieldwork itself, there then follows the tedious analysis of the various potential hosts, both for the presence of the virus and their taxonomic identification.

Laboratory

Rapid sample analysis (blood samples of patients, samples of other body liquids, etc.) and rapid transmission of the results is recommended. Logistical problems can hinder this. Investment in research and cooperation will pay dividends.

Looking for isolated cases

The maximum known incubation period is 21 days. After the end of the epidemic (no more cases for a minimum of 6 weeks), surveillance can be carried out locally. It is possible that isolated cases and limited outbreaks occur. In order to obtain a better understanding of this disease, long-term surveillance is necessary. Regular flare-ups of the disease in the aftermath of the devastating epidemic in Guinea, Sierra Leone and Liberia, were seen due to sexual transmission, even several months after the declaration of the end of the epidemic.

Future prevention

We do not know how all epidemics started, but several followed the consumption of infected apes. The risk of nosocomial transmission is clear. Owing to modern rapid means of transportations, Ebola fever can emerge anywhere in the world. Naturally this does not only apply to Ebola, but to the whole spectrum of communicable diseases.

Poliomyelitis

Summary
• Poliomyelitis: enteroviral infection with faecal-oral transmission
• There are three related polioviruses, but no immunological cross-protection
• Often asymptomatic infections
• Sometimes flu-like syndrome with muscle pain and fever
• In a minority of patients, flaccid paralysis with sensation intact follows
• Vaccination is very effective as prevention
• Wild-type poliovirus serotype 2 and 3 were last detected in 1999 and 2012 and declared eradicated in 2015 and 2019, respectively
• Emergence of vaccine-derived (reverse mutation) pathogenic viral strains can cause outbreaks in under-vaccinated populations
•Anno 2024, the Global Polio Eradication Programme has not yet achieved the final goal of eradication

General

Poliomyelitis is a disease caused by a picornavirus (family enteroviruses). There are 3 different polioviruses, i.e., serotypes 1, 2 and 3. They do not exhibit immunological cross-reactions.
Chimpanzees, Rhesus monkeys and cynomolgus monkeys (syn. Macaca fascicularis) can be infected orally and suffer paralysis as a result, but in practice, man is the only reservoir. Chronic latent carriers are very rare, but healthy and immune-compromised long-term virus shedders were identified, excreting poliovirus for more than 20 years. The epidemiological importance of these people is not yet known. The virus does not survive in the wild –ex. sewage and surface water- for more than a few months, and then only under conditions of low temperatures and high humidity.

Historical Note

Poliomyelitis has been known since ancient times. An Egyptian carving from 1580 BC shows typical sequelae of poliomyelitis. At present, it is in the Carlsberg Glyptothek Museum in Copenhagen, Denmark. A 3,500-year-old Egyptian limestone funeral stela represents a man called Roma, giving offerings to the Goddess Astarte. He was a doorkeeper of the Eighteenth or Nineteenth Dynasty. He is portrayed with a wasted and shortened leg accompanied by an equinus foot deformity. The most likely diagnosis is that these are sequelae from poliomyelitis acquired in childhood. The person appears with a stick which could be used as a crutch. His disability had clearly not prevented his attaining high office, marrying and having at least one child.

In 1916 there was a polio epidemic in the USA with more than 10,000 cases. It was noticeable that most victims with paralysis were found among those groups of the population that observed the greatest possible hygiene precautions. It was referred to as a disease of cleanliness. At that time, the washing of the whole body (rather than just the face and
hands) and the installation of bathrooms in the houses of wealthy citizens was greatly on the increase. The improved hygiene ensured the infection did not develop at a very youthful age. The disease follows a more serious course when it occurs at a more advanced age when

children are no longer protected by maternal antibodies. Under poor hygiene conditions, children are infected at a very young age, a small proportion of whom will develop paralysis ("infantile paralysis"). In 1952-53 Europe experienced a very severe epidemic. North America also was not spared, with 55,000 cases in 1953.

In the early 1950s, Jonas Salk developed the first formalin-inactivated injectable vaccine. This became available in the USA in April 1955. This was followed in 1960 by the oral vaccine developed by the Polish clinician Albert Sabin. Important work was also undertaken by Dr Hilary Koprowski, head of the Wistar Institute in Philadelphia, in relation to an experimental oral poliomyelitis vaccine ("CHAT" vaccine). The incidence of the disease has declined very markedly since the introduction of vaccination. Because the only reservoir is the human being with no persisting infection and because of the efficiency of vaccination, it is an eradicable disease.

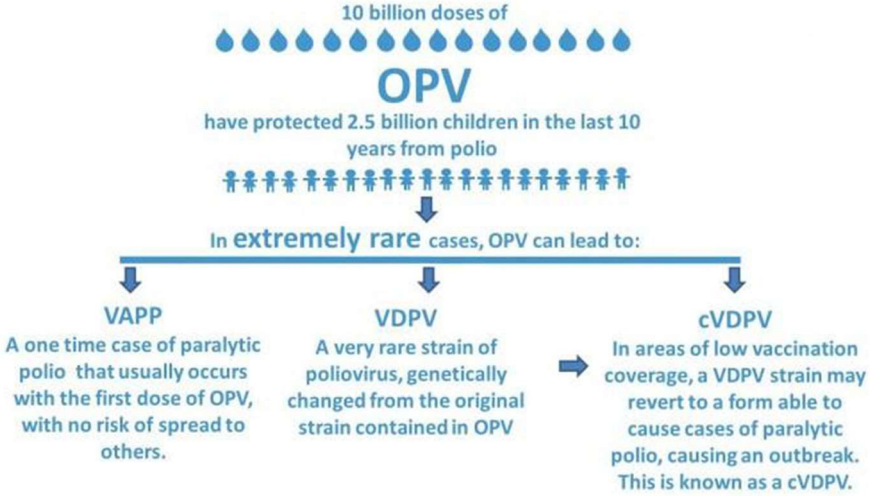
Though rare persistent carriers exist mostly due to an underlying immune defect. In May 1988, the WHO adopted a resolution to eliminate poliomyelitis ("Global Polio Eradication Initiative"). Since then, huge progress against the disease has been achieved, and until 2016 the number of paralytic cases was reduced by 99.99%, with 42 cases in that year worldwide. As of 2021, the wild-type virus has been found to circulate in Afghanistan and Pakistan only (and one single case in Malawi in
2021).

The hope that it would be possible to achieve the total eradication of poliomyelitis by the turn of the century has not become a reality. As long as a single person remains a carrier of poliovirus, children in all countries are at risk of contracting the disease. The poliovirus can easily be imported into a polio-free country and can spread rapidly amongst unimmunized populations. In 1988, 350,000 cases (1000 children per day) were officially reported in 125 countries. The number is steadily declining. In 1996, the figure was still 4,000 and there were less than 2,000 registered cases in 2002. The last countries where wild type poliovirus type 2 occurred were Afghanistan in 1997, Nigeria in 1998, and India in 1999. The last known reservoirs of type 2 were in Bihar, Uttar Pradesh and West Bengal. In 2001 the WHO reported that wild-type poliovirus type 2 had been eradicated worldwide. The last poliovirus serotype 3 was seen in 2012 in Nigeria.

In late 2005 however, **vaccine-derived polio virus type 2** reappeared in Nigeria. It was not the wild-type virus that reappeared but a pathogenic reverse mutant from the oral polio vaccine. The emergence of serotype 2 circulating vaccine-derived poliovirus (cVDPV) has complicated the epidemiology of polio. The type 2 component in trivalent OPV accounts for more than 90% of all cVDPV cases. By 2020, 24 cVDPV outbreaks have occurred in 21 countries, resulting in more than 750 cases of paralytic polio. The biggest risk factor for cVDPV emergence is low vaccination coverage. It will take many months for a cVDPV to emerge and cause an outbreak. These cVDPV outbreaks can become endemic and spread further in under-vaccinated communities and even to other countries.

Of note: **cVDPV** (circulating vaccine-derived poliovirus) is not the same as VAPP (vaccineassociated paralytic polio). The latter is caused by a strain of poliovirus that reverts to a neurovirulent variant following OPV administration. This causes a paralysis clinically

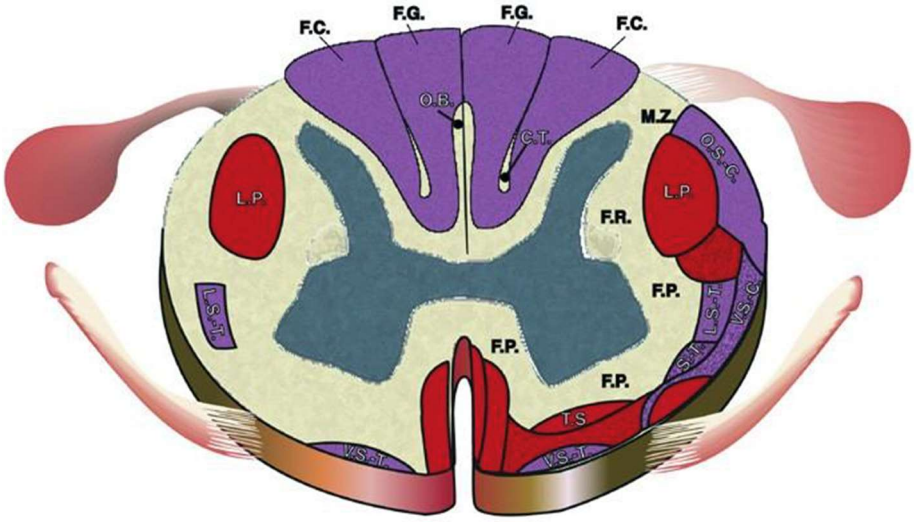
indistinguishable from poliomyelitis caused by the wild-type poliovirus. VAPP occurs in recently vaccinated patients and sometimes in close contacts of recently vaccinated persons (contact VAPP). The weakened virus in VAPP does not cause outbreaks. It is estimated to occur in 1 out of 2.34 million administered first doses of OPV.



Source: WHO, Polio Global Eradication Initiative

A recent successful event in the polio eradication program was the declaration of a world free of wild-type poliovirus type 3 in October 2019. Since 2012, the only wild-type poliovirus is type I, and today it is only circulating in Afghanistan and Pakistan. In 2019 176 WPV1 infections were diagnosed in Afghanistan and Pakistan, and 365 cases of cVDPV were diagnosed worldwide of which 40 in Afghanistan and Pakistan and 325 in non-endemic countries, mainly in sub-Saharan Africa. The militant Taliban claim that oral polio vaccination is a Western plot to sterilize Muslim children shows that success can be hampered if the 2019 numbers are compared with 2017: that year only 22 WPV1 cases were reported.

Pathophysiology



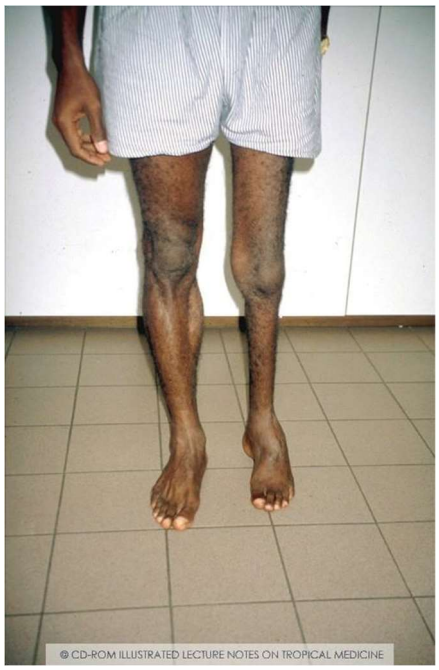
Polioviruses can attack motor neurons in the anterior horn of the spinal cord (shown) and in the bulbar area.

Transmission is fecal-oral by ingestion of contaminated food or water, and the incubation period is, on average, 3-6 days. The viruses proliferate in the intestinal mucosa. The virus can

still be found in the feces up to a few months after infection. The virus does not cause diarrhea. During the acute phase, it is also found in the throat. After passing into the body from the intestinal tract, the virus becomes localized in various tissues, such as the lymph nodes. On the cell membrane, the virus attaches itself to a specific protein: human poliovirus receptor (CD155). This protein belongs to the immunoglobulin superfamily and occurs in several tissues (brain, spinal cord, kidneys, heart, etc.). However, some cells that express the receptor appear not to suffer any adverse effect, probably because one of the subsequent stages in the intracellular replication of the poliovirus is blocked.

In a small percentage of cases, dissemination occurs to the central nervous system (it is “neurotropic”), where it can cause non-paralytic aseptic meningitis. In about 1 percent of infections, the virus spreads to the grey matter in the ventral spinal cord (“polios” = grey, “myelon” = marrow), where the motor neurons are found. These are nerve cells that transmit impulses to the muscles. These cells become damaged or destroyed. Damage often occurs at other sites but is usually less pronounced (medulla, cerebellar vermis, midbrain, thalamus, cerebral motor cortex).

Clinical aspects



Poliomyelitis, atrophy of a leg. Such lesions tend to be asymmetric.
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The incubation period is 9 to 12 days (rare extremes 3-35 days). The infection can follow a variety of courses: asymptomatic, flu-like syndrome, aseptic meningitis or paralytic. The most common is an asymptomatic infection (72%). About a quarter of infected cases will develop a short-lasting flu-like syndrome with slight muscle pain: abortive poliomyelitis. In a small minority of cases (1%), non-paralytic aseptic meningitis occurs after this phase of minor signs and symptoms. Fever and muscle weakness can occur initially, sometimes with severe muscle pain. Highly characteristic is the fact that the muscle pain improves on movement. The reason is unclear. Between 1 in 50 and 1 in 500 infections subsequently progress rapidly to paralysis,

sometimes within a few hours: “morning paralysis”. This is an asymmetrical, flaccid (no tendon reflexes) and often ascending paralysis (exacerbation over a few days). Sensation is not affected. Sometimes the lesions may be localized at a higher level from the onset. Bulbar involvement (10% of the total number of those paralyzed) damages cranial motor nerves such as the glossopharyngeal nerve (9th cranial nerve, palate, swallowing problems), the vagal nerve (10th cranial nerve, including the recurrent laryngeal nerve) and the facial nerve (7th cranial nerve).

Occasionally the ocular muscle nerves – the 3rd, 4th and/or 6th cranial nerves – are involved. Hypoxia can occur as a result of involvement of the diaphragm and intercostal muscles. Even though the virus may affect muscles on both body sides, the paralysis is usually asymmetrical. Cerebrospinal fluid contains an increased number of lymphocytes. Recovery can take months, in the course of which marked hypotrophy of the muscles occurs. Mortality during the acute disease can reach 10% due to respiratory paralysis. The sequelae of spinal polio are often permanent if the affected nerve cells are completely destroyed leading to severe disability in a quarter of patients and mild disability in another quarter. In half the patients with spinal polio, cells are not completely destroyed and full recovery will take place with maximum possible improvement occurring within 6 months.

Bulbar versus pseudobulbar paralysis

Bulbar paralysis is caused by injury to the lower motor neurons (motor nuclei of the throat muscles), for example due to poliomyelitis or Guillain-Barré syndrome. Speech is nasal and the tongue is flaccid, atrophic and exhibits fasciculations. The masseter reflex is normal or absent. In pseudobulbar paralysis, there is damage to the higher motor neurons. Speech is monotonous and contains many high tones: spastic dysarthria ("Donald Duck" speech). The tongue is spastic and the masseter reflex increased. There is no atrophy of the tongue.

Dysphasia versus dysarthria

In the case of dysphasia, speech is abnormal in content, usually as a result of a cerebrovascular accident. The disorder may be motor-related in the event of injury to the posterior-interior part of the dominant frontal lobe (Broca aphasia: the patient recognizes an object but cannot say its name) or sensory as a result of damage to the temporal lobe (Wernicke aphasia: the patient does not understand the meaning of words).

In dysarthria there are difficulties of articulation, but the content of the speech is correct. Injuries to the 9th, 10th and 12th cranial nerves can result in dysarthria, dysphagia and nasal regurgitation.

Post-poliomyelitis syndrome

In some patients, progressive muscle weakness recurs several years after acute paralytic poliomyelitis in the muscle groups involved in the previous episode. Rapid fatigability, swallowing disorders, respiratory difficulties, muscle atrophy, discomfort and pain in muscles and joints can occur. This post-poliomyelitis syndrome affects one in three people who suffered paralytic poliomyelitis forty or fifty years earlier. It is apparently not caused by the reactivation of dormant polioviruses. The exact etiology is unknown. One hypothesis is that the symptoms are due to the natural progressive deterioration of the remaining motor

neurons. An as-of-yet unelucidated immunological mechanism might play a role. There is no specific therapy apart from muscle-strengthening exercises. Evidently, further study is necessary to understand this condition better.

Diagnosis

Most victims are children less than 5 years old. The diagnosis is clinical. A predominantly asymmetrical flaccid paralysis of sudden onset with decreased or absent tendon reflexes with normal sensation, normal level of consciousness and preceded by muscle pain is suggestive. Often there is fever and meningeal irritation. Routine laboratory tests show few abnormalities. Lumbar puncture suggests viral meningitis (lymphocytic pleocytosis). In a patient with symptoms suspect for poliomyelitis two stool samples and two oropharyngeal swabs should be obtained at least 24 hours apart in the two weeks after symptom onset. Reverse-transcriptase polymerase chain reaction (RT-PCR) for polio and nonpolio enteroviruses will be performed. Cell cultures will also be done if available. Serological tests with type-specific IgM on serum or on liquor can be performed but, like viral culture, are rarely available. WHO installed worldwide equipped labs for the investigation of acute flaccid paralysis cases (Collaborating Center for Reference and Research on Poliomyelitis). Confirmed cases are usually thoroughly examined with PCR to establish whether it involves a “wild type” virus or whether it is a reverse mutation of a vaccine strain. For every diagnosed wild-type poliovirus infection, about 2000-3000 asymptomatic carriers exist, underlining the importance of determining the source of the ‘outbreak’.

Initial differential diagnosis includes:

1. Guillain-Barré syndrome or acute inflammatory demyelinating polyneuropathy is characterized by an ascending symmetrical paralysis with sensory involvement (reduced sensation and paresthesia). A form predominantly affecting the cranial nerves also exists (Miller- Fisher syndrome). Respiration becomes affected in the late stages. Determination of the vital capacity of the lungs is important. In Guillain-Barré syndrome, the cerebrospinal fluid is very typical: large quantities of protein and only a slight increase in cell count. In early stages, the CSF can still be normal. In such cases, repeated lumbar puncture is essential one week later. In the case of poliomyelitis, there is pleocytosis (large numbers of lymphocytes) in the cerebrospinal fluid and a slight elevation of protein.
With poliomyelitis there is usually no progression of the paralysis after the 4th day, in contrast to Guillain-Barré.

2. Tick paralysis: some ticks secrete a paralytic substance in their saliva. This causes anascending symmetrical paralysis with paresthesia, often around the mouth. The paralysis gradually increases over 5 to 6 days. Removal of the tick produces a dramatic improvement within a few hours or days. There is no fever or pain.

3. Paralytic rabies: previous history of infected bite (sometimes the victim is unaware of the bite: role of bats in South America). Ascending flaccid paralysis with moderate sensory disorders and with a fatal outcome.

4. Acute transverse myelitis: transverse lesion with bilateral sensory and motor disorders below a certain level (spinal cord segment) with back pain and flaccid paralysis initially, and subsequently spastic paralysis and major sphincter disorders. The cerebrospinal fluid often

exhibits pleocytosis and a raised protein content. Consideration should also be given to trauma, herniated disk, acute schistosomiasis, compression by a spinal abscess or tumour (possibly acute symptoms from bleeding in a tumour), complications of brucellosis and Pott's disease.

5. Diphtheria: Caused by toxins secreted by Corynebacterium diphtheriae. Aerogenic transmission. Incubation period 2 to 5 days. Mostly throat infection, often with pseudomembranes, dysphagia, airways obstruction, markedly enlarged cervical lymphnodes and leukocytosis. This is followed by heart failure (myocarditis) around the 2nd week and sometimes peripheral paralysis (neuritis) around the 3rd to 6th weeks. The nerve damage often occurs first in the throat, palate and ocular muscles and may subsequently become generalized. Sometimes the infection is localized in the nose or skin, which then tends to follow a less aggressive course. Untreated patients remain infectious for 2 to 3 weeks. Treatment consists of antibiotics [(neo)macrolides or penicillins] and diphtheria antitoxin.

6. Buckthorn poisoning. The clinical picture which follows ingestion of the berries of buckthorn (*Karwinskia humboldtiana* and *K. calderoni*) resembles poliomyelitis and Guillain-Barré syndrome. The neurotoxic effects of this plant are well known in Mexico and Central America (Nicaragua!) and consist of an ascending symmetric flaccid paralysis, often leading to bulbar paralysis and death.

7. Botulism: Intoxication by neurotoxins (type A, B or E) secreted by a Gram-positive anaerobic bacterium Clostridium botulinum. The toxins (zinc endopeptidases, cf. tetanus) bind presynaptically, interfere with the neurotransmitter vesicles and thus prevent the release of acetylcholine. Botulinum toxins B, D, F and G cleave synaptobrevin. Botulinum toxins A and E cleave SNAP-25 and botulinum toxin C1 cleaves syntaxin (all vesicleassociated proteins). The organism (or heat-resistant spore) can be found in a wound, the colon or in food. The role of food is reflected in the name of the disease: "Botulus" = sausage, after an incident in the 18th century in southern Germany. Bacteria can proliferate in anaerobic conditions and secrete toxins. If this happens and people eat contaminated food, disease will follow. After 12-36 hours a bilateral symmetrical and descending flaccid paralysis occurs with hypotension, dry mouth, ptosis, diplopia with dilated pupils and no light reflex, constipation and often distended abdomen (ileus) and urinary retention. Bulbar paresis with dysarthria and dysphagia may be particularly apparent and result in aspiration pneumonia.

Respiratory paralysis can follow. Tendon reflexes are impaired or absent. There is normal sensation, no fever, normal consciousness, normal cerebrospinal fluid. There will be normal base line laboratory test results. Wound botulism causes no intestinal symptoms. A rapid improvement of the symptoms is obtained within a few hours with anti-ABE antitoxin (horse serum; 1 ampoule IV and 1 IM, repeated if no improvement after 2-4 hours), but complete recovery is usually very slow (weeks to months). It is a rare condition and it is difficult to diagnose. Specific EMG patterns can be detected (including a reduction in muscle action potentials at low frequency stimulation and posttetanic potentiation, i.e. increase in muscle action potentials after high frequency stimulation or maximum voluntary muscle contraction for 30-60 seconds). An EMG as well as specific bacterial cultures and bioassays in mice to detect toxins are in general not available in developing countries. Even in well-equipped

medical centres, confirmation of botulism is difficult. Botulinum toxin is used in medicine in a number of different indications, such as for the control of spasms in superficial muscles (e.g. around the eye, blepharospasms), in focal dystonia, in chronic anal fissures, in achalasia and Chagas' disease and even in axillary hyperhydrosis. In this last case, injections of botulinum toxin A are used. The toxin blocks the release of acetylcholine at the neuromuscular junction but also at the cholinergic autonomic nerve endings (reduced sweat production as a result).

8. Myasthenia gravis and Lambert-Eaton myasthenia syndrome (e.g. in bronchial carcinoma) can also cause paralysis of the ocular muscles, ptosis, facial muscle weakness and swallowing difficulties, but the course is slower. If available, an EMG and an edrophonium test (Tensilon®) are useful.

9. Viral meningitis. If the clinical presentation is that of acute viral meningitis, mumps, Coxsackie A7, Enterovirus 71 and echoviruses should be considered in addition to infection with poliovirus. Naturally this can only be established by a sophisticated laboratory.

10. Enterovirus 71 deserves some additional comment. It was first isolated in a cell culture from a child with encephalitis in California in 1969. It is easily transmissible, which is important for contact persons within a family. The virus can be isolated from stools. There were large epidemics in Eastern Europe in 1975 and 1978 and in Southeast Asia (Malaysia, Singapore, Taiwan) between 1997 and 2000. In most outbreaks hand, foot and mouth disease has been the dominant clinical presentation, although herpangina, interstitial pneumonia, myocarditis, intrauterine infection and neonatal hepatic necrosis occur sporadically. The virus is also neurotropic. In contrast to other enteroviruses, it can invade the ventral brainstem, cerebellum and spinal cord. This results in a spectrum of serious neurological syndromes, ranging from acute flaccid paralysis of one or more extremities, to cranial nerve paresis, tremors, myoclonus and ataxia. Acute pulmonary oedema is thought to result from the destruction of medullary respiratory and vasomotor centres, leading to central sympathetic activation with severe systemic vasoconstriction and pulmonary vascular overload. The nervous damage is due to direct invasion of the neurons by the virus, as well hypoxia. Children who had encephalitis and cardiopulmonary failure have a high risk of poor neurodevelopment and cognitive outcome. Nowadays epidemic paralytic disease is more likely to be caused by EV-71 than by polioviruses.

11. Acute beriberi (thiamine deficiency). Develops more slowly with muscle weakness and often also heart failure. Good response to thiamine. See chapter on nutrient deficiencies.

Differential diagnosis of Acute Flaccid paralysis, summary

1. Polio: CSF, lymphocytic meningitis, PCR, viral culture
2. West Nile Fever virus polio–like: CSF, lymphocytic meningitis, serology, PCR, viral culture
3. Enterovirus 71 polio-like: CSF, lymphocytic meningitis, PCR, viral culture
4. Guillain-Barré / Fischer: CSF, protein-cellular dissociation
5. Diphtheria: inflammation throat or nose, LN, cardiomyopathy
6. Botulism: dry mouth, constipation, blurred vision, mydriasis, toxin assay
7. Rabies, paralytic: CSF variable, consciousness variable, agitation, relentless progression till death, IF, PCR

8. Myasthenia crisis, Eaton: Tensilon (edrophonium) or neostigmine test, EMG, antiacetylcholine-receptor antibodies
9. Beriberi: cardiac, subacute, poor nutrition, vomiting, ethanol, thiaminases
10. Periodic paralysis: recurrent, induced by effort or sugars, K+ concentrations variable
11. Hypophosphatemia: alcoholism or rapid feeding after starvation, TPN
12. Tick paralysis: specific hard tick present, better after removal
13. Neurotoxic snake: history, ptosis, dysphagia, bite wound
14. Organophosphates: hypersalivation, cramps, diarrhoea, sweat, miosis, wheezing, bradycardia
15. Shellfish poisoning (PSP, NSP): ingestion shellfish, paresthesia, diarrhoea, ataxia, mydriasis
16. Buckthorn poisoning: ingestion *Karwinskia* berries, New World
17. Curare poisoning (medication): peracute, only parenteral, New World, consciousness OK
18. Fugu poisoning (TTX = tetrodotoxin): ingestion fish, Asia; paresthesiae lips, peracute paralysis, consciousness OK
19. TTX, other source: e.g. bite of blue-ringed octopus, see fugu poisoning
20. Thallium poisoning: gastro-intestinal, painful polyneuritis (esp hands and feet), diplopia

Treatment

There is no specific treatment for poliomyelitis. Symptomatic and supportive measures are necessary. Bed rest is compulsory at the beginning of the disease as physical activity may aggravate the nerve damage. Moist heat packs relieve muscle pain. Attention should be paid to the possibility of urinary retention due to bladder paralysis. Physiotherapy should be instituted 3 to 4 days after the regression of the fever and if there is no further progression of the paralysis.

Physiotherapy does not prevent the muscular atrophy that occurs as a result of denervation (destruction of the motor neurons). It does, however, maintain the muscles in a good state for the few regenerating neurons. In 1927, Philip Drinker and Louis Agassiz Shaw Jr invented the iron lung at the Harvard School of Public Health. After the widespread use of iron lungs from 1939 onwards and during the largest polio outbreak in the US in the 1950s, the death rate did not show the same strongly correlated increase anymore. This respirator allowed care for patients with paralysis of the respiratory muscles till the paralysis faded on average after one or two weeks. Although improving the survival of many patients, the iron lung also led to dramatic situations where paralyzed people had to remain immobile inside the respirator for the rest of their lives.

Prevention

General

In the first half of the 20th century, poliomyelitis was a major problem in the West. The development of vaccines had a dramatic effect in a very short space of time. In many countries, it has proved possible to eliminate poliomyelitis by means of a routine vaccination program.
Within a generation, the disease has virtually disappeared in the developed countries. In some countries where poliomyelitis still occurs, annual national vaccination days are held in addition

to the usual vaccination programs. In 1988, the Global Polio Eradication Initiative was launched and is led by five organizations: the WHO, the United States Centers for Disease Control and Prevention, the UN Children's Fund, Rotary International and the Bill and Melinda Gates Foundation.

Four pillars form the global eradication program:
• Routine infant vaccination
• Supplementary immunization activities in at-risk middle and low-income countries: doorto-door campaigns, eg a national campaign targeting all <5 years without regard to prior OPV immunization status
• Surveillance for acute flaccid paralysis (AFP)
• Mop-up campaigns: if a poliomyelitis patient or circulation of wild type virus of cVDPV is found, house-to house vaccination ("mopping-up" vaccination) is conducted over a large area.

As poliomyelitis is becoming increasingly rare, the importance of good surveillance is increasing. As poliomyelitis is becoming increasingly rare, the importance of good surveillance is increasing. Patients with acute flaccid paralysis form the basis for the detection of "possible poliomyelitis".

Acute flaccid paralysis surveillance is the gold standard for surveillance in the polio eradication initiative. Cases of acute flaccid paralysis (AFP) must be officially reported. It may be assumed that every year in a population approximately 1/100,000 persons under 15 years of age will develop an acute flaccid paralysis (non-poliomyelitis). Where possible, a stool specimen should be obtained for virus isolation in a regional Global Polio Laboratory Network laboratory. On top of the AFP investigation, environmental surveillance sampling sewage effluents in high-risk areas complements AFP surveillance. Organisms that are found here (e.g., mutated vaccine strains) can be tested for neurovirulence in transgenic mice that are carriers of the human poliovirus receptor. The rationale for environment surveillance is based on the characteristic poliovirus excretion pattern. Infected individuals excrete poliovirus in feces for periods of up to several weeks, whether or not they are symptomatic. Occasionally very long-term excretors will be encountered. As fewer AFP cases are to be expected, environmental surveillance may become more important to ensure early response, even before clinical cases re-occur.

In 1995, the "Global Commission for the Certification of the Eradication of Poliomyelitis" was established. This commission defined the principles, criteria and the process by which certification is to take place. All countries have to be able to show that they have stopped circulation of wild type virus. Certification, cannot be granted in less than three years from the last report of poliovirus. Each country should set up a national certification commission which should collect the necessary documentation. The national commission is not authorized to declare its own country poliomyelitis-free.

Vaccines
There are two types of vaccine:

Inactivated Polio Vaccine (Salk)

The injectable Salk vaccine (1955) or IPV (Inactivated Polio Vaccine) can be administered IM or SC and has the advantage of being heat-stable, which is important under field conditions. It can also be given to immune-depressed patients. It is more expensive than the Sabin vaccine. These vaccines protect a person against paralytic poliomyelitis but will not combat an asymptomatic infection in the intestinal tract. Vaccinees are still able to pass on the wild-type virus to their environment. With the killed vaccine, no reverse mutations can occur. In most high-resource countries, IPV is integrated in the routine childhood vaccination program.

Oral Polio vaccine (Sabin)

The oral Sabin vaccine (1960, OPV = oral polio vaccine) contains live attenuated viruses. It should be stored in a refrigerator. It is very efficient and cheap. It has the enormous advantage of being able to be administered without a syringe. While IPV only protects the CNS, the OPV also protects the digestive tract, thereby preventing the spread of poliovirus more effectively. If there is an intestinal infection (diarrhea) during the vaccination, the vaccine has less chance of success. The first dose may be given immediately after birth. This is then followed by three doses at intervals of one to two months. A booster at the age of 18 months and at 5 years is indicated. A return to neurovirulence is possible in the case of specific reverse mutations. After vaccination with OPV, the viruses can be excreted for a while and may protect other children as well. As an anecdote, the poliomyelitis virus was isolated from the preflight stools of the Apollo 11 crewmen after the crewmen had been given poliomyelitis boosters.

Vaccination risk and strategic implications

Questions are raised about the putative risks of poliomyelitis vaccination. In the 1950s, there was the so-called “Cutter” incident (named after the Cutter laboratory). Children who received the supposedly inactivated vaccine subsequently developed the disease. This was due to a problem in the filtration process in the preparation of the vaccine. Aggregates that still contained pathogenic viruses were not inactivated by the formalin and were not filtered out. This problem has obviously now long been corrected.

Between 1957 and 1960 the oral “CHAT” vaccine was used, particularly in Central Africa. The developer of this vaccine was accused of having used contaminated chimpanzee kidneys for this vaccine and, therefore of having started the AIDS epidemic. This, however has never been formally confirmed and this hypothesis has been rejected. Strains that were preserved from that time have been studied for the presence of HIV and all found to be negative.

The live viruses in the OPV differ from the pathogenic viruses through a small number of mutations. Occasionally reverse mutations occur, as a result of which a vaccine virus can recover its pathogenicity. Paralysis in receivers of the OPV vaccine can occur in two ways:

- Vaccine-associated paralytic poliomyelitis (VAPP): refers to the regeneration of neurovirulence that occurs spontaneously in a recently vaccinated person. This is very rare: for every million doses of OPV, there are between 0.09 and 25 cases of VAPP.

- Vaccine-derived poliovirus (VDPV): refers to the regeneration of neurovirulence over a longer period. There are different types of VDPVs:

• iVDPV: these are VDPVs that come from people with primary immunodeficiencies
•cVDPD: these are VDPVs that have been transmitted between people in the community and cause new outbreaks. They are found in individuals who are not direct contacts of poliomyelitis cases, or they can be detected in environmental samples (e.g., sewage water).

In populations with a high polio vaccine coverage, the risk of cVDPVs is very low. In the vaccines against serotypes 1 and 3, there are many genetic mutations that distinguish them from wild-type poliovirus strains. However, in vaccines against serotype 2, there are fewer genetic mutations distinguishing them from the wild-type virus, so the vast majority of cVDPVs come from OPV against serotype 2. cVDPV2 outbreaks occur mostly in communities with low coverage of vaccines for poliovirus serotype 2.

The prospects for eradication are good, but setbacks do occur. In the autumn of 2003, there was a dramatic increase in poliomyelitis cases in Kano, Nigeria. Early November 2003, there were already 217 cases on a global total of 491. Vaccination had been stopped due to false rumors, fear and mistrust between the Muslim population and the government. This created a very dangerous situation, even threatening the accomplishments of the global eradication campaign.
Over a year time, the virus spread far and wide to 20 different countries, from countries bordering Nigeria to even Indonesia. As an example, Somalia reported 73 cases in December 2005, after a three year period where not a single case was detected. Later it became clear that a small number of cases in Nigeria were due to the spread of a mutated vaccine strain. The financial price to quell this outbreak was very high, but we have to be prepared to go all the way to eradicate this disease from our planet.

Details on the Polio Eradication and Endgame Strategic Plan can be found at: http://polioeradication.org/who-we-are/

Switch from tOPV to bOPV and the future of OPV

Nearly all vaccine-derived polioviruses in circulation are of type 2. Administration of the trivalent oral polio vaccine (tOPV, containing the three living vaccine strains) led mainly to an immune response against type 2, but the OPV type 2 in tOPV decreased immune responses to type 1 and 3 polioviruses. In April 2016 a new bivalent oral polio vaccine (bOPV) has replaced tOPV in all OPVusing countries, which will decrease cVDPV cases and VAPP cases due to type 2 poliovirus. Along with this, WHO recommended the use of IPV towards serotype 2 instead of the OPV. However, OPV vaccines are much cheaper and easier to administer than IPV vaccines.

Given the WPV1 and WPV3 eradication, the use of all OPV in routine immunizations should be stopped and all countries will rely on IPV alone to prevent poliovirus after OPV withdrawal. As an intermediate step, high-risk countries can give ≥1 IPV dose in the routine immunization program on top of the bOPV immunization to maintain immunity levels to type 2 polio. Since 2019, all 126 countries that previously used OPV now use at least one dose of IPV.

Because of an uncertain risk of circulating vaccine-derived poliovirus (cVDPV) reemergence from residual oral poliovirus type 2 circulation and from immunodeficient, long-term

VDPVexcretors, two inactivated poliovirus vaccine (IPV) doses at 14 weeks and 9 months of age have been added to the recommended Expanded Program on Immunization (EPI) schedule for oral polio vaccine (OPV)-using countries in addition to routine bivalent OPV (bOPV) dosesat 6, 10, and 14 weeks of age.

This schedule provides seroconversion rates ≥97 percent of all three poliovirus serotypes by nine months of age.

At the same time, stockpiles of monovalent OPV type 2 are maintained to respond to future cVDPV2 outbreaks. Of course, the use of mOPV2 in response to VDPV2 outbreaks increases the risk of seeding new VDPV2 outbreaks. Therefore, since 2020 the novel oral poliovirus (nOPV2) is being rolled out to countries with cVDPV2 cases or outbreaks. nOPV2 is much more genetically stable and less likely to regain neurovirulence. It received a WHO Emergency Use Listing in November 2020 and has since been deployed in 21 countries.

Prevention, comparison of vaccines

Table: Advantages and disadvantages of dead and live attenuated poliomyelitis vaccine

Dead vaccine (Salk)	**Live vaccin (Sabin)**
Antibodies in the blood	Antibodies in the intestinal tract and blood
Since there is no immunity in the intestinal tract, wild type virus can still be transmitted faecal-orally	Vaccine can spread to the family: beneficial for immunity. Sometimes transmission of virulent mutant.
No mutation towards new virulense possible	Vaccine virus can mutate to neurovirulent type
Use permitted in immune deficiency	Possibly dangerioous for immune deficient subjects
Injection necessary	Oral administration
Booster vaccinations necessary for long-term immunity	Immunity for life
Higher seroconversion rates than OPV in low-income settings where enteric pathogens/pathology reduces the OPV efficacy	

Future

The GPEI (Global Polio Eradication Initiative) implemented the “Polio Eradication and Endgame Strategic Plan” and hopes to finance the last stretch of vaccination campaigns where polis is still endemic today: Afghanistan, Pakistan and Nigeria (Nigeria is declared free from wild-type poliovirus but remains endemic for cVDPV2). If poliomyelitis is successfully eradicated in the early 21st century, no wild-type virus will be present anywhere on the planet (except possibly for a few stocks in protected laboratories). However, OPV strains can revert to the wild-type form. Will the oral poliomyelitis vaccine ultimately have to be destroyed? How long should the injectable, dead vaccine be used and/or kept? Will silent long-term excretors start an epidemic decades after stopping vaccination? Some immunocompromised

people excrete wild-type or vaccine-type poliovirus for years (possible for life?). As long as this situation exists, it cannot be stated that the battle against polio has been won.

Rabies

Summary
- Rabies is caused by several closely related Rhabdoviruses
- Infection of the central nervous system: meningo-encephalitis
- Transmission via saliva of infected animals, very rarely aerogenic transmission
- Often long incubation time, usually 20-90 days, leaving a window for curative vaccination
- Muscle spasms, salivation, intermittent delirium, fever, hydrophobia
- If symptoms, 100% fatal
- Prevented by wound cleaning, antiserum and vaccination

General

The genus of Lyssaviruses (Gr. "lyssa" = madness) includes rabies virus and a few other related viruses (Mokola, Duvenhage, Lagos bat virus, European bat lyssavirus 1 and 2, Australian bat lyssavirus, Irkut virus, Aravan virus, West Caucasian Bat virus, Bokelo bat lyssavirus, Ikoma Lyssavirus, rabies virus, Shimoni bat virus). Mokola virus was originally found in Nigeria in various shrews (Crocidura).
Duvenhage virus was isolated from insectivorous bats in South Africa and Zimbabwe. They are all rhabdoviruses (Gr. "rhabdos" = rod, hence “rod-shaped viruses”), a term which is derived from their cylindrical, bullet shape under electron microscopy. Various subtypes can be distinguished with monoclonal antibodies and so the source of an infection can sometimes be traced.

Rabies is a viral infection that affects the central nervous system. It is a zoonosis affecting many mammals (dog, cat, fox, squirrel, ferret, skunk, raccoon, sheep, cattle, bat, etc.). Those which are mainly responsible for transmission to humans are dogs (Africa, Asia) and vampire bats (Central and South America). The latter feed mainly on cattle blood. Rabies infection produces a form of paralysis in cattle, sometimes leading to large losses of livestock. The virus is distributed world-wide except in New Zealand, West Malaysia and a number of islands such as Borneo, New-Guinea, Bali, Hawaii and Great Britain (although there was an endemic case in the UK in the early 21st century). Australia was rabiesfree until 1995, when a rabies virus was discovered in flying foxes in that country. The number of cases of rabies is estimated as >50,000 per year. Four million people annually receive post-exposure prophylaxis.

Transmission

Transmission occurs via the saliva of an infected animal as a result of a bite or of licking damaged skin or mucous membrane. Infected dogs, raccoons, cats, etc., can be responsible for transmission. In dogs, the saliva becomes infectious at least 2 days before symptoms of the disease appear. In extremely rare cases, asymptomatic dogs can excrete the virus for years. In principle, infection can also occur via aerosol, but this is rare (limited to bat-infested caves, laboratories). Transmission by eating contaminated meat has been described in animals but is not (yet) known in humans. To date, a few cases of human-to-human transmission have been described, 1 ascertained bite transmission. Transmission via corneal transplantation and organ transplantation has occurred. Successful transmission experiments were conducted as early as the beginning of the 19th-century, when saliva from a person with rabies was

introduced into a healthy dog. Rabbits primarily exhibit paralytic rabies, which was of importance in the search for a vaccine (these animals were easier to study than convulsing rabid dogs).

The type of exposure according to WHO

Category 1 exposure: touching, stroking or feeding a suspect or sick animal or being licked on intact skin

Category 2 exposure: nibbling of unprotected skin, minor scratches without bleeding, being licked on damaged skin

Category 3 exposure: transdermal bites or scratches, exposure of mucous membrane to saliva; exposure to bat bites or scratches

Vampires

Bats are classified in their own order within the mammals. The Chiroptera (Gr. "cheiro" hand; "pteron" wing) includes approximately 900 species. An animal requires ± 20 ml of blood each day (half its body weight). In the wild, a vampire lives on average 10 years. Like its mythical human counterpart, the animal hunts at night. It lands in the vicinity of its victim (usually a
cow) and then carefully creeps up close. The bat licks the hair of the pelt of the cow and then cuts away a small area with its teeth. It makes a 5 mm shallow wound with its razor-sharp, self-sharpening incisors (only in Hollywood do vampires bite with
their canines). The animal licks up the blood that is discharged. The saliva contains anticoagulants (including a plasminogen activator that is being studied as a new thrombolytic). Rabies virus may be found in the saliva. Desmodus rotundus can also sometimes drink blood from humans and then usually bites the nose, ears or lips. While feeding, the bat urinates on its prey.
This probably helps it to find the animal again the following night. Vampires must have blood very regularly. They die if they cannot eat for two or three days. Vampires, are social animals and often regurgitate and share blood meal with their young and with fellow members of the same colony that have been unable to find any prey. As a result of this social behaviour, rabies
virus spreads to other animals within the colony. Rabid bats can attack other animals without provocation, including other bats and humans. Bats can be asymptomatic carriers or become ill, exhibit aberrant behaviour and die from the infection. Bats can also spread histoplasmosis through their faeces.

In addition to their known role as biologic vectors of rabies to humans and domestic animals and surra (Trypanosoma evansi) to horses and cattle, vampire bats can also be temporary biologic as well as mechanical vectors of Venezuelan equine encephalitis virus and foot-and-mouth disease. They are likely to be effective mechanical vectors if not biologic vectors of any bloodborne pathogen, including HIV. Besides rabies virus, other viruses ascribed to bats have proven pathogenic or fatal to people and domestic animals. Four species of Australian Pteropus bats in Queensland carry Hendra virus without developing symptoms. These bats disseminate virus in urine or amniotic fluid during birthing, and the virus is later ingested by pregnant horses that amplify the virus, which then spreads to people and causes a fatal pneumonia (13/20 horses were infected in a 1994 outbreak, which resulted in two human deaths).

Nipah virus, identified in urine and saliva of Pteropus bats in Malaysia, spreads the virus to pigs and destroyed that country's swine industry in 1998. The virus spread from pigs to hundreds of industry workers; approximately 40% of these workers died of severe viral encephalitis caused by the agent. The symptoms are similar to Japanese encephalitis.

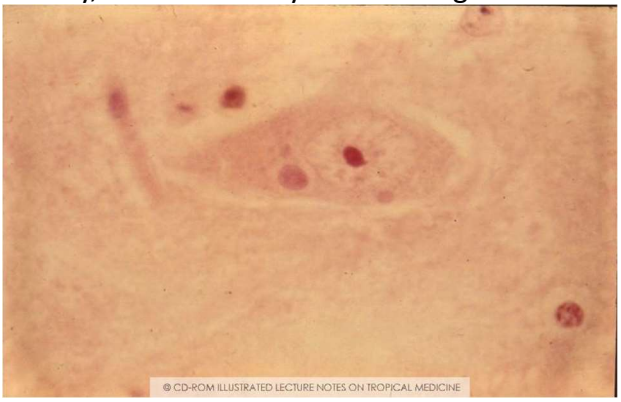
Pseudorabies

In veterinary medicine, rabies should not be confused with pseudorabies or Aujeszky's disease. This viral condition (Suid herpes virus) predominantly attacks pigs (the only natural host), but can occasionally affect cattle, sheep, goats, dogs, cats and wild animals. Infections can be latent or clinically manifest, including involvement of the central nervous system with
symptoms that include abnormal gait, intense scratching, self-mutilation, convulsions and death.

Pathogenesis

After the virus has entered the body, it multiplies locally in myocytes and afterwards crosses the neuromuscular junction to penetrate a peripheral nerve. The virus enters nerve cells through nerve spindles of sensory nerves or neuromuscular junctions of motor nerves. Subsequently it spreads along the nerve by retro-axonal flow to the spinal cord and brain. In the central nervous system, the virus proliferates further. The nucleocapsid of the virus can be detected by microscopy in some neurons as spherical inclusions in the cytoplasm: Negri bodies [Adelchi Negri 1876-1912, assistant to Camillo Golgi in 1900]. From there, the virus again spreads to almost all organs in the body. The saliva contains high concentrations of infectious virus.

The patient develops a very aggressive viral encephalitis. This is supposed to be 100% lethal, although there may be some exceptions. Very occasional cases of rabies survivors –with and without treatment according to the Milwaukee protocol- have been published, be it with severe sequelae. In 2012, it was reported that in a remote part of the Peruvian Amazon where rabies secondary to vampire bats is common, unvaccinated people had antirabies antibodies in their blood. It is still unclear what this finding means exactly. It is not known if these people developed minor symptoms and recovered, asymptomatically seroconverted after a very small inoculum with or without the help of bat oral flora, specific genetics in an isolated community, natural immunity, cross-reactivity with other germs or still something else.



Rabies. Some neurons contain intraneuronal inclusions which consist of viral nucleocapsid.

Clinical aspects

General

Incubation lasts 20 to 90 days (extremes of 4 days and 6 years have been described). Bites close to the face and with a large inoculum (severe wounds) are associated with the shortest incubation times. A prodromal phase lasting 2 to 10 days then follows. The first symptom is an influenza-like syndrome with moderate fever and malaise lasting a few days. This can be associated with severe local pruritus leading to scratching and excoriations, headache, pain or paraesthesia at the site of the bite. Sometimes there is moderate muscle weakness. Local myxedema after muscle percussion can occur. Agitation and insomnia can occur at a very early stage. Afterwards the disease can take two different courses, depending on which features predominate: furious rabies on the one hand (more involvement of the brain) and paralytic rabies (extensive involvement of the spinal cord) on the other.

Furious rabies

This form is more common accounting for about two thirds of the cases. There is increasing anxiety, excitation, hyperactivity, hyperventilation, disorientation and/or hallucinations. Symptoms occur intermittently and persist for 1 to 5 min, followed by a period of mental calm. Hyperstimulation occurs as a result of destruction of inhibitory centers in the brain stem. In approximately half the patients, painful spasms of the larynx and throat muscles occur (swallowing and vocal cord spasms). These are triggered for instance by seeing or wanting to drink a glass of water. This is associated with painful convulsive contractions of the respiratory muscles. The patient is therefore afraid of this situation (hydrophobia or fear of water). The spasms can also be induced by blowing air over the face (aerophobia) or by other, often minor, stimuli (compare with tetanus). The spasms develop into generalized convulsions. There is no trismus or muscle rigidity between convulsions (in contrast to tetanus). Neck stiffness can occur but is usually not pronounced. There is profound dysautonomia. The patient may sweat and weep profusely, as well as displaying hypersalivation, hypothermia, hypertension and tachycardia (involvement of the autonomic nervous system). Fever can occur. There is a pronounced thirst. The patient is in agony. Hypothalamic involvement can result in diabetes insipidus (insufficient ADH) or hypersecretion of antidiuretic hormone (SIADH). Myocarditis can cause cardiac arrhythmias. Coma follows within 10 days after the onset of the acute neurological symptoms and can persist for hours to months (mostly short-lasting). Finally, cardiac and respiratory arrest follow.

Death occurs in nearly 100% of cases, in general 2-7 days after the onset of the disease. Medical management can prolong survival up to 133 days.

In the whole of the medical literature (up to 2016), about 15 people have been described who survived clinical rabies. Of these survivors, several received immune prophylaxis. In 2004, a 15-year-old girl who was bitten by a bat in Wisconsin survived rabies after treatment with coma induction, ketamine, midazolam, ribavirin, and amantadine. Later on, several patients have recovered with this regimen, but many failures of this new regimen have also been seen.

An immune response is essential for recovery from rabies, although vaccine would not need to be given if at the time of diagnosis- a patient had developed already rabies virus-specific antibody (controversial).

Differential diagnosis of furious rabies:

Delirium tremens: chronic alcohol misuse and sudden abstinence, signs of hepatic injury (spider naevi, flapping tremor, gynaecomastia, collateral circulation, etc).

Reaction to some hard drugs (crack, speed). This occurs more often in some large cities.

Strychnine poisoning. This plant product suppresses nerve impulse inhibition and thus causes convulsions. All types of sensory stimuli can cause convulsions. Consciousness is clear if no asphyxia has occurred. It is sometimes used as a rodent poison. If the patient survives the first 24-hours, the prognosis is good. In the event of death, the rapid onset of rigor mortis is characteristic.

Acute psychosis and hysteria. Very common in developing countries. Hysteria: no hydrophobia if the patient is unaware of the existence of this sign.

Tetanus: portal of entry, trismus, muscle stiffness, convulsions on sudden stimulus, clear consciousness, mostly shorter incubation, no encephalitis, clear CSF.

Bacterial meningitis: lumbar puncture. Note that several organisms can cause lymphocytic pleiocytosis (*Brucella, Listeria, Treponema pallidum* (syphilis), *Borrelia,* tuberculosis, *Coxiella burnetii,* various rickettsiae, etc.). Various systemic fungal infections, sarcoidosis, auto-immune diseases (S.L.E.) with cerebral vasculitis etc. can produce abnormal cerebrospinal fluid.

Cerebral abscess. As a result of septic emboli (subacute bacterial endocarditis) or from penetration of a collection of pus (sinus, middle ear, etc.). Cerebral toxoplasmosis is common in AIDS.

Viral encephalitis due to herpes simplex or an arbovirosis such as Japanese encephalitis, West Nile fever, tick-borne encephalitis or Venezuelan equine encephalitis. Often no virus can be found. There are no lucid periods and no typical spasms. For arboviral infections, serology is important. Infections with Herpes virus B (Herpes simiae virus) are rare. This virus can be transmitted via a bite, scratch or via body fluids from an infected monkey. Mucocutaneous lesions and encephalitis can follow inoculation. (Val-)acyclovir or ganciclovir can be tried in treatment, but the infection provokes dramatic neurological symptoms.

Cerebral malaria (Plasmodium falciparum)

Post rabies-vaccination encephalitis if vaccination has been given with the old nerve tissue based vaccines.

Bite of a cobra or other elapid snake: saliva will dribble out of the mouth as a result of throat paralysis (not from spasms). Ptosis, swelling, pain and tissue injury at the site of the bite.

Paralytic rabies

This is the most frequent form after a vampire bite (South America). There is a flaccid paralysis (no tendon reflexes). There are often mild sensory disorders. The paralysis often begins in the bitten part of the body and then ascends further. Death follows from general paralysis. The course is less rapid than in the furious form.

Differential diagnosis paralytic rabies:

Polio: initially fever and muscle pain, asymmetrical paralysis, clear consciousness.

Guillain-Barre syndrome: ascending symmetrical paralysis, typical cerebrospinal fluid with large amount of protein but few cells. Early in this syndrome, the CSF might still be normal. Control lumbar puncture some days (up to a week) later then shows the albuminocytological dissociation. There are variants in which the cranial nerves are primarily affected (Fischer syndrome). It should be noted that initially the cerebrospinal fluid can be normal, but very quickly the protein level in the CSF will raise substantially. Often the syndrome follows one or more weeks after *Campylobacter* enteritis or another infection.

Botulism: descending paralysis (ocular muscles, throat muscles, neck, other muscles, progressive respiratory paralysis), no fever, dry mucous membranes, large pupils. Is caused by toxins produced by a specific bacterium (*Clostridium botulinum*), related to the organism that causes tetanus. The organism can be found in a wound or more often in spoilt food.

Diphtheria: is rare but poses few diagnostic problems in general in case of throat, nose or laryngeal infection. Extensive membrane-like coating in the throat ("diphthera" = leather) with marked cervical lymph node enlargement. This is followed 1 to 2 weeks later by carditis and progressive paralysis, sometimes also with sensory disorders (peripheral neuropathy). Cutaneous diphtheria produces painful wounds but rarely paralysis.

Bite of an elapid snake (e.g. cobra): rapidly occurring descending paralysis + local reaction at the site of the bite.

Metabolic / hypoxic / toxic encephalopathy

Reye syndrome: sudden onset, often after an initial viral syndrome. Vomiting is frequent. There is hepatomegaly in 40% of cases and liver function tests are abnormal. A liver biopsy is diagnostic.

Differential diagnosis of dysphagia:

Determine whether there is fever, whether pain occurs on swallowing and whether the dysphagia is high (throat area) or low (retrosternal). Include visual mouth examination.

Foreign body in the throat or oesophagus: sudden onset, history of swallowing fishbone, chicken bone or hard piece of food, feeling that "something is stuck in the throat", no fever if no complications. Beware of perforation of the oesophagus by sharp objects such as toothpicks. Mediastinitis can follow.

Neurotoxic snake bite: progressively worse, ptosis, cough and speaking becomes difficult, history of snake bite, often pain and swelling at the site of the bite. No fever if no complications.

Infection of the mouth or throat (viral, Candida, streptococci, abscess, aphthous stomatitis, etc.): painful, acute, visibly red throat/tonsils/abscess, fever, lymphadenopathy.

Diphtheria (is reported separately because of its important nature): inflamed throat with grey membranes (sometimes skin wound), lymphadenopathy, neuritis, sometimes with regurgitation of drink or food through nose, visual problems, paralysis and/or carditis, often no history of immunization.

Rabies: history of animal bite, signs of encephalitis with episodic hyperactivity and paralysis, sometimes hydrophobia. Fluctuating consciousness.

Tetanus: begins over the course of several days, often recent wound, no immunization, the mouth cannot be opened wide, muscle spasms over the rest of the body. Temperature fluctuating. Normal consciousness.

Neurological disorders with paralysis of the palate (e.g. bulbar poliomyelitis, bulbar tumour).

HIV with candidiasis of the oesophagus: check other clinical indicators, positive HIV test.

Oesophageal disorders such as **Chagas disease, stenosis, achalasia, tumours.**

Diagnosis

The diagnosis is clinical. Rabies must be suspected in someone who develops neurological symptoms a week or more after an animal bite. The number of white blood cells in the peripheral blood is normal or slightly raised, with a slight elevation of monocytes. Albuminuria can occur. An EEG shows abnormalities consistent with encephalitis. A CT scan or NMR scan of the brain can show surprisingly few abnormalities. Hydrophobia occurs in approximately half the patients and is pathognomonic (i.e.: highly specific). Investigation of contact with animals is important, but no history of exposure can be found in 20% of patients. The protein content in the cerebrospinal fluid is usually normal and in the first week of the disease the white blood cell count in the CSF is raised in 70% of cases (highly fluctuating differential count). Antibodies in serum and cerebrospinal fluid cannot be detected before there are symptoms. Antibodies against rabies virus cannot be detected in most laboratories in the tropics. The virus may be detected in corneal smears. The test is highly specific, but there are many false negatives and in most cases the technique is not available (fluorescein-conjugated antirabies serum). The virus is sometimes detectable by immunofluorescence in a skin biopsy, which is best taken from the neck (many hair follicles surrounded by nerve endings). Isolation of the virus from saliva, urine and cerebrospinal fluid (not from blood) is possible, but in tropical practice not feasible. The best technique is reverse transcriptase PCR on saliva (detection of rabies RNA). After death, the diagnosis can be established retrospectively by a brain biopsy. Negri bodies (intraneuronal inclusions consisting of viral nucleocapsid) are detectable in 80% of patients. All in all, rabies is a clinical diagnosis, but this has to be

supported with arguments, such as:

1. RT-PCR on saliva for rabies RNA
2. Virus isolation from saliva or cerebrospinal fluid
3. Corneal smear for rabies virus antigen
4. Antibodies in serum
5. Skin biopsy for immunofluorescence

Rabies in the animal

The incubation period in dogs is 2 weeks to 6 months. Rabies in dogs (and also in cats and horses) leads to changes in behaviour, aggressiveness, running away from home, difficulty in swallowing with hypersalivation, and convulsions. The animal can exhibit a more paralytic presentation with dysphagia and a drooping lower jaw (more so in the fox and cattle). Sometimes the hind legs give way. The animal usually dies within 7-10 days. Rabies in animals is not universally fatal. In case of a bite from a dog suspected of rabies, the dog should be observed for ten days. If it exhibits abnormal behaviour, the animal's brain can be analyzed. Negri bodies will only be present if the animal has shown clear signs of rabies. If the animal is killed immediately, the opportunity of making or excluding a diagnosis via this method is lost. If state-of-the-art technical facilities are available, however, it is better to kill the animal immediately and to detect rabies virus in the spinal cord or brain. This will rarely be possible in tropical regions. If the animal cannot be found (e.g. after a scratch by a bat), treatment of the human victim should follow on the assumption that it was infected.

Cow dying of rabies. Copyright ITM

Treatment

Since there is quasi 100% mortality once symptoms have occurred, only palliative therapy can be given at that time: pain relief (morphine) and reduction of spasms (myorelaxants e.g. diazepam). In most cases, barbiturates and chlorpromazine are also given. Although no case of transmission from patient to medical personnel has yet been described, it is recommended that the patient should be isolated and staff should wear masks, goggles and gloves during the provision of care. Staff should also preferably be vaccinated, but that is not obligatory.
In 2004, Jeanna Giese became the first patient ever to recover from rabies without the vaccine. She survived with a treatment based on a chemical-induced coma and intense anti-excitotoxic strategy combined with antivirals. The treatment –called the “Milwaukee

protocol"- comprised ketamineinduced coma, together with high doses of benzodiazepines (midazolam) and supplemental barbiturates, ribavirin and amantadine. Normally, ribavirin penetrates little into the cerebrospinal fluid, but in rabies the permeability of the blood-brain barrier is higher. Ribavirin might also protect against rabies myocarditis. Amantadine (200 mg/day) has in vitro activity against rabies virus, and has intrinsic anti-excitotoxic properties. Ketamine is a non-competitive N-methyl-D-aspartate (NMDA)-receptor antagonist with specific activity against rabies in animal models. It is possible that the NMDA-receptor may be one of the rabies virus receptors. Ketamine inhibited the genome transcription of rabies virus and restricted viral spread in an experimental rat model. Benzodiazepines and barbiturates are gammaaminobutyric acid (GABA)-receptor agonists.

Unfortunately, attempts to replicate the successful (modified) Milwaukee protocol have been discouraging. In 2019, about 14 adequately documented survivors of rabies have been reported worldwide, five of them from India. Most survivors had received at least 3 anti-rabies vaccines initiated on the day of the bite. Almost all survivors have moderate to severe neurological sequelae with poor functional outcomes. The focus on treatment and management of rabies should not draw away attention from the core objective, which is unarguably the "prevention" of human rabies.

What to do after a potentially infected bite?

Clean the wound with a detergent (soap). It is extremely important to wash the wound immediately with soap and water for 15 minutes, because the virus is very sensitive to cleaning agents. Afterwards the should be disinfected with iodine/isobetadine or 60-80% ethanol. Oxygenated water or mercurochrome are not indicated. Leave the wound open afterwards (no primary closure of bite wounds).

Tetanus vaccination status should be checked and tetanus vaccination +/- immunoglobulins should be given if needed.

Antibiotics: All bites are by definition bacterially contaminated but do not always become infected.
Wound infection with Pasteurella multocida or Capnocytophaga canimorsus is frequent after dog or cat bites. Routine administration of antibiotics after bite wound is not recommended. Antibiotics should be given if there are clinical signs of surinfection (dolor, tumor, calor, rubor) or in severely contaminated wounds.

Evaluation of the contact of the animal with the skin
The measures to be taken following a contact with a possible rabid animal depend on the type of contact, the immune status of the patient, the endemicity of the region and the type animal.

WHO EXPOSURE RISK CATEGORIES	
Category I	• Tactile contact (stroking) or feeding the animal • Licking of the intact skin *In other words: no exposure*
Category II	• Gnawing the uncovered and originally intact skin • Superficial lesions from scratches or grazes, without bleeding. • Licking of non-intact skin
Category III	• Single or multiple bites or scratches that penetrate the dermis • Contact with the mucous membranes via the saliva after licking • Licking a grazed or broken skin • (Possible) scratches and bites of bats: often no visible lesion or the feeling of a bite

ANIMALS	CATEGORY I	CATEGORY II	CATEGORY III	IMMUNE SUPPRESSION CATEGORIES II and III	Rabies-PrEP in good order
WILD LAND MAMMALS SUCH AS FOX, WOLF, RACOON ...					
Endemic	None	SCHEDULE 2	SCHEDULE 3	SCHEDULE 3	SCHEDULE 1
Non-endemic: Suspected	None	SCHEDULE 2	SCHEDULE 3	SCHEDULE 3	SCHEDULE 1
Non-endemic: Not suspected	None	None	None	None	None
MONKEY (WILD)					
Endemic	None	SCHEDULE 2	SCHEDULE 2	SCHEDULE 3	SCHEDULE 1
DOG, CAT, FERRET					
Endemic	None	SCHEDULE 2	SCHEDULE 3	SCHEDULE 3	SCHEDULE 1
Non-endemic: (Imported fewer than 12 months previously)	None	SCHEDULE 2	SCHEDULE 3	SCHEDULE 3	SCHEDULE 1
Non-endemic: Suspected	None	SCHEDULE 2	SCHEDULE 3	SCHEDULE 3	SCHEDULE 1
Non-endemic: Not suspected	None	None	None	None	None
BAT					
Endemic and **non-endemic:**	None* or Schedule 3	SCHEDULE 3	SCHEDULE 3	SCHEDULE 3	SCHEDULE 1

• Schedule 1: vaccination Day 0 and Day 3 or intradermal vaccination (4x 0.1 ml) on Day 0
• Schedule 2: vaccination Day 0 (2x), Day 7, Day 21
• Schedule 3: RIG Day 0 + vaccination Day 0, 3, 7, 14 and 28
• Rabies PrEP in good order: rabies pre-exposition prophylaxis before the bite (Day 0 and 7)

Human anti-rabies immunoglobulins (RIG (Rabies Immune Globulin) – e.g. Berirab®, Imogam RabiesHT®) are administered as soon as possible after the bite, whereby the largest possible amount is administered via a deep local injection in and around the bite with the aim of locally neutralizing the virus. The amount of MARIG depends on the anatomical location or locations of the injury and the size of the lesions, with a maximum dose based on body weight (20 IU/kg). MARIG administration at an anatomical site different from the site of the bite is no longer recommended. Following mucosal contact with saliva of a potentially rabid animal without injury (category III), MARIG is no longer indicated.

Vaccination in humans was first carried out by Louis Pasteur in 1885. Vaccination is possible in view of the long incubation time. Antibodies are present after 7-14 days. There are several

vaccines and therapeutic post-exposure vaccination regimens. WHO currently strongly recommends the safer modern cell-culture or embryonated-egg vaccines (CCEEV's). CCEEVs contain inactivated rabies virus that has been grown in embryonated duck or chicken eggs or in cell culture (e.g. primary chick embryo cells, Vero cells or human diploid cells). The viral harvest is concentrated, purified, inactivated and lyophilized. In some CCEEVs, human albumin or processed gelatine is used as a stabilizer.

If rabies immunoglobulins are not available, schedule 2 is always the preferred schedule (2 injections on day 0, 1 injection day 7 and 21). If it is not possible to complete the full schedule with the same brand of vaccine, another brand may be used. The WHO has documented the interchangeability of Verorab®, Rabipur®, HDCV Rabiës®.

Because preventive vaccination (pre-exposure prophylaxis) does not provide complete protection, PEP booster vaccinations are still necessary for vaccinated individuals following a type II or III contact: rabies PEP schedule 1.

Prevention

Do not touch any sick, paralyzed animals, or better still: simply never touch animals in the wild.

Kill stray dogs (sometimes problematical in Buddhist countries).

Vaccinate dogs (pets).

Vaccination of wild animals: for example in Switzerland and Germany foxes are vaccinated with oral live vaccine incorporated in fishmeal pellets or other bait. Vampire bats can be vaccinated by catching some and applying the live vaccine to the skin. The animals often lick one another and it would be possible in this way to vaccinate a colony of animals. The vaccine could also be applied to cattle.

Persons in high-risk occupations (e.g. veterinarians, certain laboratory personnel, medical personnel in the infectious diseases departments of hospitals), and certain travellers to high endemic regions) should receive PrEP vaccination. It could also be considered in populations living in rabies endemic areas, where the dog bite incidence is high. WHO currently recommends a 2-day intradermal regimen at Day 0 and Day 7.

HTLV-1 Infection

Summary
•HTLV-1 : chronic retroviral infection
•Importance in certain geographical foci.
•Transmission from mother-to-child (breast feeding), sexually or via blood
•Clinical aspects: 95% of infected people remain asymptomatic, 5% become symptomatic
•Opportunistic infections: Norwegian scabies, tuberculosis, Strongyloides stercoralis, etc.
•Inflammatory syndromes: uveitis
•Evolution to neoplastic diseases in a minority of patients: adult T-cell leukaemia / lymphoma
•Neurological syndrome with abnormal gait pattern and urinary incontinence (HAM/TSP)

General

The name retroviruses refers to the unique manner in which these viruses reproduce. Their genetic information is coded in RNA. This is not in itself unusual. However, they also possess an RNAdependent DNA-polymerase (reverse transcriptase) which produces DNA from the RNA strand. This DNA can be integrated at random into the host genome. Using this new DNA as template, mRNA can be transcribed and then translated into viral proteins. Such a flow of genetic information (from RNA to DNA) does not occur in other organisms so far as is known.

The family of Retroviridae contains three subfamilies: the Oncovirinae (with HTLV-1 as the most important representative), the Lentivirinae (with HIV as the most important virus) and the Spumavirinae ("foamy viruses"). The group of viruses known as the Primate T-lymphotrophic viruses (PTLVs) is composed of simian and human T-lymphotrophic retroviruses (SLTVs and HTLVs respectively). The viruses are genetically closely related. It has been shown that hunter-gatherers, when hunting monkeys and/or apes, are regularly exposed to the simian viruses. In urban bushmeat markets in Cameroon, about 10% seroprovalence was found in the hunted wild monkeys. Studies have shown that the diversity of HTLVs is directly related to the genetic diversity of the STLVs from which the primary zoonotic infection originated. The ease with which STLVs seems to be able to cross species barriers warrants increased surveillance of these viruses. HTLV-1 has spread to many parts of the globe and is associated with adult T-cell leukaemia and myelopathy/tropical spastic paraparesis. In 1982 HTLV-2 was isolated in a patient with hairy cell leukaemia. HTLV-2 is less pathogenic than HTLV1. More than 99% of infected individuals will remain asymptomatic but a minority will develop myelopathy/tropical spastic paraparesis. HTLV-3 and HIV turned out to be the same virus. Less is known about HTLV-4, which was identified in 2005 in Cameroon.

HTLV-1 is genetically very stable. This stands in marked contrast with HIV which is so variable that it is sometimes called a quasi-species.

Epidemiology

HTLV-1 was first isolated in 1980 from a T-cell lymphoma cell line, originating from a patient with adult T-cell leukaemia/lymphoma. It was the first time human retroviruses were shown to exist. Africa is the only continent where all different primate T-cell lymphocytotropic viruses have been found, from HTLV types 1 to 4, and the simian retroviruses STLV types 1 to 3. This

suggests that the virus spread from Africa to the rest of the world. On the other hand, HTLV-1 genetic sequences have been found in a 1500-old Chilean mummy.

The seroprevalence fluctuates widely from region to region. HTLV-1 is endemic in certain geographical areas, such as Taiwan and the Southwest of Japan, Okinawa, Papua New Guinea, Melanesia (Solomon Islands, Vanuatu), Australia (in Aboriginals), the Caribbean, West and Central Africa and the northeast of South America including Peru. In certain endemic areas, more than 1% of the population can be infected (e.g. Togo, Guinea-Bissau, the southern part of Cameroun). The highest prevalence is found in Southern Japan (up to 10%). Apart from Japan, Taiwan, a Chinese mainland province near Taiwan and Iran (seroprevalelce 0.1 to 1%), the infection seems to be rare in other parts of Asia, although more study is needed. For many African regions, there are no good prevalence data available at present. The virus also occurs in some other regions such as Italy, Romania, Israel and the Arctic, but is less common there. It is rare in the rest of Europe and North America, although there are some small foci among Native Americans. In the first decade of the 21st century, it is estimated that 10 to 20 million people are infected worldwide. However, few population-based studies have been performed therefore prevalence data may be lacking. Studying the prevalence in blood donors, pregnant women or other groups might bias the data. One also must check the diagnostic criteria. Different techniques and strategies can give rise to different results.

The virus

HTLV-I is a round, enveloped retrovirus which contains reverse transcriptase and integrase. Its genome is composed of positive single-stranded RNA. As with all retroviruses, this is converted to double-stranded DNA which is integrated into the host cell chromosomes. The virus exists predominantly as a cell-associated provirus and is transmitted as such. The plasma viral load is therefore often undetectable. Cytotoxic T-cells destroy infected cells by lysis. This results in the simultaneous production of inflammatory cytokines. The balance between these two processes leads to a more-or-less steady state in any given individual. HTLV-1 does not contain oncogenes. However, one of the viral encoded proteins induces abnormal cell growth. It blocks transcription of certain genes that are important for the control of the cell cycle, apoptosis and DNA repair. This results in mitosis without checking for chromosomal abnormalities. Genetically damaged cells with unstable chromosomes will not apoptose helping clonal outgrowth of these cells.

Transmission

The virus is transmitted by at least three different mechanisms:

From mother-to-child. Transmission via breastmilk is the major route. The infection is transmitted via infected lymphocytes in the milk. Intra-uterine and peripartum transmission appears to be rare (less than 5% of children with infected mothers). Children of seropositive mothers have an approximately 15 to 20% risk of infection if they receive long-term breastfeeding, as is normal in many regions.

Via sexual intercourse. This is bi-directional, yet transmission from man to woman is much more common than the reverse. After 10 years of sexual intercourse with an infected man, a

woman has a 60% risk of becoming infected herself. The risk in the reverse situation is only 0.4%. The presence of genital ulcers increases the risk. The risk for men who have sex with men increases greatly depending on the number of years that there has been sexual contact and on condom use.

Via infected blood transfusions or infected medical material, chiefly when cellular elements are present (plasma-derived products do not represent a risk). The infectious titre in the cell-free plasma is very low. Blood for transfusion which has been stored for longer than one week has nearly zero percent chance of transmitting infection, due to the lack of viable T-cells. Transfusion of contaminated blood results in seroconversion in more than 40% of patients. In endemic areas, candidate blood donors are screened for HTLV-1 antibodies.

Via contaminated syringes and needles. HTLV-1 infections are common among intravenous drug users in Brazil and New York, although in other North American and European IV drug users, HTLV-2 is more prevalent.

Clinical aspects

General

Several cell types may be infected, but T-cells are the most important of these. After infection various scenarios are possible:

1. latent infection without symptoms (the most common)
2. evolution to lymphoma / leukaemia
3. neurological syndrome with abnormal gait pattern and urinary incontinence
4. dermatitis
5. uveitis, arthropathy and other inflammatory processes, possibly with an auto-immune component
6. opportunistic infections

Latent infection

Infection may leave a person with a latent disease. He or she is infectious to others, but exhibits no symptoms or problems. During his or her life there is an approximately 90 to 95% chance that no complications will arise. If not tested specifically for this virus, the person in question will have no idea that he or she is infected. A number of seropositive individuals will be found by chance e.g. during the control of donor blood. There are some preliminary data suggesting that infection with HTLV-1 is associated with a lower risk for development of stomach carcinoma in Japanese patients.

Lymphoma/ leukaemia

ATL (adult T-cell leukaemia/lymphoma). There are several histological subtypes, but the diffuse large cell lymphoma is the most common. The lifetime cumulative risk is roughly 2% (1 to 5%). The tumours consist of monoclonal proliferation of CD4-positive T-cells. The clinical course may be acute, lymphomatous, chronic or smouldering. A fifth form, primary cutaneous tumoral ATL, has also been described.

If the course is acute and aggressive, nearly all patients will have lymphadenopathy and 50% will have hepatosplenomegaly. Skin lesions can resemble those of mycosis fungoides (cutaneous T-cell lymphoma). The dermal abnormalities include nodules, papules and diffuse infiltrative lesions. About 70% of patients develop hypercalcemia and osteolytic bone lesions. Approximately 10% exhibit involvement of the cerebral meninges resulting in muscle weakness, disturbed behaviour and/or headache. Oddly enough the protein content in the cerebrospinal fluid may still be normal, while at the same time containing ATL cells. Peripheral blood contains pleomorphic atypical lymphoid cells with basophilic cytoplasm and convoluted nuclei (so-called flower cells). During the leukemic phase, leukocyte count may increase dramatically. Acute ATL has a poor prognosis, with a median survival time after diagnosis of 6 months.

The lymphomatous form occurs in approximately 20% of symptomatic patients. The course is the same as in the acute aggressive form. There is also lymphadenopathy. Neoplastic cells are found in the blood, yet there is no lymphocytosis. The average survival time is 10 months.

In the chronic form the disease lingers for two years on average, without bone lesions, hypercalcemia or neurological involvement. There is lymphocytosis. There may be hepatosplenomegaly, lymphadenopathy, skin and lung lesions.

In the smouldering form the disease lasts for more than 5 years. In this form skin lesions, and to a lesser extent pulmonary lesions, are prominent, while hypercalcemia, hepatosplenomegaly and lymphadenopathy are absent. Transformation from a smouldering or chronic form to an acute form may occur suddenly.

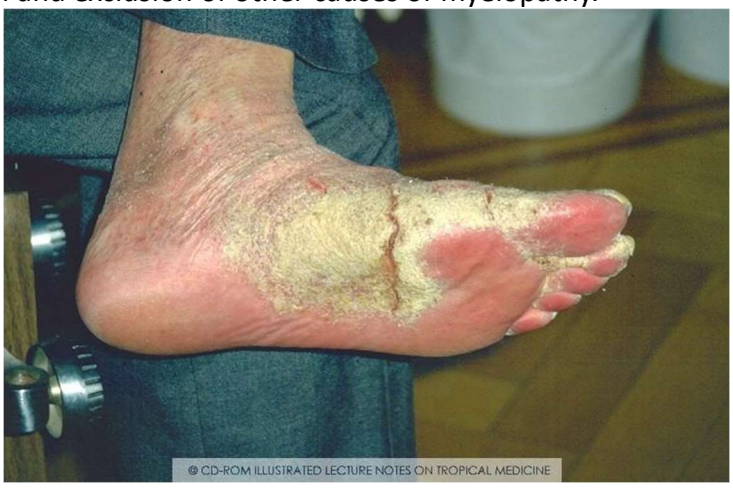
HTLV-1 associated myelopathy (HAM)

HTLV-1 associated myelopathy (HAM) is also known as chronic progressive myelopathy or tropical spastic paraparesis (TSP). This is a progressive hypertonic and ataxic myelopathy. The cumulative risk to develop this after infection with HTLV-1 is 2%. The disorder is more common in women. The main pathological feature of this condition is chronic inflammation of the white and grey matter of the spinal cord. Mononuclear cells, mainly T-cells, cause perivascular cuffing and infiltrate the spinal cord, which in a later stage will lead to atrophy. It is possible that there is an auto-immune component in the destruction of nerve cells (cross-reactivity between HTLV-1 antigens and tissue antigens). Patients with rapidly progressing HAM/TSP have a higher proviral load than those with slow progression.

Most damage occurs in the lower thoracic spinal cord. Weakness and stiffness of the legs, back pain and urinary incontinence together with abnormal gait pattern, are characteristic of the disease. Th sensory disturbances are usually limited, but there may be polyneuropathy with dysesthesia. Bladder disorders are an important cause of impairment among HAM/TSP patients. The course is progressive, so that many patients need to use a wheelchair and/or are bedridden within 10 years.

There is typical hypertonic symmetrical paraparesis or paraplegia with hyperreflexia and pronounced ankle clonus, for example when testing the Achilles tendon reflex or in sudden dorsiflexion of the foot. Babinski's sign can be elicited (spreading and extension of the toes instead of the normal plantar flexion upon stimulation of the sole of the foot). The reaction

corresponding to this in the hands is Hoffman's sign. The cerebrospinal fluid may show an increased protein content and may contain mild pleiocytosis with "flower cells", anti-HTLV-1 antibodies and oligoclonal bands. Definite diagnosis of HAM/TSP requires the demonstration of HTLV-1 infection and exclusion of other causes of myelopathy.



Norwegian scabies in HTLV-1 patient. Copyright Alexander von Humboldt Institute, Peru.

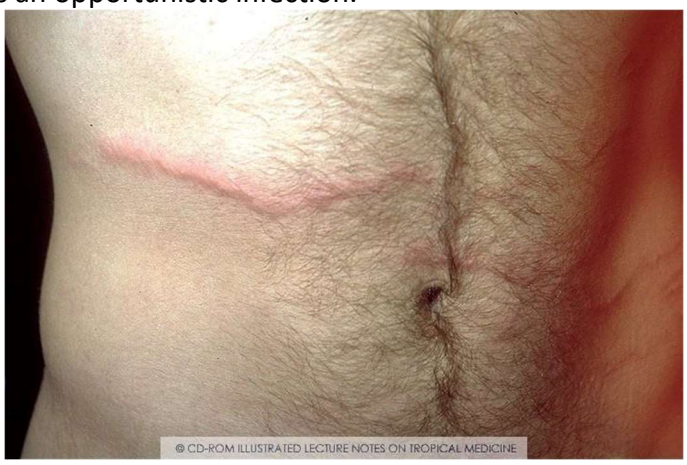
HTLV-1 dermatitis. Copyright Alexander von Humboldt Institute, Peru

Clinical aspects, miscellaneous

People infected with HTLV-1 have a high risk of dermatitis, often with superinfection by Gram-positive bacteria (*Streptococcus pyogenes* and *Staphylococcus aureus*). Lesions are often eczematous, and tend to be localized on the scalp, face (paranasal skin), ears, eyelids, neck, axillae and groins. Infective dermatitis is a chronic relapsing syndrome that mainly affects children. Co-morbidities include glomerulonephritis, bronchiectasis, lymphocytic interstitial pneumonia and anaemia.

Norwegian scabies present with massive crusted skin lesions, mainly in pressure areas. Opportunistic infections due to immunosuppression are common, including *Pneumocystis jiroveci* and systemic fungal infections. There is a risk of hyper-infection with *Strongyloides stercoralis*, especially in those who are

being treated with corticosteroids. Since the larvae mechanically carry bacteria from the colon, sepsis is common. Relapse after treatment with ivermectin is common. Herpes zoster is not so common as an opportunistic infection.



Strongyloides stercoralis, larva currens.
Copyright ITM

People infected with HTLV-1 have an increased risk for tuberculosis, and patients tend to have more severe lesions due to tuberculosis.

In regions where HTLV-1 is endemic, various inflammatory and auto-immune disorders, including uveitis, the sicca syndrome, pneumonitis, arthropathy and thyroiditis are attributed to this virus.

However, more research is needed into these matters. Patients with uveitis often present with blurred vision with floaters. Iritis and vitreous opacities are almost always present, often in association with retinal vasculitis, and sometimes with retinal exudates and haemorrhages. Bilateral lesions are as common as unilateral inflammation. The prognosis is good, since it tends to resolve spontaneously within weeks. Topical or systemic corticosteroid treatment hastens recovery. More than 90% of cases recur within 3 years. Complication include retinal degeneration, glaucoma and steroid-induced cataracts.

Differential diagnosis:

The differential diagnosis is broad. HAM / TSP is similar to multiple sclerosis, with a slow, gradual onset.

The disorder should be differentiated from lathyrism and konzo, both of which have an acute onset and are caused by toxins in the diet. The cauda equina syndrome, various neurodegenerative disorders such as amyothrophic lateral sclerosis, as well as infections such as syphilis, HIV, neurobrucellosis and tuberculous meningitis may be included in the differential diagnosis. The skin lesions are similar to mycosis fungoides (cutaneous T-cell lymphoma), leukemic skin lesions and those of non-HTLV-1related lymphoma.

Diagnosis

HTLV-1 is usually detected by carrying out serological tests because of clinical suspicion, screening at the blood bank or due to concerns by family members of HTLV-1 positive patients. Sometimes the diagnosis is made when a patient has a persistent Strongyloides stercoralis

infection (faeces with larvae, cutaneous larva currens or signs of hyperinfection). In the family history, which is important due to the mother-to-child transmission, it is often possible to find maternal family members who suffered from lymphoma or who were wheelchair users.

The antibodies can be detected by enzyme immunosorbent assay (EIA). Polymerase chain reaction (PCR) can provide a definite diagnosis. With real-time PCR the proviral load can be quantified as the number of HTLV-1 DNA copies per fixed number of peripheral blood mononuclear cells. This is often used as a marker for prognosis and diseases progression.

The test for HTLV-1 also detects the majority of HTLV-II infections. MRI [magnetic resonance imaging] or a CT scan shows speckled white abnormalities in the spinal cord. In chronic ATL absolute lymphocytosis is found (more than 3.5 x 109/liter). In the acute form of ATL the blood count will suggest leukaemia. Blood smears may contain abnormal lymphocytes with a highly wrinkled nucleus, called "flower cells". A skin biopsy shows the malignant lymphocytes.

Treatment

Oral or intravenous corticosteroids are used in the early phase of HAM/TSP, when inflammation is more prominent than demyelination. Motor disability, pain, and urinary dysfunction may be ameliorated, but improvement is not sustained in many patients. Valproic acid is an anti-epileptic with histone deacetylase inhibiting activity. It activates viral gene expression and exposes virus-infected cells to the immune system. Preliminary data show that the proviral charge initially increases, but subsequently decreases. Its exact place in therapy needs to be further clarified. Other drugs, such as interferon-alpha, daclizumab (humanized anti-Tac), plasmapheresis and intravenous immunoglobulins (IVIG) are used or being studied. The place of nucleoside analogues is unclear.
Hypercalcemia can be treated with cortisone and antineoplastic drugs. Calcitonin, for osteoclast inhibition, and etidronate or other bisphosphonates (also osteoclast inhibitors) will not usually be available. The tumour is initially highly sensitive to chemotherapy (e.g. CHOP [cyclophosphamide, hydroxydaunomycin/doxorubicin (Adriamycin), oncovin, prednisone]), but there is a serious risk of opportunistic infections. Relapse is common. Physiotherapy is important. For the hypertonicity, tetrazepam (Myolastan®), dantrolene (Dantrium®), baclofen (Lioresal®) and/or tizanidine (Sirdalud®) may be used. However, sometimes patients use their spastic legs as crutches and are able to walk. Antispasmodics can have the undesirable effect that walking in these patients suddenly becomes more difficult. In case of bladder hypertonia the patient is advised to begin bladder training. The idea is that the patient urinates at regular times, even if at that moment there is no urge to urinate. The intervals are gradually lengthened. In this way the bladder can become accustomed to retaining ever larger amounts of urine.

Prevention

Breastfeeding by infected mothers should be discouraged. Blood donors should be screened for the virus. As with HIV, safe sex also has a role to play here. The repeated use and certainly sharing of needles should be avoided. Correct cleansing and sterilization of medical equipment should be obligatory.

Bacteria

Regionally relevant pathogens

Anthrax

Summary
- Anthrax is caused by a large Gram-positive bacterium, Bacillus anthracis
- Bacterium can survive adverse environmental conditions as a resistant spore
- Can be used as a biological weapon
- There is no human-to-human transmission
- Pathology caused by powerful exotoxins
- Cutaneous anthrax: skin ulcers with oedema
- Respiratory anthrax: fulminant mediastinitis / pneumonia, meningitis, septicaemia
- Treatment with penicillin, ciprofloxacin, rifampicin, clindamycin
- Neutralisation toxin with antitoxin, e.g. raxibacumab or analogues

General

Anthrax is a widespread zoonotic infectious disease caused by a **large Gram-positive rod-sha**ped bacterium: *Bacillus anthracis.* Anthrax is usually a disease of herbivores. The animals are infected by grazing in an area contaminated with bacterial spores. Mortality in these animals is high and the carcasses will in turn contaminate the soil. This animal disease also affects man. People die not so much from the invasion of this pathogen but from the toxins that are secreted.

The causative agent of anthrax was identified by French biologist Casimir-Joseph Davaine in 1863 and by German bacteriologist Robert Koch, who isolated the organism in pure culture in 1876.

Toxin

The bacterium is surrounded by a polypeptide capsule (polyglutamic acid) that protects the pathogen against phagocytosis. As in other toxin-dependent diseases caused by Gram-positive bacteria such as tetanus or diphtheria, the pathogenesis of anthrax is attributable in the first place to **exotoxins** that are produced. Strains that cannot produce toxins are avirulent. The principal virulence factors of *B. anthracis* are coded on two **plasmids**; one involved in the synthesis of the capsule and the other coding the exotoxins.

The vegetative pathogen releases toxins that have a complex action. The exotoxins are binary and consist of a **B (binding) protein that is necessary for cell penetration and an A (active) protein that causes metabolic dysfunction**. There are three proteins: PA (protective antigen), LF (lethal factor) and EF (oedema factor).
LF is a zinc metalloprotease which kills cells by proteolytic cleavage of several members of the MAP kinase signal transduction pathway. EF is a calmodulin-dependent adenylate cyclase which catalyses the conversion of ATP to cAMP, causing an elevation in cAMP levels. This leads to pronounced oedema, inhibition of neutrophils and monocytes.

Anthrax spores

In certain circumstances *B. anthracis* can form an **endospore**. Spores like this are **very resistant to unfavourable environmental conditions**. The pathogen survives as a spore in the soil for many years, but seemingly less easily in acid soil than neutral soil.

Anthrax spore survival

This long survival was shown very clearly by experiments in the Second World War when Gruinard Island to the north-west of Scotland was deliberately contaminated with the pathogen in order to establish the effects on experimental animals such as sheep. Many years later viable spores of the bacterium were still found in the soil. This required a very aggressive
decontamination of the whole of the island in 1986.

In April 1979 there was a notorious accident in Sverdlovsk (now Ekaterinburg) in Russia, in which 66 people died from inhalational anthrax. It is now certain that the cause was an accident in a biological weapons installation of the Russian BioPreparat Programme. About 10 kg of anthrax spores (4 different strains) were released because someone failed to replace
a filter on an air vent. People were infected up to a distance of 4 km away from the installation. There were even cases in animals 50 km further away. All the cases occurred in a period of 6 weeks after the incident.

In the period 1979-80 there was an epizootic among cattle in Zimbabwe with about 10,000 infections (epizootic = "epidemic in animals"). Human cases were generally limited to cutaneous anthrax.

Clinical aspects

Cutaneous anthrax

If someone has contact with animal fur or skin in which there are anthrax bacteria, the skin can become infected. Infection can also follow a bite by an infected horsefly (mechanical transmission of the pathogen).

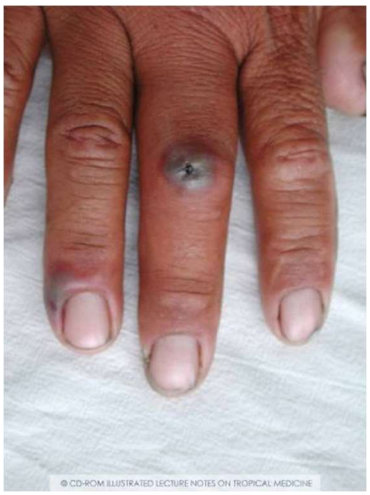
After a **short incubation period of 2 to 3 days**, a **small red skin wheal** occurs at the inoculation site. This can itch at first. Over the course of the next week vesicles form around the central lesion. Occasionally there are atypical cases without vesicles. A central painless ulceration follows. The ulcer is dry, with minimal or no pus. There is often a black crust, hence the name "anthrax" = charcoal. The ulcer is surrounded by red gelatinous local oedema, which sometimes becomes massive (e.g. lesions in the face / neck). Regional lymphadenopathy with lymphangitis and moderate fever can occur, but often the patient is afebrile. The pathogens can multiply in the lymph nodes. The regional lymph nodes are often painful. Superinfection by pyogenic pathogens is rare. There is no peripheral leucocytosis. The skin lesion heals slowly (2-6 weeks) in more than 90% of cases but in rarely there is progression of the infection, with systemic involvement. Without antibiotics mortality can be as high as 20 percent.

Cutaneous anthrax

- The lesion can be similar to the consequences of a bite by a *Loxosceles* spider. It is usually easy to distinguish from orf, since there is no oedema in this viral infection.
- Cowpox generally leads to less oedema.
- Herpes simplex can resemble cutaneous anthrax.
- Cat-scratch disease has a slower course.
- Cutaneous tularaemia can occur in similar circumstances (contact with an infected animal).
- A pyogenic lesion such as a furuncle is usually caused by *Streptococcus pyogenes* or *Staphylococcus aureus* suppurates and is painful.
- Ecthyma gangrenosum may occur in patients with neutropenia and/or Pseudomonas aeruginosa bacteraemia.
- Cutaneous leishmaniasis develops much more slowly and is not so painful

Diagnosis

A **Gram stain of a smear of the lesion** shows the typical large Gram-positive rods (1-1.5 x 4-10 µm). The bacterium is noticeably larger than most other pathogens. *Bacillus anthracis* is morphological very similar to *Bacillus cereus* and *B. subtilis*. These latter two pathogens do not however cause any lesions that may be confused with anthrax. If a person is infected; spores are not produced during the disease.
An alternative to Gram stain is polychrome methylene blue (M'Fadyean's stain). This stain is based on the use of an alkaline methylene blue solution in which progressive oxidative demethylation occurs on ageing. With this stain the bacterium is coloured blue-black. The **large size** and a somewhat **square, blocky appearance** are typical. A rose-coloured capsule can be seen with M'Fadyean's stain. A culture confirms the identity of the pathogen (wound culture, blood culture, CSF, biopsy). The bacterium grows easily under aerobic conditions on sheep blood agar. Bacillus anthracis forms typical large greywhite, tenacious, non-haemolytic colonies. If anthrax is suspected **the lab should be notified** as spores can form in a Petri dish, with risk for **transmission in the lab**. It is detected in a blood culture within 24 hours. It should be noted that the pathogen is Gram-positive in young cultures but can become Gramvariable afterwards. In aerobic cultures the pathogen soon loses its capsule. The absence of a capsule on for example sheep blood agar is therefore not an argument against *B. anthracis*. Other specific culture methods are necessary in order to demonstrate the capsule. Culturing in the presence of 5% CO2 on basal media with 0.8% NaHCO3 shows densely encapsulated bacteria, visible with India ink stain. The bacterium is not motile and can develop central or subterminal spores if the nutrients in the medium are exhausted. The bacterium **is usually sensitive to penicillin**, with a clear inhibition zone on an agar plate around the antibiotic disc. Serology (e.g. ELISA) can be carried out in order to detect antibodies against lethal toxin and oedema toxin but has **no place in acute diagnostics**. Serology is clearly less sensitive in cutaneous anthrax (67%) than in inhalational anthrax (94%). PCR and related techniques can be used for rapid identification. Using immunohistochemical techniques the pathogen, the capsule and polysaccharide cell wall antigens can be detected in tissue slices. There is a "Direct Fluorescent Antibody" (DFA) test that is used for rapid diagnosis of anthrax in exudates from skin lesions. The technique is not very sensitive for inhalational anthrax. Research into fast detection techniques of anthrax spores in micro-samples and dust clouds is ongoing, especially after 9/11 in the USA.



Cutaneous Anthrax. Copyright ITM

Pulmonary anthrax

If anthrax spores are **inhaled** ("woolsorters' disease"), after a short incubation period, high fever and dyspnoea occur. Once the bacteria have produced enough toxin (after 1-3 days), antibiotics are less effective. The primary lesion in inhalational anthrax is rarely in the nasal mucosa. For the first three days the symptoms are atypical; with fever, malaise, myalgia, a dry cough, chest pain, abdominal discomfort, nausea and vomiting. Then the disease develops dramatically. B. anthracis spores are phagocytosed by alveolar macrophages and transported to mediastinal lymph nodes. There they germinate, multiply, and release toxins, causing haemorrhagic necrosis of the thoracic lymph nodes draining the lungs, which results in a haemorrhagic mediastinitis and, in occasional cases, a necrotizing pneumonia. The organisms then become bloodborne, causing bacteraemia and in some cases meningitis. The clinical picture is that of a **fulminant pneumonia or mediastinitis** comparable with plague pneumonia (*Yersinia pestis*), pulmonary hantavirus, severe pulmonary leptospirosis, SARS, influenza or pulmonary tularaemia (*Francisella tularensis*).

A high fever, dyspnoea, stridor, cyanosis and shock characterise the course of the disease. Stridor is caused by extrinsic compression of the trachea by enlarged lymph nodes, mediastinal widening and subcutaneous emphysema of the chest and neck. Haemorrhagic necrosis of the hilar lymph nodes and mediastinitis follow. On a chest X-ray, **pleural fluid and a typical widening of the mediastinum** can be seen (DD of this important observation: post-surgical infection, rupture of an aortic aneurysm and contused chest trauma such as with deceleration lesions). Petechiae and splenomegaly occur. On a CT scan of the chest a widened mediastinum, pleural fluid and enlarged hilar lymph nodes are seen. These lymph nodes have about the same density as the aorta, which reflects the haemorrhagic-necrotic nature. **Mortality used to be very high** (almost 100%) but can be reduced to below 50% by starting aggressive antibiotic therapy quickly (see below) and raxibacumab.

Meningeal anthrax

The cerebral membranes can be affected, leading to a black haemorrhagic discolouring of the meninges. Red blood cells and many neutrophils, as well as the bacterium itself are found in the CSF.

This complication occurs with haematogenous dissemination in about **50% of inhalational** anthrax.
Only the vegetative pathogens (not the spores) are found in the CSF. Mortality is 75% within the first 24 hours after presentation and overall survival only 6%.

Gastrointestinal anthrax

Gastrointestinal anthrax tends to occur in family clusters or point-source outbreaks. After eating foodinfected with anthrax (for example an animal that has died from anthrax), infection of the throat or intestines can follow. Gastrointestinal anthrax is characterised by fever, **ulcerative intestinal lesions** in the caecum or terminal ileum, bloody diarrhoea and the development of shock. Hematemesis can be caused by bleeding stomach ulcers. Haemorrhagic mesenteric lymphadenitis with prominent ascites can occur. There is high mortality. Intestinal anthrax is rare. Differential diagnosis includes campylobacteriosis and yersiniosis. Necrotic enteritis (infection with toxicogenic Clostridium perfringens) or pigbel might be considered in malnourished patients presenting with acute necrosis of the jejunum, or more rarely ileum, caecum or colon.

The bacterium rarely causes inflammation of the throat (oropharyngeal anthrax), which can resemble diphtheria or plague with oedema, tissue necrosis and lymphadenopathy. On draining, the pus has a notably foul-smelling odour. Occasional cases of anthrax have been reported in IV heroin users. In these cases the heroin was apparently mixed with infected diatomaceous earth, resulting in soft tissue infections with a similar clinical picture to gas gangrene and mimicking necrotising fasciitis. Other soil bacteria such as *Clostridium novyi* have also been found in the same group of patients.

Treatment

For severe cases the **combination of several antibiotics which have complementary working mechanisms such as ciprofloxacin, rifampicin and clindamycin** has been suggested. With this combination therapy, mortality from pulmonary anthrax in the USA has been reduced to 40%. Meropenem can be added if the meninges are involved and linezolid is favoured over clindamycin because it is likely to have better CNS penetration. Other fluoroquinolones and doxycycline can also be used. Because of inducible beta-lactamase activity, monotherapy with penicillin G, ampicillin or amoxicillin is not advised. The pathogens may contain a natural cephalosporinase, so cephalosporins such as ceftriaxone or ceftazidime are not a good choice. The pathogen is resistant to aztreonam. Since the morbidity is largely toxin-mediated, there is a possibility that systemic administration of steroids may be beneficial but good data is lacking. Pleural fluid should be drained early and aggressively since it is associated with improved survival by reducing the toxin level and by decreasing mechanical lung compression.

In 2009, a single dose of **raxibacumab** (ABthrax), a human monoclonal IgG1 antibody directed against protective antigen, the binding part of the tripartite anthrax toxin, was shown to improve survival in two animal models of inhalational anthrax, where rabbits and monkeys were exposed to approximately 200 times the lethal dose of inhalational anthrax spores. This monoclonal antibody binds protective antigen with high affinity and blocks binding of the toxin to its receptor. Safety studies of IV raxibacumab 40 mg/kg in healthy volunteers showed a half-life of about 3 weeks. It had a good safety profile. **Obiltoxaximab** is a monoclonal

antibody against the protective antigen of B. anthracis. It is effective in animal models. Hyperimmune serum from vaccinated volunteers is beneficial in animal studies and seems promising in human infection: nineteen patients with anthrax were treated with anthrax immunoglobulin and antimicrobial therapy under an expanded access program. Three had inhalation anthrax, one had gastrointestinal anthrax, and 15 had injection anthrax caused by contaminated heroin. Of these patients, 13 survived, including two of the three patients with inhalation anthrax.

Cutaneous anthrax is treated for 7-10 days with fluoroquinolones or doxycycline, although wound cultures are often already negative after 24 hours. This rule of thumb applies to people who have contracted the infection for example by handling an infected animal.

Prevention

Cutaneous anthrax because of bioterrorism (where there is a possibility of aerogenic exposure) is treated for fully 60 days. If spores may have been inhaled, antibiotics should be used prophylactically for a period of two months. Ciprofloxacin 500 mg bid is a good first choice and doxycycline 200 mg/day is an alternative. These antibiotics do not kill the spores but the vegetative forms. It should be emphasised that the decision to administer preventive antibiotics is determined by the probability of exposure, and not by the laboratory results of the potentially infected person. In patients requiring post-exposure prophylaxis, vaccinations spread over 3 dose (0,2 and 4 weeks) can be considered. The carcasses of infected animals should be burnt, not buried. Vaccination for animals can be carried out with an acapsular, low-virulence strain (Sterne vaccine). There is a vaccine for humans, and it is used for example for vaccinating soldiers (USA). Six injections are needed, spread over more than a year. It is given at weeks 0, 2 and 4 with subsequent injections at 6, 12 and 18 months. Thereafter, annual boosters are needed. The effectiveness of this vaccine has been demonstrated by aerosol exposure of monkeys, where full protection was established after 8 weeks falling to 88% protection after 10 weeks.

Biowarfare and bioterrorism

Anthrax can be used as a weapon for **biowarfare and bioterrorism.**

Use of Anthrax in Wars

In the First World War an attempt was made in Scandinavia to infect horses and reindeer with sugar lumps containing anthrax spores. The animals were used for transporting the allies' supplies. Baron Otto Karl von Rosen was arrested in 1917, suspected of sabotage and spying for Germany. It was only 80 years later that it was discovered that the sugar lumps in his bag contained anthrax. So many years after the incident, the bacterial spores were still alive.

In World War II the American forces prepared thousands of small hay balls impregnated with anthrax spores. These were shipped to England with the intention of dropping them over cattle-breeding areas of the Axis countries in order to disrupt meat supplies in Germany. The weapon was never used.

Before the first Gulf War, Iraq made large quantities of anthrax. Here too the weapon was not used.

The toxicogenic bacteria can be cultured in vitro. To obtain a weapon that can be used in aerosol form, the formation of spores from the cultures must be promoted. The mass that is obtained is then freezedried and ground to a fine powder. Weapons-grade powder would be characterized by high spore concentration, uniform small particle size, particles with a certain electrostatic charge to promote mutual repulsion and an agent to prevent clumping. The spores display a tendency to stick together so that quite **large particles** are formed. Large particles do not stay airborne for a long time. Because the greatest danger comes from spores between 1 and 5 µm, which can reach the alveoli quickly, the spore powder has to be treated in order to prevent its forming larger particles.
After they have been dispersed or whirled up, the pathogens can reach the pulmonary alveoli **by inhalation, without being exhaled again** immediately or being removed by mucociliary clearing. The inoculation dose for inhalational anthrax for a person is estimated at 10,000 (2,500-55,000) spores.

This is **quite high** and explains why formerly "woolsorters' disease", even among furriers who used goat's wool was rare.

Anthrax is not spread from person to person and medical personnel do not need to use additional protective equipment apart from the usual standard hygiene precautions, an important difference compared with plague pneumonia.

Bioterrorism

In 1993, members of the Japanese Aum Shinrikyo sect repeatedly spread anthrax in Kameido, Tokyo. There were however no cases of disease, because the sect had used a non-virulent strain (vaccine strain without capsule), low spore concentration, ineffective dispersal, a clogged spray device and probably also because of inactivation by sunlight (on a bright summer day, B. anthracis spores have an estimated survival time of less than 150 minutes).

The fear that anthrax would be used in bioterrorism became reality after the attacks of September 11, 2001 on the World Trade Center, New York and the Pentagon, Washington DC, USA. A week after the turmoil of September 11, letters containing anthrax spores were mailed to various people, government departments and news agencies in the USA. Twentytwo people developed anthrax infections, including people working in mail-sorting centres. Eleven people developed inhalation anthrax, and five of those victims died. The powder in the envelopes contained high concentrations of finely dispersed anthrax spores, made of different grades in different envelopes.

What to do if such a scenario would be repeated? The government must be informed of any incident where release of anthrax is suspected. Samples are taken for bacteriological examination from the area in which the spores are released. Afterwards, decontamination is carried out with a strong hypochlorite solution. For the people involved, who may still be asymptomatic, nasal swabs are taken and potential victims are advised to immediately wash thoroughly with soap in a shower and then to take ciprofloxacin 500 mg bid (adults) until

the full result of the laboratory examination is known. The accuracy of a nasal swab culture in predicting exposure is not known, and its value is greatly disputed. There is really no good method for determining whether someone has or has not been exposed to an aerosol that contains B. anthracis. If the infection is confirmed and there are still no symptoms, ciprofloxacin PO is taken for two months. Vaccination can be considered but has never been used in these cases. The vaccine is not routinely available.

Biological weapons and Rebirth Island

Vozrozhdeniye Island, or "Rebirth Island" in English was located in the Aral Sea, which divides the Central Asian countries of Uzbekistan and Kazakhstan. (The recent drying out of the Aral Sea makes the place no longer an island). During the Soviet era, the island was an open-air testing site for the Soviet biological weapons program. From 1936 to 1991, field tests carried out on the island involved the release of "weaponized" pathogens: microorganisms specially developed by military scientists to be virulent, hardy, and antibiotic-resistant. Among the biological warfare agents tested on the island were special strains of
Bacillus anthracis (the causative agent of anthrax), Yersiniapestis (plague), and Francisella tularensis (tularemia) that had been rendered resistant to multiple antibiotics and environmental stresses. It is likely that viral agents, including the smallpox virus, were also tested on the island.

The Red Army's Fifteenth Directorate which ran the test site, operated a year-round command post in Aralsk, on the Kazakh mainland. All of the key facilities on the island, however were located south of the Uzbek border. At the barracks and headquarters area, up to 800 scientists and troops were deployed at the peak testing periods from April to August.

The Aral Sea was once the world's fourth largest inland body of water. During the Soviet testing program, deadly germs released experimentally were unable to escape from the island because a large expanse of open water separated it from the mainland. Beginning in the 1960s the Soviet authorities diverted the sea's feeder-rivers into concrete irrigation canals, with the aim of growing large amounts of cotton. After a few successful harvests, the desert soil became exhausted, the rivers silted over, and desiccation and pesticide contamination turned the area into an environmental wasteland, with serious health consequences for the local populations. The diversion of the rivers has also caused the Aral Sea to shrink dramatically and ended the former isolation of Vozrozhdeniye Island. By the late 1980's the sea's level had dropped so much that the lake had separated into two distinct bodies: the Small Aral (north) and the Large Aral (south). By 2007 the south had split into a deep Western basis, a shallow eastern basis and a small isolated gulf. The Large Aral's volume had dropped from 708 to 75 cubic kilometers, accompanied by a rise in salinity. In 2001 Vozrozhdeniya united with the shore in the South. By 2008 the initial small landbridge become a broad base, transforming the island into a peninsula connected to the Uzbek mainland. The implications of rodents carrying infected fleas leaving this former testing ground can only be guessed at present.

In 1988, after the Soviet BW program was supposedly shut down. Large quantities of anthrax spores had been produced at the military microbiology facility in Sverdlovsk and

then stockpiled near Irkutsk. Because the volume of the anthrax material was too large to autoclave, it was shipped to Vozrozhdeniye Island for decontamination and burial. The anthrax spores were mixed with bleach in 250-liter stainless steel containers and then buried in 11 pits within a total area of less than a football field. Because the spores tended to clump together, some were protected from the bleach and remained viable in the soil.

In 1992, Kanatjan Alibekov, a senior Soviet bioweapons scientist, defected to the United States and revealed that weaponized anthrax had been buried on Vozrozhdeniye Island. The U.S. intelligence community was able to determine the locations of the burial sites from historical satellite images taken while the pits were being dug. A Department of Defence team then travelled to the island and took soil samples, which revealed the presence of viable spores of weaponized anthrax.

In the aftermath of the September 11 attacks, the U.S. government recognized the urgency of decontaminating the anthrax burial sites to eliminate the threat of terrorist access. Moreover, because oil companies are interested in drilling on the island for petroleum and natural gas, these activities could stir up contaminated dust that could blow across to the mainland. The special decontamination solution was used to soak the anthrax-contaminated soil in situ. The soil was dug up and passed through the solution again to make sure that all the spores were killed. The anthrax pits decontamination ended in late 2002.

Tularemia

Summary
- Tularemia: bacterial infection by Francisella tularensis
- Contact with infected animals (e.g. wild rabbits), contaminated dust and water
- Fever, skin lesions and lymphadenopathy
- Other presentations include ocular, septicaemic and pneumonic forms
- Diagnosis: clinical presentation, culture and/or antibodies

General

Tularemia (syn. tularaemia) is an infectious disease caused by a small, pleomorphic, aerobic, non-motile and non-spore-forming Gram-negative coccobacillus, ***Francisella tularensis*** (formerly *Pasteurella tularensis*). The generic name refers to Edward Francis, a scientist who devoted many years of his life to studying the disease. The species name refers to Tulare County in California, an area where tularemia occurs regularly. There are three biovars, *F. tularensis tularensis* (biovar A, syn. nearctica), *F. tularensis holarctica* (biovar B, syn. palearctica) and *F. tularensis novicida* (biovar C).

In humans, infection with type A has a much more serious course than with type B. Type A is mainly found in rabbits and rodents. Type B is found more in animals that live near water and is predominant in Eurasia. Type A is predominant in North America, although it is sometimes found in Central Europe.

Biovar C is a germ with low virulence, found in North America. **Infections in Europe or Russia tend to have a much milder course than infections in the New World.** Type A is fatal to guinea pigs and rabbits, unlike type B. Serologically there is no difference between the three forms. Both phagocytosing cells and non-phagocytosing cells can be invaded. **Intracellular multiplication** occurs.

Specific exotoxins such as in anthrax have so far not been demonstrated. There is however an **endotoxin,** similar to other Gram-negative pathogens. The disease has been studied for possible use as a **biological weapon.**

Transmission

The infection is restricted to certain areas and **only occurs in the Northern Hemisphere**: Mexico, USA¸ Canada, Scandinavia, eastern Europe and in Russia as far as Siberia. Cases which occurred in Utah led to the name "Pahvant Valley fever". There are few infections in Japan, where the disease is known as "yatobyoo". In 1939 some 2300 cases were reported in the USA, but since then the number of infections has fallen substantially. In 1966-67 there was an epidemic with more than 600 cases in Sweden. In the period 1999-2000, 327 cases were reported in post-war Kosovo. In the New World, cottontail rabbits and jackrabbits form an important reservoir, hence the common name "rabbit fever". Other animals such as dogs and cats, sheep, squirrels, skunks, beavers, muskrats and even birds can be infected. Prairie dogs can become chronic carriers. **Various occupations** are at an increased risk of tularemia: hunters, butchers, veterinary surgeons, and furriers. There have been no reports of personto-person transmission. Transmission is by **inhalation, ingestion, inoculation or contamination**

through direct contact with infected material, including water. Although the pathogen does not form spores -unlike anthrax- the bacterium can **survive for 2-6 months in mud, water and carcasses.**

Transmission can be by the bite of hard **ticks, fleas** or **horseflies** such as tabanids ("deer fly fever"). These arthropods first infect themselves by sucking the blood of an infected animal. With ticks there is transovarian transmission. The pathogen is present in small numbers in tick saliva and in greater numbers in tick faeces. The ticks that are notorious for transmitting Francisella tularensis in the USA are *Dermacentorandersoni* (Rocky Mountain wood tick), *D. variabilis* (American dog tick), *D. occidentalis* (Pacific coast dog tick) and *Amblyomma americanum* (Lone Star tick). Skin contact with the infected tissue of an animal that has for example been hunted and skinned is dangerous. The disease can occur after **eating infected meat**. Racoons, snakes or coyotes can carry the bacteria in their mouths.

Domestic animals or wild animals that have had direct contact with an infected animal can cause infection in man. Transmission by **aerosol** is possible. Transmission can occur by breathing in contaminated **dust** that has been whipped up, such as by a grass cutter or brush cutter. By this route the pathogen is extremely infectious. This was one of the reasons why tularemia was studied as a bio. warfare agent. Fewer than 50 bacteria are enough to cause pulmonary infection. The infectious dose by the oral route is much higher: 108 organisms.

Clinical aspects

The disease occurs in different clinical forms. Its presentation depends on the route of infection, the size of the inoculum, the virulence of the organism and the immune status of the patient.

Ulceroglandular form. About 80-90% of cases are of this form. The point of entry may be the site where an arthropod has bitten. Microtraumata with small tissue defects in the skin form a point of entry. After an incubation period of 2-4 days (1-10, exceptionally 21) there is **suddenly high fever with rigors**, together with headache, nausea, vomiting and pronounced malaise and fatigue. A primary red, slightly itching and slightly painful skin **papule** is observed. This soon becomes pustular and necrotic. The ulcer is usually on the hands. Afterwards there is **local lymphadenopathy** (buboes) with swelling of the epitrochlear and/or axillar lymph nodes. If inoculation occurs on a leg, there are swollen inguinal/femoral lymph nodes. Oral infection results in cervical lymphadenopathy. The lymph nodes may **suppurate** and drain to the skin. A non-specific roseola-like maculopapular rash appears in 20% of cases. Rarely there is erythema nodosum.

Oculoglandular form (1%). With inoculation in the conjunctiva, for example due to dirty fingers, severe painful conjunctivitis develops, followed by swelling of the ipsilateral lymph nodes. Keratitis and corneal ulceration may follow. If the pre-auricular nodes are swollen, this is called **Parinaud's oculoglandular complex**. This is to be distinguished from cat-scratch disease, tuberculosis, sporotrichosis, sarcoidosis and syphilis. [P.S. Do not confuse the term with Parinaud's syndrome, a neurological entity with vertical gaze abnormalities due to lesions in the dorsal part of the midbrain, the colliculi superior.]

A **purely glandular** form can occur, but this is rare (2%). It is a form consisting of local lymphadenitis without a primary skin lesion. Sometimes there is cervical adenopathy, which suggests oral ingestion of the pathogens.

Oropharyngeal form, with stomatitis and/or severe inflammation of the throat (pharyngitis, tonsillitis) that can resemble diphtheria, together with cervical lymphadenopathy.

Gastrointestinal form follows eating infected meat. Mesenterial lymphadenopathy, abdominal pain, nausea, vomiting, diarrhoea and intestinal blood loss from intestinal ulcers occur.

Typhoidal form. Here sepsis with abdominal pain predominates. Myalgia and joint pain may occur but are unspecific. **Disseminated necrotic foci** are found throughout the body (1 mm to 8 cm in diameter). The systemic toxicity is pronounced. Delirium can occur. Splenomegaly and perisplenitis can arise. A full blood count reveals a normal or raised leukocyte count. Mediastinitis, meningitis, peritonitis and lung abscess can occur as complications but are rare. Tularemia is a rare cause of "fever of unknown origin".

Pulmonary tularemia. Tularemia is a rare cause of **atypical pneumonia as well as fulminant** pneumonia. Primary pulmonary tularemia progresses rapidly with fever, cough, dyspnoea and a burning feeling under the sternum. Pleural effusions and pleuritic pain can occur. On a chest X-ray there are poorly defined infiltrates and the concave lining of pleural fluid can be seen. Mediastinal lymphadenopathy can occur. Pneumonia does not always have to be primary but can be secondary (cfr similar situation in plague).

Differential diagnosis:

Depending on the clinical presentation, several other diseases can also be considered. The clinical picture of a **febrile syndrome of sudden onset with a skin lesion and swollen lymph nodes after contact with a possibly infected animal**, could be:

- ulceroglandular tularemia (*Francisella tularensis*), but also
- bubonic plague (*Yersinia pestis*) or
- cutaneous anthrax (*Bacillus anthracis*).
- Skin infection with pyogenic bacteria such as *Streptococcus pyogenes* and *Staphylococcus aureus* are in most cases not difficult to diagnose.
- Rat bite fever, also known as "sodoku" is caused by Spirillum minus and can follow a bite from an infected rat. Relapsing fever, skin lesions and joint pain are important.
- Dog bites are often infected with *Capnocytophaga canimorsus*.
- Scrub typhus (*Orientia tsutsugamushi*) occurs in Asia (geographically different area from tularemia). Here the lymphadenopathy is less pronounced.
- Swimming pool granuloma caused by *Mycobacterium marinum* may be a possibility, but its course is less rapid, and the general condition is excellent.
- Cat-scratch fever (Bartonella henselae) is a more difficult differential diagnosis.
- Sporotrichosis can mimic tularemia.

Oropharyngeal tularemia must be distinguished from diphtheria, severe streptococcal pharyngitis, actinomycosis, lymphoma, tuberculosis and Plaut-Vincent pharyngitis.

Atypical pneumonia due to tularemia can resemble infections caused by *Coxiella burnetii, Legionella pneumophila, Chlamydia psittaci, Chlamydia pneumoniae, Mycoplasma pneumoniae* and even *Histoplasma capsulatum.* Fulminant pneumonia can resemble anthrax, pneumonic plague, SARS and pulmonary hantavirosis caused by the Sin Nombre virus.

Typhoidal tularemia may resemble typhoid fever (Salmonella typhi), brucellosis (Brucella sp), typhus (rickettsioses such as Rocky Mountain spotted fever) and erhlichioses. The latter two should be especially considered if it is known that the person has been bitten by a tick. If granulomata are present tuberculosis and sarcoidosis can be brought into the differential diagnosis of tularemia. Haverhill fever is caused by *Actinobacillus muris* (= Streptobacillus moniliformis) and can follow a rat bite or by drinking milk infected with rat urine. In practice the diagnosis of Haverhill fever can only be confirmed by identifying the pathogen in a culture.

Diagnosis

Francisella tularensis type A is a **level 3 pathogen**. As the bacterium is highly infectious, it is dangerous to try to isolate it in a standard laboratory (culturing skin lesions, sputum, pleural fluid, blood culture). Laboratory infections have been described. It is not an easy bacterium to culture. Clinical samples can be examined quickly with fluorescing antibodies.
Serology is important. In some patients antibodies are positive after one week but in other patients it takes three weeks before antibodies can be detected. This can lead to false-negative results early in the disease. In the right context a single raised value of 1/160 can suggest the diagnosis. There is a limited cross-reactivity with *Brucella* and *Legionella* bacteria. These antibodies play a minor role in protection. It is predominantly primary (polymorphonuclear) and cellular immunity which is responsible for protection. The T-lymphocyte-dependent protection develops over the course of 2-4 weeks.
Initially a lesion contains many neutrophils. A biopsy of a cutaneous lesion may be pathologically similar to tuberculosis, but the evolution of tularemia is far more rapid. There is **granuloma** formation with epitheloid cells, lymphocytes and polynuclear giant cells. PCR exists for the bacterium.

Treatment

The pathogen is sensitive to **gentamicin, streptomycin** and to **fluoroquinolones** and **doxycycline**. Tularemic meningitis can be managed with an aminoglycoside combined with chloramphenicol or doxycycline. If the patient is pregnant gentamicin is still the recommended treatment. If treatment is given soon after infection, mortality remains low. Skin wounds require local care. In the case of ocular tularemia moist dressings, eyedrops with homatropine and dark glasses are recommended.

Prevention

Avoid **ticks and insect bites** (protective clothing, repellents, permethrin). Wear gloves and masks **when touching wild animals** (e.g. the fieldwork of a biologist) particularly if these are rabbits in an endemic area. Shot game must be very thoroughly cooked before it can be eaten. The previously used vaccine prepared from the live vaccine strain (LVS) of *F. tularensis* subspecies *holarctica* is no longer available because of concerns about its unknown

mechanisms of attenuation and stability. Using leaf blowers to clear gardens, streets or parks in areas with tularemia is not advised (airborne transmission via contaminated dust).

Plague

Summary
•Plague: infection with Yersina pestis, a Gram-negative bacterium
•Isolated cases or epidemic
•Transmission via fleas (importance of rat population), body lice (hygiene) or aerogenically (cough)
•Lymphadenitis (bubonic plague), pneumonia (pneumonic plague) with septicaemia and bleeding
•Isolation of cases, flea and lice eradication
•Aminoglycoside (gentamicin), fluoroquinolone or tetracycline
•Tetracycline for immediate contacts

General

Plague is an infection caused by a **Gram-negative bacterium: *Yersinia pestis.*** This organism was isolated in 1894 by the Japanese researcher Shibasaburo Kitasato (a co-worker of Koch) and the Swiss bacteriologist Alexander Yersin (a student of Pasteur) during an epidemic in Hong Kong. The organism has a characteristic shape when stained with Giemsa or Wayson stain: a bipolar rod with a safety pin appearance. The organism is non-motile and forms no spores. The organism grows well on various tissue media. In 1897, the Japanese doctor Masaki Ogata reported that plague was transmitted by rat fleas. In 1898, Paul-Louis Simond during his work in Bombay suspected that the rat flea Xenopsylla cheopis might be the vector. This was confirmed experimentally in 1914 by Bacot and Martin.

Yersinia

Do not confuse Yersina pestis with Yersinia enterocolitica or Yersinia pseudotuberculosis. These bacteria can provoke enteritis and mesenterial adenitis (swollen lymph nodes in the mesentery, especially near the terminal ileum and the ileocolic junction). Y. pseudotuberculosis is maybe the cause of Izumi fever (pseudoscarlatina).

Historical perspective

There have been various well-known pandemics in history. The **Athenian "plague"** (430 BC) at the time of the Peloponnesian War (431-404 BC) was described by the Greek historian Thucydides, but the precise aetiology of this epidemic is uncertain.
The profusion of different hypotheses (Ebola, Rickettsia prowazekii, ergotism, epidemic recurrent fever, smallpox, Bacillus anthracis, Yersinia pestis, arbovirosis, robovirosis, a variant of "Spanish" flu, etc.) shows that, in the absence of essential data, a correct diagnosis after the event is not easy.

In 542 AD, at the time of the Roman emperor Justinian, an epidemic occurred in Pelusium, in Egypt, a seaport at the mouth of the eastern branch of the Nile delta. The epidemic subsequently struck Turkey and Europe (**Justinian plague**). The consequences and terrors were described by the Byzantine historian Procopius, secretary to Belisarius, one of the most important generals under Emperor Justinian. The epidemic ended about 767.

In 1346 there **were cases of plague in Astrakhan**, situated at the mouth of the Volga (north of the Caspian Sea). Afterwards, spread occurred via the River Don to the Sea of Azov and subsequently to the shores of the Black Sea. In 1347 there were **Genoese** traders in the city of Caffa (now Feodosiya), in the south of the Crimean peninsula in the Black Sea. It was the terminus of the northern branch of the Trans-Asiatic silk route. The city was besieged by Janiberg, leader of the Kipchak Tartars, in whose camp an epidemic of plague broke out. The Tartars catapulted bodies of their own comrades who died of the disease over the walls of the city. To what extent this contributed to the spread of plague is open to question. Anyway, the plague appeared in Caffa city. Twelve Genoese ships withdrew with cases of plague on board. Their crews went ashore at various places in Constantinople, Cyprus, Messina (Sicily), Southern France and Italy, after which a major epidemic broke out in December 1347. In June 1348 the plague reached Paris. In December it arrived in England. In May 1349 a ship with a cargo of wool sailed from London to Bergen in Norway. A few days later it was found drifting with the crew dead off the Norwegian coast. The cargo was brought on land and by the end of 1349 the plague had spread throughout the whole of the country. In 1351 the plague came to Poland. The Black Death in the 14th century wiped out approximately a quarter of the population of Western Europe. Together with the other terrors of the 14th century (e.g. the Hundred Years' War between England and France, 1339-1453), this meant that the **European population declined from 73 million to 45 million**.

The term "**quarantine**" stems from 1370, when seafarers arriving in the Republic of Ragusa in Southern Italy were isolated for 40 days (quaranti giorni).

Plague also raged from the 15th to the 17th century in Europe. The Great Plague of London in 1665 totalled 70,000 deaths. The epidemic was possibly stopped by the Great Fire of London in 1666, but according to English demographic data ("Bill of Mortality") mortality had already declined before the Great Fire.

Subsequently other smaller outbreaks happened (Marseilles in 1720, Egypt in 1834). The decline of the plague has been associated with the reduction in the number of black rats and their replacement by brown rats which have less close contact with humans.

In 1860, a new epidemic arose in Yunnan, China, which later spread, first to the town of Pakhoi and then to Canton (Guangzhou), before subsequently travelling downstream and reaching Hong Kong in 1894. It was then that the organism was isolated. From this port there was further spread via ships' rats (e.g. to San Francisco 1903, Auckland, Bangkok, Manila, Rangoon, Saigon, Batavia, Tokyo, Sydney, Cape Town, Buenos Aires, Mauritius and Glasgow), which caused huge mortality, especially in India. Between 1898 and 1918, 8 to 12.5 million people died in India. The epidemic was brought to a halt in the first half of the twentieth century. In North China there was also a major epidemic. This resulted from the intensified hunting of marmots. These mammals had a valuable pelt and were also very susceptible to plague. The local Mongols knew the risk of this only too well and shot the animals instead of catching them. They also always avoided touching sick or dead animals. When the price of pelts quadrupled in 1910, there was a large influx of inexperienced amateur Chinese who hunted without precautions in search of rapid profits. The hunters also often kept warm together in underground shelters, which was ideal for transmission.

Pneumonic plague broke out in Hailar and spread along the railway line to Harbin and afterwards to Vladivostok.

In the Second World War, Japanese Imperial Army's Unit 731 killed thousands of Chinese and Russians held prisoner in Japanese-occupied Manchuria, in experiments to develop chemical and biological weapons. Japanese doctors tested the use of plague among others. Infected *Pulex irritans* fleas were cultured and released in a few Chinese towns, resulting in small epidemics of bubonic plague.

After an absence of 50 years, plague reappeared in 2003 in Oran and in other foci in Algeria. New foci were discovered in 2008, including one in Libya. The rodent species Meriones shawii (Shaw's jird) was shown to be present in the transmission area. The animal is plague-resistant and forms an efficient reservoir for *Yersina pestis*.

Spread of plague throughout Europe during Middle-Ages

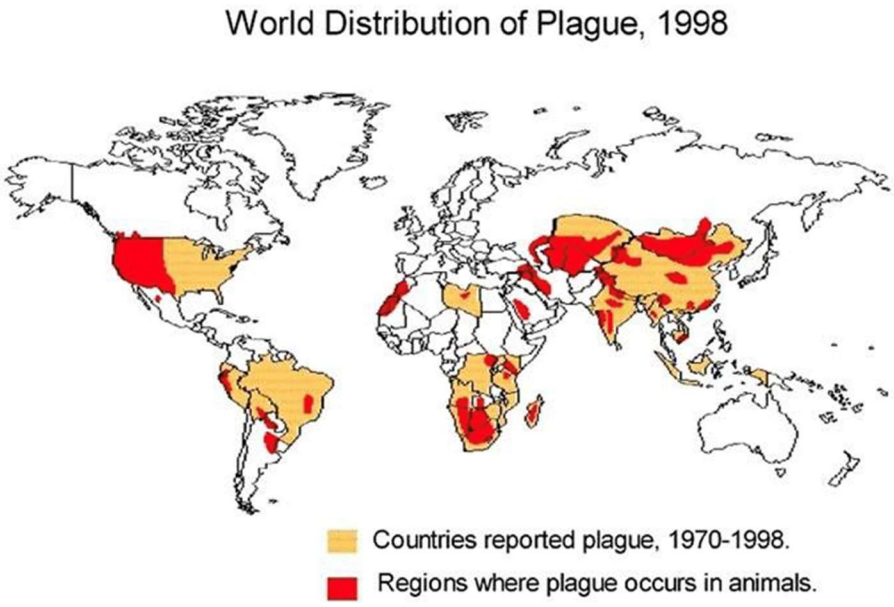
Plague = plague?

How do we know so positively that the "plague" in earlier centuries was in fact "the plague"? Naturally, there are numerous historical descriptions that are suggestive, but there still remains questions. In the case of the Athenian plague there are many question marks regarding the aetiology. There have also sometimes been epidemics of diseases with high mortality which disappeared as quickly as they had appeared and which do not resemble any disease that we now recognise (e.g. the epidemic of lethal "sweating sickness" (1485-1551) which in the summers of 1508, 1517, 1528 and 1551, claimed many victims in England and elsewhere). The nature of the organism that caused "sweating sickness" is still unknown. In 1998, Didier Raoult (Marseilles) studied the dental pulp of non-erupted teeth from people who had died in the 16th and 18th century from plague and were buried in large graves in Lambesc and Marseilles. Using PCR technology it was possible to detect a few genes of Yersinia pestis in the dentition. Control teeth were negative. This technique opens new avenues for study and for obtaining a better understanding of historical epidemics.

Present situation

Plague is at present a rare, cosmopolitan disease which still persists in **various foci in several parts of the world**. From 2000 to 2009, a total of 21,725 cases of plague with 1612 deaths (7.4 percent fatality rate) were reported worldwide from 16 countries. A further 3248 cases of plague were reported to the World Health Organization (WHO) between 2010 and 2015, with 584 associated deaths. Since 2000, more than 95 percent of reported cases have been from Africa. Outbreaks of human plague, with numbers of cases ranging from 100 to more than 1000, have occurred since 1992 in DRC, Peru, India, and the Congo. Plague reappeared in Malawi, Mozambique, and India in 1994, in Algeria in 2003, and in Libya in 2009, raising concern that the disease may re-emerge as a worldwide public health hazard.
Available data may be underestimates because diagnostic facilities and surveillance systems are inadequate in many areas of the world where plague is endemic or occurs in focal outbreaks.

In the Western World, the rate of plague is low, probably because the affected areas are rural and largely uninhabited. In the United States, a total of 91 cases of human plague were reported in the United States from 2000 to 2015, over 80 percent of which were the bubonic form.



Map showing areas where plague occurred in the period 1970-1998

Transmission and epidemiology

Plague is first and foremost a disease of **wild rodents (zoonosis).** Mammals from at least 73 genera can be infected and approximately 30 species of fleas can transmit the organism. This does not mean that they are all equally important. Many of these animals are relatively resistant to the infection. Only a few are of importance for maintaining enzootic and epizootic cycles. In a focus of infection, it is possible to obtain an idea of the local situation (plague surveillance) by serological surveys of various wild animals.

Sometimes an epizootic occurs (an epidemic in animals).

Paul-Louis Simond

French researcher Paul-Louis Simond (1858-1947) helped in Bombay to combat the Indian plague epidemic of 1897. At that time, it was thought that rats caught plague by cannibalising dead rats, and that people caught plague through tiny cuts and cracks in their feet. Simond showed it was rather difficult to infect rats by feeding them infected material. Also, mere physical contact with infectious material did not seem to infect the rats. However, pricking the feet of rats with a plaguecontaminated needle infected them rather easily. Rubbing plague material on the surface of an intact rat paw produced no infection. If rats could get plague via tiny prick injuries, what might be causing them in their natural habitat? Simond considered insect bites. He knew rats were often infested with fleas. He also knew rat fleas would bite humans (fleas are less discriminatory of food sources than lice). In a critical experiment, he showed that rats did not get plague in the absence of fleas. Simond noted that not only were there large number of dead and dying rats in the streets and buildings, but that 20 laborers in a wool factory who had been cleaning the floor of dead rats had died of plague, but none of the other factory workers who had no contact with rats had become ill. He found that healthy rats groomed themselves and had few fleas, while sick rats unable to groom their fur had many. When the rats died, the fleas moved on to other hosts. Simond began to suspect fleas as intermediaries. In an experiment, he placed a sick rat at the bottom of a jar and suspended a healthy rat in a wire mesh cage above it. Although the healthy rat had no direct contact with the plague-infected one, it did become infected. Simond determined that rat fleas could jump 10 cm high without difficulties. As a control he placed a sick rat without fleas together with healthy rats in a jar. None of the healthy rats became sick (which ruled out air-borne transmission). When he introduced fleas into the jar, they developed plague and died. On 2 June 1898 he wrote Pasteur that the problem of plague transmission had been solved. It would be several years before he was believed.

The bacteria can survive for a long time in the burrows of various rodents. The infection is transmitted **from animal to animal by fleas**. When a flea sucks blood from an infected animal it ingests bacteria.

These organisms then proliferate in the insect's proventriculus and stomach. The bacteria attach to the wall if they carry a specific gene, the "haemin storage locus". At the same time, they secrete an enzyme (coagulase) that coagulates the aspirated blood. This causes an **obstruction in the flea's stomach**. The flea then becomes increasingly hungry and bites more often. As a result of the obstruction, **the blood with bacteria is regurgitated**. The flea can only digest the clots at temperatures higher than 28°C ("cold fleas digest poorly"). At high environmental temperatures (>28°C) a plague epidemic will therefore spread less rapidly and sometimes stop because the flea can digest the blood and there is much less regurgitation into the bite wound. The proventriculus of the flea in fact contains internal projections which make regurgitation difficult in "usual" circumstances. The bacteria can also be introduced into a wound by flea faeces or by crushing the insect (scratching an itchy fleabite!).

Cat flea. *Ctenocephalides felis*. Occasional vector of *Yersinia pestis* (plague) and vector of *Rickettsia felis*. Copyright ITM

An isolated case of plague can occur when a human is bitten by an infected flea from wild rodents such as sand rats or desert rats [gerbils] (e.g. Meriones sp, Tatera sp, Rhombomys sp, Gerbillus sp). This is then referred to as **sylvatic transmission** ("sylva" = wood). This happens for instance to hunters, wood cutters, etc. Other animals, such as Mastomys sp, Arvicanthis, Otomys sp, etc., are also involved in transmission but are less important. Carnivores of the cat and dog families and species belonging to the weasel family naturally have a high probability of **being contaminated by their prey** as a result of their hunting behaviour. There are regular cases of transmission via a sick domestic cat or dog. **These animals can cough and infect humans aerogenically.** Contamination can also occur through wounds and direct contact with contaminated body fluids. Consumption of contaminated meat and liver (e.g. sick camel) can result in active infection with Y. pestis.

Sometimes **rodents that live close to humans are infected**. Rats, principally the brown rat (*Rattus norvegicus,* also called the Norwegian, grey or sewer rat; little contact with humans) and the black rat (*Rattus rattus*, also known as the house rat, lives close to humans) constitute the main reservoir. These rats are much more susceptible to infection than gerbils. **The plague bacterium usually kills the rat**, after which the flea *Xenopsylla cheopis* – the oriental rat flea - has to search for another source of blood, often humans. There are other fleas (e.g. *Pulex irritans* [human flea], *Nosopsyllus fasciatus* [brown rat flea], *Oropsylla montana* [rock squirrel flea], *Oropsylla silantievi* [tarabagan flea]) that can transmit plague, but these are of minor epidemiological importance. It is possible that transmission via *Pulex* was very important during the period of the Black Death in Europe.

Y. pestis may have a **reservoir in the soil.** It has been shown that *Y. pestis* can survive for at least 24 days in contaminated soil under natural condition. The upper limit is unknown at present.

The presence of *Y. pestis* in the fleas affects their behaviour, such as their preferred optimal temperature. Infected fleas appear to prefer a mean environmental temperature that is 1.6°C lower than that of non-infected fleas. Healthy rats have a body temperature of ± 38.5°C. Sick rats develop fever (i.e. >38.5°C). Thus, **infected fleas are unlikely to remain on an infected rat.** They move on to the next available host. If this is a human, then the bacterium is transferred at the same time. This has important consequences in the epidemiology of the infection with the massive release of contaminated fleas in the event of extensive rodent die-

off ("ratfall"). **Humans are then accidental "hosts" to the fleas.** In this case, human-to-human transmission still does not occur.

Epidemic plague can occur e.g. via bites from the human flea ("Pulex irritans"). A patient with bubonic plague can develop **secondary pneumonic plague**. When humans develop the pulmonary form of plague, the disease **can be further transmitted from person to person by cough droplets** without further intervention by fleas or rats.

In the USA, there are several cases of plague every year following contact with sick or dead wild animals (mice, squirrels, prairie dogs, rabbits, etc). *Oropsylla montana* is an important vector in the USA. Monitoring rodent populations and their predators (e.g. coyotes) is important for predicting imminent outbreaks. It should be noted that domestic cats, dogs and other animals can also be infected with plague and develop the disease.

Historical data seem to imply that rat-die offs were not associated with human epidemics in the 1300's. The rodent's fleas might not have been active during the cold European winter months. Still cases of bubonic plague occurred (besides pneumonic plague) during the cold periods, very suggestive of transmission via biting arthropods. It was demonstrated that **body lice** can also transmit plague. Since they stay in human clothing, transmission during winter can be expected. Body lice can be infected when living on a septicaemic patient and stay alive for a week, producing infectious faeces. The exact role of body lice is still not well defined, but further work might clarify the epidemiology of this disease.

Yersinia pestis

Three biotypes of the bacterium are currently recognised based on the capability of glycerol fermention and nitrite to nitrate conversion. Ribotyping of the various isolates supports the recognised division of these biotypes. These are the Antiqua, Mediaevalis and Orientalis biotypes. The Antiqua biotype occurs in Africa, Southern Russia and Central Asia. The Mediaevalis biotype is found around the Caspian Sea. The Orientalis biotype is predominant in Asia and is the only one that occurs in the New World. A fourth biotype, Microtus, refers to Medievalis isolates lacking arabinose fermentation. In 1951, Devignat proposed that each of the first 3 biotypes determined each plague pandemic.

However, at present there are strong arguments to suppose that the three historic pandemics were caused by the Orientalis biotype (studies based on PCR-analysis of ancient dental pulp of victims).

Clinical aspects

Some cases are asymptomatic. After a flea bite, a local pustule or ulcer occurs, sometimes with a black crust. The bacterium spreads via the lymphatics. Some cases have clinical features of minor lymphadenitis.

Bubonic plague

The **incubation period is short (2-7 days).** In a minority of cases (6%), there is a pustule or a carbuncle at the site of the flea bite. In most cases, no ascending lymphangitis is noted.

Sudden high **fever with chills** occurs, associated with hypotension, headache and severe general malaise. The **regional lymph nodes** draining the site of the bite enlarge rapidly and are very painful. In most cases, the femoral and inguinal lymph nodes are affected, followed in terms of frequency by the axillary and cervical nodes.

Plague nodes differ from other lymphadenitides through their rapid development, severe pain and accompanying toxaemia. Mild forms however also occur ("pestis minor"). The swollen lymph nodes are known as buboes, from which the term "bubonic plague" is derived. The buboes rapidly break open, discharging dirty, foul-smelling, necrotic tissue. There is high fever and the patient's general condition is poor, blood pressure low and the liver and spleen can be enlarged. Subcapsular splenic bleeding is not unusual. **Mortality is high** (50-90%). With rapid treatment it can be reduced to 1-2%. Blood vessels are damaged and contain clots. Subcutaneous bleeding occurs, which takes the form of petechiae, purpura and ecchymoses. Subsequently, the skin lesions become necrotic and gangrene can set in ("Black Death"). If treatment is incomplete, meningeal invasion can occur (plague meningitis). When pustules or ecthyma gangrenosum are the predominant clinical features, this is sometimes referred to as cutaneous plague.

Septicaemic plague

Sometimes **sepsis/septic shock** is clinically apparent before the lymph nodes have time to enlarge: septicaemic plague. This is an incorrect term since septicaemia also occurs in the other forms of plague. **Bacteraemia** can be very high so that sometimes bacilli can be seen in a thin or thick blood smear. Often the patient presents initially with gastro-intestinal symptoms, such as nausea, vomiting, diarrhoea and/or abdominal pain, which can lead a clinician astray. In most cases the patient dies very rapidly (1 to 2 days) in a condition of septic shock with refractory hypotension, renal failure, stupor, ARDS and DIC (petechiae, bruising, bleeding tendency and acral gangrene).

Pneumonic plague

These days, pneumonic plague is rare. The infection can be **primary** as a result of contamination via an aerosol of plague bacteria or **secondary** through haematogenic spread to the lungs. Primary pneumonic plague has an incubation period of 2 to 4 days. The onset is acute, and the course is fulminant with fever, chest discomfort, general malaise, hypotension and severe pneumonia, with a productive cough and bloody sputum. This is usually associated with pleural effusion. Patients who cough are very contagious. At this point another person can be infected by direct person-to-person transmission. It takes the form of a **very rapidly progressive pneumonia** with almost 100% mortality within a few days. Secondary pneumonic plague initially takes the form of interstitial pneumonia with a small amount of thick, viscous sputum, subsequently progressing to the symptoms described above. It is striking how unremarkable the auscultatory findings are. It is possible but not formally proven, that *Yersinia pestis* increases its virulence after repeated passage via the lungs.

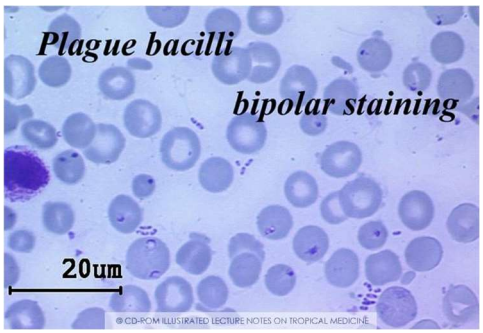
Oropharyngeal plague

Oropharyngeal plague, in which the portal of entry is the throat (ingested flea, consumption of contaminated meat, dirty hands after touching contaminated animal tissues), takes the form of a serious disease with throat pain, severely enlarged painful cervical lymph nodes and local oedema (DD diphtheria, anthrax, tularemia).

Diagnosis

Consideration should be given to the possibility of plague, particularly if there is a sudden increase in **rodent mortality in an endemic region.** The diagnosis should be considered in healthy subjects who **suddenly become very severely ill with fever, extremely enlarged painful lymph nodes, brutal pneumonia or if a rapid succession of deaths occurs within one family.**

Extensive **leukocytosis** is present. **Microscopic examination** of aspirated fluid from a bubo, sputum, cerebrospinal fluid and/or peripheral blood shows bipolar Gram-negative bacilli. The buboes do not contain liquid pus. Some sterile saline (1 ml) is injected into a bubo in order to obtain an aspirate. In the words of Yersin, the fluid contains "une véritable purée de microbes". Sometimes the bacteria can be detected in a thick or thin **blood smear**. They then have the appearance of a "safety pin" (bipolar granules). A staining method that reveals this clearly is the Wayson stain (based on basic fuchsin mixed with methylene blue in 95% ethanol and phenol). The organism is then light blue with darker terminal granules.
Culture is desirable for formal proof in view of the implications of a potentially threatening epidemic.
Serology is possible in specialised laboratories (e.g. ELISA for detecting antibodies to the F1 antigen).
Approximately 5% of survivors do not seroconvert. Serology permits a retrospective diagnosis, but is not useful for the acute, individual patient.
There is also a technique available involving a dipstick coated with antibodies which can be used to detect **the F1 antigen**. This rapid test can use sputum or serum, as early as the second day of the disease. The result is known in 15 minutes and is thus clinically very useful for the individual patient and any contacts. F1-deficient mutants occur very rarely and cannot be detected with this dipstick method.
Presumptive identification of Y. pestis can also be made by polymerase chain reaction (PCR). PCR testing has been used to detect *Y. pestis* in skeletons which are hundreds of years old.



Blood smear with *Yersina pestis* bacteria. Copyright ITM

Differential diagnosis:

Bubonic plague, with its principal characteristic feature of acute buboes, need to be distinguished from:
• lymphogranuloma venereum (much slower progression)
• chancroid (slower, ulcers, fluctuating bubo)

• streptococcal/staphylococcal adenitis (general condition is good)
• filarial adenitis (progression, microfilaria, eosinophils)
• strangulated inguinal hernia.

Pneumonic plague takes the form of a rapidly progressing pneumonia. It can resemble
• a brutal bacterial pneumonia (e.g. pneumococcal)
• legionellosis, tularaemia
• anthrax, SARS (Coronaviral pneumonia)
• or hantavirus pulmonary syndrome (Sin Nombre virus).

An isolated case can be easily missed. In epidemics, there is the possibility that all pulmonary symptoms of all patients are attributed to pneumonic plague (e.g. patients with pneumococcal pneumonia may be viewed as having pneumonic plague).

Septicaemic plague develops very rapidly and resembles meningococcal septicaemia or other severe forms of Gram-negative sepsis. Confusion with acute rickettsioses (epidemic typhus) and louse-borne relapsing fever is possible.

Therapy

All patients should be **isolated**, including those with bubonic plague, because secondary pneumonic plague can develop. In 1948 it was discovered that **streptomycin** was active against the plague bacillus and this antibiotic still remains the first choice. In view of the high mortality and rapid progression, treatment must be initiated as soon as possible. The dose of streptomycin for adults is 2 x 1.5 g IM daily. If streptomycin is not available, gentamicin constitutes a good alternative. For gentamicin, a dose of 2 mg/kg tid is used. Hypotension should be treated, preferably with IV fluids. Improvement is rapid and most patients are afebrile after 3 days. It is not necessary to combine antibiotics. It is important to maintain therapy for at least 10 days.

Tetracyclines are an alternative to aminoglycosides: 2 to 4 g orally for 10 days. They are also very useful in epidemics. Quinolones are also active however not as effective and often are more expensive.

Chloramphenicol is indicated in plague meningitis and/or endophthalmitis. Initially it is given IV. After a few days, in most cases it becomes possible to switch to oral medication. Sulphonamides are also used as prophylaxis, but they are not the first choice. **Penicillins, cephalosporins and macrolides are inactive** against *Yersinia pestis.* Resistance to the common antibiotics is infrequent. Sometimes tetracycline-resistant strains are isolated. In 1995, a **multiresistant strain of *Yersinia pestis*** was isolated in Madagascar (resistance to streptomycin, kanamycin, chloramphenicol, tetracyclines, sulphonamides, ampicillin and spectinomycin). The resistance was coded by a plasmid. *Yersinia pestis* probably acquired the plasmid via horizontal transfer from another Gram-negative organism of the *Enterobacteriaceae family*.

Surveillance

Surveillance can be conducted in several ways. Carnivores can be regularly tested serologically and constitute a sensitive sentinel system of rodent plague in a specific area. *Yersinia pestis* can be detected in animals found dead in a region. The fleas can be collected from abandoned rodent nests, identified and tested. Live rodents can be captured and these animals and their fleas examined.

Prevention

Plague is a disease for which **international quarantine is mandatory** and cases must be **notified**. All patients with plague, irrespective of the presence of cough or pneumonia, should be treated in strict isolation for at least 48 hours (risk of secondary pneumonic plague with subsequent aerogenic transmission). The room should be decontaminated and sprayed with insecticides. Masks, goggles and protective clothing are indicated. Gloves should be worn when handling bubonic aspirates and blood. Contacts may take tetracyclines (4 x 500 mg) or vibramycin for 1 week (ciprofloxacine or sulphonamides are an alternative). They should be closely monitored for 7-10 days.

Vaccination gives temporary protection against bubonic plague, but the vaccine is very difficult to obtain. Soldiers in the American forces during the Vietnam War were routinely vaccinated with a dead cell vaccine (3 primary injections followed by boosters, depending on the antibody titre in the blood). There was a much lower incidence in vaccinated than in the South Vietnamese forces (1/3000 cases per year of exposure).

Urban plague can usually be controlled by **quarantine** and by **rat control and flea eradication. Sylvatic plague cannot definitively be eradicated** in view of its animal reservoir. In combating urban plague, **fleas should be controlled first and then the rats.** Otherwise a large number of fleas are suddenly released (since they no longer have any animal host) and then transfer to humans. It is important to have an idea of the susceptibility of the insects to various insecticides. As strains of Xenopsylla cheopsis and Synosyllus fonquerniei (flea vectors in Madagascar) have been found which were resistant to the insecticides DDT and dieldrin (organochlorine compounds), malathion or phenitrothion (organophosphates) and propoxur (carbamate). Such resistance data are useful if there is an outbreak.

Rat control involves the use of various methods, including rodenticides such as anticoagulants (warfarin, fumarin, bromadiolone, chlorophacinone), zinc phosphide, sodium fluoroacetate and strychnine. Rats are very social and intelligent animals and can learn to avoid poison, as well as teaching their nest mates to do so.

The concern about plague as a bioterrorism agent has led to the development of several newer vaccines, some of which are undergoing clinical testing.

Brucellosis

Summary
•Gram-negative coccobacilli (Brucella spp.) with a tropism for the reticulo-endothelial system
•Zoonosis, through infected dairy products and animal contact (goats, sheep, cattle)
•Chronic granulomatous infectious disease
•Chronic fever and wide range of symptoms
•Diagnosis by serology and culture
•Treatment by rifampicin, doxycycline, aminoglycoside for at least 6 weeks

General

Brucellosis is a **chronic granulomatous infectious disease** caused by small, facultative intracellular, Gram-negative coccobacilli. *Brucella melitensis* (goats, sheep, camels, chamois, ibex), *B. abortus* (cattle, buffalo, bison, zebra, impala, waterbuck, hippopotamus), *B. suis* (pigs) and *B. canis* (dogs) are the causative agents of this zoonosis, in descending order of importance. There are several biovars. For example; pigs are infected by *B. suis* biovars 1, 2 and 3, European wild rabbits by biovar 2. Biovar 4 is found in caribou and reindeer. Humans are accidentally infected and play no role in the survival of these organisms in nature. Animals are the only source of infection and **there are no known vectors**. *B. ovis* (sheep) and *B. neotomae* (desert rats) are not known to cause disease in man. Other species (*Brucella pinnipediae, B. maris, B. cetaceae*) infect marine mammals, such as seals, dolphins, porpoises, minke whales, etc. There have been rare cases of human infection with some of these marine strains.

Historical note

The condition was known as **Malta fever** as a result of a persistent epidemic at the end of the 19th-century in British soldiers on the island. The disease was studied intensively by David Bruce of Trypanosoma fame. He studied 91 cases and found two features: splenomegaly and undulating fever. In 1887 he isolated the organism from splenic tissue of dead soldiers and named it "Micrococcus melitensis". This organism was capable of infecting healthy chimpanzees. In 1897, Wright described a serum agglutination test for the diagnosis of this disease. In 1904 the Brucella Committee was established, as a result of which it was possible to undertake large-scale epidemiological research. In 1905, Themistocles Zammit discovered that the blood of many, apparently healthy goats agglutinated Brucella organisms. Bruce identified the organism in goat blood and milk and as such discovered the reservoir of the organism. Up to 10% of animals had Brucella in their milk. Monkeys which received infected goat's milk to drink developed the disease.

After some hesitation, specific measures were implemented. **Pasteurization** was introduced as a legal requirement in Malta in 1938. The transport of goats was restricted, infected goats had to be killed and milk had to be boiled or pasteurised, including the milk used for the preparation of cheese. The ban on using fresh milk resulted in a dramatic fall in the number of cases in the British Army, but the reduction of cases in the island population was much less spectacular because the indigenous population did not accept the idea of boiling milk. The last documented outbreak of brucellosis on the island occurred in 1995.

In 1895-1897 the Danish doctor/veterinarian Bernhard Bang (1848-1932) identified *Brucella abortus* in cows, the pathogen of infectious abortion in these animals. A previous name for brucellosis was "Bang's disease". In 1921, a substantial problem of brucellosis was seen in Rhodesia in people who had had no contact with goats. However, there was often infectious abortions seen in livestock. Apparently *Brucella abortus* could also infect humans. So, there appeared to be more than one organism that caused undulating fever.

In 1914 Traum identified B. suis in pigs. Carmichael and Bruner discovered *B. canis* in 1968 in dogs. *B. pinnipediae* and *B. cetaceae* were only discovered in 1994 by Ewalt and Ross.

Transmission

Transmission of brucellosis occurs mainly through **eating or drinking contaminated unpasteurized animal-milk products** such as raw milk, soft cheese (cottage cheese), butter and ice cream. Hard cheese, yogurt and sour milk are less dangerous because of the fermentation which has taken place. Eating undercooked infected animal products (spleen, liver) are occasionally responsible for infection. A low pH in the stomach is partially protective (importance of antacids, ranitidine, omeprazole, etc.).

Direct contact (inoculation through skin wound, conjunctiva) with secretions and excretions of infected animals (e.g. placenta, aborted foetuses) can also cause disease. Pregnant infected animals usually develop placentitis. Inhalation of **infected aerosolized particles** can occur (personnel working in microbiology labs!). This has been studied in the context of biowarfare. Brucellosis is an **occupational disease** in farmers, livestock producers, herdsmen, butchers, veterinarians, shepherds, abattoir workers, dairy-industry professionals and lab workers. There is almost no human-to-human transmission although in rare cases sexual transmission has been suspected. The organism has been isolated from human breast milk and from sperm. In animals the disease is commonly transmitted sexually.

After entering the human body and being taken up by local tissue lymphocytes the bacteria migrate via the regional lymph nodes into the general circulation. They display a tropism for the **reticuloendothelial system**. Brucella bacteria replicate intracellularly without affecting cellular viability. They switch off cellular apoptosis rendering the host cell immortal.

Clinical aspects

The clinical features are **very varied and often non-specific**. The incubation period is usually **two to four weeks** but can be as short as one week or as long as several months. The temperature is often only raised in the evening. General malaise, various symptoms such as sweating, headache, muscle pain, abdominal pain, tiredness, depression, etc., may occur. Sometimes the clinical presentation is that of **fever of unknown origin.** Chronic febrile arthritis should point to brucellosis (and tuberculosis). Some patients try to explain their joint or bone lesions as being due to local trauma, whereas the real cause is a Brucella infection. Osteomyelitis of the vertebrae can resemble tuberculosis (Pott's disease).

Sacroiliitis, arthritis of the sternoclavicular joints and involvement of the large joints (hip, knee) is not unusual. The fever can occur in waves ("**undulant fever**"). Uveitis, both posterior and anterior, can be found. Brucellosis can mimic various other diseases and is one of the

great "imitators" in the world of infectious diseases. Rarely peripheral neuritis, orchitis, meningitis, cholecystitis, aortitis or endocarditis can be seen as a consequence. Neurobrucellosis is a feared complication. The risk of abortion in women is thought to be much lower than in animals.

On physical examination, **splenomegaly is observed in 25%** sometimes with enlarged lymph nodes in the groin and neck. Skin abnormalities (papules, erythema nodosum, fine erythematous rash) can occur, but is found only in a minority of cases (5%). There can be signs of arthritis in general large joints (hip, knee, or the sarcoiliac joints). The clinical findings in neurobrucellosis depend on the localisation of the lesions. A slitlamp eye examination and ophthalmoscopy should always be included in any physical examination.

Physical examination usually does not provide pathognomonic findings. Above all **the possibility of brucellosis** should be considered in the differential diagnosis. With the cluster of orchitis arthralgiaeye problems, consideration should first be given to Reiter's syndrome rather than to brucellosis, although brucellosis can lead to such symptoms.

Diagnosis

Leukopenia or a normal white blood cell count is more common than leukocytosis. Normocytic **anaemia** is frequently present. Sometimes there is thrombocytopenia. Liver tests may be abnormal and a liver biopsy or bone marrow specimen can often (± 75%) show **granulomatous lesions**. If granuloma are large enough, they can display fibrinoid necrosis. The cerebrospinal fluid can be abnormal with an **increased lymphocyte count,** raised CSF protein and normal glucose concentration.

Brucellosis can be suspected **serologically**, but the antibodies cross-react with, for example, *Yersinia enterocolitica, Francisella tularensis, Salmonella* and other organisms. Serologically, *B. canis* infections can be detected only with difficulty. False negative results are common early in the course of infection.

A prozone effect can also occur (negative serology at low dilutions becoming positive at higher dilutions). There are rare cases of active Brucella infections in which the standard serology is negative ("blocking antibodies"?). Many laboratories use the so-called "**Rose Bengal**" test, an agglutination test which gives results within 5 minutes. If positive, a **Wright** serological test can be performed but this test needs a longer time (serum agglutination test with overnight incubation). After successful therapy, the IgG titre falls.

Isolation of the organism from blood, tissue, urine, bone marrow, cerebrospinal fluid, require specific culture media. It is a **slow-growing organism**. It is best to notify the laboratory beforehand. Bone marrow cultures have a higher sensitivity than blood cultures. With some rapid automated commercial methods, misidentification of the organism as *Moraxella phenylpyruvica* is possible. Because the organism is a coccobacillus, a laboratory can wrongly describe the organism as a coccus on one occasion and as a rod-shaped bacterium on another.

Radiographs, bone scans, computerized tomography (CT), magnetic resonance imaging (MRI), and echocardiography may be helpful in evaluating focal disease but do not provide a definitive diagnosis.

Localized snowflake calcification in chronic hepatosplenic brucellosis is the only specific radiographic finding that may be used to distinguish brucellosis from other diseases. PCR is a promising tool for rapid and accurate diagnosis of human brucellosis.

Treatment

Rifampicin (600-900 mg/day) and **doxycycline** (200 mg/day) are often used as first line. If possible an aminoglycoside should be added (minimal dual regimen; optimal tritherapy which includes streptomycin or gentamycin). Sometimes combination treatment includes cotrimoxazole (children, pregnant women) or ofloxacin. It is recommended that a specialist with experience in brucellosis be consulted. Treatment lasts **at least six weeks**, but sometimes must be continued for many months. In general, longer courses of therapy (at least 12 weeks) are warranted for treatment of spondylitis, neurobrucellosis, endocarditis or localized suppurative lesions. Clinical relapse sometimes occurs, usually within 6 months of discontinuing the antibiotics. Relapse is usually not a consequence of antibiotic resistance, but due to the persistence of a focus (drainage sometimes necessary). Naturally, patients can still complain of pain following correct treatment due to the consequences of joint involvement, for example.

It was found (with real-time PCR) that in the majority, *Brucella melitensis* DNA will persist in the human body for several years despite appropriate treatment and apparent clinical recovery. It has not been formally shown that this DNA is from dead or living bacteria, but it strongly suggests that *B. melitensis* is a noneradicable persisting pathogen.

Prevention

Detection and destruction of infected animals must be implemented. Brucellosis may be prevented via **vaccination**, which is effective for cattle, sheep and goats (not for humans), but requires a sustained vaccination program over several years. **Proper pasteurisation of milk** and avoidance of cheese made from potentially contaminated milk are important. If for example cottage cheese is used in cooking, it needs to be heated long enough (the centre heats less quickly than the outside; the centre of the lumps needs to be heated above the minimum temperature to destroy Brucella bacteria). Gloves are to be used when working with potentially infected animals and their secretions.

Uveitis

Uveitis is a general term for inflammatory disorders of the uveal tract. Anterior uveitis is the term which encompasses iritis and iridocyclitis. Posterior uveitis is the preferred term for choroiditis and chorioretinitis. In the non-granulomatous form, the onset is characteristically acute, with pain, injection, photophobia and blurred vision. There is a circumcorneal flush caused by dilated limbal blood vessels. Fine white deposits on the posterior surface of the cornea can be seen with a slitlamp. The pupil is small and there may be a collection of fibrin with cells in the anterior chamber. If posterior synechiae are present, the pupil will be irregular in shape. In granulomatous uveitis, the onset is usually insidious. Vision gradually becomes blurred and the affected eye becomes diffusely red with circumcorneal flush. Pain is minimal and photophobia is less marked than in the nongranulomatous form. Fresh active lesions of the choroid and retina appear as yellowish-white patches seen hazily with the ophthalmoscope through the cloudy vitreous. As healing

progresses, the vitreous haze lessens and pigmentation occurs gradually at the edges of the yellowish-white spots. In the healed stage there is usually considerable pigment deposition. If the macula is not involved, recovery of central vision is complete. The patient is usually not aware of the scotoma in the peripheral field corresponding to the scarred area.

There are various causes, including several infectious diseases, but also auto-immune disorders. A wider range of diagnoses must be considered for patients in developing countries. Expert advice from an experienced ophthalmologist and a specialist in internal diseases is essential to save the patient's sight. If for example toxocariosis of the eye were to be treated with anthelminthics only, the larva would die and release a large quantity of antigen. This would cause the intra-ocular inflammation to increase, resulting in cloudiness of the vitreous humour and total blindness.

Infectious causes of uveitis include:

1. Parasitic: toxoplasmosis, Toxocara infection (infection by the larva of a canine nematode), cysticercosis (larval Taenia solium), Onchocerca volvulus microfilaria
2. Bacterial: syphilis, tuberculosis (with granulomata on the retina), leprosy, bartonellosis with cat scratch disease, leptospirosis, Q fever, Lyme disease, brucellosis
3. Viral: CMV (think of HIV), herpes simplex, HTLV-1, measles
4. Fungal: Candida (usually panophthalmitis), cryptococcosis, histoplasmosis

Non-infectious causes include:

1. Sarcoidosis
2. Systemic lupus erythematosus (i.e vasculitis).
3. Traumatic and sympathetic ophthalmia.
4. Reiter's syndrome. In addition to anterior uveitis, conjunctivitis, urethritis, balanitis, oral ulcers, low fever and joint pain cann also be present. There is often a recent history of infected sexual contact (Chlamydia trachomatis) or enteritis. Hyperkeratotic lesions on the palms of the hands and soles of the feet resembling pustular psoriasis can occur.
5. Associated with juvenile rheumatoid arthritis, Still's disease.
6. Associated with ankylosing spondylitis - HLA B27 (Bechterew's disease).
7. Behçet's syndrome.
8. Vogt-Koyanagi-Harada syndrome (uveo-encephalitis) with cutaneous and neurological symptoms in addition to ocular lesions (birdshot retinopathy).
9. Unknown cause, e.g. heterochromic uveitis (Fuch's cyclitis)

Melioidosis

Summary
- Environmental bacterium (soil, water): Burkholderia pseudomallei
- Southeast Asia and Northern Australia are hotspots
- Infection is through skin and inhalation
- Diabetes and other immune depressed at risk
- Acute or chronic disease
- Skin infection – pneumonia - blood stream infection – deep abscesses, high mortality
- Treatment: ceftazidime or meropenem followed by co-trimoxazole or co-amoxiclav (at least 3 months)

General

Burkholderia pseudomallei (formerly *Pseudomonas pseudomallei*) is a facultative intracellular Gramnegative rod-shaped bacterium also known as Whitmore's bacillus. The organism is responsible for infections in sheep, goats, pigs, cattle, horses, rats, cats and dogs. **Soil and stagnant water (rice fields)** form its natural reservoir. Humans are infected by **contaminated soil via skin** abrasions. **Swallowing and inhalation** of the bacilli can also result in clinical infection. Neonates can be infected on rare occasions (via placental micro-abscesses?). The disease is endemic in **Southeast Asia and northern Australia**. Very rarely cases are diagnosed in Central and South America and also in Africa.

B. pseudomallei has two chromosomes. Together they contain more than 7 megabasepairs, making it a very complex bacterial genome. Genotyping of multiple *B. pseudomallei* colonies from several tissue sites showed substantial genetic diversity within a single patient, illustrating the capacity of the bacterium to evolve rapidly within a host. It can invade and survive in a range of phagocytic and nonphagocytic cells. It replicates in the cytosol after leaving the vacuole.

Historical perspective

Glanders is a chronic disease of horses associated with involvement of the nasal mucosa with mucus production, as well as local lymph node enlargement. Glanders in animals is caused by the immotile Burkholderia mallei (formerly Pseudomonas mallei). Human infections are rare.

In 1911, the British pathologist Captain Alfred Whitmore and his assistant C.S. Krishnaswami discovered that ill-nourished and neglected inhabitants of Rangoon, Burma, exhibited the same sort of lesions as horses with glanders. They also performed autopsies on emaciated morphine addicts. About one in every twenty post-mortem examinations in Rangoon Central Hospital was on a case of the disease. The organism which was recovered from the numerous and widespread abscesses observed at post-mortem examination in these cases could be grown on peptone agar or on potato slopes (the bacteriological tools of the day). The organism isolated from humans, however exhibited some differences from the one that caused glanders in animals. The new bacterium was motile (glanders is caused by an immotile bacterium) and caused a slightly different reaction after inoculation in guinea pigs.

The bacterium was initially called Bacillus pseudomallei. The term "pseudoglanders" is sometimes used in English. In 1913 there was an outbreak of an unusual "distemper-like" disease in the veterinary department of the Institute for Medical Research in Kuala Lumpur, Federated Malay States. Dr Fletcher isolated the organism during this outbreak, but he was unable to identify it. In 1917 Stanton isolated the bacterium during an outbreak among Tamil rubber tappers, and saw it was identical to Whitmore's bacillus. In the following years Stanton and Fletcher conducted research on this organism and named the disease meioidosis (Gr. "melis", referring to glanders-like disease of asses).

The occurrence of infections in Vietnam in French colonial soldiers involved in a car accident led to the hypothesis that the organism could enter the body via mud-soiled wounds or via aspiration of muddy water. Guinea pigs with a scarified abdomen could be infected by immersion in muddy water. Finally, the organism was cultured in vitro from soil. It was shown that the organism produced a heat-labile exotoxin. During the Vietnam War several cases occurred in wounded soldiers, but there were also abnormally large numbers of cases among helicopter pilots, which suggested that aerogenic transmission was possible. Several American veterans developed active melioidosis up to 26 years after their stay in Vietnam. An 82-year-old U.S. veteran held as a Japanese prisoner of war in Indochina during World War II developed an infected ulcer on his right hand as symptom of melioidosis. This was 62 years after his exposure. No-one knows the anatomical site where the bacterium survives or how the immune system is evaded. All in all, our knowledge about melioidosis is clearly inadequate. There is a strong association between melioidosis and rainfall (80% of cases occur in the wet season). Heavy rain and wind, such as in monsoon season seems to cause a shift from inoculation towards inhalation of Burkholderia pseudomallei.

In 1950 there was an epidemic in Aruba - an island off the coast of Venezuela. In 1970 an outbreak in France was linked to the zoo in the Jardin des Plantes near the Musée National d'Histoire Naturelle. It was assumed that the epidemic was caused either by an infected giant panda imported from China or an infected horse introduced from Iran.

Clinical aspects

The incubation period can last **weeks, months or years.** Subclinical infections can occur. The disease can be latent for years. Often the clinical presentation is that of an acute febrile respiratory infection **(pneumonia), but acute localized skin infection** (skin abscess with or without drainage sinus, necrotizing fasciitis, lymphangitis), **blood stream infection** with or without a clear focus, genitourinary infection, synovitis with or without septic arthritis, osteomyelitis, neurological involvement (myelitis, brain-stem encephalitis with cranial-nerve palsies) and chronic disease with **disseminated organ abscesses** also occur. Suppurative parotitis seems to be common in Thailand and Cambodia but is very rare elsewhere. **Pure cutaneous** forms without systemic features exist, from a primary solitary lesion to multiple lesions (secondary spread). Pustular rash can be found during septicaemia. Respiratory tract infection is sometimes difficult to distinguish from tuberculosis (both classical and miliary). Pulmonary cavities can appear. Splenomegaly is regularly present. During pulmonary melioidosis, urticaria, flushing and/or cyanosis can occur. In some areas, such as northern Thailand, it is the most important cause of community-acquired bloodstream infection.

Meloidosis is one of the "great imitators" due to its wide -ranging clinical presentation.

Melioidosis tends to have a **protracted course** and cure is difficult without a **prolonged course** of appropriate antibiotics.

Risk factors include alcoholism, malnutrition, renal failure, chronic pulmonary disease, corticosteroid use, cancer and especially diabetes. There is insufficient data about a possible interactions with HIV.

Mortality in active disseminated disease is high, about **40-80%,** especially when additional risk factors are present. With early diagnosis and institution of therapy with ceftazidime or meropenem and access to state-of-the-art intensive care therapy, the overall mortality from melioidosis can now be as low as 10 percent.

Diagnosis

Patients tend to be from Southeast Asia (esp Northeast Thailand, Cambodia) or Northern Australia.
The infection can be suspected from a chest X-ray. The diagnosis is established by culture (blood, urine, skin, sputum). The organism grows on several media but should be distinguished from *Pseudomonas* species. Growth can be quite slow, as compared with other bacteria that cause blood stream infection. In view of the risk which this organism presents, culture and isolation is best left to well-equipped laboratories. Gram stain of sputum and abscess pus may reveal gram-negative bacilli of *B. pseudomallei*. The organisms often have a characteristic bipolar staining with a "safety pin" appearance.
Antibodies can be detected serologically. A positive serology can point to an active infection or a previous (including subclinical) melioidosis. Most seropositive patients have no overt clinical disease. A latex agglutination test which can be used with urine has been developed. The main differential diagnoses are tuberculosis, disseminated fungal infections and chronic pyogenic osteomyelitis but melioidosis is one of the "great imitators". It is clear that more research is needed.

Treatment

Burkholderia pseudomallei is **intrinsically resistant to numerous antibiotics**, including aminoglycosides, penicillin, ampicillin, first- and second-generation cephalosporins, chloramphenicol and fluoroquinolones. First line treatment for severe cases is IV **ceftazidime** (Glazidim®, a beta-lactam belonging to the third generation cephalosporins) combined with cotrimoxazole. Dosage is ceftazidime 2 grams TDS for a minimum of 2 weeks. Beta-lactams belonging to the carbapenems such as imipenem in combination with cilastatin (Tienam®) or meropenem (Meronem®) are (expensive and often difficultto-access) alternatives.

For mild ambulatory cases, amoxicillin with clavulanic acid (co-**amoxiclav,** Augmentin), also in combination with high dose **cotrimoxazole** forte 3 to 4 tablets per day for an adult (one tablet containing trimethoprim 160 mg + sulfamethoxazole 800 mg) is often used.
The optimal duration of maintenance treatment (cotrimoxazole or doxycycline) is not known but 3-6 months is often recommended.

Relapse can occur after several years, especially during immunosuppression. This means that lifelong follow-up is indicated.

There is currently no vaccine available.

Rickettsiosis and related infections

Rickettsiosis

Summary
- Rickettsioses: bacterial infections of varying degrees of severity
- Transmission of typhus via lice, flies, ticks or mites
- Basic lesion is vasculitic
- Fever, rash, sometimes chancre and multi-organ involvement
- Hepatosplenomegaly, neurological signs, heart failure, renal insufficiency, bleeding
- Diagnosis clinical and often difficult; serological tests and PCR often not available
- Treatment with tetracyclines (1st choice)

General

Rickettsiae are **very small bacteria** (0.8 x 0.4 µm) that belong to the alpha-group of purple bacteria. The Rickettsiacea family contains the genera Rickettsia and Orienta. These coccobacilli are closely related to *Bartonella, Wolbachia, Cowdria* and *Anaplasma*. They multiply intracellularly. They have a Gram-negative cell wall structure, but cannot be detected by Gram staining, although they can be by Giemsa staining – with difficulty.

They derive their name from the American researcher, Howard Ricketts, who discovered them in 1909 in Montana, USA, as the source of a serious disease (Rocky Mountain Spotted Fever = RMSF). He himself died from typhus in an epidemic in Mexico some years later.

Rickettsia discovery

They bacterium derives its name from the American researcher, Howard Ricketts, who discovered them in 1909 in Montana, USA, as the source of a serious disease (Rocky Mountain Spotted Fever = RMSF caused by Rickettsia rickettsiae). Originally the disease was called Black Measles due to the spotted rash throughout the body of infected patients. Howard himself died from typhus in an epidemic in Mexico some years later. In 1916, Henrique da Roche
Lima discovered Rickettsia prowazecki, the bacterium that causes epidemic typhus. He named it after his colleague Stanislaus van Prowazek, who had died from typhus whilst investigating the diseases in a prison hospital in Hamburg.

The historical role of Typhus in various armed conflicts

The Grande Armée of Napoleon Bonaparte lost many soldiers from epidemic typhus during the invasion of Russia in 1812.
Of the invading 422,000 soldiers of the Grande Armée, only a few ten thousand (numbers vary according to source) would return due to decimation by epidemic typhus, extreme cold, hunger and to a lesser degree battle. Several decades later during the Crimean War (1854-56) between Russia and England and France on the other, typhus took a high toll. Florence Nightingale was famous for her help to the wounded during this dreadful conflict. In the 1915 Serbian epidemic, it is estimated that nearly all the country's 400 doctors contracted epidemic typhus and more than a 100 of them died. The scale of the massive epidemics in Eastern Europe and Russia between 1918 and 1922 can hardly be imagined,

with an estimated 20-30 million cases and at least 3 million deaths. Now there are occasional flare-ups of epidemic typhus, as in 1997 in Burundi withan estimated 24,000 cases in the first half of that year.

Classifications

Different classifications may be found in many textbooks and manuals, e.g. the "Spotted Fever" group (transmitted by ticks), the typhus group (transmitted by fleas and lice, no outer membrane protein OmpA) and scrub typhus. The division is based on **intracellular growth characteristics** and on antigenic differences between the various micro-organisms. Organisms of the spotted fever group cause rapid cell lysis and spread rapidly from cell to cell, while *R. prowazekii* - belonging to the typhus group - grows to enormous numbers intracellularly before causing the host cell to burst.
Spotted fever group Rickettsiae are found in both the nucleus and the cytoplasm, whereas *R. prowazekii* is found in the cytoplasm only. In practical terms these divisions are not useful. They can give rise to confusion rather than clarification.

New Rickettsiae and various subtypes are still regularly being discovered. It is easier just to state that there are various sorts of Rickettsiae and that they cause a range of diseases of varying severity.
Furthermore, not all Rickettsiae occur everywhere. Thus RMSF is not found in Asia, nor does scrub typhus exist in America.

Another way to classify rickettsioses is according to the transmitting vector, but the patient is often unaware of the ectoparasite that bit him. It is probably more useful to classify Rickettsioses according to their clinical picture's severity:

Mainly very serious course

Species	Disease	Vector	Distribution
R. prowazekii	Epidemic typhus	Louse	Worldwide
R. rickettsii	Rocky Mountain SF	Tick	America
O. tsutsugamushi	Scrub Typhus	Mite	SE-Asia, Australia

Mainly mild to moderately severe course

Species	Disease	Vector	Distribution
R. typhi (mooseri)	Endemic typhus	Flea	Worldwide
R. felis	Flea typhus	Flea	Europe, Americas, Africa, Thailand, New Zealand
R. conorri	Fièvre boutonneuse	Tick	Mediterranean, Africa (India?)
R.africae	African Spotted Fever (SF)	Tick	Africa, Carribean
R. sharoni	Istraeli SF	Tick	Middle East
R. sibirica	North Asian SF	Tick	Siberia, Mongolia
R. japonica	Japanese SF	Tick	Japan
R. australis	Queensland SF	Tick	Australia
R. honei	Flinder Island SF	Tick	Australia

R. mongolotimonae	Atypical fièvre boutonneuse	Tick	Asia, Europe, Africa
R. helvetica	Influenza syndrome	Tick	Europe
R. slovaca	Tick-borne lymphadenopathy	Tick	Europe
R. akari	Rickettsialpox	Mite	USA, Africa

Transmission

With the exception of epidemic typhus, rickettsiosis are **zoonoses**. Transmission to humans occurs via **arthropods.** Ticks and mites infect humans through their bite. Lice and fleas infect humans through their faeces. Louse faeces can remain contagious for months. Ticks and mites transmit the organisms to their progeny (transovarial transmission). Mites and ticks are thus both vector and reservoir. In mites, infection with *Orientia tsutsugamushi* causes a shift in the sex-ratio in the offspring of the mites so that the female mites predominate in the following generation. This can be prevented by treating mites with tetracyclines.

Typhus transmission via lice

In 1906 Charles Nicolle demonstrated that **infection can be transmitted by body lice** (head lice and public lice are not known to transmit pathogens). Afterwards it was shown that **louse faeces** were infectious. Transmission is also possible when dry louse faeces are **inhaled** via aerosol.

Charles Nicolle

At the time Charles Nicolle was working at the Pasteur Institute in Tunis. There were numerous cases of typhus and the hospitals were over-full. In 1909 he observed that personnel in the laundry became infected when they had washed the clothing of people who had been admitted. There was however no secondary infections originating in the over-full hospital
wards. Hospitalised patients were given a hot bath with soap and clean hospital clothing on their admission. Dr Nicolle suspected a pathogenic agent in the patients' dirty clothing and underwear. He injected a chimpanzee with a patient's blood.
After a few days he collected some lice from the animal and introduced these insects into another, non-injected healthy chimpanzee. This second animal in turn became ill after ten days. Control experiments confirmed the results.

People who have previously survived epidemic typhus (R. prowazekii) often harbour the bacteria in their body for life, even though they are asymptomatic (**chronic carriers**). In the event of immunosuppression, this can result in a mild flare-up of the infection, even after many years (**Brill-Zinsser disease**). When such a person is in the "right" circumstances, this can cause epidemic louseborne typhus. As transmission of epidemic typhus occurs through lice, epidemics occur in conditions of poverty, overpopulation and poor hygiene (war, prisons, starvation, natural disasters, the homeless, refugee camps). The louse takes a contaminated blood meal and the bacteria proliferate in its intestinal epithelium. After 3-5 days, the infected cells burst. The intestine and faeces contain very large numbers of the bacteria. The haemolymph of the insect turns red from the passage of the intestinal contents (blood) into

the body cavity. The **louse itself does not survive infection** with *R. prowazekii* and dies after 1 to 3 weeks. **It does not form a reservoir.** The American flying squirrel (*Glaucomys volans*) is a sylvatic reservoir for *R. prowazecki* with occasional transmission to humans after aerosolization of its faeces containing infected fleas and lice. Squirrel fleas (Orchospea howardii) will bite humans and transmit epidemic typhus to humans if their normal host, the flying squirrel, is unavailable.

Note: the body louse is also the vector of recurrent fever (see borreliosis) and trench fever.

Pediculus humanus capitis. Head louse. Copyright ITM

Typhus transmission via fleas

The reservoirs of endemic or so-called murine typhus (*R. typhi*) are rodents (mice, rats). The infection is transmitted to humans by rodent fleas such as the oriental rat flea, *Xenopsylla cheopis*. In certain circumstances, e.g. markets, grain stores and forest fires, there is increased contact with rodents and their fleas and transmission can occur. In contrast to R. prowazekii, R. typhi does not kill the vector. A closely related organism, *R. felis* is transmitted by cat fleas (*Ctenocephalides felis*). One of the reservoirs for this bacterium is the opossum (California), but the organism has also been detected outside the USA, in Latin America, Africa, Europe, Thailand and New Zealand.

Cat flea. *Ctenocephalides felis.* Occasional vector of *Yersinia pestis* (plague) and vector of *Rickettsia felis.* Copyright ITM

Rickettsia transmission, via ticks

Female hard tick. *Rhipicephalus* dorsal view. Notice the small dorsal shield, typical of females. Copyright ITM

Rickettsial spotted fever and **Rocky Mountain spotted fever** are transmitted by the bite of hard ticks. Dermacentor variabilis (American dog tick) is notorious in the eastern USA, while in the western USA Dermacentor andersoni is the principal vector (Rocky Mountain wood tick) for Rickettsia rickettsii. Besides those main vectors, Rhipicephalus sanguineus, the brown dog tick, also plays an important role in transmitting the infection to humans in the USA and Mexico. Amblyomma cajennense and A. aureoloatum play a role in Latin America and Brazil. In Africa Rhipicephalus species are responsible for transmission of R. conori and Amblyomma species for R. africae. A wide variety of **mammals constitute the reservoir.**

Queensland Spotted Fever, Japanese SF, Astrakhan SF, Israeli SF, Flinders Island SF and Siberian SF are also transmitted by **hard ticks.** Rickettsia slovaca was first identified in Dermacentor ticks from Slovakia and has subsequently been found in Dermacentor marginatus and D. reticulatus in France, Switzerland, Portugal, Spain, Armenia and Germany. The geographical areas where certain species occur, is not well known. E.g. in 2002, the first case of infection with R. aeschlimannii was detected in South Africa.

Transmission of this bacterium can occur via the bite of Hyalomma ticks and Rhipicephalus ticks. This bacterium must of course have existed before but was previously not identified.
Rickettsia heilongjiangensis was isolated in 2002 from Dermacentor sylvarum ticks in the Heilongjiang Province of China, near the Russian-Chinese border. This rickettsia is closely related to *R. japonica.*

The bacteria enter the tick as part of its blood meal and multiply. The organisms are transmitted with the saliva during the next bite. **Transovarial transmission** in ticks can be 100%, but other factors also play an important role in determining the final infectious state of the vector. In the USA <1% of Dermacentor ticks in the wild are infected with *R. rickettsii*. This may be explained by an interference phenomenon in which infection of the tick with the very commonly occurring, non-pathogenic *R. peacockii, R. belli, R. montana* or *R. rhipicephali* prevents *R. rickettsii* from becoming established in tick ovaries. Naturally occurring double infections (two species of Rickettsia in 1 tick) have yet to be observed. Vertical transmission occurs when a female tick has infected ovaries, which ensures infected tick progeny. However, it is known that *R. ricketsii* takes a substantial toll on the tick, since few larvae emerge from infected eggs, and even fewer survive and mature into adults. Horizontal transmission

depends upon transient rickettsaemia in a nonimmune host, on which uninfected ticks feed, creating newly infected ticks. Feeding adjacent (in time and space) to an infected tick allows for the acquisition of *R. rickettsii* without the presence of infection in the host (uninfected tick ingests saliva from the infected tick).

Typhus transmission via mites

Scrub typhus is caused by *Orientia tsutsugamushi* [Japanese "tsutsuga" = sick; "mushi" = insect]. The organism was classified in the past as *Rickettsia tsutsugamushi.* There are several antigenic variants (Gilliam, Karp, Kato, Shimokoshi, Kuroki, etc...). The organism is only transmitted by the **bite of mite** larvae known as "chiggers" (Leptotrombidium sp.). In nature the larvae feed on rats and other rodents while the adults feed on small invertebrate animals and insect eggs. The infection occurs focally in Asia where there is a specific ecological habitat of **transitional vegetation** (sides of roads, overgrown agricultural areas, disturbed rain forests, river banks, etc.). The larvae secrete an enzyme that dissolves animal tissue, after which the mite can suck up the fluid. This causes local irritation. When *Orientia tsutsugamushi* is introduced into the skin an inoculation chancre occurs in 50% of infections.

Infections with *R. akari* are not often seen in clinical practice and the condition "**Rickettsialpox**" is more of a curiosity. Transmission occurs via **mite bites:** *Liponyssoides sanguineus.* These mites parasitise mice.

Ticks that serve as vectors for Rickettsia from Eurasia, Australia and Africa.

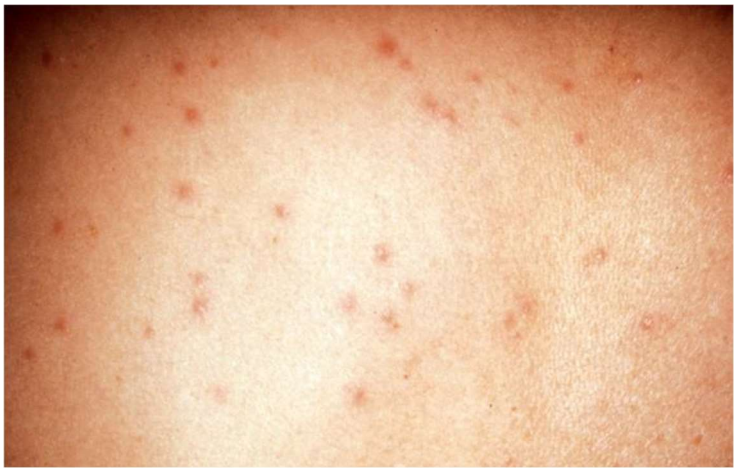
R. conorri	*Rhipicephalus sanguineus*	Mediterranean
R. sibirica	*Dermacentor sp*	Europe, former USSR, China
R. heilongjiangensis	*Dermacentor sylvarym*	China
R. australis	*Ixodes holocyclus*	Queensland
R. japonica	*Haemaphysalis longircornis*	Japan
R. honei	Insufficient data	Flinders Island
R. africae	*Amblyomma variegatum*	Ethiopia, Southern Africa
R. mongolotimonae	*Hyalomma sp.*	France, Inner Mongolia, Africa
R. slovaca	*Dermacentor Marginatus*	Europe
R. monacensis	Suspected *I. ricinus*	Europe
R. helvetica	*Ixodes ricinus*	Africa
R. aeschlimannii	*Rhipidephalus appendiculatus, htalomma marginatum*	Africa
Astrakan fever agent	*Rhipicephalus sanguineus, R. pumilio*	Astrakhan region of ex-USSR

Clinical aspects

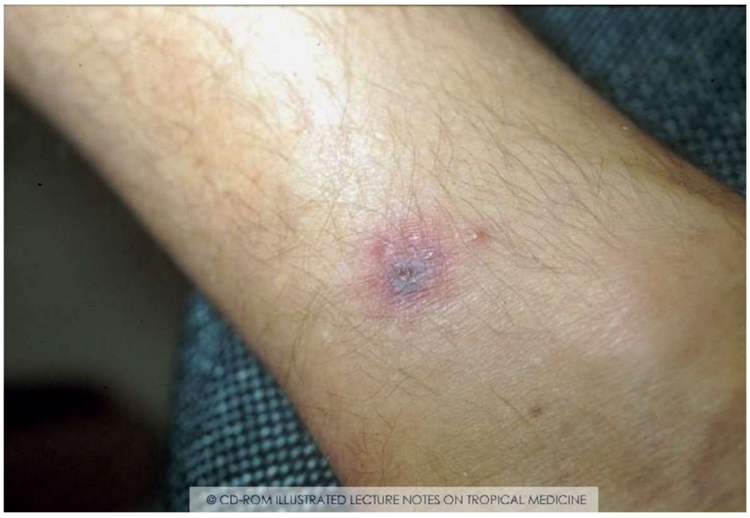
General features rickettsial disease

As there are several diseases that are caused by *Rickettsiae*, a general description is difficult. The **incubation period is 1 to 3 weeks**. After inoculation, *Rickettsiae* proliferate intracellularly in the **endothelium of small blood vessels**. Endothelial damage results in **focal occlusive**

endangiitis in small venules and arterioles. Histologically this is identified in tissue sections in the form of typhus nodules (**Wolbach nodules**; not to be confused with typhoid nodules in the liver in typhoid fever!). In this way a **generalised, multifocal, multi-organ vasculitis occurs.** This can lead to thrombosis and vascular occlusion, possibly with oedema and local necrosis. As practically every organ in the body can be affected, the symptoms are extremely diverse. The various symptoms can be better understood if the localisation of the vasculitis lesions is borne in mind.



Skin rash during infection with *Rickettsia conori*. Copyright ITM



Inoculation chancre, *Rickettsia conori*. Copyright ITM

The lesions appear in:

• **Skin:** At the site of the arthropod bite there is sometimes a papulovesicular lesion with local necrosis: inoculation chancre (tache noire [black spot]). The regional lymph nodes can enlarge subsequently. A chancre occurs in fièvre boutonneuse, South American RMSF ("Sao Paulo tick fever") and frequently in scrub typhus (but not necessarily). The chancre is almost always absent in North American RMSF and never present in epidemic and endemic typhus. The rash should be distinguished from severe measles, severe dengue and septicaemic purpura, e.g. due to meningococci. With a mild rash, a distinction must be made from typhoid fever (treatment differs).

• **Brain:** Meningo-encephalitis with confusion ("tuphos"), delirium and coma. Distinction from cerebral malaria is important. Often occurs with scrub typhus, epidemic typhus and RMSF.

Hemiplegia can occur. In general there are features of aseptic meningitis, but in RMSF there can also be an increase in the number of neutrophils in the cerebrospinal fluid. Deafness may persist for months in scrub typhus

• **Myocardium:** Myocarditis, heart failure, hypotension and shock. Hypovolaemia as a result of bleeding and increased vascular permeability contributes to low blood pressure.

• **Blood vessels:** Occlusion of arteries results in gangrene, possibly late onset (toes, fingers) and occurs predominantly in epidemic typhus and RMSF. Thrombophlebitis occurs as a result of vasculitis and stasis in severely ill patients.

• **Kidney:** kidney failure from vasculitis and interstitial nephritis, promoted by hypotension; albuminuria, oliguria.

• **Eyes:** Conjunctivitis, papilloedema (with cerebral involvement). Enlargement of the blind spot and scotomas occur frequently in scrub typhus.

• **Lungs:** Cough, tachypnoea, dyspnoea.

Clinical aspects of epidemic typhus, scrub typhus and RMSF

These infections usually have a very serious course. The incubation period is 5-10 days for scrub typhus and RMSF and ± 12 days for epidemic typhus. After a few days of generally not feeling well, a **high fever** occurs. It is associated with severe general malaise, severe headache, muscular pain, conjunctivitis, cough, hypotension, meningeal irritation, vomiting, epistaxis, confusion or coma.

Hepatosplenomegaly occurs occasionally but is rare. Lymphadenopathy occurs in approximately one in four patients. **Rash appears around the 3rd to the 7th day** after the onset of fever. The absence of a rash in the first few days often makes it difficult for the diagnosis to be suspected at an early stage. The skin rash in RMSF begins on the wrists, palms and soles and spreads **centripetally** to the trunk. In epidemic typhus and scrub typhus it is the reverse: beginning on the trunk (axilla), it spreads **centrifugally** over the rest of the body, sometimes sparing the face, hands and feet. The rash may develop into purpura and can rapidly become haemorrhagic. **Gangrene** of the fingers and toes can occur. Because of diffuse intravascular coagulation (fibrinogen consumption), there may be a pronounced bleeding tendency. Rocky Mountain fever sequelae include deafness, amputations and permanent learning disabilities.

O. tsutsugamushi has several subtypes and repeated infections with scrub typhus are possible. Untreated scrub typhus fever can persist for more than 2 weeks and is often accompanies by intense headache and diffuse myalgias. In about 50% of patients an eschar is present.

Brill-Zinsser disease is defined as the recrudescence of epidemic typhus years after the initial episode.
In contrast to acute primary infection Brill-Zinsser disease is generally a mild illness.

Clinical aspects of endemic typhus

Endemic flea-borne typhus follows the **same course as epidemic typhus, but milder**. Reaching a clinical diagnosis is difficult and the disease is often missed. Rash occurs in half the cases. There is no chancre. Similar symptoms are present in infection with *R. felis*. Differential diagnosis includes typhoid fever, ehrlichiosis, dengue and other arboviroses.

Clinical aspects of tick-borne rickettsioses

Rickettsia spotted fever

This disease follows the **same clinical course as mild RMSF**. The **rash** is generalised. The inoculation **chancre is characteristic** here. During physical examination, a search for this chancre often leads to the correct diagnosis. **Subcutaneous vasculitis** can result in the formation of subcutaneous nodules (fièvre boutonneuse). *R. africae* occurs predominantly in Southern Africa. Skin rash is more confined or absent in infection with *R. africae.*

Clinical signs of infection consist of a skin lesion at the site of the tick bite and regional lymphadenopathy it is often painful. Fever and rash develop subsequently. The acute disease can be followed by fatigue and **residual alopecia** at the bite site.

Rickettsialpox

Rickettsia akari causes rickettsialpox. It is a rare infection which manifests as a self-limiting, febrile, vesicular skin rash, often **confused with varicella.** The differential diagnosis includes monkeypox, a viral pox disease which to date is endemic in Central Africa and is known to cause epidemics in men having sex with men.

Diagnosis

Clinical

In developing countries, the diagnosis of typhus is predominantly clinical. If scrub typhus (Southeast Asia) or boutonneuse fever (Africa, Mediterranean Sea basin) is suspected, a chancre should be sought.

Eschars are may be overlooked easily when a careful clinical exam including inspection of genitalia and skin folds under the breast is not performed.

In RMSF, the cerebrospinal fluid is usually normal, although sometimes the neutrophil count is slightly raised. Scrub typhus and murine typhus can cause an increased number of lymphocytes in the cerebrospinal fluid in meningo-encephalitis so that the infections can resemble also (arbo)viral infections and leptospirosis. In the blood, the white blood cell count is normal or reduced.

Diffuse intravascular coagulation often occurs which is accompanied by thrombocytopenia.

Serology

The diagnosis can be confirmed at a **late stage by serology**. A 4-fold increase in titre between acute and convalescent sera must be detected. Serologic testing is helpful for a retrospective diagnosis of rickettsiosis but will not assist in clinical decision making. IgM and IgG antibodies typically appear 7 to 10 days after the onset of the illness, the optimal time to obtain a convalescent antibody titre is 14 to 21 days after the onset of symptoms. Treatment must be started early without waiting for laboratory confirmation.

Culture

Isolation of the organism by blood culture is usually not performed. **Culture of rickettsia is difficult**, laborious and dangerous (tissue culture or isolation on embryonated eggs). Rickettsia

is an obligate intracellular parasite and in only a few reference labs in the world it is cultured on cell culture monolayers. Growth is confirmed using specialized stains (e.g. Gimenez). Guinea pigs can be inoculated with blood from a patient. After 4-5 days, the animals develop fever and male guinea pigs develop a swollen scrotum (Neil-Mooser reaction). There is a significant risk of laboratory infection.
PCR technology has become very important in identifying rickettsial species and strains: this is usually done on blood or skin biopsies of eschars.

Treatment

Untreated, the mortality of RMSF is 20 to 40% and of epidemic typhus ± 20%. General status (malnutrition, etc) plays a role here. Scrub typhus has a mortality rate of 6%, endemic typhus follows a milder course (mortality 2%) and fièvre boutonneuse has a low mortality (< 1%). It is not necessary to wait for confirmation of the diagnosis for treatment. If the course is fulminant, antibiotics are relatively ineffective.

Antibiotics

Tetracyclines are active against the organisms and are the first line treatment. The organisms are not 100% eliminated from the body. Recovery is determined by the patient's immunological resistance.
Doxycycline is very useful in epidemics of louse-borne typhus and for scrub typhus. RMSF and endemic typhus should be treated for at least 1 week. In epidemic typhus an improvement may be expected within 24 to 72 hours.

Chloramphenicol is second choice, e.g. in pregnancy, notwithstanding the risk of "grey-baby" syndrome. **Ciprofloxacin** has some activity against Rickettsiae, but is inferior to doxycycline.
Azithromycin has been used for mild forms in pregnancy. Erythromycin is not a good choice. Often the initial differential diagnosis includes bacterial meningitis caused by Haemophilus influenza or Neisseria meningitidis. Chloramphenicol is also active against these organisms. **Penicillins, ampicillin and streptomycin are inactive against *Rickettsiae***. Traditionally it is assumed that scrub typhus is highly susceptible to tetracyclines (this is sometimes used as a diagnostic test). In Thailand in 1996, scrub typhus infections were observed which clearly exhibited reduced susceptibility to doxycycline (both clinically and in vitro). Azithromycin or rifampicin 900 mg daily for 1 week is used as treatment in these cases.

Vector control

All patients and their clothing should be free from insects, ticks and mites. Delousing is of major importance in epidemics. The patient should be washed (removal of louse faeces on the skin and in the hair). Clothes and sheets should be decontaminated.

Prevention

- Epidemic typhus: Delousing (e.g. 1% permethrin or 1% malathion puffs in/on clothing, heat sterilisation of clothing), treat cases, improve general hygiene.
- Endemic typhus: Rodent control
- Scrub typhus: Preventive antibiotics, rodent control. DEET or permethrin on clothing and skin. In endemic areas vegetation must be cleared.
- RMSF and rickettsia spotted fever: Protective clothing, tick repellents in infested areas.

- Manual removal of ticks.

Weigl vaccine

The so-called Weigl vaccine was produced from 1920 to 1930. Lice were inoculated intrarectally with viable R. prowazekii. The lice fed on Dr Weigl and on the bodies of his colleagues so that the rickettsia was able to proliferate. Some of his colleagues died from typhus. Some 100 lice were necessary for one dose of vaccine. Subsequently it was decided to culture a louse strain ("Orlando") that sucked blood from rabbits. This strain is still the reference strain for study of these insects.

Q-Fever

General

In some textbooks, Q fever is included among the rickettsiosis for historical reasons, but clinically the condition differs fundamentally from "typhus" presentations. In 1937, Derrick described a new and unusual febrile illness affecting abattoir workers in Brisbane, Australia. When the blood of these febrile patients was injected into guinea pigs the animals developed mild fever and splenomegaly. Burnet identified small, filterable rickettsial-like micro-organisms in the spleens of these infected animals. Cox cultured the bacteria in yolk sacs of embryonated hen's eggs (the bacteria cannot be cultured on cell free media). Davis and Cox isolated the organism from ticks collected near Nile Mile Creek in Montana, USA. The disease is now called Q fever, where the Q refers to query because of the initial mysterious nature of the disease. Cox and Burnet have been honoured for their discoveries in the designation of the causal agent *Coxiella burnetii.*

Coxiella burnetii is a small (0.3-1.0 µm) pleomorphic **strict intracellular Gram-negative bacterium** that originally was classified among the Rickettsiaceae. More recent phylogenetic studies show that taxonomically the organism is only distantly related to the Rickettsiae. Gene-sequence analysis (16S rDNA) now classifies it in the order of the *Legionella*, family Coxiellaceae.

C. burnetii proliferates **intracellularly in an acidic vacuole** (phagolysosome, pH 4.8). Infection with this bacterium inhibits the normal final phagosome maturation step, and therefore the bacterium will survive. Interferon-gamma reverses this and allows intracellular killing of the bacterium. Interferongamma also induces the killing of C. burnetii through apoptosis of infected macrophages. The organism can survive for a long time as a spore (small-cell) in very unfavourable conditions in the environment. The small-cell and large-cell variants can be distinguished by electron microscopy. The large-cell variants multiply in host cells. These variants should not be confused with antigenic states phase I and II (see further).

Epidemiology

Reservoir

The reservoir is found in animals. Q fever is a **worldwide zoonosis**, although no endemic cases have occurred in New Zealand. Arthropods, fish, birds, rodents, marsupials, horses, dogs, cats, cattle, goats and other animals can be infected. The most important sources of infection for humans are cattle, sheep and goats. In these animals, the uterus and mammary glands are primary sites in the chronic phase of infection. Infected mammals shed bacteria in urine, faeces, milk and birth products. High concentrations of *C. burnetii* (up to 109 bacteria per gram of tissue) can be found in the **placenta of infected mammals**. Bacterial spores can remain viable in **dust and dried faeces** for a very long time (years).

Transmission

Transmission **between animals often occurs through ticks**. There is often reactivation of an infection in pregnant animals. During parturition, an infectious aerosol can be formed. Inhalation of contaminated aerosols from parturient fluids of infected lifestock is important. Animal-to-human transmission of the infection then occurs **aerogenically**. There is apparently no human-to-human transmission. Very rarely transmission occurs from drinking

contaminated milk. Inhalation of stirred up contaminated dust (e.g. sleeping in stables previously occupied by sheep, manure) is another risk factor. Persons most at risk for infection are farmers, people living downwind from farms and contaminated manure, straw or dust, laboratory personnel working with *C. burnetii* and abattoir workers.

The largest outbreak ever recorded started in Herpen, in the south of the Netherlands in 2007. It soon spread to two Dutch provinces Noord Brabant and Gelderland. Before 2007, about 15 cases per year were diagnosed in the country. This increased to 2357 human cases in 2009, luckily with "only" 6 deaths in this year. The epidemic continued in 2010. A considerable number of cases were urban. An official ban to spread manure from goat and sheep farms did not seem to achieve significant results. Other hygienic measures, particularly pregnant women avoiding contact with small ruminants have been applied. Limited vaccination of milking sheep and goats was undertaken in 2008. A massive vaccination program was undertaken in 2009 (see further, under prevention).

Q fever was studied by the military for its **potential as a biological weapon.**

Clinical aspects

Primary infection *with C. burnetii* is commonly asymptomatic. HIV patients appear to have a higher risk for symptomatic disease. The **incubation period is rather long** (14-26 days with an average of 15 days). Q fever does not cause direct vasculitis and the infection manifests itself differently from typhus.

However, circulating immune complexes may occur which can lead to glomerulonephritis and leukocytoclastic vasculitis. There is no such thing as "classic Q fever". Most symptomatic patients have a **self-limiting, febrile syndrome**, possibly with headache, nausea and losing weight ± atypical pneumonia; similar to *Mycoplasmapneumoniae, Chlamydiaepneumoniae*, legionellosis or viral pneumonia. With pulmonary involvement, there is often no cough (cough occurs in only 25%), but in general the chest X-ray will be abnormal. **Hepatitis and endocarditis** also occur, as well as -albeit rarely- thyroiditis, orchitis, pancreatitis, myocarditis, pericarditis, SIADH, haemophagocytosis or erythema nodosum. Various **neurological** problems can occur, including optic neuritis and aseptic lymphocytic meningitis. In Q fever cerebrospinal fluid is often normal even though *C. burnetii* can be isolated from patient's cerebrospinal fluid. Encephalitis and/or cerebellitis can occur (often with ataxia). Severe headache and chronic tiredness are also frequently present. There is rarely rash and there is no chancre. Sometimes slight leukocytosis is present, but in most cases (75-90%) the white blood cell count is normal. Thrombocytopenia is present in approximately 1/3 of patients. Liver enzymes and creatine kinase levels can be elevated.

Cases of Q fever have been reported in pregnancy. Intrauterine transmission has been documented. The placenta can develop necrotic foci (vasculitis) and fetal infection is known. There is an increased risk of oligamnios, fetal miscarriage, abortion, prematurity, low birth weight and neonatal death. There is also a risk to the obstetrician delivering the baby. Long-term treatment with cotrimoxazole protects against maternal chronic Q fever, although it is only bacteriostatic and carries the risk of neonatal hyperbilirubinemia if used just before delivery.

Chronic Q fever develops in a minority (1-5%) and is defined as infection lasting for 6 months or more. The organs most commonly affected are heart, arteries (vascular aneurysm), bones (beware prothesis, osteomyelitis) and liver. In rare cases mixed cryoglobulinemia can occur. Chronic disease may develop insidiously months or years after the acute disease. In chronic Q fever with cardiac valve involvement, vegetations are only rarely found on echocardiography. Q fever endocarditis carries a high mortality and tends to occur in patients with pre-existing valvulopathy. Chronic Q fever is most likely to develop in those who are pregnant, immunocompromised (eg, patients receiving prolonged or high-dose corticosteroid therapy or tumour necrosis factor-alpha inhibitors), have underlying valvular or vascular disease or a prosthetic joint. In such patients, C. burnetii multiplies in macrophages and produces a prolonged bacteraemia; the resulting high levels of antibodies and immune complexes directed at the organism contribute to many of the symptoms.

Diagnosis

The diagnosis is extremely difficult and based on **specific serology**. The best approach is to look for **seroconversion**. IgM can remain positive for a very long time, even longer than one year in this infection (low titres). The serological response in acute infections is mainly IgM against phase II antigens, followed by IgG antibody to phase II antigen. In chronic infection there is a serological response (IgG and to a lesser extent IgA) to phase I and II antigens. Phase I antigens are less immunogenic than phase II antigens. In patients convalescing from acute disease, phase I antibodies decrease rapidly. In patients with chronic disease, antiphase I titres remain raised as a consequence of constant antigenic stimulation. Immunofluorescence titres to phase I antigen of 1/800 or more are very suggestive of endocarditis, but the cut-off titres used in different labs are variable. As such a positive serology is a major criterion in the "modified Duke criteria" for endocarditis. Because of cross reactivity between *Coxiella* and *Bartonella* antibodies, a positive Bartonella-serology in a patient in whom Q-fever endocarditis is suspected, paradoxically favours the diagnosis of Q-fever. Remember: cats are sources of both *C. burnetii* and *B. henselae.* PCR can be performed on excised heart valve tissue or serum in the initial stage of the infection when serology reveals no or low level antibodies.

Diagnosis chronic Q fever:
1. Phase I IgG larger than or equal to 1/4096, or
2. Phase I IgG larger than phase II IgG, or
3. PCR Coxiella burnetii positive after one month of illness

Antigenic variation

Coxiella burnetii displays antigenic phase variation, similarly to the smooth and rough colonies of certain bacteria when they are cultured in Petri dishes. In animal or human infection, *C. burnetii* exhibits phase I and is very infectious, but after repeated passage in cells or embryonated eggs, it converts to the non-infectious phase II. This transition is associated with a chromosomal deletion. Phase I antigen is a polysaccharide component of lipopolysaccharide. When some carbohydrates are lost, phase II antigen appears. In acute Q fever antibodies against phase II predominate, but in chronic fever the highest titres are found against phase I antigens.

Suggestive but transient **"doughnut"-shaped granulomas** (fibrinoid ring formation) are sometimes detected by liver biopsy. In practice, most cases of Q fever are missed unless serology (IgG and IgM) is available. Culture is possible in embryonated hen's eggs and in various cell lines (human embryo fibroblast cells, green monkey kidney cells and others). However, in view of the infectious and dangerous nature of the organism, in vitro isolation is rarely performed. People who work (e.g. research) with *Coxiella burnetii* have an increased risk of becoming infected.

Treatment

The aim of treatment is different in **acute and chronic Q fever.**

In acute infection, bacteriostatic treatment will usually suffice for a clinical cure. Doxycycline is a good choice here (200 mg/day x 2-3 weeks). Clarithromycin or azithromycin are alternatives.

In chronic Q fever, a bacteriostatic treatment will probably control the disease but not cure it. Bactericidal therapy is preferable. Since the organism lives in a very acidic environment (pH of the phagolysosome = 4.8), an attempt may be made to alkalinise the vacuole, for example by the simultaneous administration of hydroxychloroquine. This will raise the pH from 4.8 to 5.7. In this way it is possible to render doxycycline bactericidal. The preferred treatment for chronic Q fever is hydroxychloroquine combined with doxycycline for at least 18 months (longer if antibody titre IgG remains > 1/800). QTc-time prolongations should be monitored.

In case of Q fever endocarditis, cardiac surgery will often be required. In pregnancy, treatment with cotrimoxazole will prevent fetal death and miscarriage, but this treatment will not prevent the development of chronic infection in the mother. Once the child is delivered, treatment with doxycycline plus hydroxychloroquine for one year will enable normal subsequent pregnancies.

Prevention

When an outbreak is identified, transport of manure in the area will be prohibited.

A formalin-inactivated whole cell vaccine from the Henzerling strain (Q vax) has been used in Australia.

In November 2005, CSL Ltd in Australia (Commonwealth Serum Laboratories, the only producer in the world) announced to stop production of the vaccine for economic reasons, but the Australian government subsequently prevented this. In the Soviet Union, an avirulant variant of the Grita strain has been studied as a vaccine. However, the general public does not need to be vaccinated. Vaccination of people at risk (e.g. lab personnel) is useful. Prevaccination testing is advised, and includes history, serology and a skin test with dilute vaccine. In order to stem the large Dutch outbreak of 2007-2011, the Dutch government provided 400,000 vaccine doses in 2009 (Coxevac, a killed vaccine based on the Nile Miles strain).

Vaccination for humans are reserved for high risk professions (e.g. slaughterhouse workers) and patients with:

1. previous endocarditis (non-Coxiella)
2. heart valve prothesis
3. congenital heart disease
4. aortic or mitral valve problem
5. aortic aneurysm
6. aorta prothesis

Contraindications for vaccination with Q-vax include pregnancy and previous Q-fever.

Ehrlichia and Anaplasma

Ehrlichia and Anaplasma are bacteria related to Rickettsiae. They are obligate intracellular bacteria that grow within membrane-bound vacuoles in human and animal leukocytes. The obligate intracellular bacteria proliferate in white blood cells: monocytes (E. chaffeensis, HME: human monocytic ehrlichiosis) or granulocytes [Ehrlichia sp. related to E. equi (horses) and E. phagocytophila (cattle)].

Historical note

The generic name refers to Paul Erhlich (1854-1915), the famous German bacteriologist (Nobel Chemistry Prize 1908), the discoverer of salvarsan, an arsenical preparation active against syphilis. In 1954 the first human ehrlichiosis was described in Japan, caused by E. sennetsu. Since this initial report, several tick-borne infections have now been recognized. Human monocytic ehrlichiosis (HME) was first described in 1986 and is caused by Ehrlichia chaffeensis. The name refers to the American army base Fort Chaffee in Arkansas.

Human granulocytotropic anaplasmosis (HGA) was described in 1993 and is caused by Anaplasma phagocytophilia. Ehrlichia ewingii was described in 1999 as an agent of human ehrlichiosis. E. ewingii provokes "human granulocytic ehrlichiosis ".

The organisms are transmitted by ixodid ticks. Amblyomma americanum (Lone star tick) is the main vector for E. chaffeensis. In the USA white-tailed deer and coyotes form the most important reservoir. It has been shown that ticks on migrating birds can be infected with Erhlichia sp. and can thus be transported over long distances. Anaplasma phagocytophila in the broad sense is found in rodents such as the dusky-footed wood rats and mice. The reservoir of E. ewingii is still unknown. Transmission occurs predominantly by the bite of infected ticks, but mother-to-child transmission and transmission by blood transfusion or slaughtering of infected animals is reported.

Common symptoms include fever with or without chills, headache, myalgia, arthralgia, weakness, nausea, leukopenia and thrombocytopenia. Rash is uncommon. Liver test abnormalities can be found in about 50% of cases. In rare cases human monocytic ehrlichiosis can be associated with neurological lesions or meningitis. Post-infection asthenia can continue for months. In HIV patients infection can be overwhelming.

Diagnosis

White blood cell and platelet abnormalities are almost always present, so normal values virtually rule out this infection. Anemia is commonly present, so pancytopenia can be suggestive of anaplasmosis or ehrlichiosis.

Probably many infections are missed since laboratory testing is not widely available. The diagnosis of human granulocytic anaplasmosis is made by microscopic examination of a peripheral blood smear or serologic testing. A 4-fold rise in antibody titer between the acute an convalescent phases of infection confirms the diagnosis. Microscopy is labor intensive and the sensitivity of microscopy ranges from 20. to 80% depending on the degree of expertise: bacteria are observed in the cytoplasm of leukocytes as 0.5 to 1.5 µm large inclusions which are combined in groups (morulae). Today, PCR has gained diagnostic importance in high

resource settings. Culturing of this intracellular bacteria is complex and it's the most accurate method, and it is reserved for research purposes.

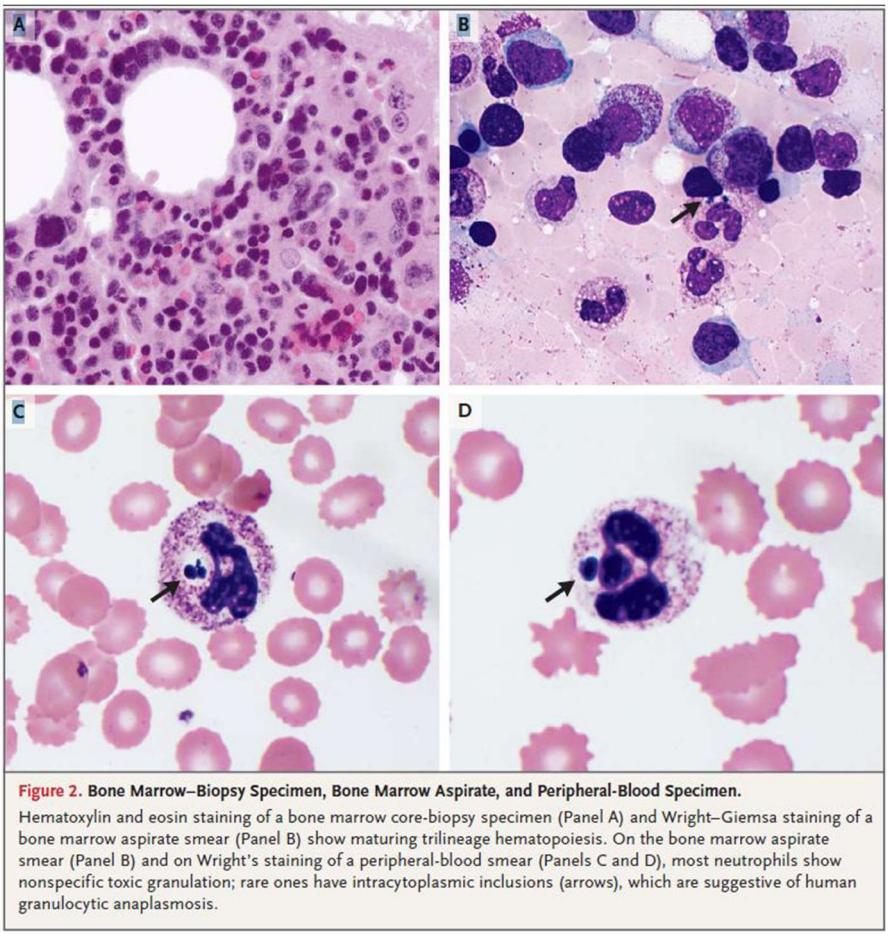
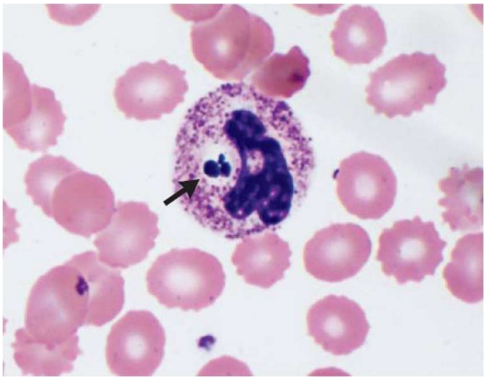


Figure 2. Bone Marrow–Biopsy Specimen, Bone Marrow Aspirate, and Peripheral-Blood Specimen.
Hematoxylin and eosin staining of a bone marrow core-biopsy specimen (Panel A) and Wright–Giemsa staining of a bone marrow aspirate smear (Panel B) show maturing trilineage hematopoiesis. On the bone marrow aspirate smear (Panel B) and on Wright's staining of a peripheral-blood smear (Panels C and D), most neutrophils show nonspecific toxic granulation; rare ones have intracytoplasmic inclusions (arrows), which are suggestive of human granulocytic anaplasmosis.



Human granlulocytic anaplasmosis: Wright's staining of a peripheral blood smear. Neutrophils show nonspecific toxic granulation and some have intracytoplasmic inclusions (arrow). Source: N Engl J Med 2020;382:1258-66. DOI: 10.1056/NEJMcpc1916250

Differential diagnosis:

The differential diagnosis includes rickettsiosis, typhoid fever and several arboviral infections, such as dengue.

The diagnosis of HGA can be overlooked if there is simultaneous infection with B. burgdorferi. In such cases, the typical rash of early Lyme disease (erythema migrans) may mislead the

clinician into ignoring possible coinfection with ehrlichia or anaplasma. Findings that may suggest coinfection include leukopenia, thrombocytopenia, and high fever (all relatively uncommon in Lyme disease) and abnormal liver enzyme tests accompanying the erythema migrans.

Treatment

Treatment is based on administration of tetracyclines, e.g. doxycycline 100 mg twice daily for 7 days.

Bartonellosis

Summary
•Bacterial infection with bacilliformis
•Occurs only in certain areas of South America
•Transmission via sandflies of the genus Lutzomyia
•Treatment with ciprofloxacin or chloramphenicol
•Acute disease: febrile haemolytic anaemia, hepatosplenomegaly, lymphadenopathy, bleeding
•Chronic disease leads to chronic angiomatous skin lesions

General

The genus *Bartonella* currently contains 19 species of bacteria which infect erythrocytes of vertebrate hosts. It is expected that new species will be identified in the future.

In 1992, the *Bartonella* genus consisted of a single species but by 2007, this had increased to 19 officially recognised species. At present, humans are the sole reservoir for only two species: *B. quintana* and *B. bacilliformis.* Exceptionally, infections with other Bartonella species occur and result in bacteraemia or endocarditis (*B. elizabethiae, B. clarridgeiae, B. vinsonii vinsonii, B. vinsonii arupensis* and *B. vinsonii berkhoffii*). In 2007, a newly recognized *Bartonella* species was isolated from a patient with bacteraemia. It grew slowly in BACTEC bottles (blood culture bottles) could not been visualised with Gram staining but stained with acridine-orange. The proposed name is *B. rochalimae*. The infected patient had recently travelled to Peru where she visited places in the Andes mountains. It is possible that some cases of Oroya fever are actually due to infection with this new bacterium.

Bartonella baciliformis	Oroya fever, verruga peruviana, asymptomatic carriers
Bartonella quitana	Trench fever, bacillary angiomatisis, endocarditis, chronic bacteraemie
Bartonella henselae	Cat-scratch disease, bacillary angiomatosis, visceral peliosis, endocarditis, septicaemia
Bartonella clarridgeiae	Cat-scratch disease (rare)
Bartonella elizabthea	Endocarditis
Bartonella washoensis	Cardiac disease
Bartonella grahamii	Neurorentinitis
Bartonella vinsonii	Endocarditis, fever and neurological disease

Bartonella bacilliformis or Carrion's disease

History

South American-bartonellosis, (Carrión's disease, Oroya fever, verruga peruana, verruga peruviana) results from infection with the bacterium Bartonella bacilliformis and is transmitted by sandflies. The infection manifests itself in **two very different clinical forms** with the causal connection being recognised by the young Peruvian doctor Daniel Alcides Carrión.

Pre-Columbian mummies with histologically confirmed verruga lesions have been discovered in Peru and bartonellosis occurred in Francisco Pizarro's army (1471-1541). During the Inca era, the disease was called "Sirki," which means "warts in blood." In Peru between 1869 and 1873 more than 7000 workers building the Lima-La Oroya railway died from the disease at Cocachacra, 65 kilometers from Lima, 1600 meters above sea level. The name "Oroya fever" refers to this, although in the mining town of La Oroya (altitude 3800 m), strangely enough there was no transmission of Oroya fever. In 1936 a large epidemic was seen in the Guaitara valley on the border between Colombia and Ecuador. An epidemic occurred in 1980 in Ecuador and another in 1987 in Peru with a death rate of 88% in the untreated patients. Now and then there have been isolated cases or small outbreaks. In 1997 there was an outbreak in the area of Cuzco, Peru. In an outbreak in Zumba, Ecuador (1995-96), large numbers of dead rodents were found around the places where the cases had occurred. This finding led to the hypothesis that bartonellosis could have an animal reservoir.

Daniel Alcides Carrión

Daniel Alcides Carrión (1858-1885) was a medical student in Lima, Peru. He was required to prepare an original thesis and choose to study the epidemiology and clinical manifestations of verruga peruviana. His home was in Cerro de Pasco, a mining town high in the Andes where he had seen many cases. These left a deep impression on the young man. He told a classmate that he hoped "to make an important contribution to aching humanity". He became concerned with the difficulty in diagnosing verruga peruviana before the typical eruption started. The appearance of the skin lesions was preceded by fever and anaemia, but there was a lot of confusion between the prodromal phase of verruga and other febrile disorders such as malaria. Carrión wanted to determine the incubation period and early symptoms of verruga, so he decided to inoculate himself with some fluid from a chronic skin lesion of a verruga patient. Many friends and professors tried to dissuade him. On the morning of August 27, 1885, Carrión was in the Nuestra Senora de las Mercedes ward of the Dos de Mayo Hospital in Lima. A 14-year-old boy named Carmen Paredes was admitted with verruga on his right eyebrow. Assisted by Dr Chavez, a young ward physician, Carrión used a lancet to inoculate his own arm with blood taken from that verruga. He kept a diary afterwards. The first symptoms started after 21 days, with discomfort and pain in his left ankle. Two days later he developed fever, chills, abdominal pain and generalised pain in bones and joints. He had anorexia and noted severe thirst. His urine became dark red and scanty. He developed jaundice. A week later, he became too ill to continue his diary. His classmates took over this task and were surprised at how quickly anaemia developed. A systolic heart murmur developed and grew in intensity. A few days later, muscle fasciculations appeared in his arm muscles. He said to his friends: "Up to today, I thought I was only in the invasive stage of the verruga as a consequence of my inoculation, that is, in the period of anaemia that precedes the eruption. But now I am deeply convinced that I am

suffering from the fever that killed our friend, Orihuela. Therefore, this is the evident proof that Oroya fever and the verruga have the same origin, as Dr Alarco once said." This insight was the essence of Carrión's experiment. He had not set out to prove the single cause of verruga peruviana and Oroya fever. He only intended to study the incubation period and prodrome of verruga. When a completely different disease developed, he was lucid enough to understand the full meaning of his experiment. On October 3, he became delirious and two days later he fell into a coma and died at midday. He became a hero of Peruvian medicine and is remembered to this day. The day of his death, October 5, is celebrated yearly as the "Dia de la Medicina Peruana". His burial vault - where doctors pay tribute- is in the Hospital Nacional Dos de Mayo in Lima. The Peruvian National University in Cerro de Pasco carries his name.

Puente 'Verrugas' in the Andes, a railway bridge on the trail Lima- La Oroya (Peru). The name refers to a bartonellosis epidemic in 1869-1873.
Copyright ITM

Picture of Dr Daniel Alcides Carrion, on the road Lima - La Oroya. In this area of the Western Andes in Peru, there was a bartonellosis epidemic in 1869-1873. The disease is also known as Oroya fever or Carrion's disease.
Photo Dr Van den Enden. Copyright ITM

Aetiology

Barton described the pathogen in 1909, but he thought that it was a protozoon. The Japanese bacteriologist Hideyo Noguchi demonstrated the bacterial nature of the pathogen. Bartonella

bacilliformis is a small Gram-negative coccobacillus (0.6-1 µm), which takes Giemsa and Warthin Starry stain. The pathogen has one or more polar flagella. It replicates within the vascular endothelium and erythrocytes. The bacterium is related to rickettsiae. The bacillus grows quickly (extracellularly) on non-living culture media with blood or on chicken embryos at 25-28°C. Numerous related organisms are animal pathogens.

Distribution

The disease caused by Bartonella bacilliformis only **occurs in certain narrow high valleys of the western-most slopes of the Andes at altitudes between 500 and 3200 meters in Peru, Ecuador and Colombia**, between 2° N and 13° S. Whether endemic cases occurred in Chili, Bolivia, Guatemala and Honduras is very doubtful. Sporadic cases of so-called "bartonelloses" have been reported in Africa, (Niger, Sudan), in Asia (Pakistan) and in the USA, but it is still not clear whether there is a connection with Carrión's disease. Our knowledge about Bartonella and related bacteria has largely increased in recent years but is still very incomplete.

Transmission

A sandfly, *Lutzomyia verrucarum*, and perhaps a few related species, is responsible for transmission.
Transmission only occurs at night and is seasonal, particularly during the rains. It was formerly assumed that the reservoir was purely human, but this was recently cast into doubt (there may be a rodent reservoir). In some of the inhabitants in the endemic valleys bacteria can be found in the blood, but these carriers are usually without any symptoms. These latent infections which are likely to have been contracted in childhood probably give stable immunity. It is only if non-immune populations enter the endemic area that epidemics occur, sometimes on a large scale, such as in wars or when large public works are being carried out. Tourists may be at risk for the disease.

Clinical aspects

The **clinical range is wide,** going from **asymptomatic infections** via serious febrile forms with acute **haemolytic anaemia**, to the **angiomatous skin lesions** which can be present from the onset or can be preceded by the febrile stage. The mortality of untreated cases varies between epidemics and ranges from 10-40% after 2-3 weeks. The disease is less severe in children and the mortality is far lower. If the course of the disease is favourable, **the fever can last for 3 to 4 months**. In 40-50% of cases of Oroya fever, **concurrent salmonellosis** (generally Salmonella typhimurium) complicates the illness and makes the prognosis less favourable. The superinfection causes fever with gastrointestinal symptoms and a deterioration of the patient's general condition.

Oroya fever or Acute Carrion's Disease

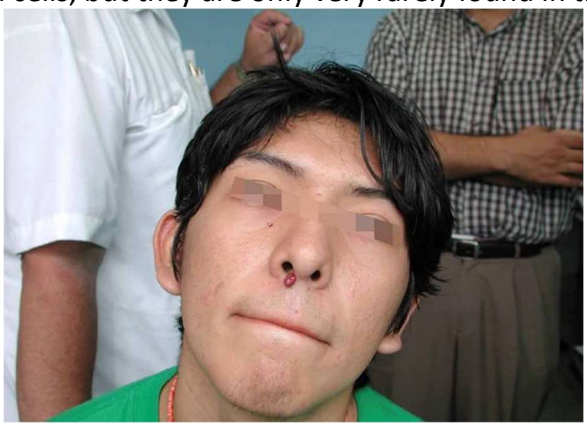
Key clinical aspects

1. Incubation takes approximately **3 to 8 weeks** (range 10-210 days). It begins insidiously with:
2. Irregular **intermittent febrile** attacks with shivering
3. Rapidly worsening **anaemia** with tachycardia, pallor and (sub)icterus
4. Severe **headache** with bone and joint pain. This may persist after the fever has ended

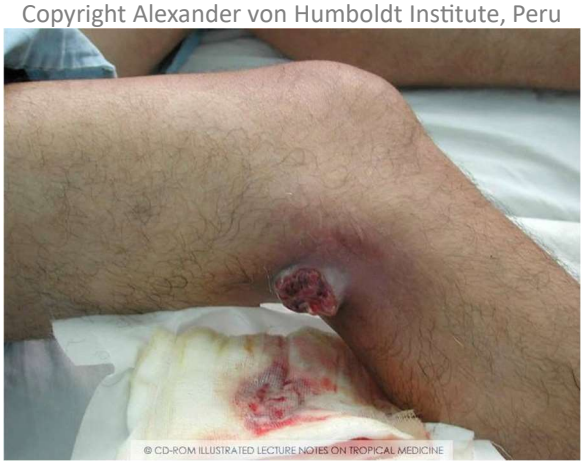
5. Enlargement of the **liver and spleen**, slightly painful on palpation
6. Generalised painful **swollen lymph nodes**
7. Myocarditis, pulmonary oedema and anasarca (generalised oedema)
8. **Haemorrhagic diathesis** as a result of the endothelial lesions: petechiae and tendency to thrombosis. Necrotic foci are found in the liver, spleen and bone marrow.
9. **Neutrophilia**
10. Spontaneous abortion, foetal death or transplacental transmission can occur.
11. **Neurobartonellosis** due to involvement of the CNS takes the form of meningo-encephalitis with or without convulsions and with high mortality. Myelitis also occurs with spastic or flaccid paraplegia with sequelae which can be permanent. There is pleiocytosis of the CSF. More focal and transient lesions of the spinal cord or of the cranial nerves are seen at the verruga stage.

Verruga peruana or Chronic Carrion's Disease

This is the chronic eruptive stage of infection with Bartonella bacilliformis. The painless wart-like skin eruption results from the abnormal growth of blood vessels with the appearance of haemangiomas and the formation of angioblastic nodules. At this stage Bartonella can still be found in the endothelial cells, but they are only very rarely found in the erythrocytes.



Verruga peruviana in chronic bartonellosis (infection with *Bartonella bacilliformis*). Do not confuse this lesion with a granuloma pyogenicum.
Copyright Alexander von Humboldt Institute, Peru



Bacillary angiomatosis. Ulcer due to infection with *Bartonella henselae.*
Copyright Alexander von Humboldt Institute, Peru

The skin eruption usually **appears 6 to 14 weeks after the acute stage.** Both pathological conditions can be present at the same time. The skin eruption may initially be accompanied by a mild fever and arthralgia. The eruption is polymorphic. Some lesions disappear quickly,

others persist or grow for some time only to shrivel and disappear, generally without leaving scars. There are three forms:

Miliary form: the lesions are small (< 0.5 cm), very numerous and mainly found on the face, on the extensor surface of the limbs and on the trunk. They are initially macular and grow to small vascular, sometimes pedunculated and protruding nodules. Lesions are also present on the digestive and genito-urinary mucosa. Dysphagia, haematemesis, melaena, haematuria and metrorrhagia can occur.

Nodular form: the nodules are larger, less numerous, deeper, chronic and mainly found around the elbows and knees. The mucous membranes are spared. The lesions appear in cycles for 2 to 3 months.

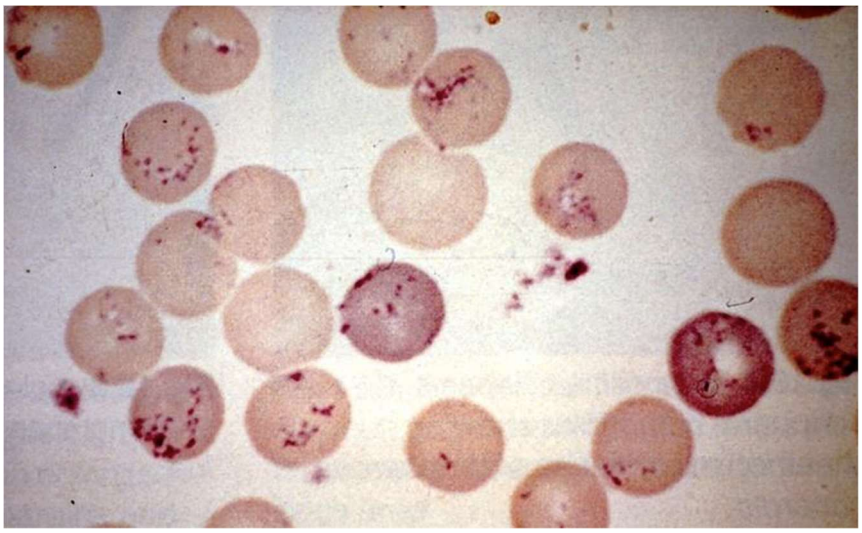
Mular form: there are isolated pseudotumoural haemorrhagic nodules which macroscopically resemble granuloma pyogenicum.

Immunity

Immunity is **gradually acquired during the acute stage**, so that the disease becomes **limited to the wart-like lesions** of the skin and mucous membranes which subsequently heal completely and permanently. In experimentally infected monkeys the **disease can be reversed** from the verruga stage to the febrile haemolytic stage by splenectomy. The same probably occurs in humans. The prognosis of verruga is good. They evolve in spurts and the lesions generally heal spontaneously in less than 6 months.

Diagnosis

The diagnosis is based on the endemicity, the clinical characteristics, full blood count, the presence of *Bartonella* in blood smears (Giemsa stain), blood cultures or tissue cultures from skin lesions or even the histological examination of the latter. More than 70 percent of patients with acute Oroya fever have a positive blood culture for *B. bacilliformis*, although there may be a delay of more than 14 days for the organism to grow in culture. *B. bacilliformis* is fastidious and requires Columbia agar, an enriched blood medium, for growth, which occurs most readily at 25 to 28°C.



Bartonella bacilliformis in red blood cells

In the differential diagnosis, consideration is given to malaria, dengue, viral hepatitis, babesiosis, bacillary angiomatosis in AIDS patients, typhus, typhoid fever, Yaws, Kaposi's sarcoma, haemangiomas, pyogenic granuloma and various skin tumours. In mild forms, the number of Bartonella in the blood smear can fall below the detection limit. The degree of haemolysis is then very limited, and the infection is extremely difficult to diagnose if no serology is available. PCR, Immunofluorescence, ELISA and Western Blot among others are used for diagnosis.

Treatment

Until recently **chloramphenicol** was the drug of choice but **ciprofloxacin** has now been shown to give better results. Both are also effective in Salmonella infections (in absence of resistance).

Chloramphenicol is administered at doses of 4 g/day for 5 days. Ciprofloxacin is given as 500 mg BD.

The fever disappears in less than 48 hours. The mortality rate of Oroya fever can be largely reduced with antibiotic therapy. Late development of the verruga stage is possible despite correct treatment.

Ciprofloxacin or rifampicin for 2 to 3 weeks can be used in the verruga stage.

Disease control

Spraying with insecticides, especially those which retain their activity for long periods interrupts transmission. However, control measures are not essential in endemic areas because of the immunity of the adult population. Individual protection consists of avoiding spending the night in exposed biotopes and the use of insect repellents or mosquito nets treated with permethrin/deltamethrin.

Although theoretically possible vaccination is not used.

Bartonella quintana or Trench fever

General

Bartonella quintana is a very small Gram-negative intracellular rod-shaped bacterium responsible for a range of clinical presentations. Infections with this bacterium are **linked to louse infestation** and occur where **people lack access to adequate water to maintain personal hygiene, such as homeless encampments in high-income countries and refugee camps and remote rural areas in low-income-countries**. The bacterium is **not recognized by routine bacterial culture**. **Trench fever** was the first clinical manifestation of infection with *Bartonella quintana* to be recognized. The name refers to its association with the German and Allied troops in the First World War. It is estimated that more than one million people were infected during the war. British troops took the disease to Mesopotamia during Lawrence of Arabia. After the war, the incidence fell very sharply. The disease broke out again during the Second World War with large-scale epidemics. As the taxonomic understanding improved over the years, the pathogen underwent several name changes: *Rickettsia quintana, Rickettsia weigli, Rochalimaea quintana* and finally, *Bartonella quintana.*

The 1.6 Mb genome of *Bartonella quintana* has been sequenced. It is closely related (maybe a degenerative form) to *B. henselae,* which can be considered a shortened version of the *Brucella melitensis* genome.

Transmission

The natural reservoir is still uncertain. The **body louse** *Pediculus humanus corporis* is the **predominant vector**. These insects bite an average of 5 times per day. The bacteria multiply in the lice. *Bartonella quintana* survives up to a year in louse faeces. Since *B. quintana* propagates in the intestinal lumen of the body louse, not in the intestinal epithelial cells, infection probably results from contact with contaminated louse feces. **Wounds caused by scratching** facilitates the entry of the **bacteria in louse faeces**. *Bartonella quintana* has also been detected in *Pulex irritans* fleas, cat fleas, cat dental pulp, monkey fleas, and has been isolated from *Pediculus humanis capitis*, the human head louse. The significance of this latter finding is still unclear, but recent genomic studies link head lice infestation to *B. quintana* bacteremia in low-resource settings (e.g.; rural Senegal). Recent studies identify *B. quintana* in various macaque species, but more studies of possible reservoir hosts are needed.

Pediculus humanus, human louse. Copyright ITM

Clinical aspects

The clinical spectrum of trench fever was described in 1919 via experimental infections in volunteer soldiers. In 1949, an accidental epidemic among 104 laboratory workers resulted in 90 symptomatic cases, which were described in detail. The incubation period varies from **15 to 25 days** (sometimes extremes of 3-38 days are mentioned). Infection can lead to several distinct clinical forms:

The patient **may have no or very few symptoms while having *B. quintana* bacteremia (bloodstream infection).** They may be afebrile. People can be asymptomatic carriers and act as a reservoir. *B. quintana* bacteremia is chronic may last many months or years (the most extended duration recorded is 8 years, though more recent studies describe a period of up to one year). In 1995 *B. quintana* was found in the blood in 14% of people without homes in Marseilles, who presented without symptoms or with general, vague unspecific symptoms.

Chronic endocarditis can occur. The main characteristics are fever, splenomegaly and heart murmurs. The symptoms can be divided into (a) symptoms of infection such as fever, weight loss, malaise, nocturnal sweating, clubbing, enlargement of the spleen, anemia and mycotic aneurysms, (b) heart murmurs and heart failure, (c) embolic phenomena such as CVA or a peripheral arterial embolism, (d) vasculitis such as microscopic haematuria with or without renal failure, splinter hemorrhages under the nails, Osler's nodules (painful lesions on the fingers), Roth's spots on the retina. As the bacterium is not identified by routine 5-day bacterial culture, *B. quintana* endocarditis is referred to as a common type of **culture-negative infective endocarditis.**

Classical trench fever. The patient develops a fever which persists for 5 days. This is accompanied by severe headache and muscle pain, particularly in the legs (“shin pain”). Retro-ocular pain, red conjunctivae, spleen enlargement, and leukocytosis can occur. After a fever-free interval, the fever can return. These cycles can recur 3-5, even up to 8 times. The term "quintan fever" derives from the recurring five-day attacks. Mortality is very low. The pathogen may be present in the human body long after the symptoms have disappeared. Classical trench fever is rarely described in contemporary times.

Continuous fever can develop for several weeks (typhoidal form), accompanied by splenomegaly.

The pathogen can be isolated from cutaneous angioproliferative skin lesions in patients with bacillary angiomatosis (*Bartonella henselae* can also be cultured from similar lesions). Many of these patients are immune-deficient (HIV). The pathogen is phagocytosed by endothelial cells and survives in a vacuole. Angiogenic factors are secreted by the pathogen or the host's response to infection, leading to the proliferation of endothelial cells, with typical neovascularisation. **Bacillary angiomatosis** is characterized by the emergence of a few to hundreds of skin lesions, from a few mm to several cm in diameter. They are reddish-purple and may be ulcerated, resembling a pyogenic granuloma or Kaposi's sarcoma. The lesions bleed heavily when injured. They can also affect the lymph nodes, bone, bone marrow, liver and spleen. The growth of new blood vessel cells resembles the late stages of the skin lesions of verruga peruviana triggered by *Bartonella bacilliformis*. The pathogen can be detected by Warthin-Starry staining.

Diagnosis

The pathogen is not identified by routine bacterial culture (5-day incubation), but can be cultured axenically which takes a long time (up to 45 days) and requires special techniques. It is best to use a combination of cultures on solid medium, liquid medium and cell cultures. Since *Bartonella* is a facultative intracellular bacterium, to release the bacterium from the erythrocyte, lysis techniques such as “freeze-thaw” or the lysis-centrifugation system (Isolator) are recommended for the cultivation of *Bartonella* sp. from blood. Inoculation of material from the Isolator tube and tissue onto freshly made chocolate agar plates facilitates the growth of the organism. For isolation, incubation in a humid atmosphere with 5% to 10% CO2 for several weeks is required. Serologically, antibodies display a great deal of cross-reactivity. Indirect immunofluorescent antibody (IFA) testing is the reference serologic method. IgG of > 1/50 indicates *Bartonella* infection. Endocarditis patients usually have titers of > 1/800. It is sometimes possible to reveal the bacteria in biopsy material using a Warthin-Starry stain (a complex silver stain) or immunohistochemistry. At present, PCR has a central role.

Treatment

Not much is known about the treatment of this pathogen. To treat classical trench fever and bacillary angiomatosis, administration of doxycycline or azithromycin is recommended. In treating endocarditis and chronic bacteremia, it is preferable to use doxycycline with either gentamicin or rifampicin, as well as considering surgery in cases of endocarditis. Bacillary angiomatosis takes 4-12 weeks to treat.

Not much is known about this pathogen. In vitro it is susceptible to beta-lactam antibiotics and it can also be killed in vitro by gentamicin, doxycycline, rifampicin, erythromycin and the new macrolides. To treat classical trench fever, once-daily administration of azithromycin or doxycycline is recommended.

In treating endocarditis, it is preferable to use doxycycline with gentamicin or rifampicin as well as considering surgery. Bacillary angiomatosis takes 4-12 weeks to treat.

Bartonella henselae or Cat-scratch disease

Key clinical aspects

This disease manifests itself mainly as a rather slow-healing ulcer with chronic lymphadenitis (98%) or rarely as a systemic condition (2%). An **ulceroglandular syndrome** which must be distinguished from tularemia, mycotic and mycobacterial infections. Sometimes there is **Parinaud's oculoglandular** syndrome (which can resemble sarcoidosis) or one of the rarer forms, such as retinitis with papilloedema.

The condition is caused by *Bartonella henselae* and very rarely by *Afipia felis*. The latter pathogen derives its name from the "Armed Forces Institute of Pathology in the USA, where the bacterium was first identified in 1988. Infection is contracted by cat scratches or bites and possibly also by infected cat fleas. *Bartonella henselae* has also been recovered from ixodid ticks, though the role of ticks in transmission of bartonellosis is not clear yet. It is useful to

know that cat bites can also transmit other dangerous infections such as plague, tularemia, sporotrichosis, nocardiosis and infections with *Pasteurella multocida* and *Capnocytophaga canimorsus.*

Bacteraemia with B. henselae can persist in cats for months (asymptomatic for the animal). A biopsy of the skin lesion or an affected lymph node can help to cement the diagnosis. Antibodies *against B. henselae* can be detected serologically.

In lymphadenitis azithromycin for 5 days is first line treatment, alternatively clarithromycin, ciprofloxacin or doxycycline for 7-10 days can be used.

Spirochaetal diseases

Summary
•Spirochaetes are very thin, spiral shaped organisms.
•There are a number of species.
•The bacteria take their name from various sources: Borrelia (after the French bacteriologist Amédée Borrel), leptospires (meaning "fine coils"), treponemes ("turning, drilling").
• Spirilla are usually classified separately.
• As yet there is no definitive nomenclature for the various subspecies.

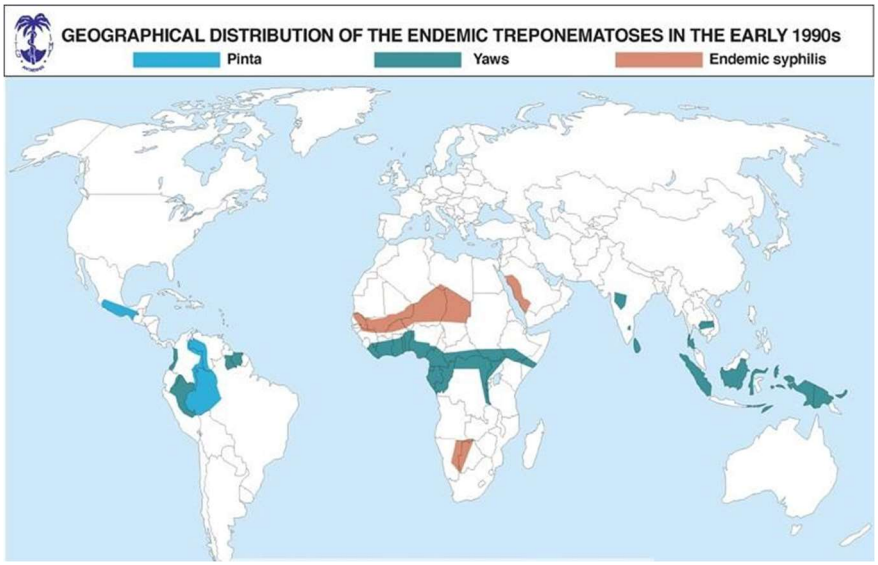
T. pallidu	syphilis, bejel (non-venereal syphilis)
T. pertenue	framboesia (= yaws, = pian)
T. carateum	pinta
L. interrogans	Weil's disease and more mild forms
B. recurrentis	louse-borne borreliosis
B. duttonii, B. hispanica, B. persica and others	tick-borne borreliosis
B. burgdorferi sl	Lyme disease
B. vincenti	tropical ulcer, Plaut-Vincent's angina, cancrum oris, Fournier's scrotal gangrene, trench mouth (necrotising ulcerative gingivitis)
Spirillum minus Streptobacillus moniliformis	sodoku or rat bite fever Haverhill fever

There are Treponema diseases:

1. Venereal syphilis or Lues
2. Non-venereal syphilis or Bejel
3. Framboesia or Yaws or Pian
4. Pinta

Non-venereal treponematoses

Treonematoses are diseases caused by treponemes. These are bacteria with a spiral structure ("trepo" = turn; "nema" = thread). They belong to the *Spirochaetaceae*. They cause 4 different chronic exclusively human diseases. There is **no animal reservoir.** The various treponemes cannot be cultured in vitro (*Treponema pallidum* can be cultured with some difficulty in tissue culture and in rabbit testicles). **Morphologically they cannot be distinguished one from another** and all give positive results on so called syphilis serology. They are all sensitive to penicillin. Prevention varies.



Geographical distribution of non-venereal treponematoses.

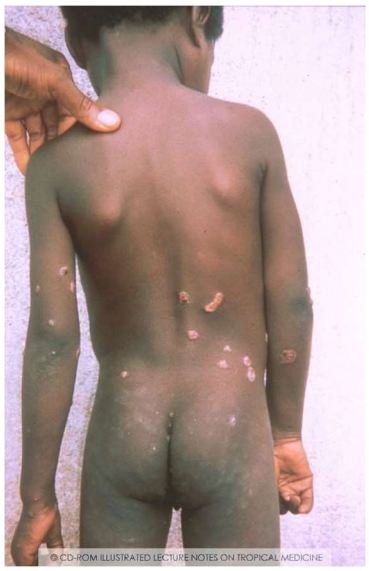
Bejel or Njovera or Treponarid

Bejel is caused by ***Treponema endemicum*** *(Treponema pallidum endemicum*). The disease occurs (occurred) in foci in sub-Saharan Africa, in the Middle East, central Australia and in Asia, in temperate to warm dry climates (e.g. Sahel area, Zimbabwe, Botswana). The disease formerly also occurred in Bosnia. Between 1950 and 1960 there were large-scale campaigns to control the disease in the Sahel countries. At present the disease has become rare.

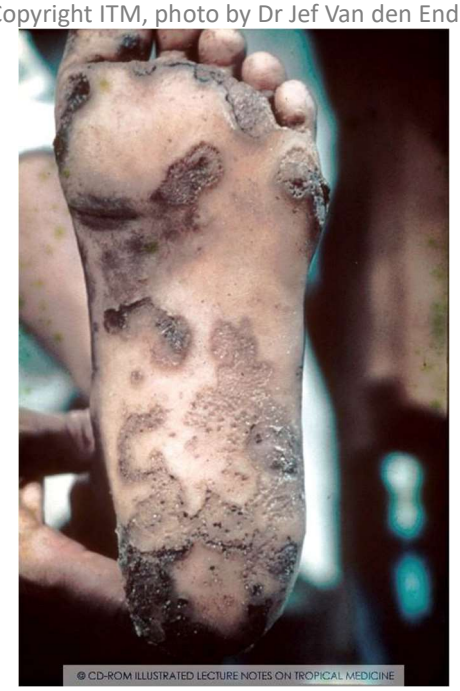
Infection mainly results in skin and skeletal abnormalities. Transmission is not via sexual intercourse but through contact. The incubation time is unknown. As a rule, non-venereal or endemic syphilis occurs in childhood. The **oral mucosae are the most important source of infection**. Children are mainly infected by objects they use such as contaminated beakers (bacteria entering through the mouth). In this way they probably acquire immunity against *T. pallidum* before puberty and are protected against later venereal syphilis.

There is an **early stage** which lasts some 5 years. This is characterised by skin lesions and oral mucosal lesions which occur intermittently. **Osteitis and periostitis** can occur. In rare cases there are **delayed lesions (gummata).** Gangosa is characterized by destruction of the nose, lip and palate and can lead to severe mutilation. Treatment consists of a single IM administration of 1.2 or 2.4 million units of longacting benzathine penicillin. A single dose of azithromycin can also be used for treatment but some guidelines prefer to safeguard azithromycin as reserve antibiotic. Tetracyclines can be used as an alternative. Plastic reconstructive surgery is often needed to repair mutilations.

Framboesia or Yaws or Pian



Framboesia, yaws, pian. Infection with Treponema pallidum pertenue.
Copyright ITM, photo by Dr Jef Van den Ende.



Framboesia, yaws, pian. Infection with Treponema pallidum pertenue, resulting in plantar hyperkeratosis with painful cracks and fissures.
Copyright ITM, photo by Dr Jef Van den Ende.

Yaws is caused by ***Treponema pertenue*** or *Treponema pallidum pertenue*. The transmission of yaws in man through inoculation was demonstrated by Paulet in 1848 and by Charlouis in 1881, predating the discovery of *T. pertenue* by Castellani in two Ceylonese patients with the disease (called "parangi" there).

This treponematosis is transmitted from person to person via **direct skin and mucous membrane contact** (small scrapes). It is a disease of poor isolated rural communities in warm, humid, tropical areas of Africa, Central and South America, and some islands in Southeast Asia. There is hardly any congenital transmission. Framboesia has currently become rare and

has been eliminated in some areas (e.g. in Esmeraldas, Ecuador) but may be re-emerging in some areas. This is explained by the deterioration in clinical medical care in certain areas (it is easy to diagnose and the treatment is cheap and simple) and the lack of large-scale treatment campaigns. *T. pertenue* can infect baboons, chimpanzees and some other monkeys, but the importance of this is not clear. It is unlikely that an animal reservoir plays an important epidemiological role as far as can be judged at this time.

Clinical Aspects

The **skin and skeleton** are affected, deep organs are always spared. The disease is characterised by **wart-like skin lesions with the appearance of strawberries** (hence the name; yaw = strawberry). The skin lesions return periodically.

The primary lesion is extragenital. It may consist of one warty lesion but sometimes there is an initial parent lesion with various satellite lesions. In most cases the lymph nodes are swollen. If the hypertrophic, papillomatous epidermis is removed an exudate with a crust forms. There is no deep ulceration. These early lesions heal without leaving scars. After healing some residual skin discoloration may remain.
A few weeks to months after the primary lesion, more scattered secondary macular or papillomatous lesions occur. The early skin lesions which contain a great number of treponemes, tend to be multiple and moist. They occur in flare-ups which last weeks or months in each case. Without treatment this can last 3 to 5 years. When there is a flare-up, there can be general malaise together with joint pain and fever. The skin lesions may persist for 3-6 months. On the palms of the hand and the soles of the feet the skin can thicken, become **hyperkeratotic** and itchy and painful fissures appear. These result in the characteristic gait, the so-called **"crab gait".** A severe infection with *Tunga penetrans* (sand fleas) can sometimes produce a similar picture, but on closer inspection the difference is clear. Sometimes there is involvement of the skeleton. Chronic inflammation of the bones of the fingers (dactylitis) should be distinguished from the more acute dactylitis seen in sickle cell anaemia. Since the general availability of penicillin occasionally mild forms of yaws are seen with only one or just a few small lesions, a few papules or limited hyperkeratosis. It is not known whether the pathogen has a reduced sensitivity to penicillin.

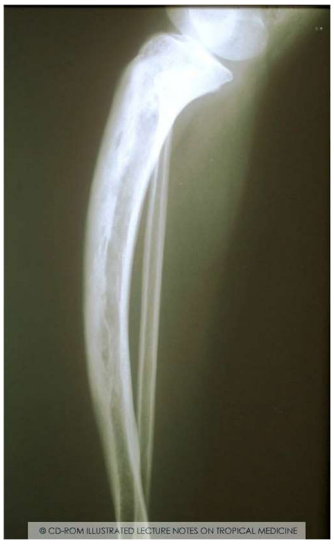
Late-onset framboesia occurs in 10% of patients (after > 5 years). Characteristic of this condition are **sporadic gummata in the skin**; deep crater-like ulcers which later heal with the formation of scars covered by a thin skin. Treponemes are very rare here and the lesions are therefore not particularly infectious. Contracture of the affected limb may occur. Joints may stiffen and chronic osteitis and periostitis can lead to bent legs (sabre tibiae).

A number of secondary lesions occur in framboesia:
• **Nodules:** mainly around joints. Hard nodules which are loose from the skin and the deep tissue on the extensor side of elbows, wrists also on trochanters, ankles and sacrum. The aetiology is unclear and a differential diagnosis has to be made with onchocerciasis.

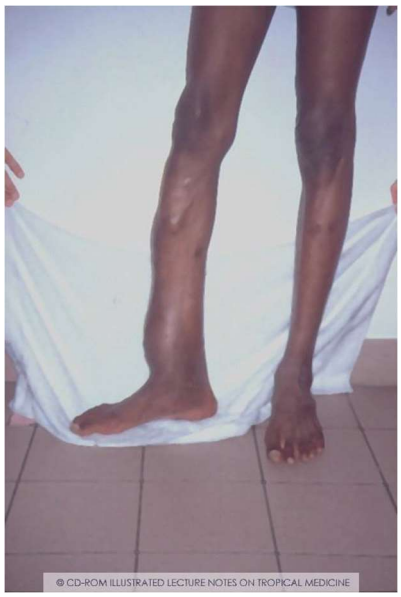
• **Gangosa:** this is rapid tissue loss from the nose, palate and upper lip, caused by a gumma in this area. To be differentiated from espundia (mucocutaneous leishmaniasis), deep mycosis (e.g. blastomycosis), leprosy and noma (= cancrum oris associated with among other things, malnutrition caused by infection with *Borrelia sp.* and fusobacteria).

• **Goundou:** swelling of the nose and upper jaw bones due to inflammation of the bones of the nose (osteitis). The rare fungal infection rhinoentomophthoromycosis can sometimes be confused with this.

• **Gumma:** a subcutaneous gumma can manifest itself as a cold abscess.



Framboesia, yaws, pian. Infection with *Treponema pallidum pertenue*. Deformed tibia, the so-called sabre tibia. Copyright ITM



Melorheostosis can resemble *Treponema pertenue* sequellae, such as sabre tibiae. The radiological lesions often look like dripping candle wax. Copyright ITM

Framboesia, yaws, pian. Infection with *Treponema pallidum pertenue*. Notice the deformed tibiae, the so-called sabre tibiae. Copyright ITM, photo by Dr Jef Van den Ende

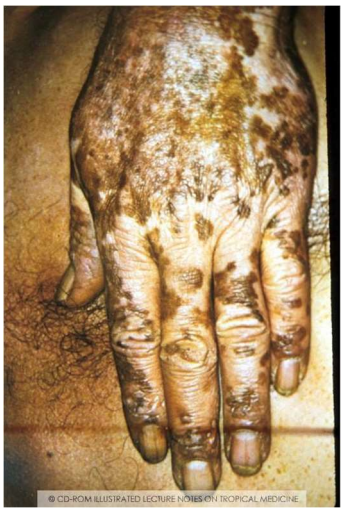
Framboesia, infection with *Treponema pertenue*. The name gangosa refers to the ulcerative destruction of the centre of the face. If a child survives noma, similar lesions can be found in adults.

Treatment

In patients over 10 years of age, **a single IM injection of 2.4 million units of benzathine penicillin or a single dose of azithromycin 30 mg/kg (max 2 gr)** is sufficient. Half the dose of penicillin should be used in younger children. In the early stages this produces fairly spectacular results. All individuals who have been in contact with the patient should also be treated. Doxycycline can be used for one week as an alternative. Erythromycin is less active. Azithromycin has been successfully used in mass treatment programs to enable yaws elimination. In certain areas the eradication of framboesia has been followed by an increase in venereal syphilis.

After successful treatment titers of nontreponemal serological tests become negative within less than 2 years.

Pinta



Pinta, depigmented skin lesions. Infection with Treponema carateum. Photo Cochabamba, Bolivia

Pinta is caused by *Treponema carateum*. This treponematosis is limited to a few foci in Central America, Colombia and southern Mexico. Cases of pinta are becoming less and less frequent. Only the skin is affected. Transmission is through contact. The primary lesion is a scaling papule which is often itchy. This appears within ten days after exposure. The papule increases in size over the following 2 to 3 months and forms a flat, scaly plaque. There is no latency period, unlike other treponematoses. A few months to more than one year later, a mild itchy maculopapular rash develops. The spots are distributed randomly over the whole of the body. They have abnormal changing pigmentation: initially blue to purplish then brown. They still contain treponemes. Later the lesions become atrophic and fade. After treatment with penicillin the lesions remain discoloured. The main problem is cosmetic, to be distinguished from other causes of hypopigmentation such as vitiligo and leprosy. There are no ulcers and no bone lesions. Pinta does not protect against the other treponematoses.

Summary

	Syphilis	**Bejel**	**Yaws**	**Pinta**
Point of entry	Genitalia	mouth	skin	skin
Congenital	Yes	No	No	No
Bone lesions	sometimes	sometimes	Often	never
Visceral lesions	yes	No	No	No

Leptospirosis

Summary
- Leptospirosis: bacterial zoonosis
- Transmission via contact with contaminated fresh water
- Fever, muscle pain, cough, red eyes,
- Hepatomegaly, icterus, haemorrhagic tendency, meningitis, nephritis
- Difficult clinical diagnosis: water contact, leukocytosis, urine analysis, lumbar puncture
- Serology and direct detection of bacteria are difficult to carry out
- Treatment tetracyclines, penicillin

General

Leptospirosis is the most widespread zoonosis worldwide, caused by the spirochetes of the genus Leptospira. An estimated one million people are infected annually, with 60.000 deaths. It is most prevalent in tropical regions, but there are occasional cases in Belgium and the Netherlands.

Leptospires are the only pathogenic spirochaetes that **are free-living in the environment**. In comparison, *Treponema pallidum* is only found in humans, and Borrelia spirochaetes are only found in arthropods and mammals.

The severe form of leptospirosis was described in 1886 by the German Adolf Weil, Professor of Medicine at the University of Heidelberg. It is therefore still called **Weil's disease**. In 1907 Stimson discovered the organism in kidney tissue from a patient who died during a yellow fever epidemic (see Clinical aspects).

Clinically it is indeed **tough to differentiate** between yellow fever and leptospirosis. In regions where scrub typhus and hantavirosis are endemic, differentiation between *Orientia tsutsugamushi*, hantavirus infection and leptospirosis on clinical criteria alone is impossible.

Taxonomy

The bacteria are very delicate and spiral-shaped. They have a typical terminal hook (Gr. leptos = delicate, slender, speira = spiral, interrogans = question mark). The bacteria are so thin that they cannot be detected with normal light microscopy. They can be seen using phase contrast or darkfield microscopy (urine) and using silver staining of tissue sections. Leptospires have a characteristic **double membrane architecture with features of both Gram-positive and Gramnegative bacteria.**

Traditionally, the genus Leptospira contained two species: Leptospira interrogans sensu lato, which was pathogenic and L. biflexa sensu lato which was non-pathogenic for man. However, the taxonomy of Leptospiro has undergone significant changes due to large-scale whole-genome sequencing. There are currently 64 species, split into two clades (pathogenic 'P' and saprophytic 'S') and four subclades. 17 Pathogenic species are classified in subclade P1 (*L. mayottensis, L. alexanderi, L. kirschneri, L. kmetyi, L. alstonii, L. adleri, L. barantonii, L. ellisii, L. dzianensis, L. gomenensis, L. putramalaysiae, L. tipperaryensis, L. borgpetersenii, L. interrogans, L. noguchii, L. santarosai, L. weilii*). Subclade P2 comprises 20 species of

intermediate or unclear pathogenicity (*L. broomii, L. licerasiae, L. fainei, L. venezuelensis, L. wolffII, L. haakeii, L. hartskeerlii, L. saintgironsiae, L. neocaledonica, L. perolatii, L. zoumogneensis, L. fletcheri, L. fluminis, L. johnsonii, L. koniamboensis, L. langatensis, L. sarikeiensis, L. selangorensis, L. semungkisensis, L. andrefontaineae, L. inadai*). The previously categorized saprophytes are subdivided in subclades S1 (*L. terpstrae, L. vanthielii, L. yanagawae, L. brenneri, L. harrisiae, L. levettii, L. kemamanensis, L. bandrabouensis, L. bourretii, L. bouyouniensis, L. congkakensis, L. ellinghausenii, L. jelokensis, L. kanakyensis, L. montravelensis, L. mtsangambouensis, L. noumeaensis, L. perdikensis, L. biflexa, L. meyeri, L. wolbachii, L. idonii)* and S2 *(L. ilyithenensis, L. kobayashii, L. ognonii, L. ryugenii*), with 22 and 5 species, respectively. In the older classification, 300 serovars, which can be differentiated by cross-agglutination absorption testing, are grouped into 32 serogroups.

Transmission

The pathogenic bacteria can **survive in fresh water** but die in seawater. Infected animals retain **bacteria in their kidneys for a long time and eliminate them in the urine**. Transmission follows contact with fresh water contaminated with the urine of infected animals. **Rats** form the main reservoir, but other animals such as cattle, dogs, cats and pigs may also become infected. It is an important animal disease.

Leptospires are killed by gastric acid and bile salts. They penetrate the body via wounds and via the mucosa of the mouth, nose and eyes (conjunctivae). **Water** is the most important transmission route, but direct contact with infected animals may also be significant (slaughterhouse workers, veterinary surgeons). It is a disease associated **with certain occupations**, e.g. workers in paddy fields or on sugar cane plantations, farmers, workers in sewers and canals, gold prospectors (gold dust obtained from water courses). People who bathe or swim in infected surface water are at increased risk of this zoonosis. Now that rafting, kayaking and adventure sports in tropical regions have become popular, there is an increase in leptospirosis in tourists. Ideal conditions for transmission are produced when dirty streets with large rat populations are flooded. Heavy rainfall or flooding in endemic areas can lead to large outbreaks of leptospirosis, especially in areas with poor housing and sanitation. Outbreaks have also been reported in triathlon participants where the swimming was in fresh water.

Clinical aspects

Given the many species of leptospires, **a broad spectrum of diseases is possible**. Symptoms range from mild fever with a 'flu'-like syndrome to atypical pneumonia, myocarditis, aseptic meningitis or the severe Weil's disease with liver and kidney failure, meningitis and hemorrhage.

The disease course has **three phases:** the first **septicaemic,** the second with leptospiruria (leptospires in the urine) and the third **convalescence** phase. During the first phase, the leptospires are present in the blood in low numbers (too low to be detected in a blood smear using phase contrast microscopy). Subsequently, the organisms disappear from the blood due to the formation of antibodies. The cellular defense also clears the bacteria from the various tissues. Leptospires persist in the kidney. In the renal tubules, the organisms can multiply and cause renal damage. Bacteria are eliminated with the urine, although the concentration is

quite low: < 104/ml urine. The bacteria may remain in the kidneys for months, even after clinical recovery. Leptospires might also persist in the choroid plexus of the brain.

Most cases of leptospirosis are mild and self-limiting or asymptomatic. Mild forms are often atypical and are generally missed unless they are specifically sought for. The acute phase of leptospirosis usually starts 5 to 14 days after exposure (maximal incubation range 2 to 30 days).

Fever, rigors, myalgias (mainly in the calves and lower back), headache and general malaise usually last two to nine days. Patients can sometimes pinpoint within the hour when the illness began. Next, the fever may subside for a few days and then increase once more (biphasic fever) during the "immune" phase. The absence of this fever pattern does not rule out the disease.

Significant muscle pain is almost always present. If it is absent, the diagnosis is improbable. There is sometimes a sore throat and a dry cough, later possibly hemoptysis. In 10 to 30% of patients, the lower legs have a spotty skin rash. [This was initially described as "Fort Bragg Fever" caused by L. interrogans autumnalis]. The eyes are often bloodshot due to dilation of conjunctival blood vessels causing conjunctival erythema. Pus discharge is absent, unlike in purulent conjunctivitis. Subconjunctival hemorrhage can occur on top of the conjunctival suffusion. During the immune phase, anterior uveitis presenting as acute onset pain and redness of the eye(s) may occur. Posterior uveitis (chorioretinitis) is less common and presents with decreased vision or floaters. Two-thirds of patients suffer nausea and/or vomiting. Swollen lymph nodes are only present in a minority of patients. The spleen is swollen in 20% of cases.

Marked elevation of CK levels indicates **muscle damage** and occurs only in severe cases. Muscle pain, predominantly in the calves, can lead to local swelling, which can be so severe that patients cannot walk anymore. Pectoral, back and abdominal muscles may also be involved.

Palpation of the calves tends to be painful. The injured muscles heal without scarring. CK levels and muscle symptoms usually diminish in the second week of illness. Rhabdomyolysis seems to be secondary to direct muscle cell invasion with cell necrosis and small intramuscular hemorrhages.

Myocarditis occurs and often leads to congestive heart failure and cardiogenic shock. Electrocardiographic abnormalities are common.

Weil's disease is a syndrome characterized by icteric leptospirosis with fever, jaundice and renal failure. Lung bleeding with ARDS, myocarditis and rhabdomyolysis may accompany this syndrome.

Involvement of the liver is characterized by hepatomegaly, jaundice and a hemorrhagic tendency. Scleral icterus and jaundice are accompanied by a marked conjugated bilirubin elevation with normal or slightly elevated aminotransferases. The gall bladder may become inflamed (acute cholecystitis). Liver failure is rare.

Atypical pneumonia with possible blood-tinged sputum can be expected in severe cases. Pulmonary lesions are primarily hemorrhagic rather than inflammatory. Patients are at risk for secondary bacterial pneumonia.

Kidney damage leads to proteinuria, hematuria and uremia. Hypovolaemia and poor renal circulation may further exacerbate renal damage. Hypovolaemia is characterized by oliguria, low blood pressure, diminished skin turgor and flat neck veins. If it is not corrected by giving fluids, tubular necrosis will follow. Temporarily hemodialysis is needed in severe renal failure. Sterile pyuria, proteinuria, granular casts, myoglobinuria and enlarged kidneys occur in some patients. The 'haem' part of myoglobin separates form the globin moiety in an acid environment (pH < 5,4). Renal tubular obstruction due to the precipitation of myoglobin is dangerous. Myoglobin is less toxic if there is no dehydration or acidosis. Therefore alkalinization of the urine is essential.

Meningism may occur early but is more frequent in the immune phase. Neck stiffness is present in half the patients with aseptic meningitis. The CSF typically has a neutrophilic or lymphocytic pleocytosis with mild proteinorachia. CSF pleocytosis may last for up to three months. Meningitis is attributed to the immune response rather than a true CNS infection. However, recent studies could Leptospira in the CSF by polymerase chain reaction.

In severe leptospirosis, the total period of illness is approximately three weeks to one month. The mortality is between 5 and 30 %; severe icterus has a poor prognosis. If the patient survives, there is usually no residual damage. A long convalescent period is typical.

Differential diagnosis:

This is **very broad in view of the variable symptoms**. It includes Hantavirus infection, influenza, gastro-enteritis, meningitis, malaria, hepatitis, cholangitis, rickettsiosis (e.g. scrub typhus), borreliosis, typhoid fever, Reye's syndrome, arboviroses such as yellow fever, Rift valley Fever, Crimean-Congo haemorrhagic fever and West Nile fever as well as arenaviroses. In the case of haemorrhagic tendency; Gram-negative bloodstream infection and the various viral haemorrhagic fevers should be considered.

Diagnosis

Diagnosis is quite **difficult**. This disease is often missed. The disease may be clinically suspected in patients exposed to endemic or outbreak settings who present with systemic febrile illness without an alternative explanation. **Exposure** to potentially contaminated water (occupation, accident, swimming, recent travel to flooded areas etc.) and rat exposure should be enquired.

Aseptic meningitis, uveitis, jaundice, acute febrile kidney injury, pulmonary hemorrhage and conjunctival suffusion should raise the suspicion for leptospirosis.

Microscopy of *Leptospira sp.,* bacteria that cause leptospirosis. Photo Cochabamba, Bolivia

There is proteinuria, pyuria and microscopic haematuria. The cerebrospinal fluid initially contains neutrophils. Later, lymphocytes predominate, together with elevated protein and normal glucose.

In general, there is significant leukocytosis, but this is not constant. Thrombocytopenia is common. Early in the disease, leptospires can rarely be found in the blood, urine or cerebrospinal fluid (the tests are not very sensitive). Subsequently, the bacteria are only found in the urine.

Since these are very thin organisms (0.1 µm diameter), a dark-field microscope is needed to detect them in a blood smear. Indirect illumination is used in this method instead of direct illumination so that fine structures can be detected which are not visible with the traditional microscope. This method is not very sensitive and has been responsible for many errors (many false positives and false negatives).

Serology can be performed. The traditional serology using micro-agglutination test or MAT requires a well-functioning laboratory, which will not be available in practice in low resources settings. A single positive or negative IgM or IgG cannot confirm or rule out infection, even though a single IgG titer (>1:800 on MAT) strongly supports infection. A second sample 7 to 14 days after the first antibody test should be obtained, and a four-fold increase in IgG titer confirms infection. A second sample 7 to 14 days after the first antibody test should be obtained, and a four-fold increase in IgG titer confirms infection. Antibody tests that do not detect all serovars may produce false negative results. The interpretation of MAT serology results to identify the responsible serovars is rather difficult because the highest titer does not necessarily correlate with the actual serovar responsible for the infection.

Culture of the bacteria is the gold standard but is not practical in most settings. The culture of leptospires is complex and requires non-standard equipment. Special media such as Fletcher's, Ellinghausen's, or polysorbate 80 media are required for isolation. Blood and CSF specimens are positive during the first ten days of the illness. Urine cultures become positive during the second week of illness and remain so for up to 30 days after the resolution of symptoms.

In high-resource settings, PCR is used on blood samples, urine, CSF and tissue biopsies. Whereas PCR detects leptospires during the first week of symptoms, urine samples are

particularly valuable beyond the first week of illness. The sensitivity of PCR ranges from 40 to 60 percent in blood samples; the specificity exceeds 95 percent.

Antigen detection using a monoclonal antibody-based direct ELISA (anti-LipL32 antibodies) on blood has shown promising results in Sri Lanka but needs validation in larger international studies.

Treatment

Most patients with leptospirosis will recover without antibiotics. Although robust evidence is lacking, early treatment initiation might prevent evolution to severe disease. Antibiotics such as tetracyclines within the first 4 days are assumed to shorten the illness. Sometimes leptospires persist in urine, despite the correct treatment. Oral doxycycline 200 mg per day for 1 week is the preferred regimen for mild infections. If there is vomiting, IV penicillin is used. For severe infections, ceftriaxone can also be used. This allows for once-daily dosing, which is more practical than the multiple dosing schemes using penicillin. Azithromycin and ampicillin are also active against leptospires. Chloramphenicol is not. Most patients with critical illness will have been placed on an empirical antibiotic treatment. However, since the pathophysiology suggest an exaggerated immune reaction, the beneficial effect in severe disease remains controversial. The immune-triggered second phase suggests a role for corticoids in Weil's disease. Some studies suggest a possible benefit, but more studies are needed. Symptomatic and supportive therapy is vital. If there is myoglobinemia, alkalinization of the urine is essential to limit renal damage. In severe disease, hemodialysis, mechanical ventilation and blood products can be life-saving.

Prevention

Since **rats** form the main reservoir and contaminate surface water and drains, their control is important for prevention. Nevertheless, it should not be forgotten that the animal reservoir is much broader (e.g. dogs etc.) and **cannot be eradicated completely**. Avoiding sources of infections, such as water contaminated with animal urine, is advised. Wearing boots when working in stagnant water is advisable. **Chemoprophylaxis** of 200 mg doxycycline per week may be taken as a preventative in high-risk situations like flooding in an endemic region. After infection, there is protection against the infecting serovar but no cross-immunity. Human vaccines have been developed, but they are serovar-specific, and none of them is widely available. Animal vaccination can provide variable levels of protection for animals and humans.

Borreliosis

Relapsing fever

Summary
•Spiral shaped bacteria, transmitted by ticks (endemic) or lice (epidemic)
•Recurrent fever, rash, hepatosplenomegaly, red eyes, haemorrhagic diathesis, muscular pain,
coughing, confusion, neurological complications
•Thick film test positive, esp. in beginning of attack
•Treatment with penicillin or tetracyclines (e.g. doxycycline)

General

Borrelia sp. are very thin, spiral shaped bacteria. They are **larger, longer and have looser coils** than treponemes or leptospires. They are responsible for major diseases, including **recurrent or relapsing fever**. In 1868 the German Otto Obermeier identified the microorganisms during an epidemic in Berlin.
The pathogenic potential was demonstrated in 1874 by Gregor Münch, who inoculated himself with *Borrelia recurrentis* and survived the subsequent relapsing fever. The French microbiologists Sergent and Foley identified the body louse as the vector. The British pathologist Joseph Dutton (famous because of *B. duttoni*) discovered an alternative vector: the Argasid soft tick *Ornithodoros moubata*. He injured himself while performing an autopsy on a patient who had died from borreliosis and died himself from relapsing fever. During his research into East Coast fever in East Africa, Robert Koch discovered that transovarial transmission took place in these ticks. Charles Nicolle and co-workers established that *Borrelia recurrentis* disappeared from the intestine of the louse 24 hours after a blood-meal, to appear again suddenly in the haemolymph of the insect after 6-8 days. Experimental animals such as rats and mice can be inoculated successfully. Borrelia recurrentis can be grown in chicken embryos and since 1994 in-vitro.

There are two types of borreliosis: relapsing fever, **louse-borne borreliosis** (***Borrelia recurrentis***) and **tick-borne borreliosis** (***Borrelia duttoni*** and many other varieties, depending on the geographical region). The bacteria are morphologically identical. The name "tick-borne borreliosis" sometimes causes confusion, as *Borrelia burgdorferi* is also transmitted by ticks, but this organism does not cause relapsing fever.

Epidemic, louse-borne relapsing fever

In the **epidemic** form of borreliosis the bacterium ***Borrelia recurrentis* is transmitted by lice**. The vector is the common body louse (*Pediculus humanus corporis*). [The body louse is also the vector of epidemic typhus and of Bartonella quintana. This insect is not to be confused with the pubic louse (*Phthirus pubis*)]. The head louse (*P. h. capitis*) hardly ever plays a part in transmission. There is no transovarial transmission of Borrelia recurrentis in the louse. **Humans are the reservoir** of the disease.

The louse is infected by sucking blood at the time the patient has an outbreak of fever. At this time the levels of bacteria in the blood are at their highest. The bacteria penetrate the insect's

intestine and multiply in the haemolymph ["blood"] of the louse. The bacteria do not penetrate the salivary glands.
The disease is not transmitted by the bite itself. **If an infected louse is crushed on the skin when scratching, the bacteria can penetrate into the skin.** Lice do not like high temperatures and will readily leave a person who has a fever. In the event of poor hygiene and close physical contact between people lice can pass from a sick person to a healthy person.

The disease is **rare but can occur all over the world**. The geographical distribution of LBRF has declined due to improvements in living standards. Currently the disease is primarily found in limited endemic foci in Ethiopia but also in Somalia and Sudan. The disease has also been recorded in the rural Andean community in Peru and in northern China. Epidemics occur in conditions of poor hygiene, overcrowding and malnutrition, such as in floods, mass migration, earthquakes, concentration camps and refugee camps, war, and in the slum districts of large towns. Body lice multiply rapidly and a population can increase by 11% per day. Infection is more frequent in the cold months. People live closer together then, wear more clothes, so there are more lice and consequently more transmission. **Mortality can be very high (30 to 80%)**. Between 1910 and 1945 there were 7 large epidemics in Africa, Eastern Europe and Russia with 15 million cases and 5 million dead.

Endemic, tick-borne relapsing fever

This is a sporadic, endemic disease in a number of areas caused by *Borrelia duttoni* and related bacteria.
The vectors are **soft ticks** (*Ornithodoros sp*.). In West Africa *O. erraticus* is responsible for the transmission of *B. hispanica.* In Central, Eastern and Southern Africa *Ornithodoros moubata* is the main vector (*B. duttoni*). These latter ticks infect people through their saliva and through coxal fluid. It is mainly an infection of rodents. These animals are the principal reservoir. Because the bacterium in ticks passes from one generation to the next by transovarial transmission, the ticks themselves also form a reservoir. People can be infected by ticks for example when walking through grass or bushes. In Central Africa there is a domestic variety whereby the ticks live in cracks in the walls of mud huts and are therefore more likely to bite humans. The people who are infected are then the main reservoir. Ticks can live for a number of years (exceptionally up to 15 years) unlike lice (a maximum of 2 months). They can survive for a long time without a blood-meal. **Mortality in man is lower with tick-borne borreliosis (2 to 5%) than with the epidemic form.** The local population builds up immunity from repeated infections; they usually have a mild form. The bacteria can cross the placenta to the fetus.

Over the course of an infection in a single human host *Borrelia sp.* regularly display **antigenic variation**, mainly by changing various surface proteins ("variable large proteins and variable small proteins").

Clinical Aspects

After an incubation period of **4 to 14 days (1 week on average)**, the patient suddenly develops a **violent fever** (39° to 41°C). This is accompanied by a high bacteraemia: 106-8/ml. The concentration of bacteria is so high that they can be detected with the thick film test or a thin blood smear (in classical Gramnegative bacteraemia (e.g. E. coli) **the concentration of bacteria**

is much lower). The patient suffers from headache, muscular pain and pain in the joints. There is often a dry cough and dyspnoea, which can be quite severe. The patient sometimes suffers from abdominal pain and diarrhoea. The patient is frequently jaundiced. The spleen, the liver and the lymph nodes are often swollen. Neurological abnormalities occur. The conjunctivae are often red. Sometimes (in 4 to 50% of cases) there is a discrete rash which usually appears when the first fever peak subsides. Diffuse intravascular coagulation (DIC) and thrombocytopenia, petechiae and haemorrhaging can occur, e.g. epistaxis (nose bleeds). Sometimes (1/3) a considerable leucocytosis can be present, but leukopenia can also occur.
The cerebrospinal fluid can contain an increased number of lymphocytes (mainly in endemic tick-borne borreliosis). The fever suddenly disappears after 2 to 8 days on average 5 days. This is usually accompanied by an aggravation of the symptoms, hypotension and sometimes death. The prognosis is worse with louse-borne borreliosis, when there is manifest jaundice, hypotension and high bacteraemia (which can be objectivised in a thin blood smear). There is high neonatal mortality (50%).

The first febrile episode is followed by a period of **3 to 30 days (on average 9 days) without fever**. In 60% of patients this is followed by a second febrile period, which is somewhat less severe than the first and also lasts for a shorter time (on average 2 days). This can be repeated a number of times: maximum 4 times in case of louse-borne borreliosis, maximum 11 times in case of tick-borne borreliosis. This characteristic explains why it is called "relapsing fever".

Complications are meningo-encephalitis with as sequelae facial paralysis, deafness and paralysis of the eye muscles (mainly endemic tick-borne borreliosis). Most spirochaetes are neurotropic. Myocarditis and abortion may also occur. If a pregnant woman has relapsing fever she has around a 50% risk of going into labour.

Diagnosis

The **clinical signs and symptoms are not specific** apart from the **recurrent bouts of fever**. At the beginning of a febrile episode bacteria are found in the blood. These very thin spiral shaped bacteria (0.5µm) can be seen in an unstained unfixed preparation because of their typical mobility. They can also be stained with Giemsa and Wright stain. Staining with Diff-Quik (xanthene thiazine stain) is an alternative. They are found between the red blood cells. The fact that the bacteria can be seen in peripheral blood is explained by the very high density of the bacteria. Borrelia spp can be cultured through animal inoculation or in vitro cultivation in a Barbour-Stoenner-Kelly (BSK) medium. PCR and serology are only available in a few reference laboratories.

The differential diagnosis includes **many febrile conditions** including malaria, typhoid fever, hepatitis, amoebic hepatic abscess, leptospirosis, rat bite fever, septicaemia, arbovirosis, ehrlichiosis and anaplasmosis, babesiosis, rickettsial diseases (can also be transmitted by lice and ticks).

Treatment

Tetracyclines are the first choice, e.g. doxycycline. A single administration is often sufficient.
Alternatively erythromycin can be given. In the case of louse-borne borreliosis, in ± 90% of patients a spectacular deterioration in the symptoms is seen 1 to 3 hours after starting

therapy: headache and muscular pain, tremor, very high fever, tachypnoea, tachycardia and initial hypertension. This is followed shortly after by excessive perspiration and hypotension and sometimes shock. This is a socalled "**Jarisch-Herxheimer**" reaction which usually lasts 6 to 12 hours. This reaction rarely occurs (1%) with tick-borne borreliosis. The reaction was first described in syphilis patients who were being treated with mercury chloride or penicillin. It can also occur when treating other infections caused by intracellular bacteria (such as Brucella, Q fever). It has a mortality rate of about 5%. It is thought that it develops from various substances being released from the destroyed bacteria, together with high concentrations of certain cytokines (e.g. TNF alpha, IL-6 and IL-8). Steroids are not effective in preventing the reaction. It has been shown that treatment with anti-tumour necrosis-alpha antibodies mitigates the Herxheimer reaction. The patient must be kept under close supervision (bed rest, IV infusion). Penicillin is less frequently associated with Herxheimer reactions but is less effective (often further recurrences).

Prevention

There is no vaccination and no lasting immunity after a patient has had the infection. In the case of an epidemic (louse-borne borreliosis) mass delousing is often carried out (2 x with an interval of 2 weeks) for example in refugee camps. This is based on the use of insecticides and hot sterilisation (boiling and washing) of clothes.

Borrelia vincenti

It is not clear whether this bacterium is itself a pathogen or whether it is present as a saprophyte in necrotic material. The bacteria can, unlike the other *Borrelia* be cultured in an anaerobic environment. In combination with certain anaerobic bacteria (fusobacteria = anaerobic Gram-negative "fusiform bacteria") this bacterium is suspected of causing ulcerative damage in the:

- **throat:** Plaut-Vincent's angina. This results in a major throat infection with localised necrosis. DDx: diphtheria of the throat, local anthrax or plague.
- **gums:** Trench mouth or Vincent's stomatitis, a necrotising and ulcerative gingivitis of the cheek. This occurs in malnourished children and sometimes after herpes simplex.
- **cheeks/lips:** Cancrum oris (noma) is characterised by pain and extensive tissue destruction. Treatment consists of penicillin, correct nutrition and treatment of any underlying disorder (e.g. kala-azar, etc). Plastic surgery will be needed.
- **scrotum:** Gangrene of the scrotum (Fournier's gangrene).
- **skin:** Painful (in the acute stage), purulent, foul-smelling ulcers, mainly on the legs or feet (phagedenic or tropical ulcer). Ulcers such as this can drag on for years or sometimes heal spontaneously. In some patients a spinocellular carcinoma develops which is invasive locally and can metastasise to the local lymph nodes. Treatment consists of penicillin and metronidazole. Local wound cleaning, antiseptics and non-adhesive dressings are important. Dry dressings should be avoided because they prevent the forming of new epithelium (when the dressing is removed the new cells are pulled off)

Rat Bite Fever

Summary
• Infection by bacteria: Streptobacillus moniliformis or Spirillum minus
• Rate bite fever is named sodoku in Asia, caused by S. minus
• Haverhill fever is rat bite fever caused by Streptobacillus moniliformis after ingestion of food or water contaminated with rat faeces
• Rat bite wound followed by fever, lymphadenopathy, migrating arthralgia, skin rash and muscle pain
• If transmitted via infected drink: episodic fever, throat pain, rash, muscle and joint pain
• Systemic complications possible: myocarditis, pneumonia, abscesses, meningitis
• Treatment with penicillin

General

Rat bites may give rise to infection with various bacteria but two deserve special attention. *Spirillum minus* is a systemic zoonosis occuring mainly in Asia. Rat bite fever caused by *Streptobacillus moniliformis* has a more cosmopolitan distribution and is mainly recognised in Europe and North America. A third species causing rat bite fever – *Streptobacillus notomytis* – has only been reported rarely. Infection in third world countries will probably be discovered as soon as better diagnostic facilities are available. Rat bite fever may trigger intermittent fever which may make it similar to other infections.

Clinical aspects

Spirillum minus is a small spiral-shaped bacterium and is usually classified as a spirochaete and is unable to be cultured. The bacterium has flagellae and moves quickly unlike *Streptobacillus moniliformis*.

Streptobacillus moniliformis is a difficult to culture pleomorphic non-motile Gram-negative rod-shaped bacterium. Its name refers to the necklace like morphology exhibited by the bacteria that form thin branched filaments.

The disease caused by *S. minus* is known as sodoku in Asia (a Japanese name: so: rat, doku: poison). Infection may follow a rat bite or the consumption of water or milk contaminated by rat urine or faeces.

Streptobacillus moniliformis infection occurs after ingestion of food or water contaminated with infected rat faeces. The disease is known as **Haverhill Fever.** The name Haverhill refers to a small town in Massachusetts where an epidemic broke out in 1926 following the consumption of contaminated unpasteurized milk. The bacteria occur naturally in the nasopharynx of rats and are found in 50 to 100% of rats living in the wild. The risk of rat bite fever due to *S. moniliformis* after a rat bite is estimated to be 10%. Not only rats, but also other rodents such as mice, gerbils, squirrels or carnivores or omnivores which eat rodents (cats, dogs, pigs, weasels, ferrets) can transmit the bacteria. People who work with animals (laboratory staff, some biologists) are at increased risk.

The incubation time is 1 to 30 days, usually approximately 1 week. If infection (*S. minus* and *S. moniliformus*) is transmitted orally, there are no skin wounds. A bite wound of S. Minus causes local inflammation and even tissue necrosis with enlarged regional lymph nodes and its initial wound may reappear at the onset of systemic illness. *Streptobacillus moniliformis* bite wounds heal spontaneously.

After the wound has healed, intermittent chills, extreme fatigue, vomiting, diffuse muscle and joint pain and headache follow. Arthritis is not common in *S. minus* infection. *S. moniliformis* infection may give rise to an asymmetrical non-purulent poly-arthritis in up to 50% of patients. Generally the large joints are affected, such as the knees, ankles, elbows, wrists, shoulders and hips. Purulent arthritis is rare. If a patient is bitten on a finger, a neighbouring interphalangeal joint may exhibit impaired function.
Approximately two to four days after the beginning of the fever a skin rash occurs. This may have a morbilliform, pustular or petechial character. The rash is most pronounced on the hands and feet.
Desquamation may occur. Somewhat later the patient develops painful pharyngitis. After an average of five days spontaneous improvement is seen. The fever disappears and the other lesions improve over the course of a few weeks.
After an irregular period of time there might be a relapse which resembles a picture of fever of unknown origin. This recurrence may persist for two years.
Complications include ulcerative endocarditis, subacute myocarditis, pericarditis, meningitis, pneumonia, amnionitis and anaemia. Abscesses may occur in any organ. In epidemics the name erythema arthriticum epidemicum is used.

Differential diagnosis:
Differential diagnosis includes coxsackievirus (hand-foot-mouth syndrom) or an unspecific viral exanthema, meningococcal septicaemia, leptospirosis, erythema multiforme, secondary syphilis, rickettsiosis (RMSF [Rocky Mountain spotted fever]), tularaemia, *Bartonella henselae* (cat scratch disease) and infections which typically occur after bites, such as *Capnocytophaga canimorsus, Eikenella corrodens* or *Pasteurella multocida* infections. If joint problems are prominent, Lyme disease, acute rheumatic fever, brucellosis, gonococcal infection, septic arthritis, infectious endocarditis and autoimmune disorders may have to be excluded.

Diagnosis

A diagnosis may be reached clinically: unexplained (relapsing) fever or sepsis, maculopapular rash and/or polyarthritis in patient with rat exposure. But even if there has been a rat bite, this will not always be reported when taking the history. Nevertheless this detail will be an important guiding factor.
Some patients have a normal blood count, while others have significant leukocytosis (to 30,000) with left shift. Confirming a diagnosis microbiologically is extremely difficult: *Spirillum minus* can be demonstrated using dark-field microscopy of a little fluid from the site of the bite but cannot be cultured yet in vitro. *Streptobacillus moniliformis* can be cultured on specially enriched anaerobic media.

Serology (ELISA) may be carried out in specialised laboratories.

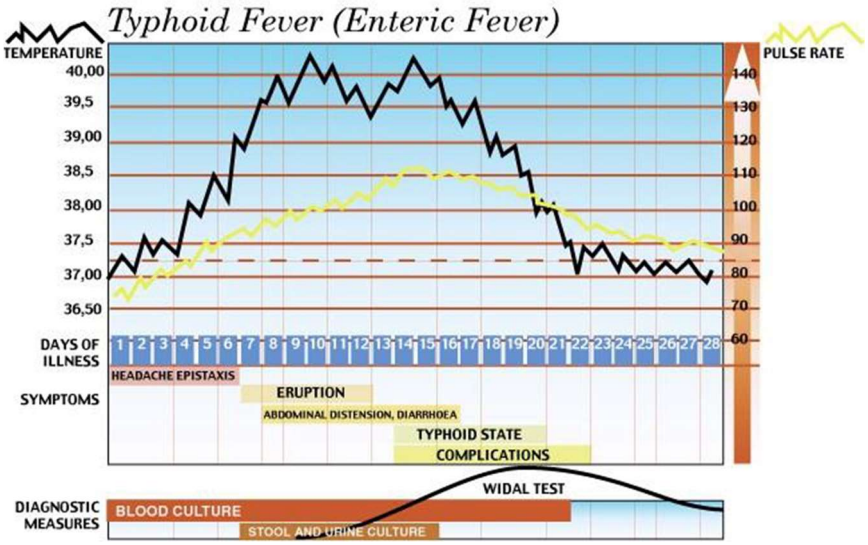
Treatment

Empirical therapy should be started instantly if rat bite fever is suspected since mortality may reach 13% in untreated patients and laboratory confirmation is strenuous and time consuming. The treatment is based on penicillin (or a tetracycline in patients allergic to penicillin) preferably given for 14 days. There may be a Jarisch-Herxheimer-like reaction at the beginning of treatment. Ceftriaxone is also effective.

Trachoma

Summary
• Trachoma: important cause of blindness
• Chronic follicular keratoconjunctivitis caused by serotype A, B, Ba and C of Chlamydia trachomatis
• Inflammation of the upper eyelid, followed by pannus of the cornea, entropion and trichiasis
• Treatment by tetracyclines or azithromycin
• Prevention by better hygiene, water, soap and fly control

General



Trachoma, map. *Chlamydia trachomatis.* Copyright WHO

The three most important diseases which lead to blindness in the tropics are onchocerciasis, vitamin A deficiency and trachoma. Other frequent causes are trauma, diabetes, leprosy, cataract, macular degeneration and chorioretinitis. The name trachoma refers to the raw appearance of the eyelid (Gr. "trachoma" = rawness). The term was first used by the Greek Pedanius Dioscorides (AD 50-70).

Trachoma is a chronic form of conjunctivitis which is caused by some serotypes of *Chlamydia trachomatis.* Repeated reinfections are probably important in the ultimate pathology. The infection is characterised by progressive exacerbations and remissions, with follicular hyperplasia, corneal neovascularisation and scarring of the conjunctivae, cornea and eyelids. The disease occurs predominantly in dry areas of Africa (except for Congo), the Middle East, India and Southeast Asia. The disease is rare in the New World. The lack of water and soap for elementary hygiene plays an important role in transmission. Transmission takes place by hand-to-eye contact. Even sharing infected utensils can lead to transmission. The role of flies (Musca sp.) was underlined by Jones, who showed that fluorescein-labelled eye secretions can be transmitted from child-to-child by these insects.

Chlamydia trachomatis

Chlamydiae are very small bacteria which have to live intracellularly. They were originally considered to be viruses, but it is now known that they contain both DNA and RNA and are structurally related to Gram-negative bacteria. Several species are known in the genus Chlamydia: *C. psittaci, the pathogen of psittacosis; C. pneumoniae* (old name TWAR), which provokes atypical pneumonia; and *C. trachomatis,* which has many serotypes. Serotypes A, B, Ba, and C cause trachoma. Serotypes D to K cause inclusion conjunctivitis in the newborn ("paratrachoma"), Reiter's syndrome, non-gonococcal urethritis, epididymitis, cervicitis and P.I.D. (pelvic inflammatory disease). Neonatal conjunctivitis and pneumonia can be caused in the newborn by these bacteria. Serotypes L1 and L2 cause the sexually-transmitted disease lymphogranuloma venereum. L3 causes pneumonia in mice*. C. trachomatis* is considered to be responsible for 20% of the pharyngitis symptoms in adults.

Clinical aspects

After an incubation period of approximately 7 days, four different clinical stages can be distinguished.
These stages overlap. Reinfection can occur and makes the classification rather artificial.

Stage 1: there is bilateral redness of the conjunctivae. Photophobia, eyelid oedema and lacrimation follow. Small (2-3 mm) lymphoid follicles develop on the tarsal conjunctivae which increase in size over the course of one month. The inner side of especially the upper eyelid then becomes granular. This follicular-papular hypertrophy stage can last from several months to years.

Stage 2: After several months small blood vessels begin to grow into the uppermost part of the cornea. This process starts in the upper limbus of the cornea. The combination of blood vessels and infiltrate is known as a pannus. The mucus-producing cells in the conjunctiva are destroyed, leading to "dry eye" (sicca syndrome). Corneal ulcerations can occur. If left untreated the cornea becomes cloudy with functional blindness as the ultimate result. In rare cases the corneal neovascularisation regresses without treatment.

Stage 3: Linear scarring appears in the tarsal conjunctiva. Follicles are replaced by small white lines. The conjunctiva becomes smooth, white and avascular. The conjunctiva of the lower eyelid may take on a milky appearance. The craters of the ruptured follicles are lined with epithelium and form a series of lacunae in the limbus, known as Herbert's pits. The pannus regresses.

Stage 4: In this stage there is no longer any active infection. The scar tissue contracts and deforms the upper eyelid so that entropion follows. Due to the turning inward of the eyelid, the eyelashes scratch the cornea (trichiasis) and cause mechanical trauma. Bacterial superinfection can occur. The epithelium of the cornea becomes dull and thickened, which is made even worse by chronic exposure to dust and sand. This promotes further neovascularisation.

Diagnosis

In most endemic areas trachoma will be a clinical diagnosis. Chlamydia trachomatis can be cultured but the infrastructure for this is beyond the capabilities of most hospitals. PCR is more sensitive than culture. In the early stages small basophilic cytoplasmic inclusions can be seen with Giemsa staining in scrapings of the tarsal conjunctival epithelium. In clinical practice it is not necessary to provide formal proof of infection. Trachoma has to be distinguished from chronic allergic conjunctivitis. This is not always easy but eosinophilia and milky flat-topped papillae are present whereas basophilic inclusions are not found. Under field conditions the diagnosis of trachoma is likely to be correct if at least two of the following criteria are present:

1. Follicles on the upper palpebral conjunctiva in the mid-tarsal region
2. Linear scars of the tarsal conjunctiva (Arlt's syndrome)
3. Active keratitis
4. Follicles in the limbus or their sequelae (Herbert's pits)
5. Pannus in the upper third of the cornea.

Treatment

The treatment used to rely on the administration of tetracycline eye ointment or taking doxycycline 100 mg bid for 4 weeks (erythromycin for children). Currently the treatment of choice is a single administration of azithromycin (Zitromax®), which greatly simplifies treatment. At present WHO recommends annual mass azithromycin treatment for 3 years in communities in which the prevalence of "trachomatous inflammation - follicular" in children between 1 and 9 years of age is 10% or more.

However the presence of clinical trachomatous follicular inflammation disappears more slowly than the implied by PCR results of conjunctival swabs. Further field-based study of estimating the prevalence of active infection is needed. Deformities of the eyelid, such as entropion or trichiasis have to be treated surgically. Reinfection can occur and further treatment forms part of a control programme.

Inclusion conjunctivitis (serotype D-K), a sexually transmitted disease has to be treated in the child and the mother as well as her sexual partners. It is important to make people aware of the fact that removing eyelashes which face inwards may bring some temporary relief, but that it can make the situation worse. The eyelashes grow back and the short stubby hairs scratch the cornea resulting in still more damage.

Trachoma is disappearing in many parts of the World even in the absence of specific control programs, probably due to the high background of antimicrobial drug use for other reasons.

Typhoid fever and other salmonellosis

Summary
• Typhoid: important disease in terms of frequency and mortality.
• Over and under diagnosis are common
• *Salmonella typhi* : Human reservoir, causing systemic illness, hotspot Asia
• Non-typhoid Salmonellae: zoonosis , causing enteritis (and invasive disease), hotspot Africa
• Typhoid fever: fever, abdominal pain, diarrhoea/constipation, dry cough, splenomegaly, relative bradycardia, rarely roseola typhosa
• Complications: ileal perforation, organ abscesses
• Clinical diagnosis: leukopenia, faeces/urine/blood/bone marrow cultures
• Widal test : serology has lack of specificity and sensitivity
• Treatment: (quinolones), ceftriaxone, azithromycin. Resistance is increasing worldwide.
• Importance of relapse, antibiotic resistance, chronic carriers, gallstones, schistosomiasis.

General

Typhoid fever is caused by infection with Salmonella typhi, a Gram-negative facultative intracellular bacterium. The genus is named after the American physician Daniel Salmon. Recently the bacterium has been named *Salmonella* enterica subsp. enterica serotype Typhi. However, the older name will be used in this text. It causes disease only in humans and has no animal reservoir, unlike non-Typhi *Salmonella* spp.

"Typhos" means smoke, obscurity, stupor in Greek and refers to the apathy, confusion, stupor and neuropsychiatric symptoms which are often seen in severe infection. The word also reflects the earlier belief that illnesses were caused by all kinds of emanations (miasmas). The disease is sometimes difficult to differentiate from spotted fever, caused by Rickettsiae (in typhus the rash is more pronounced).

Paratyphoid fever is infection with the closely related bacteria *Salmonella paratyphi* A, B and C. Clinically the course of these is similar although rather milder. Gastro-enteritis caused by other animal Salmonella species should not be given the name paratyphoid. *Salmonella paratyphi* A and B have humans as their reservoir. The term "enteric fever" is a collective term that refers to both typhoid and paratyphoid fever.

Infections with non-Typhi *Salmonella enterica* can be invasive (i.e. positive blood cultures) and occur overall in about 5% of invasive cases. The invasiveness is age-dependent and varies according to serotype. For serotype Enteritidis and Typhimurium it increases by 10x above the age of 65 years.

Bacterial structure

The bacterium has flagella, structures which should not be confused which those of eukaryotic organisms. Anti-H antibodies bind to the flagella. Many *Salmonella sp.* can form two different H antigens. They sometimes undergo a phase change and possess either one or the other H antigen.

The **bacterial wall structure is typical of Gram-negative bacteria**. Around the cytoplasmic membrane lies a thin layer of peptidoglycans. This so-called murein layer consists of long chains of repetitive disaccharide links. Oligopeptide bridges connect the sugar chains. External to this second layer is a third; outer membrane. It consists of a phospholipid double layer in which complex lipopolysaccharides (LPS) are anchored. These fatty sugars have the following components, seen from the inside out: a fatty part (lipid A) anchored in the membrane, a core and an external sugar part consisting of repeating oligosaccharide chains. The latter form the so-called O antigens. The structure and sugar composition of the O antigens vary between different *Salmonella* species. However they all have the same basic structure, there are many serological cross-reactions. Lipid A is very toxic (endotoxin) and causes a broad spectrum of effects such as fever and shock during Gram-negative septicaemia. Septic shock in infections with Gram-negative bacteria is mainly secondary to the effects of endotoxin. Shock in infections with Gram-positive bacteria is mainly due to the effects of secreted exotoxins. Endotoxin acts on the proteins of the complement pathway and on various cytokine networks.

Specific antibodies are produced by the body: **anti-O and anti-H** (also called TO and TH). The humoral antibodies result in little protection. Protection is based on cellular immunity. The O- and H-antigens are used in serological tests (Widal) [named after the French physician Ferdinand Widal, 1862-1929].

Since all *Salmonella* (not only *Salmonella typhi*) and all bacteria related to *Salmonella* possess similar antigens, there are many cross-reactions (the test is not specific). The test also has low sensitivity. This means that the contribution of serology is limited in many clinical situations.

The **Vi-antigen** (virulence antigen) is a part of the capsule that surrounds the cell wall. It consists of a polymer of a single sugar. The Vi antigen physically covers the O antigen and thus protects it from antiO antibodies. If the Vi-antigen is present, phagocytosis is more difficult and the bacterium will be more virulent. The infectious dose (ID50) for strains that possess this antigen is 107, which is 10 to 100 times lower than the IG50 of strains without the Vi-antigen. The Vi-antigen also occurs in *Salmonella paratyphi C* and S*. dublin* (a subtype of S. enteritidis).

The bacterium produces and excretes a protein known as ‘invasin’, which allows non-phagocytic cells to take up the bacterium where it is able to live and replicate intracellularly.

Epidemiology

Historical note on Typhoid fever

It was quite a long time **before typhoid fever was differentiated from other febrile disorders**. Many scientists have contributed to our knowledge about typhoid fever. The French physician Pierre Charles Alexandre Louis first proposed the name "typhoid fever". Between 1822 and 1827 he studied a total of 138 patients with typhoid fever, 50 of whom died. The post-mortem findings were compared to post-mortem results from 83 people who died from other causes. These ideas of meticulous documentation, the use of controls and numerical analysis of the data were an important milestone in medical history. Early ground-breaking work on the germ theory of disease and on the concept of water-borne transmission of illnesses water was done by Dr. William Budd (1808-1882). He investigated

an outbreak of typhoid fever in the small Welsh border town of Cowbridge. In 1853, during the local race week, there were balls on two successive nights; eight of those who celebrated subsequently died of "typhoid fever." The diagnosis of typhoid fever was confirmed at autopsy. Dr. Budd noticed that the local well was close to the septic pit of the inn, suggesting water contamination. He also noticed that a patient who was recovering from typhoid fever had left the inn two days before the parties took place. All the people who became ill had been given lemonade, prepared with water from the contaminated well.

The water from the well was the only possible source of infection common to all those who died. He reinforced his theories in 1866 when he and a colleague, Dr. Grace, traced a similar outbreak in several farm cottages. The fever was brought there by the father of one of the families. No one else was ill at the time he arrived and it was obvious that he had contracted the disease elsewhere, probably in nearby Bristol. Dr. Budd and his colleague noted that 4 weeks later several other cases of typhoid fever occurred in persons who lived in cottages which lay a quarter of a mile below the original outbreak. Those who lived in cottages at a higher level escaped entirely. They found that the drains from all the cottages were linked to the same stream and that the second outbreak had occurred downstream.

They reasoned that the agent which provoked typhoid fever was carried there, contaminating the drinking water of the second group of cottages. Dr Budd claimed that **typhoid fever was disseminated via the faecal-oral route**, a new concept. Nevertheless, the community and the medical world were not yet ready for this theory. The earlier hypothesis that typhoid fever could be caused by "rotting material" (pythogenic fever).

This was disproved in 1858-1859, when the great "Thames Stink" occurred in London. Due to certain hydrological and meteorological circumstances there was a huge wave of stench in London. The enormous amounts of rotting material in the river should have produced ideal conditions for typhoid fever, yet there were noticeably few cases during those years.

The famous Canadian physician Sir William Osler (1849-1919) campaigned for a long time against the term "typhomalaria" which had been introduced by Dr Woodward to describe difficult febrile cases. In 1911, Elie Metchnikoff (1845-1916) fulfilled one of Koch's postulates reproducing disease in chimpanzees after throat inoculation with *Salmonella typhi.*

Military impact

Throughout history epidemics of infectious illnesses have often played an important part in military conflicts. Famous examples are the American Civil War (1861-1865) with 75,361 cases of typhoid fever, of which 27,056 died. Note that these data are from the time before the bacterium had been isolated. In the Boer War in South Africa (1899-1902) 56,686 cases of typhoid fever were recorded, with 8,225 dead, compared to 7,582 who died from battle wounds. In the brief Spanish American War (1898) there were 20,738 cases out of a total of 107,973 soldiers, with 1,500 deaths. In those days, there was total disregard of the most elementary hygiene. This is in sharp contrast to the Russian-Japanese War of 1904-5, in which the Japanese boiled their water, tested their drinking water wells, covered latrines, disinfected excreta and sterilised cooking utensils, plates and mess tins. The low number of infected soldiers was probably due to these innovations. As well as typhoid fever, the role of epidemic typhus, epidemic borreliosis and bacillary dysentery in these conflicts should not be overlooked.

Transmission

Transmission is mainly via **contaminated water and food**. The bacteria survive for a varying number of weeks in water, ice, dust and can multiply in food. In many regions the infection is endemic. Sometimes there may be local epidemics. One classic mechanism is the contamination of a drinking water reservoir with the contents of a septic tank. In the past this was checked with a fluoresceine test. The bacteria only infect humans. **There is no animal reservoir** unlike the majority of the other Salmonella species.

Recent convalescent patients form the most important reservoir. People can be healthy carriers and **excrete *Salmonella typhi* for prolonged periods** (concept published in 1903 by Robert Koch : the healthy chronic "Typhusbazillenträger").

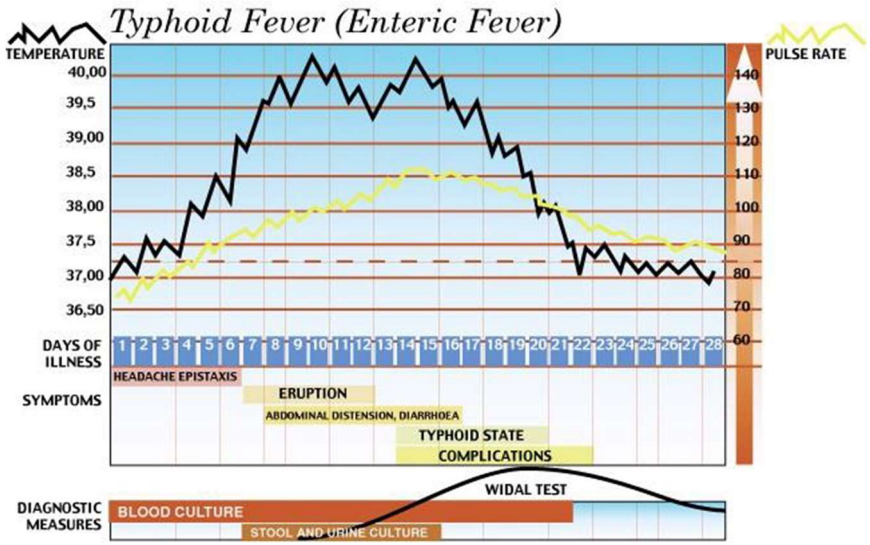
Typhoid Mary

A classic example of a healthy carrier is the case of "Typhoid Mary" the nickname of an Irish woman who became very famous at the beginning of the 20th century. In 1904 there was an epidemic of typhoid fever in a district of Long Island, New York. It was discovered that patients belonged to households where Mary Mallon had been cook. When she was tracked down by George Soper in 1907, she initially refused to cooperate. She was taken by the police, tested positive for S. typhi and was subsequently forced to stay at Riverside Hospital on North Brother Island. After three years she was released after pressure from the media. She then caused further cases including some at the Sloane Maternity Hospital where she worked in the kitchen under a false name. Overall it is certain that there were 53 cases, with 3 deaths, but possibly there were many more (possible role in the outbreak in Ithaca of 1903, with >300 cases).

Pathophysiology

After infection the bacterium penetrates the intestinal mucosa via **M cells that overlie the ileal Peyer's** patches. M cells are phagocytic cells in the mucous membrane whose function is to sample microbes from the intestinal lumen and pass them on to the lymphoid tissue of the Peyer's patch in order to activate the immune defences against intestinal microbes. Once inside the M cell the Salmonella **replicate within the phagosome,** subsequently killing the cell and spreading to adjacent cells. The bacteria are then taken up by mononuclear cells in the intestinal lymphoid tissue. There is intracellular **multiplication in the mesenteric lymph nodes.** From the lymphatic tract the bacteria pass into the blood (**bacteraemia**) and are disseminated through the whole body (spleen, liver, gall bladder, etc.). The intestine will be re-infected through the bile. The seeding of extra-intestinal organs can result in **extra-intestinal complications** virtually anywhere.

Clinical aspects



Overview of the symptoms during "classic" typhoid fever. Copyright ITM

Early clinical

Symptoms are quite variable. The **incubation period is usually 10 to 14 days.** This is considerably longer than the incubation time of 1-5 days for most other intestinal bacterial pathogens. One of the factors that determines the incubation period is the number of bacteria in the inoculum. There is always fever, which rises progressively. Initially there may be a brief episode of diarrhoea. Inflammation of the lungs leads to a dry cough. The combination of cough with fever sometimes leads to an assumption that the illness is a respiratory tract disorder. General malaise and headache are prominent. The illness may initially be confused with malaria. The patient is severely ill and sometimes apathetic or confused (typhoid = stuporous).

Half of the patients will subsequently develop abdominal pain. Diarrhoea -often described as pea soupor constipation occur in roughly equal proportions (40 %). One third of patients vomit. The intestinal mucosa of the small intestine at the antimesenteric border becomes inflamed. The lymph follicles (Peyer's patches) that are present in this location become infected and necrotic. Intestinal ulcers result which may subsequently perforate. If this does occur, the perforation is found in the final 60 cm of the ileum. Invasion of the liver and spleen leads to mild or moderate hyperplasia of the reticulo-endothelial system resulting in hepatosplenomegaly. Small red spots (2 to 5 mm) which recede when pressed can be observed on the trunk on white skin in a small number of patients. These "roseola typhosa" are quite difficult to see on a white skin and almost impossible to make out on a darker skin. The skin rash disappears after a few days. The heart rate is sometimes relatively slow for the fever (Faget's sign, French physician Jean Faget 1818-1884). Tachycardia would be expected when the temperature is 39.5°C or 40°C. Relative bradycardia is not a constant finding however and is also non-specific. For example, it also occurs in yellow fever. If a liver biopsy is taken, very typical lobular aggregates of Kupffer's cells are seen in the parenchyma (typhoid nodules). They simulate granulomas and illustrate the hyperplasia of the reticulo-endothelial system.

Complications

If untreated the fever remains high for two weeks, after which there is progressive improvement during the third week. If ileum perforation occurs, it is usually during this period. Generalised peritonitis results. There is then a sudden deterioration of the general condition: tachycardia, hypotension and pain in the right iliac fossa. A similar deterioration occurs in the case of gastrointestinal bleeding.

Without antibiotics the mortality in typhoid fever is 10%, chiefly due to intestinal perforation, internal bleeding, septicaemia with toxaemia and the formation of abscesses in other organs. If there are no complications (deep-seated abscesses, cholecystitis, osteomyelitis, etc.), the fever disappears in the third week. Spontaneous abortion may be triggered by this severe illness. Hair loss may be extensive.

Often the bacteria can still be detected using coproculture or urine culture, after the symptoms have disappeared. This is still possible one year after the illness in patients who become chronic carriers (16%, on average 3 % of patients). Carriers are more frequent in patients with gallstones or schistosomiasis. Prolonged salmonellosis in schistosome-infected patients is due to an association of *Salmonella sp.* with the schistosome worms themselves through pili which specifically recognize and bind glycolipids on the surface of the worms. The worms thus provide a multiplication focus for these bacteria in the portal mesenteric system, with a persisting blood stream infection following. Most carriers are asymptomatic.

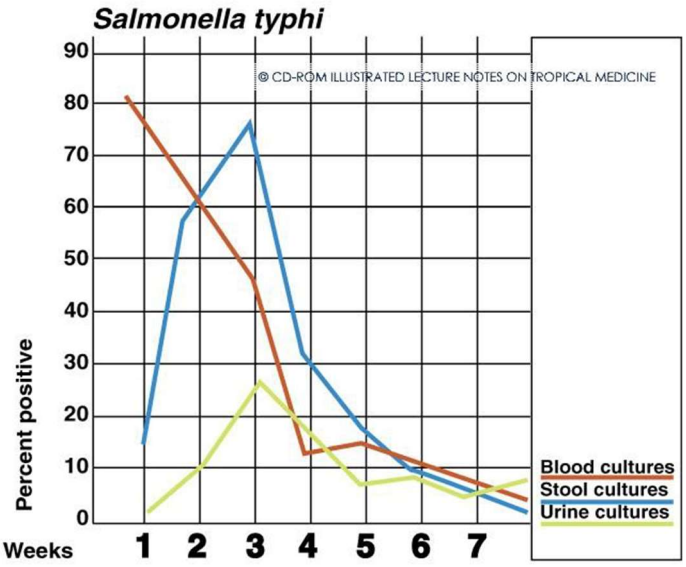
Relapse

Relapse occurs in 2 to 10% of patients 5 days to 2 weeks after the fever has subsided. This usually has a milder course than the first episode. The relapse is caused by multiplication of reactivated persistent intracellular bacteria which were previously "dormant". It is not due to antibiotic resistance; so that treatment of a relapse is the same as that for the first attack. If there is a lack of clinical improvement in the first disease episode notwithstanding antibiotic treatment, the bacteria are likely resistant and the antibiotic must be changed.

Differential diagnosis:

Differentiation from other febrile disorders is initially difficult. The differential diagnosis should include: respiratory tract infection (clinical, chest X-ray), brucellosis (undulating fever pattern, vertebral involvement, blood cultures for which specific media need to be used), malaria (thick smear, thrombocytopenia), subacute bacterial endocarditis (heart auscultation, splinter haemorrhages, embolic problems, painful Osler's nodes at the finger tips, Roth's spots on the retina, blood cultures), kala azar (chronic splenomegaly, bone marrow amastigotes), deep pyogenic abscesses (elevated neutrophil count, ultrasound, aspiration of pus), liver amoebiasis (leukocytosis, clinical examination, ultrasound, serology, aspiration), typhus (often pronounced rash, sometimes chancre, meningeal signs, DIC [disseminated intravascular coagulation]). Differentiation from viral infections may be very difficult. Differentiating typhoidal ileal ulcers from those caused by tuberculosis or Crohn's disease is usually easy.

Diagnosis



Blood cultures in typhoid fever have higher sensitivity than coprocultures in the early stages of the disease. Later, the inverse applies. Copyright ITM

Clinical

The diagnosis of typhoid fever is usually based on clinical criteria and in the majority of cases it will be made without formal proof. Perforation of the terminal ileum is quasi pathognomonic for typhoid fever, but the diagnosis should be made before this complication arises. In clinical practice there are actually few disorders that cause perforations in the terminal ileum: typhoid fever, tuberculosis, trauma (e.g. ingested tooth pick) and Crohn's disease. In many developing countries, two diseases often act as default diagnoses: malaria and typhoid fever. This illustrates the difficulties and uncertainties with which clinicians are confronted, together with the fact that both diseases are relatively frequent and are treatable (low threshold for diagnosis). Further too much importance is attached to a Widal test and the interpretation of a thick smear is often not reliable in a local laboratory (the problem is not the thick smear itself but the reading of it).

Bacterial culture

Positive cultures still form the gold standard for diagnosis. Cultures (bone marrow, blood, faeces, urine, duodenal aspirate or string test) will often be positive, but are often not feasible in practice. The chance of obtaining a positive culture is higher if repeated cultures are taken while culturing a sufficient volume of blood per culture. Blood cultures are positive in 40 to 80% of patients. In untreated patients there are ten times more bacteria per ml bone marrow than per ml blood.

Serology

Serological tests for antibodies to O and H antigens can be carried out (Widal or newer anti body-based rapid tests). A Widal test is only positive in 50 % at the beginning of hospitalisation, and may be positive due to salmonellosis suffered previously (e.g. due to *Salmonella* enteritidis) or due to an earlier vaccination. Routinely requesting this test under third world conditions makes no sense. The test can be used to detect a rising titer

(seroconversion). Antibodies to the O antigen rise swiftly, and return to negative or to low titers in a couple of months (in particular type IgM antibodies). Anti-H antibodies rise more slowly but will stay positive for longer (in particular type IgG antibodies). If the presence of advanced typhoid fever is suspected on clinical grounds and if malaria is ruled out and a single Widal test is carried out (preferably using O antigen), then a high titer of these antibodies is a relatively strong argument that the patient does indeed have typhoid fever. Nothing can be decided from a negative result.

Other arguments

A complete blood count and differential often shows normal or reduced white blood cells. The eosinophils will be low or zero. In intestinal perforation there is leukocytosis, and in intestinal bleeding there is significant anaemia. A chest X-ray is often normal, in spite of the frequent presence of respiratory symptoms.

Treatment

Salmonella Typhi in many parts of the world have become **resistant** against chloramphenicol and other first line antibiotics (e.g. ampicillin, co-trimoxazole).

In addition, chloramphenicol has no effect on the relapse rate and is of no benefit to carriers. Ceftriaxone and quinolones (ofloxacin, ciprofloxacin) have subsequently become first line choice but are more expensive and there is quickly growing resistance for fluoroquinolones. The resistance of *S. typhi* to antibiotics varies from region to region but is increasing everywhere. Azithromycin or ceftriaxone are the drug of choice in areas with high levels of fluoroquinolone resistance such as Southeast-Asia. In many patients the time to defervescence may take from several days up to more than a week.

Adjunctive treatments include laparotomy in case of intestinal perforation and drainage of abscesses is recommended.

Prevention

Hygiene

General sanitary provisions such as clean drinking water, toilets and availability of soap to wash hands play a central role. If an epidemic occurs, in the first instance the source of infection should be sought.

When treating patients with typhoid fever attention should be given to the disinfection of linen, disposal of faeces and hand washing. Treatment of chronic carriers, in particular those involved in the preparation of food is important (cf. Typhoid Mary), but opinions vary on this. Patients who are ill or convalescing are the chief source of bacteria in the community. A second problem is that in the tropics carriers cannot usually be traced due to the lack of infrastructure. Most patients stop excreting bacteria in the weeks following typhoid fever, and no new antibiotic treatment should be started in the first months after the acute illness, unless the patient is working in food preparation. Chronic carriers with gallstones often harbour bacteria in the biofilm on the surface of the stones. If treatment is needed, a cholecystectomy is suggested together with a quinolone for a longer period (not chloramphenicol). If there is

urinary schistosomiasis, this should also be treated with praziquantel. The worms may harbour bacteria in their intestinal systems or in their tegument.

Vaccination

The old TABC vaccine had quite a number of side effects and no longer used nowadays. At present there is an oral live vaccine (Vivotif®), which uses an attenuated strain of S. typhi. This vaccine contains no Vi-antigen. Vivotif® is administered as follows: 1 capsule taken on an empty stomach on days 1, 3 and 5. This provides protection in 70 % of individuals for 3 years. The vaccine containing Vi-antigen (Typhim Vi®) is injectable (1 injection) and provides the same degree of protection. The production of a Salmonella typhi Vi-conjugated vaccine (Vi-rEPA) is a new development. In this; the immunogenic polysaccharide of the bacteria is conjugated with non-toxic recombinant Pseudomonas aeruginosa exotoxin A. Trials have shown efficacy of 91% in children between 2 and 5 years and may confer longer immunity.

Non-typhoid Salmonella blood stream infection

Non-typhoid *Salmonella* bacteraemia is most likely to occur in immunocompromised hosts such as those who are at either extreme of the age spectrum or those who have diabetes, cancer, HIV positive, or who use immunosuppressive medications. When bacteraemia occurs, extra intestinal signs and symptoms may include osteomyelitis, abscess formation, and meningitis. Salmonellae may adhere to endothelial surfaces, resulting in cardiovascular infections, such as infectious endocarditis and endarteritis. Although atherosclerotic blood vessels are more susceptible to bacterial adhesion, infection of normal endothelial surfaces can also occur. The organisms may infect pre-existing aneurysms or atherosclerotic plaques, leading to arterial-wall necrosis and rapid aneurysm formation.
The most frequently involved site is the infrarenal abdominal aorta.

Antibiotic resistance levels are even higher and mostly combined. Third generation cephalosporins and azithromycin have become drugs of choice.

Cholera

Summary
- Toxin from intraluminal intestinal bacteria, Vibrio cholerae O1 and O139
- Acute profuse to catastrophic watery diarrhoea with severe dehydration and ion loss
- Low or no fever and limited abdominal cramps
- Rehydration essential; preferably Ringer's lactate
- Antibiotics are of secondary importance

General

Cholera is an acute infectious disease, characterised by profuse watery diarrhoea. It is caused by a Gram-negative bacterium: *Vibrio cholerae* O1 (the characters O1 indicate the serogroup). It is a very small, motile, curved bacterium (vibrio is the Greek word for comma). Various subtypes exist, with classification according to biological and biochemical behaviour (biotypes) and serological characteristics (serotypes). Until 1992 it was thought that bacteria causing cholera must belong to *V. cholerae*, serogroup O1 and that they must be toxicogenic (must possess and express the genes for toxins). It was known that non-O1 *Vibrio cholerae* could sometimes cause mild gastro-enteritis or even bloodstream infection in immune depressed patients, but not cholera. In October 1992 in Madras (India), a mutated pathogenic bacterium (a new serogroup) was discovered, which also causes cholera.

The isolate was given the name *Vibrio cholerae* O139 (nicknamed Bengalen). After a short bloom, the traditional strains (O1) have become more common again. A few years later, *V. cholerae O139* Calcutta was identified.

Antibiotic resistance genes in *V. cholerae* are often positioned on plasmids and can be transmitted to vibrios from non-pathogenic intestinal flora.

V. cholerae can only cause disease if there are pili [Lat.: "hairs"] present. Pili are shorter and thinner than flagella. The pili adhere to the intestinal mucosa.

Sometimes **other Vibrio species** are responsible for diarrhoea, e.g. *Vibrio cholerae* non-O1, *V. parahaemolyticus, V. hollisae, V. minicus* and *V. fluvialis*. Our knowledge of these latter bacteria is clearly insufficient. *Vibrio vulnificus* is an aggressive species present in seawater and filter-feeding organisms such as oysters. This bacterium may cause bloodstream infection and wound infections, certainly in patients with liver cirrhosis.

Biotypes

There are 2 biotypes: classic *Vibrio cholerae* and *V. cholerae* biotype El Tor. Biotype El Tor agglutinates chicken erythrocytes and causes lysis of sheep erythrocytes, unlike the classic biotype. The name El Tor originates from the Egyptian town and quarantine camp El Tor in the Southern Sinai dessert, where the bacterium was isolated for the first time in 1905 (during the 6th pandemic) from an asymptomatic Hajj pilgrim from Mecca. The importance of this germ was long disputed (until 1961). At present El Tor has replaced the classic variant in most places, except in the Ganges and Brahmaputra delta. El Tor may also survive longer in the environment, is less dependent on transmission via water and produces more asymptomatic infections (symptomatic/asymptomatic infections = 7/100).

Serotypes

V. cholerae O1 of both biotypes can be subdivided into **serotypes according to the structure of the O antigen**. If only O antigen A and C are present, the bacterium is known as serotype Inaba. If only A and B are present, the bacterium is known as serotype Ogawa. If A, B and C are present, the name Hikojima is given. Serotype shift seldom occurs (from Ogawa to Inaba and vice versa).

The difference between these serotypes is only of importance for epidemiological studies. For example: in 1991 all cases of cholera in South America were caused by toxin-producing *Vibrio cholerae*, serogroup O1, biotype El Tor, serotype Inaba. The cholera epidemic in the Rwandan refugees in DRC (July and August 1994) was caused by El Tor, serotype Ogawa. These bacteria were resistant to tetracyclines, cotrimoxazole, chloramphenicol and ampicillin. The outbreak in Haiti in October 2010, 9 months after an earthquake was due to *V. cholerae* O1, Biotype El Tor.

Flagella, pili and fimbriae

Most motile bacteria move about with structures called flagella (spirochaetes move with the help of axial filaments). Do not confuse active bacterial movement with random Brownian movement. Do not confuse a bacterial flagellum with the flagellum of a eukaryote such as Giardia (cf. also the remark concerning cilia in Balantidium coli). The flagella are too thin (0.2 µm) to be observed with a standard light microscope. The bacterial flagellum carries out a rotating movement. Some bacteria have several flagella. When the flagella rotate anti-clockwise, they form a coherent bundle, so that the bacterium moves in a straight line. On the other hand when the flagella turn clockwise, there is no longer any co-ordination and the bacteria move randomly. By timing the duration of clockwise and anti-clockwise spinning, this mechanism can be used in chemotaxis. The motor is in the membrane and the immediate driving power is not ATP, but a proton gradient. The bacterial flagella must not be confused with fimbriae, thread-like appendages which have no function in movement, but play a part in adherence to cells or tissues (important for virulence). Flagella rotate, fimbriae do not. Pili (singular pilum) are important in conjugation, the bacterial equivalent of sex. These hollow rigid tubes permit DNA transfer between bacteria. F-pili [Fertility] are important in the spread of resistance to antibiotics. Pili may also act as receptors for bacteriophages.

Epidemiology

Epidemics, pandemics

Cholera has always been endemic in India and Bangladesh, in the huge delta formed by the confluence of the Ganges, Brahmaputra, Jamuna and Meghna rivers. Probably there was no cholera in Europe or America before the 19th century.

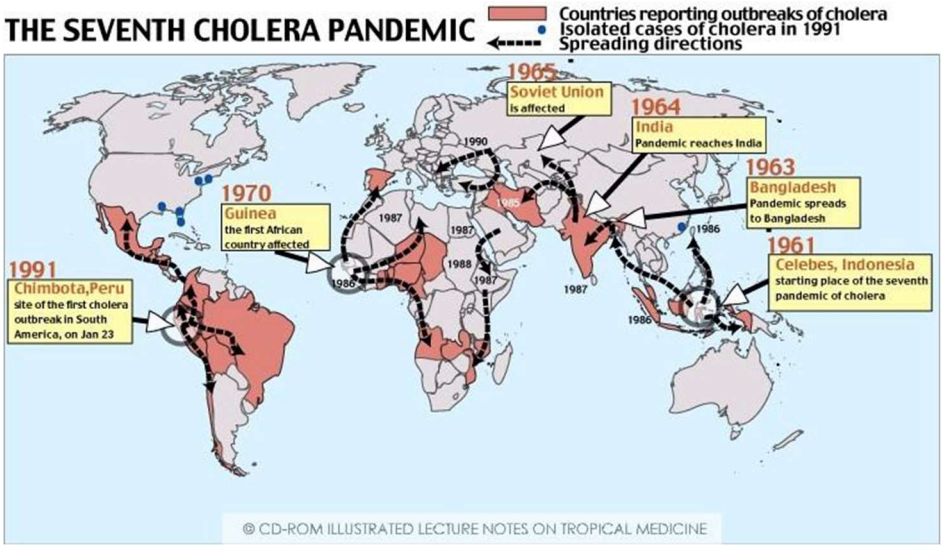
Between 1817 and 1923 there were various great pandemics, probably caused by the classic V. cholerae (there is no certainty as to the exact strain). The first pandemic which started in 1817 did not reach Western Europe. In 1829 the bacterium was introduced into the countries around the Persian Gulf via a British army unit stationed in India. From Iran the infection spread to Iraq, Syria, Georgia and Astrakhan (north of the Black Sea). It then travelled towards Odessa, Moscow, Vienna, Warsaw and Hamburg reaching England via the

port of Sunderland. The first cases in London were seen in February 1832. The third pandemic merged with the second and was amplified by the miserable conditions during the Crimean war.

When each pandemic began and ended is rather unclear. There was cholera in Belgium in 1832, 1848, 1854, 1859, 1866 and 1892. In 1866, 1 in 100 Belgians died of cholera.

The pathogen was discovered in 1884 by Robert Koch during the fifth pandemic (first work in 1883 in Alexandria, Egypt, confirmation followed by research in India in 1884, with isolation of the bacterium in culture). In fact the bacterium had already been described in 1849 by Pouchet and in 1854 by Filippo Pacini, an Italian physician. However the latter's work on this was not known outside Italy. The germ theory and in particular the work of Koch were attacked by Pettenkofer. Pettenkofer was a proponent of the "ground water theory" believing that the fermentation of organic matter in the subsoil ("miasma") released cholera into the air (no transmission from person-to-person) which then infected the most susceptible e.g. those with poor diet, constitution, etc. Both Pettenkorfer and his loyal student Emmerich drank a vial filled with cholera bacteria as proof against Kochs type transmission of V. cholerae. Amazingl, Pettenkofer did not then get cholera, but Emmerich suffered severe diarrhoea for 48 hours.

After the sixth pandemic there was a strange silence for about 40 years, for which no good explanation exists. The seventh pandemic was caused by El Tor. It started in 1961 in Celebes (Sulawesi), Indonesia, reached India in 1964 and Africa in 1970. In 2 years the infection passed through 29 African countries. In 1973 it arrived in the Gulf of Mexico. Early in 1991 the infection spread rapidly in Peru. In 3 weeks there were 30,000 cases. The bacterium then spread further into South America, causing 360,000 cases within the year. In the summer of 1992 a second, less severe outbreak occurred. Nevertheless by August 1992 "only" 5,000 deaths had been reported (from an estimated total of 600,000 cases), thanks to the wide-spread use of rehydration therapies. The case-fatality ratio varied depending on the region. After 1993 the disease assumed an endemic character in several countries, sometimes with local outbreaks. At the end of 1993 the cumulative total amounted to 900,000 cases in three years (1991-1993), with a cumulative mortality of 8,000. According to one hypothesis cholera bacteria infected the marine plankton off the Peruvian coast via the ballast water from a Chinese freighter. The possible role of changes in the nutrient-rich von Humboldt current is still unclear.



Spread of the seventh cholera pandemic

About 80% of the cholera in 1997 occurred in Africa, chiefly in the horn of Africa (118,000 cases were reported officially). The increase in cholera in this region followed heavy rains and flooding (possibly associated with the El Niño weather phenomenon).

Since 1992 V. cholerae O139 is recognised as a cause of a disease which is clinically identical to classic cholera, but which also occurs frequently in adults. Classic cholera in India, on the other hand, is common in children. There is no cross immunity with V. cholerae O1. Bacteria of the O139 serogroup have a polysaccharide capsule (unlike V. cholerae O1), which may explain the increased risk of bloodstream infection.

Cholera O139

After 1992 this new serogroup spread across Bangladesh, India, Pakistan and Southeast Asia. By the end of March 1993 more than 100,000 cases had been reported in Bangladesh. Further spread continued, but somehow diminished again, as the classic form and El Tor took over, reducing the incidence of the new serogroup. The reason is unknown. Therefore it is difficult to make then new Bengalen serogroup responsable for an 8th pandemic. It was observed in India that, after the first spread of V. cholerae O139, new variants (clones) of V. cholerae O1 El Tor once more gained the upper hand.

Cholera also surfaces regularly in Madagascar. From the beginning of December 1999 until the end of February 2000 more than 12,400 cases were reported. **The disease can thus certainly not be regarded as an entity which only existed "in the past".**

Recent epidemics

At the end of 2008 a large cholera outbreak appeared in Zimbabwe. By February 2009, this led to more than 60,000 cases with a mortality of more than reflecting the general degradation of the nation's basic infrastructure and the crumbling Zimbabwean health care system. By mid-April 2009 the official count was 96,591 cases with 4,201 deaths.

In 2010, more than 38,000 cases of cholera were identified in Nigeria.

In January 2010, a devastating earthquake hit Haiti, with its epicentre 25 km from Port-au-Prince, the capital. A couple of weeks before Nepalese United Nations peacekeepers arrived in Haiti, a cholera outbreak occurred in Kathmandu, the Nepalese capital. The forces were stationed in Mirebalais, 60 km north-east of Port-au-Prince. Late October 2010, patients with cholera were recognized in some Haitian rural areas**. In less than 6 weeks, more than 10,000 cholera cases were identified.** The disease quickly spread to the capital, where many people were still living in temporary shelters and tents, without access to safe drinking water or proper sanitary facilities. By January 1, 2011, the Ministry reported **171,304**, with cumulative mortality of **3651**. The hospital case fatality rate was too high, and a target of hospital CFR of < 1% should be achievable. A possible epidemiological connection with the Nepalese forces was suspected and created tension between the local population and the UN forces. The current Haitian strain of cholera was identified as a **virulent hybrid of the El Tor O1 biotype and the classic type, serotype Ogawa**.

Transmission

Cholera is spread **by the faecal-oral route**, via contaminated water and food. The infectious dose of bacteria required to cause clinical disease varies according to the mode of transmission and varies according to bacterial strain, with hyper-infective strains occurring immediately after gut passage. In people with normal gastric function and if ingested with water, **the infectious dose is one thousand to one million vibrios**. When ingested with food, it is lower about one hundred to ten thousand vibrios.
The low pH of stomach acid kills most vibrios. When a person uses antacids, proton pump inhibitors or ranitidine, a lower infectious dose is required to trigger infection. The same applies to chronic atrophic gastritis and status post-gastrectomy. Asymptomatic infections are common, especially in case of El Tor. People excrete bacteria for about 10 days. This is sufficient time to ensure continued contamination of the environment. Chronic carriers are very rare, but occur, sometimes with vibrios lodging in in the biliary tract.

In third world countries many people have no chlorinated, filtered, treated, pure **drinking water**. The **lack of good toilets and sewers** leads to contamination of the surface or ground water. Too often untreated sewage water is still poured into surface water. Sometimes sewage pipes and drinking water pipes are laid in the same trench, which may result in contamination if there are leaks or greatly varying water pressures in the pipes. If drinking water is contaminated in this way, bacteriological checks of the drinking water when it leaves the pumping station will not show anything amiss. In houses, drinking water containers with a wide openings often become contaminated, because people are inclined to scoop up water in their (dirty) hands. Containers with a small spout, from which water must be poured are safer.

There is also **direct transmission from person to person, but it is rare**. The number of bacteria on dirty hands is usually lower than the minimum infectious dose necessary for direct transmission. Health workers who respect basic hygiene are at extremely low risk. Filter feeders such as mussels or oysters (especially in estuaria) concentrate the bacteria in their bodies. When the organisms adhere to food particles (e.g. the chitin of crustaceans) and in the case of hypochlorhydria, lack of gastric acid due to gastric surgery, antacids, anti-ulcer drugs or atrophic gastritis, the number of organisms needed to trigger infection is much smaller. Food may be infected by **dirty hands** during or after preparation. The **bacteria can**

survive and reproduce in food such as cereals, rice or lentils and crustaceans. This intermediate replication step is very important. If someone dies of cholera and a meal is made for the mourners at the funeral by the persons who have washed the corpse, the risk of further transmission is very real. The bacteria are very sensitive to drying out, sunlight and acid. Meals which contain acid e.g. tomatoes and/or lemon, are much less dangerous than neutral or alkaline meals. Vegetables and fruit on the market are often sprayed with water to make them appear fresher and more attractive. If this is done with contaminated water, transmission may occur.

Historical note: John Snow and contaminated water

In the first half of the nineteenth century a cholera epidemic occurred in London. In 1848, a cholera outbreak started which would kill more than 14,000 people in London. Another outbreak in 1853 killed more than 10,000 people. The physician John Snow, already well known in 1853 as anaesthetist to Queen Victoria. Dr Snow had also a special interest in cholera. In 1854 he examined the various families presenting cases and calculated that the mortality in the houses that were supplied with water by the Southwark and Vauxhall Water Company was 31/1000 houses. This was 8.5 times higher than in houses supplied by the rival Lambeth Company. Although neither of the two companies offered purified water, the first company took its water from the Thames near London Bridge, downstream from the city sewage outlets while the second company pumped its water upstream from the city at Thames Ditton. In 1849 there had been no difference in mortality between the families that received water from Lambeth or Southwark. Before 1851 the Lambeth Company drew its water from a highly contaminated stretch of the Thames near Hungerford Market. It was this spectacular change (1849 compared to 1854) which made Snow conclude that contaminated water had a causal connection with cholera. Although both companies delivered water in the same streets, the water used in any particular house could be identified by its salt content (London Bridge is closer to the sea and its water is saltier than that at Thames Ditton). Adding silver nitrate leads to precipitation of silver chloride, proportionate to the amount of salt in the water. This was the basis of a simple test that could be carried out in every house.

Similar findings were made in Hamburg in 1890. The incidence of cholera was 34/1000 in Hamburg, where the drinking water was drawn from the river Elbe, and 3.9/1000 for the surrounding areas where other sources were used. In Altona, to the west and downstream from Hamburg, contaminated water was also taken from the Elbe, but there was less cholera. How could this be explained? If anything, more cholera would be expected in Altona. The difference was that in Altona the water was first filtered slowly through sand before being supplied for consumption. These observations led to attempts to provide cities with clean drinking water and to construct adequate sewers. Cholera was the first disease for which surveillance was set up and because of this the disease still has code number 001 in the international classification list of diseases.

Reservoir

Humans are the only vertebrate hosts. Vibrio cholerae can survive long-term and probably permanently in brackish water, especially if there is a neutral or slightly alkaline pH and the water contains minerals and organic material. The bacteria are concentrated in phytoplankton

(certain algae) and zooplankton which live in this water. Among the latter, copepods, a group of crustaceans, are important.

Cholera is clearly seasonal. A chronic aquatic reservoir is likely and this might be independent of continuous human faecal pollution. *V. cholerae* excreted by humans can be cultured in the laboratory.
These bacteria however may assume a living form which cannot be cultured in vitro and which do not multiply in the environment However, those bacteria are not dead as they are known to multiply when instilled in a rabbit's ileum. They are called 'viable but not culturable'. It may revert to a replicating form in its natural environment when there are favourable environmental factors and this has important epidemiological implications. The living, but non-reproducing form of *V. cholerae* can probably cause disease. Traditional culture methods for tracing *V. cholerae* in water miss these "dormant" bacteria.
Tests based on fluorescent antibodies may offer a practical solution, as they stain both dormant and active bacteria.

Physiopathology

The bacterium **multiplies in the small intestine**, where it adheres to the mucosal brush border. The bacterium is **not invasive**, in other words it does not penetrate the intestinal wall or pass into the blood. It excretes a **very powerful toxin** which causes active fluid secretion towards the lumen. This fluid is isotonic, ion-rich and protein free. There are no intestinal ulcerations and the faeces do not contain blood. There is little if any fever. There is no tenesmus**. The faeces contain significant amounts of sodium, potassium and bicarbonate.** Because of this the intestinal content is slightly alkaline (V. cholerae thrives best in a slightly alkaline environment and the bacteria are therefore producing the conditions which are optimal for their own survival). The loss of large amounts of alkaline faeces results in metabolic acidosis. People with blood group O have an equal risk of infection but are at a significantly higher risk of clinically severe cholera if they become infected. The reason is unknown.

Hypervirulent and hyperinfective strains play an important role in epidemics. Passage of *Vibrio cholerae* through the gastrointestinal tract results in a short-lived, hyperinfectious state of the organism that decays in a matter of hours into a state of lower infectiousness. Such strains have a much lower ID50 (the number of micro-organisms that will disease 50% of a population in normal conditions = measurement for virulence) than strains occurring in natural water reservoirs. The classic strain is associated with more severe illness. Faecal excretion of V. cholerae for up to two weeks has been documented and occasional asymptomatic carriers occur. Asymptomatic patients typically shed bacteria in their stools at about 1000 *V. cholerae* bacteria per gram of stool, which is a low level of shedding, compared with the 100 million bacteria per gram in case of ricewater stools.

Toxins

Vibrio cholerae produces several toxins: **cholera toxin (Ctx**), the zona occludens toxin (Zot) and the accessory cholera enterotoxin (Ace). The role of the two latter toxins is not entirely clear. The Ctx enterotoxin of *V. cholerae* consists of 2 parts: **A and B, where A stands for active and B for binding.**

They stimulate adenylate cyclase. Adenylate **cyclase increases intracellular cyclic-AMP, which**

inhibits salt absorption by the microvilli and promotes active chloride excretion by the crypt cells.

Water and potassium bicarbonate passively follow the chloride. In the end there is an overall water loss to the intestinal lumen. Fluid loss originates in the duodenum and upper jejunum, the ileum is less affected. The colon is insensitive to the toxin and cannot absorb the large amount of fluid quickly enough. Catastrophic diarrhoea follows.

Cholera toxin

Part A is a monomer, while part B consist of 5 identical subunits (a pentamer). The polypeptides of part B bind to a receptor (Gm1 ganglioside, a glycolipid) on the epithelium of the small intestine, after which part A can penetrate the cell. Part A binds covalently to an intracellular protein (Gs-protein; s for stimulatory) which irreversibly activates it, leading to the persistent stimulation of another intracellular enzyme, adenylate cyclase.

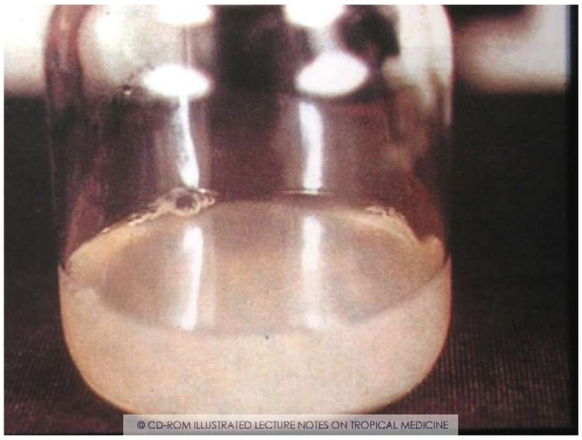
The toxic A-subunit also has other effects such as disturbing the expression of some genes, increasing inflammatory cytokines and inhibiting antigen presentation by macrophages. On the other hand, the B-subunits of cholera toxin have antiinflammatory properties. These are under intense study at present for possible therapeutic use in immune abnormalities. While cholera toxin adheres to the intestinal villus cells and disables the cellular saltwater pumps, the Zot toxin loosens the junctions that binds intestinal epithelial cells together. This contributes to the loss of water to the intestinal lumen.

The in-vivo detailed mechanism is probably more complicated. Cholera toxin also stimulates the nervous system in the intestinal wall, the myenteric plexus. This results in the release of 5-hydroxytryptamine (serotonin) from the enterochromaffin cells, leading to in additional fluid loss to the lumen. Granisetron, a 5-HT3 receptor blocker, partially reverses this effect. More research is needed to determine the role of this mechanism in the physiopathology.

Clinical aspects

The **incubation period is brief**: sometimes only hours, more commonly 1 to 5 days (average 2 days). It is one of the few infectious diseases where -in case of a very severe infection- you can be well in the morning and dead by sunset the same day. Asymptomatic infections are common (about 93%), but chronic carriers are very rare. Sometimes there is an initial transient fever (more seen in children).

Massive watery diarrhoea starts suddenly. The faeces very rapidly look like water in which rice has been boiled: watery with flakes of mucus. The faeces have a fish-like smell. The volume of faeces may rise to 500 ml per hour. Vomiting is common, but abdominal cramps are unusual. The onset of thirst, oliguria or anuria and weakness is rapid. In a short time the patient develops severe dehydration and can die within 24 hours. In other cases the diarrhoea is less severe, especially with infections with El Tor. As the patient's condition deteriorates, hoarseness of the voice and temporary deafness are often observed. Children with severe cholera may present with drowsiness or coma.



'Cholera feces which look like ''rice water''. Copyright Alexander von Humboldt Institute, Peru

The signs of dehydration are thirst, dry mouth and lips (if the patient has not vomited recently), hollow eyes and sunken fontanel in children. The skin turgor diminishes. The skin becomes wrinkled (washerwoman's hands). Often, the voice becomes weak and hoarse, the pulse quickens and is difficult to feel. The radial pulse might be impossible to detect. Blood pressure falls. There is little or no urine production (prerenal failure). Respiration becomes faster due to metabolic acidosis secondary to loss of bicarbonate in the faeces (bicarbonate is alkaline). This acidosis causes vomiting and muscle cramps.

There is also significant potassium loss in the faeces. If rehydration is carried out using fluid without potassium, severe hypokalaemia may result. Nevertheless, quite often normokalaemia is found, together with an increased anion gap. The increase in anions (= negative ions) is multifactorial due to the hyperproteinaemia (hemoconcentration), hyperphosphataemia (internal shifts and renal failure) and lactate acidosis (shock). Ketones play little if any role. An elevated hematocrit (hemoconcentration) can be found in in nonanemic patients, as can neutrophil leukocytosis in severe cases.

The mortality from classic cholera may reach 50 %, but can be brought down to < 1 % with correct therapy. Mortality is chiefly due to dehydration with kidney failure, hypokalaemia, hypoglycaemia and aspiration pneumonia during vomiting.

Diagnosis

Cholera should be suspected in acute massive rice-water diarrhoea, certainly if there have been several cases in a short time (epidemic). The clinical picture of severe cholera is so spectacular that differential diagnosis does not present many difficulties. Milder cholera may be similar to other forms of gastro-enteritis (but not to dysentery). A child above the age of five years who develops acute dehydration, or dies as the result of acute diarrhoea, is always suggestive for cholera.
The vibrios are very small and can best be seen in a fresh faecal specimen with the help of dark field microscopy. There is characteristic motility ("star shooting") which stops immediately after adding anti-O1 antiserum. This does not give any information on possible toxin production.

Confirmation is best made via a bacteriological culture.

Culture of Vibrio cholerae

Culturing should preferably be on a special medium in a bacteriology lab, e.g. TCBS-agar [=Thiosulphate-Citrate-Bile saltsSucrose], polymyxin mannose tellurite agar (PMT) or another selective medium. TCBS agar is green before inoculation; sucrose-fermenting organisms such as *V. cholerae* turn it yellow. TCBS agar is important for rapid isolation and identification, but V. cholerae also grows on routine agar media. For routine media, large numbers of bacteria per gram of stool should be present to allow detection. Patients or carriers with low burden of bacteria will be missed with routine culture media.
Overgrowth by normal faecal flora limits recovery of colonies. In order to identify the serogroup and the serotype one subsequently finds out to which antibodies (antiserum) the colonies obtained exhibit an agglutination reaction. It is also possible to find out whether the vibrios are toxicogenic (produce toxin), e.g. by a PCR variant called a loop-mediated isothermal amplification (LAMP) assay. Definitive identification is made in a reference laboratory.

Specimens may be transported in a transport medium, e.g. Cary-Blair. This is a kind of mild alkaline buffered gelatine in seawater with low redox potential in which the bacteria will survive for 4 weeks. If it is not available, a filter paper can be soaked with faeces and transported in an airtight bag to a wel-equipped laboratory. A sample treated in this way remains usable for 1 week, but the recommendation is "the faster the analysis, the more reliable". Blotting paper, soaked with liquid faeces and if possible placed in a 1% saline solution, can be kept for several weeks at 37°C (not in the freezer). This is useful if there are nitial transport problems. Nevertheless it is better to have a fresh faecal specimen. For specimens from the environment or from food, in which the number of bacteria is much lower than in faeces, enrichment is necessary. The specimen can be incubated for 8 hours in alkaline peptone water, after which a TCBS agar is used.

About 10 days after infection with V. cholerae O1 the patient produces vibrocidal antibodies. They start diminishing after only one month and disappear within the year. Antibodies against cholera toxin are produced more slowly and remain for years. However these cross-react with enterotoxin produced by ETEC bacteria [enterotoxic Escherichia coli]. The immune response to *V. cholerae* O139 is not well understood. The detection of antibodies is not important for the urgent care of the individual patient but does permit retrospective diagnosis.

Other Vibrios

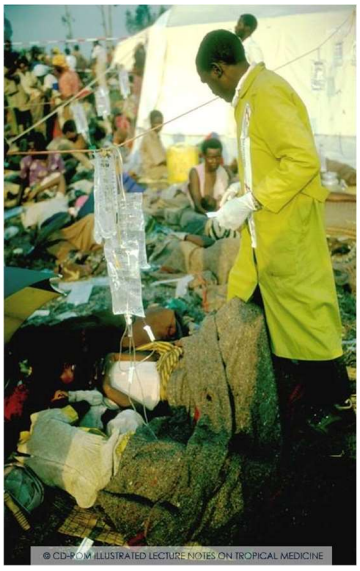
Sometimes other Vibrio species are responsible for diarrhea, e.g. *Vibriocholerae* non-O1, *V. parahaemolyticus, V. hollisae, V. minicus* and *V. fluvialis*. Our knowledge of these latter bacteria is clearly insufficient. Vibriovulnificus is an aggressive speciespresent in seawater and filter-feeding organisms such as oysters. This bacterium may cause bloodstream infection and wound infections, certainly in patients with liver cirrhosis or otherwise immune depressed.

Treatment

Rehydration is essential and must be instituted as soon as possible. **Two phases** are distinguished.
First it is important to replenish what has been lost in the previous hours or days. Then one must compensate the persistent fluid loss (e.g. the amount of fluid that is lost every hour). In mild cholera without vomiting oral rehydration may suffice. In severe forms IV fluids should be administered.

There are several possible compositions of rehydration fluids. **Solutions containing salt, sugar, potassium and bicarbonate are recommended.** Lactate is also good because it is converted in the body to bicarbonate. In cholera it is preferable to use Ringer's lactate (= Hartmann's solution). Normal physiological saline is second choice because it does not correct the acidosis nor does it contain potassium. Severe hypokalaemia may occur, with cardiac arrhythmias, kidney damage, paralytic ileus and significant muscle weakness with reduced or absent tendon reflexes. Dextrose (= glucose) 5 % without electrolytes is not advised as a rehydration fluid. A reminder: 1 gr KCl = 13 mEq KCl. **So: Hartmann = Ringer's lactate >Ringer> physiological saline>>> not glucose infusion if there is an alternative.**

In severe cholera (fluid loss > 10 % of weight) the missing fluids should be administered quickly, e.g. 6 litres over 4 hours for a patient weighing 60 kg. The first 3 litres may each be administered in 10 minute boluses (total therefore 30 minutes). After administration of the lost volume, losses are compensated with further IV and/or PO fluids (faeces volume + urine volume + 500 ml). Vomiting may make oral administration of fluids difficult**. Generally a total of 6 to 10 litres per patient is necessary.** When patients start to drink and stop vomiting, it is advised to leave IV lines in place for a while until you are sure rehydration will not pose any more problems.



Cholera epidemic in Congo. Refugees live in very poor conditions. Photo courtesy Els De Temmerman

Cholera bed used by AIDS patients with chronic diarrhoea. With special thanks to Prof. Dr R. Colebunders. Copyright ITM

Special cholera beds are useful: they have a central opening to allow the liquid faeces to pass through, and they can be collected in a bucket. This makes it possible to quickly determine the amount of fluid loss. During an epidemic people who can still hold themselves upright can sit on a bucket and try to drink as much ORS [oral rehydration solution] as possible. Children quickly develop convulsions and coma. It is important that hypoglycaemia should be considered. For an adult 50 ml of a 50% glucose solution is given IV, for a child 2-4 ml/kg 25% glucose or 10 ml/kg of a 10% glucose solution.

Antibiotics may be useful because they reduce the duration and thus the total volume of the diarrhoea and may therefore reduce the need for rehydration fluids. On the other hand, they are not essential (given the non-invasive character of the infection) and resistance often occurs. At present a single dose of azithomycin 1 gram (child 20 mg/kg) or a single dose of doxycycline 300 mg (child 4 mg/kg) are possible treatments. Doxcycline is usually contraindicated in pregnant women and children under 8 years. However, the administration of a single dose should not provoke major adverse effects. Singledose ciprofloxacin may also be effective. *V. cholerae* O139 is often resistant to cotrimoxazole (sulphamethoxazole-trimethoprim). There is insufficient data examining the effect of antibiotics on secondary transmission of cholera. However in published studies to date antibiotics have not been shown to decrease secondary transmission of cholera within households. Anti-peristaltic drugs such as loperamide may cause accumulation of fluid in the intestinal lumen with unfavourable consequences and should be avoided.

Prevention

In the industrialised world a patient with cholera will remain a sporadic case. In developing countries one case can lead to several secondary cases. Therefore ‘enteric contact precautions’ are essential in health care settings, focusing on very strict hand hygiene and thorough environmental cleaning and disinfection. The contamination of clothing and bedding is unavoidable. Boiling in water for five minutes is sufficient for disinfection. Mattresses and blankets can be dried in the sun. It is better to do this before washing them, to prevent infection of the washing area.

After surviving cholera a patient is probably immune for homologous biotypes for more than 3 years. There is some controversy: infection with the classic biotype seems to protect against recurrent infection by either biotype, but El Tor does not. No cross-immunity *between V. cholerae* O1 and V. cholerae O139 is seen, although they produce the same toxin. Immunity relies on antibodies in the intestinal lumen (the bacteria are not invasive). Systemic vibriocidal as well as anti-toxin antibodies develop during illness. Babies which are being breast-fed receive protective antibodies in their mother's milk.

Vaccination

Former parenteral vaccination with dead V. cholerae bacteria (IM administration) did not lead to sufficient formation of protective antibodies in the intestinal lumen. Only about 50-65% of people living in endemic areas were protected for 3-6 months. The IM vaccine was associated with local reactions in 50% and systemic reactions (fever, malaise) in 10-30%. Advice to vaccinate with this type of vaccine was discontinued in 1972 by the WHO [World Health Organisation]. Parenteral vaccination, mass chemoprophylaxis and "cordon sanitaire" (= restrictions on travel and trade) are not effective in preventing or limiting outbreaks. A **newly developed oral cholera vaccine** is based on a killed whole cell cholera vaccine combined with the recombinant B subunit of cholera toxin (**Dukoral®**). The vaccine contains 1 mg of recombinant B subunit, as well as 25 x 109 bacteria each *of V. cholerae* O1 classic Inaba, *V. cholerae* O1 classic Ogawa, *V. cholerae* O1 El Tor Inaba (heat-inactivated), *V. cholerae* O1 El Tor Inaba (formaline inactivated). Dukoral does not contain the A subunit of cholera toxin and therefore, no pathogenic toxin is present. Two to three doses need to be given. Two recent studies showed an effectiveness of 86% and 40% respectively; the latter study indicating a 63% protection against severely dehydrating cholera episodes. Lower levels of protection continue for 3 years. Protection wanes rapidly in young children. **A herd immunity effect is expected in areas where vaccine coverage is more than 50%.** Because the risk of cholera for most travellers is extremely low, vaccination should be considered only for those working in relief or refugee settings or for those who will be travelling in cholera-epidemic areas and who will be unable to obtain prompt medical care. WHO recommends that current available cholera vaccines be used as complements to traditional control and preventive measures in areas where the disease is endemic and should be considered in areas at risk for outbreaks.

Mass (antibiotic) chemoprophylaxis is not effective because (1) the infection spreads faster than the organisation of drug distribution, (2) the effect of a drug only lasts 2 days, after which re-infection may occur, (3) the whole population needs to be treated simultaneously and people should then be isolated and (4) it is difficult to convince asymptomatic people to take a drug. In addition, the selection of highly resistant strains has been observed in settings using mass-administration of antibiotics.

Correct eating and drinking habits, safe stool habits and personal hygiene are the most effective means for individuals to limit their risk of cholera. Improved sanitation is the pre-eminent method of eliminating cholera and many other faecal-orally transmitted infections. This is directly linked to the degree of poverty in a region. Boiling drinking water is often difficult since fuel may be scarce and expensive. Since a significant proportion of Vibrio cholerae can adhere to plankton, the drinking water can be filtered through a fine cloth, which removes both plankton and a lot of bacteria in a single operation. This is of course less effective than obtaining water from a clean pipe or pump but it is cheaper. Chlorination of

drinking water may be important (piped water or via water trucks). This is difficult to accomplish in rural areas. Chlorination is much less effective if the water is turbid due to organic debris.

Eating raw fish, shellfish (e.g. oysters, mussels) and crustaceans (such as crabs, shrimps) should be avoided. Washing hands is important for transmission control within a household. Infected faeces should not be disposed of in a poorly functioning drain (hospital: e.g. in pit with unslaked lime = CaO).

When large groups of people come together (funerals, festivals, etc.) there should be latrines with facilities for washing hands and plenty of soap.

An attempt must be made to trace the source of small, local outbreaks (see Historical note on John Snow). Contaminated water is the chief suspect in a sudden local epidemic, while in isolated cases the cause should be sought in contaminated food. This is of course not an absolute rule. Food cooked by street vendors and in restaurants poses specific problems. Flies probably play an underestimated part in transmission, but their numbers also reflect the sanitary conditions in a region.

The following points should be emphasised during information campaigns:

1. Drink only clean water (boiled or chlorinated)
2. Cook food completely and eat it while it is hot
3. Avoid uncooked food, unless it can be peeled
4. Wash hands after a bowel movement
5. Wash hands before preparing food
6. Wash hands before eating
7. Correct use of a good latrine (also for children)
8. With correct treatment cholera is rarely fatal
9. If cholera is suspected medical help should be sought immediately
10. In diarrhoea, give plenty of fluids (e.g. ORS)
11. Cholera vaccines should be used as complements to traditional control and preventive measures in areas where the disease is endemic and should be considered in areas at risk for outbreaks

In case of an epidemic, it is important to have **a large stock of IV rehydration fluid available** as well as the means of preparing **large amounts of oral rehydration fluid.** Normally such buffer stocks should be stored at various strategic points. The stocks for cholera treatment should not be segregated in storage, but should be rotated during normal use to avoid stock expiring. As soon as an epidemic is suspected, use as much oral rehydration as possible first so that stocks of the IV solutions last as long as possible. Cholera beds should be made ready. In a normal epidemic an attack rate of 0.2% can be taken as a rule of thumb (i.e. 200 cases can be expected in a population of 100,000). This is useful for estimating the size of stocks that will be needed. Sometimes the attack ratios are higher (e.g. the Rwanda-Zaire border in 1994). All this requires a solid epidemic preparedness.

Diarrhoea in the tropics

Summary
- A common and major problem; a major cause of mortality in children
- Mortality due to dehydration and invasive bacteria
- Etiology: viruses, Shigella sp., Vibrio cholerae, Giardia lamblia, Entamoeba histolytica, ...
- Clinical: degree of dehydration, ± blood in the faeces, ± fever, ± acute/chronic
- Rehydration (PO or IV); nutrition, sometimes aetiological treatment necessary

General

Diarrhoea is very common in the tropics. It is often self-limiting, but its general significance cannot be overestimated. It is a major cause of malnutrition and is one of the main causes of death particularly in children.

What precisely is meant by diarrhoea varies between patients:
1. an increased number of defecations per day (e.g. more than 3)
2. a decreased consistency of the faeces or
3. an increased volume of stools (e.g. > 200 g/24h)
all are used to define the problem.
The WHO definition of diarrhoea is at least 3 evacuations every 24 hours of unformed faeces. Unformed means here that they take the shape of any container into which they are evacuated. WHO emphasises the importance of change in stool consistency rather than frequency, and the usefulness of parental insight in deciding whether children have diarrhoea or not.

Diarrhoea causes fluid loss resulting in **dehydration**. The patient also loses electrolytes, which can lead to ion imbalances, such as hypokalaemia. Acidosis develops due to the loss of bicarbonate in the stools, to reduced renal function (less acids are excreted) and to ketosis (breakdown of body fat due to reduced food intake). Often the patient has no appetite and the nutritional status which is sometimes already poor deteriorates further. Sometimes the mother thinks she is doing good by "letting the intestines rest" and temporarily not giving food. Moderate undernourishment can then develop into severe malnutrition (marasmus and kwashiorkor). The latter is often seen if a patient has had a number of episodes of diarrhoea in quick succession.

Dysentery is a severe form of diarrhoea. Fever is common in bacillary dysentery, but rare in amoebic dysentery. Dysentery has three characteristics:
1. Abdominal pain
2. Tenesmus (pain due to cramps in the rectum) and false defecation need
3. Frequent evacuation of small quantities of faeces that are mixed with blood, mucus and/or pus

Steatorrhoea or fatty diarrhoea is characterised by large quantities of faeces with an increased fat content (the stools float on water). This occurs in certain malabsorption syndromes. The cause usually lies in disorders of the pancreas or small intestine.

Aetiology

Diarrhoea is usually caused by infections. Of the nearly 11 million deaths that occur annually among children under five years of age, diarrhoeal disease is the second leading cause (after respiratory tract infections). The most common cause of severe gastroenteritis worldwide is rotavirus which accounts for 29 to 45 percent of nearly 2 million deaths. Bacterial intestinal infections (especially dysenteria) also contribute to the high mortality.

The most common causes of diarrhoea include (non-exhaustive list):

1. **Preformed bacterial toxins,** with the bacterium itself being no longer active in the intestine. Examples include staphylococcal diarrhoea (*Staphylococcus aureus* toxin), the ingestion of *Clostridium perfringens* toxins after eating contaminated meat (pigbel) and Bacillus cereus toxins (contaminated rice, among other things). Incubation time very short (hours).
2. Bacteria which multiply in the intestines: *Salmonella, Shigella, Yersinia enterocolitica*, a whole zoo of related *Escherichia coli* strains, toxicogenic *Vibrio cholerae, Campylobacter jejuni,* toxicogenic *Clostridioides difficile*
3. Protista: Giardia, Entamoeba histolytica, Balantidium coli, Dientamoeba fragilis, microsporidia, various coccidia (Isospora belli, Cryptosporidia, Cyclospora, Sarcocystis). Sometimes malaria is accompanied by diarrhoea!
4. Worms: only in case of serious infections, e.g. *Schistosoma mansoni, Capillaria philippinensis, Strongyloides stercoralis, Trichinella spiralis;* rarely by other worms. *C. philippinensis* and *S. stercoralis* can remain several decades in the body and are able to multiply inside the human host, something that most other worms cannot achieve. They can be lethal. Since worm infections are so common in the tropics, worm eggs are often found in the stools. However, there is not necessarily an etiological connection between the presence of helminth eggs and diarrhoea.
5. Viruses: Rotavirus, Astrovirus, HIV, Noroviruses (Noroviruses cause gastro-enteritis, with important vomiting accompanying the diarrhoea).
6. Non-infectious causes such as laxative abuse, animal or vegetable toxins, marine biotoxins, mycotoxins, irritable bowel syndrome and inflammatory bowel diseases (Crohn's disease, ulcerative colitis) are much less common. Endocrine problems (hyperthyroidism) and related problems (vipoma, carcinoid, etc) exist but are present in a minority of persons who present with chronic diarrhoea.

It is not always important to discover the exact cause of an episode of diarrhoea: for example, it is important to distinguish between amoebic colitis and bacillary dysentery, but the difference between Rotavirus and Norwalk virus(= Norovirus) enteritis is at present not clinically relevant in the tropics.

Intestinal infections caused by protista occur everywhere but are more prevalent in tropical climates.

The climate helps protista to survive in the outside world and poor hygiene promotes their transmission. Diarrhoea is often found together with a parasitic infection, but **the causal connection must always be assessed critically.** It is important to distinguish between infection and disease. Of the many protista that are found in faeces, only a few types are potentially

pathogenic. Occasionally, *Plasmodium falciparum* and *Leishmania donovani* can cause digestive symptoms. The diarrhoea then displays no particular characteristics.

Patients with diarrhoea may be classified based on the disease duration, aspects of the stool and other clinical symptoms.

Acute diarrhoea

Acute non-bloody diarrhoea with little or no fever

If the diarrhoea is very watery and very abundant, the possibility of **cholera** must always be considered (see separate lecture notes on cholera).

Food poisoning by **bacterial toxins** (including staphylococci) may also causing this type of diarrhoea, resulting in explosive diarrhoea shortly after a meal. The bacteria reproduce in food and produce a thermostable toxin. The bacteria are usually killed when food is cooked or left over food is reheated.

The toxin however is not destroyed by the heat and enters the intestine, where it causes massive diarrhoea, probably by neurotoxic action on the autonomous nervous system. **Antibiotics are therefore of no value here.** Symptomatic treatment is indicated. Toxins produced by *Bacillus cereus* (often present in contaminated rice) can produce a similar picture or the "emetic syndrome". Some milder infections, such as traveller's diarrhoea, produce hardly any fever. In these cases bowel motion inhibitors (loperamide) can be given.

Acute non-bloody diarrhoea with fever

In children any infection of any type can be associated with diarrhoea, e.g. otitis media, tonsillitis, pneumonia, urinary infection, etc. The main pathogens are viruses, some *Escherichia coli* (ETEC is the most common pathogen in traveller diarrhoea) and mild forms of bacillary dysentery (*Salmonella, Shigella, Campylobacter* and *Yersinia*). The possibility of malaria and typhoid fever must be considered.

Acute diarrhoea with fever but without bloody stools, **generally requires no antibiotics**. The emphasis is on administering fluids and electrolytes. In small children a bacterial infection of the intestine can rapidly give rise to bloodstream infection. Antibiotics may therefore be indicated in small children (<1 year), other vulnerable patients or in patients with persisting and/or deteriorating symptoms.

Acute bloody diarrhoea with fever

This is the picture of a **bacillary dysentery**. Pathogens are *Shigella, Salmonella, Campylobacter* and some *Escherichia coli.*

Some bacteria are very aggressive, while others give rise to milder infections.

Complications can occur:
- toxic megacolon
- rectal prolapse
- bloodstream infection

• haemolytic-uraemic syndrome. TTP-HUS, often triggered by Shiga toxin produced by *Escherichia coli* O157:H7 or other verotoxin producing bacteria (VTEC). If HUS occurs, antibiotics are contraindicated because otherwise still more toxins are released from the bacteria that have been killed, which aggravates the clinical status.
• reactive arthritis
• Reiter's syndrome [urethritis, arthritis, conjunctivitis, uveitis, hyperkeratosis of the palms of the hand (keratoderma blennorrhagicum) and painless ulcers in the mouth and on the glans (balanitis circinata)].
• After using antibiotics an overgrowth of *Clostridioides difficile* can occur in the intestine. The toxins that are produced by this bacterium cause a severe inflammation of the colon (pseudomembranous colitis) which can develop into toxic megacolon.
• A very serious complication after *Campylobacter* enteritis is the Guillain-Barré syndrome, which is characterised by ascending paralysis caused by a demyelinating process of the spinal roots.

In case of bacillary dysentery, examination of the faeces under the microscope shows numerous **white blood cells (pus) and red blood cells**. Bacillary dysentery is associated with a marked disappearance of the normal bacterial intestinal flora. It is not possible to distinguish between the different bacteria by microscopy alone (culture is needed for this). Testing for different pathogenic *Escherichia coli* strains is difficult. E.g. testing for enterotoxicogenic E. coli requires recovery of individual bacterial clones from an agar plate inoculated with a stool sample. This should be followed by molecular evaluation for detection of specific genes. This approach is not available in most laboratories, including most labs in the West.

As always, **fluid and electrolytes** form the basis for treatment. With bacillary dysentery, **antibiotics are an important part of therapy**. The resistance of the various bacteria varies. Multi-resistant bacteria are becoming more common, especially in South and Southeast Asia. Depending on the local conditions and resistance patterns, a quinolone (e.g. ciprofloxacin) or a neo-macrolide (e.g. azithromycin) should be used. The use of diarrhoea-inhibitors (loperamide) is not recommended.

Acute bloody diarrhoea but little or no fever

The main causes are **amoebic dysentery** and to a lesser extent **mild bacillary dysentery**. Examination under the **microscope of fresh (still warm) faeces** is important in order to identify motile trophozoites.

The normal bacterial intestinal flora is maintained in amoebic dysentery. In case of severe amoebic colitis fever may be present. Amoebic dysentery is treated with medication against the trophozoites (tinidazole = Fasigyn®, metronidazole =Flagyl®) followed by medication against any remaining intestinal cysts (paromomycine = Gabbroral®, diloxanide furoate= Furamide®). Other less common causes of bloody diarrhoea without fever are acute schistosomiasis (eosinophilia, worm eggs), massive trichuriasis (microscopy), ulcerative colitis (rare in the tropics) and *Balantidium coli* (microscopy).

Ileocaecal intussusception can present with acute bloody diarrhoea followed by intestinal obstruction.

Sometimes **malignant tumours** can present with acute bloody diarrhoea.

Food poisoning with Clostridium perfringens causes necrotising enteritis. After the Second World War this became known as "darmbrand". In the dialect of Papua New Guinea the disorder is known as "pigbel". Pigbel has been recognised in Papua New Guinea since 1961. The disorder is seen mainly in undernourished and parasite-infested children after eating a rich meal with sweet potatoes and infected pigmeat (pig intestines are also eaten). Meals such as this are sometimes prepared on the occasion of a great feast at which the host expresses his social standing by slaughtering and serving a large number of pigs. The illness can therefore occur in epidemics.

Clostridium perfingrens

The anaerobic Gram-positive bacterium (Clostridium perfringens) is frequently present in the flora of the colon, so there must be other factors present to cause the onset of the disease. The bacterium, better known as the causative agent of gas gangrene, can produce various toxins. The bacterial strains which produce toxins can be classified into types A, B, C, D and E. All types produce alpha-toxin, which is a lecithinase (phospholipase C). Clostridium perfringens type C, responsible for pigbel, produces alpha- and beta-toxins. The toxins in the intestine are usually destroyed by proteases. In case of undernourishment there is an important deficiency in proteases such as trypsin, and as a result the toxins can remain active. If there are trypsin inhibitors present as well, such as are found in sweet potatoes, the remaining small amount of trypsin is neutralised. Adult Ascaris worms produce trypsin inhibitors. If the intestine has reduced motility, the toxin remains in contact with the wall for a prolonged period of time and causes transmural necrosis. The lesions tend to be more prominent in the jejunum, although lesions of the ileum also occur. Besides supportive therapy, treatment is based on antibiotics (chloramphenicol, benzylpenicillin or other, broad-spectrum antibiotics), type C antiserum and mebendazole. Sometimes surgery has been performed. Vaccination against type C toxin is useful.

Chronic diarrhoea

The great majority of diarrhoea episodes last less than one week, however when diarrhoea persists for 14 days or longer, it is called persistent diarrhoea. Some authors use the term "chronic" for diarrheal illnesses lasting 30 days or longer.

Chronic non-bloody diarrhoea without fever

In the tropics protista must be looked for in the first place: *Giardia, E. histolytica, Dientamoeba fragilis, Balantidium coli,* chronic intestinal capillaria, microsporidia, various coccidia (*Isospora belli, Cryptosporidia, Cyclospora, Sarcocystis)*. There is a long list with other diseases causing chronic watery diarrea: pellagra (niacin, vit B3 def), hyperthyroidism, irritable bowel syndrome, lactose intolerance, food allergies, coeliac disease, malnutrition, laxative abuse, neuro-endocrine tumours, intestinal lymphoma, collagenous colitis, AIDS, protein loosing enteropathy.
Campylobacter infections and some strains of *E. coli* occasionally cause persistent diarrhoea.

Chronic non-bloody diarrhoea with fever

Chronic diarrhoea, emaciation and persistent fever are important criteria for the clinical diagnosis of **AIDS**. Other clinical signs should be searched for, such as oral candidiasis, Kaposi's sarcoma lesions, chronic pruritus, severe or repetitive shingles. Serology can confirm the diagnosis. Intestinal parasites must be searched for.

Tuberculosis of the intestine is predominantly sited at the ileocecal transition. A mass can sometimes be felt there on palpation. There is sometimes ascites due to concomitant involvement of the peritoneum. Pulmonary lesions can be present, but these are certainly not a requirement for the diagnosis of intestinal TB. Intestinal tuberculosis occurs predominantly in immunocompromised individuals. It is difficult to differentiate intestinal tuberculosis from Crohn's disease because of similar clinical, pathological, radiological, and endoscopic findings. Histological interpretation of biopsies is of limited diagnostic value in the differentiation of intestinal tuberculosis from Crohn's disease, except when caseating granulomata are found. Mycobacterial culture (isolation of *Mycobacterium tuberculosis*) and PCR are helpful in making the distinction between intestinal tuberculosis and Crohn's disease.

Chronic bloody diarrhoea without fever

One has to consider persistent amoebic dysentery, severe schistosomiasis (S. mansoni, S. japonicum), inflammatory intestinal diseases, intestinal tumour and repeated intestinal invagination. Be aware of diarrhoea due to other causes together with bleeding haemorrhoids.

Chronic fatty diarrhoea

Causes of steatorrhoea include abnormalities of the **small intestine and insufficiency of the exocrine pancreas**. Calcification of the pancreas in chronic pancreatitis can be seen in 50% of cases (X-ray of the abdomen). Concomitant diabetes mellitus should be searched for.

Non-infectious causes of intestinal abnormalities such as coeliac disease (hypersensitivity to gluten) and intestinal lymphoma are rare. Coeliac disease is associated with antibodies against gliadin (a component of gluten) and autoantibodies against tissue transglutaminase (and/or anti-endomysium antibodies). Tropical sprue is a disease of unknown origin, common in Asia but less so in Africa. The disease responds to treatment with tetracyclines and folic acid.

Some infections may result in malabsorption:

1. *Giardia lamblia:* microscopy of the faeces. These are often asymptomatic infections, so their importance should not be overestimated. Giardia can also give rise to secondary lactosemalabsorption: dairy products can no longer be tolerated.

2. *Capillaria philippinensis:* occurs mainly in the Far East but is rare. Infection is caused by eating raw fresh water fish. Like Strongyloides, this worm also leads to endogenous reinfection. It can therefore reproduce in the body unlike most other worms. The eggs and larvae can be found in the stools (repeated analyses are necessary). Treatment of intestinal capillariasis is with mebendazole. It is a potentially fatal infection.

3. *Strongyloides stercoralis:* serious infections cause diarrhoea, eosinophilia, pruritus and larva currens. The stools contain seldom eggs but larvae are present.

4. *Cryptosporidia* can cause malabsorption. The possibility of AIDS must be excluded in chronic cases. The parasite can be demonstrated using Ziehl stain.

5. *Cyclospora* can be compared with "large cryptosporidia" with variable acid-fastness on Ziehl stain. Treatment with cotrimoxazole is usually effective.

6. *Tropical Sprue: the* exact cause is not known, but an infectious origin seems probable. Macrocytic anaemia, glossitis, hypo-albuminemia and signs of vitamin-deficiencies (ADEK) are common. Treatment includes tetracyclines and folic acid.

Assessment of a patient with diarrhoea

The assessment of a patient with diarrhoea includes a thorough medical history on the disease duration, stool characteristics and other relevant clinical signs and symptoms as well as an estimation of the degree of dehydration**. Medical history** should focus on:

1. How long has the patient been suffering from diarrhoea? Is it acute (<14d) or chronic (>14d)?
2. Is there fever? Weigt loss? Night sweats?
3. Is there blood or pus in the faeces, or is it watery diarrhoea ?
4. Is the diarrhoea volume large (more likely small intestine) or small (more likely colon)?
5. Is there tenesmus? Suggests that the rectum has been affected by inflammation or ulceration. Diarrhoea or rectal discharge? (suggests proctitis)
6. Is there abdominal pain? Not with cholera.
7. Is the patient vomiting? Makes dehydration worse and makes therapy more difficult.
8. Are there a number of people in the area with the same symptoms? An epidemic?
9. Is the patient immunocompromised, or does he have major co-morbidity? Any (new) medication?

Acute diarrhoea is often caused by self-limiting infections (beware exceptions). Chronic diarrhoea is more often than not caused by non-infectious causes (beware exceptions, especially in immunocompromised patients). Two intestinal helminths which as a rule persist (probably for life) even in untreated immunocompetent persons are *Strongyloides stercoralis* and *Capillaria phillipinensis.*

Chronic diarrhoea can be further classified by volume, where small frequent stools are suggestive of a distal colonic disorder. Large volume watery stools are suggestive for conditions involving the small intestine (but beware of a secreting villous colonic adenoma). Steatorrhea or fat-malabsorption suggests problems located in pancreas, bile ducts and/or small bowel.

The presence of faecal leukocytes has a sensitivity of 70% for inflammatory diarrhoea. A test for faecal lactoferrin has a higher sensitivity but is rarely available. Continuation of diarrhoea during fasting is suggestive for a secretory process. Features that suggest an organic cause as opposed to a functional cause, include a duration less than 3 months, nocturnal diarrhoea, abrupt onset, weight loss (> 5kg for an adult), stool weight more than 400 g/24h.

Clinical assessment (degree of dehydration)

The **assessment of dehydration is most important.** Dehydration is due to an insufficient intake of liquids (drinking, IV fluid) and/or excessive loss of fluid (vomiting, diarrhoea, polyuria, sweating). If loss of gastro-intestinal fluid is the cause, the patient will urinate less (oliguria) in order to minimise the loss.

If a child has lost **< 5%** of its body weight, the general condition is still quite good. The child is alert and thirsty. The mucous membranes (eyes, tongue, mouth) are moist and the turgor of the skin (elasticity) is maintained. Breathing is normal. Urine production is normal and if the child cries there are tears. The fluid deficit is < 50 ml/kg of body weight.

If 5-10% of body weight is lost the eyes are sunken, the fontanelle is hollow, the skin is no longer elastic, the lips and mouth are dry and sometimes cracked. The child is miserable, restless and cries.
There are no tears. Breathing becomes more rapid (acidosis). This must be distinguished from an accompanying pulmonary infection. Urine production decreases. The fluid deficit is 50-100 ml/kg.With a fluid loss of **>10%** the child is quiet and cold. The pulse is rapid and difficult to feel (circulatory collapse), especially the radial pulse. Skin folds do not disappear, the mucous membranes are very dry, the abdomen is hollow, the eyes are deeply set and the fontanelle is deeply sunken. Usually there is no more urine. The fluid deficit is >100 ml/kg.

A rapid clinical dehydration evaluation can make use of the following Items: general appearance, skin, eyes, tongue and tears. A more detailed evaluation can determine the following items:

Table: Evaluation dehydration for children up to 36 months

Appearance	**Normal**	**Thirsty-restless-irritable**	**Drowsy-limp**
Capillarly refill	<1.5”	1.5-3”	> 3”
Skin turgor	Instant recoil	<2 seconds	>2 seconds
Fontanelle	Normal	Slightly sunken	Very sunken
Eyes	Normal	Slightly sunken	Very sunken
Tongue	Moist	Sticky	Dry
Tears	Present	Decreased	Dry
Breathing (< 1y)	< 40/’	40-50/’	>50/’
Breating (1-3y)	< 30/’	30-40/’	>40/’
Heart rate (<6m)	<175/’	175-185/’	>185/’
Heart rate (6-36m)	<150/’	150-165/’	>165/’
Urine specific gravity	<1.015	1.016-1.030	> 1.031

Treatment

General

Two things must always be considered:(1) the degree of dehydration/rehydration needs, (2) is **drug** treatment necessary? The most important thing with acute diarrhoea is to deal with dehydration and in the second place to correct protein and calorie deficiency. Etiological treatment will only be possible in a minority of cases, but should not be disregarded.

Children are very sensitive to dehydration. Fluid loss can occur very quickly with vomiting and diarrhoea: 500 ml of fluid in a child weighing 5 kg means a loss of 10% of body weight and implies a high risk of death.

IV rehydration is not always possible nor even desirable. An important development has been the discovery that many cases of dehydration of whatever origin can be counteracted by oral rehydration.
This is possible because despite the diarrhoea, the mechanisms for absorbing water, sodium and glucose in the intestine are maintained. The minimum ingredients for this oral rehydration solution (ORS) are clean water, glucose and salt. While this can indeed bring about rehydration or prevent dehydration, a disadvantage is that the diarrhoea itself continues. The volume of stools is not reduced.
Alternatives to glucose are ordinary sugar (sucrose; this is a glucose-fructose disaccharide) or rice powder. Rice powder is better because it reduces the volume of stools. In ideal circumstances potassium (against hypokalaemia) and bicarbonate or sodium citrate (against acidosis) can be added.
Citrate is easier to store than bicarbonate. In the future there may perhaps be better formulae which also contain neutral amino-acids (glycine and alanine) and perhaps dipeptides.

There are several formulae for ORS. The WHO has developed a standard formula in which each litre of water should contain:

KCL	1.5 gram
Trisodium citrate dihydrate	2.9 gram
NaCl	2.6 gram
Glucose	13.5 gram

Treatment, in practice

- Always weigh the child and assess its general condition.
- Assess whether the weight loss is <5%, 5-10% or >10%.
- Is it dysentery or not? If yes, is it amoebic or bacillary?

With mild to moderate dehydration use ORS. The volume that should be given is 1-2 times the fluid deficit. ORS is best given by the mother and should be given over a 4 to 6 hour period. It is best if it is given with a small cup and spoon. With very small children a syringe can be used to drip the fluid into the mouth. If the child vomits a few times the treatment should be continued nevertheless. Administration using a nasogastric drip infusion is rarely necessary. The success of the treatment should be monitored by assessing the general condition of the child and its weight.

With severe dehydration (>10%) or if the treatment with ORS is not successful, IV rehydration should be used. If it is not possible to inject into a vein and a venous cut-down is not feasible and the situation is desperate, the intraosseous route can be used: the fluid enters the bone marrow of the tibia and is taken up in this way. The infusion can be rapid at first (70 to 100 ml/kg over 3 hours). If the pulse can be felt clearly again and the child has generally

improved, the treatment can then be switched to oral therapy. Potassium chloride should be added in severe diarrhoea.

Newborn children with a low birth weight are very sensitive to hypernatremia. Rehydration is achieved best with 2/3 ORS and 1/3 extra salt-free water.

Food must continue to be given while the patient has diarrhoea. It used to be thought that a period of fasting (24 to 48 hours) was good for the child, but this is counterproductive. Breastfeeding should not be stopped. A balanced diet, low in residue and semi-solid is indicated. During episodes of diarrhoea, patients are catabolic (they break down their own muscle proteins for energy).

Medication

1. Antibiotics for bacillary dysentery.
2. Antiparasitic agents for amoebiasis, giardiasis, malaria, isosporiasis, Strongyloides, capillariasis, etc.
3. Zinc, folic acid and vitamin A supplements, especially in malnourished children.
4. Antimotility products loperamide (Imodium) or opiates: codeine, paregoric (= opium tincture) or laudanum reduce intestinal cramps and the frequency of bowel movements. They are only indicated for uncomplicated diarrhoea. They do not reduce fluid loss. Anti-diarrhoeal drugs must be avoided in children because they can aggravate dysentery and can easily be given to children in too high a dose resulting in paralytic ileus and sedation interfering with oral rehydration.
5. Sometimes the main complaints is nausea. Domperidone can be used, though its use should be restricted to severe cases, especially when combined with other QTc-proloning drugs as
6. fluoroquinolones (ciprofloxacin, levofloxacin, moxifloxacin) or(neo-)macrolides (clarithromycin, azithromycin).
7. Lactobacillus and saccharomyces boulardii concentrates are probably of little benefit but more research is needed.

Prevention

Most diarrhoea is transmitted by the faecal-oral route. The prevention of these infections will therefore depend on improved general hygiene, which is determined by the general level of poverty (standard of living).
Rotavirus disease kills approximately half a million children annually in developing countries and accounts for one third of hospitalizations for diarrhoea worldwide. In 1999, the first licensed rotavirus vaccine (RotaShield) was withdrawn from the U.S. market less than a year after its introduction because it was associated with an uncommon but potentially life-threatening adverse event, intussusception, at an estimated rate of 1 incident per 10,000 vaccine recipients. The manufacture of the first licensed rotavirus vaccine was halted. In 2005, results of large clinical trials of two new vaccines, Rotateq from Merck and Rotarix from GlaxoSmithKline, were published. These are both live oral vaccines intended to be given to infants at the same time as their immunizations for diphtheria, pertussis, and tetanus, but they differ in their approaches, strains, and formulations. Rotarix is given in 2 doses with minimum 4 weeks interval. Rotateq is given in 3 doses with minimum 4 weeks interval. Both

vaccines demonstrated an impressive efficacy profile and a reassuring safety profile, particularly with respect to intussusception.

A few general tips and precautionary measures for avoiding diarrhoea are recommended:

1. Food should be completely cooked/boiled.
2. Drinking water should be protected. This can be achieved in a village context (sand filters, protection of water-wells, etc). Water can be boiled and filtered, but boiling requires a lot of fuel, which is usually expensive.
3. Wash hands with soap.
4. Sanitary provisions: toilet and drinking water should be kept separate. Inexpensive, simple, build-it-yourself, ventilated, odour-free, fly-free latrines that do not require any water can be made (the Blair latrine for example).

Diarrhoea: prevention for travellers

Food: avoid raw vegetables, fruit you cannot peel yourself, unpasteurised dairy products, fish, shellfish and meat that is raw or not cooked through. (Cook it, boil it, peel it or leave it). Avoid food from street stalls. Food should be protected against flies.

Drink: drink tea, coffee or bottled water, preferably sparkling (less risk of having been tampered with). Beer can quench the thirst, but large quantities of alcoholic drinks are not recommended. Avoid bottles sealed with reused crown caps. Ice cubes are not to be trusted. Drinking water can be filtered. This can be done in a number of ways (large porcelain filters such as Berkefeld, active charcoal filters, portable Katadyne filters). Afterwards the water can be boiled or purified chemically with silver salts such as Micropur®, Drinkwell® (not active against viruses), Chloramine (250 mg per 10-50 litres) or sodium hypochlorite (Javel, Drinkwell chlorine®, Hadex®). An unpleasant taste of chlorine can be removed byadding the non-toxic sodium thiosulphate (Drinkwell-antichlorine® drops) work in for an hour. Lugol or 2% tincture of iodine (eight drops per litre) can also be used and is more active against amoebic cysts. Long-term use (more than 3 months) is not recommended. Thyroid disorders and pregnancy are contra-indications.

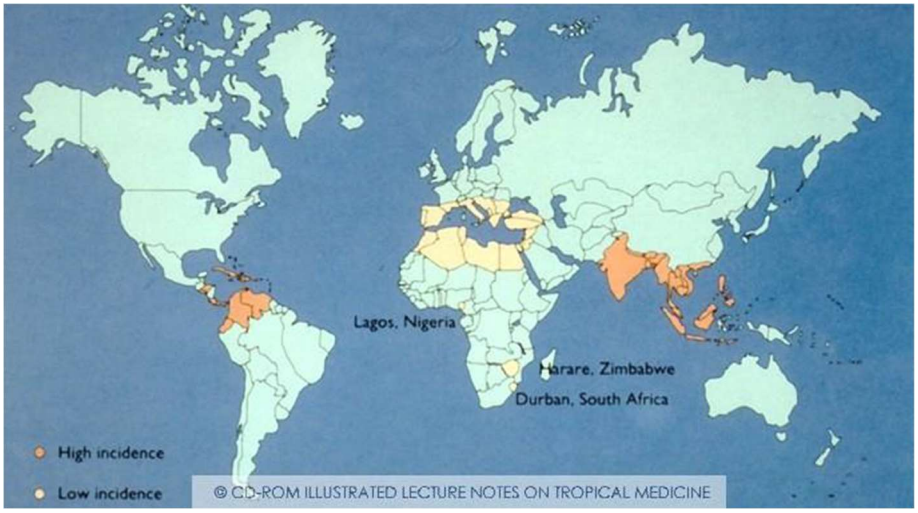
Chemoprophylaxis: This is normally not advised routinely, but does provide partial protection (e.g. ofloxacin). Only to be considered for short journeys where absolutely nothing should go wrong.

Tropical sprue

General

Tropical Sprue is largely limited to within about 30 degrees north and south of the equator. It was responsible for one-sixth of all casualties sustained by the Allied forces in India and Southeast Asia during World War II. Tropical sprue is an acquired disease of unknown origin. An infectious origin appears probable and the term "post-infectious malabsorption" is also used. Possibly there is an initial insult at the level of the jejunal-ileal enterocytes, followed by bacterial overgrowth with enterotoxic strains. The disease is characterised by abnormalities of the mucosa in the small intestine with chronic malabsorption, multiple nutritional deficiencies and anaemia. The malabsorption is generalised and affects absorption of proteins, fat, carbohydrates, minerals and vitamins (typical is iron and folate deficiency). Good response to treatment with doxycycline and iron-folate supplements is seen.

Tropical sprue occurs chiefly in the Caribbean, India, Nepal and Southeast Asia, in both the indigenous populations and immigrants. Cases have been reported from Mauritius, Fiji, southern Italy, Guyana and Central America. In Africa the disease is apparently very rare, although cases have been reported from Zimbabwe.



Map of areas with post-infective malabsorption, also known as tropical sprue. Copyright Wellcome History

Clinical aspects

Tropical sprue can have an insidious onset or can start acutely. The symptoms are those of chronic malabsorption. Generally it presents as a clinical triad of painful tongue, weight loss and persistent abdominal discomfort with diarrhoea. Patients are noticeably tired, both physically and mentally.

Amenorrhoea is very common. There is loss of weight with muscle atrophy. Hypoalbuminaemia leads to oedema. Due to malabsorption of carbohydrates there is increased gas production in the intestines, with borborygma, a bloated feeling in the abdomen and intestinal cramps. The D-xylose absorption test is abnormal in more than 90% of cases. Fat malabsorption leads to steatorrhoea with more than 10 g of fat in the faeces. This occurs in 95% of patients. The stools are pale, very odorous and quite voluminous, up to 5 times the

normal amount. Dehydration, hyponatraemia and hypokalaemia are very common. Calcium deficiency may lead to tetany with positive Trousseau's sign. Hypokalaemia leads to reduced tendon reflexes and U-waves on the electrocardiogram. There is usually a deficiency of vitamin B12, folic acid and sometimes also iron. Anaemia occurs and is typically macrocytic with megaloblastic bone marrow. In long-term cobalamine deficiency there may be peripheral neuritis and involvement of the spinal cord, chiefly of the dorsal columns (proprioception). The tongue is red and painful. As well as glossitis there may be stomatitis with superficial erosions. Deficiencies in fat-soluble vitamins (A, D, E, K) lead to prolongation of the coagulation time and osteomalacia. Vitamin A deficiency is characterised by a dry, rough skin with follicular hyperplasia and Bitot's spots on the conjunctivae. In severe deficiency night-blindness and xerophthalmia may occur.

Differential diagnosis:
Tropical sprue is a pan-enteric inflammatory process often mistaken for gluten-sensitive enteropathy.

The differential diagnosis is that of chronic malabsorption. It includes persistent giardiasis, isosporiasis, strongyloidosis, intestinal capillariasis, gluten enteropathy (coeliac disease), chronic pancreatitis, intestinal tuberculosis, intestinal amyloidosis, Whipple's disease, the blind-loop syndrome, bacterial overgrowth, diverticula and jejunocolic fistulae. Crohn's disease is rare in developing regions.

Diagnosis

Tropical sprue should be suspected in anyone with megaloblastic anaemia and malabsorption who has lived in an endemic region or has visited these regions. Biopsy of the jejunum shows typical abnormalities. Intestinal villi become shorter and broader (blunting without flattening as in gluten enteropathy). In the intestinal wall there is an inflammatory infiltrate, chiefly consisting of lymphocytes, plasma cells and a few eosinophils. The enterocytes exhibit large vacuoles. Radiography of the small intestine shows non-specific changes. There is flocculation of the contrast material and segmentation of the barium column, distension of the lumen and thickening of the mucosa. The mucosal folds in the small intestine are irregular and thickened, which gives the impression of a stack of coins. In advanced cases, no mucosal folds at all can be seen. A flat mucosa is very unusual and should lead to suspicion of a different disease (e.g. gluten enteropathy).

Treatment

Treatment is based on tetracyclines 250 mg QDS or doxycycline 100 mg daily for 3 to 6 months. Folic acid supplements (5 to 10 mg daily) and multivitamins and if necessary iron should be added to the treatment. Response to treatment is generally swift with an initial improvement within three days. Further recovery takes place in the course of the following three months.

Diphtheria

Summary
• Caused by the gram-positive bacillus Corynebacterium diphtheriae
• Infection leads to respiratory or cutaneous disease or an asymptomatic carrier state
• The pseudomembranes in combination with neck swelling can cause life threatening croup
• Diagnosis in most low-resource settings is clinical
• Treatment with erythromycin or penicillin
• Antitoxin and airway protection in severe cases
• Worldwide vaccination lead to a significant decrease in diphtheria cases

General

Diphtheria is an infectious diseases caused by the gram-positive bacillus Corynebacterium diphtheriae. Symptoms range from mild to severe. Whereas in the 1980s about 100,000 cases were reported worldwide, in 2015 this number had dropped to 4,500 cases with >80 percent vaccination rates worldwide. Regions mostly affected are sub-Saharan Africa, the Indian subcontinent and Indonesia where mostly children are affected. In 2015, 2,100 deaths were reported, down from 8,000 in 1990. The disease has become rare in high-income countries thanks to widespread vaccination but reemergence is a threat when vaccination rates decrease. Diphtheria death rate in those diagnosed varies from 5% to 10%.

There are four types of *C. diphtheria: gravis, intermedius, mitis* and *belfanti*. All four can cause Diphtheria, although mitis strains cause less severe disease. Symptoms are caused by bacterium's toxin. In rare occasions toxigenic strains of other *Corynebacterium* species (*C. ulcerans, C. haemolyticum, C. pseudotuberculosis*) evoke respiratory symptoms.

The name comes from the Greek word "diphthera" which means "leather" referring to the appearance of the pseudomembrane in the throat.

History of Diphtheria

The disease was first described in the 5th century BC by Hippocrates In 1613, Spain experienced an epidemic of diphtheria. The year is known as El Año de los Garrotillos (The Year of Strangulations) in the history of Spain.
Before 1826, diphtheria was known by different names across the world. In England, it was known as Boulogne sore throat, as it spread from France. In 1826, Pierre Bretonneau gave the disease the name diphthérite (from Greek diphthera "leather") describing the appearance of pseudomembrane in the throat.

In 1878, Queen Victoria's daughter Princess Alice and her family became infected with diphtheria, causing two deaths, Princess Marie of Hesse and by Rhine and Princess Alice herself.
In 1883, Edwin Klebs identified the bacterium causing diphtheria and named it Klebs-Loeffler bacterium. The club shape of this bacterium helped Edwin to differentiate it from other bacteria. Over the period of time, it was called *Microsporon diphtheriticum*, Bacillus

diphtheriae, and Mycobacterium diphtheriae. Current nomenclature is Corynebacterium diphtheriae.

Friedrich Loeffler was the first person to cultivate C. diphtheriae in 1884. He used Koch's postulates to prove association between C. diphtheriae and diphtheria. He also showed that the bacillus produces an exotoxin.

Joseph P. O'Dwyer introduced the O'Dwyer tube for laryngeal intubation in patients with an obstructed larynx in 1885. It soon replaced tracheostomy as the emergency diphtheric intubation method.

In 1888, Emile Roux and Alexandre Yersin showed that a substance produced by C. diphtheriae caused symptoms of diphtheria in animals.

In 1890, Shibasaburo Kitasato and Emil von Behring immunized guinea pigs with heat-treated diphtheria toxin. They also immunized goats and horses in the same way and showed that an "antitoxin" made from serum of immunized animals could cure the disease in non-immunized animals. Behring used this antitoxin (now known to consist of antibodies that neutralize the toxin produced by C. diphtheriae) for human trials in 1891, but they were unsuccessful. Successful treatment of human patients with horse-derived antitoxin began in 1894, after production and quantification of antitoxin had been optimized. Von Behring won the first Nobel Prize in medicine in 1901 for his work on diphtheria.

In 1895, H. K. Mulford Company of Philadelphia started production and testing of diphtheria antitoxin in the United States Park and Biggs described the method for producing serum from horses for use in diphtheria treatment.

In 1897, Paul Ehrlich developed a standardized unit of measure for diphtheria antitoxin. This was the first ever standardization of a biological product, and played an important role in future developmental work on sera and vaccines.

In 1901, 10 of 11 inoculated St. Louis children died from contaminated diphtheria antitoxin. The horse from which the antitoxin was derived died of tetanus. This incident, coupled with a tetanus outbreak in Camden, New Jersey, played an important part in initiating federal regulation of biologic products.

In the 1920s, each year an estimated 100,000 to 200,000 diphtheria cases and 13,000 to 15,000 deaths occurred in the United States. Children represented a large majority of these cases and fatalities. One of the most infamous outbreaks of diphtheria was in Nome, Alaska; the "Great Race of Mercy" to deliver diphtheria antitoxin is now celebrated by the Iditarod Trail Sled Dog Race.

In 1926, Alexander Thomas Glenny increased the effectiveness of diphtheria toxoid (a modified version of the toxin used for vaccination) by treating it with aluminium salts. Vaccination with toxoid was not widely used until the early 1930s.

In 1943, diphtheria outbreaks accompanied war and disruption in Europe. The 1 million cases in Europe resulted in 50,000 deaths.

In 1974, the World Health Organization included DPT vaccine in their Expanded Programme on Immunization for developing countries About a million cases a year are believed to have occurred before the 1980s.

Transmission

Diphtheria is airborne and spreads between people by coughing and sneezing. In rare occasions, direct contact with diphtheria skin lesions can transmit the bacteria. Indirect transmission is possible when an infected person touches an object on which the bacteria can remain viable. Asymptomatic carriers exist and they can still spread the infection to others. Immunity from past infection or vaccination does not prevent carriage of the bacterium.

Diptheria toxin

C. diphtheria produces and exotoxin when it is infected with a bacteriophage that integrates the toxin-encoding gene (tox+) into the bacteria. Diphtheria toxin is composed of two peptide chains: fragment A and fragment B. Fragment B facilitates toxin entry into host cells by binding the heparin-binding EGF-like growth factor on the cell membrane. Once inside the cell's endosome, a trypsin-like protease splits the toxin in the A and B fragments. The low pH in the endosome causes fragment B to create pores in the endosome membrane through which fragment A can enters the cytoplasm. Fragment A catalyses ADP-ribosylation of elongation factor EF-2, a protein that moves tRNA from the A-site to the P-site of the ribosome during the translation step in protein synthesis. The final result is a disturbed protein synthesis leading to cell death.

Clinical aspects

The incubation period is usually two to five days and the disease starts with a gradual onset of sore throat with pharyngeal erythema and fever. In more severe cases diphtheria destroys the respiratory tract tissues with dead tissues forming a thick, grey, friable and tightly adhering coating in the throat. This is called a pseudomembrane which is composed of necrotic fibrin, white- and red blood cells, epithelial cells and bacteria. The pseudomembrane may expand from the nose to the tonsils, the throat up to the bronchial tree. This can lead to dysphagia and can obstruct the airways provoking hoarseness, stridor and sometimes suffocation when membranes are aspirated. This can be exacerbated with extreme neck swelling ("bull neck") due to enlarged lymph nodes causing external pressure on the airways. This clinical picture is referred to as "diphtheritic croup" or "true croup" (= laryngotracheobronchitis caused by diphtheria). Children who have smaller airways are more vulnerable to the complications of diphtheritic croup. Nowadays, croup is mostly related to viral infections causing milder respiratory symptoms.

Diphtheria can be complicated by myocarditis (in two-thirds of severe cases) and nerve inflammation (in up to 75 percent of severe diphtheria). Paralysis of the soft palate and posterior pharyngeal wall can occur, as well as cranial nerve paralysis. Cardiac and neurological symptoms often arise from the moment respiratory symptoms are improving. A peripheral polyneuropathy can develop weeks or months after the acute illness.

Cutaneous diphtheria presents as chronic, non-healing ulcers with a grey membrane. The infection is often preceded by local trauma. Epidemics of cutaneous diphtheria have occurred in populations living in poor hygienic conditions. The ulcers can serve as a reservoir from which the infection spreads to others.

Diagnosis

Diagnosis is often made considering the setting and clinical manifestations in a non-vaccinated person. Positive cultures confirm the diagnosis, but the need for special culture media (Loffler's or Tindale's media), the need for appropriate transport media and the necessity of quick inoculation, make the confirmation challenging, even in high-resource settings. Toxin detection with PCR is possible and confirms that the strain is toxicogenic.

A probable case is a clinically compatible case that is not laboratory-confirmed nor epidemiologically linked to a confirmed case. A confirmed case is a clinically compatible case that is laboratory confirmed or epidemiologically linked to a laboratory-confirmed case. It is important that the antitoxin and antibiotics are administered prior to confirmation when diphtheritic croup is suspected. Cases of diphtheria should be reported to the World Health Organization (WHO).

Differential diagnosis

Several diseases can give a clinical picture that can resemble pharyngitis with pseudomembranes: infectious mononucleosis, group A streptococcal tonsillopharyngitis, epiglottitis, viral pharyngitis, Vincent's angina (= acute necrotizing ulcerative gingivitis), oral candidiasis, pertussis (100-day cough).

Treatment

When diphtheria is suspected, prompt initiation of antibiotics is needed since severe untreated diphtheria has a mortality rate of 40% to 50%. Erythromycin (500 mg 4 times daily, 14 days) and penicillin G (300,000 IU IM daily for patients < 10 kg and 600,000 IM IU daily for patients > 10 kg) followed by penicillin V (250 mg 4 times daily, oral) for a total of 14 days are the antibiotics of choice. In severe diphtheria with pseudomembranes or cardiac involvement, diphtheria antitoxin is indicated.

These antibodies are produced in horses that have been challenged with diphtheria toxin. The antitoxin does not neutralize toxin that is already bound to tissues, hence a delay in administration increases mortality rates. In about 10 percent of patients receiving antitoxin hypersensitivity or serum sickness arises.

In case of (threatening) respiratory failure airway protection with intubation is necessary. This procedure can be difficult if there is extensive throat oedema and mucosal friability. There is a risk of dislodging the pseudomembranes into the bronchi. In rare occasions a tracheotomy is needed. After recovery, vaccination is still needed since pharyngeal infections do not protect against future infections. Skin infections are an exception since they evoke a strong antibody response.

Prevention

An effective vaccine exists with different available formulations. In childhood, three or four doses are given along with tetanus and pertussis in a penta-, sexta- or heptavalent vaccine. A booster vaccination, together with tetanus, is recommended every ten years.

Close contacts can be given prophylaxis with a single dose of penicillin G benzathine (1.200.000 IU IM) or oral erythromycin 500 mg 4 times daily for 1 week.

Tetanus

Summary
- Tetanus: symptoms caused by a powerful toxin from anaerobic bacteria
- Pathogenic organism present in wounds, umbilical stump infections
- Prevention by vaccination including pregnant women
- Clinical diagnosis
- Painful muscle spasms (spontaneous and after provocation), normal consciousness
- Treatment: wound care, antitoxin, anti-spasmodics, clear airways, supportive measures
- Avoid or treat complications

General

Tetanus is a disease caused by the toxin produced by an anaerobic bacterium: *Clostridium tetani*. This disease is completely preventable by vaccination therefore it is particularly tragic that is still occurs.
The disease cannot be transmitted from human-to-human. *Clostridium tetani* is a strictly anaerobic Gram-positive rod-shaped bacterium, in cultures or in tissue it can be Gram-variable. It forms a characteristic spore at one end (exclamation mark, tennis racket). These spores are very resistant: they resist boiling, short autoclaving, alcohol and phenol. They are destroyed by autoclaving at 121°C for at least 12 minutes (better 15'). The bacterium occurs widely in nature for example in the soil and in the intestinal tract (especially of cattle and horses). Approximately 10% of people have C. tetani in their colon.

Neurotoxin

If the organism infects a wound where the oxygen concentration is low (interrupted vascularization, foreign body, tissue necrosis, umbilical stump) the bacterium can multiply. The bacterium itself is not invasive. The pathogenic organism produces a neurotoxin-tetanospasmin. This is released when the organism lyses. This protein is responsible for all the clinical manifestations of tetanus. The toxin is cleaved outside the cell by a bacterial protease into a heavy and a light chain. The toxin enters the neuromuscular junction. Once internalized, it migrates via the fast retrograde axonal transport pathway of the peripheral nerves towards the nerve soma located in the spinal cord. Another pathway which is hypothesised is via the lymphatics and the blood to the central nervous system. The neurotoxin inside the motor neurons translocates (crosses the synapse) to inhibitory interneurons.

There the toxin cleaves the protein synaptobrevin which is present on the presynaptic vesicles which contain the inhibiting neurotransmitters GABA and glycine. Due to the removal of synaptobrevin on the exterior of the vesicles the latter can no longer fuse with the synaptic membrane. Therefore the reflex arc cannot be inhibited. The consequence of the removal of the normal inhibition of the motor neurones is increased muscle tone at rest and tonic spasms. The toxin is also active on the sympathetic nervous system. The role of a second toxin-tetanolysin is still unclear.

GABA (gamma- Aminobutyric Acid)
Throughout the central nervous system, GABA is an inhibitory neurotransmitter. GABA receptors open channels for negatively charged chloride ions, hyperpolarizing the neuronal membrane and making it less likely that action potentials can be generated in output neurons.

Tetanospasmin is one of the most powerful toxins known to man (botulinum toxin is the undisputed leader). The toxin is present in the body at such low doses that it does not trigger an immunological response. **Tetanus can therefore be contracted more than once.** That is one reason why people with clinical tetanus should still be vaccinated.

Most cases of tetanus occur after wounds (lacerations, bites, burns, pricks, IM injections, umbilical infections in neonates, infected abortions, a sand flea burrowing under a toenail, infected Guinea worm). Sometimes the focus is a middle-ear infection (otitis media with perforated ear drum). In 20 to 30% of tetanus patients no entry point or wound can be found.

Clinical aspects

Neonatal tetanus with opisthotonus. Photo Cochabamba, Bolivia

Tetanus, adult man. Notice the slight opisthotonus. Copyright ITM

The incubation period varies: the shorter it is the more serious the infection. Neonates who contract tetanus before they are 7 days old almost never survive. The incubation period varies between a few days and a few weeks. Three clinical forms can be distinguished:

Localized tetanus: rigidity and painful spasms in a group of muscles in the area of the wound, without general involvement. This form is rare. It is sometimes prolonged for months. The mortality rate is < 1%.

Generalized tetanus, including neonatal tetanus: first there is a short period of restlessness, irritability, dysphagia and sweating. Trismus frequently occurs (spasms of the masseter = jaw muscle). Patients are no longer able to open their mouths wide. Another name for tetanus is "lockjaw", which refers to the trismus. If the spasms spread to the other muscles of the face a spastic grimace sets in: risus sardonicus ("bitter laugh"). The disease typically descends; after the jaws and the face to follow the neck, back, abdomen and finally the extremities. Back muscle spasms lead to arching backwards (opisthotonus). Successive attacks of opisthotonus are characteristic. The spasms are very painful and last a few seconds to a few minutes. They can occur spontaneously or are elicited by all kinds of stimuli (sudden noises, touching, sudden bright light). Because the latter is a well-known phenomenon, the patients are sometimes placed in a dark room. This sometimes leads to insufficient nursing care with
serious consequences. The body temperature, heart rate and blood pressure are variable because the autonomic nervous system is also affected. In most cases there is rather low to moderate fever but hyperpyrexic periods do occur.

Cephalic form: Occasionally a true cephalic form occurs, with symptoms affecting the head, throat and neck; while sparing the rest of the body.

Differential diagnosis:
Generalized tetanus
Bacterial meningitis and subarachnoidal haemorrhage: lumbar puncture

Epilepsy: no muscle rigidity between spasms, history of previous episodes

Extrapyramidal reactions and dystonias while on neuroleptics, such as phenothiazines e.g. chlorpromazine (Largactil®) or metoclopramide (Primperan®).

Cerebral malaria: thick film test, no muscle rigidity between convulsions

Acute strychnine poisoning resembles tetanus very closely, and an old proposed name for strychnine was "tetanine". This bitter colourless alkaloid is obtained from the ripe seeds of *Strychnos nuxvomica* and related plants, such as Saint Ignatius beans (Strychnos ignatia) and snake wood (*Strychnos colubrina*). The plant seeds are sometimes used in traditional medicine (e.g. in Cambodia). It is a competitive antagonist of glycine, an inhibitory neurotransmitter. There are face spasms followed by hyperreflexia in the legs and arms. This is followed, a little later by painful generalised convulsions, triggered by sudden sounds or stimuli. The patient may be conscious. Finally breathing difficulties and coma follow. Upon death, rigor mortis sets in very quickly. If the patient survives recovery is fairly quick unlike tetanus.

Hypocalcaemic tetany after accidental parathyroidectomy or in primary hypoparathyroidia is rare. The parathyroid glands secrete parathyroid hormone which increases the concentration of calcium in the blood. If there is a shortage of parathyroid hormone, the calcium levels in

the blood fall and convulsions may occur. There may be spasms of the hands and feet as well as tingling around the mouth. Trismus is rare. Chvostek's and Trousseau's sign may be present.

Rabies: hydrophobia, periods of confusion, brain stem symptoms and cranial nerves being affected.

Trismus
•Dental abscess, peritonsillar abscess

• Pharyngeal diphtheria

•Fracture of the mandible

•Mumps

Diagnosis

The diagnosis is purely clinical. Repeated tonic spasms with muscle rigidity between the convulsions are typical. Spasms can be triggered by sudden stimuli: e.g. clapping the hands. The patient is fully conscious. *Clostridium tetani* can be found in wounds in less than 30% of cases, but a microbiological diagnosis via culture is less important than making a clinical diagnosis. The cerebrospinal fluid is normal.

Treatment

Tetanus is a disease which can drag on for weeks. There is high mortality. Treatment consists mainly of neutralising toxin and preventing convulsions and complications. Thorough cleansing of the wound and good nursing care are the most important factors in determining whether the patient survives or not.

1. The pathogenic organism, *Clostridium tetani*, has to be eradicated: by wound cleansing (hydrogen peroxide, povidone iodine [Iso-Betadine®], debridement) and penicillin G preferably IV, e.g. 1 to 12 million units per day. However, it is possible that penicillin G may act on GABA transmission and exacerbate the toxin's effect. Therefore the use of penicillin is controversial. Metronidazole is sometimes recommended instead.

2. The toxin which is still circulating must be neutralised with antitoxin. Human hyperimmunoglobulin is best: one single IM injection of 3000 to 6000 IU in two different sites (or 10,000 to 50,000 IU hyperimmune horse serum). Sometimes lower quantities are recommended. Human antiserum has a half-life of 25-28 days therefore it must not be given repeatedly. The half-life of horse antiserum is somewhat less than 2 weeks. Toxin which has already bound to nerve cells, cannot be removed and is responsible for the repeated spasms. Some guidelines use tetanus immunoglobulins intrathecally.

3. The infection does not produce any immunity so that the patient must also be vaccinated. The vaccine must not be mixed with gammaglobulins and must be injected at another site.

4. Prevention of muscle spasms is important because the spasms are very painful, and they interfere with breathing. They can lead to gastric reflux with aspiration pneumonia. The repeated violent convulsions can even result in patients breaking their own bones. Diazepam (Valium®) is better than barbiturates and is often used as the drug of first choice. Sometimes very large quantities have to be given (50 to 500 mg/day). Respiratory depression can occur.

Midazolam (Dormicum®) is an alternative. In the case of depression of the central nervous system, flumazenil (Anexate®) can be used as an antidote. Dantrolene (Dantrium®) can be used but it is very expensive. Chlorpromazine (Largactil®) is also useful. Baclofen (Lioresal®) is a GABA B receptor agonist that inhibits pre-synaptic acetylcholine release and synaptic medullar reflexes (i.e., lowers excitability of motor neurons), which results in an antispastic action. It is rarely available in low resource setting. If possible, baclofen can also be administered intrathecally.

5. Trismus, dysphagia, laryngeal spasms, respiratory muscle spasms, gastric reflux and sedatives can lead to pulmonary complications. Aspiration of secretions to clear the airway is necessary. Oxygen will often be given. Sometimes tracheostomy (severe laryngeal spasms) is performed. The indications for tracheostomy are acute airway obstruction due to laryngeal spasms that interfere with respiration, or to facilitate mechanical ventilation. If the means are available, curare (muscle relaxant, e.g. pancuronium = Pavulon®, vecuronium) and mechanical ventilation can be used.

6. The use of magnesium sulfate infusions in the management of tetanus enables one to minimize sedation and reduce the need for mechanical ventilation, and thereby greatly simplifying the care of the tetanus patient. Magnesium is also able to minimize sympathetic overactivity associated with tetanus. Furthermore, magnesium sulfate is already a well-known entity due to its extensive use in the management of pregnancy induced hypertension. As a guide line for an adult, a loading dose of 5 gram is given, followed by 2-3 gram per hour afterwards.

7. The patient must be regularly turned to prevent pressure sores. The risk of pulmonary embolism decreases with subcutaneous heparinisation. Low-molecular heparin can be given prophylactically, but this is often not available in the tropics. Feeding is performed mainly via a thin flexible nasogastric tube (the patient cannot eat for weeks), his is sometimes overlooked. Urinary catheterisation is necessary to prevent urine retention.

8. Septicaemia occurs frequently in neonatal tetanus (umbilical stump as the point of entry) and must not be ignored. In third world countries, it is not unusual for the umbilical stump to be covered with various contaminated herbs, animal droppings or fats.

9. Beta-blockers such as labetalol can be administered in cases of excessive sympathetic tone. In the case of hypotension, IV fluid and vasopressors should be administered if available.

Example of "Adult tetanus protocol"
1. Start metronidazole intravenously 500mg three times a day.

2. Give tetanus human immune globulin IM 3,000-6,000 iu if available. If not available Equine ATS 10,000 iu IM.

3. Admit ICU, commence oxygen, IV access and monitoring.

4. Alert surgeon to do radical debridement. Nasogastric tube may be passed during surgery.

5. Slow loading dose diazepam IV to control spasms. Up to about 40mg may be required. Give a loading dose of 5g magnesium sulphate slowly over 20 minutes IV.

6. Start diazepam 10mg 6 hourly and increase to hourly if required. Titrate to symptoms.

7. Start magnesium 2.5g IV 2 hourly and increase to hourly if required. Titrate to symptoms. Stop diazepam if symptoms controlled by magnesium alone.

8. Phenobarbitone up to 200mg IV twice a day for breakthrough spasms using 50mg doses.

9. Tracheostomy if airway compromised by above treatment.

10. Intermittent positive pressure ventilation with muscle relaxants if respiration compromised by treatment or uncontrolled spasms.

Prognosis

Incubation period < 7 days:
1. The course of the disease is always very serious.
2. The interval between the first symptoms and generalized spasms is 3 days or less.
3. Mortality rate > 80 %

Incubation 7 to 10 days:
1. Moderately severe course with the symptoms developing over 3 to 6 days.
2. The mortality rate varies from centre to centre.

Incubation > 10 days:
1. Milder course with the usual symptoms setting in slowly.
2. Generalized convulsions are sometimes absent.
3. If a baby survives neonatal tetanus there is an increased risk of permanent brain damage, with behavioural and developmental problems as well as difficulties with fine motor movements.

Prevention

In the case of a wound which is likely infected with C. tetani, prior to symptoms development; in addition to wound care and tetanus vaccination, human hyperimmunoglobulins are given intramuscularly, i.e. 250 to 500 IU once only. Hyperimmune horse serum can be used but this sometimes leads to anaphylactic reactions and serum sickness. Tetanus toxoid (toxin inactivated by formalin) is used for vaccination. The vaccine is administered intramuscularly on 3 occasions with a minimum interval of one month between each injection. There is a

booster after 1 year and then every 10 years (or after 5 years if injured). It is best if children are vaccinated at the age of 2, 4, 6 and 15 months of age. This series is completed with a dose between 4 and 6 years. Additional boosters are given every 10 years after that. A serum antitoxin concentration of 0.01 IU/ml is regarded as protecting against tetanus. This determination can only be carried out in a few laboratories.

The antibodies (particularly subclass IgG1) cross the placenta from mother to child and protect the neonate from neonatal tetanus. These antibodies gradually disappear from the child's blood over the following months. Vaccination of the mother is therefore part of the prenatal consultation. The vaccine is very efficient and very safe. It is part of the EPI (extended programme of immunization) of the WHO.

Leprosy

Summary
•Chronic infection with Mycobacterium leprae
•Bacteria multiply in the macrophages and Schwann cells of peripheral nerves
•Clinical spectrum: from tuberculoid (paucibacillary) to lepromatous (multibacillary)
•Thickened nerves with neuritis: trophic, motor and sensory disturbances
•Neuropathy leads to paralysis, trophic ulcers, blindness, mutilations
•No central nervous system lesions
•Skin: numb white area with elevated edge (tuberculoid) to diffuse infiltration with nodules (lepromatous).
•In lepromatous leprosy also involvement of deeper tissues (testes, tongue, eyes, etc.)
•Diagnosis: clinical, modified Ziehl staining of smears (skin lesion, nose, earlobe)
•Treatment of leprosy with dapsone, rifampicin and clofazimine
•Leprosy reactions: type 1 (change in immunologic defense) and type 2 (immune complex)
•IRIS reaction possible in HIV patients within 4 months of starting HAART

General

Hansen's disease, or leprosy, was previously present in most parts of the world. Now, it is a problem in regions of extreme poverty. The number of registered cases is falling: 5.37 million in 1985, 3.1 million in 1992, 1.8 million in 2000, 249,007 in 2008, and 174,087 new cases in 2022, according to WHO. At the end of 2022, the prevalence was estimated at 154,459 cases, and more than half came from India. There are probably as many patients who have not yet been diagnosed. The number of severe infections (with disability) is decreasing, reflecting earlier detection. It is hoped that the general incidence of the infection will be below 1/10,000 soon. A prevalence of less than 1/10,000 is regarded as the goal for eliminating leprosy as a public health problem, this is not the same as eradicating the disease. By 1999, 80% of all leprosy cases occurred in 6 countries: India, Brazil, Bangladesh, Indonesia, Myanmar and Nigeria. HIV-infected patients usually die of infections caused by faster-growing bacteria (e.g., tuberculosis) and not from the slow-growing *Mycobacterium leprae*. The AIDS epidemic has, therefore, had little effect on the incidence of leprosy, but immune reconstitution after starting HAART can lead to florid lesions in a patient who has subclinical asymptomatic leprosy. The illness is characterized by skin and nerve lesions, which, together with progressive tissue destruction, causes mutilation.
Resistance to dapsone became a significant problem around 1980. Thus, combination therapy has been used since that time.

Historical note

Due to the mutilations which can occur in leprosy, since ancient time there has been a lot of prejudice and stigmatization. Sufferers were usually banished from the community. Apart from the physical handicap, the emotional, economic and social consequences were often very severe. The hypothesis was that leprosy was a hereditary disease and/or a punishment from God. One argument in favour of hereditary transmission or rather against the hypothesis of leprosy being an infectious disease was the result of transmission experiments, in which Dr Daniel Danielssen in Bergen, Norway, injected himself and four helpers with material obtained from skin nodules from leprosy patients, without further

consequences. The pathogen of this chronic disease was discovered in 1873 by the Norwegian Gerhard Henrick Armauer Hansen. At that time there were several thousand leprosy sufferers in Norway. Following the example of John Snow (see Cholera) he followed the course of each illness over time. Families of which the members lived physically close together had a higher incidence of the disease, compared to families of which the members lived apart. In this way he came to the idea that this could be an infectious disease. In 1871-72 he observed small vague intracellular rods in skin nodules. This information was published in 1873. A staining method was discovered in 1880 by the German Albert Neisser. The pathogen proved to be a bacterium: Mycobacterium leprae. It was the first time that a bacterium had been considered responsible for causing a human disease. Regrettably, Dr Hansen carried out an unethical experiment, in which he introduced material from a leprosy nodule into the cornea of another person in an attempt to prove its infectious nature. He was suspended from practicing for life by the courts.

In 1873 Jozef de Veuster, better known as Father Damian arrived on Molokai in the Hawaiian archipelago. There he found 800 leprosy patients who were living in miserable conditions. He decided to stay and to devote the rest of his life to improving the fate of his fellow human beings. In 1876 he developed lesions on his arms and back (an illustration of the long incubation period). In 1881 he developed nerve pain and in 1883 his left foot lost all sensation. He died on 15th April 1889.

Mycobacterium leprae

Mycobacterium leprae is an obligate intracellular, slow-growing acid-fast bacillus (0,5 x 3-8 µm). On Gram-staining, it is Gram-variable. The *Mycobacterium leprae* genome project sequenced the entire genome in 2001. The genome is relatively small (3,27 Mbp) and contains about 1600 genes and more than 1100 pseudogenes. In comparison, *Mycobacterium tuberculosis* contains about 4000 genes. This seems to imply massive gene decay in the leprosy bacillus and the absence of critical enzymatic pathways, thereby requiring host parasitism for survival.

Biological information

Do not confuse Mycobacterium leprae with Mycobacterium lepraemurium, a natural pathogen of rats and mice. The disease caused by Mycobacterium lepraemurium is sometimes used as a model for human leprosy. In 2008, Mycobacterium lepromatosis was identified (analysis of 16s rRNA gene) as a related but distinct mycobacterium which might be responsible for diffuse cutaneous leprosy and Lucio's phenomenon in humans. Additional research still has to identify the place in the overall pathology of the disease.

Phenolic glycolipid-1 (PGL-1) is a glycolipid in the capsule of Mycobacterium leprae. PGL-1 contains an antigenically distinct trisaccharide unit that is not found in any other bacteria. PGL-1 makes up to 2% of the total bacteria mass, suggesting that the function of the sugar chains may be related to functions unique to Mycobacterium leprae. PGL-1 binds to laminin-2, which facilitates PGL-1 binding to the basal lamina of axons on Schwann cells and the resulting invasion of the cells. This might explain the neurotropism of these bacteria. Because this invasion can occur even when the bacteria are dead, the invasion seems not to be driven by the bacteria, but by passive interaction between glycolipids in the capsule

of the cell wall and molecules in the basal lamina of Schwann cells. If this binding can be blocked, a new therapeutic avenue may become possible. However, laminin-2 is also present in the basement membrane of other tissues. The basement membrane in muscle is composed of laminin, type IV collagen, entactin/nidogen and heparan sulphate proteoglycan. One major component of the basement membrane in muscle is laminin-2, which is composed of a heavy chain laminin a2 and two light chains, b1 and laminin g1. Other factors must also play a role in the fact that Mycobacterium leprae has a predilection for neural tissue.

It has long been suspected that leprosy has a strong genetic component. A leprosy susceptibility locus on the long arm of chromosome 6 (region q25-q26) was discovered in 2003/4. This DNA stretch included the Parkinson's disease gene PARK2 and the co-regulated gene PACRG. The PARK2 gene is expressed by human Schwann cells and macrophages, which are the primary host cells of Mycobacterium leprae.

Mycobacterial culture

It has not been possible to date to culture the bacterium in vitro, which has made research extremely difficult. This can be circumvented to some extent by making use of animal experiments. However, the bacterium multiplies very slowly (generation time 12 days). It was assumed that the bacterium had a preference for cooler parts of the body. In 1960 the American Charles Shepard (CDC) discovered that it is possible to culture the bacterium in the footpads of mice (average 30°C). In this way it was possible to obtain 106 bacteria from each footpad. More severe infection could be obtained by using immune deficient mice (e.g. athymic nude mice). It became possible to test the efficacy of drugs against the mycobacterium.
In 1971 Waldeman Kirchheimer and Eleanor Storrs discovered that the nine-banded armadillo, Dasypus novemcinctus, could also become infected. This species was selected because it has a low body temperature (approximately 34°C) and a primitive immune system. The animal develops a generalized infection with involvement of the internal organs, especially the liver and spleen. After intravenous inoculation, between 1010 and 1012 mycobacteria per gram of tissue can be obtained (chiefly from the liver and spleen). In this way it became possible for researchers to analyse large amounts of proteins and DNA, which accelerated research. Latest data suggest that in South America armadillos might function as a natural reservoir for this infection but more study is required, clarification of this would be very important regarding the possibility of eventual eradication of the disease.

Transmission

Humans form the reservoir. Infection with M. leprae is possible in chimpanzees, Rhesus monkeys, mangabey monkeys and wild armadillos, but the epidemiological importance of this is unknown and is probably very small. Further research is required to understand its significance. In 2011 genetic analysis showed that in some cases in the Southern USA, leprosy might be acquired from infected armadillos. Leprosy, in such cases, can be considered as a zoonosis.

The transmission route (or routes) is (are) not known with certainty. There is probable transmission via nasal secretions from humans with multibacillary leprosy. Unlike chronic skin wounds, the affected nasal mucosa in these patients contains large quantities of bacilli. Mycobacterium leprae is identified in the oral mucosa of paucibacillary and multibacillary leprosy patients. Speaking, coughing and sneezing produce aerosols (droplet clouds). The most crucial port of entry is probably the lungs: the bacteria are breathed in. Direct contact is probably of much lesser importance. Long-term close contact with leprosy sufferers increases the risk of infection. Nevertheless, most cases occur without known contact with leprosy patients. The risk of the disease for leprosy health workers is minimal. Leprosy is possibly a highly infectious disease with low disease expression. Most people exhibit no symptoms after infection while others have brief cutaneous lesions. The susceptible individuals are in the minority: fewer than 10 % of infected people become ill. In hyperendemic regions, the proportion of people with symptoms is not more than 4 %, and the ratio is usually smaller (1/1000). Transplacental infection in untreated multibacillary pregnant patients has been described but is rare (in approximately 1% of the children in this situation).

Leprosy epidemic?

Leprosy epidemics do not occur, although a single unusual exception has been recorded. In 1912 a woman with leprosy arrived in the Oceanic island state of Nauru. This had presumably never happened before. In 1920 the first secondary case was diagnosed in the indigenous population. In 1924 there were 284 cases in a population of 1250 people. In 1929 there were
438 cases (34% of the population), after which the incidence decreased. More than 90% of the lesions were tuberculoid and deformities were rare. The extent to which genetic inbreeding within an immunologically naive population was important in this case, has not been investigated. (Compare with the ravages caused by measles in isolated island dwellers when they were first contacted by Western seafarers; see also the results of smallpox in the Aztec kingdom after the arrival of Cortez in the 16th century).

Physiopathology

It is assumed that after inhaling the bacterium multiplies within macrophages and Schwann cells (myelin-producing cells around peripheral nerves) and spreads very slowly. This occurs chiefly at the relatively cooler superficial body parts: the skin, superficial nerves, eyes, nasal mucosa and testicles. Mycobacterium leprae seems to be a thermophobic germ. Very rarely (in lepromatous patients), the bacteria spread to the deeper tissues (lymph nodes, muscles, bone, and even kidneys). The central nervous system is never affected. Nystagmus, ataxia or the presence of Babinski's sign cannot be attributed to leprosy. The human body defends itself against this mycobacterium employing specific defense cells (lymphocytes). Few bacteria and a strong Th1 immunity response characterizes tuberculoid leprosy. Patients with lepromatous leprosy have lesions with many bacteria and a strong Th2 immunity (with reciprocal repression of the Th1 response). If the Th1 reaction is strong, there are few bacteria and well-defined granuloma. If minimal, the bacteria can multiply virtually unhindered and granuloma formation diminishes. The reason why a person develops a Th1 or Th2 response to M. leprae is not yet clear. In lepromatous cases, the high bacillary load leads to an abundance of mycobacterial antigen, leading to immune complexes bound to antibodies. These circulating immune complexes bind complement to opsonize them and facilitate phagocyte uptake.

Leprosy classification

The symptoms vary greatly. This has led to considerable confusion in the past. A fundamental breakthrough was achieved by Ridley and Jopling (1962, 1964). They concluded that the patient's cellular defences determine the clinical expression. They proposed a classification for the disease with tuberculoid leprosy at one extreme and lepromatous leprosy at the other and a spectrum of borderline (B) forms in between:

TT <--> BT <--> BB <--> BL <--> LL

In practice, this classification is complex and requires high expertise and experience. Even so, consensus is difficult to reach in a single patient. Some classification schemes include polar forms (TTp and LLp). The WHO promoted a simpler, more straightforward pragmatic division into paucibacillary and multibacillary forms for operational reasons and accepted in 1987 (pauci = few; multi = many). If at least 1 acid-fast rod is found, the patient is referred to as multibacillary. A disadvantage of this very simple classification is that if the microscopy is poorly executed, a multibacillary case may be classified as paucibacillary and will then remain under-treated. Therefore, WHO has harmonazid pauci- and multibacillary leprosy treatment in 2018, recommending the same 3-drug regimen for both presentations. The only difference is the treatment duration. Leprosy can be classified on clinical grounds only. Patients with 1 to 5 skin lesions and maximally 1 trunk nerve affected are considered paucibacillary. If more than 5 skin lesions or more than 1 trunk nerve are involved, the patient is regarded as multibacillary.
Paucibacillary: Indeterminate, TT, BT (with no acid-fast rods on the smear)
Multibacillary: BT with bacteria visible on a smear, BB, BL, LL

The Ridley-Jopling classification reflects the cellular resistance of the patient:
A patient with the tuberculoid form (TT) has high cellular resistance. There are few bacteria, the lesions are localised, and the patient is not very infectious. If leprosy bacillus antigen (lepromine) is injected into the skin, the lymphocytes react strongly. A local reaction is observed. The lepromine test is positive in this case. The reaction is read after 28 days (Mitsuda reaction): diameter > 5 mm is highly positive (cf. Mantoux reaction in tuberculosis). An earlier reaction (Fernandez reaction: 48 hours) can also be read but it is non-specific. There is no cross reaction between Mantoux and the lepromine test. There is quite a poor correlation between the Fernandez and Mitsuda reactions.
There is little immunological resistance in lepromatous form (LL). There are countless bacilli and the lesions are diffuse. Patients are infectious for their environment. The lack of resistance is reflected in the negative lepromine test. The patient produces antibodies but these are not protective.

Clinical aspects

Indeterminate leprosy

Most infections do not give rise to symptoms (only to a positive lepromine test). After infection, there is an incubation period of 2-15 years (the mycobacteria multiply slowly). If the patient does not recover spontaneously, a transient indeterminate lesion appears. It consists of one or more grouped hypopigmented non-pruritic macules, which are well delineated. On white skin, they are red or hyperpigmented. There will rarely be any reduction of sensitivity (hypo-aesthesia). Sometimes, somewhat reduced sweating is seen. Nerves never become

thickened at this stage. Bacilli are practically never found in this lesion. After the initial lesion, there is evolution towards recovery or towards one of the forms in the spectrum TT - LL, which usually occurs within 2 years. Approximately 75% of indeterminate leprosy cases recover spontaneously. This indeterminate stage is often not diagnosed. Some indeterminate leprosy infections can be diagnosed on clinical grounds alone, especially in family members of an untreated leprosy patient. In other situations, diagnosis is often only possible via histology.

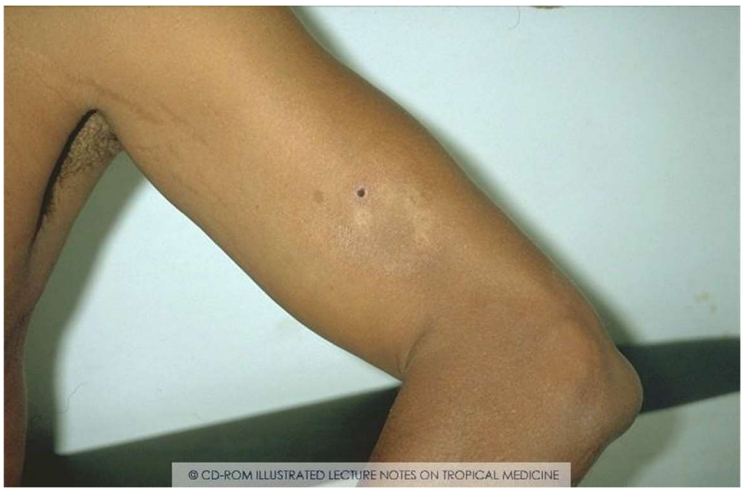
Tuberculoid leprosy

In tuberculoid leprosy, there are only one or a few asymmetrical skin spots on not more than two body parts. They are sharply delineated, sometimes with a slightly elevated border and central healing. There are often papules on the edge. The lesion is rather hypopigmented (on dark skin) or erythematous (on white skin), and sensitivity is lost. First, the sensitivity to temperature decreases, then the sense of touch, then pain and finally, deep sensitivity. There is hair loss, and the skin is dry. One or two peripheral nerves are affected (thickened) at the areas of predilection or in the region of the skin lesion.

The consequences of neural dysfunction appear early (muscle weakness and atrophy, reduced sensitivity to pain, sense of touch and sweating). Paralysis is common. It sometimes occurs before the loss of sensitivity. There is no direct involvement of other tissues. Leprosy is the only infectious disease which causes thickening of the nerves. Purely neurological involvement also sometimes occurs without skin abnormalities (= neural leprosy). This slow form of neural dysfunction stands in sharp contrast to the swift neurological damage that occurs during leprosy reactions. In leprosy, autonomic symptoms such as bladder or bowel problems, postural hypotension, impotence, etc, are rare. Patients with amyloidosis tend to have pronounced autonomic neuropathy.

Peripheral neuropathy, mononeuritis multiplex and polyneuropathy

Most cases of leprosy present with skin and neurological signs. However, pure neuritic leprosy also occurs. In the tropics, leprosy should, therefore be considered in the differential diagnosis of any peripheral neurological symptom,. This tends to be predominant axonal (lower amplitudes on EMG), probably due to intraneuronal edema with compression of the axons, but occasionally accompanying demyelination is found (slower conduction speed on EMG). Here, the differential diagnosis becomes much more complex, and sometimes, it can only be reached through nerve biopsy.



Indeterminate leprosy, small lesion on left upper arm. The lesion was initially treated for suspected mycosis. ©ITM


Tuberculoid leprosy, hypopigmented skin lesion. Photo Dr Brouwers. ©ITM

An individual peripheral nerve can become damaged by direct trauma, invasion by a tumor, but also via entrapment e.g., carpal tunnel syndrome, repeated compression, such as prolonged leaning on an arm in a particular position (N. radialis) or repeated pressure, e.g., at the level of the fibula head while seated on the ground (N. fibularis).

The peripheral nerve damage in leprosy (outside of leprosy reactions) is due to a slow-evolving mononeuritis multiplex, i.e., dysfunction of individual named peripheral nerves. Evolution is much faster in leprosy reactions, which can lead a clinician astray, especially if skin lesions are few. Sometimes the distinction between polyneuropathy with typical symmetrical distal gloves and stocking distribution is unclear. The differential diagnosis of mononeuritis multiplex is vast. Many systemic diseases associated with mononeuritis multiplex cause nerve damage by affecting the vasa vasorum.

Inflammation of these structures should be looked for in a biopsy when vasculitis is possible. Mononeuritis multiplex occurs in several forms of vasculitis (polyarteritis nodosa, Granulomatosis with polyangitis, systemic lupus erythematosus, livedoid vasculopathy), other connective tissue diseases (mixed forms), anti-phospholipid antibody syndrome, cryoglobulinemia, sarcoidosis, amyloidosis, diabetes and as a paraneoplastic entity. Nerve lesions secondary to chronic hypereosinophilia will orient the clinician in a very different direction. Neuropathy due to diphtheria occurs about a month after infection, mainly

demyelination of motor fibers, e.g., of motoric cranial nerves, leading to visual symptoms. Lyme disease can give acute neuritis, and so will usually not enter the differential diagnosis.

Peripheral neuropathy differential diagnosis

Lepromatous leprosy is a cause of peripheral neuropathy, leading to glove-and-stocking paresthesia. The differential diagnosis is large: many cases of polyneuropathy are secondary to metabolic disturbances and intoxications, ethanol being the prime example. Some metabolic diseases can be interpreted as autointoxication, e.g. uraemia. The clinician has to consider diabetes mellitus, hypothyroidism, vitamin 12 deficiency, dry beriberi (thiamine deficiency without cardiac failure), dysglobulinemia including multiple myeloma and Waldenström macroglobulinemia, primary and secondary amyloidosis, chronic hepatitis, heavy metal intoxication (lead, arsenic, thallium, mercury), solvents (hexacarbon solvents and CS2), buckthorn toxin (used as tea), chronic ethylene oxide poisoning. Check for possible side-effects of medication, such as isoniazid (vitamin B6 antagonism), vincristine, cisplatinum, nitrofurantoin.

Rarely mononucleosis, typhoid fever and mumps are mentioned as causes but the pathogenesis here is unclear. Guillain-Barré syndrome is an acute ascending inflammatory demyelinating polyradiculoneuropathy and in its acute form the distinction with leprosy is straightforward. Chronic inflammatory demyelinising polyneuropathy (CIDP) however is more difficult. It resembles a chronic form of Guillain-Barré and can occur in isolation or in AIDS.

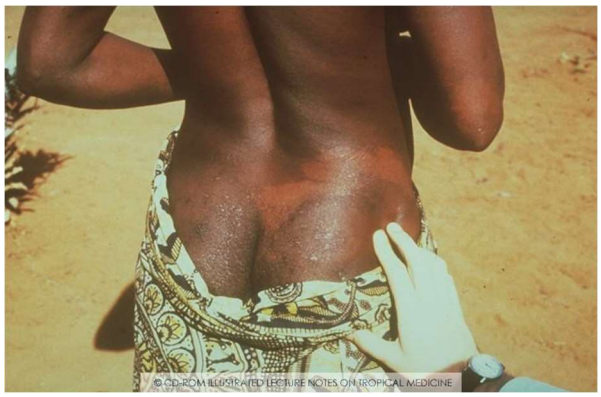
A paraneoplastic origin of polyneuropathy is often difficult to prove early in the disease (lung, pancreas, ...) but as times passes, the presence and identity of the tumour will become clear. CIDP is a chronic progressive or relapsing symmetric sensorimotor disorder, leading to generalized thickening of the brachial and lumbar plexi and peripheral nerves (including sciatic nerves and others), as can be demonstrated on whole body magnetic resonance neurography.

A number of hereditary conditions can lead to neuropathy, e.g. porphyria, Tangier's disease (genetic disorder of cholesterol transport), Bassen-Kornzweig syndrome (vitamin E deficiency due to abetalipoproteinemia), Fabry's disease (lysosomal storage disease: check family history and look for corneal opacities and spoke-like cataracts), Refsum's disease (phytanic acid accumulation often with deafness and retinitis pigmentosa). Hereditary polyneuropathies such as the different types of Charcot-Marie-Tooth (early drop-foot, hammer toes and peroneal atrophy with thin "stork legs" with familial clustering), Déjerine-Sottas (more rapid and severe than "classic" Charcot-Marie-Tooth), Friedreich's disease and hereditary pressure neuropathy fall need the assessment of a specialist in neurology. Finally, many polyneuropathies are idiopathic.

Nerve thickening

Leprosy is the only infectious disease which causes nerve thickening. Nerve thickening may also occur in rare non-infectious disorders such as certain forms of primary amyloidosis of the nerves and inherited muscular and nervous diseases. DéjerineSottas disease is a rare form of hypertrophic neuritis which usually leads to severe disability in childhood. Here, the skin is normal. In some cases of Charcot-Marie-Tooth disease (hereditary sensimotor neuropathy type I), hypertrophic neuritis occurs. In Refsum's disease, an autosomal recessive familial disorder, there is a defect in the degradation of phytanic acid, which sometimes causes thickening of nerves, together with cerebellar ataxia, progressive

deafness, heart problems, skeletal deformations, retinitis pigmentosa and dry skin (ichthyosis). Neurofibromatosis can also be included in the differential diagnosis (including café-au-lait patches). Traumatic injury may sometimes cause local thickening, as may amyloidosis.
Chronic inflammatory demyelinating neuropathy can lead to generalized diffuse thickening of plexi and peripheral nerves, as mentioned above. The technique to demonstrate this in a non-invasive way is whole-body magnetic resonance neurography using diffusion-weighted whole body imaging with background signal suppression (DWIBS) is at the onset of the second decade of the 21st Century only available in a few medical centres in the West.



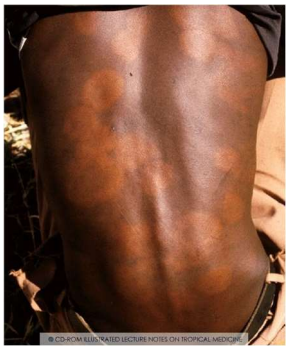
Tuberculoid leprosy, hypopigmented skin lesion. Photo Dr Brouwers. ©ITM

Multiple well-demarcated hypopigmented skin lesions in leprosy. Photo Dr Brouwers. ©ITM

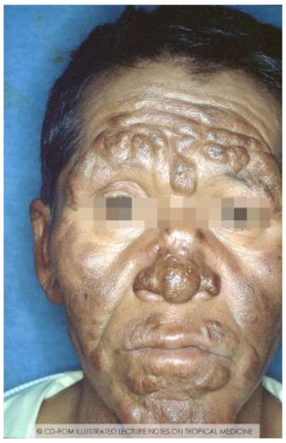
Borderline leprosy

Patients with borderline leprosy have lesions that fall between the tuberculoid and lepromatous forms. Multiple skin lesions exist, and nerve lesions are common. Three types of borderline leprosy are described: borderline tuberculous, mid borderline and borderline lepromatous leprosy. The spectrum varies from >3 well-defined, dry, firm and rough, anaesthetic, asymmetric lesions with autonomic dysfunction (loss of hair and sweat) in borderline tuberculous leprosy towards more generalized, ill-defined, smooth, shiny and soft, non-anaesthetic, symmetrical lesions without autonomic dysfunction in borderline lepromatous leprosy.

Lepromatous leprosy



Lepromatous leprosy, photo Cochabamba, Bolivia



Multibacillary leprosy, numerous. hypopigmented skin patches. ©ITM

There are countless disseminated macules and/or skin nodules, with blurred outlines sometimes joining to form larger plaques. There is no central healing tendency and no hypopigmentation, although sometimes a "copper colour" is present. The infiltrated skin nodules exhibit less or no anesthesia, but numbness develops in the hands and feet. The infiltration may lead to diffuse skin thickening, chiefly of the ears, lips and forehead ("lion's face" in LLp). In Mexico, the diffuse variety of leprosy is sometimes called "pretty leprosy" (lepra bonita) because it tends to iron out the wrinkles in the skin, restoring a youthful appearance to the patient.

Infiltration of the mucosa leads to chronic rhinitis with epistaxis, septum perforation and destruction of the nasal cartilage. The tongue is thickened, and there may be hoarseness. The upper incisors become loose and often drop out. There is often loss of the eyebrows (madarosis) and eyelashes. The central portion of the forehead (frontalis muscle) is more affected than the lateral portion. This sign is quite characteristic of leprosy and was first described by Monrad-Krohn. The sensory loss on the forehead can be quite marked (since the skin is relatively cold), but at the hairline, there tends to be an abrupt increase in the sensitivity to pinprick. Testicular atrophy leads to gynecomastia.

The nerves are not severely thickened, but the involvement of the nerves is extensive, generalized, gradual and symmetrical. The consequences of this loss are evident later in the disease, and sensory dysfunction, rather than motor defects, are foremost. Deep tendon reflexes are preserved for a long time, distinguishing this disease from many other neuropathies (except amyloidosis). Vibration sense and position sense remain intact for a long

time. With the progression of the disease, the motor branches of small nerves are invaded so that there are distal atrophy, especially in the hands.

Clinical aspects, specific problems

Blindness

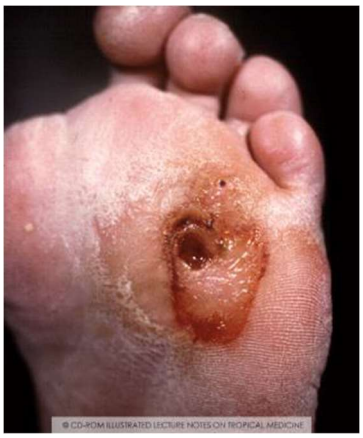
Blindness can occur in tuberculoid as well as in lepromatous leprosy. Blindness may be caused by:

1. Involvement of the facial nerve. This causes peripheral facial paralysis. Most often, the zygomatic branch is affected, and the eye can no longer close (lagophthalmia), which leads to drying out of the cornea. The patient attempts to draw the eyes upwards under the eyelids to moisten them. The lower eyelid may exhibit paralytic ectropion. Artificial tears may prevent corneal dehydration. Sometimes, the eyelid needs surgical correction to prevent blindness.
2. Involvement of the supra-orbital branch of the trigeminal nerve leads to an insensitive cornea. The patient does not feel dehydration or the presence of dust, resulting in keratitis. Artificial tears, as used in sicca syndrome, may be beneficial. The patient should be taught to consciously blink """("Think blink""""").
3. Infiltration of the eye by countless bacilli with possible formation of lepromas (nodules full of bacteria). The latter only occurs in lepromatous leprosy.
4. Iridocyclitis, for which eye drops with steroids are indicated. It is seen in lepromatous leprosy. Cataract formation and phtysis bulbi are late complications.
5. Beware of glaucoma in patients treated with cortisone for leprosy reactions, as cortisone can increase intraocular pressure. Topical cortisone administered as eyedrops is more dangerous than systemic cortisone.

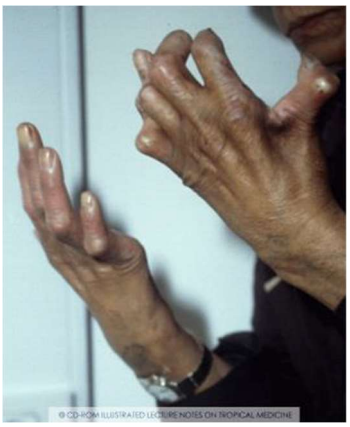
Mutilations

Mutilations may occur due to:

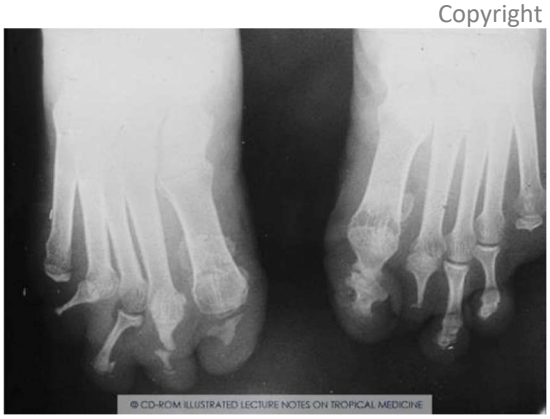
1. Paralysis with muscular atrophy and contractures.
2. Loss of sensitivity leads to not noticing wounds or burns and maintaining postures that would otherwise be painful.
3. Failure of autonomous nerves resulting in trophic skin disturbances. Dry skin with cracks occurs.
4. Untended wounds with secondary infections may lead to chronic ulceration. Tissue destruction and bone resorption lead to mutilation of the fingers, hands, toes and feet. Most mutilations can be avoided. E.g., to avoid claw hands, the patient should passively stretch their fingers daily.
5. Direct destruction of tissues, e.g., the nose. Bone lesions in LL are often more attributable to direct destruction than to bacterial infiltration. Injuries are made worse by anesthesia, superinfection, atrophy and ankylosis following disuse. The phalanges of fingers (dactylitis) and toes are most frequently affected, shorten gradually and become thinner due to lateral bone resorption.



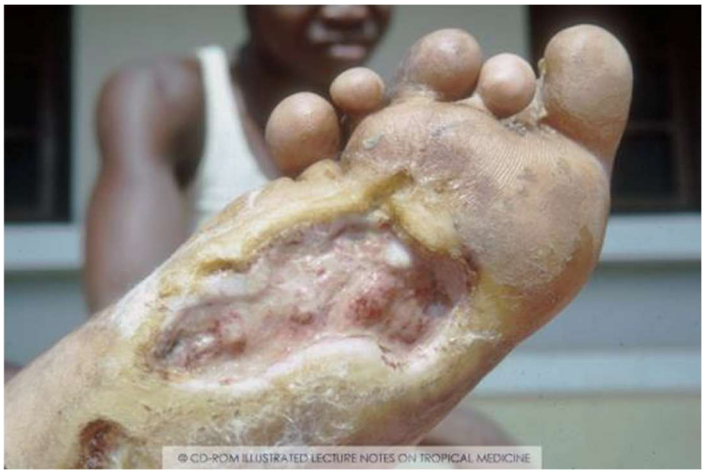
Multibacillary leprosy. Ulcus penetrans on foot. Copyright ITM



Multibacillary leprosy, hand mutilation. Copyright ITM



Leprosy with mutilations of the feet. Bone destruction is visible on this X-ray. Notice the so-called pencil shape of some affected phalanges. Copyright ITM



Leprosy. Foot ulcer.

Leprosy reactions

The host's immune responses to the leprosy bacillus create some of the pathology associated with the disease. Reactions in leprosy can occur before, during or after treatment, though they are most often seen in the months following treatment. Beware: A leprosy type 1 reaction has nothing to do with the hypersensitivity-anaphylactic type I reaction.

Type 1 or reversal reactions

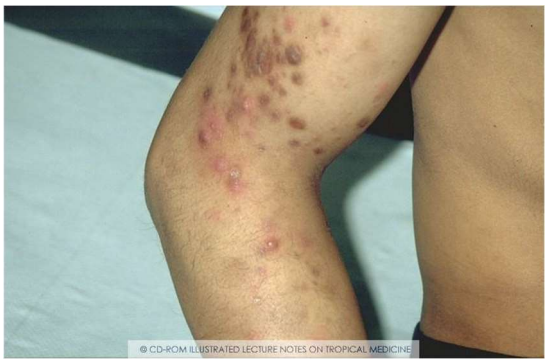
Lesions caused by a change in the immunologic defense of the patient are called type 1 reactions. These reactions may be triggered by: treatment, pregnancy, inter-current illness or vaccination or are sometimes due to spontaneous changes in immune defense. Polar tuberculoid or lepromatous forms are generally immunologically stable and do not develop type 1 reactions (only the 3 borderline stages are unstable). In the central part of the clinical spectrum, there are fluctuations in the number of bacilli and in the patient's resistance. If the patient's immunity increases, many leprosy bacilli will be destroyed. The body may react strongly to proteins released from these dead bacilli. This type of reaction (previously called an **upgrading reaction:** increase in cellular immunity towards TT) may cause damage to the body itself. Existing skin lesions become inflamed, discolored, red and painful. [Signs of inflammation of the leprosy patches are only found in leprosy reactions]. There will rarely be new lesions. Paralysis may occur quickly with a sudden increase in size and tenderness. Treatment of such a reaction must be swift to limit the damage: anti-inflammatory therapy (aspirin, indomethacin, corticosteroids), immobilization of the affected body part and sometimes decompressive nerve surgery. The leprosy therapy is not discontinued. Side effects of steroid therapy include Cushing's syndrome with weight gain, moon facies, steroid acne, osteoporosis, gastritis, diabetes and steroid cataract.

Sometimes, the immune response may be reduced. Nowadays, this is seldom seen with combination therapy. Progressive leprosy lesions develop (previously called a **downgrading reaction:** decreased cellular immunity towards LL). They take a less dramatic course than upgrading reactions.

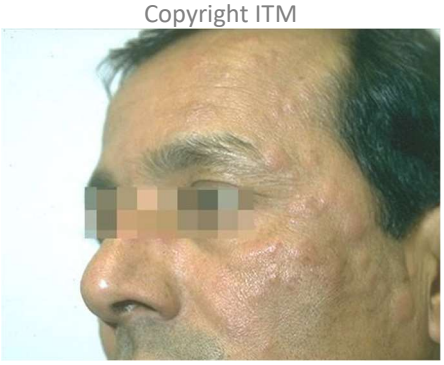
Type 2 reactions

Patients with LL and BL have almost no cellular defense against the leprosy bacillus. They do produce many antibodies, but these are not protective. The antibodies may precipitate in the body through immune complexes and cause a different set of lesions (type III hypersensitivity reaction). This type of reaction is called a type 2 leprosy reaction. It is also called Erythema Nodosum Leprosum (**ENL**). ENL shows a high relapse tendency. Leprosy reactions are an important cause of mutilation. These reactions appear as sudden new red, painful skin nodules on the legs and arms, which may form sterile pustules or ulcers. The symptoms are usually generalized, such as fever and general malaise, accompanied by muscle and joint pain, proteinuria, inflammation of the eyes and, swelling and pain in the nerves, acute epididymo-orchitis. Approximately half of LL and BL patients develop ENL a few months after beginning chemotherapy. Patients with TT are spared this complication.

Differentiation between type 1 and 2 reactions is not always easy, nor is the distinction between leprosy reaction and disease relapse. For treatment of Type I and Type II reactions, see the chapter on treatment.



Lepromatous leprosy with leprosy reaction type 2. Copyright ITM



Lepromatous leprosy with leprosy reaction type 1 in the face. Copyright ITM

Lucio's phenomenon

In 1852, Lucio and Alvarado described a necrotizing skin reaction associated with non-nodular diffuse leprosy. Lucio's phenomenon occurs in diffuse lepromatous leprosy (Lepra bonita) and can be considered an extreme type II reaction. It occurs in untreated patients and is mainly known from Mexico and other countries in Central America. It is characterized histologically by ischemic necrosis of the epidermis as a result of necrotizing vasculitis of small blood vessels whose endothelium is massively invaded by Mycobacterium leprae. Clinically, one can recognize eruptions of crops of small erythematous lesions with central necrosis. The eschar may be shed, revealing ulceration, with eventual scar formation.

Large painful haemorrhagic skin infarcts and vasculitis lesions can occur. The resultant ulcers are large with undermined edges and necrotic bases. Smears from the bases generally show large numbers of acid-fast bacilli. This condition is treated with wide surgical excision with skin grafts. The ulcers will not be cured by chemotherapy alone.

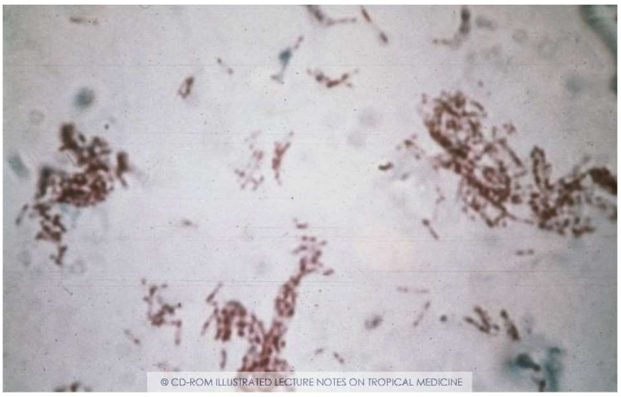
IRIS reaction in HIV patients

In the first decade of the 21st century, antiretroviral medication became more widely available in developing countries. In the first four months of therapy, there is a danger of immune reconstitution syndrome (IRIS). The rapid recovery of cell-mediated immunity triggers an immune response to foreign antigens. This presents with the first, often dramatic manifestation of an existing subclinical infection or the deterioration of existing lesions. Acute reactions in leprosy lesions can result in severe skin inflammation, ulceration and rapid loss of nerve function. The patient might mistake the HAART as responsible for leprosy symptoms.

Strange at first sight, but prolonged immunosuppressive therapy may be necessary while the patient's immune system recovers from the suppression by HIV.

Diagnosis

General



Lepromatous leprosy, skin biopsy. Numerous acid-fast mycobacteria are visible. They typically cluster in globi (small groups), which strongly suggests leprosy.

The diagnosis of leprosy is based on clinical and microscopic examination. Leprosy cases are often cared for by specialized teams, previously more so than nowadays. The role of the first-line health workers should not be underestimated: recognizing the illness and following up with the patient (leprosy reactions, eyes, wounds, foot care).

There are 3 cardinal signs for leprosy diagnosis. At least 1 of the 3 must be present to make the diagnosis of leprosy in an endemic area:

1. Anesthesia over the skin lesions
2. Enlargement of peripheral nerves with or without tenderness with evidence of nerve damage: loss of sensation, muscle paresis or paralysis of hand, feet or eyes
3. Demonstration of *Mycobacterium leprae* in the skin smears

Clinical aspects

The reason for an initial consultation is often the observation of painless traumas, burns or chronic skin abnormalities. Sometimes the initial presentation is an acute problem, e.g., ENL triggered by pregnancy, delivery, concomitant illness or vaccination.

Skin lesions

1. Check the texture, color, hair growth, sweating. Anhydrosis occurs quite early due to trophic and vasomotor disturbances (chiefly in tuberculoid leprosy). Loss of hair occurs, and the skin is often atrophic. However, edema also occurs, even progressing to elephantiasis of the feet and legs.
2. Loss of eyebrows and eyelashes (madarosis) in lepromatous leprosy.
3. Macules, papules, plaques or nodules. A leprosy macule is never completely colorless, has never been present since birth and does not flake or itch unless there is a leprosy reaction.
4. Open wounds are complications, not primary signs.

Diminished sensitivity (numbness)

Examining sensitivity reliably is not easy. Use two basins with cold and hot water, a cotton wool ball, a feather, and a needle. A tuning fork of 128 Hertz can be used for proprioception. One technique for testing feet sensitivity is using a Semmes-Weinstein monofilament. This monofilament is a supple thread of artificial material, such as nylon, mounted on a holder. The thread is pressed perpendicularly against the foot until it assumes a C-shape. In this way, a standardized pressure can be created. If the patient does not feel this, there is neuropathy leading to an increased risk of foot ulcers. When the soles of the feet are hyperkeratotic, the test is more difficult to interpret. When seeing a patient suffering from sensory loss, one has to try to detect an underlying pattern during the neurological examination. Typical patterns include:

1. Moneuropathy, when isolated, damages an individual nerve, affecting the sensation in the area of the nerve.
2. Mononeuritis multiplex. Similar to mononeuropathy but,several peripheral nerves are affected.
3. Polyneuropathy in a glove-and-stocking distribution of impairment. The longest nerves tend to be involved first in metabolic or toxic causes, e.g., diabetes, or alcohol.
4. Dermatomal distribution. Sensory loss corresponds to the cutaneous distribution of a spinal nerve root, stressing the importance of knowing the dermatomes.
5. Sequence of failure: t° > fine touch > pain > deep pressure.

Thickening of superficial nerves

Examine and palpate peripheral nerves systematically. Some of the most important are the supraorbital nerve (above the eye socket), great auricular nerve (in the neck, arises behind the sternocleidomastoid, ascends, curving diagonally across that muscle, and courses forwards and upwards), the ulnar nerve (at the elbow), median nerve (ventral side of the wrist) radial nerve (the superficial branch at the wrist), lateral peroneal nerve (the knee, at the head of the fibula), posterior tibial nerve (behind the medial malleolus) and near a skin lesion.

Neural dysfunction

1. Painless wounds, risk of burns due to the lack of pain sensation.
2. Peripheral facial paralysis with the risk of eye lesions due to lagophthalmia with cornea drying.
3. Trigeminal nerve involvement with risk of eye lesions due to insensitivity of the cornea. Test with a cotton wool stick.
4. Atrophy of the thenar (common digital nerve) and hypothenar eminence.
5. Claw hand with atrophy of the interossei (ulnar nerve). Reminder: there are seven interosseous muscles, 3 palmar (adduction of fingers) and 4 dorsal (abduction of fingers). They assist the lumbrical muscles to bend the metacarpophalangeal joints and extend the interphalangeal joints. They are all innervated by the ulnar nerve. An excellent clinical test for these muscles is to spread and then adduct the fingers. A sheet of paper between the adducted fingers must be firmly held. Froment's sign tests for palsy of the ulnar nerve, and more specifically, the action of adductor pollicis. To perform the test, a

patient is asked to hold a piece of paper between the thumb and his flat palm. The paper is then pulled away. If the ulnar nerve is intact, the patient can maintain and hold the paper without difficulty. In the case of ulnar nerve palsy, this will be difficult. The patient might compensate by flexing the flexor pollicus longus of the thumb (flexion of the DIP joint of the thumb), a muscle innervated by the median nerve.

6. Opposition of the thumb. If the median nerve is affected, the m. abductor pollicis brevis, the m. flexor pollicis brevis and the m. opponens pollicis become dysfunctional, and opposition of the thumb is compromised.
7. Wrist drop (radial nerve). Dorsal wrist extension is weak or not possible.
8. Foot drop (fibular nerve = peroneal nerve). Heel gait is not possible.
9. Claw toe (paralysis of flexors) and loss of sensation at sole of foot (posterior tibial nerve). The patient cannot walk on his or her toes.

Painful peripheral neuropathy can also occur: leprosy is not always a painless disease! Gabapentin and especially pregabalin (Lyrica®) are helpful against neuropathic pain. Pregabalin is active on calcium channels that are central to neuropathic syndromes. In general, pregabalin is preferred over tricyclic antidepressants and anti-epileptic drugs.

Electromyography

Nerve-conduction studies provide two basic measurements. The first is of the total number of units that respond on either the motor or the sensory side. The total sensory or total motor potential (sensory action potentials and motor action potentials) indicates the number of axons that have reached their destination and are still functioning. In axonal neuropathies,
such as those due to vincristine, alcoholism, diabetes or uraemia, an early reduction in sensory action potential is recorded from the distal parts of the extremities. Amyloid neuropathy gives similar results. The second measurement is of the conduction velocity which reflects Schwann-cell or myelin function. It is a measurement of preservation of saltatory conduction down the nerve fibre. A few diseases affect primarily myelin in the peripheral nerves, e.g. Guillain-Barré syndrome and its variants. Chronic pressure, such as in carpal tunnel syndrome, leads to pressure lesions and can result in prominent slowing of the conduction velocity. Mixed lesions are common. In leprosy, conduction velocities are reduced in a spotty fashion.

Microscopy

Acid-fast bacilli in smear

Preferably, several smears should be taken from the ear lobes, forehead, chin, buttock and from the raised edge of active skin lesions. The latter is sometimes forgotten. The skin is pinched between the thumb and finger of one hand. Make a small incision with the other hand (5 mm long, 2 mm deep) using a scalpel, scrape a little tissue away, then smeared onto a slide (slit-skin smear). Try not to include any blood in the smears. Smears are also sometimes made from the nasal mucosa.

The smears are stained with a modified Ziehl-Neelsen stain (e.g., Kinyoun stain). It is a cold stain. Discoloration is with a low concentration of acid (1% HCl). The mycobacteria are less acid-fast than *Mycobacterium tuberculosis* and bleach too much with standard Ziehl-Neelsen,

which uses more concentrated acid. *M. leprae* is a weak Gram-positive or Gram-neutral acid-fast bacillus measuring 0.3x2-7 µm. The bacteria are often grouped in clusters (globi). The hydrophobic character of the waxy mycobacterial capsule plays a part here.

The morphological index has increasingly been abandoned. It is the percentage of live bacteria to the total number of bacteria. For this, 200 free-lying bacteria are examined. Live bacteria are homogeneously stained. Dead bacteria have a granular staining pattern. The resorption of dead bacilli into the tissues is very slow (1 log decrease per year). The presence of acid-fast bacilli in a treated patient does not necessarily mean the therapy has failed. The morphological index is a better measure of recovery than the bacterial index. The disappearance of bacteria during treatment can be partly attributed to the loss of their acid-fast nature. In some biopsies that test negative with the Fite-Faraco stain, bacteria can still be detected using Gomori methenamine-silver staining.

Ridley proposed the bacterial index. He developed a logarithmic scale from 0 to 6+. Using an oil-immersion objective, the scale is based on the average number of bacilli per microscopic field. Infections with a high bacterial load usually take 5-8 years from the beginning of therapy before the bacterial index is negative. [A rule of thumb is 1+ per year].

Bacterial index

0 0 bacilli in 100 oil-immersion fields

1+ 1 to 10 bacilli per 100 fields

2+ 1 to 10 bacilli per 10 fields

3+ 1 to 10 bacilli per field

4+ 10 to 100 bacilli per field

5+ 100 to 1000 bacilli per field

6+ > 1000 bacilli per field

Biopsy

If the smears are negative, a skin biopsy is performed, which must penetrate the subcutaneous tissue. The biopsy should preferably contain a superficial nerve branch (hypodermic). In multibacillary leprosy, there is a zone of healthy tissue between the superficial epidermis and the infiltration with bacilli in deeper dermis. This does not apply to tuberculoid leprosy. The linear distribution of an infiltrate consisting of neutrophils and vacuolated macrophages follows nerves and blood vessels. The foamy characteristic of the histiocytes is an important clue. Bacilli in a tissue biopsy can most easily be detected with a modified Ziehl stain (Fite-Faraco stain). *Mycobacterium leprae* stains poorly with Ziehl on sections from paraffin tissue blocks. The acid fastness may be restored by impregnating the tissue segments with peanut oil or turpentine beforehand. In TT, it will rarely be possible to detect acid- fast rods, but the diagnosis can be made based on typical histological appearance (non-caseous granulomas

around a nerve branch). In indeterminate leprosy, minimal nonspecific chronically inflamed infiltrate around nerves, blood vessels and skin nodes. Bacilli are very seldom present in indeterminate leprosy, and generally, none are found. A sural nerve biopsy can be performed when the diagnosis is unclear. The presence of neutrophils and edema of the papillary dermis is also an essential clue to a Type 2 reaction. Type 1 reactions, by contrast, are lymphocyte-rich.

Lepromine

Lepromine is not used as a diagnostic aid for the individual patient. In the time before armadillos could be used, lepromine was prepared from skin nodules from multibacillary patients. It was called lepromine H (human), with 160 million bacilli/ml as standard. At present lepromine is prepared from armadillos and is known as lepromine A (armadillo). Both human and armadillo lepromine contain varying amounts of tissue. A more purified preparation is Dharmendra lepromine, which is used in India. This produces a weak Mitsuda reaction. Sometimes another purified version is used, called Convit's soluble antigen.

LTT

Lymphocyte transformation tests have been developed. They are more specific than lepromine tests. It was observed that many healthy people who had had contact with lepers reacted positively in this test, unlike people who had not been exposed to leprosy. This is an argument for the hypothesis that leprosy is highly infectious but has a very low disease expression.

Serology

Antibodies can be detected using serology, but this produces many practical problems. One of the better-studied antibodies is called phenolic glycolipid-I antibody (PGL-I). The titers are proportional to the bacillary load. Newly lepromatous patients are always positive, but the diagnosis can be made simpler. Up to 50% of tuberculoid patients are negative in this test. At present, the technique (ELISA or dipstick assay) is abandoned more and more. Leprosy sufferers often have circulating auto-antibodies so that their plasma often gives false-positive results for various other disorders (e.g., positive RPR or VDRL suggesting syphilis). Cross-reactivity with Leishmania is described as an important differential diagnosis in regions where both diseases are endemic. The cerebrospinal fluid in leprosy is normal.

PCR

A PCR [polymerase chain reaction] has been developed to test for *M. leprae*. In view of the inherent problems with this technique due to contamination in the laboratory, the results from various studies must be interpreted with caution. In many people, positive PCR results from nasal swabs (e.g., 33% in contact persons in the same household and 20% of persons who work with leprosy sufferers) are found. There may be indeed many asymptomatic carriers. As in many diseases, PCR is gaining importance in diagnosing leprosy.

Culture

If there is doubt concerning resistance, an in-vivo culture can be carried out in research centers (injection in the food-pad in mice). The inoculated test animals then receive food mixed with various concentrations of dapsone, rifampicin or clofazimine. Maximum growth

of the resistant bacteria is reached in approximately 6-9 months. To bypass the problems associated with experimental animals, attempts are being made to develop in-vitro techniques. Using in-vitro radiorespirometry (14CO2 production from 14C-labelled palmitinic acid) as in the Bactec or Buddemeyer systems, an attempt can be made to measure the metabolism of the bacteria, and in future it should be possible to use this to examine the viability of the mycobacteria, e.g. during treatment. These techniques have no place in daily clinical practice. It must be stated that no long term in-vitro cultivation technique is available.

Differential diagnosis

Initially, the differential diagnosis must take into account a large number of other diseases. Fixed drug eruption, morphea (localized scleroderma), dermatophytosis, dermal filariasis, eczema, scars, nodular cutaneous leishmaniasis, post-kala azar dermatitis and keloids may exhibit clinical similarities.

Diffuse cutaneous leishmaniasis often resembles lepromatous leprosy and can be similar to cutaneous lymphoma (mycosis fungoides).

Lobomycosis or Lobo's disease is rare and occurs almost exclusively in the Amazon and Orinoco basins, although some cases have been known from Surinam and Central America. The disease is caused by a fungus, *Loboa loboi,* and may be clinically very similar to lepromatous leprosy or keloids. The diagnosis is with a skin biopsy.

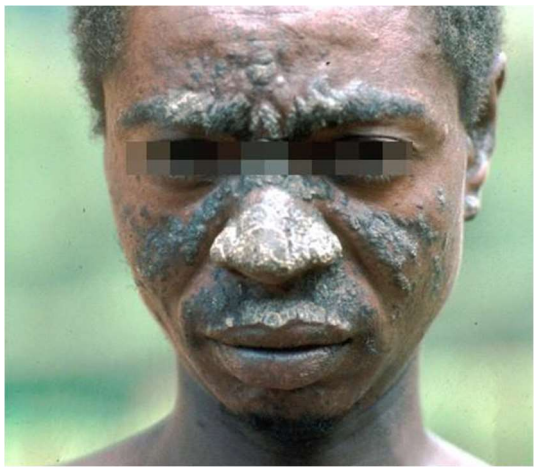
Systemic lupus erythematosus (SLE) may be mistaken for leprosy. Skin and mucosal lesions of lupus erythematosus discoides, necrobiosis lipoidica (check for hyperglycemia) and porphyria cutanea tarda (lesions chiefly on the hands and face, where exposed to the light) may pose diagnostic problems.

Neurofibromatosis (Recklinghausen's disease) sometimes causes a problem in differential diagnosis. [In neurofibromatosis type 1, 100% of the children have café au lait patches before they are 2 years old, 70% have freckles in the skin folds (axilla), and 90-100% of patients also have hamartomas in the iris (Lisch's nodules) as well as neurofibromas by the time they are 20 years old. In the rarer type 2 (NF2), café au lait patches only occur in 1%, and the freckles are absent. In NF2, the peripheral nerves may develop schwannomas, but in these patients, acoustic neurinomas are the most common.

Annular skin lesions which are similar to tuberculoid leprosy may also occur in tinea corporis, cutaneous sarcoidosis (lupus pernio), granuloma annulare, granuloma multiforme, syphilis, actinic granulomas and Jessner-Kanof 's lymphocytic skin infiltration (pseudolymphoma; etiology unknown). A Sutton's naevus is generally easy to recognize (ring-shaped depigmentation with central hyperpigmentation).

Annular psoriasis is characterized by the presence of thick scales that usually exhibit symmetrical distribution, with enlarged blood vessels in the dermis. There may be pustules or pitting of the nails and/or arthropathy. Köbner's phenomenon may occur.

Granuloma annulare is more challenging to differentiate. It is a benign skin disorder characterized by a granulomatous inflammatory process, which manifests itself in a ring or annular configuration of papules. The lesions usually occur in the region of a joint (the hands, elbows) but may also occur elsewhere. There is no neural dysfunction. An aetiological association with sunlight is assumed, but this is only one hypothesis. Most lesions (75%) heal spontaneously in 1-2 years. It is possible that granuloma multiforme is a variant of granuloma annulare. Biopsy is usually central to the diagnosis. Given the strong similarity to leprosy and since granuloma multiforme is regularly seen and treated as leprosy, it is advisable to study a number of photographs of people with this disorder, or better still, the patients themselves, in order to become familiar with the clinical picture.



Systemic lupus erythematosus with butterfly rash on the face. This patient was wrongly diagnosed as having leprosy and treated as such for one year before the correct diagnosis was made. Photo Prof Gigase, copyright ITM.

Pityriasis alba is an eczema variant (slightly scaly, on skin exposed to light). Gibert's pityriasis rosea is another condition that is easier to differentiate.

Pityriasis versicolor (Gr. "pityron" = bran; refers to the light skin scaliness) is a widespread skin infection with a fungus: *Pityrosporum ovale* (yeast stage) or *Malassezia furfur* (mycelium stage). This lipophilic fungus forms the tyrosinase inhibitor azelaic acid from sebaceous fats, a substance that inhibits melanin synthesis, explaining the white appearance of the skin spots. Account must be taken of the fact that depigmented skin spots can also be caused by damage to the melanocytes (pigment cells) after an ordinary infection, wound or burn (post-inflammatory hypopigmentation).

Vitiligo is easy to differentiate because most depigmentation is complete (never complete in leprosy) and the texture of the skin with this condition is otherwise normal)

Endemic treponematosis and syphilis (the differential diagnosis is often difficult here). It is important to know that people with leprosy often have a false positive VDRL (screening for syphilis). TPHA [the *Trepanoma pallidum* haemagglutination test] permits differentiation.

Trichoepithelioma is a condition resembling leprosy with numerous rounded, skin-coloured, firm papules and nodules. It is a benign tumor originating in the hair follicles.

There are not many neuropathies where temperature and pain sensation are diminished while sparing vibration and position sense, as well as sparing deep tendon reflexes. In these cases, one should consider **primary amyloidosis** and **syringomyelia** (lesion of the crossing fibers of the central grey matter of the spinal cord) in the differential diagnosis. Less than 10% of leprosy cases develop secondary amyloidosis. Patients with primary/hereditary amyloidosis usually have pronounced autonomic neuropathy from the onset, with episodic diarrhea, impotence, decreased sweating, postural hypotension and other evidence of impaired vasomotor control.

Treatment

Before discussing the antibiotic treatment of leprosy, it is essential to mention that the following steps are critical in the management of Hansen's Disease:

1. Assess nerve damage severity and duration
2. Treat type I and type 2 reactions
3. Manage neuropathic hands and feet. Surgery can have an essential role in mitigating mutilations and neuropathic lesions
4. Educate the patient about the disease and adverse effects of medication
5. Explain the psychological issues that accompany leprosy
6. Address the stigma of leprosy

Because of the increasing resistance to dapsone, in 1982, the WHO proposed using only combination regimens. With modern therapy, the infectivity falls very swiftly (in a few weeks). People are being cared for more and more in their home environment. This requires vast efforts in follow-up. Rehabilitation, orthopedic aids, good shoes and eye care are crucial. Surgical reconstruction, tendon transplantations etc., have their place but require specialized physicians. Instructing patients, chiefly concerning checking wounds and foot hygiene, is very important. Prompt treatment of wounds can prevent much suffering.

Historical note

In the past, leprosy sufferers were strictly avoided or isolated in a leprosarium. This completely disrupted the social lives of the people affected. Patients hid themselves and withdrew from care.

Dapsone was first synthesised by Fromm and Whitmann in 1908, but it was used exclusively in veterinary medicine for streptococcal mastitis. In 1941 it was discovered that Promin® (sodium glucosulphone) PO and IV could produce an improvement in leprosy. Diasone, another sulphone, was better tolerated but was later replaced by dapsone.

The first cases
of dapsone resistance were reported in 1964. In the '60s the efficacy of clofazimine was discovered. In 1965 the activity of thalidomide in ENL was ascertained. In the late '60s and early '70s rifampicin was developed and this exhibited exceptional efficacy.

Typical regimens

Paucibacillary leprosy (TT and BT)

For 6 months

Rifampicin 600 mg/once per month under supervision Clofazimine 300 mg/once per month under supervision and Clofazimine 50 mg/day without supervision

Dapsone 100 mg/day without supervision

Then keep under supervision for a further 2 years for late leprosy reactions and any relapse.

Multibacillary leprosy (smear-positive BT, BB, BL and LL)

For 1 year Rifampicin 600 mg/once per month under supervision

Clofazimine 300 mg/once per month under supervision and Clofazimine 50 mg/day without supervision

Dapsone 100 mg/day without supervision

Then, keep under supervision for 5 years (or life-long in LL).

These regimens are usually quickly accepted and have little toxicity. Relapses seldom occur (< 5% after several years).

Dapsone

The anti-leprosy activity of this sulphone was ascertained in the '40s and until 1980 it was often used in monotherapy (initially IM, later PO). It is safe during pregnancy. Dapsone (=DDS; Diamino Diphenyl Sulphone) is a slow-acting bacteriostatic product. It is swiftly absorbed from the intestine and undergoes enterohepatic circulation. A steady-state serum concentration is reached approximately eight days after beginning the treatment. It has a half-life of 28 hours and can be taken once daily. Dapsone resistance is presently spread world-wide. Dapsone is generally well tolerated.

1. Pharmacological predictable adverse reaction to dapsone
- peripheral neuropathy
- hemolytic anemia (even if there is no G6PD deficiency)
- methaemoglobinaemia with nonspecific nausea, vomiting, fatigue, dizziness, weakness, headache

2. Allergic/idiosyncratic reaction: the dapsone hypersensitivity syndrome. This usually starts within 6 weeks after beginning dapsone. If there is no alternative, desensitization may need to be carried out. Symptoms:

- hepatitis with icterus
- eosinophilia
- fever
- skin eruption, including exanthema, pustular lesions and even Stevens-Johnson syndrome
- lymphadenopathy
- agranulocytosis
- nephritis
- pneumonitis
- hypothyroidism

Other medical uses of dapsone

Dapsone is also used in the treatment and prevention of Pneumocystis jirovecii, in the treatment of toxoplasmosis, in dermatitis herpetiformis, in Loxosceles bites (see the chapter "spiders") and several other rare disorders. Dapsone is contained in Lapdap® and Maloprim®, agents for malaria prophylaxis.

Rifampicin

Rifampicin (Rifadin®, Rimactan®) (id. Rifampin) is a highly active but expensive bactericidal agent. It interferes with the synthesis of nucleic acids by inhibiting DNA-dependent RNA polymerase. Due to its high sterilizing activity and the slow growth of *M. leprae* it can be given once monthly if combined with other drugs. This reduces the cost and toxicity significantly without compromising efficacy and makes supervision of adherence easier. Rifampicin sometimes causes liver damage. See also tuberculosis. It may be used during pregnancy, although there are isolated reports of congenital deformities. Spina bifida and hare lip were observed in the progeny of rodents when the product was administered at high doses during pregnancy.

Clofazimine (Lamprene®)

Clofazimine is a weak bactericidal agent. It has anti-lepromatous and anti-inflammatory properties. This lipophilic drug is best taken after a meal for better absorption. It accumulates slowly in the skin, where it may cause dryness and hyperpigmentation. The latter may sometimes cause stigma in patients. The urine, tears and sweat are also stained red. Sometimes, there is nausea. In rare cases, there is severe enteritis with paralytic ileus. The tissue half-life is very long (70 days). If clofazimine is used in type 2 leprosy reactions, the effect is usually only noticeable after 4 to 6 weeks. Clofazimine passes the placental barrier and is present in breast milk. Neonates may then also exhibit hyperpigmentation. It is probably safe during pregnancy.

Other

In 1987, it was discovered that minocycline, ofloxacin (Tarivid®) and clarithromycin (Biclar®) possess bactericidal properties against *Mycobacterium leprae*. The therapeutic place of all these drugs in the treatment of leprosy still needs to be determined. They may be used if, for example, rifampicin is not tolerated. Shorter therapies (single dose and 6 weeks) are being studied but have been abandoned due to too many failures in follow-up.

New experimental regimens

Monthly treatment with rifampin 600 mg, minocycline 100 mg and moxfloxacin 400 mg (RMM) is a promising treatment being studied.

Pregnancy and lactation

There is very little data on leprosy and pregnancy. During pregnancy there is progressive reduction of the cellular resistance, but humoral immunity is probably stimulated. In theory fewer type 1 reactions would be expected during pregnancy. On the other hand, type 2

reactions may be more frequent. Possibly, there is an increase in the bacillary load in untreated patients. Since the disease may become worse during pregnancy, the medication is continued unchanged. The use of thalidomide for leprosy reaction type II during pregnancy is, of course, forbidden.

Neuropathic pain in leprosy

Leprosy patients often suffer from neuropathic pain. Carbamazepine (can be used but can result in a lupus-like syndrome. Pregabalin is approved for chronic neuropathic pain (leprosy, diabetes, shingles). It can be administered orally, for example, 150 mg in the morning and 300 mg in the evening.

Treatment of leprosy reactions

Treatment of type I leprosy reactions

Treatment of such a reaction must be swift to limit the damage: anti-inflammatory therapy (aspirin, indomethacin, corticosteroids) and immobilization of the affected body part. The leprosy therapy is not discontinued. Contrary to tuberculosis, prolonged steroid use does not seem to increase the risk for severe leprosy nor to re-activate asymptomatic infections.

Treatment of type II leprosy reactions with erythema nodosum leprosum

The treatment of ENL consists of analgesics, clofazimine (which also has anti-inflammatory characteristics) at higher doses than normal leprosy therapy (100 mg 3 times daily for 1 to 3 months). However, it is a slow-acting drug, corticoids systemically and if necessary eye drops. If needed, the fast-acting drug thalidomide can be used (Softenon®, 100 to 400 mg/day for 10 days, then reduced to 50-100 mg daily). **Methotrexate** seems a good alternative in patients with poor response to steroids who cannot take thalidomide or had poor response to thalidomide.

Thalidomide is not an immunosuppressive but is immune-modulating drug. It changes the balance of several cytokines. For example, it is an antagonist of TNF-alpha and increases the action of IL-2. Contraception is mandatory during the use of thalidomide (men and women) since it is highly teratogenic, probably due to interference with angiogenesis in the fetus, not due to induction of mutations. It causes phocomelia, heart, ear and eye abnormalities, autism and embryopathy.
Thalidomide was officially taken off the market in 1961. In 1965, Dr Jacob Sheskin, an Israeli dermatologist discovered fortuitously that thalidomide in leprosy patients improved ENL. In 1998, thalidomide was approved by the FDA for treating ENL and in 2006, for treating multiple myeloma.

Thalidomide is now used in erythema nodosum leprosum and some immunologically mediated diseases, such as refractory mucosal aphthosis (common in AIDS), Behçet's syndrome, severe erythema multiforme and severe prurigo nodularis (Hyde's disease).
Apart from teratogenicity, side effects include peripheral neuropathy (risk higher when the cumulative dose is greater than 20 grams), somnolence, constipation, nonspecific skin rash and dizziness.

Lenalidomide

Lenalidomide (Revlimid) is a 1:1 racemate and chemically related to thalidomide. It is studied in myelodysplastic syndromes. About two thirds of patients with the 5q- syndrome (myelodysplasia with anaemia and thrombocytosis) benefit from lenalidomide. At present it is used in Kahler's disease (multiple myeloma). It might replace thalidomide in the future for certain indications. Lenalidomide is 50,000 times more potent than thalidomide in inhibiting tumour necrosis factor-alpha.Because of its resemblance to thalidomide, it is contra-indicated in pregnancy. It’s place in leprosy reactions is not yet clear today.

Cyclosporin A in leprosy

Cyclosporin A acts primarily to suppress T-cell activation, especially the CD4-Th1 helper cell, which play a central role in reversal reactions. Such reversal lesions contain high numbers of CD4-lymphocytes, especially Th-1 helper cells. This is in contrast with ENL, where an influx of CD4-Th2 cells and deposition of immune complexes occurs. Prednisone remains the drug of choice in reversal reactions, but in case of failure, cyclosporin A can be used as an alternative.

Prevention

Basic hygiene is important for staff and patients: washing hands, wearing a mask if the patient has rhinitis, and wearing gloves to take samples. *Mycobacterium leprae* is found in breast milk, but this is not sufficient reason to stop breastfeeding. It is thought that infectivity quickly drops to zero after the start of combined chemotherapy. Examination of the people in contact with leprosy patients is indicated. The risk of leprosy in the family of lepromatous patients is 5-8 times higher than in the general population.

Since 2020, WHO recommends prophylaxis with a single-dose rifampicin (SDR-PEP) for both household and social contacts. The protective effect in preventing leprosy is 55-60% with a higher efficacy when combined with BCG vaccination at birth. In the case of contact with multibacillary patients, check-ups for 5 to 7 years, once per year, including looking for the "numb spot", are advised.

Due to the complex bacterial cell wall combined with the difficulty of cultivating M. leprae in vivo, no suitable antigen for vaccine production has been found today. BCG vaccination provides partial protection.

Miscellaneous skin diseases

Buruli ulcer

Summary
- Skin ulcers caused by Mycobacterium ulcerans
- Role of mycolactone, the Buruli toxin secreted by the organism
- Extensive involvement of subcutis and underlying tissue
- Little pain
- Surgical intervention is the first choice for treatment
- Add rifampicin plus streptomycin if early diagnosis/lesion

Historical note

In 1897, a disease was noted in Africa by Sir Albert Cook that is most likely to have been Buruli ulcer. Between 1923-35 the condition was also observed by Kleinsmidt in north-east Congo. The disease was seen in 1940 and subsequently (1948) described by MacCallum in Australia as Bairnsdale ulcer. Afterwards similar ulcers were found in Africa, Papua New Guinea and
other parts of the world. In 1961 a focus was discovered in Uganda along the White Nile in Buruli County near Lake Kyoga, hence the name Buruli ulcer which has since been used extensively. After 1980, important new foci were discovered in West Africa. Since December 1997, the condition has been recognised by the WHO as an important emerging disease. The "Global Buruli Ulcer Initiative" was launched in February 1998 with the intention of improving knowledge and control of this disease.

Geographical distribution

The geographical range of the disease is still incompletely known. In the year 2000, the condition was known to occur in:

- Africa: Benin, Burkina Faso, Cameroon, Ivory Coast, Ghana, Guinea, Liberia, Nigeria, Sierra Leone, Togo, Angola, Congo, Gabon, Sudan, Uganda
- Oceania: Australia, Papua New Guinea
- Asia: China, India, Indonesia, Japan, Malaysia
- Southamerica: Bolivia, French Guyana, Mexico, Peru, Surinam

Aetiology

Buruli ulcer is caused by Mycobacterium ulcerans, an organism that is closely related to *M. tuberculosis*. These bacteria are acid-fast rods, 3-7 µm long. The generation time is 20 hours (slow-growing organism). The reservoir and the route of transmission remain unknown. Regular reference is made to the presence of the disease in marshy areas along large rivers. *M. ulcerans* grows best at low oxygen concentrations, such as are found in the mud of marshy ground. The clinical history often includes a report of minor trauma, an insect bite or a hypodermic injection at the site of the original solitary lesion.

It is suspected that transmission might occur via the bite of infected water bugs. These insects are possibly infected by filter feeding on micro-organisms in the water, subsequently serving as mechanical vectors. This however is still only a hypothesis. The mycobacteria are detectable in those insects by PCR. Mosquitoes were suspected in a large outbreak in Australia (PCR-positive). As a rule, attempts to isolate the organism from the environment (e.g. streambeds of slow-flowing rivers or marshes) fail. The interval between sampling and culture, the transport media, the temperature and the aggressive decontamination procedures that are used possibly play a part in this.

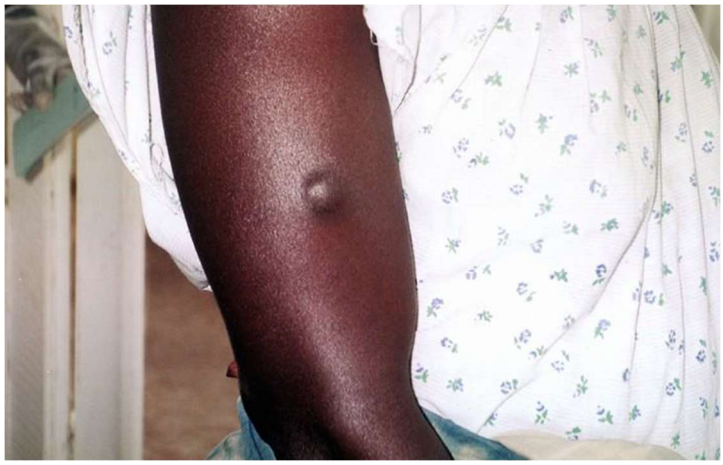
Pathology

M. ulcerans is a mycobacterium that grows extracellularly in the human body. The earliest lesion is a necrotic zone in subcutaneous fatty tissue. There is typically surprisingly little inflammatory reaction in the surrounding tissues. Clumps of acid-fast bacilli are found in the necrotic fatty tissue ("steatonecrosis"), sometimes in huge numbers. Calcifications can also form. Eventually the lesion ulcerates as a result of necrosis of the overlying skin. Necrosis of the fatty tissue is always more extensive than the ulcer itself so that the edges are undermined and become detached over a considerable distance.

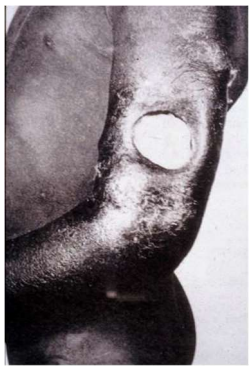
Multiple ulcers can form, connected at the deeper level by necrotic subcutaneous channels. From the edges of the ulcer there is a tendency to re-epidermalisation of the lowest level of the detached skin, which is pathognomonic for this disease. The base of the ulcer is coated with a layer of necrotic, purulent material in which for the most part no *M. ulcerans* is found. In contrast to tropical ulcers, these ulcers show no tendency to malignant degeneration.

The tissue necrosis extends further than the colonies of acid-fast rods. Following injection in experimental animals, a sterile ultrafiltrate of M. ulcerans can cause lesions that are very similar to Buruli ulcers. A cytotoxic necrotic toxin that is responsible for the steatonecrosis is found in the culture medium of *M. ulcerans*. This substance probably also has a bacteriostatic effect, which would explain the rarity of secondary infection. The toxin is a polyketide macrolide: mycolactone (C44H70O9). *M. ulcerans* strains that produce no mycolactone are avirulent to guinea pigs. Mycolactone is probably locally immunosuppressant.

Clinical Aspects



Infection with *Mycobacterium ulcerans*. Subcutaneous lesion on arm. There is no break-through (yet) to the surface. © ITM



Buruli ulcer results from infection with *Mycobacterium ulcerans*. Notice the undermined edges.

It is estimated that the incubation time is 6 weeks or longer. The ulcers are predominantly found on the limbs, more above the elbow and knees, but in 10% of cases it can be found the trunk and the abdominal wall and very rarely on the face or scalp.

The disease course can be divided into 4 stages: nodule, cellulitis, ulceration, scar. It begins as a pruritic, painless or slightly painful subcutaneous swelling that gradually becomes attached to the skin. A papulonecrotic or vesicular lesion then appears on the skin that progresses to an open ulcer with a gelatin-like coating. The skin around the ulcer is dark, sometimes with slight desquamation or with a deep reddish-purple colour (Caucasians) or hyperpigmentation (darker skin). The edges are slightly raised and rolled. The undermining of the wound edges can be established by probing. Satellite lesions and metastatic lesions in the skin or bone sometimes occur. In addition, there can be numerous lesions at the time of the first examination. The general state of health remains excellent, without fever or malaise, irrespective of how extensive the ulcer is.

When the ulcer is finally formed, it remains and becomes generally painless unless a secondary infection is involved. Sometimes localised pain is present. At the deeper level muscle, bone and joint tissue are destroyed with the accompanying formation of sequesters.

Calcifications can be detected radiologically:

- in any lesion, irrespective of its location or whether it is ulcerative.
- in the skin near a lesion either before ulceration or in the subsequent scars.

In the long-term, after months or years, the ulcer tends to heal, but extensive deformities, ankylosis or lymphoedema remain. The scars are reminiscent of old burns or the consequences of late treponematoses.

Diagnosis

The diagnosis of the ulcerative form is somewhat easier than that of the non-ulcerative form. The undermining of the wound edges is a characteristic of Buruli ulcer. Radiologically, subcutaneous fat calcifications and/or osteomyelitis are observed in a large percentage of patients.

The acid-fast rods are examined with Ziehl stain in smears of curettage products from the edges of the ulcer (preferably from the underside of the skin edges and not from the centre

of the ulcer). The Ziehl stain of a smear demonstrates bacilli in ± 75% of cases. The histological features on biopsy are characteristic on condition that the sample has been taken sufficiently deeply to include the necrotic fatty tissue. Punch biopsies are usually not sufficient. Serodiagnosis is still experimental. Culture is possible but slow (several months). The organism grows optimally at 32°C. Higher temperatures inhibit the organism (important when transporting). Semisolid transport media such as PANTA (polymyxin B, amphotericin B, nalidixic acid, trimethoprim, azlocillin) can be used, although growth is not always obtained. The organism cannot be frozen although storage at 4°C is possible. Löwenstein-Jensen medium is best used as a culture medium in an atmosphere with little oxygen. Additionally, clinicians with Buruli ulcer experience state that the ulcers have a characteristic unpleasant smell, which can contribute to the diagnosis.

There are a few other non-tuberculous mycobacteria that can cause skin abscesses and ulcers, e.g. *Mycobacterium avium intracellulare* in AIDS patients, as well as *M. szulgai, M. terrae, M. fortuitum, M. chelonae, M. malmoense* and *M. xenopi. M. abscessus* is a fast-growing organism that can cause tissue necrosis after accidental contamination of a deep inoculation (injection). Of course tuberculosis and leprosy need to be ruled out.

Infection induces cross sensitivity with tuberculin. It is possible that the opposite is also true, and that tuberculosis provides partial protection against *Mycobacterium ulcerans*. Patients with active lesions often have no local skin reaction after injection of *M. ulcerans* antigen (burulin). After recovery they test positive (cell immunity).

There are various PCR methods for detecting *M. ulcerans* but the technique is expensive and only available in a few places. False positive results can be reduced by developing a meticulous technique. False negatives (e.g. as a result of the presence of PCR inhibitors) are detected by carrying out simultaneous controls with known positive samples.

1. Cutaneous tuberculosis: scrophulus, lupus vulgaris
2. Atypical mycobacteriosis e.g. Swimming pool granuloma(M. marinum), M. abscessus (postsurgery or deep injection), M. avium-intracellulare in AIDS-patients
3. Leprosy (less ulceration)
4. Cat scratch disease
5. Tropical ulcer
6. Tertiary syphilis (gumma)
7. Framboesia (= Yaws = Pian): Treponema pertenue
8. Rat-bite fever or sodoku: Spirillum minus
9. Ecthyma: Streptococcus pyogenes, β-haemolytic (also known as Group A Strep)
10. Cutaneous diphtheria
11. Actinomycosis or mycetoma (incl. phycomycosis), deep mycosis: histoplasmosis, blastomycosis, chromomycosis, maduramycosis, sporotrichosis
12. Cancrum oris (= Noma)
13. Cutaneous leishmaniasis
14. Cutaneous amoebiasis (Acanthamoeba, Entamoeba histolytica)
15. Pyogenic abscess with e.g. pyomyositis
16. Fistula of classic osteomyelitis
17. Trauma, residual foreign body and burns, decubitus

18. Cancer: spinocellular carcinoma (also secondary to chronic ulcer), Marjolin ulcer, Kaposi, melanoma, basocellular
19. Arterial, diabetic or venous ulcer
20. Haematological abnormalities, e.g. sickle cell anaemia
21. Vasculitis (leukocytoclastic, Behçet, microscopic polyangitis, Churg-Strauss, cryoglobulinemia)
22. Pyoderma gangrenosum. This can be difficult to distinguish from Buruli. Both have undermined edges. Pyoderma gangrenosum is often secondary to chronic inflammatory conditions such as ulcerative colitis, Crohn's enteritis, rheumatoid arthritis, pulmonary abscesses, paraproteinemia. Acid-fast bacilli will be absent of course and the infiltration will be mainly neutrophilic.
23. Botryomycosis: S. Aureus or other bacteria
24. Inoculation chancre: tryponosomiasis, rickettsia (tache noir)
25. Dracunculiasis (Guinea worm)
26. Anthrax
27. Tularemia
28. Snake bite (viperidae)
29. Loxosceles bites (spider)

Prognosis

The prognosis is unfavourable because of the severe skin and bone lesions, scars, tendency to infectious metastases and the problems of surgical treatment. Many lesions heal spontaneously, although with severe sequelae.

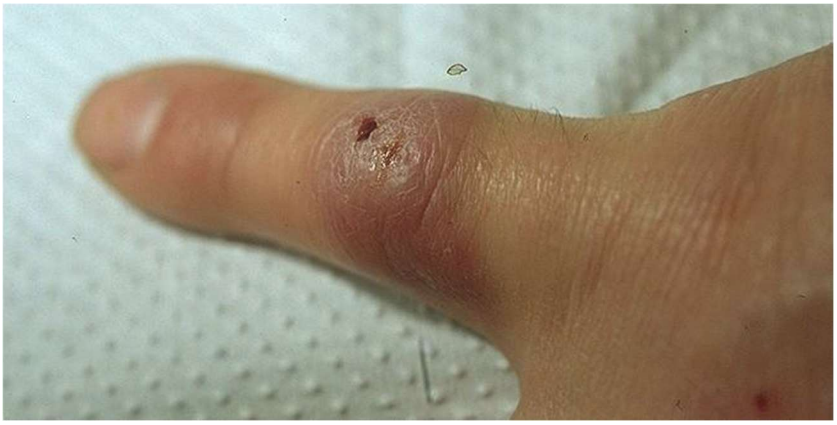
Treatment

Drug treatment is disappointing in the late stages. In vitro *M. ulcerans* is susceptible to rifampicin, clarithromycin, amikacin and streptomycin. Cycloserine, dapsone and clofazimine are active, but the organism is resistant to isoniazid. Clinical results however are often disappointing, possibly because the antibiotics do not diffuse to the bacillus itself. Treatment therefore is principally surgical: excision of the tissue followed by curettage, followed by immobilisation in a functional position. In most cases, excision of the tissues is carried out under broad-spectrum antibiotic cover. The previously mentioned antimycobacterial antibiotics can be administered at the same time to prevent the emergence of metastatic lesions. The combination rifampicin, clarithromycin and amikacin is practical. Studies
suggest that an antimicrobial regimen of rifampicin plus streptomycin may be effective against early forms of Buruli ulcer. After the formation of healthy granulation tissue, skin transplants are applied (split skin grafts). Amputation may sometimes be the only possible treatment. Tetanus vaccination should not be overlooked. Good results can be obtained with local thermotherapy by surrounding the ulcer with water bottles at 40°C. This can cause logistical and technical problems. Healing of ulcers is obtained after an average of 41 days. There is little experience with hyperbaric oxygen therapy.
Intensive physiotherapy can improve the function of a mutilated limb. Relapse of Buruli ulcer is not exceptional. Follow-up is important to rapidly identify those cases. Delays in seeking medical advice can lead to severe complications, including dissemination of disease and especially the development of bone lesions.

Prevention

In two studies in Uganda, BCG vaccination was shown to have about 50% efficacy against M. ulcerans. Protection nevertheless was temporary, on average lasting only a year. The ulcers that developed in vaccinated patients were smaller than those in controls. Possibly this merely involves non-specific immunostimulation by BCG.

Mycobacterium marinum



Mycobacterium marinum, ulcer on finger. Copyright ITM

Mycobacterium marinum (M. balnei) causes swimming pool granuloma. The condition was first described in Sweden and was later observed in most Western countries. It involves papules with central ulceration which heal spontaneously after a few months with the formation of a small scar. Infection occurs during bathing by rubbing the skin against the rough cement lining of a swimming pool or aquarium or by touching tropical fish. For treatment, a combination of rifampicin (600 mg/day on an empty stomach) with minocycline or doxycycline (100-200 mg per day) is used, together with clarithromycin (500 mg twice daily), cotrimoxazole (twice 800/160) or ethambutol (max. 2.5 g/day).

The disease must not be confused with Erysipeloid (Rosenbach's disease), an infection caused by the Gram-positive bacterium *Erysipelothrix rhusiopathiae*. Infections with this organism also occur frequently in fishermen and people who handle crabs. Pig slaughterers represent another risk group. Cat scratch disease, leishmaniasis and sporotrichosis are to be considered in the differential diagnosis.

Tropical ulcer

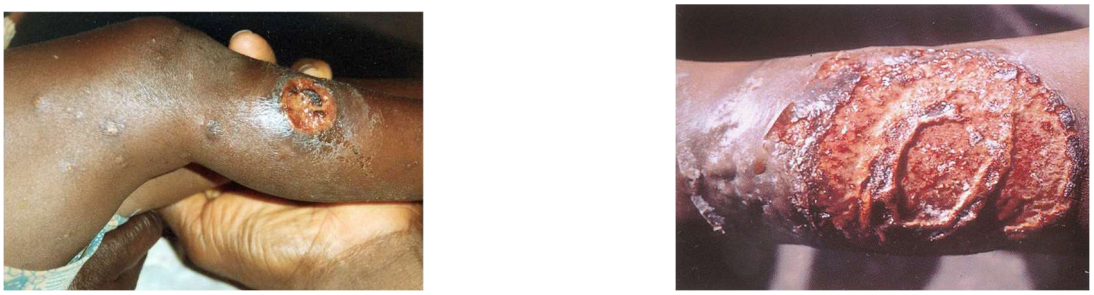
Summary
- Fusospirillary association (Fusobacterium + Borrelia)
- Initially very painful, subsequently painless ulcer on feet or lower leg
- Bad smell in early stage
- Very chronic course with frequent relapses
- Treatment with antibiotics, local care and skin grafts

Introduction

Tropical ulcer or phagedenic ulcer is a disease of warm and moist geographical regions. There is an association with poor living conditions: lack of clean water, lack of basic health services, carelessness in the treatment of small wounds, abundance of flies, etc. The role of malnutrition and lack of hygiene is clear. For example, in 1942-1945 the disease was extremely common and severe in Western prisoners of war in Japanese camps in Southeast Asia.

In early lesions, Vincent's fusospirillary bacterial association is usually detected: ***Fusobacterium fusiformis*** and ***Borrelia vincenti.*** The same organisms are isolated from the mouth in a third of the patients, from which it is deduced that the cause of tropical ulcer might probably be transmitted to small wounds by saliva. In 1989, two new species of Fusobacterium were isolated from tropical ulcers but their exact role in the aetiology has not been determined. In more chronic cases the flora is nonspecific. The histological presentation is non-specific. It is possible that tropical ulcer is initially caused by a trivial infection or secondary infection with streptococci or staphylococci in an undernourished person.

Clinical aspects



Tropical ulcers

The primary localizations are on the lower leg, the front of the ankle and the dorsum of the foot. These are sites where the bone lies immediately beneath the skin and where the blood supply is less extensive. In this respect they resemble stasis ulcers in venous insufficiency. In tropical ulcer there are no signs of venous insufficiency. Ulcers occur less often on other parts of the body. Schematically, the disease progresses in three stages:

Acute stage: Local swelling of the skin, oedematous, violently painful and pruritic, sometimes with general symptoms such as fever. A blister with serous or bloody content forms and rapidly bursts. The small ulcer then extends both peripherally and inwards. The patient sometimes reports a recent minor trauma e.g a thorn prick or an insect bite at this site.

Subacute stage: On the ulcer, a superficially necrotic, evil-smelling, purulent, yellow-green or haemorrhagic black coating forms. The base is granular and bleeds easily. Deep in the ulcer the tendons, aponeuroses and periosteum can be seen. The edge of the ulcer is raised but with little if any undermining (in contrast to Buruli ulcers). After a few weeks, the ulcer's diameter is on average 10-12 cm. The form is or becomes regular, round or oval. Painful lymphadenitis may be present.

Chronic stage: After approximately one month, the swelling and pain decrease. The edge becomes flatter. The base is now coarsely granular, less haemorrhagic and forms less exudate, but the odour persists. Bacteriologically, the flora is now non-specific. Beneath the base of the ulcer there is reactional periostitis in chronic cases. The ulcer gradually heals spontaneously. The longer the disease course, the more difficult healing becomes and the more readily a relapse occurs, as the scar always consists of a small amount of connective tissue lined with fragile, smooth, shiny, often depigmented and atrophic skin. If the lifestyle is not changed, the ulcer flares up again at the first opportunity.

Complications are numerous:
Malformations and functional disorders. Scars with fibrosis of the deeper muscles and stiffness of the ankle joint cause all kinds of problems, of which the most common is retraction of the Achilles tendon with club feet of the equinovarus type.

Secondary infection can lead to tetanus, gas gangrene or cellulitis. Thrombosis of the large arteries can result in distal gangrene. Bleeding can occur as a result of erosion of blood vessels.

Osteomyelitis. There is often a limited cortical reactional osteitis. Extensive destruction of the bone under the ulcer is suggestive of cancer.

Carcinoma. Almost always involves spinocellular epithelioma of the skin with a starting point in the border of the ulcer ("Marjolin' ulcer"). Cancer occurs after a prolonged course, whether as the gradual degeneration of an active ulcer or in a scar after one or more recurrent episodes of the ulcer. The cancer then develops in the scar itself but also sometimes in the apparently healthy skin. The edges are partially or completely raised. The base is irregular and bleeds readily. There is induration and the ulcer becomes irregular. Spontaneous fractures and spontaneous complete amputation of the lower leg can occur. In 85% of cases the ipsilateral lymph nodes are enlarged, but only a third of these by metastases, the remainder as a result of lymphadenitis. Histological examination provides formal diagnosis. The biopsy site must be carefully chosen as not all the ulcer is necessarily degenerated. Metastases in the lymph nodes can also only be confirmed by biopsy.

Tropical ulcer, differential diagnosis

See differential diagnosis ‘Buruli Ulcer’

Prognosis and social importance

The importance of this rural disease is usually underestimated. Allowance must be made for the following factors:

1. High prevalence, which is rapidly reduced as living conditions are improved: better nutrition, clean water, primary health care services, etc.

2. Numerous health centre consultations for tropical ulcer. The disease takes up much of the personnel's time for treatment, disinfection and bandages.

3. Multiple and long-term admissions.

4. Frequent relapse.

5. Severe invalidity in many patients.

6. High incidence of cancer formation, which is a potentially fatal complication. The risk of cancer formation in a poorly treated or untreated tropical ulcer is estimated at 10-15%.

Treatment

Acute cases

Local and systemic treatment with penicillin is indicated. The results are good if the ulcer is recent and its diameter is less than 2.5 cm. Some tropical ulcers heal in 2-3 weeks after administration of metronidazole for 7 days. Metronidazole is effective against anaerobic organisms.

Chronic ulcers

Antibiotics improve the case but do not heal the ulcer. Immobilisation and local treatment e.g. by bathing with Dakin's solution (aqueous sodium hypochlorite solution) and parenteral antibiotics can result in healing after a few weeks. Effective treatment of a chronic tropical ulcer involves complete excision followed by skin transplants. This can be performed under either general or epidural anaesthesia. The ulcer is curetted until there is diffuse bleeding from the whole underlaying surface.
The skin is cut away for up to 0.5 cm at the edges of the ulcer. The underlying bone is vigorously curetted in order to remove sequesters and irregularities and to obtain a flat area. Powder with sulphonamides or antibiotics is then sprinkled on the wound and a pressure bandage applied on top. If the ulcer is next to a joint, this is immobilised with a plaster of Paris. At the same time antibiotics are administered parenterally. After one week the bandage is removed, the wound cleaned, and skin grafts applied. These are obtained with a dermatome from the heterolateral thigh.

In this way up to 90% of tropical ulcers can heal in less than 3 weeks and leave an acceptable scar.

Malignant degeneration

Treatment consists of conservative amputation with adaptation of the stump for a simple prosthesis.

The inguinal lymph nodes are removed for histological examination. These tumours metastasise haematogenous and the prognosis is unfavourable.

Prevention

Peripheral health centres should provide proper wound care. It is important to promote:

1. Decentralisation of primary health care services which can tend small wounds effectively: antiseptics, simple, clean, non-hermetic bandage, penicillin if necessary

2. Proper diet with sufficient animal proteins

3. Good water supply

4. Health education

5. Monitoring at the workplace of people with tropical ulcer scars or who suffer a deterioration in their nutritional or health status

Noma

Noma (Gr. numein: to devour) or cancrum oris is a terrible gangrenous disease which leads to severe soft and hard tissue destruction in the face (mouth, teeth, lips, nose, cheeks) with lasting disfigurement. It is associated with a high mortality.

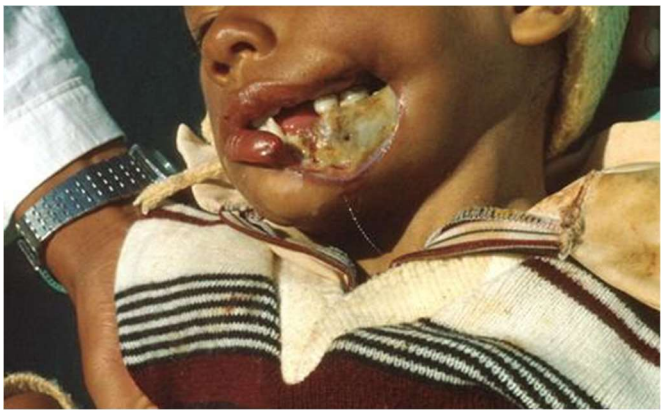
The exact aetiology is not yet known, but it is thought that several factors contribute to this devastating illness. It is clearly a disease of poverty and social deprivation. Improvements in general socioeconomic status, public health and nutrition made that noma disappeared from all places except the most desperately poor and where severe malnutrition occurs. Several factors contribute, such as malnutrition with associated vitamin and trace element deficiencies, poor oral hygiene, a compromised immune status (malnutrition, measles, CMV infection, blood dyscrasia such as leukaemia), a lesion of the gingival mucosal barrier, a (bacterial?) trigger and inappropriate initial treatment. They probably act together to cause noma. Bacteria such as spirochaetes, *Prevotella intermedia* and *Fusobacteriumnecrophorans* are suspected to play a role in the acute pathology. However, it should be remembered that at present, most bacteria in the mouth cannot be cultured in vitro.

Although the disease existed in Europe and other parts of the globe, at present it is most common in Africa. The disease affects mostly children between 2-6 years but can occasionally appear in older children and even in debilitated adults (Auschwitz!).

The disease starts as an acute painful necrotising gingivitis ("trench mouth"), evolving to a necrotising stomatitis with ulceration and oedema of the cheek. The lesion tends to start at the alveolar margin in the premolar-molar region. It spreads very fast (1-2 days). Within a couple of days, a greyish area appears on the cheek. This becomes black and necrotic and has well defined margins. There is an offensive odour. The necrotic zone penetrates the cheek and has a typical cone shape ("cône gangréneux"). After the necrotic tissue has sloughed away, bone is exposed. Large bone sequesters may form, sometimes with destruction of maxilla and/or mandibule. It should be distinguished from pyogenic abscesses and Burkitt's lymphoma. Secondary infection occurs rapidly, as can be expected.

Fever occurs in some patients. Many patients die due to starvation, septicaemia, or aspiration pneumonia. Because of the high mortality in acute noma and the fact that it occurs in the poorest among us in areas with inadequate reporting, the burden of disease is difficult to determine for epidemiologists.

Noma, cancrum oris. Photo Cochabamba, Bolivia



Noma, cancrum oris. Face ulcer. Photo Cochabamba

The tissue defects are classified in 4 types:

1. Type I is the most common and consists of a localised cheek and commissural defect. It can be bilateral.

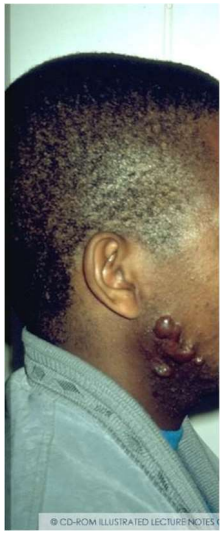
2. Type II includes the upper lip, and in some cases the nose and the palate.

3. Type III is located on the lower lip ± the mandible and floor of the mouth.

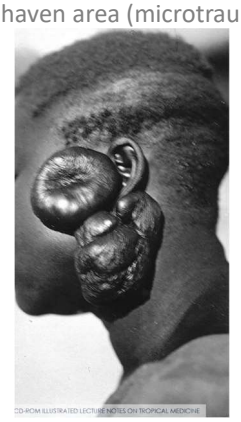
4. Type IV consists of major defects of the whole cheek, lips, palate, maxilla and can extend up to the orbit, eyelids and nose.

Treatment in the acute phase encompasses proper oral hygiene, mouth rinses with chlorhexidine, antibiotics including penicillin and metronidazole against anaerobic bacteria, proper nutrition and vitamin/trace element supplements and treatment of any underlying medical conditions. The healing is characterized by ugly scars with fibrous tissue which tends to provoke strictures. After the acute phase, physiotherapy should be initiated to limit the strictures, fibrous scarring, trismus and to avoid bony ankylosis (bridging) between upper and lower jaw. Bundles of wooden spatulae in the mouth or more sophisticated devices (e.g. the Therabite) are used. At least a year after the initial disease, reconstructive craniomaxillofacial surgery for the sequelae can be considered. This should be done by experienced teams including specialised surgeons and anaesthesiologists (tracheostomies, fiberoptic intranasal intubation). Each case will require an individual approach.

Keloids



Keloids on the face, shaven area (microtraumata). Copyright ITM



Keloid of the ears, as reaction to perforations for aesthetic reasons. Copyright ITM

Keloids are nodular, often lobulated, firm to hard but movable, non-encapsulated masses of hyperplastic scar tissue. It is a result of an overgrowth of granulation tissue (collagen type 3, early) at the site of a healed skin injury which is then slowly replaced by collagen type 1 (late). The pathogenesis is complex and involves both genetic and environmental factors and the exact mechanism is still unknown. Growth factors like VEGF, TGF-β1, TGF, β2, CTGF and PDGF-α play are overexpressed, but it remains unclear if this is the cause or the consequence of the excessive scarring. Keloids can closely resemble lobomycosis but can also be confused with lepromata and less likely with lesions of diffuse cutaneous leishmaniasis. Africans are particularly susceptible to keloids. The tribal scar pattern following scarification is based on this property. Keloids occur in all types of conditions, for example after burns, cauterisation, vaccinations, on in-growing beard hair, folliculitis or even spontaneously.
Keloids are raised and sharply delineated. The overlying skin is reddish and shiny. The lesion can be itchy or painless and the dimensions can be unexpectedly large. Keloids can develop later, up to years after the initial trauma. Treatment is difficult. Treatment options include resection, cryotherapy, intralaesional corticosteroids, 5-fluorouracil or bleomycin. Complete excision is followed by recurrence in 70% of cases. Excision within the edges of the lesion is recommended but the result is aesthetically unsatisfactory. Corticosteroids have no effect on the fixed lesions, but can prevent their recurrence by injections localised around the site of

the original lesion if started 3 weeks after surgery and repeated weekly for the following 8-12 weeks. Bigger and horizontally growing keloids are more likely to recur after treatment.