



# Lecture Notes on Tropical Medicine

## Part II



Lecture Notes on  
Tropical Medicine  
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# Helminthiasis

## Summary

Roundworms or nematodes:

- Separate sexes
- Adult in intestine lumen or larvae in tissue
- Some species have lung passage of larvae
- Transmission faecal-oral (directly or indirectly, e.g. via food), transcutaneous
- Filaria are part of the nematodes

Tapeworms or cestodes:

- Hermaphrodite adults in the intestine or larvae in the tissues
- Transmission faecal-oral or via food

Flukes or trematodes:

- Most are hermaphrodite, except blood flukes (= schistosomiasis)
- Are found in blood vessels, the intestine, biliary tract, lungs
- Transmission via food (distomatoses) or transcutaneous (schistosomes)
- First intermediate host is always a freshwater snail

## Worms, Life cycles

**All intestinal roundworms** (nematodes) have a fairly complex cycle, but almost always without an intermediate host (*Capillaria philippinensis* is an exception = mainly through the ingestion of raw fish).

The lack of an intermediate host which can only live in a well-defined ecosystem, explains the cosmopolitan character of intestinal nematodes. All intestinal nematodes have separate sexes and lay eggs which can be found in faeces. Sometimes only the female survives in the intestine. In *Strongyloides* larvae hatch before they arrive in the outside world. The larvae of nematodes have several consecutive development stages.

**Larval tissue nematodes:** The larvae of some nematode species infect various human tissues. These are accidental infections and do not represent the natural life cycle of the parasite

\* The larvae of canine and feline roundworms (*Toxocara* sp.) and also those of *Gnathostoma* may penetrate humans "by mistake" and cause visceral larva migrans. The larvae migrate through the liver, eyes, brain and so on, where they cause a granulomatous inflammatory reaction.

*Trichinella* larvae are found in the muscles and the heart.

• Filaria are a separate group. They are live-bearing (do not lay eggs) and generally their intermediate hosts are insects.

All flukes (trematodes) have a cycle with an obligatory intermediate host. The first intermediate host of these flatworms is always a freshwater snail. The larvae which comes



from the snail then, depending on the species either infects a second intermediate host (fish, crab), encysts on certain plants or penetrates the final host directly through the skin. It is precisely the presence of the intermediate host which determines whether a particular fluke can be present or not in any given area. All food-borne trematode infections are zoonoses. Infestations by flukes are always via larval forms, never via eggs. Except for schistosomes all trematodes are hermaphrodite (no separate sexes).

All tapeworms (cestodes) are parasites which are found in the intestinal lumen as adults. They are hermaphrodites. Each animal has both testes and ovaries. They have a head (scolex) and body segments (proglottids). There is generally only one adult worm in the intestinal tract (Fr.: ver solitaire = tapeworm) but multiple infections do occur. The larval forms of these worms (hydatid, cysticercus) may be located in various organs.

## Worms, Transmission

Several ways of infection are possible:

### *Oral transmission*

**Human faeces.** Faecal-oral transmission is important in several worm infections. Soiling by infected human faeces is responsible for infestation by *Ascaris*, *Enterobius*, *Trichuris*, cysticercus larvae (larval *T. solium*). Larvae from hookworms and *Strongyloides* may also be ingested orally.

**Animal faeces.** Humans become infected with the eggs of *Toxocara* (visceral larva migrans) and *Echinococcus granulosus* (hydatid cysts) by eating products which have been contaminated by animal excreta.

**Infected meat.** Eating raw or insufficiently cooked meat which contains larvae, leads to infection by *Trichinella*, adult *Taenia* and *Gnathostoma*.

**Infected fish.** Eating raw or insufficiently cooked fish [Latin American ceviche, Japanese sushi and sashimi, Dutch maatjesharing (herring), Norwegian gravlax (salmon), Hawaiian lomi-lomi (raw salmon), Spanish boquerones (anchovies in vinegar)] may lead to infection with: (1) nematodes such as *Anisakis* or *Pseudoterranova* larvae, *Capillaria philippinensis*, *Gnathostoma*; (2) cestodes such as *Diphyllobothrium* (fish tapeworm) and *Diplogonoporus*; (3) trematodes such as *Metagonimus* and *Heterophyes* (small intestinal flukes), *Clonorchis* and *Opisthorchis* (liver flukes).

**Infected crabs and crayfish.** Eating larvally infested, raw or insufficiently cooked crabs may lead to paragonimiasis (lung fluke).

**Contaminated plants.** Infection with the giant intestinal fluke (*Fasciolopsis*) occurs via the consumption of several kinds of raw plants e.g. water nut and water chestnut on which larvae are encysted. *Fasciola hepatica* (liver fluke) is transmitted via contaminated water cress.

Contaminated water. Drinking water containing *Cyclops* (small crustaceans) infected with *Dracunculus* leads to Guinea worm infection.

### *Skin Penetration*

Larvae of *Strongyloides* and hookworm enter through the skin from the soil. They then penetrate deeper. The hookworm *Ancylostoma braziliense* also penetrates skin but cannot go deeper. It stays in the skin and give rise to cutaneous larva migrans. *Schistosoma* cercariae penetrate the skin when humans come into contact with infested water.

### *Through a vector*

Filaria are transmitted by the bite of various Diptera: mosquitoes and flies. *Dracunculus* has *Cyclops* as its vector a small crustacea.

## Diagnosis

### *General*

It is important to bear in mind that many worm infections may be diagnosed by simple examination of the faeces, sputum, urine, blood or skin. Helminths which produce a large numbers of eggs or larvae are naturally easier to identify than infections with only a few eggs or larvae. In the latter case it is helpful to enrich the volume of the parasitic material to be examined, by means of concentration techniques. In this way it is possible to make a diagnosis in many patients who have a low worm load.

The tests mentioned above cannot however produce a diagnosis in the following cases:

1. Infection with immature parasites. In acute Katayama fever no eggs are found early in the disease.
2. Infections with male worms. This is why it is important to know whether or not a parasite is hermaphrodite e.g. in infections with male *Ascaris lumbricoides*.
3. Infections with adult worms which are located in an enclosed space such as the brain.
4. Infections with larvae where the human is the intermediate host e.g. cysticercosis, echinococcosis and visceral larva migrans. Trichinellosis may also be included here.
5. Infections with old or damaged worms e.g. after use of anthelmintics.
6. Many patients with loasis do not have microfilariae in their blood

### *Microscopic recognition of worm eggs*

Recognition of worm eggs requires training, practice and experience. Otherwise it is possible to interpret a certain microscopic structure wrongly for years (quality control is important).

Size. Since infections with *Ascaris lumbricoides* are so common the size of a fertilised egg (60 µm) can be used as a reference measure. If no special microscopic eyepiece is available to carry out measurements, the relative size of a structure can be compared to a fertilized *Ascaris* egg.

1. Eggs much larger: *Fasciola hepatica*, *Fasciolopsis buski*, *S. mansoni*, *S. haematobium*
2. Eggs somewhat larger: *Paragonimus*, *S. japonicum*, *Trichostrongylus orientalis*, *Hymenolepis diminuta*, *Ascaris* unfertilised egg
3. Same dimensions: hookworms eggs, *Hymenolepis nana*, *Diphyllobothrium latum*
4. Eggs somewhat smaller: *Trichuris*, *Enterobius*, *Taenia solium*, *T. saginata*
5. Eggs much smaller: *Clonorchis*, *Metagonimus*, *Opisthorchis*.

Shape. Most eggs are symmetrical. The exceptions are those of *Enterobius*, *Trichostrongylus orientalis*, *Dicrocoelium dendriticum* and unfertilised *Ascaris*. The eggs of hermaphrodite trematodes often have an operculum this small structure is not always easy to see. Some other worms also have it (*D. latum*).

Polar caps occur in *Trichuris trichiura* and *Capillaria sp.*, giving them a lemon-like appearance. Some eggs, such as various schistosomes, have a spine. These may be large or small compared to the egg, and protrude either terminally or laterally.

Colour. Many eggs have a rather yellowish brown colour due to bile salts. Some are more or less colourless (hyaline), such as those of hookworms, *T. orientalis*, *E. vermicularis* and *Ascaris* (if there is no protein mantel on the egg).

Egg shell. This may be surrounded by a knobbly protein layer, as in *Ascaris*. In some worms the egg shell is thin, as in hookworms. In others it is thick, as in lung flukes.

## Intestinal Nematodes

### Summary

- *Ascaris*: common, lung passage, sometimes intestinal or biliary obstruction
- *Trichuris*: common, symptoms only in severe infection (diarrhoea, anal prolapse)
- *Enterobius*: common, anal itch, exogenous auto-infection
- Hookworms: common, lung passage, anaemia if worms are numerous
- *Strongyloides*: common, chronic, larva currens, lung passage, endogenous re-infection, fatal hyperinfection
- *Capillaria philippinensis*: rare, diarrhoea, endogenous re-infection, sometimes fatal

The first five species are currently aggregated under the term “soil-transmitted helminthiasis” (STH).

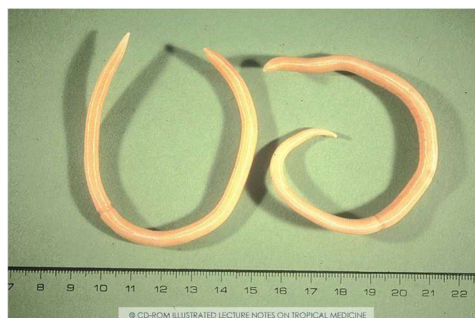
More than a billion people are infected with a least one species.

## *Ascaris lumbricoides*

### Summary

- A very common parasite, 15 to 40 cm long – jejunum (small intestine)
- Lung passage may cause transient asthma-like symptoms
- Generally atypical symptoms, or asymptomatic
- Sometimes obstruction of hollow organs (intestine, pancreas and biliary tract) causing severe complications

## Life cycle



Adult *Ascaris lumbricoides*. ©ITM

Cosmopolitan but much more common in the tropics. The eggs pass on to the ground via the faeces. Fertilized eggs require 10 to 40 days in the outside world to mature before they become infectious.

Direct self-infection is thus ruled out. Once they are mature the eggs are taken up once more (faecaloral transmission) via contaminated food, drink (fluids), dirty fingernails or hands. In the intestine small larvae emerge from the eggs and these bore through the intestinal wall. In this way they reach the blood (portal vein system). They are carried with the blood, through the liver to the lungs. Lung passage occurs 3 to 14 days after ingestion. In the lungs the larvae make their way to the bronchial lumen and ascent via the respiratory branches into the throat. They are subsequently swallowed and in this way they again reach the intestine. They grow into adult worms in the jejunum. They do not damage the intestinal wall. Adult worms do not

multiply in the human host; the number of adult worms in an infected individual depends on the degree of exposure to infectious eggs over time. Egg laying begins two months after infection when both female and male worms are present in the intestine. Each female worm produces approximately 200,000 fertilized eggs per day. The adult worm survives on average for 1 year. The creatures reach 15 to 40 cm, making them the largest nematode parasitizing humans.

There is no animal reservoir. Occasionally infections with *Ascaris suum* occur (parasite of pigs); this worm resembles *Ascaris lumbricoides* very closely and some think the parasites are identical.

## Epidemiology

This is the most common worm infection in humans. It has a cosmopolitan distribution. Children are most often infected. The eggs are very resistant, which makes it possible in certain circumstances for them to survive for a long time in the outside world (years). The number of eggs which can be found in the soil is a measure of the hygiene standard and degree of sanitation of an area (faecal pollution of the ground).

## Clinical aspects

The vast majority are asymptomatic. Any illness caused by worms depends to an important extent on the number of parasites. The total worm load is only increased by repeated exposure (exceptions are *Strongyloides stercoralis* and *Capillaria filippinensis* which can multiply inside the human body). Some people have various forms of intestinal discomfort or allergic symptoms. Serious complications are rare. Nevertheless, in view of the large number of infected persons, the morbidity and mortality should not be disregarded.

### *Lung passage symptoms*

The larvae undergo lung passage. This produces rarely symptoms of mild to severe cough, dyspnoea, thoracic pain and sometimes fever. The clinical picture is similar to asthma or pneumonia. On chest Xray migratory infiltrates are rarely observed. Eosinophilia is present. This whole phenomenon is called "Loeffler's syndrome". The sputum contains many eosinophils, Charcot-Leyden crystals and sometimes also larvae. The symptoms last for some days or max. 2 weeks. Most of the time this goes unrecognized.

### *Obstruction of, or migration in, hollow organs*

- When numerous adult worms are present they may form a tangle and cause mechanical intestinal obstruction manifested by a bloated abdomen, increased peristalsis with clangor, colicky pain, vomiting (bile, faecaloid) and dilated intestinal lumen on an abdominal X-ray.
- Migration into the biliary tract may lead to biliary obstruction (cholestasis) with possibly infection (e.g. cholangitis, liver abscess, pancreatitis).
- Sometimes there is migration to the appendix with inflammation (appendicitis).
- Sometimes an adult *Ascaris* is present in vomitus.
- Occasionally an adult can penetrate the lacrimal duct.
- Recent surgical intestinal sutures can be breached by an invasive adult *Ascaris*, leading to bowel perforation and peritonitis. Pre-operative deworming is advised in endemic areas.
- Infection with *Ascaris lumbricoides* also plays a role in the development of pigbel (clostridial

necrotizing enteritis, an often fatal type of food poisoning caused by a  $\beta$ -toxin of *Clostridium perfringens*; see chapter on diarrhoea).

### Malnutrition

*Ascaris* itself does not cause malnutrition. In borderline malnutrition the presence of numerous worms can have a negative effect, however. It is also important to know that many patients suffer from anorexia. On a population level the mass treatment (deworming) has a positive influence on the cognitive development in children.

### Diagnosis

Since an adult female lays up to 200,000 eggs per day, as a rule no concentration technique is necessary to detect eggs in the faeces. If infection is solely with one or more male worms then no eggs will be detected. Stool concentration methods for detection of *Ascaris* eggs (rarely needed in endemic areas) include Kato-Katz and FLOTAC techniques like for other intestinal worms. Charcot-Leyden crystals, which consist of lysophospholipase, an eosinophil-derived enzyme, may be seen by microscopic stool examination.

During lung passage there is significant eosinophilia. After lung passage there is no longer appreciable eosinophilia. Sputum analysis may demonstrate eosinophils and Charcot-Leyden crystals.

X-ray of the intestine with barium contrast may show one or more adult worms. The worm forms a long, thin dark area. Sometimes a central longitudinal radio-opaque line can be seen; this is the intestinal tract of the worm. Such a line is absent in tapeworms.

An ultrasound of the pancreas (Wirsung duct) or of the biliary tract and gallbladder may show an ectopic migrating adult *Ascaris*.

### Treatment

Mebendazole (Vermox®): 100 mg BD x 3 days, effective, broad spectrum (or 500 mg single dose)

Albendazole, 400 mg single dose effective, broad spectrum

Ivermectine: similar efficacy (single dose 200  $\mu$ g/kg) as single dose albendazole

(Flubendazole (Fluvermal®): 100 mg BD x 3 days, effective, narrow spectrum; Piperazine (Adiver®): narrow spectrum; Pyrantel pamoate/ oxantel (Antiminth®, Combantrin®) can be used in pregnancy; Levamisole; Tribendimine; Nitazoxanide)

Pulmonary manifestations can be treated with bronchodilators or if severe with systemic corticosteroids if *Strongyloides stercoralis* infection is ruled out.

*Drug resistance*



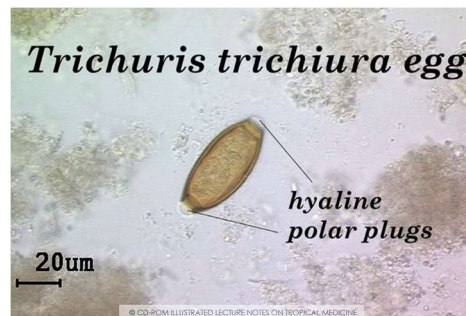
Benzimidazole drugs bind to nematode  $\beta$ -tubulin and inhibit parasite microtubule polymerisation. Drug resistance against front-line anthelmintics is widespread in nematodes of livestock due to frequent treatment of animals. Therefore, the effectiveness of drugs must be closely monitored in regions where mass anthelmintic chemotherapy is administered.

## *Trichuris trichiura*

### Summary

- Adult worms measure approximately 4 cm (sometimes seen in stool) – colon
- Faeco-oral transmission via eggs.
- Generally asymptomatic
- In severe infections diarrhoea and sometimes anal prolapse
- Role in bacterial dysentery or invasive amebiasis?

### Life cycle



*Trichuris trichiura* egg with its typical polar caps, suggesting a lemon-shape. Copyright ITM



*Trichuris suis*, related to *Trichuris trichiura*, a nematode which frequently infects humans. Copyright ITM

*Trichuris trichiura* is a cosmopolitan nematode, but is rare in subarctic areas. This is an ancient parasite and this is demonstrated in that it also occurs outside the tropics is that eggs were found in Ötzi the iceman, a bronze-age mummy discovered in the Italian Alps, and in coprolites (fossilized faeces) in prehistoric salt mines in Austria.

The eggs are eliminated with the faeces. Infection is via the oral route, after obligatory maturation in the outside world. Eggs embryonate in the external environment for 10-30 days, depending upon temperature: slower when colder; no development above 37°C. It is possible that in nature (as opposed to the lab) much longer periods are possible. Many eggs remain viable in the soil for longer than a year; depending upon local humidity. In Bangladesh, a study of 2400 houseflies discovered that 47% of the insects were carrying eggs (flies acting as mechanical transport vectors).

The embryonated eggs hatch after ingestion. It is likely that the hatching worm dissolved the polar caps with enzymes. The fate of the larvae after hatching the first 5-10 days is controversial. No studies have been done on humans. Serial necropsy of dogs infected with *T. vulpis* suggest that larvae first penetrate the mucosal duodenal epithelium, re-emerge into



the lumen 8-10 days later and settle in the caecum. However, this data is questioned and it is unclear if this can be generalized to human infections. More study is required to answer some basic questions.

Larvae will undergo four molts. Egg laying begins about 2 months after infection. Experimental infection in human volunteers showed a somewhat longer prepatent period of 120-130 days. It is estimated that 5-22% of ingested embryonated eggs develop to patency. A female worm measures 35 cm and sheds between 3000-20,000 eggs per day. Since the uterus of a female worm contains approximately 60,000 eggs at any one time, this implies that between 5 and 30% of the eggs have to be replaced on a daily basis. The adult worm has a thin whip-like head with which it buries itself in the mucosa of the large intestine especially the caecum. The worm survives for 1-4 years on average, although extremes of 20 years are known.

### Clinical aspects

Most infected humans remain asymptomatic. Only in severe infections (> 1000 worms; >10,000 eggs per gram of faeces) do symptoms occur: these include diarrhoea (dysentery type), malnutrition or anaemia. In undernourished children with chronic diarrhoea and tenesmus there is sometimes prolapse of the rectum, in which the worms can be seen on the prolapsed mucosa.

### Diagnosis

Diagnosis is based on faecal examination. No concentration technique is necessary for clinically relevant infections. The Kato-Katz technique can be used to quantify egg numbers. Sometimes the worms can be seen on the rectal mucosa (rectoscopy or during anal prolapse). Normally there is no eosinophilia (since there is no larval migration).

### Treatment

- Mebendazole 100 mg BD x 3 days, or 500 mg single dose (but less active: 65-70%)
- Albendazole 400 mg BD x 3 days (for cure rate above 90%)
- Ivermectine is also less active
- The combination treatment albendazole plus oxantel pamoate showed higher cure rates and higher egg reduction rates than mebendazole or albendazole alone.
- The new tribendimidine drug has limited activity

## Hookworms

### Summary

- Blood-sucking worms 1 cm long (but never seen in the stool); in the jejunum
- Transmission by larvae: transcutaneous and oral
- Brief local itch after skin penetration, lung passage (but very rarely noticed)
- Generally asymptomatic
- In severe infection iron deficiency anaemia

### Life cycle

There are two important hookworms: *Necator americanus* and *Ancylostoma duodenale*. [L. necator = murderer; Gr. ancylo = hook, stoma = mouth]. There are a few zoonotic hookworms which are of much less clinical importance and seldom cause infections in humans (e.g. *Ancylostoma ceylanicum*, *A. caninum*, *A. malayanum*, *Cyclodontostomumpurvisi*). The adult worms are found in the small intestine. It is estimated that the life span of adult worms is 5 to 15 years. *Necator* lives longer than *Ancylostoma*.

The adults measure approximately 1 cm. A few weeks or months after infection eggs can be found in the faeces. Once the eggs arrive in the outside world with the faeces, they take one week to mature to infectious larvae. At first they are rod-shaped = rhabditiform, later thread-shaped = filariform. They may survive for weeks or months (at an optimal temperature and humidity for as much as 2 years). A soil with neutral pH is optimal for their development, as is shade and a sufficiently high temperature (23°C to 30°C is ideal). If the faeces mix with urine the eggs die. Frost, direct sunlight and a soil saturated with salt or water are unfavourable conditions.

Infection occurs via the mouth (*A. duodenale*) or via the skin (*A. duodenale* and *N. americanus*). If they enter through the skin, the young parasites have to pass through the lungs. A new dimension in the epidemiology of hookworm disease emerged when it was found that insufficiently cooked meat from paratenic hosts (= an intermediate host in which no development of the parasite occurs) such as pigs, cattle, rabbits and sheep can be responsible for transmission.

The adult hookworms bore a hole in the mucosa of the duodenum and the small intestine and suck blood.

They adhere with hooked teeth in their mouth (*Ancylostoma*) or with two buccal cutting plates (*Necator*). *A. duodenale* sucks 5 to 10 times more blood than *N. americanus* (approximately 30 µl per day for *Necator* and 260 µl for *Ancylostoma*). Blood loss is caused primarily by parasite release of anticlotting agents -anticoagulant peptides that inhibit activated factor X and factor VIIa/tissue factor complex and that inhibit platelet activation- which causes continuous blood loss in the stool and only secondly due to actual blood consumption by the worm.

## Clinical aspects

At the site where the hookworms penetrate, the skin may rarely develop a rash and itch (called "ground itch"). This is short-lived and rarely noticed. Lung passage also rarely produces symptoms, but may be accompanied by Loeffler's syndrome. There are few intestinal symptoms. When infection with *A. duodenale* occurs by the oral route, the early migrations of third-stage larvae cause a syndrome known as Wakana disease, which is characterized by nausea, vomiting, pharyngeal irritation, cough, dyspnoea and hoarseness. Significant infections (>1000 worms) may result in pronounced anaemia. The haemoglobin level may sometimes be very low. Children and pregnant women in whom the iron supplies are already low, are particularly affected. Hypoproteinaemia may also occur and results in oedema and anasarca. Protein deficiency also has consequences for the production of immunoglobulins. Some patients exhibit geophagia. In history, certain regions in the USA were famed for their "quality" clay and people would cover great distances to eat this iron-containing soil.

### **Differential diagnosis:**

Differentiation from *Strongyloides* larvae is based chiefly on the difference in morphology of the "head" end. The mouth is elongated in ancylostomes and shorter in *Strongyloides*. Sometimes, if intestinal transit has been swift eggs of *Strongyloides stercoralis* may be found in the faeces. These too should be differentiated from hookworm eggs.

## Diagnosis

The eggs are found in fresh faeces. In an old stool (>24 hours) the eggs will have hatched and rhabditiform larvae can be seen (Gr. rhabdos = rod). There is mild eosinophilia. Since an adult hookworm lays approximately 25,000 eggs per day, as a very rough estimate 100 eggs per gram of faeces corresponds to 1 adult worm. The Kato-Katz concentration technique can be used to estimate the number of eggs per gram of faeces. The eggs of *N. americanus* and *A. duodenale* are morphologically indistinguishable.

Eggs of *Oesophagostomum* are morphologically identical to those of hookworms. Identification of the latter parasite can only be made by coproculture (identification of the typical stage 3 larvae).

## Treatment

- Mebendazole 2 x 100 mg/day for 3 days. Also give iron supplementation and folic acid in anemia.
- Albendazole may be used in treatment (400 mg single dose) and is generally effective.
- Pyrantel 10 mg/kg for 3 days or levamisole 2.5mg/kg once or twice (less used nowadays)
- *Necator* and *Ancylostoma duodenale* are less sensitive to ivermectin (cure rate around 30%).
- Tribendimidine has a promising activity on hookworms

## Prevention

Mass chemotherapy together with health education and sanitary provisions are strategies which are often used for morbidity control. The most heavily infected individuals are the chief target group.

There are however increasing concerns about long-term sustainability. Wearing footwear only partly prevents infection because oral infection is also important for *Ancylostoma duodenale*. Children are the main victims as they rarely wear shoes and their whole skin is a portal of entry.

### **Cutaneous larva migrans**

Some larvae from animal hookworms may penetrate human skin, but do not migrate deeper to the underlying tissues and organs. Their cycle thus reaches a dead end in the skin. Examples are the hookworms of dogs and cats (*Ancylostoma braziliense*, *Ancylostoma caninum*) and animal *Strongyloides* species. The migration of these larvae causes very itchy red lines on the skin which slowly move about (i.e. creeping eruption). A single oral administration of 12 mg of ivermectin (or albendazole 400 mg x 5 days) is effective.

## *Strongyloides stercoralis*

### Summary

- Infection with small worms 3 mm long (never seen in the stool) – small intestine
- Transmission by larvae is transcutaneous or oral
- Importance of endogenous re-infection and multiplication, which lead to very long-term infections
- Hypereosinophilia, larva currens with itch, chronic lung problems
- Hyperinfection in immunosuppression with steroids, HTLV-1

### Life cycle

The adult female worm is found in the mucosa of the small intestine. Males cannot penetrate the intestinal mucosa and perish. Reproduction is asexual via parthenogenesis (=development of an embryo from an unfertilized egg cell). The females lay eggs after 2-3 weeks, from which larvae are quickly produced. Initially the larvae are described as rhabditiform. These quickly develop into filariform (infectious) larvae. These larvae may:

- either penetrate back into the intestinal mucosa (*Strongyloides* is one of the rare worms which can multiply in the human body).
- or pass to the perianal skin and from there again penetrate the body (auto re-infection). In auto reinfection there is always another lung passage. In this way an infection with *Strongyloides* may persist for a very long time (more than 30 years).
- or pass to the outside world with the faeces. From there after molting, they may go in either of two directions. The larvae either again penetrate the skin of a human (sometimes even via the mouth) or they develop to adult worms in the outside world. They may then via sexual reproduction in their turn lay eggs, from which new larvae develop. The worm can thus survive without a host.

### Clinical aspects

Mild infection is generally asymptomatic. In severe infections there may be intestinal discomfort or diarrhoea. During lung passage symptoms may occur depending on the number of larvae. Auto reinfection via the skin may give sometimes rise to significant itching, chiefly peri-anal. Migration of the larvae in the skin leads to itching red swollen lines (on the rump, arms, face, etc.). These lines may occur anywhere and progress swiftly (up to 10 cm per hour). The swelling is the result of an urticarial reaction to the migrating larva (the larva itself is only 0.2 mm long). These lesion disappear spontaneously a few hours later, to reappear once more at a different site and this rather typical symptom is called “larva currens” (observed at some moment in about 20% of infected individuals).



*Strongyloides stercoralis*, larva currens. Such recurrent migrating linear urticarial stripes are pathognomonic for infection with this parasite. Copyright ITM

Immune suppression (especially HTLV-1 infection), achlorhydria (low gastric acid secretion), haematological malignancies including lymphoma, nephropathy, transplant patient taking immunosuppression (cyclosporine, tacrolimus), cytotoxic medication but especially the long-term use of systemic corticoids, all increase the risk of hyperinfection. In such cases there is extensive multiplication with spread of the larvae to all organs (including the brain) due to a dysfunction of the Th2 helper cells. Symptoms include purpura-like skin lesions (initially often peri-umbilical), severe diarrhoea, pulmonary symptoms (dyspnoea, bronchospasms, bloody sputum) and meningoencephalitis. Hyperinfection with *Strongyloides stercoralis* may be accompanied by bacterial septicaemia (with usually Gram-negative bacteria). Mixed infection may occur. This probably depends on mechanical damage to the colon wall; adhesion of intestinal bacteria to the outside of migrating larvae and excretion of bacteria from the intestinal system of the parasite. Hyperinfection has a high mortality (75%). In chronic and persistent infection an underlying infection with HTLV-1 or use of glucocorticoids should be considered. There have been fewer hyperinfections in AIDS patients than one would expect at first sight.

## Diagnosis

The eggs hatch very rapidly in the intestine and are often not found in a faecal specimen. Larvae are found in the faeces. Often the numbers are not so high and specific concentration techniques, e.g. the Baermann method or modified agar plate method need to be used. In general, the diagnosis of *S. stercoralis* infection is difficult in the tropics as well as in travellers. Larvae can also be detected via duodenal intubation. Differentiation from hookworm larvae is necessary. Eosinophilia is almost always present, except when immune suppression exists. A history of larva currens is suggestive of strongyloidiasis and is enough to start treatment even if no larvae are found in the faeces. In hyperinfection larvae may be found in the sputum or in broncho-alveolar lavage fluid. The sputum must be regarded as infectious. If this sputum is cultured on blood agar, bacterial colonies can be seen which form a curvilinear pattern, reminiscent of a pearl necklace. This follows the migration of a larva on the agar plate, with translocation of the bacteria.

PCR on a stool sample is the most sensitive test, but is not widely available.

An ELISA test detecting IgG to filariform larvae in serum, can be used in immunocompetent hosts.

However ELISA results can be falsely negative in immunocompromised hosts and in acute infection (as seen in travellers) during the window period. Cross-reactivity may occur in the presence of other helminth infections. As a whole serology is widely used in travel medicine to diagnose (past) exposure to *Strongyloides* but is almost nowhere available in the tropics.

## Treatment

- Thiabendazole was used in the past, but had many side effects. Albendazole (400 mg twice daily for 3 to 7 days) is moderately effective. Mebendazole is not active.
- Ivermectin PO (200 µg/kg single dose) is easy to use and effective and at present is the first line treatment. Some experts recommend a second course after 1-2 weeks (an RCT is ongoing to answer this question). If immunosuppression is present, the cure rate with ivermectin is lower, certainly if cortisone has been taken. In such cases, successive courses of treatment should be administered. It should be mentioned that there are parenteral ivermectin formulations for veterinary use. They are not (as yet) registered for use in humans, but anecdotal case reports mention success with them.
- In hyperinfection it is important not to forget to use antibiotics, in view of the risk of severe septicaemia.

## Enterobius vermicularis

### Summary

- Cosmopolitan distribution
- Humans are the reservoir of this 1 cm long worm
- Ileocaecal region → Anal region: anal itch

### Life cycle

This parasite is cosmopolitan. There is no intermediate host. Infection is via ingestion of eggs e.g. by eating food touched by contaminated hands or by handling contaminated clothes or bed linens. Eggs accumulate in the ileo-caecal region. After copulation the males die. The females migrate via the colon to the anus and lay their eggs chiefly at night as they creep over the peri-anal skin. This explains the nightly itching. Self-infection occurs by transferring infective eggs to the mouth with hands that have scratched the perianal area. Retro-infection or the migration of newly hatched larvae from the anal skin back into the rectum, may also occur. In rare cases there is vaginal itch because the females can also hide there. Sometimes the parasites are found in the appendix. The eggs must be sought not only in the faeces, but also on the peri-anal skin (using Scotch tape or other transparent sticky tape). In women the eggs may be found in the urine due to contamination. Apart from the itch there are few problems. There is a possible association between infection with *Enterobius* and infection with the possibly pathogenic amoeba flagellate, *Dientamoeba fragilis*. A hypothesis is that *Enterobius vermicularis* serves as a vector for *D. fragilis*, as *D. fragilis* DNA has been detected within surface sterilized eggs of *E. vermicularis*.

### Treatment

- Mebendazole 100 mg (Vermox®), to be repeated after 1 and 2 weeks. Albendazole is also effective.
- Ivermectin 12 mg single dose, to repeat after 2 weeks
- Pyrantel pamoate 10 mg/kg base once (max. 1 g); to repeat in 2 weeks
- Vanquin® (pyrvinium) may also be used as an alternative to mebendazole. The faeces may discolour red.

Since the eggs can adhere to all objects e.g. underclothing, sheets and so on, these should be changed. In a family it is best to treat all the family members, even those without symptoms.



## Capillaria philippinensis

### Summary

- Infections with *Capillaria philippinensis* are rare, but potentially fatal
- Transmission by eating infected fish
- Endogenous multiplication resulting in chronic malabsorption and diarrhoea

### Life cycle

*Capillaria philippinensis* is a nematode which causes severe infections. The parasite was discovered in 1960 in Luzon, an island in the Philippines. Subsequently it was also found in Thailand, Indonesia, Egypt, Japan, Taiwan, Korea and Iran. It is a parasite of fish-eating waterbirds. The infection occurs due to eating infected fish which live in fresh or brackish water. The larvae are found in the muscles of the fish.

It is an intestinal nematode which has an intermediate host (most nematodes don't). After developing to adult forms the parasites, which are 2 to 4 mm long, live in the mucosa of the small intestine. The worm is capable of multiplication in the human intestine (cf. *Strongyloides*). This phenomenon may lead to severe infection (high worm load). The incubation period can be very long (many months). Chronic watery diarrhoea, malabsorption and cachexia follow. The diarrhoea can be high volume (several litres per day). Ascites, pleural fluid and severe electrolyte imbalance including hypokalaemia may occur. The infection is sometimes fatal if not treated in time.

### Diagnosis and treatment

Diagnosis is made by means of faecal examination. Often it is necessary to analyse multiple stool samples before eggs are found. Intestinal biopsy can show worm fragments. Every infection must be treated promptly with mebendazole, 200 mg x 2 per day for 20 days or albendazole x 10 days. Cooking fish prevents the infection. Eating raw fish is a culinary habit in many Asiatic countries and this is difficult to change.

## ***Oesophagostomiasis***

Nematodes of the genus *Oesophagostomum* (*O. bifurcum*, *O. aculeatum*, *O. stephanostomum*) are widely distributed intestinal worms of monkeys. In some regions humans are accidental final hosts. Foci of *Oesophagostomum bifurcum* infections occur commonly in parts of West Africa (Northern Ghana and Togo) with very high prevalence in some villages. The eggs are morphologically identical to those of hookworms. The larvae develop when the eggs land on the ground, progressing through stages 1-3 in 5 to 7 days. Probably a number of stage 3 larvae can resist long periods of dehydration. Stage 3 larvae are swallowed with food or water and penetrate the human intestinal wall. They then develop further inducing abscesses with a necrotic content (helminthoma). The worms may cause severe intestinal lesions, including eosinophilic granulomas in the intestinal wall (mostly caecum) and mesentery, deep abscesses and peritonitis. Epigastric or periumbilical masses may result.

As soon as the worms become adult they return to the intestinal lumen where they attach to the mucosa and mate. Adult worms in the intestinal lumen do not cause illness. In veterinary medicine the illness is known as "pimply gut" which refers to countless abscesses under the serosa.



Oesophagostomiasis with intestinal abscess spreading to the abdominal wall. Photo prof Gigase.

## Anisakiasis

Adult *Anisakis simplex* have been found in the stomachs of whales, seals, sea lions, walrus and dolphins. Humans are incidental hosts and the human "equivalent" of anisakiasis for sea animals is ascariasis. The eggs are eliminated with the faeces. In sea water the eggs hatch after embryonation after which the released larvae penetrate small crustaceans e.g. copepods or krill, which then in turn are eaten by fish or cephalopods. *Anisakis* larvae are usually restricted to the fish viscera in vivo only infesting the muscles after the fish has been killed, particularly if the fish is not promptly gutted and cleaned after its death. Humans become infected by eating undercooked or raw infected marine fish.

The parasites which measure about 2-3 cm in length attach themselves to the gastric or intestinal mucosa by their anterior parts as far as the muscularis mucosa. This makes them visible during endoscopy.

In humans the parasites do not reach the adult stage and usually die off spontaneously after 3 weeks. The dying organism induces an inflammatory reaction and a tissue abscess develops with a predominance of eosinophils. **Gastric pain and nausea/vomiting** may occur within a few hours after eating infected fish or cephalopod but symptoms may have a late onset with abdominal pain appearing up to three weeks later. Late manifestations have rarely been described (several weeks to months) and are due to more distal intestinal infections. The infection is sometimes confused initially with appendicitis, stomach ulcer, duodenal ulcer, stomach cancer or Crohn's disease. Rarely the worms perforate the intestinal wall and are found in the peritoneum. Eosinophilia is present. Approximately 95% of all cases in the world, which amounts to some 2000 cases annually, occur in Japan. Many different species of *Anisakis larvae* are being recognized as the cause of urticaria and hypersensitivity reactions after eating fish. The worm can in fact trigger quite dramatic hypersensitivity reactions even after it is dead. The first signs of an allergic reaction usually occur 60-120 minutes after ingestion, but may be delayed for up to 6 hours later probably due to passage of the food bolus through the gastrointestinal tract. This means that urticaria and angio-oedema may occur at night. The diagnosis of allergy to *Anisakis simplex* is based on (1) a compatible anamnesis such as urticaria or angio-oedema after consumption of saltwater fish, (2) a positive skin prick test, (3) specific IgE against *Anisakis simplex* via radio-immunoassay, (4) negative reactions to the proteins of fish. There are some people who have antibodies to *Anisakis* without ever having exhibited symptoms.

Therapy of anisakiasis consists of mechanical removal by means of surgery (in case of intestinal obstruction) or endoscopic extraction. Ivermectin and albendazole therapy has been suggested.

Thorough cooking to 70°C or adequate freezing to -20°C for a minimum of 72 hours are the best preventive measures.

## Tissue nematodes

### *Trichinella* sp

#### Summary

- Trichinellosis = Trichinosis
- *Trichinella*: adult worm in intestinal wall (not in the lumen), larvae in muscles and heart
- Transmission by eating infected meat, so there is never a free-living parasite
- Hypereosinophilia, fever, muscle pain, oedema chiefly peri-orbital
- Faeces negative for parasites (no eggs)
- Muscle biopsy positive for larvae
- Filaria are part of tissue nematodes

#### Historical note

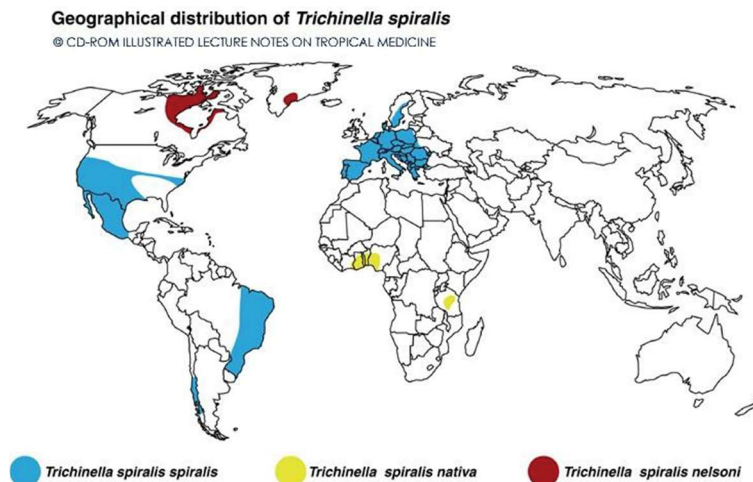
In 1835 a 51-year-old Italian bricklayer died of tuberculosis in St Bartholomew's Hospital, London. Jim Paget, a first-year student (later of "Paget's disease" or osteitis deformans fame), was present during the autopsy and observed fine hard white inclusions in the diaphragm. Similar inclusions had been observed by doctors from time to time in the past but were attributed to commonplace muscle calcification, which quickly blunted the dissecting scalpel. Paget inspected the lesions with a hand lens and with a compound microscope in the British Museum. At the time there was only one such instrument in the entire Museum and it belonged to Robert Brown, of "Brownian motion" fame. Paget quickly recognised their worm-like structure and wrote of his discovery to his brother. After the word got out, surgeon Thomas Wormald took a second piece of the "sandy" diaphragm to Richard Owen, at that time assistant conservator of the Hungarian collection in the museum of the Royal College of Surgeons. He later became a major figure in comparative anatomy and paleontology, coining for example the name "Dinosauria".

He published the discovery of the parasite ("a microscopic entozoon", but didn't give the proper credit to Jim Paget. The name "*Trichina spiralis*" was suggested. This name *Trichina* had already been given to a fly, and the name was later changed to "*Trichinella*". In 1846, the American Joseph Leidy found *Trichinella* larvae in the pork he had for dinner. He hypothesized that trichinosis is caused by consuming undercooked pork. In 1859 Rudolph Virchow carried out transmission experiments in which infected human muscle was fed to a healthy dog. After only 3 to 4 days adult *Trichinella* worms were found in the dog's duodenum and jejunum. He also discovered that heating the meat for 10 minutes was enough to stop transmission.

He started to spread the message that eating raw or lightly smoke-cured ham was dangerous. In Germany, where sausages were an important part of the daily diet, he provoked resistance from the German Veterinarian's Society. At a public meeting when denounced by a veterinarian, he showed the public an infected piece of ham and challenged his opponent to dare to eat it. In front of the crowd the veterinarian wisely declined the offer. Virchow's reputation grew quickly after this incident. When later challenged to a duel by Baron von Bismarck, he chose infected sausages as his weapon. The Baron declined to eat them after hearing what trichinosis was but the two men became friends later on. These days, Virchow is mainly remembered as the father of cell theory ("omnis cellula e cellula" or every living cell comes from another living cell).

PS. Robert Brown died in 1858, just before Charles Darwin received news from Alfred Russel Wallace's independent discovery of the role of natural selection in the evolution of animals and plants. It was Brown's death that provided the vacant slot in the Linnean Society's programme that allowed Darwin (spurred on by Wallace's findings) to describe his theory (and Wallace's) in public.

## General



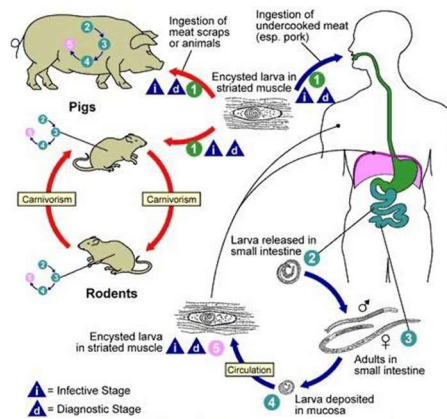
Map showing the areas endemic for the different subspecies of *Trichinella spiralis*: *Trichinella spiralis spiralis*, *Trichinella spiralis nativa*, *Trichinella spiralis nelsoni*. Copyright ITM

Trichinosis or trichinellosis is a zoonosis. It refers to infection with the larval and adult stages of a group of closely related nematodes which belong to the genus *Trichinella*. The infection is meat-borne.

Typically pork is implicated. Other meat sources such as horses and wild game, certain birds and even reptiles increase in significance as more study results are becoming available. Carnivores and omnivores represent the most important reservoirs.

### *Trichinella species*

There are 9 *Trichinella* species. All species can develop in mammals and some in birds or even reptiles. The parasites are widespread on all continents except Antarctica. *T. spiralis* occurs in temperate regions and infects mainly pigs. *T. nativa* occurs in the arctic and subarctic areas in terrestrial and marine carnivores (e.g. polar bear, walrus). These parasites are resistant to freezing which is important for meat storage. *T. britovi* occurs in temperate areas of the Palearctic region, as well as North and West Africa. *T. spiralis nelsoni* occurs in Africa and southern Europe with a reservoir in wild carnivores and wild pigs.



*Trichinella spiralis* life cycle. Courtesy of CDC, Division of Parasitic Diseases

More than 100 species of mammals are susceptible to the infection. By and large pigs and horses seem to be responsible for the majority of human infections. Horses are considered herbivores, but 32% of horses tested ate meat when offered. The feeding of animal products to horses is a practice that occurs in several countries. Eating walrus meat plays a role in the arctic. On a global scale *T. spiralis* is responsible for the majority of human infections. Rats play an important part in the transmission in pig-raising areas. It is unclear if they form a true reservoir. The use of rat pesticides can actually augment transmission as poisoned rats are easy prey for pigs.

Gravid female worms embedded in the intestinal mucosa release newborn larvae. These larvae measure about 100  $\mu\text{m}$  by 6  $\mu\text{m}$ . These immature larvae are extracellularly exposed to the humoral immune system. The larvae migrate to the intestinal lymphatics, then enter blood vessels and subsequently penetrate striated muscle cells. Then something strange happens. After entering the muscle cell, the larvae are completely intracellular. This is unique. They will convert their host cell into a so-called nurse cell. Their metabolism is mainly anaerobic, which helps their survival after the death of the host. In the muscle cells, larvae can survive several decades. They are now called infective larvae and are visible with low magnification. Larvae do not mature or become encapsulated in heart muscle.

When a new host ingests muscle tissues, the larvae are released in the stomach by digestion. In the duodenum they penetrate the villi and undergo 4 molts, developing into adults which measure about 1 mm (males) to 3 mm (females) with a thickness of about 30  $\mu\text{m}$ . Males and females copulate and 6 to 7 days post-infection, the females start to produce new-born larvae. This continues for a few weeks according to the immune response of the host. Afterwards adults are expelled. It is extremely rare to find an adult worm in a human patient.

## Clinical aspects

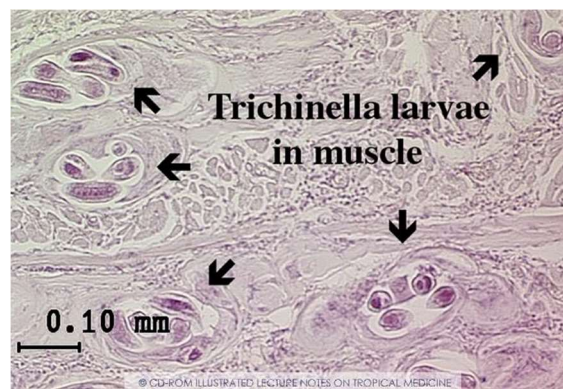
Light infections may be asymptomatic. About 70 live larvae are sufficient to provoke clinical disease. In more typical cases there is nausea, non-bloody diarrhoea, abdominal pain, vomiting and fever; a few days after eating infected meat. After 10 days the fever tends to increase. The patient is very ill, asthenic and debilitated, there are muscle pains and a typical peri-orbital oedema (differential diagnosis acute trypanosomiasis, angioedema, gnathostomiasis and nephrotic syndrome). This oedema is caused by invasion of the small muscles around the eye. In severe cases, oedema extends to arms and legs. Conjunctival and

subungual haemorrhages may occur (due to vasculitis, not endocarditis). There may be signs of myocarditis, encephalitis, urticaria and asthma. A small number of persons may develop a maculopapular rash after the onset on muscular pain. There is often very significant eosinophilia. This lasts from several weeks to three months.

A massive decrease in eosinophils in persons with severe trichinellosis predicts a severe outcome. Myositis causes an increase in the muscle enzymes (creatine phosphokinase, CK). Wandering newborn larvae can become trapped in small blood vessels leading to vasculitis and peri-vasculitis with diffuse or focal lesions in the central nervous system. Aspecific cortical and subcortical lesions (ischemia) can be identified on MRI, and much more rarely, white matter lesions (granulomatous reaction). Severe myalgia generally lasts for two to three weeks.

Dyspnoea is relatively common and is primarily caused by invasion and inflammation of the diaphragm. After a few months the symptoms are reduced or disappear, although asthenia and chronic muscle pain can persist for up to 6 months. Mild infections are self-limiting but live larvae will persist in muscles for years.

## Diagnosis



*Trichinella spiralis* in a muscle biopsy. Copyright ITM

The clinical picture is of a patient with acute fever and myalgia, pronounced asthenia, possibly diarrhoea and a swollen face. Cardiopulmonary, neurological or renal complications may be fatal. The consumption of insufficiently cooked or raw meat can often be found in the patient's history, and this is often game that the patient has hunted (e.g. wild boar) or raw meat eaten in Asian cuisine or the Arctic.

Here it is important to consider the incubation period; one week for severe disease, two weeks for moderate disease, and three to four weeks for benign forms. Sometimes the infection can be traced to infected horsemeat. There is leukocytosis with eosinophilia, although eosinophilia can be absent in immunocompromised persons (renal graft, HIV, chronic myeloid leukaemia). Muscle biopsy should be performed (deltoid muscle or other). An infection is clinically patent in humans when the number of larvae per gram of muscle biopsy is around ten and severe when above hundred. In early stages of infection, histology is more sensitive than trichinelloscopy. The larvae can be seen coiled inside myocytes. There are various serological techniques (e.g. ELISA, Western blotting) for identifying antibodies against *Trichinella* species. Serology is negative during the first days of the febrile phase

(seroconversion during second to fifth week of infection). PCR can be performed in the International Trichinella Reference Centre (Istituto Superiore di Sanita, Rome, Italy). Remember that there will be no eggs in the faeces.

## Treatment

For mild infection symptomatic treatment is often sufficient. In the early stage albendazole (800 mg/day) or mebendazole at high doses can eradicate adult worms in the intestine. Mebendazole is poorly absorbed. Albendazole 800 mg daily for 7-14 days may be used, in combination with high-dose prednisolone. With treatment the duration of the disease may be reduced to one or two weeks.

Pyrantel is sometimes used during pregnancy, but its efficacy is disputed.

## Prevention

- Meat should be well boiled or roasted through.
- Importance of meat inspection. The identification of *Trichinella* larvae in muscle samples is limited to post-mortem inspection of carcasses. Selection of muscles for sampling in meat inspection requires identification of predilection sites in a particular animal, but in low grade infection, distribution of the larvae is not homogeneous. In pigs infected with *T. spiralis*, predilection sites are the diaphragm crus, the tongue and the masseter. The diaphragm of a slaughtered animal is inspected (the piece of muscle of a certain minimum weight is flattened between two glass slides and examined using transillumination). This technique (trichinoscopy) is not so good for *Trichinella pseudospiralis* because it is not surrounded by a capsule and is easily missed. Pooled muscle samples can be inspected with a method which employs artificial enzymatic digestion to free and to look for the larvae.
- Pig food (which may include infected rats) should be boiled for 30 minutes.
- To store pork for 10 days at -25°C is generally impractical in developing countries. In the West meat is sometimes irradiated with high doses of gamma rays, which will kill any larvae.



## *Angiostrongylus cantonensis*

### Life cycle and transmission

In 1938, *Angiostrongylus cantonensis* was discovered in rat lungs by Chen in Canton, China. The first human case description dates from 1945. Recently, the taxonomical position of the worm has changed and *A. cantonensis* has been transferred to the genus *Parastrongylus*, but in this text we will continue to use the generic name *Angiostrongylus*. Infection with *A. cantonensis* is the most common aetiology of eosinophilic meningitis. Angiostrongyliasis occurs primarily in Southeast Asia, throughout the Pacific Basin, including Hawaii, Fiji, Indonesia, Philippines, Japan, mainland China, Taiwan and Papua New Guinea, but also in several Caribbean nations (Bahamas, Cuba, Puerto Rico, Dominican Republic and Jamaica). Occasionally small outbreaks occur.

#### *Final hosts*

A wide variety of rodents are final hosts, primarily in the genera *Rattus* and *Bandicota*. Eggs laid by the female worm hatch in branches of the pulmonary arteries. After hatching, first-stage larvae enter the alveoli, migrate up the trachea, are swallowed and reach the alimentary tract. Subsequently, they are excreted in the faeces of the animal. When a snail consumes these droppings, infection of the mollusk will ensue. Within about two weeks, infective third-stage larvae will appear. When ingested by a rodent, these L3 larvae migrate to the brain via the blood circulation and develop into fourth-stage larvae and then young adults within the next 4 weeks. They migrate to the subarachnoid space, enter the venous plexus, and are carried to their final destination, the pulmonary arteries.

Humans and rats become infected through eating raw slugs or snails, soiled lettuce contaminated with mollusks, eating a carrier ("paratenic") host, such as infected planarians, land crabs or freshwater shrimps. Certain freshwater as well as marine fish can become infected. Inside man, the neurotropic third-stage larvae pass from the intestinal tract to the meninges. They die 1-2 weeks after arriving in the human brain. Adult worms do not occur in humans.

### Clinical aspects

Angiostrongyliasis (infection with *A. cantonensis*, the rat lungworm) has an incubation period of 2-35 days. Symptoms are due to migration of the larvae in the brain and the inflammatory reaction which occurs. The disease presents with acute moderate to severe headache (100%). Besides the headache, patients can complain of eyeball pain. Visual problems can occur, due to involvement of one or more cranial nerves (diplopia, acute strabismus, gaze palsy) or due to migration of the larva into the eye, which can lead to retinal detachment and blindness. Nuchal rigidity occurs in about 66% of patients and Brudzinski's sign is present in  $\pm$  66%. Facial nerve paralysis, transient ataxia, delirium, seizures, cognitive dysfunction, hyperesthesia in various dermatomes and paraesthesia of arms and legs, trunk or face may occur and some symptoms may persist for months, although chronic disease is rare.

Vomiting and nausea are self-limited and stop after a few days. Fever occurs in less than 50% of patients. The disease tends to be more serious in children. The disease is self-limiting. Most

symptoms disappear spontaneously within 4 weeks of onset (range 2-8 weeks). Mortality is less than 1%.

## Diagnosis

Eosinophilia of peripheral blood or CSF is not always present on initial laboratory testing. Pleocytosis may be absent early in the course of infection. Larvae are rarely detected in the CSF. The CSF can be clear or cloudy, but does not contain blood (except in case of a traumatic tap). The absence of focal lesions on CT or MRI-scanning of the brain distinguishes *A. cantonensis* infections from most other helminthic infections of the brain. Immunodiagnosis (ELISA, Western Blot) is possible in some centers.

There is a poor correlation between the serological results of serum and CSF. Since in most cases, larvae will not be recovered in the cerebrospinal fluid and an autopsy will not be performed (the infection is not lethal in general), the diagnosis will be a tentative one, relying on the history, positive serology and exclusion of other causes.

## Treatment

Analgesics are usually needed. Steroids (e.g. prednisolone 60 mg/day x 2 weeks or dexamethasone) shorten the duration of the headache. When performing a spinal tap, the opening pressure is increased in about 60% of patients. Repeated spinal taps to reduce the intracranial pressure are sometimes performed. Antihelminthics are thought by some not to be effective and considered to worsen the symptoms, probably because of the inflammatory reaction to antigens released by dying worms. Some clinicians use mebendazole or albendazole, but controlled studies are lacking.

## *Gnathostoma sp.*

Nematodes of the genus *Gnathostoma* belong to the *Gnathostomatidae*. At least 13 species have been identified, with 5 recorded in humans. Various species may cause severe infections in humans: *G. spinigerum* (in several geographical areas), *G. doloresi*, *G. hispidum*, *G. nipponicum* (all 3 only in Japan), *G. binucleatum* (only in the Americas). Humans are infected by eating raw or undercooked shellfish, freshwater fish, frogs or chicken. The male worms are 10-25 mm long and the females' measure 25-55 mm. The third-stage larvae, which are responsible for disease in humans, measure about 3 mm. The final host for *G. hispidum* is the pig. The usual final hosts for *G. spinigerum* are dogs and cats. The eggs reach the outside world in the faeces. If they are dropped into water they will hatch 10 days later.

Freshwater copepods (*Cyclops*, belonging to water fleas) are the first intermediate hosts. Fish, amphibians and various mammals may become infected by eating the infected *Cyclops*. There is low host-specificity and humans can also become infected.

The incubation time can be as long as 10 years. The larvae cannot develop into adult worms in humans. They migrate through the body and in doing so may trigger itching, transient subcutaneous swelling with local erythema and possible discrete pain. These symptoms occur after an interval of days to weeks. The swellings are caused by local oedema, necrosis and haemorrhages within the migration path. If the larvae penetrate vital organs (e.g. the brain) the situation may become life-threatening.

Gnathostomiasis is an important cause of eosinophilic meningitis and myelitis. Almost all cases of neurognathostomiasis are reported from Thailand and result from infection with *G. spinigerum*. *Gnathostoma* larvae typically enter the spinal cord along the nerve roots resulting in radiculomyelitis.

The worm can ascend the spinal cord and reach the brain. This journey can take several years. Spinal cord disease result in radicular pain followed by ascending paralysis of legs or quadriplegia with bladder dysfunction and eosinophilic pleocytosis in the cerebrospinal fluid. Diagnosis can be confirmed via serology. Most often ELISA is performed, followed by Western Blot if positive; a positive 24-kD band is nearly 100% specific for gnathostomiasis. Mechanical / surgical extraction of the larva is possible in a minority of patients (11% in one series).

No randomized trials of antihelminthic therapy have been conducted. The treatment is symptomatic and if possible / necessary also surgical. Albendazole 400 - 800 mg daily for 21 days is often used as an etiologic treatment. An alternative is two repeated doses of ivermectin. Corticosteroids have been used to treat cerebral and spinal oedema.

## *Toxocara sp.*

Nematodes of the genus *Toxocara* belong to the Ascarididae. *Toxocara canis* and *T. cati* are parasites which can cause a visceral larva migrans syndrome (fever, hepatitis, pneumonitis, urticarial, eosinophilia) with or without asthma. If they reach the retina, they may lead to chorioretinitis with blindness as a possible outcome. Infection occurs by swallowing an egg that has reached the outside world via the feces of a dog (*T. canis*) or a cat (*T. cati*). A *Toxocara* parasite may become adult and eggs may appear in the faeces of humans in exceptional circumstances (e.g. HIV infection AIDS).

Definitive diagnosis is established via detection of larvae in a tissue biopsy, though symptoms rarely justify such an aggressive diagnostic approach. ELISA is useful for visceral larva migrans, but not for ocular larva migrans. Cross-reactivity with other parasite antigens is common. Treatment is based on steroids and albendazole or diethylcarbamazepine (DEC). Nevertheless the effect of the anthelmintic treatment on the extra-intestinal larvae is limited. Intra-ocular infection resulting from *Toxocara* larvae may lead to traction upon the retina resulting in retinal detachment. Vitreoretinal surgery has a good chance of leading to an improvement in vision in approximately 50% of cases.

## Filariasis

### Summary

Major filariasis

Lymphatic: *Wuchereria* (90%) and *Brugia* (10%)

- Lymphangitis + adenitis + superinfection
- Lymphatic obstruction with hydrocele, lymphoedema and elephantiasis
- Chyluria, intermittent fever and asthmatic syndrome.
- Microfilariae principally at night in blood
- Transmission via mosquitoes

Onchocerciasis: *Onchocerca volvulus*

- Skin nodules, pruritus, eye lesions, swollen lymph nodes, sometimes lymphoedema
- Microfilariae in skin samples (snip, scarification) and eye (anterior chamber)
- Transmission via simuliids

Loiasis: *Loa loa*

- Calabar oedema, subconjunctival migration across the eye, subcutaneous migration
- Microfilariae in blood during the day
- Transmission via fly which bites during daylight

"Minor" filariasis

- Mansonellosis (*M. perstans*, *streptocerca*, *ozzardi*)
- Dirofilariasis (*D. immitis*,...)
- Dracunculiasis (*D. medinensis*)

Filariae are nematodes that live as adults in various human tissues. They do not lay eggs, but constantly produce enormous numbers of larvae (microfilariae) in humans. These are found in the skin or blood.

Human-to-human transmission occurs via insects: the parasites are thus "arthropod-borne". Animal reservoirs play no role of significance in most places, except in subperiodic *Brugia malayi*. Filariasis only exist in warm climates because of the high temperature necessary for the development of the worm in the vector.

There are several species of filariae, but 6 are commonly pathogenic:

- *Wuchereria bancrofti*
- *Brugia malayi*
- *Brugia timori*
- *Loa loa*
- *Onchocerca volvulus*
- (*Mansonella streptocerca*)

There are 2 filariae that are often well tolerated by humans: *Mansonella perstans* and *Mansonella ozzardi*.

The reason for this tolerance is not known; however it should be recognised that not all people infected with these filariae are asymptomatic. Insufficient is known about these parasites.

Dirofilariasis is for the most part only of anecdotal importance and no microfilariae are found in humans.

Dracunculiasis is traditionally included among the filariasis, although there are marked clinical differences between this parasite and the other filariae.

## Lymphatic filariasis

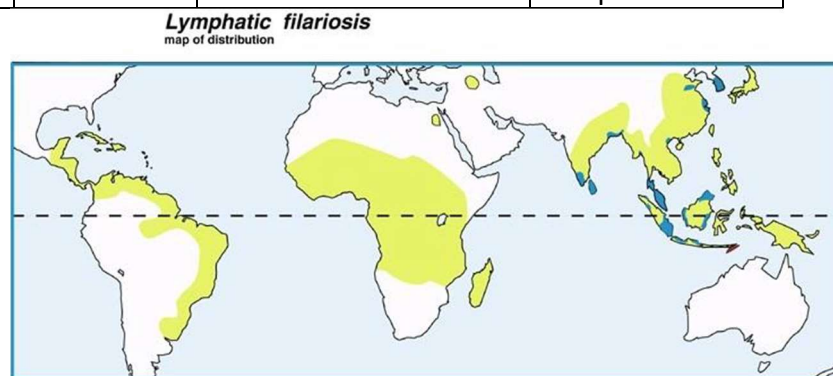
### General

*Wuchereria bancrofti*, *Brugia malayi* and *Brugia timori* cause lymphatic disorders. *Wuchereria bancrofti* is the most widespread of the human filariae in the world. The majority of infections occur in Asia, but this parasite also causes considerable problems in Africa and the north-west of South America. There is a periodic and a subperiodic form.

*B. malayi* occurs in Southeast Asia. There are two forms: a periodically transmitted form (without animal reservoir) and a subperiodic form (animal reservoir in monkeys). Consequently, subperiodic *B. malayi* infection is a zoonosis.

*Brugia timori* is limited to a few islands around Timor.

Parasite	Rhythm	Reservoir	Main vector
<i>W. bancrofti</i>	Periodic	Humans	Culex, Anopheles
<i>W. bancrofti</i>	Subperiodic	Humans	Aedes
<i>B. malayi</i>	Periodic	Humans	Anopheles
<i>B. malayi</i>	Subperiodic	Humans, monkeys, cats	Mansonia
<i>B. timori</i>	Periodic	Humans	Anopheles



@GDPOM ILLUSTRATED LECTURE NOTES ON TROPICAL MEDICINE  
■ *Wuchereria bancrofti* (periodic) ■ *Wuchereria bancrofti* (subperiodic) ■ *Brugia malayi* (periodic) ● *Brugia timori*  
 Lymphatic filariasis, geographical distribution

### Transmission

It became clear that the parasites are transmitted via the bite of infected mosquitoes, primarily by the night-biting *Culex (quinquefasciatus)* and *Anopheles* mosquitoes. This biting behaviour is important as the numbers of microfilariae in the peripheral blood systematically fluctuate over a 24-hour period reaching their highest levels at night. There is a remarkable



periodicity of the microfilariae. The density of parasites is greatest at the time when the chance of transmission is greatest (at night).

*Wuchereria bancrofti* becomes adult in human lymphatics and lymph nodes. The adult female worms are 0.2 mm wide and can be up to 10 cm long. The males are shorter (40 x 0,1 mm). *Brugia* adults are about half this size. They can survive for up to 20 years, but the average life time is 5 years. Approximately 8 months after infection *W. bancrofti* microfilariae appear in the circulation. For *Brugia* sp, this prepatent period is about 3 months.

#### *Historical note*

In 1866, the German doctor Otto Wucherer discovered numerous microfilariae in patients with haematuria and chyluria in Bahia, Brazil. In 1872 the Briton, Lewis, in Calcutta discovered that patients with elephantiasis were infected with filariae. Bancroft was the first to discover the adult worm in an abscess and later in fluid which he tapped from a hydrocele during his surgical practice. He was one of the first to suggest that disease was transmitted by mosquitoes, although it was Patrick Manson (1844-1922) who reported the development of filarial embryos in the mosquito. Because microfilariae were periodically detectable in the blood, the Scottish doctor Patrick Manson suspected that night-biting mosquitoes might be responsible for transmission.

### Pathogenesis

The adult worm induces an immunological reaction in humans. The basic lesion is a sterile inflammation around the worm; in and around the lymph nodes and lymph vessels. In the case of lymphangitis, there is often retrograde inflammation (centrifugal spread). This inflammation leads to obstruction of lymph vessels, resulting in temporary lymphostasis and lymphoedema. Following repeated attacks, irreversible damage to the lymphatics occurs with permanent "non-pitting" lymphoedema. Sometimes abscesses occur at the site of dead adult worms. There is also evidence that indicates adult worms can themselves directly attack the lymphatics (irrespective of the immunological response).

In humans with severe symptoms, low or no microfilaraemia is often found, whereas humans with high microfilaraemia often have no symptoms. The reasons for this apparent paradox, that the resulting pathology is caused by the patient's own immunological response to the adult worms. If the reaction is violent, few adult worms and microfilariae survive but considerable inflammation will occur with sequelae. During infection with the filariae the immunological response evolves. Down-regulation can occur and some patients do not produce any interferon-gamma after exposure to parasitic antigen.

This is currently the subject of intense study. It is likely that this influence on the immune system explains the many asymptomatic patients in endemic areas. Both infected amicrofilaemic and microfilaraemic patients display lymphangiectasis on ultrasound or scintigraphy. The adult worms seem to induce multiplication of endothelial cells and dilatation of lymph vessels, even in the absence of inflammation. On the opposite side, when there is a violent immune reaction against the microfilariae, Weingarten syndrome appears (see below).

## Worm load

There is no multiplication of adult parasites in humans so that the worm load and the degree of illness is proportional to the number of infective larvae transmitted by infected insects. The number of insect bites is directly proportional to the duration and intensity of exposure in a filariasis region. In most cases; severe disease is only seen in humans who have lived for a long time in an endemic area. The patient's individual immunological response has a significant role in the development of the various symptoms.

## Endosymbiont

The intracellular *Wolbachia* endosymbiont is apparently an obligate parasite of these worms. These bacteria are related to *Rickettsiae*. Their presence appears to be favourable for the growth and fertility of the nematode. This has become a target for therapeutic intervention, after it was observed that *Wolbachia* is susceptible to tetracyclines.

## Clinical aspects



Severe myxedema as complication of hyperthyroidism. This needs to be distinguished from lymphatic filariasis

The majority of infected people exhibit no or few obvious clinical signs even though they can have microfilariae in their peripheral blood. Although these people are asymptomatic or paucisymptomatic, almost all have subclinical disease with microscopic haematuria or proteinuria, dilated tortuous lymphatics and, in males, scrotal lymphangiectasia. Among the more obviously symptomatic the acute temporary signs and symptoms caused by inflammation should be distinguished from those resulting from chronic lymph tract obstruction.

## Signs of inflammation

Adenolymphangitis: Acute pain and inflammation in one or more lymph nodes (groin, axilla, elbow, neck). This is associated with fever and general malaise. Retrograde lymphangitis often occurs after 4 to 8 hours. There is centrifugal redness, pain and heat over the course of the lymph vessels. Pyogenic lymphangitis proceeds centripetal, not centrifugal. In most cases, the symptoms last 3-4 days. Each episode results in several days of incapacity for work.

Inflammation of testis and spermatic cord: acute pain, swelling and fever. Repeated funiculitis (inflammation of the spermatic cord) results in thickening of this structure.

Filaria fever: Irregular fever often occurs without external lymph node inflammation, as a result of inflammation of the deeper lymphatics and lymph nodes. The fever may recur



irregularly for months or years after the patient leaves an endemic region (observed in 20,000 American military personnel who fought in the South Pacific during the Second World War, an area endemic for *W. bancrofti*).

### Signs of chronic obstruction



*Wuchereriabancrofti filariasis*, elephantiasis of the genitals. Copyright ITM



*Wuchereriabancrofti filariasis*, elephantiasis of the genitals. Copyright ITM

**Hydrocoele:** accumulation of fluid in the tunica vaginalis. Hydrocoele often occurs in orchitis (inflammation of the testis). This is very common in endemic regions. Microfilariae are often found in hydrocoele fluid. Large hydrocoeles can be very inconvenient. Sexual incapacity associated with genital filariasis is a major concern for those infected. Shame, anxiety, sexual problems and social stigmatisation are widespread. In the differential, inguinal hernia is important. Besides the fact that it can be reducible or irreducible (even obstructed), it is not possible to feel above the upper edge of the swelling. A testis tumour, tuberculosis of the epididymis and chronic lymphogranuloma venereum and chronic schistosomiasis also need to be ruled out.

**Lymphoedema and elephantiasis:** Chronic lymphostasis can lead to lymphoedema. The first sign is the loss of contour around the ankles. Later, a reversible pitting oedema appears. After this has turned into non-pitting oedema, the skin will thicken. Lymphoedema is most striking in the legs, scrotum, breasts and arms. The labia and penis are somewhat less frequently affected. If the lymphoedema persists for a long time, elephantiasis can occur. The skin is then markedly thickened and can become wart-like. The oedema is "non-pitting" because there is also a proliferation of connective tissue. The tissue is fibrotic and hard. Recurrent erysipelas (bacterial superinfection) causes the elephantiasis to increase still further. Entry points for bacteria are; fissures caused by athlete's foot, traditional scarifications, insect bites and small scratch wounds. *Brugia* infections mostly cause elephantiasis confined to lower legs and lower arms.

**Lymph leakage:** The rupture of swollen lymphatics into the renal pelvis can cause chyluria (milk-like pale pink urine). This can have an insidious or sudden onset. The prevalence is low. It is often recurrent.

The chyluria is often more pronounced in the morning and after a heavy fatty meal. This sort of fistula can follow a very chronic course. Rupture of lymphatics in the abdominal cavity or thorax results in chylous ascites and chylothorax (chyle = lymph). A protein-rich white fluid is obtained on aspiration.

Lymph leakage into the area of the tunica vaginalis results in chylocoele. Clumping of lymph proteins in the ureters can cause obstruction. Long-term extensive chyluria results in hypoproteinaemia. The rupture of numerous small skin lymphatics in the scrotum can lead to a constantly wet, sticky scrotum which is particularly unpleasant.

#### *Tropical pulmonary eosinophilia, Weingarten's syndrome.*

Tropical pulmonary eosinophilia is particularly common in India and Southeast Asia. In other geographical areas it seems to be rare. Pulmonary symptoms are predominant: cough, dyspnoea, asthmatic syndrome. Chest X-rays consistently show patchy infiltrates, in contrast to Loeffler's syndrome in which they are more fleeting. Microfilariae can be detected on lung biopsies. Sometimes the lymph nodes swell and splenomegaly occurs. The erythrocyte sedimentation rate increases and there is marked eosinophilia (usually >3000 cells/mm<sup>3</sup>). There are no microfilariae in the peripheral blood. Serological tests for filariae are strongly positive. This condition responds very well to therapy with DEC (in contrast to Loeffler's syndrome). Usually 6 mg/kg/day x 21 days is given. Steroids can be given if other diagnoses (e.g. strongyloidosis) can be excluded. If not treated it can lead to pulmonary fibrosis. Tropical pulmonary eosinophilia needs to be differentiated from classic asthma, tuberculosis, chronic strongyloidosis, schistosomiasis (does not occur in India), repeated *Ascaris* migrations and toxocariosis.

#### *Endomyocardial fibrosis*

Chronic hypereosinophilia can cause cardiac lesions such as endomyocardial fibrosis or fibroplastic endocarditis. The contents of the eosinophilic granules (including major basic protein) are toxic to the endocardium and the adjacent myocardium. A restrictive cardiomyopathy develops.

#### **Clinical difference between *W. bancrofti* and *Brugia* infections.**

*Brugia* infections rarely lead to genital lesions or chyluria. The elephantiasis tends to be limited to below the knee. A filarial dance sign cannot be obtained on ultrasound.

#### **Diagnosis**

Microfilariae should be searched for in blood: thin smear, thick smear, concentration method (Knott's test, microfilter). Because of the periodicity, it is best to take the blood at night. A small dose of DEC can increase microfilaraemia during the day: the microfilariae are then expelled from the pulmonary vascular bed; it is optimal to sample 45 mins after administration of 100 mg of DEC (adult dose). This does not work for people who have a disturbed sleeping pattern. Do not use DEC in an onchocerciasis area (Mazotti reaction). Albendazole and

ivermectin do not provoke a release of microfilariae into the peripheral blood. Microfilariae are sometimes detected in chylous urine, hydrocoele fluid and ascites fluid.

Indirect diagnosis can be made by serological detection of antibodies. Serological testing is of limited sensitivity and specificity.

Lymph node biopsies should be avoided as they obstruct lymph drainage still further.

Live adult *Wuchereria* worms can be detected by Doppler ultrasound of the scrotum. Dilated lymphatics are observed in which moving worms are found ("filarial dance sign"). This tool has however limited sensitivity; strangely enough adult *Brugia malayi* are almost never detected by ultrasound.

Circulating antigen of adult *W. bancrofti* can be detected with an antigen-capture ELISA but this is not practical in the field (although it is in a central laboratory). Several ICT tests have been developed which are very simple (like an ICT-test for malaria). There is no diurnal variation in the concentration of free circulating antigen so that nocturnal blood sampling is not necessary. It is a powerful test for studying the efficacy of chemotherapy. People who have no microfilariae in the blood but who do harbour live *adult W. bancrofti* test positive. People who recover no longer have circulating antigen in the blood. For population surveys, the previous standard parasitological techniques (nocturnal blood samples) were cumbersome, time-consuming, expensive and very intrusive. The use of rapid tests detecting circulating antigen has entered the clinical practice in endemic areas.

There is still no commercially available antigen test for detecting *Brugia* infections, which is a problem in *Brugia* endemic regions.

PCR tests have been developed that detect DNA of *W. bancrofti*, *Brugia malayi* and *B. timori*. They are used predominantly in epidemiological work and research settings.

#### **Differential diagnosis lymphedema:**

- Primary lymphedema: aplasia or hypoplasia lymphatics, tendency towards symmetrical lesions
- Lepromatous leprosy
- Chlamydia trachomatis, LGV
- Contact dermatitis with recurrent erysipelas
- Pretibial myxedema
- Chromomycosis
- Mycetoma
- Loiasis: Calabar swelling
- Onchocerciasis: hanging groin
- Kaposi sarcoma
- Lymphatic damage (surgery, radiation, burns, TB, malignancy)
- Podoconiosis

*Podoconiosis*

Podoconiosis (syn. lymphatic siderosilicosis or lymphoconiosis) is a chronic disorder characterised by the very slow onset of oedema, subsequent lymphoedema and later elephantiasis (mostly limited to below the knee). The disease is caused by immunological response to certain minerals (silicates, zirconium or beryllium-containing minerals). When walking barefoot on ground containing these minerals, dust particles can be absorbed through the soles of the feet via small wounds. They are then transported via the lymphatics to the inguinal lymph nodes where they cause a local inflammatory reaction. Atrophy and fibrosis of the lymphatics occur subsequently (in contrast to bancroftiasis where dilatation occurs). The disorder occurs in well-defined areas (specific mineral composition of the soil!) in people who walk about barefoot, such as Ethiopia, Kenya, Rwanda, Uganda, West Africa and India. Whereas lymphatic filariases occur predominantly in lower-lying areas (vector biotope), podoconiosis is characteristic of higher-lying zones. This is not absolute. Other signs of bancroftiasis are absent (hydrocoele, eosinophilia, and nocturnal microfilaraemia). Confusion with mycetoma ("Madura foot") or with classic Kaposi's sarcoma is possible.

## Treatment

### *General*

Self-help groups in a community can play an important role. In an acute situation, pain relief, antipyretics and anti-inflammatories are indicated. In chronic lesions of the scrotum, surgery can be performed. Elephantiasis of the limbs is relatively treatment-resistant. Physical methods (lymph drainage by massage) should be continued for a long time and will not in any way alter the fibrotic component of the swelling. Permanent compressive bandages are not practical in a warm, moist environment. A firm, compressive dressing (elastic bandages) may be applied centripetally. These are then changed daily and relatively good results can be achieved in this way, particularly if elastic compressive stockings can be worn afterwards. Microsurgery with the creation of several lymphovenous anastomoses is difficult.

### *Hygiene and antibiotics*

Good, enforced hygiene can dramatically reduce the number of complications. General cleanliness, washing with soap and disinfection of wounds are crucial. If bacterial superinfection is present, this should be treated appropriately. There is often a fungal infection between the toes (athlete's foot), which acts as a portal of entry for various bacteria. Simple hygiene is important and should be stressed:

- Washing the affected part of the body twice daily with soap and water
- Elevation of the affected limb at night
- Keeping nails clean
- Wearing shoes
- Disinfecting skin wounds rapidly and properly
- Systemic antibiotics for superinfection (erysipelas)
- Treating athlete's foot
- Daily physical exercise to improve lymph drainage
- Physiotherapy
- Diethylcarbamazine or DEC

### Diethylcarbamazine (= DEC)

Was introduced in 1947. In 1967 Frank Hawking, father of the famous physicist Stephen Hawking, published the results of a study in Brazil of the effect of enriching cooking salt with DEC on lymphatic filariasis. DEC (Notezine<sup>®</sup>, Hetrazan<sup>®</sup>, Banocide<sup>®</sup>) has a rapid, but indirect microfilaricidal effect on *Wuchereria* and *Brugia*. DEC somehow modifies the microfilariae so they can be destroyed by the immune cells. If it is taken for prolonged periods, there is also an incomplete microfilaricidal effect. The dose conventionally given as monotherapy is 72 mg/kg (in total) over 10-14 days (e.g. 50 tablets of 100 mg), although often it will be stated that 3 divided doses after meals will be better. There is evidence to show that lower doses for shorter periods are as effective (e.g. single dose of 6 mg/kg). DEC in monotherapy has an efficacy of  $\pm$  90% (against microfilariae). Pregnancy is a contra-indication for the administration of DEC. This medication is fairly well tolerated, but systemic reactions can occur, caused by the massive and sudden death of microfilariae. Recent studies suggest that these reactions are due to the abrupt release of *Wolbachia* in the human tissues (substantial increase in *Wolbachia* DNA by pCR). Symptoms may include malaise, pruritus, urticaria, fever, headache, vomiting and asthmatic crisis (cf. Mazzotti reaction in onchocerciasis and the Jarish-Herxheimer reaction in spirochaetosis).

This usually happens in the first 48 hours. For mild reactions antihistamines can be used, in severe reactions steroids are indicated. Local tissue reactions can also occur around dead microfilariae (lymphangitis, abscess, funiculitis). Those local reactions can occur up to several weeks after therapy.

Most microfilaraemic patients have a transient increase of haematuria and/or proteinuria after starting DEC. Because of these potential side effects, treatment is started with a low dose and increased progressively. It sometimes needs to be repeated. The microfilariae are not killed immediately by DEC, but their phagocytosis is facilitated. The indirect effect of DEC means that microfilariae can remain alive in cavities (e.g. hydrocoele). This can give rise to confusion, but otherwise is not important as microfilariae are not pathogenic. If the adult worms are not killed, microfilariae reappear in the blood 36 months later.

### Ivermectin (Mectizan<sup>®</sup>, Stromectol<sup>®</sup>)

This drug became available in 1984 for the treatment of onchocerciasis. It is also active as a microfilaricide against *W. bancrofti*. It has the enormous advantage that it can be given in one oral dose and has few side effects. It is not microfilaricidal, even if repeated at high doses. It is useful in the control of bancroftiasis (suppressing microfilaraemia stops transmission to the vectors) but probably not in helping individual patients. The combination of single dose DEC with single dose ivermectin is much more effective (99% of decrease of microfilariae load at least for 12 months) than each medication alone.

### Albendazole

Albendazole has a very limited microfilaricidal effect. It has been shown however that the combination of single doses of ivermectin with albendazole suppresses microfilaraemia by 99% for at least 15 months. This combination is more effective than each medication used separately. This combination also has the great advantage of eliminating diverse intestinal worms and of treating scabies (ivermectin).

## Tetracyclines

Tetracyclines are active against the endosymbiotic *Wolbachia*, and their eradication results in longterm sterility and eventual death of macrofilariae. This has become a new therapeutic point of attack, and this is the first effective microfilaricidal treatment (although indirect and slow). Initial clinical studies showed a favourable effect of a 8-week course of tetracycline on the clinical symptoms and the number of adult worms as reflected by the decrease/suppression of worms detected by ultrasound, decrease/suppression of circulating antigen load. In addition, a much lower rate of adverse reaction was observed when compared to the classic DEC treatment. Subsequent studies showed that a 4-week course of doxycycline has a similar efficacy as a 8-week course, but treatments of shorter duration do not seem to provide the same clinical benefit (although a microfilaricidal effect was also observed). **Nowadays, a 4 to 6-week course of doxycycline (200 mg/day) is the first-line treatment for the patient diagnosed with acute or chronic lymphatic filariasis.**

Of note, azithromycin does not deplete the *Wolbachia*, so that there is still no therapeutic option for children and pregnant women. A 2 to 4-week course of rifampicin (but not a 1-week course) has substantial anti-*Wolbachia* activity, but its therapeutic implications need to be further studied.

## Combination therapy

Combination therapy (albendazole 400 mg + ivermectin 200µg/kg) or (albendazole 400 mg + DEC 6 mg/kg) is largely used in mass drug administration at present to stop transmission. The second regimen is not used in countries where onchocerciasis occurs (risk of Mazotti reaction with acute blindness).

For the individual symptomatic patient, some experts recommend to combine a 6-week course of doxycycline with 14 days DEC, although the clinical superiority of this combination over doxycycline monotherapy has not been demonstrated so far.

## Prevention

At the end of the 20th Century, it was estimated that about 120 million people were infected and about 43 million were symptomatic. In May 1997, the WHO adopted a resolution to eliminate lymphatic filariasis as a public health problem. The programme "Global Programme to Eliminate Lymphatic Filariasis" or "Global Lymphatic Filariasis Initiative" was launched in the year 2000. It is based on two approaches:

- annual single dose, two-drug treatment allowing suppression of microfilaraemia for a year and which can be given as mass treatment for 5-6 successive years [albendazole + ivermectin in onchocerciasis areas; albendazole + DEC in other areas],
- simple diagnostic tests that can be performed by a finger prick at any time of the day.

An alternative used in some countries (China, Taiwan) is DEC 0,3% medicated salt x 12 months. It is hoped to eradicate lymphatic filariasis by about 2020. Good financial backing has been provided but this still needs to be extended. Technical training, logistics and management support should be organised. Continuous monitoring and evaluation should constitute an integral part of the programme.

Of note, a recent small study in Papua New Guinea suggests that a triple-drug therapy (DEC + albendazole + ivermectin) would suppress the microfilaremia for up to 2 years (and should be considered in heavily infected patients). This requires confirmation in larger trials.

The focus of disease control has been mass drug administration programs as described above. Vector control with insecticide-treated bed nets is useful where *Anopheles* (night biting mosquito) transmit the parasite. Repellents as personal protection tools prevent on an individual level. Currently, no vaccine exists.

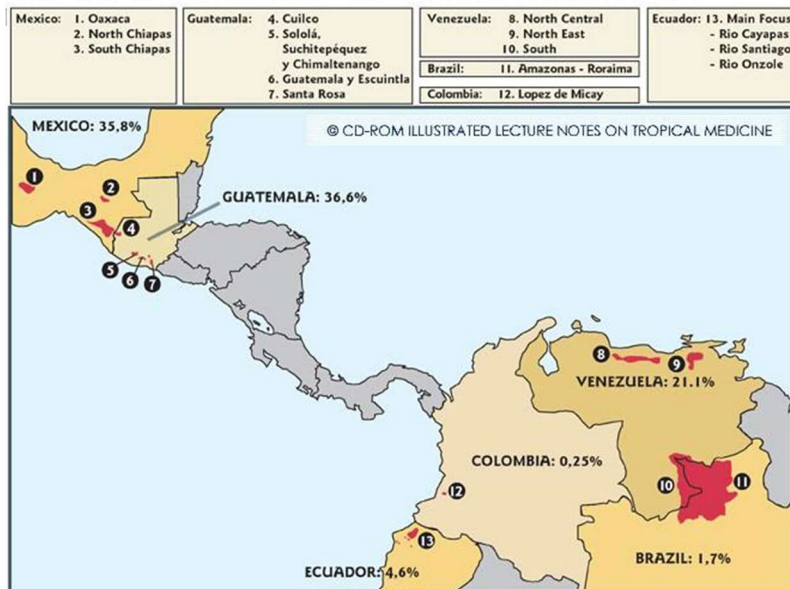
## Onchocerciasis

### Introduction

Onchocerciasis (syn. onchocercosis) is a disease resulting from infection by the nematode *Onchocerca volvulus*. The principal characteristics of the condition are pruritic dermatitis, subcutaneous nodules and ocular lesions. It is also known as "river blindness" because the blackfly vector breeds near fastflowing streams and rivers.

### Distribution

#### ONCHOCERCIASIS IN LATIN AMERICA



Map of onchocerciasis endemic area in Latin America. Adapted from publication of 'Programa para la Eliminación de la Onchocercosis en las Américas - OEPA', with special thanks to Dr Juan Martín Moreira.



Map of endemic onchocerciasis areas.

The disease occurs principally in large parts of Africa, especially West and Central Africa (including both Congos and Angola), but also in Sudan, Ethiopia, the north of Uganda and even Tanzania. About 99% of the cases are now limited to Africa, the remaining being observed in a few foci in Latin America (disease about to be eliminated there).



## Vector

The infective larvae are transmitted by *Simulium* mosquitoes ("blackflies"). They reproduce in rocky rivers with fast stream and can cover large distances. The fact that the insects are good fliers makes vector control difficult. Sometimes there are only a few ecologically very suitable places, where thousands of eggs are then laid. This can lead to the sudden simultaneous appearance of massive numbers of adult insects when the environmental conditions are right.

Only female insects suck blood (from humans as well as animals). This happens during the day in the open at well-defined times. The bite is painful.

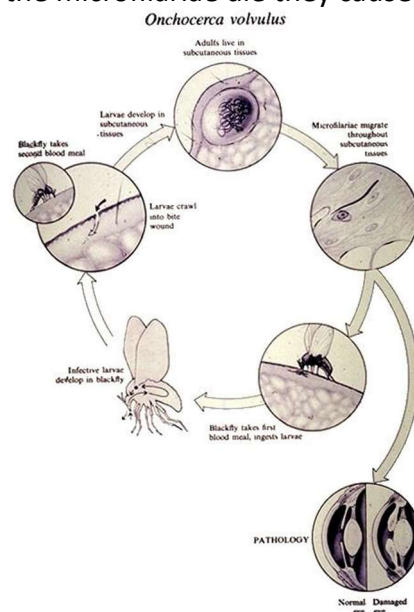
## Life cycle

There is no significant animal reservoir. *Onchocerca volvulus* microfilariae are more tissue parasites than blood parasites.

The more insect bites someone suffers over the course of the years, the greater the worm load. After a bite from an infected insect, the infective larvae develop in humans to become adult worms (=macrofilariae) that live subcutaneously. The prepatent period (time between infection and detection of microfilariae) is 3 to 15 months.

The macrofilariae lie coiled subcutaneously in nodules and can live for up to 15 years. These nodules are predominantly located on the scalp and upper body in people living in Central and South America but occur more on the pelvis and legs in Africans. This has to do with the biting habits of the vector.

*Simulium damnosum* (Africa) tends to bite on the lower half of the body (98% of bites below the belt) and *Simulium ochraceum* (America) preferably bite on the upper part of the body. A female lays on average 1600 microfilariae per day. The microfilariae concentrate in the skin, eyes and lymph nodes. When the microfilariae die they cause a local inflammatory reaction.



Life cycle of *Onchocerca volvulus*.

### *Onchocerca volvulus* : Endosymbiont

Intracellular bacteria can be detected by electron microscopy in adult *Onchocerca volvulus* and also in the microfilariae. The bacteria belong to the genus *Wolbachia* of the Rickettsiales (Alphaproteobacteria) and are closely related to *Ehrlichia*, *Cowdria* and *Anaplasma*. The *Wolbachia* bacteria are transmitted transovarially, have a preference for oocytes and the lateral tissues ("lateral chords") in the macrofilariae and have a development cycle that resembles that of the *Chlamydiae*. As for *Wuchereria* and *Brugia* filariae, they have also become a new point of attack for therapy. It was possible to render the macrofilariae in onchocercomata bacteria-free by means of a 6-week treatment with doxycycline.

At the same time parasitic embryogenesis was totally impaired. *Wolbachia* bacteria are also susceptible to rifampicin and this might become an option (like for lymphatic filariasis) for children less than 8 years and pregnant women. How far these findings are relevant for clinical practice has not been fully elucidated. *Wolbachia*-antigen released in the circulation contributes to the discomfort which is seen soon after administration of classic anti-filarial medication, but these symptoms do not seem to occur with tetracyclines.

## Clinical aspects

### *Skin abnormalities*

Pruritus occurs locally or systemically. There are scratch lesions often with bacterial superinfection. The chronic itching has given rise to the terms "gale filarienne" and "craw crawl". If untreated the dermatitis assumes the form of a pruritic papular dermatitis, progressing to a chronic rough, coarse, papular dermatitis, often with postinflammatory hyperpigmentation, followed by lichenification, atrophy and finally patchy depigmentation (leopard skin).

Pea- to plum-sized subcutaneous nodules are found predominantly over bony protuberances such as the hip, pelvis, ribs, shoulder blades and skull. These need to be distinguished from cysticerci.

Though not always found (in Africa in only 30 to 60% of positive people), enlargement of the inguinal nodes is sometimes also present, resulting in what is known as "hanging groin". Onchocerciasis causes localized elephantiasis (lymphoedema) in a number of cases.

### *Ocular lesions*

Ocular lesions only occur after many years of severe infection and are therefore usually not present before the age of 30. They are more frequent in savanna regions than in the rainforest.

In onchocerciasis patients with heavy infections, microfilariae can be seen in the anterior chamber with a slit lamp. When microfilariae die, opaque fine 0.5 mm wide corneal lesions occur: keratitis punctata.

This is corneal inflammation with small spots on the cornea accompanied by redness of the conjunctiva. Sclerosing keratitis occurs later (hazy cornea with pannus formation) as well as iritis and uveitis, resulting in blindness (river blindness!). More rarely, there is involvement of the posterior part of the eye: chorioretinitis and optic nerve atrophy. Ocular lesions can be

exacerbated by DEC therapy (**which is therefore formally contra-indicated**) but not by ivermectin.

There seems to exist an epidemiological link between onchocercosis and epilepsy, but more study on this subject is needed to confirm a causal relationship.

*The world's leading causes of blindness are:*

- cataract (clouding of the lens),
- trachoma (eye infection with the bacterium *Chlamydia trachomatis*),
- glaucoma (increased intra-ocular pressure with damage to the optic nerve),
- xerophthalmia (secondary to vitamin A deficiency with initial night blindness, followed by dry eyes and corneal softening),
- onchocerciasis,
- diabetes,
- leprosy,
- maculopathy and
- trauma.

## Diagnosis

### *Detection of microfilariae in a skin snip*

Various techniques may be used for detecting microfilariae in the skin. A skin snip is often used. A needle is used to raise the skin and a fine piece is shaved off with a razor blade. A standardised punch biopsy is also possible. The piece of tissue is placed in physiological saline. The specimen is then examined 15 minutes to 3 hours later to see whether or not microfilariae have emerged. In early infections (first 15 months) not enough microfilaria are present in the skin to be detected with a skin snip.

### *Detection of microfilariae in skin fluid*

This is done by means of scarification with a sterile razor blade. Preferably several sites are examined (often 4 sites are chosen). The fluid obtained can be collected on a glass slide and stained with Giemsa to allow identification.

### *Detection of microfilariae at other sites*

Occasionally *O. volvulus* microfilariae are found in the blood and in the urine.

### *Nodulectomy*

This is both diagnostic and curative if all the nodules are resected. However the palpable skin nodules are often outnumbered by deeper lying subcutaneous nodules. The microfilariae are found in the nodule.

### *Slit lamp examination*

This is a non-invasive test, but requires considerable experience. It is best to get the patient to lay his/her head on his/her knees for at least 2 minutes before the examination to allow more microfilariae to come into the anterior eye chamber.

### *Mazzotti test*

If the diagnosis is doubtful, the patient may be given 50 mg DEC orally. If microfilariae are present, a severe itching reaction will occur within 2 hours. This is caused by an allergic reaction to the proteins released after the rapid breakdown of microfilariae. Because this is very unpleasant, this test should be used only when strictly necessary. An alternative tool, a patch with DEC, is better used (if available) because it causes a localised reaction on the skin.

### *Serology*

Serology cannot distinguish between the various species of filariae. The antigen used is usually extracted from a different worm: *Litosomoides sigmodontis*. It is useless for patient care in endemic settings.

## Treatment

### *Ivermectin (Mectizan®, Stromectol®)*

Ivermectin is a fast-acting, safe and effective microfilaricide. Ivermectin has a broad spectrum and is active against various worms and arthropods (ectoparasites). In onchocerciasis, it is active against the free microfilariae and those that are still in the uterus of the female.

It can be given in a single oral dose (4 tablets of 3 mg for an adult; 200 microg./kg in children). It must be given repeatedly. The ideal frequency of administration (once a year or more frequently) still remains to be determined. Ivermectin does not penetrate the aqueous humour. Consequently it does not cause intra-ocular inflammatory reactions that might exacerbate ocular lesions. It was initially thought that pregnancy constituted a contra-indication to treatment with ivermectin but no increase in the incidence of abnormalities has been observed in neonates when the product has accidentally been taken by the mothers during pregnancy. In areas of *Loa loa* a slight risk of neurological side-effects of ivermectin exists (see below). In 1987 the manufacturer of ivermectin (Merck Company) announced that the company would make the medication freely available to combat onchocerciasis.

### *Moxidectin*

A promising new drug moxidectin, has been shown to have significant macrofilaricidal activity in animal studies and could be capable to interrupt transmission within six annual rounds of treatment. This drug might replace Ivermectin in the future.

### *Tetracyclines*

Tetracyclines such as doxycycline and vibramycin can kill *Wolbachia* endosymbionts of microfilariae.

According to initial findings, the subsequent suppression of embryogenesis by ivermectin lasts much longer (at least 18 months) and results eventually to the death of the adult worms (macrofilaricidal effect). A four-week course of doxycycline is very effective in killing microfilariae and is increasingly used in non-endemic settings to avoid repeated annual treatment with ivermectin for many years. In endemic countries however annual administration of ivermectin is still preferred for its fast effect on microfilariae (the pathogenic

form in onchocerciasis) and its simplicity of use (mass drug administration of a 4-week doxycycline treatment is unfeasible).

### *Nodulectomy*

This involves the removal of superficial nodules and is (was) popular in Central America.

### Prevention

In 1968, the WHO decided to start a large scale onchocerciasis control programme (OCP). The emphasis of the programme is on vector control in areas where the disease is often associated with blindness (savanna-type onchocerciasis). Initially it involved 7 West African countries (Benin, Burkina Faso, Ivory Coast, Ghana, Mali, Niger and Togo).

After a few years of preparation, the vector control programme started in 1974. In 1978, and subsequently in 1986, the programme was extended to the west and south (including Guinea, GuineaBissau, Senegal and Sierra Leone) to a total of 11 countries. It covers an area of 1,235,000 km<sup>2</sup> with 50,000 km of river. Initially 30,000,000 people lived there. In view of the fact that an adult female worm lives on average 11 years, it was estimated that a minimum of 14 years of insect control would be necessary to eradicate the human reservoir of onchocerciasis. Later, after a successful (but costly) period of intensive vector control, activities focused on regular administration of ivermectin (after 1987 and the free access to this drug) through vertical programs and subsequently primary care settings in order to decrease both morbidity and transmission. At present interruption of transmission has been achieved in large areas and onchocerciasis no longer constitutes a public health problem there.

# Loiasis

## Introduction



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Areas endemic for *Loa loa* filariasis in Africa

*Loa loa* is a nematode that is solely present in the rainforest area of Central and West Africa. Adult *Loa loa* worms are 3 to 7 cm long and 0.5 mm wide. They live 4 to 17 years. The microfilariae appear 6 to 12 months after infection. They measure 230 to 300  $\mu\text{m}$  by 7  $\mu\text{m}$ .

The microfilariae are found in the blood and exhibit periodicity with the highest density occurring around midday. The adult filaria do not contain a bacterial endosymbiont, as opposed to *Onchocerca volvulus* and *Wuchereria/Brugyia* filaria where *Wolbachia* is endosymbiotic.

Transmission occurs via the bite of female Chrysops flies. They are insects with beautiful and often yellowish-gold iridescent eyes when they are alive (chrysos = gold). Chrysops flies belong to the Tabanidae, which suck blood of mammals and are active during the day. There is no animal reservoir.

## Clinical aspects

The adult worms migrate through the subcutaneous tissues. This migration or the intermittent discharge of large quantities of microfilariae causes transient local oedema: Calabar swellings (Calabar is a place in Nigeria close to the border with Cameroon). There is also local redness and itching.

Generalized itching is also described.

When the worm passes under the conjunctiva, it can be observed and removed (*Loa loa* is for this reason known as the eyeworm). There is no intra-ocular invasion and there is no risk of blindness. The adult worm migrates through the loose-meshed subconjunctival connective tissue. This migration can thus be observed macroscopically, in contrast to the migration of microfilariae of *Onchocerca volvulus*.

The subcutaneous passage of the worms can sometimes be perceived as an itchy and rapidly moving linear swelling. Dead worms can calcify and thus be radiologically visible (e.g. in the

hands and wrists). In general, loiasis is accompanied by hypereosinophilia. This increases the risk for endomyocardial fibrosis.

## Diagnosis

Serology is aspecific, useless and not performed in endemic areas.

*Clinically:* Calabar swellings and worm passage across the eye may be considered as pathognomonic for the disease and sufficient to establish a diagnosis in the absence of *Loa loa* microfilariae in the blood.



Above: Dead and calcified adult *Loa loa* filaria, visible on a radiograph of the hands. Copyright ITM

Detection of microfilariae in peripheral blood (during the day) is obtained via a thin blood smear, thick smear or preferably via a concentration technique (Knott or nucleopore filter).

The number of *Loa loa* microfilaria in the peripheral blood can be very high. The higher the number, the higher the risk of neurological complications, especially when drug treatment is started. In order to diminish the risk apheresis can be performed.

## Treatment

Treatment of loiasis is based on administration of DEC for 3 weeks. The dose of DEC should be gradually build up over the course of 4 days, up to 400 mg/day. DEC is both micro- and macrofilaricidal against *Loa loa* although often several treatments are sometimes necessary.

Before starting with DEC, simultaneous onchocerciasis should be excluded in view of the risk of extremely unpleasant/severe Mazzotti reactions in the patient.

Ivermectin causes a marked but transient reduction in microfilaraemia. One week after administration on average 10% of the original microfilaraemia still persist.

With high microfilaraemia (>2000/ml; especially if >50,000/ml) there is an increased risk of neurological complications (headache, confusion, gait disorders, hypertension, incontinence, encephalopathy, coma) when DEC is administered. In such cases it is advised to associate prednisone 1 mg/kg for 4 days. Hospitalization for 4 days is advised since most side effects of starting treatment occur in this time frame. In very high microfilaraemia, even the

administration of ivermectin (sometimes used to decrease the microfilariae load before DEC treatment) may be risky. In such a situation apheresis may be necessary although it requires complicated and expensive apparatus and specialised personnel (out of reach of most endemic settings). In low-resource settings, a 3-week course of albendazole can be used instead of apheresis in order to reduce microfilaraemias.

Removal of the adult worms during their migration beneath the conjunctiva (local anaesthesia with cocaine or tetracaine) is possible. But if this is not done, the worm creeps on spontaneously and leaves the eye. While extracting the worm from the eye care has to be taken not to rupture the worm, as this leads to a severe inflammatory reaction.

## Prevention

DEC 5 mg/kg, 3 days per month can –rarely- be used as prevention in an endemic region. DEC 300 mg per week (dose for adults) is also effective. Vector control is problematical as the breeding sites are very diffuse and widespread and the insects bite out of doors.

## “Minor” filariasis

- Mansonellosis (*M. perstans*, *streptocerca*, *ozzardi*)
- Dirofilariasis (*D. immitis*,...)
- Dracunculiasis (*D. medinensis*)



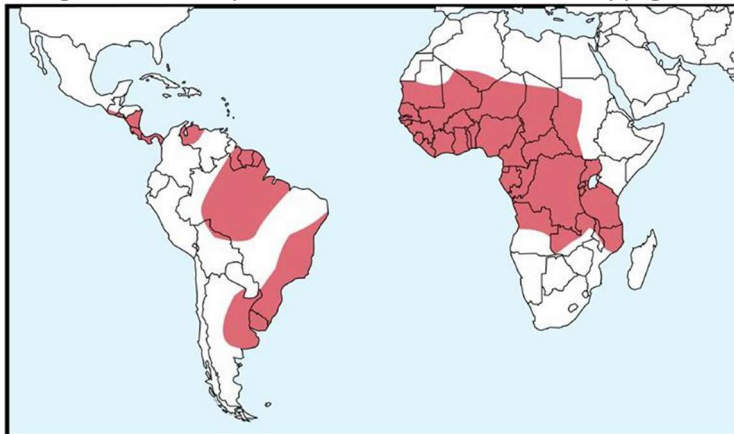
## *Mansonella perstans*

*Mansonella perstans* (formerly *Dipetalonema perstans*) is a nematode transmitted by Culicoides insects.

These midges have an aggressive and very annoying biting behaviour principally at dusk but also to a lesser extent at night and during the day. They bite predominantly on hands, arms and head. They often fly in swarms around the face. In view of their large numbers, they can form a very severe plague. Control is difficult.



Midges. *Culicoides* sp. Vectors of *Mansonella filaria*. Copyright ITM



Map showing areas endemic for *Mansonella perstans* filariosis. Copyright ITM

The infection is widely distributed in Africa but is more localised in Central and South America. *M. perstans* does not occur in Asia.

The adult worms live in body cavities (peritoneum, pleura and pericardium) and in perirenal fat. Most patients infected with *Mansonella perstans* are asymptomatic. A number of different symptoms and allergic reactions are sometimes ascribed to this parasite, but the disease spectrum has not yet been fully established.

The diagnosis is established by detecting the typical small microfilariae in the peripheral blood.

If asymptomatic, no treatment is necessary. A 6-week course of doxycycline with 200 mg/day resulted in a 97% cure-rate at 12 months in a study in Mali. Ivermectin, albendazole and DEC are inactive.

## *Mansonella streptocerca*



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Map showing areas endemic for *Mansonella streptocerca* filariosis. Copyright ITM

Streptocercosis is caused by *Mansonella streptocerca* (formerly *Dipetalonema streptocerca*). This nematode is confined to Central and West Africa. The parasite is transmitted by *Culicoides* midges. It may be a zoonosis as morphologically identical parasites are found in chimpanzees. Adult worms live in the skin. Live worms' cause no lesions but a local inflammatory reaction occurs when they die, with papules and possibly subsequent fibrosis. There are no eye lesions. Differentiation from onchocerciasis is necessary.

Many infected people are asymptomatic. The most frequent symptom is chronic pruritus. The skin is thickened and there are papules. Hypopigmented patches can occur which must be distinguished from leprosy, endemic treponematoses and onchocerciasis. Lymph nodes can be enlarged.

The microfilariae are found in the skin. Detection is as for onchocerciasis (skin snip, scarification with collection of dermal fluid). In the event of doubt or suspicion of leprosy, a biopsy is useful. DEC causes a Mazzotti reaction as in onchocerciasis.

DEC is micro- and macrofilaricidal for *Mansonella streptocerca*. Ivermectin is highly active against this parasite.

**Table: Overview of characteristics of microfilariae**

Species	location	Sheath	Period	Length	Tail nucleus
<i>Loa Loa</i>	Blood	+	Day	275 µm	+ terminal
<i>W. bancrofti</i>	Blood	+	Night (periodic strain)	260 µm	-
<i>Brugia malayi</i>	Blood	+	Night (periodic strain)	220 µm	+ isolated
<i>Brugia timori</i>	Blood	+	Night	290 µm	+ isolated
<i>M. ozzardi</i>	Blood	-	-	200 µm	-
<i>M. perstans</i>	Blood	-	-	<200 µm	+ double row
<i>M. streptocerca</i>	Skin	-	-	210 µm	+ and hook
<i>O. volvulus</i>	Skin	-	-	250 µm	-

## ***Dirofilariasis***

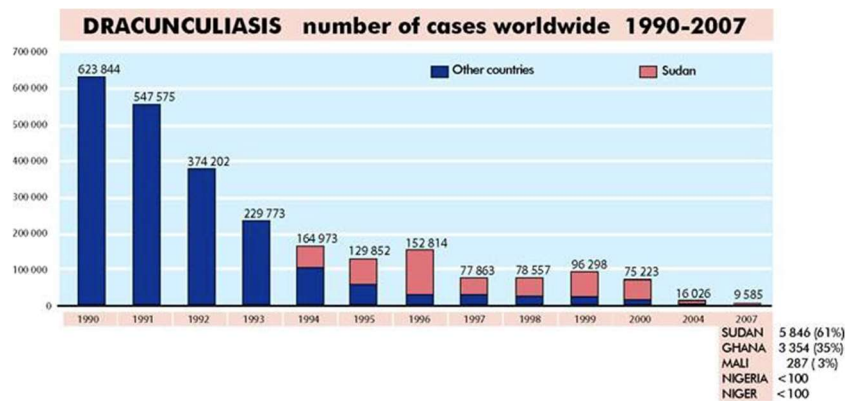
Occasionally humans can be infected by species of filariae which normally have other vertebrates as the final host. The most well-known belong to the genus *Dirofilaria*.

*Dirofilaria immitis* is a worm that parasitises dogs and cats (so-called heart-worm). The adult worms are 10-30 cm long and are sometimes found in hundreds in the dog's right heart chamber and/or pulmonary artery. The microfilariae are found in the dog's blood and are transmitted via the bite of infected *Aedes* mosquitoes. Man is an accidental host in which further development of the parasite is not possible. Consequently there are no microfilariae in humans. The immature worms die in the branches of the human pulmonary artery which can cause coin lesions in the lung. These are asymptomatic round nodules, 2-3 cm in diameter, which are sometimes found by chance on a chest Xray (coin lesion). As differential diagnosis with tumour is difficult, the diagnosis is often established on the basis of a lung biopsy. No treatment is necessary.

Other *Dirofilaria* (*Nochtiella*) species (*D. repens*, *D. tenuis*) are sometimes found subcutaneously in a nodule in humans. These nodules can migrate which is clearly different from nodules caused by cysticercosis. *Dirofilaria repens* causes swelling in the subcutaneous tissues in general around the eye, although various other locations are possible including spermatic cord and omentum. Treatment consists of surgical removal. It should be noted that these filariae produce no microfilariae in humans.

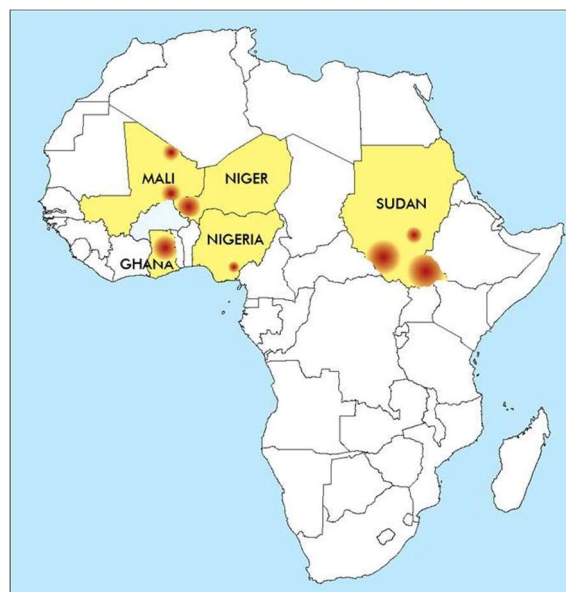
The diagnosis is usually curative (resection biopsy).

## Dracunculiasis



Graph showing the decrease in number of cases 1990-2007. Copyright ITM

*Dracunculus medinensis* or Guinea worm, is a nematode that was distributed in the past in several African countries north of the Equator as well as in Central Asia, India and the Arabian Peninsula. During the slave trade cases were introduced into the New World but subsequently the disease disappeared spontaneously. In 1986 the total number of active cases was estimated to be around 3,500,000 and the infection occurred in 20 countries. In 1993 there were 23,735 cases. The last foci in India and Yemen were eradicated in 1996 and 1997, respectively so the disease is now only found in Africa. In 2004, a total of 16,026 cases were reported. Southern Sudan, Northern Ghana and eastern Mali (regions Mopti, Kidal, Gao and Timbuktu) are the three last strongholds of the disease.



Map of the area endemic for Guinea worm.

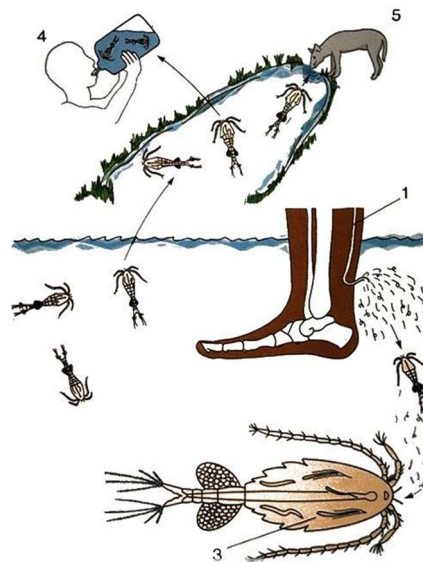
Year after year, the map changes due to progress in the eradication programme.



Guinea worm



Guinea worm removal



Guinea worm (*Dracunculus medinensis*), life cycle

Humans acquire the infection by drinking fresh water containing infected small water fleas (*Cyclops* sp). After several moults in the water flea, infective larvae are produced. When humans drink water containing infected copepods, the vector is digested in the stomach. The infective larvae penetrate the stomach or the intestine and start maturation in the peritoneum. After 3 months copulation occurs. The male dies and the female grows further

to reach her adult length of 60 to 100 cm after approximately 1 year. The long maturation period of approximately one year is required to coincide with the annual peaks in *Cyclops* numbers.

A female worm tends to be localised subcutaneously and causes a painful blister on the skin, usually on the lower legs or feet. The lesion occurs as a result of toxic secretions from the papillae around the parasite's mouth. When the blister bursts it creates an ulcer. When the human host wades in fresh water, the female parasite discharges several hundreds of thousands of larvae. Each time the ulcer comes into contact with water, the female slides out further and releases more larvae a process that repeats itself over a number of weeks until the whole worm is "used up". This process is slow, painful and disabling. The pain is alleviated by contact with cold water. This can be seen as a mechanism for promoting the survival of the parasite as a species because the victim looks for water to relieve the pain. The adult worm dies approximately 3 weeks after its emergence. After its death it will calcify and become visible on an X-ray, for example as an irregular calcified coiled string of about 2 mm diameter which should be distinguished from vascular calcifications.

The best approach is to remove the adult worm in its entirety. The adult worm can be coiled around a stick and one turn made daily. To accelerate expulsion, it is best to keep the wound constantly moist, for example with wet compresses. This however requires approximately 14 days. Surgical removal under local anaesthesia has been described.

Prevention is based on three approaches: safe drinking water, health information (people with wounds should not enter the water) and control of the vector (temephos (Abate®) is an organophosphate harmless to humans but kills the vector *Cyclops*).

# Cestodes (Tapeworms)

## Taeniasis

### Summary

- *Taenia saginata*: infection only via beef with larvae, resulting in an adult intestinal worm
- Infection with *Taeniasolium* larvae present in pork results in an adult intestinal worm: vague abdominal symptoms or asymptomatic
- Feco-oral infection via human feces containing *Taenia solium* eggs results in cysticercosis: epilepsy, subcutaneous nodules, nodules located in muscles, etc.
- *Taenia asiatica*: resembles *Taenia saginata*, but is transmitted via pigs. No cysticercosis in humans.

### Life cycle



*Taenia saginata*. The adult cestode can grow to several meters in length. Copyright ITM

Eating insufficiently cooked infected beef (*Taenia saginata*) or pork (*T. solium*) leads to infection with adult tapeworms. Humans are the natural final host and the only carriers of these cestodes, and thus also the only distributors of their eggs. The adult worms live in the small intestine and are several meters long. The pre-patent period is approximately 3 months.

A third species of human *Taenia* has been described in Asia (*Taenia asiatica*). The clinical importance of this has still to be determined. At present insufficient is known about *T. asiatica*. The adult worm is morphologically very similar to *T. saginata*. The life cycle of this cestode is different, however. Unlike *T. saginata*, which causes infections in the skeletal muscles of cattle, *T. asiatica* affects the liver, omentum, serosa and lungs of pigs. At present, *Taenia asiatica* does not seem to cause neurocysticercosis in humans, but more study is needed.

### Clinical aspects

Below, the symptoms present due to infection with an adult worm are described.

Most carriers of adult worms are asymptomatic. The length of an adult worm is usually  $\leq 5$  m for *T. saginata* (however, it may reach up to 25 m) and 2 to 7 m for *T. solium*. Some people



present nausea, anorexia or epigastric pain. The loose segments of *T. saginata* (not of *T. solium*) may actively creep outside through the anus, and cause local discomfort. Each segment contains approximately 60,000 eggs. *Taenia* may have a role in malnutrition (5 to 7 cm of worm has to be produced every day, for which food is needed), but only if there are also other reasons for malnutrition. In only 15% of patients peripheral eosinophilia is present. Note that while many humans can carry *T. solium* adult worms without any apparent effect, these people are the only source of eggs. When ingested, these eggs can produce larvae both in the natural host and in humans. The larvae are the cause of cysticercosis in both pig and human. Human-to-human transmission can therefore take place so that cysticercosis can occur in people who do not eat pork or who have no pigs in their surroundings.

### Diagnosis of infection with an adult worm

Finding proglottids in the feces, or a history of motile proglottids crawling out of the anus is important.

Eggs are sometimes found in the stools. The eggs are sticky and easily get onto the peri-anal skin. They can be detected in the peri-anal region with a Scotch tape test. There is no morphological difference between the eggs of *T. saginata* and those of *T. solium*. Differentiation can be made by the proglottids: a uterus with 10 branches or less in the dangerous *T. solium* and a highly branched uterus (12 or more) in the harmless *T. saginata*. *Taenia* antigens may be found in the feces. Only rarely can the tapeworm's head be discovered. The undamaged scolex of *T. solium* bears two rows of hooks. The scolex of *T. saginata* is hookless. However, dysmorphic tape worms are sometimes encountered.

### Treatment

- Niclosamide (Yomesan®) 4 tablets each of 500 mg will be taken together and chewed well. If the patient should vomit there is a theoretical risk that *T. solium* eggs will pass back into the stomach, activate and subsequently give rise to cysticercosis.
- Praziquantel (Biltricide®), in a very low dose (5-10 mg/kg), is also very effective. Praziquantel in a higher dose can sometimes provoke complications - such as sudden neurological symptoms should cysticerci be present in the brain. This complication seems however extremely rare in endemic areas where praziquantel mass treatment is used to control schistosomiasis.

For successful treatment, the scolex must be destroyed and eliminated; a residual scolex can result in regrowth of the entire tapeworm. Some experts recommend purgative treatment to be associated with antihelminthic drugs to have more probability to obtain the scolex in the stool, but this method is far from being universally accepted.

## Cysticercosis

### Summary

- Infection by *T. solium* eggs followed by development of cysticerci (= larvae) in the body.
- Symptoms depending on localization of the larvae.
- Neurocysticercosis with epilepsy is a common complication.

### General

At the end of the 19th century cysticercosis was still occurring frequently in Europe. At that time cysticercosis was found in 2% of the autopsies in Berlin. Nowadays the disease has virtually disappeared in the West. There are still occasional imported cases. Approximately 50 million people worldwide are estimated to have cysticercosis infection, although subclinical infection may underestimate this number. The disease occurs in regions where pigs are kept and eaten (thus not in Muslim regions). In many poor areas pigs are not kept in a pigsty, but run about in the open. This is encouraged in some areas, so that the animals function as a kind of free waste-disposal system. These are generally also places where the sanitary facilities are inadequate. The animals can become infected from human faeces via coprophagy. Insufficient meat control is an important risk factor in endemic regions. Not cooking meat through is another risk factor. Cases of cysticercosis in non-endemic regions may sometimes occur via infection from the carriers of adult worms who have come from endemic regions. If these infected migrants are employed in a household they may cause infections in their new surroundings (e.g. Mexican women who go to work in households in the USA).

### Life cycle

When larval *Taenia solium* infect a human they develop into an adult tapeworm. In contrast, if the eggs of *Taenia solium* are swallowed (food or water infected with human feces) the larvae (oncospheres) which emerge from them penetrate the intestinal wall and spread throughout the whole body via the blood stream. Therefore note that cysticercosis is caused by infected human faeces and not directly by eating insufficiently cooked pork. People with cysticercosis do not necessarily have an adult tapeworm.

Auto-infection in humans infected with an adult *Taenia solium*, is a possibility, however. In approximately 40% of people with cysticercosis an adult worm is found in the intestinal tract. The larvae migrate to various tissues and within 2 months convert into what are known as bladder worms (cysticerci). The typical bladder worm is a small ellipsoidal bag measuring 5-15 mm surrounded by a white translucent membrane. This bag contains clear fluid and a single round head, the protoscolex.

When the cysticerci die off they are absorbed or encapsulated and calcify. Each egg produces 1 cysticercus. Larval multiplication does not occur. In the brain, cysticerci can become extremely large (many centimeters diameter) when they develop in the ventricles (racemose form, see below).

Cysticerci which are present in pork, evaginate normally in the human intestine to then grow to full adult worms. Evagination is also possible, (but rare) in the human eye and intraventricular evagination may occur in the brain. These are sites where no inflammatory capsule is formed around the parasite.

Evagination does not occur in the muscles or in the cerebral parenchyma.

## Clinical aspects

Symptoms vary greatly, depending on the location of the cysticerci and the immune response of the individual. Small cysts may be found in the muscles, subcutis, eyes and in other tissues, where they are usually asymptomatic but may cause discomfort when inflamed. They appear as small nodules (5-10 mm). Sometimes they are much larger (e.g. 30 mm or even larger). In neurocysticercosis (NCC) there are parenchymal, subarachnoidal, intraventricular, spinal and ocular forms. Racemose cysticercosis is an aberrant development form of the parasite similar to a bunch of grapes. Live cysticerci in the central nervous system often cause remarkably few symptoms. When the parasites degenerate there may be focal encephalitis and oedema. Sometimes they may trigger a severe inflammatory response within a few days, which can be fatal.

In the brain they often cause late-onset epilepsy (common in Mexico and South America). Adult-onset seizures in endemic regions are therefore highly suggestive of neurocysticercosis. This can manifest itself as focal seizures (Jacksonian epilepsy). This is often followed by postictal confusion or transient paralysis (Todd's paralysis) and/or speech  $\pm$  vision problems. Todd's paralysis usually disappears within 48 hours. Intraventricular cysts may cause obstructive hydrocephalus. According to a recent meta-analysis, the main syndromes caused by NCC by order of frequency are epilepsy (80%), focal deficits (15%), intracranial hypertension (10%) and cognitive declines (5%). Mixed locations (parenchymal + meningeal) are common, but spinal localization is rare. Chronic meningitis, paralysis of cranial nerves, spinal cord lesions and mass effects may occur.

Psychiatric symptoms, encephalitis-like, are possible in case of severe infection. All these neurological problems may be acute or delayed forms. Focal calcifications are detectable 8 months to 10 years after infection. Larvae cannot be regarded as dead, unless the lesion is completely calcified.



Radiograph of an arm, showing elongated oval calcifications, typical of cysticercosis (calcified cysts in muscle).



*Taenia solium*, calcified cysticerci in the muscles of the legs. Such lesions are typically oval and elongated when localised in a muscle.

**Table: Symptoms of neurocysticercosis**

Headache	(23-98%)
Meningism	(29-33%)
Papilloedema	(48-84%)
Convulsions	(37-92%)
Abnormal mental state	(74-80%)
Focal deficits (motor and/or sensory)	(3-36%)
Ataxia	(5-24%)
Myelopathy	(<1%)
Cerebral nerve defect	(1-36%)
Visual disturbances	(5-34%)

**Diagnosis**

Diagnosis is made by means of excision of skin nodules or by using serological techniques, including antigen detection. Antibody and antigen detection may be carried out on cerebrospinal fluid as well as serum, but are often negative if only one or two lesions are

present. Lesions can be demonstrated by radiology, such as radiography targeting the soft tissues (shoulder and thigh muscles) and X-ray of the skull. In muscles, the calcified cysts tend to be elongated ovals.

MRI scanning of the brain is clearly superior to CT from a diagnostic point of view and scolices can sometimes be visualized through MRI. Living cysticerci are seen on a CT scan as hypodense lesions which are not enhanced by IV contrast. Degenerating cysticerci are sometimes isodense or hyperdense with an edematous ring-shaped zone which can be enhanced by IV contrast. They may disappear within 3 months. Sometimes the diagnosis can only be made by stereotactic brain biopsy.

The parasite is often surrounded by an inflammatory infiltrate with plasma cells. Immunoglobulins may accumulate in the cytoplasm of reactive plasma cells, and form prominent eosinophilic inclusions (Russell bodies). They are however not specific for cysticercosis.

Serology is available and EITB (enzyme-linked immunoelectrotransfer blot assay) uses affinity-purified glycoprotein antigens fractionated by electrophoresis which performs better than ELISA. Antigenic tests have been also developed, reflecting the presence of viable cysts (if their numbers is sufficient however), in contrast to antibody detection tests reflecting only (past) exposure. Field-adapted lateral flow antigen-based diagnostics are being evaluated.

The most important **differential diagnoses of cerebral ring-shaped lesions:**

- Cerebral toxoplasmosis
- Hydatid cyst
- Ectopic worms (Paragonimus, Schistosoma)
- Amoebiasis
- Tuberculosis and cryptococcosis
- Bacterial abscesses, septic emboli, nocardiosis
- Glioma, lymphoma, metastasis
- Haemangioma

## Treatment

Therapy of neurocysticercosis is extremely complex and depends on the cysticerci stages (viable, degenerating, calcified) and localizations (intra- or extra-parenchymal). It remains a rather controversial area. Expert advice and a multidisciplinary approach are often required. The most important is to treat the symptoms first, mainly the seizures with anti-epileptic drugs (up to 2 years after the last seizure, like for other epilepsy). Medical treatment, when indicated, is based on administration of praziquantel (50 mg/kg/day) or albendazole (15 mg/kg/day) for 2 weeks, but in case of multiple cysticerci (>3), association of both drugs was found superior in seizure reduction.

In adequately selected patients (see below), antihelminthic therapy may reduce the risk of generalized seizures by 67% as well as of the number of seizures, and lead to complete resolution of the lesions at CT/MRI in a substantial proportion of patients. But in real life, the outcome is rarely this favourable. It is important to note that when the bladder worms die off

they cause a local tissue reaction. Neurological symptoms may therefore exacerbate (generally on the 2nd to 4th day of treatment) and can be very difficult to manage where there are no neurosurgical facilities or neuro-imaging. This effect can be mitigated by starting dexamethasone 1 day before the other drugs, maintained up to one month (a dose of 8 mg/day seems optimal), before it's slowly tapered down. Albendazole does not interfere with carbamazepine (Tegretol®) or phenytoin (Diphantoine®). Corticosteroids reduce the blood level of praziquantel and increase albendazole levels, but this is probably of no clinical importance.

Recently the benefit of medical therapy has been questioned. Probably many patients with infections recover spontaneously. When treating neurocysticercosis, it is important to know beforehand whether there are intra-ocular lesions. Degeneration of a cysticercus in the retina, together with accompanying inflammation, may lead to acute blindness. Surgical removal via vitrectomy should be considered, but such a procedure is not without risk.

Today, experts recommend antihelminthic drugs only in case of viable (symptomatic) cysts without inflammation and degenerating cysts with inflammation (to accelerate involution). Granulomatous cysts and those already calcified, for which symptomatic treatment and steroids are sufficient, do not benefit from antihelminics. More and more a segregation is made between intra-parenchymal NCC, where medical therapy may be carefully proposed, except when there are too many lesions (**contra-indicated** because of the high risk of diffuse cerebral oedema), and extra-parenchymal (intraventricular, meningeal, spinal, ocular,...) NCC where neurosurgery is almost always required (due to risk of intracerebral obstruction and hydrocephalus) in combination with longer antihelminthic treatment at higher dose (extraparenchymal cysticerci are usually less susceptible to medication).

Sometimes a ventriculoperitoneal shunt must be inserted in obstructive hydrocephalus. Shunt blockage is common if the cerebrospinal fluid contains large amounts of protein. Appropriate treatment of NCC is nearly unfeasible without adequate diagnostic/surgical facilities, and impossible without an initial careful neuro-imaging assessment. In remote, low-resource settings, it is often preferable to just treat the complications (epilepsy) with a symptomatic antiepileptic therapy, rather than using an etiologic treatment without possibility of monitoring or neurosurgical intervention.

## Prevention

Since humans are the only reservoir for adult *T. solium* the disease can be controlled by improved sanitation and hygiene, in particular by controlling pollution with human faeces. Human carriers should be treated. To reduce the number of carriers of *Taenia solium*, proper statutory meat inspection should be carried out. Furthermore, meat should be heated to above 56°C or stored for at least 10 days at 10°C (requiring a freezer). Eating raw or insufficiently cooked pork should be discouraged. If there is a patient with cysticercosis, it is best to investigate whether the patient, close family members, domestic staff and friends are carriers of adult *T. solium* and constitute a possible source. A faecal examination and an antigen-capture ELISA test are used for screening. If positive, a CT scan of the brain is carried out (detection of cysts in the brain).

Pigs can be treated with a single administration of oxfendazole, a benzimidazole. Vaccination of pigs is under study, and appears to be efficacious under controlled environments. There should be a strong recommendation that pigs not be allowed to run free. They are coprophagic. If humans do not compost their faeces, but use them directly as pig fodder or on the fields, the animals will become infected.

Composting kills the eggs. Washing hands with soap after using the toilet should be encouraged. Parasitic infections in which faeces play a part, are a taboo subject in some communities. A control program needs to take account of this.

There is no vaccine for human protection against *T. solium*. However, vaccines are available to prevent *T. saginata* infection in cattle and *T. ovis* in sheep.

## Cystic echinococcosis

### Summary

- Infection from eggs in dog feces.
- Larvae form large cysts with internal daughter cysts.
- Cysts in the liver and lungs, rarely in other organs.
- Often asymptomatic, sometimes symptoms due to pressure upon surrounding organs.
- Risk of rupture with anaphylaxis or dissemination.

### General

There are several tape worms in the genus *Echinococcus*: *E. granulosus*, *E. multilocularis*, *E. vogeli*, *E. oligarthus*, *E. shiquicus*. The most important and frequent one is *E. granulosus*, causing cystic echinococcosis or hydatid disease. *Echinococcus granulosus* is a small tapeworm (a few mm long) which infects dogs and other canines. Its distribution is world-wide. In some regions the problem is very important such as North Kenya around Lake Turkana and Kyrgyzstan and the surrounding central Asiatic republics. Various animals (sheep, goats, cattle, pigs) may become infected with the eggs in dog faeces. In the animal's intestine the larva (called "oncosphere") emerges from the egg. It penetrates the intestinal wall and is carried by the venous blood towards the portal vein. After development of the parasite, hydatid cysts are formed in internal organs. The cycle is completed when a dog has the opportunity to eat offal containing hydatid cysts. In the dog's intestine adult *E. granulosus* then develop, after which egg laying can begin. Each hydatid cyst leads to multiple adult worms.

Humans are accidental hosts. If humans take water or food contaminated by dog faeces, they will develop one or more hydatid cysts. The cyst contains fluid and daughter cysts and is known as a hydatid cyst. On the inside of each cyst is a germinal membrane. From this membrane countless protoscolices (small heads) develop. There is thus multiplication at the larval stage. A capsule of connective tissue is formed around the cyst. This capsule consists of the cyst wall together with the germinal membrane. The majority of cysts are found in the liver and lungs, but other locations are also possible (brain, bones, spleen, kidneys). These are often continuously growing cysts, which may produce pressure on surrounding organs, may rupture or die off and calcify. When the parasite has died and disintegrated the hooks which were situated at the former heads remain in the sandy fluid of the dead cyst, and these can be seen under a microscope. This is useful if there is doubt concerning the nature of a cystic lesion.

### Clinical aspects

Humans are generally infected faecal-orally during childhood. The cysts grow very slowly, about 1 to 2 cm per year. The carrier may remain asymptomatic for a long time and symptoms are unusual before the cyst has reached 10 cm in diameter, at least in the liver, its preferred localization. There may be mechanical consequences. Pressure on surrounding organs leads to various symptoms and complaints. Hepatic cysts may lead to an enlarged liver with local discomfort, obstructive icterus with or without cholangitis. If localization is in the central nervous system this produces symptoms of a brain tumour, epilepsy, compression of the spinal cord or brain stem and even eosinophilic meningitis if there is spillage. If situated in the skeleton there is often bone pain, sometimes with fractures. This has to be differentiated from ordinary bone cysts or tumours. Lung cysts are usually asymptomatic, but



sometimes there is a cough and thoracic discomfort. Renal cysts are sometimes found by chance and may cause unilateral kidney destruction. Allergic reactions may also occur, such as urticarial rash, bronchospasm, anaphylactic shock after rupture of a cyst (which may be spontaneous, after trauma or during surgery). After rupture there may be dissemination of the protoscolices in the peritoneum or pleura. Mechanical aspiration of a cyst may sometimes lead to rupture with allergic shock and dissemination.

## Diagnosis

Plain X-ray of the abdomen (crescentic calcifications), X-ray of the lungs or CT scan. Ultrasound of the liver shows a round or oval hypodense zone with retro-acoustic intensification. The cyst can contain septa or daughter cysts. The wall may appear split (the endocyst separated from the pericyst) or it may be partially or completely calcified. Sometimes the cyst appears heterogeneous and produces a pseudo-tumorous image. Sometimes the diagnosis is made during surgery. In case of doubt as to the nature of a cystic mass, the content of the lesions may be examined for the presence of hydatid sand or the presence of the typical small hooks which remain after the protoscolices degenerate. Serology may be negative in the case of well encapsulated liver cysts and lung cysts. Sometimes the serology is positive or the titer increases during treatment due to leakage of the cyst content and release of antigen which cause the immune response to increase.

## Ultrasound

Various types of cysts can be identified by ultrasound. The following signs are regarded as pathognomonic for cystic echinococcosis (CE):

- Unilocular, anechogenic round or oval lesions with a pronounced laminated membrane or with snow-like inclusions.
- Multivesicular cysts or cysts with multiple septa with a wheel-like appearance.
- Unilocular cysts with daughter cysts which may exhibit a honeycomb appearance.
- Cysts with floating laminated membranes (“water-lily sign”) which may also contain daughter cysts.

Ultrasound is also of utmost importance to stage the liver cysts according to the 2010 WHO classification (see Fig 2 below), between active (or early: CE1 and CE2), transitional (C3a and C3b) and inactive (or late: CE4 and CE5) lesions. This has immediate implication for the prognosis and treatment of cystic echinococcosis.

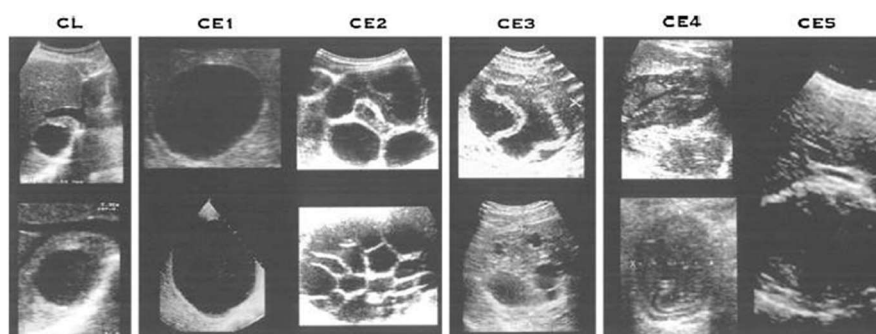


Fig. 2. WHO-IWGE standardized classification.

### *WHO Classification of hepatic hydatid cysts*

<b>CL</b>	Unilocular anechoic cystic lesion without any internal echoes and septations
<b>CE 1</b>	Uniformly anechoic cyst with fine echoes settled in it representing hydatid sand
<b>CE 2</b>	Cyst with multiple septations giving it multivesicular appearance or rosette appearance or honey comb appearance with unilocular mother cyst; this stage is the active stage of the cyst
<b>CE 3</b>	Unilocular cyst with daughter cysts with detached laminated membranes appearing as water lily sign; this is the transitional stage of the cyst
<b>CE 4</b>	Mixed hypo and hyperechoic contents with absent daughter cysts, these contents give an appearance of ball of wool sign indicating the degenerative nature of the cyst
<b>CE 5</b>	Arch-like thick partially or completely calcified wall; this stage of cyst is inactive and infertile

## Treatment

### *Waiting*

Many cysts remain stable, calcify or even involute spontaneously. Small, calcified cysts in the elderly can usually be left untreated. As a whole, a wait and see attitude is recommended for CE4 and CE5.

### *Surgery*

Pericystectomy or partial liver resection. Sometimes what is known as the "frozen-seal" method is applied. Using liquid nitrogen, a funnel is frozen onto the liver capsule to prevent accidental spillage.

The liver is opened and the cyst content evacuated. During the operation, lavage is carried out with a scolicidal agent. Surgery is the treatment of first choice for large cysts (> 10 cm), for CE2-CE3b lesions, if there is superinfection or communication with the biliary tract. For extrahepatic cysts, surgery is always the treatment of first choice. Albendazole is administered ideally prior to surgery (but optimal timing is unknown, up to 4 weeks), and praziquantel is given at the time of the operation. This is done in order to diminish the risk of disseminated infection in case of accidental rupture or spillage during operation. Post-operative complications are not unusual.

### *Medication*

Mebendazole is no longer used (only at high dose, in case of albendazole toxicity). Long-term therapy with albendazole (e.g. 800 mg daily for 6 to 9 months, blocks glucose uptake by the parasite) is usually used alone for CE1 and CE3a lesions < 5 cm and in combination with PAIR or surgery for bigger lesions or in CE2 and CE3b lesions. It is used in extended duration for inoperable and/or disseminated disease.

Previously this was given in cycles, but nowadays the medication is administered daily without interruption. The efficacy of medical therapy varies greatly (overall cure rate of 30%) and clearly leaves much to be desired. Higher levels of albendazole sulphoxide (ricobendazole), the chief active metabolite, may be obtained by higher dosage, ingestion with a fatty meal, or

by combination with praziquantel or cimetidine [cimetidine inhibits the breakdown of both albendazole and praziquantel].

Albendazole cannot be used during pregnancy. The combination albendazole (10-15 mg/kg daily divided in two doses) with praziquantel (40 mg/kg once a week) is probably more effective than either drug alone.

### *PAIR*

Percutaneous treatment with the PAIR technique (puncture-aspiration-injection-reaspiration) can be used for CE1 and CE3a lesions. Daughter cyst should be ruled out, since their presence reduces the likelihood of successful treatment with PAIR. Experienced surgeons can perform a laparoscopic variant of this technique. In hospitals where the necessary equipment is available, after detection of a cyst an endoscopic retrograde cholangiography is carried out. This permits determination of whether there is any communication between the cyst and the biliary tract. Under ultrasound or CT guidance the cyst is punctured transhepatically with a fine needle. The cystic pressure can be measured. Vital cysts have a pressure of 8-75 cm water. Dead cysts have a low pressure (0-2 cm water). Subsequently 10-15 ml of cystic fluid is aspirated. Live protoscolices are actively motile upon microscopic examination.

Biochemical analysis of the fluid for the presence of bilirubin is carried out to exclude communication with the biliary tree. If there is sufficient evidence of active echinococcosis, the remaining cystic fluid is aspirated. Afterwards a protoscolicidal agent is injected (generally 95% ethanol or 15-20% hypertonic salt). As a guideline the amount injected should be 1/3 of the volume of the aspirated fluid.

After 10 to 30 minutes the cyst content is then aspirated again. The risk of rupture, dissemination or anaphylaxis is minimal if there is at least 1 cm (preferably 2 cm) between the liver capsule and the cyst wall.

If there is a cyst-to-biliary tract fistula, the PAIR technique cannot be used due to the risk of sclerosing cholangitis. It is advisable to begin albendazole one week before and to continue administering this until 4 weeks after the procedure. PAIR cannot be used for extra-hepatic lesions. Those who have no experience with PAIR are advised to leave this to an expert as the complication rate is quite high.

## Prevention

De-worm dogs and prevent them from eating offal.

Keep dogs out of slaughterhouses.

The first results of a recombinant vaccine (EG95) administered to sheep and goats, are encouraging, and show protection of 83-100% for these animals.

## ***Echinococcus multilocularis***

*Echinococcus multilocularis* or fox tapeworm is closely related to *E. granulosus* (dog tapeworm). The parasite occurs in the northern hemisphere, often in regions with a cold climate such as Alaska, the Alps, Siberia, north-west China and central Turkey. The eggs of the parasite are cold-resistant.

Transmission by sleigh dogs is known. Treatment of these draught animals with praziquantel reduces the transmission to humans. In the wild there is a cycle between canines (including fox, wolf, etc.) and various rodents, including mice. Domestic dogs and cats may also become infected. Humans become infected accidentally by faecal-oral transmission, e.g. by eating contaminated berries, or drinking water contaminated with fox faeces. After infection with eggs the larvae develop, resulting in alveolar hydatidosis of the liver and other organs. The cysts may calcify, but usually continue to grow slowly and constantly and are similar to a malignant growth. Metastasis may occur. There may be growth through to the diaphragm and into the inferior vena cava. Treatment is difficult and involves liver surgery and/or long-term therapy with antihelmintics (even life-long in inoperable cases).

## *Hymenolepis nana*

In 1921 Saeki demonstrated direct transmission (i.e. without intermediate host) of *H. nana* in humans, in contrast with *H. diminuta* for which human infection requires ingestion of infected insects. *H. nana* occurs in foci and has a cosmopolitan distribution.

The highest prevalence of this cestode is found in hot, dry regions. People become infected by swallowing an egg (faeco-oral transmission) or by accidentally swallowing an insect (flea, weevil) which acts as intermediate host. An intermediate host is not essential for infection. Humans are the only definitive host. The adult worm is found in the lumen of the small intestine. The adult parasite is smaller than *H. diminuta*: it only measures 2-4 cm (dwarf tapeworm). The strobila contains 100 to 200 proglottids.

The course of infection is almost always asymptomatic, but marked hypereosinophilia can be present. Malignant transformation of *H. nana* has been described in an HIV-infected patient in 2015, being a novel disease mechanism of a neoplasm in invertebrates invading human tissue. The treatment of choice is praziquantel.

# Trematodes (Flukes)

## Introduction

The trematodes are flatworms which are of great importance in tropical pathology. They may affect various organs. They have at least two suckers, one oral and one ventral (*Heterophyes* has three). The oral sucker surrounds the mouth. The intestinal system has a blind ending. They have no blood circulation. Oxygen is absorbed by diffusion. Most trematodes are hermaphrodites and thus possess both male and female genitalia. They have a cirrus (penis). The function of the Laurer canal is unclear, but it is probably a vestigial vagina. Cross-fertilization and self-insemination are both possible. There are exceptions, e.g. schistosomes have separate sexes. After leaving the ovary, the eggs are fertilized and subsequently surrounded by yolk in the ootype (an extension of the vitelline duct). Several concentric eggshells are formed. The eggshells then undergo a chemical reaction, a kind of tanning process, which makes them tough and harder. In this way the egg acquires its typical form, and becomes more resistant to conditions in the outside world, which are often unfavorable.

## Localisation

<b>Intestinal lumen</b>	Large intestinal fluke ( <i>Fasciolopsis buski</i> ) Small intestinal flukes ( <i>Metagonimus</i> , <i>Heterophyes</i> )
<b>Lungs</b>	Lung fluke ( <i>Paragonimus</i> )
<b>Bile ducts</b>	Large liver flukes ( <i>Fasciola hepatica</i> and <i>F. gigantica</i> ) Small liver flukes ( <i>Opisthorchis</i> , <i>Clonorchis</i> , <i>Dicrocoelium</i> , <i>Amphimerus</i> )
<b>Blood vessels</b>	Blood flukes ( <i>Schistosoma</i> sp.)

## Intestinal flukes

Various trematodes are found as adult worms in human intestines. Examples are *Fasciolopsis buski*, *Metagonimus yokogawai*, *Heterophyes heterophyes*, *Echinostoma* sp., *Gastrodiscoides* sp. Most infections are asymptomatic or provoke vague abdominal symptoms. Only in severe infestations (high worm load) are there likely to be signs of malabsorption. Eosinophilia is common. Diagnosis can only be made by examining the faeces for parasites. As a general rule most of these infections can easily be treated with praziquantel.

## Lung flukes

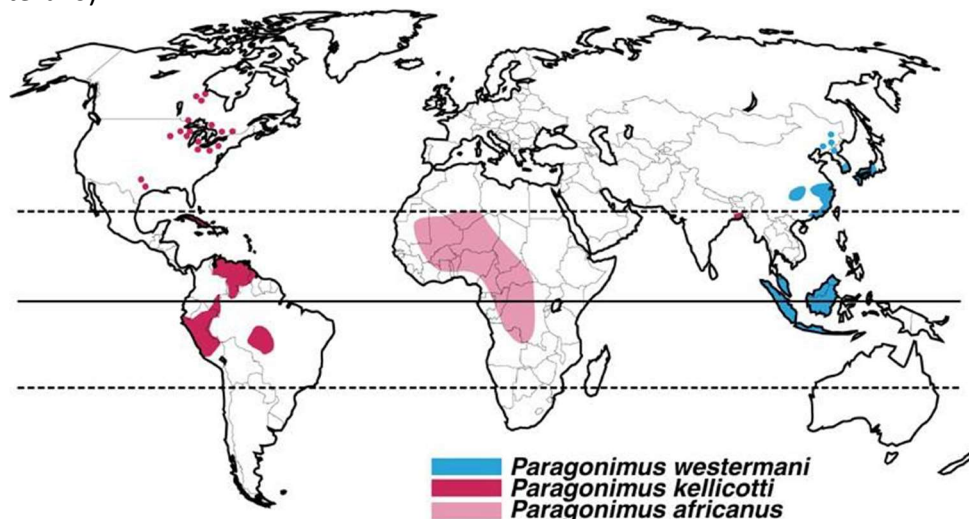
### *Paragonimus sp.*

#### Summary

- Transmission via eating infected crabs and crayfish
- Symptoms resembling pulmonary tuberculosis or chronic bronchitis
- Sometimes ectopic localization
- Diagnosis via detection of eggs in sputum

#### General

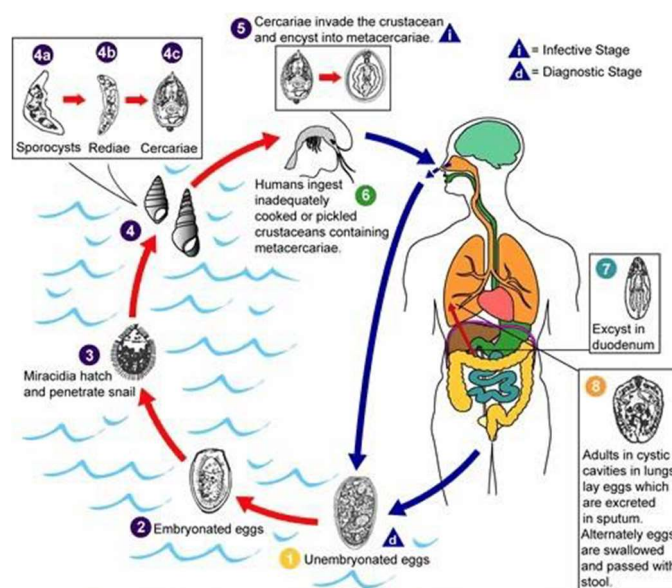
The parasite occurs in Southeast Asia and the Far East, in Central and West Africa. In America its distribution is limited to Central America and the north of South America. Usually *P. westermani* is reported, but there are a number of other species which can cause infection in humans (*Paragonimus africanus*, *P. bangkokensis*, *P. heterotremus*, *P. hueitungensis*, *P. kellicotti*, *P. mexicanus*, *P. miyazakii*, *P. ohirae*, *P. philippinensis*, *P. sadoensis*, *P. skrjabini*, *P. uterobilateralis*).



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Map showing areas endemic for *Paragonimus westermani*, *P. kellicotti* and *P. africanus*. Copyright ITM

## Life cycle



*Paragonimus westermani*, life cycle. Courtesy of CDC, Division of Parasitic Diseases.

Adult worms live in the lungs. Eggs pass to the outside with the sputum. If sputum is swallowed, eggs may also be found in faeces. Once in the outside world and in water, miracidia (first-stage larvae) emerge from the eggs. They penetrate snails, where they undergo a transformation. After 3 to 5 months cercariae (second-stage larvae) leave the snail and penetrate crabs. Here the cercariae develop into metacercariae (third-stage larvae). It is this form which is infectious for the definitive host.

Paragonomiasis is a zoonosis of carnivorous animals. Humans are only an exceptional host. They become infected by eating raw fresh-water crabs and river crayfish which contain infectious metacercariae. Excystation occurs in the duodenum. The larvae bore through the intestinal wall and migrate via the abdominal cavity and diaphragm to the lungs. There they develop into adult worms. The worms form a cavity 1 to 4 cm in diameter. Egg-laying begins 8 to 10 weeks after infection. The worms may also migrate to ectopic sites. An individual generally carries  $\leq 20$  worms, which can persist within humans for 20 years. *Paragonimus* got its name from the shape with 'gonads at the side'.

## Clinical aspects

Mild infections are asymptomatic. In the acute stage (invasion and migration of the larvae) there may be diarrhoea, abdominal pain, urticaria and eosinophilia. This is followed by fever, thoracic pain, cough, dyspnoea and malaise. The chronic illness resembles chronic bronchitis and TB. There is spasmodic cough (especially after exertion) with expectoration of blood stained sputum, as well as dyspnoea sometimes with wheezing and pleural pain. When the parasite is located in an ectopic site (brain, peritoneal cavity, liver, subcutaneous region, etc.), causing an eosinophilic abscess, the symptoms depend on the place where the worms are.

## Diagnosis

Diagnosis is by detecting the eggs. The eggs often need to be concentrated (e.g. mix sputum + water + potassium hydroxide, then centrifuge and examine the sediment). Differential



diagnosis includes tuberculosis of the lungs, Loeffler's syndrome, pulmonary abscess, chronic bronchitis, melioidosis, histoplasmosis, coccidioidomycosis, lung carcinoma and lung metastases. If sputum is swallowed, eggs may also be found in the faeces. Serology has a sensitivity and specificity > 90 percent.

### Treatment

Praziquantel 75 mg/day for 3 days is very effective. Triclabendazole is an alternative. In cases of cerebral localization higher doses must be given but only under the protection of steroids due to the risk of epileptic fits secondary to perilesional oedema.

## Liver flukes

### Summary

- Small liver flukes: eating infected fish leads to cholangitis, icterus, eosinophilia, cancer of the bile duct
- Large liver flukes: eating contaminated plants leads to cholangitis, icterus, eosinophilia

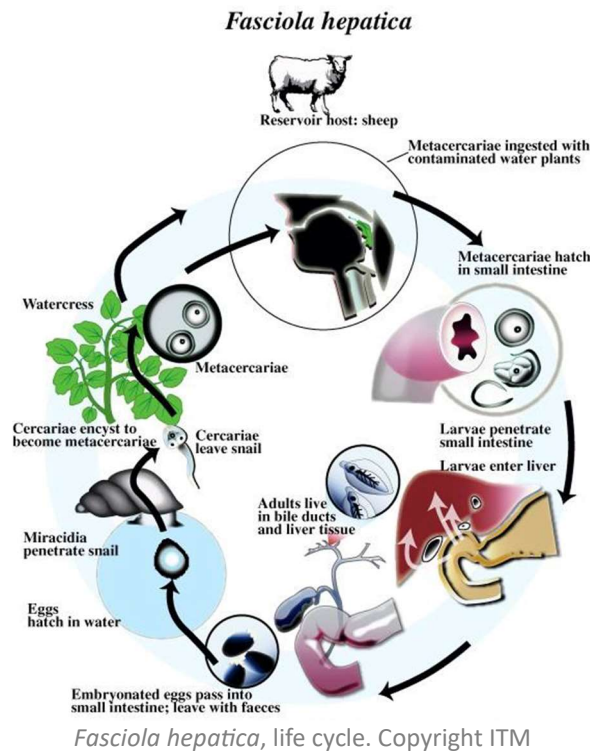
### Small liver flukes: *Clonorchis*, *Opisthorchis* and *Metorchis*

*Opisthorchis viverrini* and *Clonorchis sinensis* (= *Opisthorchis sinensis*) occur in Asia. Eggs eliminated in the bile and faeces are taken up by snails. After further development in these animals, they leave the mollusk and penetrate freshwater fish (metacercariae). Humans become infected by eating raw fish such as carp. After the larvae are released in the duodenum, they migrate directly via the main bile duct to the intrahepatic bile ducts. Thus, there is no tissue passage. The parasites are approximately 1 to 2 cm long and can live for 20 years. Dogs and cats form a reservoir.

There may or may not be symptoms, depending on the worm load and location of the worms. Intermittent pain may occur around the liver which is sometimes enlarged. If bacterial superinfection occurs, febrile suppurating cholangitis results. If impaction with obstruction of the main bile duct occurs, there will be progressive icterus. In long-existing cases of infestation with *Clonorchis sinensis*, secondary biliary cirrhosis and carcinoma of the bile duct (cholangiocarcinoma) may develop. The diagnosis is made by detecting eggs in the faeces. A concentration technique is necessary. However, if bile duct is obstructed, no eggs can be detected. Sometimes duodenal intubation is necessary (aspiration of bile containing eggs). Serology may be helpful. The treatment consists of **praziquantel**. A new drug, tribendimidine, appeared very promising for both *Opisthorchis* and *Clonorchis* in recent phase 2 trial.

## Large liver flukes: *Fasciola hepatica* and *F. gigantica*

### General



Infection with these large liver flukes is quite wide-spread among animals. For example, *Fasciola hepatica* causes liver rot in sheep. The encapsulated larvae (metacercariae) are found on all kinds of plants such as water cress (*Nasturtium officinale*), etc. After infected plants have been consumed the larvae are released in the small intestine, migrate within the hour through the intestinal wall to the peritoneal cavity and then bore through the liver capsule about 5 days later. After further migration in the liver, they reach the bile duct after approximately 7 weeks and remain there, laying their eggs.

These are transferred via the bile to the intestine, and then excreted with the faeces. A single liver fluke can lay up to 20,000 eggs a day but usually produces smaller numbers. It should be noted that fertilized eggs can be produced by a single liver fluke (they are hermaphroditic).

Eggs often remain viable for months and can overwinter. Survival for more than 2 years has been demonstrated at a temperature of 2°C. Fierce heat and drying out kills the eggs. At a temperature of approximately 25°C (the optimum temperature) eggs develop in about three weeks. There is much variation in the rate at which eggs are released, which is an advantage to the parasite, since a particular habitat will remain infectious over longer periods. Under the influence of specific stimuli a 130 µm long larva (miracidium) emerges from the egg. This is covered with cilia and is immediately mobile in water. It can easily swim for hours. The larva has eye spots and is highly phototropic (it swims towards the light).

This prevents the larva from wasting time and energy exploring the bottom of the pond, where the intermediate host (usually *Lymnaea trunculata*) is not to be found. This is unlike *F. gigantica* where the miracidium actively swims away from light to find *L. natalensis*, which

lives deeper down. If the larva does not find the correct snail within 24 hours its glycogen reserves are exhausted and the larva dies. If a miracidium arrives some 15 cm from a snail, there is pronounced chemotaxis and the larva swims directly to the host and penetrates it. The next development takes place within the snail. These snails can survive long periods of drought (via aestivation) and long-term cold (via hibernation). Inside the snail, the miracidium develops into a sporocyst and then into rediae, a stage named after the Italian physician Francesco Redi (1688). The rediae measure approximately 1-3 mm, are mobile and may cause significant damage in the snail (if the infection is severe the snail dies). After 4-7 weeks the first cercariae emerge from the rediae; they measure 250-350 µm and leave the snail. The cercariae swim around in the water, to encyst within 2 hours on particular plants. Each cercaria then changes into a metacercaria (plural metacercariae). Due to the amplification phase in the snail, a single egg can produce 4000 metacercariae. Metacercariae can survive for more than a year on pasture. They are destroyed by heat and drought (the effect of long hot summers).

### Clinical aspects

Symptoms are present mainly during the migration period: fever, pain in the liver region, hepatomegaly, urticarial, eosinophilia. After this period symptoms are generally mild or absent.

Sometimes there is cholangitis and obstructive jaundice. If raw goat's or sheep's liver is eaten, adult worms can sometimes attach to the throat, resulting in local irritation (halzoun).

### Diagnosis

The diagnosis is made by detecting the eggs in faeces or duodenal aspirate (eggs appear approximately 12 weeks after infection). Repeated specimens are often necessary in view of the small number of eggs which are produced daily. If an individual has eaten infected sheep's liver, he/she can have eggs in the faeces, although no real infection occurs (spurious infection). Ultrasound or CT scan of the liver may show a clustering of hypo reflective or hypo dense tunnels in the liver parenchyma (these are inflamed bile ducts). Sometimes it is possible to actually visualize the moving worms. Via laparoscopy, one can sometimes find slowly migrating worm tracts. The specificity of serology is lowered by cross-reactivity with other helminths.

### Treatment

The therapy is problematical at present.

- Praziquantel is not sufficiently active.
- **Triclabendazole (Fasinex®, Egaten®) 10 mg/kg taken in one dose** together with a fatty meal is becoming the treatment of choice. Triclabendazole-resistant *F. hepatica* strains are already known in cattle.
- Nitazoxanide is an alternative drug. The dose is 500 mg BD for 1 week.
- Artemisinin may become an alternative. Worm burden reductions of 99-100% were observed with a single dose of the drug.

## Blood flukes

### Schistosomiasis

#### Summary

- Schistosomiasis (or bilharziasis): infection with small blood flukes
- *S. haematobium*, *S. mansoni*, *S. japonicum* are the most common
- *S. intercalatum* (closely related to *S. guineensis*) and *S. mekongi* are of regional importance
- Hybrid species (*S. bovis-S. haematobium*) are occasionally found in humans
- Certain species of fresh water snails are intermediate host
- Transmission via skin contact with fresh water containing larvae (cercariae) released by intermediate host.
- Mainstay treatment with praziquantel, active against adult worms only. Artemisinin derivatives are active against immature schistosomes
- Symptoms depend upon worm load (number of worms), anatomical location of parasites, duration of infection and host immune system reactions.
- Short-lasting pruritus after transcutaneous infection is possible (swimmer's itch)
- Acute schistosomiasis syndrome or "Katayama syndrome" a few weeks to months after primary infection (beginning of egg production). Fever, cough, influenza-like symptoms, abdominal pain, hypereosinophilia, splenomegaly.
- Ectopic localizations with e.g. neurological lesions may occasionally occur after primary infection, but also during chronic infection
- Chronic lesions due to *S. mansoni* and *S. japonicum*: abdominal discomfort, bloody diarrhea, hepatic fibrosis with portal hypertension, esophageal varices, ascites, hepatosplenomegaly – no risk for colon carcinoma
- Chronic lesions due to *S. haematobium*: hematuria, hydronephrosis, renal insufficiency, genital lesions, right heart decompensation due to pulmonary hypertension secondary to lung fibrosis. Increased risk for bladder carcinoma.

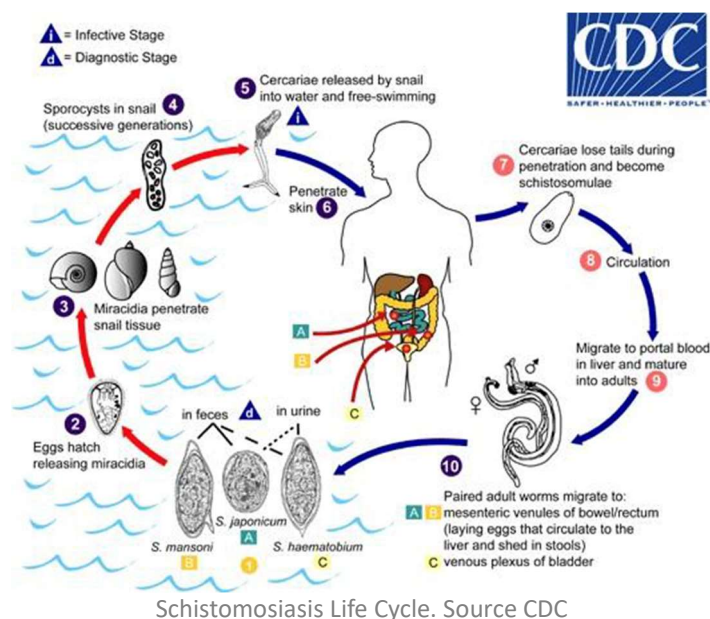
#### General

The infection was first described by Dr. Theodore Bilharz (1825-1862) when he was working in the Kasr-el-Aini hospital in Cairo. Schistosomiasis or bilharziasis (this terminology should be abandoned) is a disease caused by flatworms (trematodes or flukes). There are 3 main species which infect humans: *Schistosoma haematobium*, *S. mansoni* and *S. japonicum*. There are a few other species that infect humans, but these are less widespread: *S. mekongi* and *S. intercalatum*. Approximately 200 million people throughout the world are infected, a minority of whom are severely infected.

Both males and female worms have two suckers. The more anterior one surrounds the mouth. Bilharz mistakenly took the two suckers for two mouths and thus called the worm *Distomum* ("two mouths"). Five species of schistosomes are known to infect humans and lay eggs (complete their life cycle).

Infections with *Schistosoma mansoni*, *S. japonicum*, *S. mekongi*, and *S. intercalatum* are associated with intestinal lesions and chronic hepatic fibrosis. *S. haematobium* infection mainly results in fibrosis, strictures and calcification of the urinary tract.

## Life Cycle



Eggs reach the outside world via the faeces or urine. Each egg contains a larva that possesses numerous cilia. In a fresh microscopic preparation, a larva can be seen moving in the egg. If an egg reaches fresh water, the larva (synonym miracidium) is released. If the eggs do not reach the water, the larvae die rapidly, except in the case of *S. japonicum*. The latter can survive for up to 80 days outside the body (importance for hibernating). After swimming around for a while, a miracidium penetrates a snail (each parasite species is restricted to its own range of host snails). After two generations in the snail in the form of primary and later daughter sporocysts, a very large number of larvae each with a bifurcated tail, known as cercariae, is released. There is asexual proliferation in the snail, therefore one miracidium can produce up to 100,000 cercariae (etymology Gr. “kerkos” = tail). Cercariae live for 48 to 72 hours.

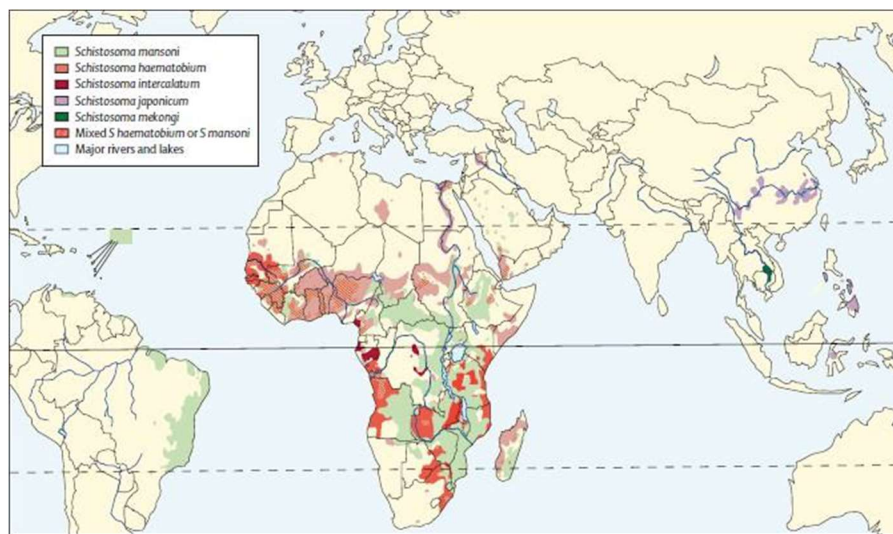
Infection is acquired via skin contact with contaminated water. These larvae can actively penetrate the skin in 3 to 5 minutes. Cercariae penetrate the skin of humans or, in the case of *S. japonicum*, humans and other mammalian hosts that act as reservoirs for infection. Once in the human body, the cercariae lose their bifurcated tail and develop further into schistosomula.

After migration through the dermis and the lungs, they reach the liver. Copulation occurs in the blood vessels of the liver (in contrast to other trematodes, schistosomes are not hermaphroditic but have separate sexes). Afterwards, the worms migrate upstream to their final destination: the superior mesenteric venules in the case of *S. mansoni*, the inferior mesenteric and superior hemorrhoidal venules in the case of *S. japonicum*, or the vesical plexus and veins draining the ureters in the case of *S. haematobium*. Egg production starts (300 to 3000 per day, depending on the species) four to six weeks after infection. The eggs mature in approximately one week and remain alive for 3 weeks (longer in the case of *S. japonicum*). The eggs contain a miracidium that is motile (cilia). The movement can be seen in fresh microscopic preparations. The adult worms are not carried away by the flow of blood because they are attached to the vascular wall with two suckers per worm. The short, wide

male contains the longer thinner female in a sort of groove, the gynaecophoric canal. It was previously thought that this was a single animal with a split body. This continuous "embrace" led to the parasite's name [schistos = split; soma = body]. The male is approximately 10 mm and the female 20 mm long. The mean life span of the adult worms is 3 to 7 years, but some can survive for up to 20 years. The theoretical reproduction potential of one schistosome pair is up to 600 billion schistosomes. For *S. haematobium* and *S. mansoni*, humans are the most important reservoir. *S. japonicum*, however, is predominantly a parasite of animals (water buffalo, dog, pigs, etc).

### Geographical distribution

<i>S. mansoni</i>	Africa, Middle East, South America	GI-tract, colon	mostly	( <i>Biomphalaria sp</i> )
<i>S. haematibium</i>	Africa, Middle East	Urinary tract		( <i>Bulinus sp</i> )
<i>S. intercalatum</i>	Central Africa	GI-tract, rectum	mostly	( <i>Bulinus sp</i> )
<i>S. japonicum</i>	Southeast Asia and Far East	GI – small intestine		( <i>Oncomelania sp</i> )
<i>S. mekongi</i>	Mekong basin	GI-tract		( <i>Tricula sp-syn Lithoglyphosis</i> )



Global distribution of countries where human schistosomiasis is transmitted. Source Bruno Gryseels and colleagues

### Vector

Each *Schistosoma* species has its own snail species as a vector: see table above. These snails require well-defined ecological conditions to thrive. The average temperature plays a role.

If it is too cold, the snails cannot proliferate. There is thus practically no transmission above 1800 meters, where the water temperature is too low. Some snail species resist periods of long-term drought. This explains the occurrence of schistosomiasis in locations where there is only abundant water during the rainy season. At these sites, there is naturally no transmission during the dry season.



*Biomphalaria alexandrina*. This snail can harbor *Fasciola gigantica* and *Schistosoma mansoni* (bilharzia).  
Copyright ITM



Snails. *Bulinus truncatus* / *Bulinus africanus*, host for *Schistosoma haematobium*, bilharzia. Copyright ITM

## Hosts

<i>S. mansoni</i>	principally humans, also baboons and rodents
<i>S. haematobium</i>	principally humans, rarely monkeys
<i>S. intercalatum</i>	only humans
<i>S. japonicum</i>	animals: water buffaloes, dogs, cats, rats, pigs, etc., also humans
<i>S. mekongi</i>	dogs, sometimes humans

## Immunologic aspects of schistosomiasis

Longstanding epidemiological and clinical observations indicate that people living in endemic areas acquire some form of immune resistance after years of exposure. However it is not clear if true immunity is acquired or if the reduction in number of infections can be explained by reduced water contact after adolescence.

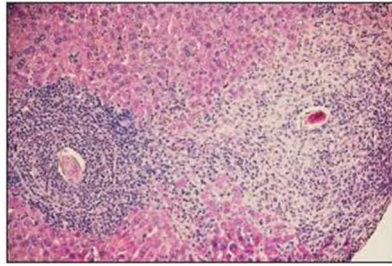
Comparative studies of reinfection after curative treatment have demonstrated that children are far more susceptible than adults and that these differences cannot be explained by quantified water contact patterns. Numerous studies in humans and in animal models suggest that acquired immunity would be mediated by IgE against larval and adult worm antigens, which stimulate eosinophils to release cytotoxines targeting schistosomulae.

Cellular immune responses against eggs and the enzymes they release are responsible for most schistosomiasis-related pathology. The granulomatous reactions around the eggs are orchestrated by CD4 T-cells and involve eosinophils, monocytes and lymphocytes. A shift from a predominantly TH1 reaction in early stages of the infection to a “modified” TH2 profile in chronic infections would account for the regression of the granulomas and their replacement by collagen. This has important implications for targeted mass treatment. Heavily infected children often present with non-fibrotic hepatomegaly due to its TH1 nature, and this pathology is usually reversible upon treatment. Likewise in early urinary schistosomiasis, haematuria seen in children promptly disappears after treatment with praziquantel.



## Clinical aspects

### Pathophysiology



*Schistosoma mansoni* egg-induced granulomas in the liver of an infected mouse. Eggs are roughly 120–180  $\mu\text{m}$  long, 45–70  $\mu\text{m}$  wide. From The Lancet

Pathology depends on the stage of the infection. Chronic symptoms are related to the total worm load (= number of worms in the body). Usually, light infections are asymptomatic. The likelihood of symptoms increases with an increase in the degree of infection. Sometimes parasites are found at ectopic sites (e.g. spinal cord), in which case there can be severe consequences, even in mild infections.

Most people in endemic areas acquire some immunity over the course of the years. Many adults exhibit few signs of infection. This immunity is directed towards new infections, against schistosomula. Adult worms are covered in an unusual skin ("tegument") that displays few parasite proteins on its outer membrane. As a result, the immune system usually takes little notice of the adults. In addition, certain human molecules, such as those which determine blood type, can stick to the surface of the worms, further shielding the parasite from the immune system. The immunological reaction directed towards the eggs produces cytokines which induce a host Th-2 immune response, leading to an eosinophilic granulomatous reaction, responsible for most pathology.

**Clinical, Swimmer's itch.** A local cutaneous itch can occur where cercariae penetrate. Slight erythema and pruritic papules develop, but will disappear spontaneously. This is well known with *S. mansoni*, *S. haematobium* and with *S. japonicum*. The itch is more frequent and violent in infections with animal schistosomes, probably because the cercariae die after penetration in humans (e.g. avian schistosomiasis, which also occurs in areas with a moderate climate).

**Acute schistosomiasis (or "Katayama fever")** is caused by primary infection with schistosomes and represents a hypersensitivity reaction to maturing schistosomules (and probably also to antigens released from the eggs). It usually occurs 3 to 8 weeks after initial infection, It can be mild or severe, with one more of the following symptoms: fever, general discomfort, abdominal pain, diarrhea, vomiting, flu-like syndrome with muscle and joint pain, severe dry cough, wheezing, urticaria and sometimes lymph node enlargement and hepatosplenomegaly. There is nearly always a marked eosinophilia of  $> 1000/\mu\text{L}$ . Katayama fever is less frequent and milder in *S. haematobium* infections.

### Diagnosis and treatment of acute (symptomatic) schistosomiasis

- Katayama fever tends to occur in patients who are not previously exposed, although in the case of *S. japonicum* this syndrome can also occur on re-infection.

- History of recent exposure, freshwater contact in endemic area < 10 weeks before symptom onset.
- Respiratory disorder and/or abdominal discomfort with fever and hypereosinophilia (DD trichinellosis, fascioliasis, filariasis, Strongyloides hyperinfection, etc.). Acute schistosomiasis is the most common cause of fever with hypereosinophilia in travelers returning from endemic areas, almost exclusively after a stay in sub-Saharan Africa.
- Eggs usually only appear in the faeces or urine after 6 to 12 weeks. Failure to detect eggs thus does not rule out acute schistosomiasis.
- Serum antibody tests may turn positive from 6 weeks after infection, but often much later.
- In acute schistosomiasis, the performance of PCR based genomic tests and of soluble schistosome antigen tests in blood and urine is still a research object.
- Treatment of acute schistosomiasis consists of steroids in the acute symptomatic phase, and additionally with praziquantel once symptoms are subdued with steroids. Repeat the course of praziquantel after a 3-4 months. Praziquantel is only active against adult worms.

## Ectopic localisations

Central nervous system schistosomiasis occurs by ectopic worm or egg dissemination via the bloodstream through retrograde venous flow into the Batson vertebral epidural venous plexus, which connects the portal venous system to the spinal cord and cerebral veins. When eggs and/or adult worms cause lesions of the spinal cord, transverse myelitis (principally *S. haematobium* and *S. mansoni*) or brain lesions (principally *S. japonicum*) ensue. These take the form respectively of spastic paraparesis and CVA or space-occupying lesion (hemiplegia, epilepsy, etc...). Urgent treatment consists of steroids for several weeks to months to limit the local inflammatory reaction. Treatment with praziquantel has to be given while continuing steroids. Sometimes eggs can reach the skin, where they can cause papular dermatitis. This rare condition can only be diagnosed by biopsy. Even rarer is localization in the vocal chords, with nodules and hoarseness.

In acute schistosomiasis, a form of localized hypersensitivity encephalopathy may occasionally occur and requires steroids for effective initial treatment.

## Symptoms in chronic infections

### General

The live larvae (miracidia) in the eggs excrete proteolytic enzymes that digest the surrounding tissues. In this way, eggs can reach the rectal lumen or the bladder after their migration through the intestinal mucosa (*S. mansoni* and *S. japonicum*) or the bladder mucosa (*S. haematobium*). In general 50% or less of the eggs are eliminated with the faeces or urine. The remaining eggs either die locally or are transported with the venous blood until they reach the liver or another organ where the blood vessels become too small for their further passage. At this point the eggs and digestive juices which they secrete can cause local inflammation. The lesions of chronic schistosomiasis can be explained almost exclusively by the local inflammatory reaction to these eggs (formation of granulomata containing numerous eosinophils). Inflammation granulomata can reach up to 100 x the size of the original egg.

Fibrous thickening and loss of elasticity of the tissues occurs. If massive infestation is present pseudopolyps occur in the intestine or bladder. The symptoms in chronic schistosomiasis differ according to the location.

### *Intestinal lesions*

Diarrhoea sometimes with some blood and mucus, can be caused by *S. mansoni* and *S. japonicum*.

Pseudopolyps can occur in the colon. There is no increased incidence of intestinal cancer. In severe chronic infections, fibrosis of the intestine can occur. *S. mekongi* and *S. intercalatum* are also found in the intestine but usually does not cause severe pathology.

### *Hepatosplenic schistosomiasis*

When *S. mansoni* eggs are carried in the portal venous bloodstream as far as the liver they cause a physical obstruction of the bloodstream. Local inflammation around the eggs exacerbates this. The result is increased pressure in the portal circulation (portal hypertension). Clinically it takes the form of:

- Collateral circulation with oesophageal varices and increased venous markings on the abdominal skin, principally around the navel (umbilicus), the so-called "caput medusa". Eggs can also reach other organs subsequently via this collateral circulation (lung).
- Ascites is a late sign.
- Because the eggs obstruct the branches of the portal venous system and fibrosis occurs around these foreign bodies, periportal fibrosis develops. This is also known as Symmers pipestem fibrosis which is seen years later in the course of the infection. The white fibrotic bands in the liver are in fact long and hollow like pipestems (in the center is a branch of the portal vein). Liver function remains surprisingly well preserved for a long time (normal blood coagulation, no severe hypoproteinemia, no hepatic encephalopathy, no gynaecomastia). This stands in contrast with chronic aggressive hepatitis B and alcoholic cirrhosis. *S. japonicum* lesions are usually more severe because the worms produce ten times more eggs per day (3000 as opposed to 300) than *S. mansoni*. People with severe, chronic infections often die from bleeding from oesophageal varices.

### *Renal and urinary tract lesions*

*S. haematobium* eggs lie grouped together in the bladder wall and surrounding organs (rectum, prostate, vagina, cervix, ovaries). This leads to the formation of very small (sandpaper-like) to a few mm large fleshy polyps and ulcerations. *S. haematobium* infection is a cause of genital lesions which are often mistaken for "warts". The lesions in the urinary tract cause blood to be passed in the urine. In endemic areas this occurs typically in children of school age. The severity of the haematuria and proteinuria is related to the degree of infection. Ureteral strictures occur mostly in the distal third (mainly in the intravesical part). Ureteral obstruction can occur with resultant hydro-ureter and hydronephrosis. Because of the impairment of the normal anatomical relations vesico-ureteral reflux can also occur. Initially these lesions are still reversible. *S. mansoni* can give rise to a deposition of immune complexes in the kidney leading to glomerulonephritis. This happens in about 0.5% of cases usually in those with severe infections. In *S. haematobium* schistosomiasis the bladder wall can thicken.

This resembles the thickening that can occur in tuberculous cystitis, chronic interstitial cystitis, radiation cystitis, chronic chemical cystitis or as a result of muscular hypertrophy with obstruction due to prostatic hypertrophy or neoplasia or with a neurogenic bladder. At a later stage in bladder schistosomiasis, calcification of the dead eggs in the bladder wall occurs which is clearly visible on X-ray film.



Bladder calcifications due to *Schistosoma hematobium*

### Bladder carcinoma

In highly endemic areas of *S. haematobium*, there is an increased incidence of bladder carcinoma. This is manifested around the 4th or 5th decade of life. It principally involves a highly malignant squamous cell carcinoma which can occur anywhere in the bladder wall (in contrast to transitional cell carcinoma that occur later in life and is located principally in the trigonum). There may be a relationship with increased carcinogen concentrations (DNA alkylating nitrosamines) due to frequent bladder infections with nitrate-reducing bacteria, due to smoking tobacco or due to the local production of nitrosamines via activated macrophages.

### Late consequences

After severe chronic infection, the following serious problems can occur:

- Reduced bladder capacity.
- Increased incidence of bladder carcinoma, especially in tobacco smokers.
- Lesions of the female genitalia with cervical erosions, papillomatous lesions, sterility, increased risk of ectopic pregnancy and sexually transmitted diseases such as HIV.
- Lesions of the male genitalia e.g. localization in the ductus spermaticus.
- Irreversible obstruction of the urinary tract with hydro-ureter and hydronephrosis.
- Renal stones can form. Recurrent bacterial urinary infections are frequent, including Salmonella.
- Nephrotic syndrome can occur, as well as hypertension.
- Finally, chronic renal insufficiency may ensue.

Remember that chronic glomerulonephritis can be caused by *S. mansoni* which is localized in the intestines (and eggs in the liver); This is secondary to the deposition of immune complexes in the renal glomeruli.

### *Cardiac and pulmonary lesions*

*S. haematobium* eggs can reach the systemic circulation and only rarely reach the portal circulation.

This parasite is not a cause of hepatosplenomegaly. These eggs then reach the lungs. The same can happen with severe portal hypertension when *S. mansoni* or *S. japonicum* eggs reach the lung via the collateral circulation. Inflammation occurs in the lungs resulting in fibrosis and pulmonary hypertension.

Right heart failure then occurs with development of cor pulmonale with congested jugular veins, peripheral edema and congested swollen liver. It used to be common in Brazil and Egypt but less so in recent years.

#### *Association with other infections*

Patients with schistosomiasis (*S. mansoni*, *S. haematobium* and *S. japonicum*) are at increased risk of being *Salmonella* carriers. These bacteria are found in the intestinal tract of the worm. Urinary schistosomiasis often results in recurrent bacterial urinary tract infections.

Genital schistosomiasis is an independent risk factor for HIV infection since eggs can induce inflammation leading to development of ulcerative lesions in the female reproductive tract.

## Diagnosis

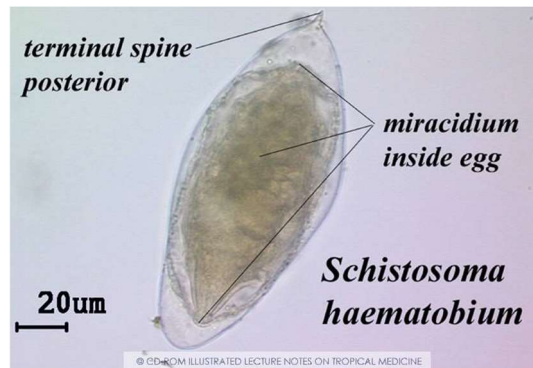
### *Microscopy*

The definitive diagnosis is established by the detection of eggs in the stools or urine with or without concentration techniques. The weight of a stool specimen that can be examined by direct microscopy is approximately 2 to 4 mg. In view of the small volume, egg excretions of up to 100,000 eggs per day can be missed. Only severe infections are detected in this way. The Kato-Katz method (cellophane impregnated with glycerol and malachite green) uses a larger quantity of stool (25 to 50 mg). The method is simple and more sensitive but more cumbersome. Low-grade infections can still be missed.

Concentration of ova in faeces or urine can be done by a range of techniques.



*Schistosoma mansoni* egg with a large lateral spine. Copyright ITM



*Schistosoma haematobium* egg with terminal spine. Copyright ITM

The **sensitivity** of the laboratory tests can be problematic. It is dependent on the quantity of sample which it is feasible to routinely examine. A sample of 10 ml **urine** is equivalent to approximately 1/100 of the daily production and thus theoretically makes it possible to detect even a mild infection if it is assumed that at least 100 eggs/worm pair/day are found in the urine. With more than 50 eggs/10 ml there is almost always (microscopic) hematuria and proteinuria and this number has therefore been taken as the accepted threshold for distinguishing between mild and more severe infections. A stool smear by contrast, examines only 2 mg out of a total quantity of feces which for an adult in a tropical environment, may be estimated as 200-400 g/day. In this case therefore only 1/100,000 - 1/200,000 of the daily quantity of stool excreted is examined.

The severity of infection is reflected by the egg load. In *S. mansoni* infection, egg loads of less than 100 eggs per gram feces (epg) are considered mild and severe infections have more than 400 epg. A mild infection is equivalent to more than 5 eggs/Kato smear. For this reason the WHO recommends the Kato method with which it is possible to examine 25 to 50 mg of stools, sufficient to discover all severe infections. It is however clear that even with this technique many milder infections are missed. It is important that a positive smear always indicates an infection which is already fairly severe.

There are several other flotation and sedimentation concentration methods which allow detection of lighter degrees of infection. For instance the FLOTAC stool concentration method is about 10 times more sensitive for *S. mansoni* egg detection than the Kato-Katz. The sensitivity is also affected by fluctuations in the quantity of eggs in the excreta. This is the case for excretion of *S. haematobium* eggs which peaks around midday. Specimens taken between 10 a.m. and 2 p.m. are therefore optimal for examination. During the evening hours and the night elimination falls to a minimum. This periodicity is the result of contractions of the muscle wall of the bladder which itself is affected by drinking/meals and exercise and not by the production of eggs (which occurs continuously). In the case of intestinal schistosomiasis this systematic factor has little if any importance.

An additional source of error is that the elimination of eggs in the same person can vary considerably from day to day, making the individual diagnosis more difficult. The consequence is that a negative parasitological examination even with sensitive techniques is only of limited value. In a severely infected person, on average high egg loads are found and only rarely low egg loads. The opposite is true for people with low worm loads. Taking fecal specimens on different days is better than examining several smears from 1 stool.

### Serology

Serology based on the detection of antibodies, does not distinguish between active and previous infections. Positive serological tests with low titers which cannot be confirmed parasitologically probably indicate either (1) an old, cured infection, (2) an infection with a very low worm load, (3) an infection by worms of a single sex or (4) cross-reactivity with other worm species. Antibodies are usually detectable before eggs are detectable.

### Antigen detection

Circulating antigen detection tests (circulating anodic and cathodic antigen CAA and CCA) have been developed, as a means to detect schistosomiasis. Antigen can be detected in serum as well as in urine (in urine also for *S. mansoni*). Many research groups are evaluating such antigenic tests in the tropical fields under rapid test formats (RDT) based on lateral flow immunochromatography. A recently developed CCA dipstick test (in urine) has been consistently found much more sensitive in detecting infection with *S. mansoni* than conventional stool microscopy (Kato-Katz concentration).

### Polymerase chain reaction

PCR based molecular techniques are currently being developed for population surveys and for clinical use. Due to sophistication and costs this technique is still restricted to research and reference labs. The sensitivity and specificity of different formats and sequence targets are being evaluated.

### Medical imaging

Complications can be detected by means of ultrasound (e.g. hydronephrosis). The degree of liver involvement can also be determined echographically. This may be of epidemiological importance for example in control programs. Ultrasound is the only possible technique for establishing a non-invasive sensitive and specific diagnosis of hepatic lesions in hepato-intestinal schistosomiasis. The lesions are pathognomonic and can even be seen in children with surprisingly low egg excretion. There is a clear relationship between the presence of ultrasound lesions and the mean excretion of eggs.

Symmers hepatic fibrosis can become symptomatic in later life when the parasite load has become low and it can even be difficult to detect that there is an infection. Clinical differences between cirrhosis and Symmers hepatic fibrosis are relative: youth of the patients, more pronounced splenic enlargement in Symmers hepatic fibrosis, general health preserved for longer even after hematemesis, hepatic enlargement predominantly of the left liver lobe.

Bladder calcifications may be visible on a standard radiograph of the abdomen and on CT-scan.

### Biopsy

**Rectal snip or rectal biopsy** consists in removing 1 to 3 fragments of superficial tissue with biopsy forceps under endoscopic control from sites where small hemorrhages or other suspicious lesions are seen. These tissue specimens are placed between two glass slides and examined immediately in a drop of water without fixation. Histological examination has a lower diagnostic yield as the section of tissue examined is much thinner. The sensitivity of a rectal snip is good and curiously, better for *S. haematobium* than for *S. mansoni*. In travel medicine this examination is more sensitive, particularly in the case of *S. haematobium* than

examination of the urine or stools because the patients involved are mainly adults with a low worm load with few eggs. Rectal snip detects eggs that have accumulated over a period of weeks, months or years under the rectal mucosa. The distinction between dead and living eggs is important. When living eggs are examined immediately after sampling in an unstained biopsy the moving cilia of the miracidia can easily be seen. The rectal biopsy technique is not used so often in endemic areas where attention is directed particularly to children. Rectal snip data are not quantitative.

**Other biopsies:** Needle biopsy or surgical biopsy of the liver cannot confirm the diagnosis in all cases and is dangerous in patients with a bleeding tendency. Cystoscopy can be used to visualize bladder lesions. Eggs are found in bladder biopsies and sometimes in cervix or skin.

## Treatment

### *Praziquantel (Biltricide®)*

Praziquantel (PZQ) is the first choice treatment. The standard dose is one 600 mg tablet per 15 kg or 40mg/kg body weight. Usually it is given as a single dose but doses can be taken 4 to 6 hours apart to minimize side-effects. A single dose reduces the parasite load by more than 80%. Treatment cost is low and makes repeated mass treatment an attractive option to reduce the parasite load.

Praziquantel kills the adult worms not the immature schistosomules or the eggs. Praziquantel is undoubtedly the most effective medication with the fewest side-effects for the treatment of all species of schistosomes. The drug seems less effective in regions of West Africa where there has been a recent invasion of the parasite possibly due to rapid re-infections. Diminished sensitivity or resistance to praziquantel are other possibilities but not a major issue so far. Apart from its activity against all human schistosomes including *S. mekongi*, *S. intercalatum* and *S. mattheei*, praziquantel is also very effective against most infections due to trematodes (except Fasciola) and cestodes but not roundworms. There are no contra-indications and no major toxicity has been reported. It may be given in hepatic insufficiency. The drug however is not recommended during the first three months of pregnancy. Side-effects include abdominal pain, vomiting, diarrhea and fever; probably caused by the reaction to the dead worms. In very severe infections cases of rectorrhagia are observed. In patients with a hemorrhagic diathesis, this could be dangerous. There is a major risk if cysticercosis is present in the region since serious complications (seizures) can occur as a consequence of the death of cysticerci in the CNS following administration of the medication. In spinal and cerebral schistosomiasis, praziquantel always follows prolonged corticosteroid treatment.

Praziquantel has no effect on eggs and immature worms. Tissue-dwelling eggs can be excreted for several weeks after treatment. During the same period prepatent or newly acquired infections can become productive. The preferred timing of follow-up is therefore 4-6 weeks after initial treatment to kill the meanwhile matured schistosomes. Katayama fever is primarily treated with corticosteroids to suppress the hypersensitivity reaction and with praziquantel to eliminate matured worms.

*Other drugs used in the past* (and still available in some countries) for schistosomiasis

- Niridazole (Ambilhar®)



- Metrifonate (Bilarcil®)
- Oxamniquine (Vansil®)

Praziquantel has become however the first-choice treatment everywhere in the world. Some of these second-line drugs might become important again if resistance to PZQ would emerge.

*It should be also noted* that the artemisinin derivatives have “preventive” effect on *S. mansoni* and *S. japonicum* (being mostly active on schistosomulae). Mefloquine is another antimalarial drug with some partial efficacy on *Schistosoma* larvae and adult worms (all species). Several small studies have demonstrated short-term reduction of egg load with both drugs in particular when they are combined.

Whether these drugs can be used in addition to praziquantel for schistosomiasis treatment or prevention (in malaria-free areas or in returning exposed travelers) still remains to be thoroughly studied before any new recommendation can be made.

## Prevention

The main objective for schistosomiasis control is the **reduction of morbidity** which is based on the reduction of the worm load. No vaccine is yet available. Attention is focused on detection and treatment and vector control. Mass treatment can be undertaken with yearly administration of praziquantel (treat everyone without screening). Selective treatment of infected people can also be carried out following active screening. Treatment can be restricted to a particular group (e.g. all schoolchildren or children from specific school years). Passive screening (all those people who attend a health center) is also possible. Control of morbidity should not be seen separately from **control of transmission**.

Health education is not effective if it is not associated with an improvement in sanitary conditions in the district. The avoidance of contact with contaminated water is only possible if there is an alternative.

Hence the importance of a technical infrastructure and its maintenance (washing areas, showers, toilets, footbridges over canals etc). It is obvious that these are only meaningful if they are accepted and used. The erection of numerous bridges over canals and the use of water pumps (drinking water, washing areas) reduce contact with potentially contaminated water. Water pumps should be of a design that allows inexpensive local repairs to be made.

It is also possible to try to **control the vectors**. Snails like mud and the presence of water plants. If there is a large amount of deep shadow in places where the vectors are present, the latter will have less food (snails eat algae and plants, which are dependent on photosynthesis). No shadow at all, however, will reduce egg production. Control of water plants and vegetation involves infrastructural changes.

Covering over irrigation channels can limit snail populations. Cleaning of canals or the use of concrete for irrigation canals can be useful. Snail control by chemical molluscicides cannot be sustained in the long-term (too expensive and too much collateral ecological damage). The efficacy of biological control of the vector still needs to be demonstrated. The use of certain plants with a molluscicidal effect is being studied, e.g. the soapberry Endod (*Phytolacca dodecandra*).

# Various Medical Mycoses

## Introduction

Medical mycology deals with the nature and the causes of the diseases occasioned directly (mycoses and allergies) or indirectly (poisoning) by fungi. Mycotoxins are toxic molecules that are present in various moulds. Reference is made to mycetism when a mycotoxin causes poisoning directly, as is the case for instance with *Amanita phalloides* (Death Cap). Mycotoxicoses are diseases caused by ingestion of foodstuffs in which toxins are released and therefore involves an indirect form of poisoning. The most well-known example is aflatoxicosis caused by aflatoxins produced by *Aspergillus flavus*. Some moulds can cause allergic syndromes such as extrinsic allergic alveolitis (e.g. cheese washer's lung, maltworker's lung). All these conditions differ from mycoses, which are a parasitic type of infection. This text deals only with mycoses.

Beware of the term "mycotic" and "mycosis", which can be misleading, as in "mycotic aneurysms" (in general due to bacteria, e.g. in endocarditis), mycosis fungoides (a neoplastic disease), and bothryomycosis (*Staphylococcus aureus* infection).

The majority of medical important fungi are land organisms in contrast to some more primitive fungi which have more in common with protista and exhibit a motile stage.

Fungi are eukaryotes. They differ fundamentally in terms of cell structure and organisation from bacteria (prokaryotes) and are not susceptible to most antibacterial antibiotics. They are heterotrophic, in other words they have to obtain their energy from already existing organic molecules.

Consequently, many fungi live in association with living plants (often as harmful parasites, but also as beneficial symbionts, cfr mycorrhiza) or as free saprophytes on dead organic substances. Like bacteria, they feed by absorption.

Two groups are distinguished among the microscopic fungi:

1. **yeasts**, unicellular organisms that proliferate by budding and identification of which is based predominantly on morphological, as well as biochemical, properties such as oxidative assimilation or fermentation of various sugars.
2. **moulds**, which are identified on the basis of morphological characteristics (filamentous).

1. An important term to understand is: Dimorphic fungi. These are fungi that can exist in the form of both mould and yeast. This is usually brought about by change in temperature. An example is *Talaromyces marneffe*, a human pathogen that grows as a mold at room temperature and as a yeast at human body temperature.

## Epidemiology

In addition to their potential pathogenicity, fungi have one fundamental characteristic in common: they are first and foremost **saprophytes**. This means that their existence as a parasite in humans or animals is entirely unnecessary for the completion of their life cycle. They are at most **facultative pathogens**, which only parasitise if they encounter **promoting factors**, whether **systemic** or **local**. Examples of the former are deep candidiasis and

aspergillosis in patients with neutropenia and superficial candidiasis and deep cryptococcosis in AIDS patients. Examples of local promoting factors are skin irritations, which predispose to cutaneous and subcutaneous mycoses.

The majority of the causative agents of mycoses are exosaprophytes. A patient only develops symptoms following exposure to the natural biotopes or ecological niches of the fungi. Knowledge of these possible sources of infection is therefore important.

Some moulds have a limited geographical distribution. An AIDS patient can catch an infection with the cosmopolitan *Cryptococcus* anywhere in the world but can only acquire an infection with *Talaromyces marneffe* (Previously *Penicillium* sp.) in Southeast Asia.

Other moulds live in or on humans.

*Candida albicans* the main causative agent of candidiasis, is an obligate **endosaprophyte**. The normal biotope of this yeast is the gastro-intestinal tract and the oral cavity in particular. Local or systemic **promoting factors** are responsible for the transition from the saprophytic to the parasitic phase.

*Malassezia furfur* (*Pityrosporum ovale*) is a lipophilic yeast present in everyone as an **episaprophyte** on the skin, which in certain circumstances can become pathogenic.

This may explain why with a few exceptions such as certain dermatophytoses and sporotrichosis, **mycoses** should **not** be considered infectious.

## Classification

### Superficial mycoses

- Tinea (synonym dermatophytoses): infections of skin, hair and nails. Tinea capitis, tinea barbae, tinea corporis, tinea cruris, tinea pedis, tinea unguium (onychomycosis). Examples of anthropophilic dermatophytes:

- o *Epidermophyton floccosum*
- o *Trichophyton mentagrophytes* var. *Interdigitale*
- o *Microsporum langeroni*
- o *Trichophyton rubrum*
- o *Trichophyton schonleini*

Examples of zoophilic dermatophytes (which can also infect humans):

- o *Trichophyton verrucosum* - cattle
- o *Trichophyton equinum* - horse
- o *Microsporum canis* - cat (dog)
- o *Trichophyton mentagrophytes* var. *mentagrophytes* – rodent

- *Malassezia furfur* (*Pityrosporum ovale*): *Pityriasis versicolor*

- Superficial candidiasis: cutaneous, oral, genital. Causative agents: *C. albicans*, *C. glabrata*, *C. guilliermondii*, *C. krusei*, *C. parapsilosis*, *C. tropicalis*, etc.

### Subcutaneous mycoses

#### - Chromomycosis

- o *Fonsecaea pedrosoi*
- o *Fonsecaea compacta*
- o *Cladosporium carionii*
- o *Phialophora verrucosa*

#### - Mycetoma

- o *Eumycetoma*
- o *Actinomycetoma* (not due to fungi)

Examples of mycetoma:

#### Red discharge

- o *Actinomyces pelletieri*

#### White or Yellow discharge

- o *Acremonium strictum*
- o *Actinomyces madurae*
- o *Aspergillus nidulans*
- o *Noctuidina rosatii*
- o *Phaeoacremonium krajdani*
- o *Pseudallescheria boydii*

#### Black discharge

- o *Aspergillus terreus*
- o *Curvularia lunata*
- o *Cladophialophora bantiana*
- o *Exophiala jeanselmei*
- o *Leptosphaeria senegalensis*
- o *Leptosphaeria tompkinsii*
- o *Madurella grisea*
- o *Madurella mycetomatis*
- o *Pyrenochaeta romeroi*

#### - Sporotrichosis

- o *Sporotrix schenckii*

#### - Rhino-entomophthoromycosis

- o *Conidiobolus*
- o *Basidiobolus*

#### - Lobomycosis

- o *Lacazia loboi*

### Deep mycoses

#### - Cosmopolitan

- o Aspergillosis : a few hundred species described. Most relevant examples:
  - *Aspergillus fumigatus*
  - *Aspergillus flavus*
  - *Aspergillus niger*

- Candidiosis, Causative agents: *C. albicans*, *C. glabrata*, *C. guilliermondii*, *C. krusei*, *C. parapsilosis*, *C. tropicalis*, *C. kefyr*, *C. lusitaniae*, *C. zeylanoides* etc.
- Cryptococcosis
  - *Cryptococcus neoformans*
  - *Cryptococcus gatii*
  - *Cryptococcus albidus*
  - *Cryptococcus uniguttulatus*
- Pneumocystosis
- Mucormycosis
- Phaeohyphomycosis

**- Exotic (≠ tropic)**

- Histoplasmosis
  - *Histoplasma capsulatum* var. *capsulatum*
  - *Histoplasma capsulatum* var. *duboisii*
- Blastomycosis
- Penicilliosis
  - *Talaromyces marneffe*
- Emmonsiosis (*Emmonsia* sp.)
- Coccidioidomycosis
  - *Coccidioides immitis*
- Paracoccidioidomycosis
  - *Paracoccidioides brasiliensis*

# Subcutaneous mycoses

## Introduction

The term subcutaneous mycosis means a disease in which the pathogen, an exosaprophyte, penetrates the dermis or even deeper during or after a skin trauma. The lesions gradually spread locally without dissemination to deep organs. However, most fungi which cause subcutaneous mycoses can also occasion deep mycoses in patients with severe underlying abnormalities (via the respiratory tract).

Mycologically the pathogens of subcutaneous mycoses have only a few common characteristics and belong to very different taxonomic groups.

Subcutaneous mycoses occur exclusively or predominantly in the tropics. This is related on the one hand to the geographical distribution of the pathogens and the ecological factors that determine their saprophytic growth and sporulation and on the other hand it is also the consequence of the medical underdevelopment in these regions. Imported or indigenous cases are only rarely found in Western Europe.

## Chromomycosis

### Clinical presentation

This chronic dermal/epidermal mycosis, also known as chromoblastomycosis is characterised by vegetative and verrucous lesions, which occur predominantly on the lower limbs. In addition erythematous, nodular or ulcerating lesions are sometimes also found.

### Pathogens

The pathogens are dark-walled fungi (*Fonsecaea pedrosoi*, *Fonsecaea compacta*, *Cladosporium carionii*, *Phialophora verrucosa* etc.), which are saprophytes on plants and wood.



Chromoblastomycosis. Copyright ITM



Chromoblastomycosis, syn. chromomycosis; hyperkeratotic lesions foot; *Fonsecaea (Phialophora)* infection, copyright ITM

### Diagnosis

Microscopic examination of crusts in KOH shows the presence of irregular, 10-20  $\mu\text{m}$  large, brownwalled elements with transverse septa, 'sclerotic cells'. The specific causative agent can only be identified by culture.

### Treatment

1. Many clinicians find chromomycosis very resistant to antifungal treatment.
2. Surgery if possible (ideal for incipient lesions)
3. Heat therapy, as well as cryotherapy (for lesions with limited extend)
4. Itraconazole : 200-400 mg/day (+ ?5-fluorocytosine: 100-150 mg/kg/day)
5. Saperconazole might be more effective than itraconazole

6. Terbinafine : 500 mg/day for 6-12 months, after 2-4 months a reduction of 70% of the sclerotic cells is seen, Cure: 40% after 4 months, 75% after 8 months, 83% after 12 months. Terbinafine might be the first choice treatment.
7. Japan: fluconazole 200 mg/day + heat therapy (improvement after 2 weeks!)
8. Some patients have responded favorable to treatment with amphotericin B
9. Dematiaceous fungi are very sensitive (in vitro) to the new triazoles voriconazole and posaconazole, but further clinical data are needed.
10. The place of the latest triazoles isavuconazole, ravuconazole and albaconazole is still unclear but if a parallel with their action against other fungal infections can be made, they might be promising.



## Mycetoma

Mycetomas are chronic, inflammatory swellings with numerous sinuses, caused by moulds or bacteria.

The causative agent can be seen in the bloody or non-bloody pus and sometimes with the naked eye in the form of granules. In 75% of cases, a mycetoma is localised on the foot (Madura foot). In addition to involvement of soft tissue; bone tissue is severely affected with osteolysis on the one hand and hyperostosis on the other.



Madura foot patient in King Saud Medical Complex. Riyadh. Saudia arabia (Image source: Haitham Alfalah, Halfalah)



Mycetoma of shoulder and back

## Pathogens

Mycetomas are caused by 2 totally different groups of organisms: the first are moulds and the second are filamentous bacteria in the order Actinomycetales. In the first case they are referred to as eumycetomas (mainly Africa), in the second as actinomycetomas (mainly Latin-America). Also in India, mycetoma is prevalent. The difference is very important for therapy. All causative agents of fungal mycetoma are exosaprophytes that have penetrated deep into the tissue with a splinter of wood or a thorn. The primary reservoir of the causative agents is believed to be the soil. The limited geographical distribution of most pathogens and their natural history explain why mycetomas occur practically exclusively in the tropics. Eumycetoma can be caused by more than 42 different fungal species.

## Diagnosis

The differential diagnosis between fungal and actinomycotic mycetomas is based on the examination of the granules and/or culture. The compact microcolonies of the causative

agents differ from one another in terms of colour, shape, dimensions and composition. Black granules are always of fungal origin (e.g. *Madurella mycetomatis*); small red granules are specific for the actinomycotic *Streptomyces pelletieri*; whitish-yellow granules can be fungal or actinomycotic in nature.

In the direct examination of a crushed granule in KOH, the distinction between fungal and actinomycotic granules can be made on the basis of the presence or absence of true hyphal fragments.

Most information is obtained from the histological examination of a deep biopsy taken from around the path of a sinus. Vesicular or filamentous elements are seen in fungal granules (Gomori-Grocott stain).

Only *Madurella mycetomatis* the most common causative agent of eumycetoma, can be detected histologically by the presence of a brown cement. With the other moulds identification should be made by culture.

New DNA-isolation techniques on fungal cultures (takes 6 weeks) or directly on the grains (immediate result) are under development. Serological tests exist but don't detect all different species and are not used in routine diagnosis.

## Treatment

Until recently only surgical removal of the whole affected area was successful in treating eumycetoma.

Itraconazole for 12 months (or longer) in combination with removal of the mass, is the current the treatment of fungal mycetomas, but only results in 37% cure rate. The newer azole derivates posaconazole, voriconazole, isavuconazole and ravuconazole have excellent in-vitro activity. Their real life efficacy is under review and isolated case studies have shown resolution of symptoms with these agents. For actinomycetoma, the first choice treatment is combination treatment of 2 drugs, such as streptomycin or amikacin IV with dapsone or cotrimoxazole for a long duration (depending mainly of the causative pathogen). New data suggest that co-amoxiclav (Augmentin™) acid can be used instead of aminoglycosides to reduce ototoxicity and kidney toxicity.

## Sporotrichosis

### Etiology

Sporotrichosis is only caused by the mould, *Sporothrix schenckii*. It is an exosaprophyte on plants, wood and in the soil (peat moss). *S. schenckii* is a dimorphic mould. At 37°C and on rich nutrient media the yeast phase is obtained with oblong yeast cells.

### Clinical aspects



Sporotrichosis lesions with spread via the lymphatics. Copyright Alexander von Humboldt Institute, Peru

The classic presentation is the lymphocutaneous form. After an initial lesion, the inoculation chancre, subcutaneous nodules appear followed by ascending lymphangitis. The nodules progressively penetrate the skin and ulcerate. The most common localisations are the hand and the forearm. In addition to this typical lymphocutaneous form there is also one with disseminated skin lesions, a local cutaneous form, often on the face which according to some authors occurs in re-infections and extracutaneous sporotrichosis with involvement of the mucous membranes, bone, muscles, lungs or systemic infection. Pulmonary localisations without involvement of other organs occur in endemic areas (South America) probably more than is thought. This chronic pulmonary disease is often mistaken for smear-negative tuberculosis or chronic pulmonary aspergillosis.

### **Differential diagnosis:**

1. *Sporothrix schenckii*
2. *Blastomyces dermatitidis*
3. *Coccidioides immitis*
4. *Cryptococcus neoformans*
5. *Histoplasma capsulatum*
6. *Mycobacterium marinum*, *M. chelonae*, *M. abscessus*, *M. kansasii*
7. *Nocardia brasiliensis* and *N. asteroides*
8. *Leishmania* sp (mainly *L. guyanensis*).
9. *Francisella tularensis*
10. *Staphylococcus aureus*
11. *Streptococcus pyogenes*
12. *Bacillus anthracis*
13. *Burkholderia pseudomallei* (melioidosis)

## Diagnosis

In contrast to all other mycoses, the diagnosis of sporotrichosis is based not on the detection of the pathogen by direct or histological examination, but solely on culture. It involves collecting a small quantity of the milky pus from ulcerated lesions after the removal of the superficial crusts and then inoculating it onto a Sabouraud nutrient medium. Growth is obtained after a few days of incubation at 25°C and the typical asexual spore formation is easily identified.

## Treatment

For cutaneous forms, oral potassium iodide (saturated solution 1g/ml) can be used. As an alternative, terbinafine 2 x 250 mg/day for maximum 32 weeks can be used. Cure can be expected after 8 weeks.

Local heat therapy (I.R. or compresses) is sometimes used. The killing rate of the fungal cells is markedly higher at 40°C than at 37°C. Itraconazole and terbinafine can be used of for small lesions, as well as for systemic cases. Amphotericine B (liposomal formulation is preferred) is preferred for severe cases. Posaconazole (Noxafil) has good in vitro activity against *S. schenckii*, but more clinical data are needed. Fluconazole (Diflucan), voriconazole (Vfend) and ravuconazole are ineffective in sporotrichosis.

## Rhino-entomophthoromycosis



Rhinoentomophthoromycosis;  
nasofacial phycomycosis;  
*Coniobolus coronatus*  
(*Entomophthora coronata*),  
copyright ITM



Rhinoentomophthoromycosis;  
nasofacial phycomycosis;  
*Coniobolus coronatus*  
(*Entomophthora coronata*), copyright  
ITM



Rhinoentomophthoromycosis;  
subcutaneous  
phycomycosis;  
*Coniobolus coronatus*  
(*Entomophthora coronata*), copyright  
ITM

Zygomycosis is a term referring to infections with zygomycetes, and more specifically infections such as mucormycosis and entomophthoromycosis (synonyms of the latter are the tongue-twisting and jaw-breaking "rhino-entomophthoromycosis" and "rhino-entomophthoramycosis"). Rhinoentomophthoromycosis is a slowly progressing tropical infection of the subcutaneous tissue or paranasal sinuses caused by *Conidiobolus coronatus* or related species. Severe mutilations with grotesque deformation of the face can ensue. Basidiobolomycosis is often considered together with rhino-entomophthoromycosis. *Basidiobolus ranarum* affects subcutaneous tissue in areas such as buttocks, thighs and arms. Localisation in the face results in severe facial swelling, with gross deformity of eyelids and cheeks. The differential diagnosis includes Burkitt's lymphoma. Lymphatic filariasis usually presents in a different manner.

Histopathology will show fungal elements, granulomata and eosinophils. Culture will confirm the diagnosis. Azoles such as itraconazole or the allylamines terbinafine - with or without surgery - should be tried as treatment, although there is insufficient clinical experience. Amphotericin B is an option for *Basidiobolus* infections.

## Lobomycosis

Lobomycosis is a very rare infection. It is a self-limited chronic fungal infection of the skin endemic in rural regions in South America and Central America. The prevalence of the disease is high among the Caiabi Indians of Brazil and among the Amoruas tribe of the Casanare state in Colombia. It was the Brazilian physician Jorge Lobo who in 1931 in Recife first described this infection. He gave the name keloidal blastomycosis. The condition was called Lobo disease in 1938, and in 1958 the name lobomycosis was applied.

The organism responsible for lobomycosis has yet to be cultured in vitro. The causative organism is a blastomycosis *Lacazia loboi*, formerly named *Loboa loboi*. The natural reservoir of the pathogen is unknown. Its likely habitat is somewhere in the rural environment because the disease occurs in humans living in rural areas. Soil and vegetation seem to be likely sources of infection. Since the pathogen has been recovered from lobomycotic lesions of *Tursiops truncatus* ("bottlenose dolphins") in Florida and in the Bay of Biscay in Europe, an aquatic reservoir seems likely. A case of dolphin-to-human transmission has been documented in 1983 in a dolphin handler. As for clinical symptoms the name keloidal blastomycosis describes the lesions very well. Lobomycosis often develops at sites of minor trauma but sometimes no history of trauma can be recalled. The disease predominately affects exposed areas and extremities. Skin lesions slowly develop over time. Only after the lesions have become large do patients tend to consult a doctor. The lesions often begin as small papules or pustules, mildly pruritic or resulting in a burning sensation. The disease leads to verrucous or lobulated keloidal nodules and crusty plaques. Lesions are well defined, smooth and painless. They are easily moved around since they lie free over the deeper tissues. Older lesions typically become wart-like and ulcerative with satellite lesions resulting from autoinoculation. The mucosae are spared. The disease does not seem to heal spontaneously. The infection may spread proximally from the extremities suggesting lymphatic dissemination. Patients lack other systemic symptoms and lobomycosis does not affect the general health of the patient although squamous cell carcinoma has been described on old scar lesions. Keloids are the most important differential diagnosis and are much more common.

Diagnosis relies on a skin biopsy. The fungus is abundant in lobomycotic skin lesions. It is a spherical intracellular yeast 6-12  $\mu\text{m}$  in diameter. The melanin-containing birefringent 1  $\mu\text{m}$  thick cell wall resists digestion by macrophages. Linear or radiating chains of 5-10, even 20 organisms linked by tubules are characteristic. The organism can be seen with haematoxylin and eosin but the best stain is Grocott silver-methenamine which will show the typical yeasts chains. Attempts at medical treatment have failed. Surgery is successful only when the lesion is small and can be fully resected. Repeated cryotherapy appears to be more successful. The present antifungals do not seem promising, but more study is needed. Cases of successful treatment with posaconazole have been described. Clofazimine and dapsone have been tried with limited success.

# Deep mycoses

## Introduction

Deep or systemic mycoses can be subdivided into

1. Cosmopolitan deep mycoses, such as aspergillosis, candidosis and cryptococcosis. They are caused by fungi that occur worldwide.
2. Exotic deep mycoses, including coccidioidomycosis and histoplasmosis and infection with *Penicillium marneffe*, the pathogens of which have a limited geographical distribution.

## Exotic deep mycoses

The exosaprophytic moulds that cause exotic deep mycoses have the lungs as their portal of entry.

They are dimorphic, i.e. they occur in two forms. In the environment they are filamentous, while in vivo they exhibit another morphology that of yeasts. These moulds are capable of causing disease in patients without predisposing factors. The severity of the condition varies according to the inoculum and the patient's immune status. In patients without predisposing factors dissemination is rare and associated with a very large inoculum. In at risk patients (AIDS) dissemination always occurs even if only a small number of spores are inhaled. Dissemination leads to the secondary form in which numerous deep foci are possible and cutaneous lesions are also often observed.

The laboratory diagnosis is based on the specific morphology which the pathogen exhibits in vivo and on the culture of the saprophytic phase. Manipulation of the cultures is dangerous! There are reliable serological tests which are of diagnostic and prognostic value. For therapy as an alternative to amphotericin B use is increasingly, and successfully, being made of the azoles (itraconazole), particularly for the secondary chronic forms or relapse prevention (cfr. cryptococcosis).

## Classic or American histoplasmosis

Histoplasmosis is also known as "**Cave disease**", "**Darling's disease**", "**Ohio valley disease**", "**reticuloendotheliosis**," "**spelunker's lung**" or "**caver's disease**". This mycosis occurs primarily in the southeast of the USA, but is not exclusively North American. Native cases also occur in South America, Africa and Asia. Apart from a handful of Romanian and Italian cases those found in Europe have been imported.

### Causative agent

The causative agent *Histoplasma capsulatum* var. *capsulatum*, is an exosaprophytic mould that is isolated in the USA from various biotopes such as chicken manure, soil under starling roosts, seagull breeding sites and bat guano in caves (cave disease or speleologist's disease).

The filamentous phase is found in the environment or in culture at 25°C characterised by round spores with a thick wall, surrounded by protrusions (chlamydospores) and small round spores with a smooth wall. In vivo, in the parasitic phase; small, ovoid yeasts of 3-4 µm are found in the cells of the RES (histiocytes, monocytes).

### Clinical aspects

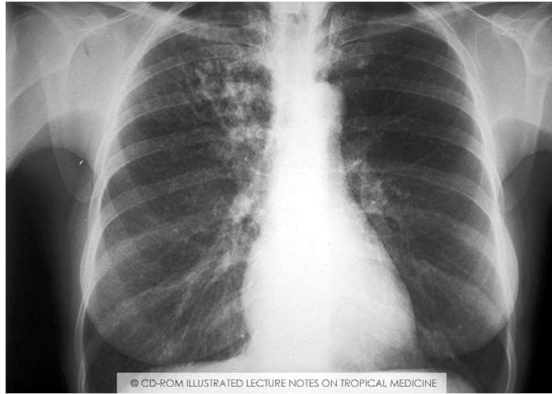
'The syphilis of the fungus world', meaning that histoplasmosis is one of the "Great Imitators". Histoplasmosis has been described as an illustration of the large variety of clinical forms.

In most cases, after contact with a low dose, an asymptomatic infection (99%) is seen or a self-limiting flu syndrome occurs (1%). At high inoculum or with temporary suppression of immunity an acute pulmonary disease can occur. After recovery calcifications in the lung and lymph nodes are seen in 1/3 of cases after 1-2 years.

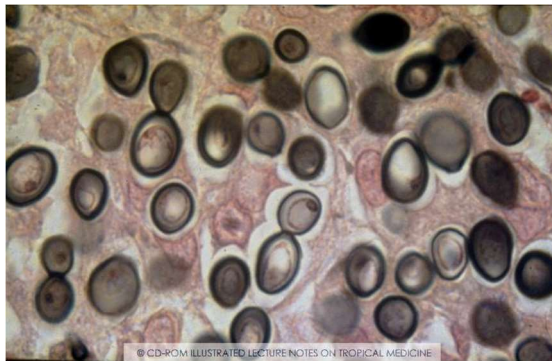
A chronic mild form is described in adults in the event of a temporary suppression of cellular immunity. This involves an endogenous re-activation, as in ex-colonial soldiers, 10-20 years after they have returned from Africa, South America or Asia, or a re-infection in people still living in endemic areas.

Oropharyngeal and nasal lesions and lesions of the vocal chords closely resembling malignant cells (nodules, ulcerations) and which may be associated with dysphagia and dysphonia are seen. In 50% of cases the adrenal glands are also involved. Systemic histoplasmosis is described in AIDS patients, sometimes with very severe cutaneous and mucosal lesions, as well as a possible cerebral involvement (meningitis). It results in a systemic granulomatous disease with preferential lesions on lips and in the adrenals.





Chest X-ray, pulmonary histoplasmosis. Copyright ITM



*Histoplasma duboisii*, histoplasmosis. Microscopic smear. With special thanks to Mr De Vroey.  
Copyright ITM

## Diagnosis

The diagnosis of histoplasmosis is made by direct examination of the parasitic phase (yeast) in sputum, bone marrow or blood. *Histoplasma* can also be found in histological preparations in RES cells. The intradermal reaction with histoplasmin is of no diagnostic value. The serological tests (immunodiffusion and complement fixation) are based on the detection of specific antibodies or on the detection of specific antigens (RIA). Both variants of *H. capsulatum* are level 3 pathogens and should only be cultured in a laboratory with the appropriate security procedures.

## ***African histoplasmosis due to H. capsulatum var. duboisii***

African histoplasmosis is geographically confined to Central Africa. Although it is established that the pathogen is also an exosaprophytic mould, the natural biotope remains unknown.

In the parasitic phase, *H. capsulatum* var. *duboisii* exhibits large, round spores of 10-15 µm. In the saprophytic phase, the two varieties are morphologically indistinguishable.

Patients with this chronic mycosis always exhibit polymorphous cutaneous lesions, bone and lymph node involvement and ultimately random deep localisations. When the disease follows an acute course (e.g. in AIDS patients), the yeast cells remain small and the infection is usually ascribed mistakenly to the variety *capsulatum*. In experimental infections also, cells exceeding the variety *capsulatum* in size are found only after a long time.

### **Histoplasmosis treatment**

In the majority of immunocompetent individuals, histoplasmosis resolves without any treatment.

Antifungal medications are used to treat severe cases of acute histoplasmosis and all cases of chronic and disseminated disease. Typical treatment of severe disease first involves treatment with amphotericin B, followed by oral itraconazole. Alternatives to itraconazole are posaconazole, voriconazole, and fluconazole.

## ***Talaromyces marneffi* (Previously known as *Penicillium marneffi*)**

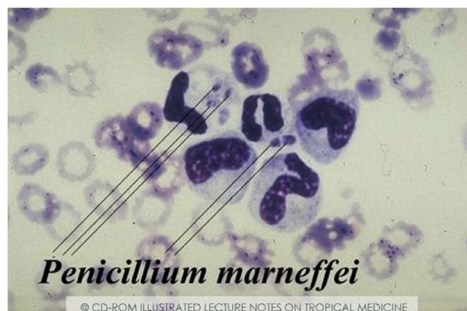
This mycosis only occurs in Southeast Asia and is usually associated with suppression of cellular immunity. The pathogen *T. marneffi* was first isolated from bamboo rats in Vietnam and has since been found in patients with a disseminated often fatal mycosis. The disease is an AIDS indicator.

### **Causative agent**

*Talaromyces marneffi* is a dimorphic mould. When it was classified as a *Penicillium* species it was regarded as the only species that can cause invasive disease, in contrast to other *Penicillium* species which are normally understood to be unimportant in human disease. In the saprophytic phase it produces a red pigment that diffuses in agar. In the parasitic phase, intrahistiocytic and- intramacrophagic elements are found which divide by fission and not by bud formation.



Infection with *Talaromyces marneffi* in an AIDS patient. Remark the typical umbilicated papular skin lesions



Microscopy of thin bloodsmear of an AIDS patient from Southeast Asia. *Talaromyces marneffi*. Copyright ITM

### **Clinical aspects**

The portal of entry is the lung and the infection spreads rapidly or otherwise via the RES. In AIDS patients, fever, marked weight loss, hepatosplenomegaly and often also cutaneous lesions are seen.

DD with cryptococcosis, histoplasmosis. This infection is now increasingly recognized as a disease in solid organ transplant recipients who travel.

### **Diagnosis**

The diagnosis of this mycosis is based on direct examination of sputum, BAL, bone marrow and material from skin lesions or on histological examination where the intrahistiocytic elements can be observed. *T. marneffi* can be cultured but the manipulation should be performed in a level 2 laboratory.

Reliable serological tests are being developed.

## Treatment

In severe disease treatment with amphotericin B IV or voriconazole IV should be started, followed by itraconazole or voriconazole po. Secondary prophylaxis with itra- or voriconazole is suggested when the CD4-count is below 100 cells/mm<sup>3</sup> in HIV-patients on HAART.

## Coccidioidomycosis

The disease is endemic in the wilderness areas of the southwest of the United States. In addition, foci have been described in Central and South America. Despite the pathogenicity of all *Coccidioides immitis* strains, it is estimated that only 0.2% of cases showed symptoms of deep localisations and/or skin granulomas in the period before 1990. Since then the number of cases has steadily increased not only because of the AIDS epidemic, but also as a result of earthquakes and sandstorms (disturbance of soil structure).

### Causative agent

*Coccidioides immitis* is a dimorphic mould found in its filamentous phase in soil. The pathogenic spores are easily dispersed from dry ground or material. In vivo (parasitic phase) no yeasts are found, but instead spherules, large, spherical elements of 20-80 µm diameter. These spherules contain numerous endospores.

### Clinical aspects

60% of those infected exhibit no symptoms or only minor respiratory disorders. These people are coccidioidin-positive. Approximately 40% go on to develop lower respiratory tract infections after 1-3 weeks, sometimes with erythema nodosum or erythema multiforme. The fungus can be spontaneously eliminated but it is preferable to administer azoles. About 5% of patients retain cavities and nodules in their lungs. Exceptionally in people with a normal immune system -but in 100% of AIDS patients- an extrapulmonary form is found with meningitis and bone involvement.

### Diagnosis

Spherules are found on direct or histological examination. The culture is relatively atypical and should only be performed in a level 3 laboratory. The coccidioidin skin test can be used for people who suffer a primary infection but anergy (absence of response) is possible in progressive disease. Spherulin is supposedly more sensitive. No cross-reactions have been described. There are other serological tests (latex agglutination, immunodiffusion, complement fixation, ELISA) for the detection of antibodies.

PCR demonstrated high sensitivity.

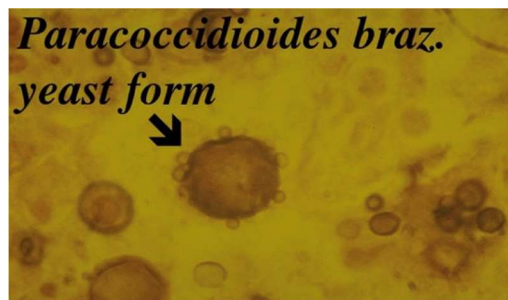
### Treatment

Many infections in healthy patients do not require treatment. Only severe infections or infections in immunosuppressed people needs treatment with itra-, keto- or fluconazole for 3 to 6 months.

## Paracoccidioidomycosis



South American blastomycosis or paracoccidioidomycosis, ulcers lips. *Paracoccidioides brasiliensis*. Photo Cochabamba



Paracoccidioidomycosis is caused by infection with *Paracoccidioides brasiliensis*. The yeasts have a typical steering wheel aspect caused by budding. Copyright ITM

Paracoccidioidomycosis or South American blastomycosis occurs in discrete foci in Latin America. It is thought that the fungus (*Paracoccidioides brasiliensis*) exists in the soil as a mold, with infections happening through the inhalation of conidia (spores). These spores convert into yeasts in the lungs and are then thought to spread to other sites haematogeneously and via the lymphatics. Most primary infections are self-limiting. The organism has the ability to remain dormant in the human host for long periods. It can cause clinical disease at a later time if host defense would become impaired (e.g. depression of cell mediated immune responses).

Overt infection results in a progressive mycosis with lesions of the skin, mucous membranes (especially mouth, lips and nose) and internal organs. Long asymptomatic periods enable patients to travel far from endemic areas before developing clinical problems. Lesions in the face, naso- and oropharynx resemble espundia (mucocutaneous leishmaniasis), lupus vulgaris (skin tuberculosis) and syphilis. Papules will ulcerate and enlarge both peripherally and deeper into the subcutaneous tissue. A hard hyperkeratotic border may surround a deep ulcerating crater on the skin. Extensive coalescent ulcerations may eventually result in the destruction of the uvula, epiglottis and vocal cords. Eating and drinking become difficult and painful. Lymphatic infections lead to painless enlargement of cervical, supraclavicular and/or axillary nodes.

Draining sinuses may form. Visceral lesions in liver, spleen and lymph nodes can lead to abdominal pain, hepatosplenomegaly and low-grade fever. Pulmonary involvement occasionally leads to mild symptoms, including cough and sputum production, although the radiograph of the chest can show dramatic involvement of the lungs. Infections tend to be very chronic, but not fatal. Diagnosis relies on demonstration of the yeasts in tissue/secretions. The yeasts are usually abundantly present in the bases and edges of slowly

expanding ulcer. In tissue specimens, the yeasts are rather large (15  $\mu\text{m}$ ) with multiple buds, thereby resembling the steering wheel of a boat. Gomori staining of biopsies is useful.

Serology by immunodiffusion is positive in  $\pm$  98% of cases. Azoles such as itraconazole are usually very effective as treatment. Amphotericin B can be used if there is no success with oral itraconazole or in case of severe infection. Sulfonamides can be used, but have only moderate activity and should be administered for very long periods.

## **Blastomycosis**

Blastomycosis -also known as North American blastomycosis or Gilchrist's disease- occurs in a geographically limited area of the south central and midwestern USA, upstate New York and southern Canada. A few cases have been described from Africa, the Middle East, India and Mexico. The fungus occurs in soil enriched with animal excreta and moist, acid, decaying organic material. Infection follows the inhalation of conidia (asexual spores) of *Blastomyces dermatitidis*. Both man and dogs can be infected. The spores will convert into yeasts, which will invade the lungs and occasionally spread haematogeneously to several organs, especially skin, bone or urogenital system. Pulmonary infection can be asymptomatic. Genital involvement such as chronic epididymitis, mimicking tuberculosis. Cough, low to moderate fever, dyspnoea and chest pain, purulent/bloody sputum, pleural fluid, weight loss and prostration occur in symptomatic patients. Radiological studies usually reveal pulmonary infiltrates and enlarged hilar lymph nodes. Progressive pulmonary blastomycosis resembles tuberculosis or a neoplasm. Raised single or multiple verrucous cutaneous lesions that tend to have an abrupt downward sloping red-purplish border are usually present in disseminated blastomycosis. The border extends slowly, leaving a central atrophic scar. Those skin lesions can resemble skin cancer.

Bones such as ribs and vertebrae are frequently affected (25-75%). Lesions appear both destructive and proliferative on radiography. Central nervous system lesions are uncommon. Acute self-limiting blastomycosis is rarely diagnosed. The organism is found in clinical specimens as a thick, double-walled cell, 5-20  $\mu\text{m}$  in diameter, sometimes even reaching 30  $\mu\text{m}$ . Some yeast cells have a single bud. Definite identification is via culture but detection of the above mentioned yeast cells in pus, sputum or urine is very suggestive. Gomori's methenamine silver stain and PAS staining are useful for biopsies. Serology is not useful. Untreated disseminated blastomycosis is usually progressive and can be fatal. Itraconazole (200-400 mg/day) is used as a first-choice treatment. If no improvement occurs the dose of itraconazole can be increased to 800mg per day or switch over to IV amphotericin B. Follow-up in order to identify a relapse should continue for several years.



## Various subjects

### Ectoparasites

#### Fleas

##### General



Cat flea, *Ctenocephalides felis*, a vector of *Rickettsia felis*. Notice the combs, which gives the animal its name.



Human flea. *Pulex irritans*. © ITM

Fleas are cosmopolitan, wingless insects. They are obligate blood-sucking ectoparasites. They are not strictly adapted to a specific host and on occasions can bite unusual hosts, including humans. Although feeding on less than ideal hosts keeps the fleas alive, it reduces their fertility. The most important jumping fleas are *Pulex irritans* (the human flea), *Ctenocephalides* species (cat and dog fleas), *Xenopsylla cheopis* (Oriental rat flea) and *Tunga penetrans* (sand flea). Adult fleas live 6-12 and sometimes even 24 months. Fertilised adult females lay 3-18 eggs per day. After 2-14 days, depending on moisture and temperature, the eggs hatch to give very active legless larvae. Under favourable conditions, the larvae will pupate and emerge as adult insects. The cocoon is spun from sticky silk, so that a wide variety of substances become attached and provides camouflage. These pupae are therefore very difficult to detect. The pupa stage usually lasts 1 to 2 weeks. Sometimes the adult insect remains in the cocoon for a long time (up to 1 year). Emergence from the cocoon is environmentally triggered (e.g. by proximity of a host: CO<sub>2</sub>, heat, vibration). This explains why people who move into a house that has been empty for a long time can suddenly suffer numerous fleabites. Adult insects can remain alive for several weeks to months without feeding if the climate is not too harsh. Optimal conditions for their survival is high moisture and temperatures around 20°-30°C. Fleas leave dead hosts and this behaviour is important in the transmission and epidemiology of

plague. Body temperature (37°C) inhibits the hatching of eggs and larval development. Reproduction occurs away from the host, on the ground, in cracks and in animal nests.

Muscles do not directly power the amazing jumps of fleas. Muscular tissue reacts too slowly. Instead, muscles are used to build up tension gradually. Fleas do not have wings but for their jumping, they use their wing stumps (their ancestors had wings). A hungry flea can jump up to 600 times per hour during three days. Fleas can jump 20 cm in height and 30 cm in distance. Bites are associated with the injection of saliva and cause a local pruritic skin irritation, principally on the legs. At night bites can occur over the whole body while people are lying down. These insects may be infected with the bacteria causing plague or endemic typhus (*Rickettsia typhi*) and *R. felis*. Other organisms can occasionally be transmitted. Fleas also transmit various sorts of minor tapeworms (*Dipylidium caninum*, *Hymenolepis diminuta* and possibly *H. nana*). Occasionally people develop long-lasting red skin lesions after insect bites. In such cases a Köbner's phenomenon due to psoriasis should be suspected. The original skin lesions themselves can be minimal (e.g. hidden on scalp, ear).

Simple hygiene is often sufficient to keep a house free of fleas. Insecticide resistance is increasing, including resistance to DDT. Organophosphates, carbamates and pyrethroids are used to eliminate flea infestation in a house. Pets can be washed with a shampoo with e.g. malathion or can wear a flea collar, i.e. a collar impregnated with dichlorvos. The latter provides a prolonged local vapour effect in the animal's fur. It should however be noted that most fleas are not present on the host, but in the bedding, on the ground, etc. For a cat with 25 fleas in its fur, there are some 500 adult insects, 500 cocoons, 3000 larvae and 1000 eggs present on the ground. Flea control should therefore also be directed towards the whole environment not just the animal. Cocoons are relatively resistant to insecticides.

## Fleas, tungiasis

Tungiasis is a superficial infection of the skin by the sand flea *Tungapenetrans* (sarcopsilla; jigger flea; chique, do not confuse with chigger, which are trombiculid mites). *Tunga trimamillata* is a sand flea species identified in 2002 and seems to be limited to Peru and Ecuador. The lesions it causes are a bit bigger than those of *Tunga penetrans*.

With a length of about 1 mm (male and unfertilized female), it is the smallest known flea species. Both sexes live on sandy ground and bite birds and mammals, particularly pigs, but also dogs, cats, sheep, goats, cattle, horses, donkeys. Newly hatched insects are very active and the larvae jump around on the ground. They seem to prefer dry sandy ground. The insects don't do well in humid environments. The insects are a poor jumpers. The fertilized female bores into the epidermis and penetrates deep into the stratum corneum. The soles of the feet, the interdigital spaces and the skin under the nails are particularly affected. Any other part of the body that comes into contact with the ground can be infected (buttocks in beggars, children and lepers). The insect bores mechanically into the stratum corneum with the head innermost and bites onto the dermal papillae. The abdomen of the female swells as a result of the maturation of the approximately 200 eggs. After ten days the flea on average measures 1 cm in diameter. The hindmost abdominal segments are not distended and protrude out as a black central spot, through which excreta and eggs are released to the outside. After the eggs have been expelled the flea dies. The hole fills with fibrin and pus and is gradually

reepidermalised. After 3-4 days larvae emerge on the ground and pupate after approximately a week.

The complete cycle takes 2-3 weeks.



Female *Tunga penetrans* under a toe nail. Photo Dr Van den Enden © ITM



Female *Tunga penetrans* burried in a finger, an uncommon site. Photo Dr Van den Enden © ITM

There is local pruritus and vague pain. In the beginning only a central black dot is visible. Later the lesion is raised, semi-transparent with a central dark spot and an erythematous halo. The number of parasites usually remains limited. However severe infestations with hundreds of sand fleas are found for example in leprosy patients, cachectic patients, alcoholics, in cases of advanced sleeping disease, in mental diseased and handicapped people or in confined communities.

Superinfection can occur during or after the primary infection, but particularly as a consequence of clumsy manipulation to remove the flea, as a result of which it breaks and parts of it remain deeply lodged. Lymphangitis can result as well as septicaemia and gas gangrene with a fatal outcome. Tetanus is a feared complication.

For treatment, the central opening in the stratum corneum is widened with a clean metal needle. The flea is removed and the remaining hole is disinfected. Prevention consists of wearing well fitting shoes instead of walking bare-foot or with wide open sandals. Socks that are left lying on the floor are to be avoided. Local basic hygiene is essential. Regular cleaning of floors using lots of water is strongly advised, together with removal of pigs from the vicinity of houses. Affected areas of soil may be burned off. Ointment with cresol or lysol protects the feet.

## Lice

### General

Lice (singular: louse) have parasitized humans since ancient times. While most primates have only one body lice species, humans have three (pubic, body and hair lice). According to genetic analysis, the common ancestor of headlice was shared by primitive hominids and primates until 6 million years ago.

Pubic lice separated about 3 million years ago. Body lice are "only" 650,000 years old (coincides with the start of hominids wearing clothing). Lice have been found on Egyptian and pre-Columbian mummies and even on bodies dug up in Pompei. The Order of lice (Phthiraptera) is divided taxonomically into sucking lice (Anoplura) and chewing lice (Mallophaga), but there are alternative taxonomic classifications. Anoplura only parasitize mammals. Only three species of Anoplura are of regular direct medical importance to humans. These wingless insects are cosmopolitan, obligate haematophages and strictly adapted to their host (there is no animal reservoir). *Polyplax spinulosa* (Anoplura) is the sucking louse of rats and acts as an occasional vector of murine typhus. Only a single species of Mallophaga (*Trichodectes canis*) is known to have medical significance. *T. canis* is the chewing louse of dogs and acts as one of the larval hosts of the dog tapeworm *Dipylidium caninum*. This insect cannot live on man.

### Pubic lice

**Pubic lice (*Pthirus pubis*)** do not themselves transmit disease. [The name *Phthirus pubis* is also used, but in 1987 the International Commission on Zoological Nomenclature decided to keep the original spelling of *Pthirus pubis*]. They occur on areas of the body with coarse hair (pubic region, peri-anally, sometimes also on legs, eyelashes, moustache, beard and even armpits and chest). Sometimes they are present on the scalp, including neonates. Sexual contact is the main method of transmission, but is not the only one (e.g. shared clothing). Any transmission involves bodily contact. They cannot live for more than 24-48 hours away from the host. If they are present in children, the possibility of sexual abuse should be taken seriously. A significant and strong correlation between the falling incidence of pubic lice infections and increase in pubic hair removal is observed. The increased incidence of hair removal may lead to atypical patterns of pubic lice infestations or its complete eradication as the natural habitat of this parasite is destroyed.



*Pthirus pubis*. Pubic louse. © ITM



Pediculus humanus capitis. Head louse. © ITM

## Body and head lice

**Body and head lice (*Pediculus humanus corporis* and *P.h.capitis*)** are two very closely related species (morphologically almost identical) but which occupy different ecological niches. The body louse *P. humanus corporis* lives in clothing and only comes onto the body to suck blood for a short time. The head louse *P. h. capitis* by contrast lives on the scalp and never on the clothing. Mutual fertilisation is possible in the laboratory, but in nature this appears not to occur and they are considered to be different species.

Fertilised females lay 6-9 eggs per day during their life. They live usually for 1 month, maximum 2. The animals are very sensitive to cold. The females attach the sticky eggs to underwear, shirts and trousers. Eggs on clothing cannot survive for more than 4 weeks (usually only 2 weeks). The eggs hatch after 6-9 days. Once hatched, the larvae suck blood five times a day, rapidly returning to the clothing after each meal. Lice avoid light. Once adult, the animals will mate repeatedly. The reason for this is that females have no spermatheca and frequent mating is therefore crucial to build up a population. All in all this means that in optimal circumstances natural populations can increase by 10% per day. This is important in order to understand the dynamics of infections such as epidemic typhus and relapsing fever. Digestion of the blood is rapid. The red blood cells lyse. *R. prowazekii* infects the cells lining the intestine. The intracellular proliferation of *R. prowazekii* causes the insect's intestine to burst, spreading its contents into the haemolymph. The haemolymph will be stained red. The red colouration can be used in the laboratory to investigate whether a louse is infected with *R. prowazekii*. The louse dies from the infection with *R. prowazekii* and this fact was used previously to investigate which antibiotics were active against this bacterium. The antibiotic that enabled the louse to survive was then studied further.

Lice are very sensitive to desiccation so that in dry environments they will not survive. Lice faeces are very dry and powder-like, with a water content of only 2%. The faeces contain a large amount of ammonium, which has an attracting effect on other lice.

*P. humanus corporis* can transmit *Rickettsia prowazekii* (epidemic typhus), *Bartonella quintana* (endocarditis, trench fever) and *Borrelia recurrentis* (epidemic relapsing fever). *R. prowazekii* is fatal for the insect after a few days. It is important for transmission and explains why people with louse borne typhus often have remarkably few lice. *B. recurrentis* proliferates only in the haemocoel of the insect and is transmitted by crushing an infected louse. This explains why "outbreaks" of louse borreliosis are rare unless there are massive numbers of lice. *B. quintana* can survive for up to a year in lice faeces.

The insects on clothing are destroyed by heat. For treatment, clothing is washed at 70°C, steam ironed or sterilised. In emergency situations (epidemic) insecticides are sprinkled (e.g. mixed with talc) between skin and clothing. Malathion or permethrin lotion or systemic ivermectin can be used.

***P. humanus capitis***. This obligate bloodsucking ectoparasite feeds three to six times per day. The female lives one month and can lay up to 300 eggs, also known as nits. The eggs are deposited very close (approximately 1 mm) to the base of the hair and are firmly attached. Given that a hair on the scalp grows about 0.4 mm per day, it follows that virtually all nits found more than 5 mm from the base of the hair are either dead or empty (in practice a figure of 7 mm is taken). The egg shells of the nits are not removed by insecticides. Their presence after therapy sometimes causes anxiety and give rise to the mistaken belief that the insects are resistant.

Larvae and adults suck blood. The irritation from the bites can lead to chronic itching and scratching, possibly with secondary infection (e.g. impetigo) as a consequence. The insects are very dependent on their host. Even fed adult lice cannot survive for more than a few days (maximum 10) without another feed. They leave a dead person or someone with high fever fairly rapidly.

#### *Head lice treatment*

There are several options: (1) wet-comb method, (2) topical organophosphate or pyrethroid insecticides, (3) topical dimethicone, (4) systemic ivermectine, (5) topical ivermectin.

**In the wet-comb method** the hair is first washed with a shampoo, followed by application of a hair conditioner to make the hair as smooth as possible. A good louse-comb has teeth 0.2 to 0.3 mm apart.

The teeth should have an angular cross-section. After application of the conditioner, the hair is finely combed from the neck towards the front hair-line. The teeth of the comb should be in contact with the skin. After each movement, the comb is cleaned on a piece of white paper. When finished, the hair is rinsed, and combing is started again, this time from the forehead hairline towards the neck. This is done 4 times in a period of 14 days. If living lice are still found after this period, another therapeutic option should be used. The wet-comb method is time-consuming and cumbersome. The success rate varies from 37% to 57%. The advantages are low price, lack of resistance and it can be used when one prefers to avoid topical insecticides (very young children, lactating women). In olden days, shaving the hair very short was sometimes used.

**Topical pediculicides.** In most cases, infestation with lice is treated with insecticides, but head lice have become more and more resistant. Treatment options are organophosphates such as 0.5% malathion [Prioderm®, Radical®] or pyrethroides such as 1% permethrin [Nix®] or depallethrine 0.66-1% in combination with 2.6-4% piperonyl butoxide (ParaShampoo®, Pyriderm®). The contact time should be sufficiently long: at least 10' for permethrin, 30' for depallethrine and 12 hours for Malathion. Lotions are better than shampoos as they have a longer contact time with the hair. If after reapplication 7 days later, living lice are still found resistance is likely (reinfestation is also possible).

**Dimethicone** 4% (Silikom<sup>®</sup>) is applied to dry hair and is rinsed off 8 hours later. This is repeated after 7 days. The idea is to suffocate lice, cutting of the oxygen supply. The cure rate is about 70% with this method, although more study is needed.

**Ivermectin** is a neurotoxin acting on glutamate-gated and gamma-amino butyric acid-gated chloride channels. Oral ivermectin (Mectizan<sup>®</sup>, Stromectol<sup>®</sup>) can be used as an alternative or in case of multiresistant lice. A dose of 200 µg/kg is given twice with a 7-day interval. Studies showed a superior efficacy (95%) as compared with topical 0.5% Malathion lotion (85%) applied with the same interval. A 0.5% ivermectin topical lotion can be applied to dry hair, left for 10 minutes then rinsed with water.

## Mites

### General

Mites are related to ticks, scorpions and spiders. In contrast to insects they do not have antennae and their body is divided into two rather than three parts. Larvae have 6 legs and adult animals 8 legs. Mites tend to be much smaller than ticks. These animals occupy the most diverse ecological niches from *Varroa* mites which are found in the respiratory tract of honey bees to *Demodex* mites which colonise the sebaceous glands of human eyelashes. In humans *Dermatophagoides pteronyssinus*, is known as house dust mite. *Sarcoptes scabiei* causes scabies.

Some mite larvae belonging to the genus *Leptotrombidium* (belong to the harvest mites) transmit *Orientia (Rickettsia) tsutsugamushi* (scrub typhus) in Southeast Asia. Adult mites are of no direct medical importance as they feed exclusively on small invertebrates and insect eggs. A female lays 1-5 eggs per day on moist ground. After the larvae appear, they begin to crawl around actively on the ground, grasses, low plants, etc. Larvae attach themselves to a host when it passes through the vegetation and seek out a piece of skin that is soft, smooth and not too thick (peri-anal, groin, ankles). The very small larvae (150-300 µm) inject saliva and suck up the digested tissues. After 2-10 days the mites fall to the ground and dig themselves in for further development. The ecological habitat of these parasites is strictly defined. Optimal moisture of the soil, the right vegetation and sufficient hosts for the nymphs and adults (arthropods of various kinds), as well as the larvae (mostly rats and mice) need to be present.

This results in a very scattered distribution and the existence of mite islands. These are areas where intense transmission of scrub typhus occurs, whereas no infections occur in places only a few kilometers away for instance. Although the potential zoonotic reservoir is not yet completely established, it is important to know that *Leptotrombidium* mites themselves serve as a reservoir for *Orientia tsutsugamushi* (transovarial transmission). Transmission of this kind can persist for several successive arthropod generations.

## Scabies



Norwegian scabies on a foot of an AIDS patient. Copyright ITM



Norwegian scabies in HTLV-1 patient. Copyright Alexander von Humboldt Institute, Peru.



Scabies, genital nodules. These nodular lesions tend to disappear more slowly than other scabies lesions.  
Copyright ITM

**Scabies** is caused by *Sarcoptes scabiei*. Morphologically similar mites are found on various animals (in dogs *Sarcoptes scabiei* var. *canis*) but do not permanently infect humans. Cat scabies is caused by *Notoedres cati*. "Milker's itch" is caused by *Sarcoptes bovis*. *Sarcoptes equi* occurs in horses and riders can suffer an itchy skin disorder from these mites. Scabies mites do not transmit any pathogenic organisms. Adult female *Sarcoptes scabiei* mites measure 400-600  $\mu\text{m}$ , while the smaller males are slightly more than half this size. The cycle from egg to egg lasts 10 to 14 days.

Human-to-human transmission occurs directly or indirectly when hygiene is poor. The majority of mites are found on the wrists and fingers, with smaller numbers on elbows and elsewhere. The face is practically always spared. The mean number of female mites per infected person is 11, most having 115 mites. Only 3% of patients have 50 mites or more. The



mites dig burrows in the stratum corneum of the skin (1-5 mm per day). These tunnels are clinically very different from larva cutanea migrans. A female lays 1-3 eggs per day in her tunnel. Besides the eggs, mite faecal matter (scybala) is present in the tunnels. Larvae appear after 3-5 days. These crawl on the skin surface and many die there. Another 5-6 days later the adult appears which remains in situ if it is female. After becoming an adult and fertilisation by a passing male, the cycle can begin again. Female mites live on a person for 1-2 months. A female can produce up to 40 eggs in her life. Scabies causes pruritus, particularly at night. A definitive diagnosis is not straightforward as the characteristic skin tunnels usually only become visible after secondary infection or eczematous reaction. Scabies may trigger "unusual" impetigo (*Streptococcus pyogenes*). Repeated application of corticosteroid cream can lead to masking: "scabies incognito".

Scabies provokes a papular, pruritic skin rash. There will be itching at sites where the mites themselves are found (e.g. between the fingers, wrists, elbows, genitalia). The rash is also seen on parts of the body that are not infested by scabies mites. Buttocks, groin, shoulders, arms, calves and ankles can become itchy. In classic scabies, the rash almost never occurs on the head, palms of the hands or soles of the feet. The rash is caused because the patient has become hypersensitive to mite allergens. In a patient who has never been exposed to scabies the rash usually occurs 4-6 weeks after infection. In previously exposed people it occurs much more rapidly, sometimes within just a few days. Despite effective treatment, symptoms and lesions of scabies can persist for weeks (e.g. scabies nodules on the scrotum and penis). Hypersensitivity to the scabies mite does not disappear immediately after the death of the parasite.

Sometimes **Norwegian scabies** occurs ("crusted scabies"). This condition is clinically totally different from classic scabies. A clinical description was first given in 1848 by Danielssen and Boeck in Norwegian leprosy patients. The condition occurs more frequently in immunosuppression e.g. AIDS and in infection with HTLV-1 than in the general population. Drug-induced immunosuppression, long-term topical steroid use and to a lesser extent a mental handicap such as Down's syndrome increase the risk.

In Norwegian scabies there are very numerous mites present in desquamating hyperkeratotic skin crusts. The latter can also occur on the face. The disorder is highly infectious. Sometimes tinea pedis, psoriasis, severe dyshidrosis, hyperkeratotic eczema, contact dermatitis or Darier's disease (keratosis follicularis; autosomal dominant inheritance) resemble it. In case of doubt, it is sufficient to examine some skin scales after treatment with 10% KOH under a low magnification microscope.

Scabies mites as yet exhibit no resistance to lindane or benzyl benzoate. For treatment, 20-30% benzyl benzoate is used, with which the whole body (except the face) is rubbed twice after washing with soap for 3 days. Lindane lotion (Quellada® = gamma-benzene hexachloride) can also be used but its use has been phased out because of toxicity concerns. This should be repeated after 7 days as the eggs are not killed by only one application. Pyrethroids are effective (e.g. 5% permethrin (Zalvor®)). Malathion is best used as a lotion not as shampoo. Crotamiton (Eurax®) is also sometimes used but is less effective. Oral ivermectin (Mectizan®) also produces relatively good results, but should preferably be repeated after a few weeks. It is the first choice in Norwegian scabies. Linen and bedclothes are disinfected at the same time by water at >60°C. Washing bedclothes and clothing and ironing them with a steam iron during

this period will also help break the cycle of transmission. Without access to a body the mites survive less than 4 days.

#### Guidelines for elimination of scabies in institutional outbreaks

- change encasings of mattresses, carpet, clothing
- cleaning rooms, furniture, couches
- topical and systemic treatment of patients (permethrin and ivermectin)
- synchronous topical treatment of all contacts with or without skin lesions
- clip nails, brush subungual folds with scabicides
- reduce social contacts, e.g. no reunions in nursing homes
- avoid pets, examine pets
- ten day quarantine of index patient
- caregivers should use gloves and protective clothing, alcohol and handwashing
- evaluate two weeks later for eventual retreatment

## Ticks

### General

Ticks are small animals related to mites, scorpions and spiders. Ticks are also known as Metastigmata.

Ticks differ from insects. Their bodies are divided into two parts rather than three. Ticks do not have antennae nor wings. The adults have eight legs instead of six. Ticks have 6 legs in the larval stage (nymphs and adult ticks gain a pair of hind legs). Ticks that have climbed onto grass or other plants become aware of their potential host by vibration, warmth, CO<sub>2</sub>, moisture and smell (all mammals secrete butyric acid). They remain attached to feathers, fur, skin or clothing, after which they seek a suitable place to suck blood.

There are two types of life cycles. In some species of tick, the larva, nymph and adult remain on the same, individual host not dropping to the ground between stages. In others, the different stages feed on 2 or 3 different individuals. The host can be identified by the origin of the blood in the tick's stomach, e.g. by PCR analysis. Ticks with a host change are usually better vectors for pathogenic organisms.

#### *Tick species*

There are approximately 840 different species of ticks which are classified into 3 families: Argasidae: argasids or soft ticks, with a tough, leathery skin and a concealed ventrally projecting capitulum ( $\pm$  170 species). There is no scutum in adult animals. A scutum is a dorsal shield.

Ixodidae: ixodids or hard ticks have a rigid scutum and a capitulum with mouthparts projecting forwards ( $\pm$  670 species). This capitulum is visible when viewed from dorsal.

Nutalliellidae

Overview of tick genera in the three families

Argasidae : *Argas, Ornithodoros, Otobius, Antricola, Nothoaspis*

Ixodidae : *Amblyomma, Aponomma, Boophilus, Cosmiomma, Dermacentor, Haemaphysalis, Hyalomma, Ixodes, Margaropus, Nosomma, Rhipicentor, Rhipicephalus*

Nutalliellidae : only 1 species, rare

### Ticks as vectors

Ticks are always obligate blood suckers, in contrast to mites which occupy much more varied ecological niches. All ticks need blood to complete each stage of their development. The chelicera dilate the skin, so that the hypostome can be inserted. This hypostome is equipped with barbs to keep them anchored in place and thus permit them to suck blood for several days in case of hard ticks. Many ticks cement their mouth parts to the skin for better attachment. This cement needs to be enzymatically broken down when they detach.

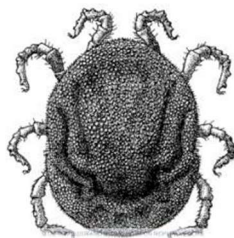
Ticks can be important vectors of various infectious organisms for humans (including protista such as babesias; viruses such as Crimean-Congo haemorrhagic fever or tick-borne encephalitis and bacteria such as *Borrelia*, *Rickettsia*, *Ehrlichia/Anaplasma*). Ticks can also transmit diseases in animals: Q fever (*Coxiella burnetii*), theileriosis (*Theileria sp.*), cowdriosis

(heartwater, *Cowdria* sp.), dermatophilosis (*Dermatophilus congolensis*, a bacterium), anaplasmosis, babesiosis, sweating sickness (toxin of *H. truncatum*) and a number of others. The micro-organisms, ticks and their usual natural hosts have developed together over the course of thousands of years with mutual adaptation as a result. As a general rule, the microbes cause little or no damage to the tick and usually persist for the whole of the vector's life. There is often trans-stadial transmission (from larva to nymph to adult) and sometimes transovarian transmission (from tick to eggs, so the following generation is born infected). The microorganisms usually have little effect on the natural vertebrate hosts. Many of these warm-blooded animals act as a lifelong reservoir and as an amplifier for both pathogens and ticks. Poorly adapted hosts such as humans, often develop diseases following accidental infection with a pathogenic microorganism. The term "adaptation" can be open to misinterpretation. It is not the case that the long-term persistence of a pathogen in a specific population automatically entails the reduced virulence of this organism.

A significant obstacle for all ticks in obtaining a blood meal is counteracting the haemostatic system of the host, such as thrombin, factor X and platelet aggregation. Ticks have several antithrombotic agents which are essential for their survival.

These products are present in salivary glands, as expected, but also in eggs and haemolymphs. It appears that their function is not only to prevent blood clot formation in the host and the blood meal, but also regulation of haemolymph coagulation in the tick itself. Besides thrombin-inhibitors, inhibitors of tissue factor and factor X or Xa, tick saliva contains a plethora of vasodilators, platelet inhibitors, fibrin (ogen)olytic agents and immunomodulators.

## Ticks, Argasidae



Soft tick, *Ornithodoros moubata*. Copyright ITM

**Argasids** take short (a few minutes) but repeated feeds. After feeding excess water in the blood meal is eliminated partly in the saliva and partly as coxal fluid (e.g. in *Ornithodoros moubata* - syn. *Ornithodoros moubata*). This coxal fluid is secreted by specialised glands between the first and second pairs of legs in the soft tick. *Borrelia duttoni* can be found in this fluid. When this fluid is rubbed into the bite wound an infection can follow. Argasids can cause persistent pruritus at the site of the bite. Some will suck blood from humans if their natural host disappears (e.g. *Argas vespertilionis*, a bat tick). *Argas reflexus* is a tick which came originally from Middle Eastern countries and has now spread throughout Europe and large parts of Asia via the domesticated pigeon, the host for this animal. Other hosts are hens and ducks. The adults can survive for months to years without a blood meal. Humans can be bitten when visiting an abandoned dovecote. The bite is often painful and the skin will swell and redden.

## Ticks, Ixodidae

**Hard ticks (Ixodidae)** are dispersed world-wide. There are 13 genera, of which *Ixodes*, *Dermacentor*, *Rhipicephalus*, *Haemaphysalis*, *Hyalomma* and *Amblyomma* are the most well-known.



Hard ticks. There are four morphological stages: (left to right) larva, nymph, adult male and female. Copyright ITM



Hard tick: female *Hyalomma aegyptium*. Copyright ITM

The ticks have a hard scutum (dorsal shield) that in the adult male covers the whole back. Males can only suck a limited quantity of blood. The scutum of the female is also hard and cannot distend. It is however smaller so that remarkable distension of the animal's body is possible when it takes a large blood meal.

They feed for 6-12 days. It is very important for the tick that during this period it should not be noticed by the host. Tick bites are painless, since a component of the tick's saliva reduces the sensitivity of the receptors in the host's skin. The males remain on the host for several weeks to months. After the adult female is sated, she falls to the ground in order to lay her eggs. After laying the eggs the female dies. A six-legged larva emerges from the egg and waits for a long time on the ground or on vegetation until a host passes to which it can attach itself. The larva takes one large blood meal over a period of 4 to 6 days.

An eight-legged nymph then develops from the larva, which afterwards develops into an adult animal following a subsequent blood meal (duration 5-8 days). The life cycle of most hard ticks lasts 2 years.

The longer the tick remains in place and sucks blood, the larger the quantity of micro-organisms which are transmitted. For example, transmission of Lyme disease is unlikely if the tick is removed rapidly.

This is in contrast to the Argasidae, where infections such as relapsing fever can be relatively rapidly transmitted as these animals have a different feeding behaviour. The attachment time

needed for transmission of *Borrelia burgdorferi* is much shorter in European ticks than in American ticks.

### Tick paralysis

The saliva of some ticks is neurotoxic and "tick paralysis" can occur. This has been described for 60 different tick species in animals, but only a few are important for humans: in the USA and Canada *Dermacentor variabilis* and *D. andersoni* and in Australia *Ixodes holocyclus*, a marsupial tick. Paralysis occurs in animals (dogs, sheep) and humans. Usually the tick needs to have been present for 4 (2-7) days before the symptoms appear. The neurotoxin is still poorly characterised, but it prevents the release of acetylcholine from the pre-synaptic membrane (cf. botulinum toxin). The condition presents as a flaccid, ascending paralysis with areflexia and with bulbar involvement and ataxia, without neck stiffness and without sensory disorders. Unsteady jerky movements of the limbs and breathing difficulties occur. The paralysis is more pronounced in children younger than 10 years, probably because of their smaller body weight. Evolution towards death is possible (respiratory failure). The disorder can resemble poliomyelitis, but motor involvement is symmetrical. Consciousness is clear. It can also resemble Guillain-Barré syndrome, including the EMG findings. On removal of the tick there is a progressive recovery over the course of the following hours to days.

### Prevention

Prevention of infections transmitted by ticks includes the avoidance of areas where there are ticks. Argasids are often found in mud huts, campsites or places where pigeons or bats nest. It is best not to sleep on the floor and if possible to avoid such places entirely. The use of concrete or plaster in houses diminishes the population of soft ticks. Hard ticks are found in places where domestic or wild animals (including birds) congregate, drink, feed or rest. It helps to tuck trousers into socks, wear dark clothing (attracts ticks less) and to wear permethrin or DEET impregnated cloths. A 'skin-check' after a walk through dense vegetation is useful.

### Removing ticks

Hard ticks are relatively difficult to remove without damaging them. They have barbs on their hypostome (a section of the mouthparts). Tick larvae are small (<1 mm) and colourless before they suck any blood. They should be removed carefully with tweezers, without squashing them. The tick should be grasped as close as possible to the site of attachment in order to minimise the risk of the mouthparts breaking off and remaining embedded in the skin. The broken-off mouthparts of a tick can cause irritation and local infection. They should be scraped out and the wound disinfected e.g. with alcohol or povidone-iodine. It is sometimes claimed that applying vaseline, butter or fat to the animal (interfering with respiration) causes the tick to react by detaching itself from the skin, after which it can be removed more easily. While this does apply to the removal of fly larvae (myiasis), it is less straightforward in ticks. Burning the animal with a cigarette is not indicated: it can cause burns (particularly in children and pets), the tick might burst, thus spreading infectious material, and finally heat encourages the tick to produce more saliva and regurgitation.

## *Diseases transmitted by ticks*

### **Soft ticks**

Relapsing fever: *Borrelia duttoni* via soft ticks such as *Ornithodoros moubata*

### **Hard ticks**

Lyme disease: *Borrelia burgdorferi*

Rickettsioses: various types such as Rocky Mountain Spotted Fever, fièvre boutonneuse, Queensland tick typhus,

Japanese spotted fever, Israeli tick typhus, Siberian tick typhus, Flinders Island spotted fever, Mongolian spotted fever

Ehrlichioses and the related anaplasmosis: monocytic (*E. chaffeensis*) and granulocytic (bacteria related to *E. equi*).

Arboviral meningo-encephalitis: TBE (FSME and RSSE), Looping ill, Powassan encephalitis, Colorado Tick Fever (= orbivirus)

Arboviral haemorrhagic fever: Crimean-Congo HF, Omsk HF, Kyasanur HF

Febrile atypical syndrome: Colorado tick fever, Kemerovo tick fever

Babesiosis, e.g. *Babesia divergens*, *B. gibonsi*, *B. microti*

Tularaemia, caused by *Francisellatularensis*

Tick paralysis: paralysis from neurotoxic substances (e.g. holocycline) in tick saliva

## **Myiasis**

### **General**

Myiasis is the invasion of the body by fly larvae. During this period, the larvae feed on live or dead tissues. Depending on the life cycle of the insect myiasis is obligatory or facultative. In obligatory myiasis, the larvae have to spend part of their life cycle on a living host. Examples are *Cordylobia anthropophaga*, *Dermatobia hominis*, *Cochliomyia hominivorax*, *Chrysomya bezziana* and *Wohlfarthia sp.*

In facultative myiasis, the larvae are normally free-living, often on corpses, rotting meat, etc., but are sometimes found on living hosts (e.g. *Calliphora*, *Lucilia*, *Phormia* and *Sarcophaga sp.*) They can infect wounds and superficial ulcers. Clinically a distinction is drawn between cutaneous, urogenital, nasopharyngeal, ophthalmic and intestinal myiasis. Obligatory intestinal myiasis occurs only in animals, not in humans.

### **Cordylobia anthropophaga**



Myiasis, infestation with the larva of a fly (*Cordylobia anthropophaga*). Copyright ITM



Myiasis, *Dermatobia hominis* and *Cordylobia anthropophaga*. Copyright ITM

*Cordylobia anthropophaga* (tumbu fly, ver de Cayor) is a thick brown fly limited to tropical Africa. The larvae are obligate parasites, among others of dogs and humans. The female lays her 100-300 eggs on shaded, polluted ground or on dirty or inadequately washed sheets or clothes with some traces of sweat or urine still on it, laid out on the ground in the shade to dry. The females never lay their eggs on clothing that has been hung up in direct sunlight and also never directly on the skin. The larvae that emerge from the eggs penetrate the epidermis as far as the subcutaneous fatty tissue and develop there for 8 to 12 days. They then crawl out of the skin and fall to the ground where they undergo pupation in 24 hours. The pupae develop into adult flies in 10 to 20 days. The larvae rapidly penetrate the skin without causing any pain. In the first few days, an itchy, painful papule appears which develops over the course of a week into a painful furuncle in the centre of which two black dots (respiratory canals) are visible. The lesions may be few or numerous. The larvae can be pressed out of the skin if their respiration is prevented by coating the lesion with vaseline. One way to avoid infection is to iron bed linen and clothes on both sides.

### Dermatobia hominis

*Dermatobia hominis* (ver macaque) occurs in scrubland and woody lowland regions of Latin America.

This large (15 mm) blue-grey fly has a remarkable life cycle. During their short life (8-9 days) adult females seize various bloodsucking insects. They then attach 6-30 eggs to the body of these arthropods, which include *Psorophora* mosquitoes and stable flies (*Stomoxys calcitrans*). Cattle flies (*Haematobia irritans* and *H. exigua*) can also act as transport hosts. In some cattle breeding districts they constitute a real plague. The larva of *Dermatobia hominis* develops in the egg. When the transport insect sucks blood the larva feels the higher temperature and breaks out of the egg and drops onto the skin or fur.

Subsequently the *Dermatobia* larva penetrates the skin relatively rapidly. The larvae cause rather large cutaneous lesions, often painful and pruritic, few in number and frequently solitary and localised on the head. Development is slow, up to 12 weeks (up to 18 weeks has been reported). Fluid is formed constantly, consisting of the excreta of the larvae, but there is rarely superinfection. If this occurs, cellulitis and lymphangitis can follow. Frequently, the larvae have to be removed surgically (the final size of the larvae is 18-25 mm). A non-invasive technique of removing larvae is based on topical application of Vaseline to cut off their oxygen supply but this does not work very well. Fresh bacon can also be tried, the white part of the raw bacon is laid on the wound for some hours until the larva has attached itself. The bacon should then be lifted up and the larva can be grasped and removed with a rapid movement.





Myiasis; *Dermatobia hominis*; infestation with fly larva; photo Dr Van den Eenden, copyright ITM

Prevention of *Dermatobia hominis* infections is difficult. The transport host *Haematobia irritans* ("horn fly") principally bites cattle and can be successfully combated by "ear tags" containing a PVC matrix with pyrethroids. They can also bite humans. When these insects form a local plague, they can be controlled in a "low-tech" fashion since *Haematobia irritans* and *H. exigua* obligatorily lay their eggs on fresh cow dung. When this is broken up mechanically, the larvae die. A shepherd with a rake can diminish a local plague and limit the exposure of humans and animals.

### Screw worms

*Cochliomyia hominivorax* ("New World screw worm"; syn. *Callitroga hominivorax*) is a fly that occurs in Latin America and the Caribbean. It belongs to the Calliphoridae ("blow flies"). It was first described in 1858 by Dr Coquerel, a French army doctor in Cayenne, French Guyana. Many of the prisoners in the penal colony of Devil's Island had infections in the nose and sinuses. The insect lays its eggs on all types of wounds. The larvae bore deep in the tissues with serious consequences, such as mutilation or even death. Although the species name translates as "man eater", the insect preferentially plagues cattle.

The name "screw worm" refers to the somewhat screw-like appearance of the larvae. They have mouth hooks in order to attach themselves firmly. Treatment consists of the mechanical removal of the intact larvae, standard wound care and tetanus prophylaxis. Antibiotics are usually necessary to combat superinfection.

*Chrysomya bezziana* ("Old World screw worm") strongly resembles *Cochliomyia hominivorax*, but does not lay its eggs on wounds. When larvae invade natural openings (vagina, nose, eyes, mouth), they can cause very painful and serious lesions. The larvae complete their development in humans in 5-6 days, after which they crawl out of the tissues and fall to the ground to pupate. *Chrysomya megaloccephala* is a facultative parasite of humans.



Myiasis. Adult *Chrysomya bezziana*, dorsal view. Copyright ITM

### *Tabanids*

Stinging flies that belong to the tabanids (*Haematopota*, *Chrysops*, *Tabanus*) can be mechanical vectors for anthrax and tularaemia ("rabbit fever"). This last infectious disease is caused by *Francisella tularensis*.

## Bed bugs



*Cimex lectularius*, bed bug. These insects are not known to transmit human pathogens. Photo copyright ITM

There are two main species of bed bugs: ***Cimex lectularius*** (the common bed bug which occurs worldwide) and ***Cimex hemipterus*** (the tropical bed bug). In West Africa, *Leptocimex bouetti* attacks man. Bed bugs are insects (4-7 mm) with rudimentary, non-functional wings. This limits their capacity for dispersion. They are not vectors of pathogenic organisms, but are primarily a nuisance because of their behaviour. They suck blood for a short time during the night or at dawn. During the day the adult insects hide in cracks and crevices. Often dirty brown spots caused by their faeces are found on sheets, walls or floors. Sometimes clusters of hundreds of 1 mm large whitish-yellow eggs can be seen on walls, under wallpaper, etc. After a bite a severe pruritic skin reaction can occur.

Spraying insecticides helps control these animals. The problem of increasing insecticide resistance among bedbugs is getting worse. DEET has a repellent effect, but makes it that blood meals are often interrupted, therefore the insect will bite several times in order to get the same amount of blood. This means that this repellent is less than ideal. Aggressive and total extermination on an infestation is the only solution for infested premises. If this is unfeasible an alternative would be to take oral ivermectin and let the bugs bite the next night. Ivermectin is a neurotoxin for these insects and will kill them.

## Beetles

### General

Although beetles have the greatest wealth of species of all insects, only a few are directly harmful to human health. A few beetles, chiefly belonging to the Scarabaeidae and Tenebrionidae, can be intermediate hosts for worms, such as the tapeworm *Hymenolepis diminuta* (the cause of non-specific abdominal discomfort).

### Blister beetles

Blister beetles are insects that cause skin lesions by direct contact. They are found on various continents. They contain highly poisonous substances such as cantharidin or pederin. Cantharidin is found in the haemolymph of the beetle and is released when the insect is crushed. A number of insects secrete the caustic fluid via their leg joints when they are disturbed ("reflex bleeding"). In *Lytta vesicatoria* cantharidin is also found in the wing sheath.



*Paederus* sp. blister beetle. Contact with the animals can result in severe dermatitis or eye inflammation. The insects contain pederin, a blistering agent. Copyright ITM

### Blister beetles toxins



Dermatitis resulting from contact with blister beetles (*Paederus* sp). Copyright ITM, Dr Van den Enden



Dermatitis secondary to contact with a blister beetle, *Paederus* sp. (fam. Staphylinidae). Contact with the eyes leads to the so-called "Nairobi eye". Copyright ITM

## Pederin

Pederin is the active vesicant of the short-winged beetle *Paederus fuscipes* and related species. It is a complex non-protein molecule. Pederin is highly toxic, more potent than cobra venom. It inhibits protein synthesis and prevents cell division.

## Cantharidin

Cantharidin binds chemically to phosphatases 1 and 2A. The toxin is very stable. Dead beetles are still dangerous. Consequently control by means of insecticides does not remove the danger. The toxin protects the beetles from predators and is found in the haemolymph and gonads.

### *Cantharidin systemic effects*

Sometimes cantharidin is swallowed. The toxin is readily absorbed from the intestine and excreted in the urine. If cantharidin is swallowed to arouse sexual appetite, in an attempted suicide, by accident, with criminal intent or to induce abortion, several symptoms may occur depending on the dose. The initial discomfort begins within 30 minutes. Dysphagia as a result of mucositis with irritation of oral, oesophageal and gastric mucosa is followed by abdominal pain, nausea and vomiting, possibly with blood. Oedema, bleeding and necrosis of the mucosa occur at an early stage. There is intense congestion of the genitourinary tract, with bleeding in the renal pyelum, ureters and bladder. Bleeding can also occur in the ovaries. Sometimes there is internal bleeding and bruising. Priapism occurs, which was the origin of the use of the substance as an aphrodisiac (Gr. Aphrodite = goddess of love). Diarrhoea occurs, accompanied by leukocytosis, haematuria, renal tubular necrosis, uraemia, shock and coma. Approximately 30-60 mg is sufficient to kill an adult person.

## Clinical aspects

On skin contact with cantharidin-containing blister beetles, local tissue irritation occurs after a few hours. In intra-epidermal blister formation, redness, oedema and vesicles can appear on the skin.

Sometimes there are "kissing lesions" on the elbow or in the hollow of the knee. In contrast, the effect of pederin is not immediately noticeable and only becomes apparent after 1 to 2 days. The erythema is much more severe and can persist for months. On contact with the conjunctiva and/or cornea, *Paederus* sp. cause "Nairobi eye". This is associated with extensive painful peri-orbital swelling and purulent conjunctivitis. Corneal erosions and blindness can follow.

## Treatment

For external lesions, the skin should be rinsed copiously as rapidly as possible. After disinfection, silver sulphadiazine cream should be applied. Subsequent care is the same as for a burn. Skin lesions caused by cantharidin practically always heal without leaving scars. An eye that is affected should be rinsed copiously. Afterwards an antibiotic- and steroid-containing eye ointment should be applied (cfr. eye lesions caused by spitting cobras).

There is no specific antidote. Steroids are not effective in controlling the ulcers in the gastrointestinal tract. Fluid, calcium supplements, analgesics and broad spectrum antibiotics should

be given. Gastric lavage should be carried out and activated charcoal administered. Cantharidin is to a large extent bound to albumin and is not removed by haemodialysis via a charcoal column. Physiological fluid should be administered IV. A blood transfusion might be necessary. Maximum diuresis must be obtained with IV fluid, mannitol and diuretics. No fat should be given orally because it increases the absorption of the toxin.

## Leeches

### General

The phylum Annelida is subdivided into three classes: Polychaeta ("bristle worms", principally marine animals), Oligochaeta (e.g. earthworms) and Hirudinida ("leeches"). Among the latter there is a subclass of Hirudinea (the true leeches) with 12 families. They include terrestrial, freshwater and saltwater species. There are approximately 650 species, but not all of these constitute a problem for humans.

Terrestrial (semiterrestrial is a better term) and amphibious species are common in Southeast Asia, the islands in the Pacific Ocean, India and South America. Aquatic species occur worldwide. They are seldom found in low-calcium water. They are good swimmers. Usually victims are people visiting marshy areas or walking in or near slow-moving small brooks or streams.

### Leeches bites

On biting leeches introduce vasodilators and hirudin, a very powerful anticoagulant into the skin. The bite causes prolonged painless local bleeding. Once sated after sucking two to five times their own weight of blood they let go and drop to the ground. They feed infrequently. After a large blood meal, the animal can go for over 6 months without feeding. The blood is then digested in the gut over a 100-day period, during which water is extracted and excreted through several pairs of ventrally located nephridia.

### Clinical aspects

Leeches can attach to the skin. With the anticoagulant, they also inject a local anaesthetic, so pain is absent. Prolonged wound bleeding can result. Removal of a leech can be facilitated by applying a little alcohol or vinegar. If necessary a burning cigarette may be held near the parasite. No attempt should be made to remove the animals rapidly because the jaws can remain behind. After wound cleaning, local pressure should be applied to stem the bleeding. The bleeding tendency can persist for many hours, sometimes even up to 2 days. This illustrates the power of the animal's anticoagulants. Aquatic species can attach to the conjunctiva, nose, nasopharynx, vagina and urethra. When they attach themselves to the epiglottis, trachea or bronchi, serious complications are likely. Internal bleeding, haemoptysis, chronic headache, dysphagia and hoarseness occur. The leeches can be loosened by local application of cocaine or lidocaine. They are removed carefully with a forceps, using a laryngoscope or endoscope. As a rule the leech itself does not transmit any pathogens, although some recent observations from Laos suggest that it might transmit *O. tsutsugamushi*. Wounds can become secondarily infected. *Aeromonas* infections can occur but is rare. Following repeated bites, hypersensitivity can occur. For prevention, protective clothing should be worn. A topical repellent such as dimethyl phthalate or dibutyl phthalate, may be applied.

# Haematology

## *Sickle cell anaemia*

### General

Sickle cells are much less flexible than normal erythrocytes. They therefore have difficulty in passing through capillary vessels, the diameter of which is often less than half the diameter of a red blood cell.

Janet Watson noted that symptoms appeared in infants only after concentrations of fetal haemoglobin (Hb F) had fallen, establishing the notion of the beneficial effect of Hb F on disease manifestations.

### Sickle cell anaemia, haemoglobin

#### *Haemoglobin structure*

Each molecule of haemoglobin consists of a tetramer consisting of 2 pairs of polypeptide chains to which a total of 4 haem groups (one haem group per globin chain) is linked. One molecule of haemoglobin therefore contains 4 proteins. Hb A contains 2 globin chains of one type (alpha) and 2 globin chains of another type (beta). Depending on which 4 chains are present in the haemoglobin tetramer, the molecule is given its name.

There are many haemoglobin variants:

#### **Normal**

Hb A:  $\alpha_2\beta_2$

Hb A2:  $\alpha_2\delta_2$

Hb F:  $\alpha_2\gamma_2$

#### **Pathological**

Hb S:  $\alpha_2\beta_2^S$

Hb C:  $\alpha_2\beta_2^C$

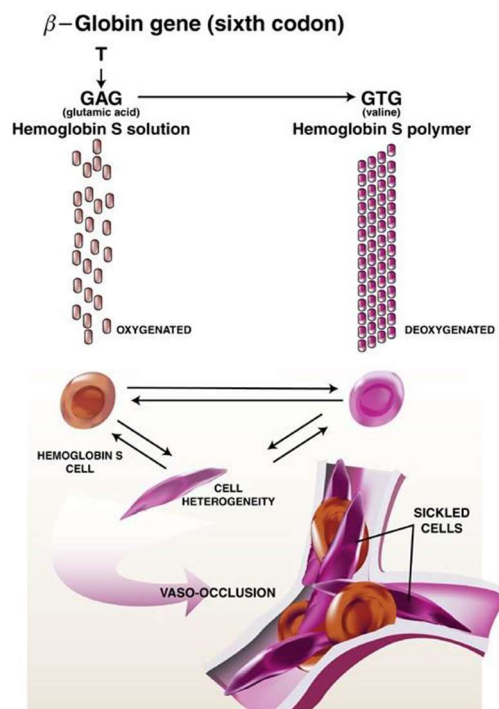
Hb E:  $\alpha_2\beta_2^E$

Hb H:  $\beta_4$  (see alpha-thalassaemia)

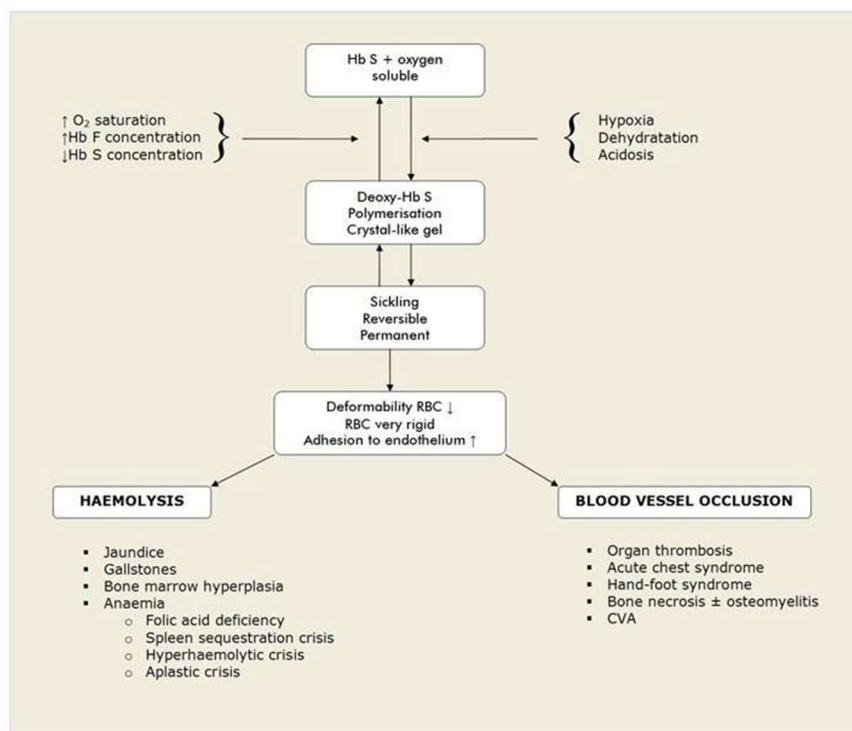
Hb Barts:  $\gamma_4$



# Physiopathology



Pathophysiology of sickle cell anaemia. Deoxy-haemoglobin S undergoes time-dependent polymerisation. This changes the shape of the red blood cells, which can block the microcirculation. Adapted from a drawing in New England Journal of Medicine, 2002.



The normal haemoglobin of a child or an adult are Hb A (97%), Hb A<sub>2</sub> (2%) and Hb F (1%). Hb A contains 2 alpha-chains and 2 beta-chains. If by mutation, the 6th amino acid of the β-chain (glutamic acid, negatively charged) is replaced by a different amino acid (valine, hydrophobic), Hb S is formed. As a result a hydrophobic site is formed on the outside of the folded mutated beta chain. With normal arterial oxygen tension there is no problem and the molecule transports the oxygen. In the capillary bed in the tissues the oxygen is released and deoxyhaemoglobin S is formed. This latter substance has several different properties. In deoxy-Hb S there is a second hydrophobic site on the surface. This site is concealed in oxy-Hb S. The site is complementary to the first. These two hydrophobic regions adhere to each other, resulting in a kind of polymerization of the deoxyhaemoglobin S molecules. The hydrophobic valine on the surface makes the haemoglobin molecule somewhat less water-soluble if the molecule is not bound to oxygen. The concentration of haemoglobin in the erythrocyte (32-34 g %) does however require a very water-soluble molecule. The deoxyhaemoglobin S molecules start to come out of solution (precipitate). At low oxygen concentrations the deoxyhaemoglobin S molecules adhere to each other, forming long, rigid strands and thus deform the red blood cells making them more rigid. The molecules stick to each other in a definite pattern (like a crystal). This polymerization reaction is relatively slow, giving a "delay time" or T<sub>d</sub>. The slower the circulation and therefore the longer the time before reoxygenation in the lungs, the more sickling occurs. Usually the transit time of a red blood cell in the microcirculation is less than T<sub>d</sub> and a major catastrophe is avoided.

The main variables that affect sickling are the intracellular haemoglobin concentration, pH, the level of oxygenation and the percentage of Hb F. Sickling is accelerated by lack of oxygen, slow blood circulation, acidification and dehydration (a situation which is common with infections). The formation of rigid Hb SS strands is counteracted by Hb F (efficiency of polymerization is reduced). People with high concentrations of Hb F have far fewer symptoms than patients with low Hb F concentrations.

The sickling process also causes damage to various membrane proteins of the erythrocyte, thus promoting adhesion to the vascular endothelium. This makes circulation even more difficult. The degree of adherence is closely correlated to the severity of the disease. If there is inflammation, this "stickiness" can increase even more.

Additional factors play a part in the pathophysiology of sickle cell disease: endothelial cells can be "activated" i.e. they can be induced to express all kinds of molecules on their membrane, after exposure to various inflammatory substances (cytokines, prostacyclin's, etc). Such cells become "sticky" and promote local haemostasis and possibly thrombosis. There is also an increase in the number of adhesion molecules on the red blood cells. The local production of Nitric oxide (NO) by the damaged endothelium falls.

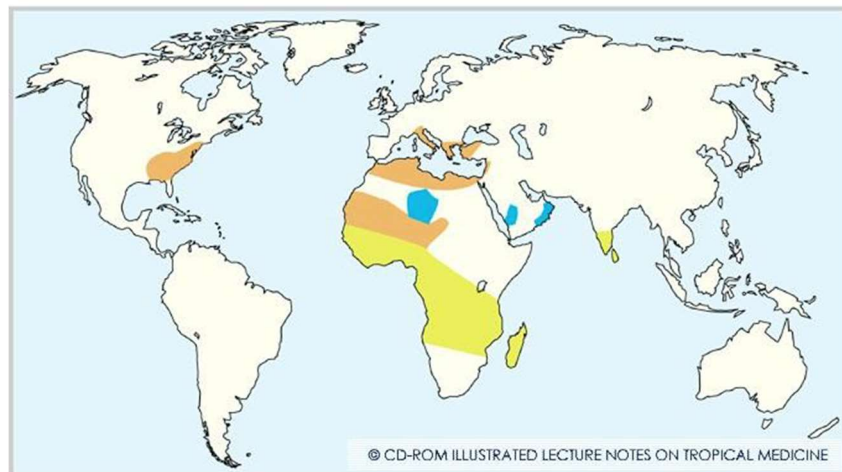
#### *Nitric oxide function*

Nitric oxide (NO) produced by endothelial cells causes vasodilatation (effect is concentration dependent). Free haemoglobin in plasma will capture NO, thereby diverting nitric oxide from its homeostatic vascular function.

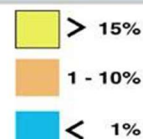
What are the clinical consequences of sickling?

- Sickle cells rapidly haemolyse. As a result, anaemia occurs: sickle cell anaemia.
- Sickle cells are rigid and obstruct the microcirculation. As a result, small or large infarctions can occur.
- Tissues with poor blood circulation can be infected more easily.
- Due to splenic atrophy, resistance to certain pathogens is reduced.

## Geographical distribution



**Geographic distribution of Hb S (1961)**



Map sickle cell disease (drepanocytosis). Due to the slave trade, the disease also exists in North America, but is especially common in areas where *Plasmodium falciparum* is frequent. Copyright ITM

The sickle cell gene occurs in large parts of Africa and to a somewhat lesser extent in the Middle East (Saudi Arabia) and India. In West Africa, 5 to 25% of the population are carriers of the gene. In Central and East Africa, heterozygotes occur with a frequency of from 20 to 40%. If 20% of the population are carriers of the gene, it follows that 1% of newborn children will be homozygous. Through the slave trade the sickle cell gene also found its way to North and South America.

Heterozygous carriers are relatively protected against fatal *P. falciparum* malaria. They are infected just as often, but are less likely to die from the infection. If the malaria parasite is present within the erythrocyte, the red blood cell acidifies slightly. This is enough to promote sickling. Because of the damage to the membrane; potassium flows out of the red blood cell, which is damaging to the parasite and the erythrocyte. The red blood cell is rapidly destroyed, for example in the spleen (heterozygotes have a normal spleen). Since heterozygotes in an endemic malaria area have a longer life expectancy than people with normal haemoglobin, it is thought that this has promoted the occurrence of sickle cell haemoglobin in Africa over the course of evolution. On the other hand, homozygous Hb S people have a very low life expectancy. There will therefore be a genetic equilibrium.

## Sickle cell anaemia, genetics and heredity

Sickle cell anaemia is a genetically determined disease. A distinction is made between three main groups: homozygotes, heterozygotes and double heterozygotes.

Hb SS disease: classic sickle cell anaemia

Hb AS: sickle cell trait, heterozygote

Hb S/Beta<sup>0</sup>-thalassemia; Hb SC: severe double heterozygote; phenotypical similar as Hb SS

## Heterozygosity ("sickle cell trait")

If someone has both a normal gene (from one parent) and a mutated gene (from the other parent), they produce both the normal haemoglobin (Hb A) and also the sickle cell haemoglobin (Hb S). One would expect a heterozygote HB AS to have about 50% haemoglobin A and about 50% haemoglobin S, but for a variety of reasons, the average patient has about 2/3 Hb A and 1/3 Hb S. The person is an asymptomatic carrier and each red blood cell contains both Hb A and Hb S. Such erythrocytes are functionally normal and have the advantage that they provide relative protection against fatal *Plasmodium falciparum* infection. Heterozygotes lead a normal life. But they may well pass the gene on to their children

Probability per child of having the different haemoglobins:

Parent Hb AA x Parent Hb AS --> 50% probability of Hb AA and 50% probability of Hb AS

Parent Hb AS x Parent Hb AS --> 25% probability of Hb AA, 25% probability of Hb SS and 50% probability of Hb AS

## Homozygosity

If a patient has two identical mutated genes (homozygote) they cannot produce Hb A. After birth, the Hb F concentration falls and after 3 to 6 months, the red blood cells contain mainly haemoglobin Hb S.

This will lead to sickle cell disease.

## Double heterozygotes

Certain double heterozygotes can display a sickle cell phenotype.

1. haemoglobin SC
2. haemoglobin SD
3. haemoglobin SO Arab
4. haemoglobin S beta thalassaemia
5. haemoglobin S with haemoglobin New York

Sometimes a child has both a sickle cell gene and also a gene for haemoglobin C. It then has both haemoglobin S and haemoglobin C (Hb SC). Doubly heterozygous people suffer a less serious course of the disease than homozygous sickle anaemia patients. They have a clearly increased risk of eye damage (retinitis proliferans), avascular necrosis of the head of the

femur, haematuria and complications during pregnancy (pulmonary infarction and risk of fat embolism after bone marrow infarction).

A little caveat: patient who are homozygous for Hb SS can have Hb A in their blood after a blood transfusion. Don't be misled by this.

## Clinical aspects

It is possible to distinguish two clinical phenotypes of sickle cell disease. The first is dominated by haemolysis and is characterized by severe haemolytic anaemia, leg ulcers (especially lower legs and around ankles) and pulmonary hypertension. The second is dominated by vaso-occlusion incidents, with episodic painful crises, acute chest syndrome, splenic infarction leading to functional asplenia, stroke and avascular necrosis of joints (hip, humerus) predominate.

### *Vaso-occlusive complications*

Pain episodes	In more than 70% of patients.
CVA	In 10% of children; "silent" lesions with cognitive damage in 50-90%.
Acute chest syndrome	In 40% of patients, more often in children.
Priapism	In 10-40% of men. Severe cases lead to permanent dysfunction.
Liver disease	In <2%. Multiple causes: hep B, C, iron overload.
Spleen sequestration	In children < 6 years of age. Often preceded by infection.
Spontaneous abortion	In 6% of pregnant women.
Skin ulcers (leg)	In 20% of adults
Osteonecrosis	In 10-50% of adults (often femur, humerus).
Proliferative retinopathy	Rare in sickle cell anaemia; in 50% with Hb SC.
Renal insufficiency	In 5-20% of adults, often with severe anaemia.

### *Complications of haemolysis*

Anaemia	Haematocrit often 15-30%.
Gallstones	In the majority of adults, usually asymptomatic.
Red bone marrow	Expansion leads to weakened cortical bone.

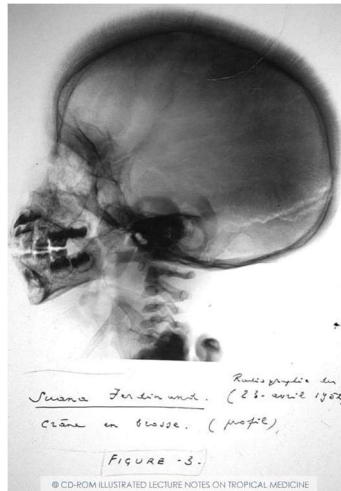
### *Infectious complications*

Streptococcus pneumoniae	Sepsis in 10% of children < 5 years.
Osteomyelitis	Often by Salmonella or Staphylococcus aureus.
Escherichia coli sepsis	In adults often originating from infection of the urinary tract.
Acute aplastic crisis	Due to parvovirus B19. Sudden severe anaemia.

Individuals with sickle cell trait are generally asymptomatic and have no abnormal physical findings. Their laboratory evaluation often shows microcytosis but is otherwise normal with no anaemia, no evidence of haemolysis and no laboratory abnormalities other than haemoglobin AS on haemoglobin electrophoresis. Complications such as splenic infarction, pain episodes and sudden death may be induced by severe hypoxia, severe dehydration, and exertion at the limits of human endurance, e.g. at high altitudes.

Homozygous (Hb SS) children with little Hb F have the clearest symptoms. The symptoms result from haemolysis, thromboses, infections and acute haematological crises. In rural Africa only a few survive beyond puberty. In the first few months after birth the baby is virtually normal (the Hb F concentration is still high). The first problems start at about 3 to 6 months.

### Haemolysis



"Hair-on-ends" image caused by bone marrow expansion in the diploic space in chronic haemolytic diseases, such as sickle cell anaemia or more commonly in beta thalassaemia major. The major trabecular spicules in the diploë are aligned perpendicular to the inner table in an effort to support the soft outer table. Photo ITM



Fish vertebrae in sickle cell anaemia. Marrow expansion makes the vertebrae more susceptible to compression, leading to this diabolo-shape. Copyright ITM

Chronic haemolysis manifests itself as pallor, mild jaundice, dark urine and retarded growth. There is hypertrophy of the bone marrow, which can often be seen in the cranium and the maxillae. But the expansion of the bone marrow is less pronounced than in homozygous  $\beta$ -thalassaemia, possibly because less erythropoietin is produced than expected due to repeated kidney damage. Due to the constant haemolysis and the production of bilirubin; bilirubin gallstones are produced at a very young age (such stones are often not radio-opaque). There is splenomegaly up to about 5 years, afterwards there is atrophy because of the repeated infarctions of the spleen. The expansion of the bone marrow can usually be seen clearly by

frontal "bossing", a pronounced curving of the forehead and by widely spaced teeth in the jaws. On an X-ray of the cranium, small canals in the diploë of the vault of the cranium and are known as a "hair-on-end" appearance (see Fig).

### *Acute haematological crisis*

Haematological crises sometimes occur. In children aged from 6 months to 3 years the spleen can sometimes swell acutely (sequestration of blood in the spleen), with sudden anaemia, hypovolemia and shock as a result. Many do not survive this. Due to infections, such as malaria for example, hyperhaemolysis can occur. After certain viral infections (for example parvovirus B19) a period may follow during which the bone marrow does not form any new red blood cells (aplastic crisis). Bone marrow arrest can also occur if there is a marked folic acid deficiency.

### *Thrombosis*

Thrombosis is manifested most commonly as episodes of pain but also can produce kidney infarctions (haematuria, papillary necrosis), priapism, atrophy of the spleen, bone necrosis (head of the femur, head of the humerus, metacarpals, vertebrae), cerebrovascular accident (CVA), including the rather rare Moyamoya syndrome (collateral circulation developing around blocked vessels, these collateral vessels are prone to bleeding aneurysm and thrombosis; moyamoya means "puff of smoke" in Japanese referring to the appearance of the collateral vessels on MRI), chronic skin wounds (mainly on the shins) and proliferative retinopathy. Hand-foot syndrome is sometimes the first clinical manifestation. The child then has acutely painfully swollen hands and feet. Chronic damage to the vertebrae leads to biconcave vertebrae ("fish vertebrae"; see Fig) with a typical appearance on X-ray.

Due to kidney damage, patients with sickle cell anaemia usually have difficulty in concentrating their urine and are susceptible to dehydration. Hyposthenuria may become evident in childhood as enuresis.

Glomerular sclerosis, manifested by proteinuria, progresses as patients age. Chronic renal failure occurs in up to 5% of patients with sickle cell anaemia. Pulmonary infarctions contribute to acute chest syndrome, with pain, dyspnoea and a poor general condition. Small cerebral watershed infarcts may be clinically silent but produce cognitive defects shown by neuropsychiatric testing. Hemiplegia can result from cerebral infarction. Most patients with brain injury require long term transfusion therapy.

### *Eye problems*

Occlusion of small retinal vessels with neovascularization is asymptomatic until haemorrhage occurs within the vitreous. Detachment of the retina, more common in late disease, is a feared complication, and an important cause of blindness, together with occlusion of the central retinal artery. The latter condition is a medical emergency, for which urgent transfusion is imperative.

### Autosplenectomy



Splenic atrophy in sickle cell anaemia; photo Dr Van den Eenden, ITM

Because of the repeated infarctions of the spleen, sickle cell anaemia patients over the age of 5 years no longer have a functioning spleen. Asplenic children are very susceptible to bacterial infections, including pneumococci (i.e. encapsulated bacteria, *Streptococcus pneumoniae*). Osteomyelitis caused by among others, *Salmonella* and staphylococci is common. Often it is difficult to distinguish between pulmonary infarction and pneumonia and between osteomyelitis and bone infarction.

### Acute chest syndrome

This syndrome consists of a collection of problems, such as acute chest pain, dyspnoea, coughing, fever, hypoxemia, leukocytosis and pulmonary infiltrates, mainly in the inferior lobes. This can develop into a full-blown ARDS (Acute Respiratory Distress Syndrome). Bone marrow infarctions followed by fat and even bone marrow embolism play a part (beware of sickle patients with first pain in limbs, followed by chest problems). Atelectasis also contributes and often develops as a result of hypoventilation that accompanies rib pain and the use of opiates. At autopsy in 75% of fatal cases bone spicules are found in the lung. In 60% of patients with acute chest syndrome, fat-loaded macrophages are found in the broncho-alveolar fluid. Because of the pain in the chest wall, patients are able to breathe less deeply ("splinting") with hypoventilation, atelectasis and perhaps superinfection as a result. Hypoxemia increases the adhesion of red blood cells to the endothelium, via the increased expression of the adhesion molecule VCAM-1 on the endothelium. Breathing in regularly, as deeply as possible, is an important part of treatment ("incentive spirometry"). The patient is asked to breathe in deeply 10 times and to do this every two hours while awake. There is a clear role of opioids in promoting to control pain with careful monitoring to avoid over-sedation and hypoventilation. The administration of oxygen, antibiotics and standard or exchange transfusion completes the treatment. In a good hospital, the mortality of acute chest syndrome is 2% for children and 5% for adults.

### Pulmonary hypertension

Pulmonary hypertension is a feared complication in chronic and severe haemolytic anaemias, such as thalassemia major, congenital spherocytosis and paroxysmal nocturnal haemoglobinuria. Pulmonary hypertension occurs in about one third of all patients with sickle cell disease. Asplenia increases the circulation of platelet-derived mediators, which promotes pulmonary microthromboses and adhesion of erythrocytes to the endothelium. Haemolysis



results in the release of free haemoglobin, which scavenges nitric oxide, causing vasoconstriction.

### *Priapism*

Priapism is a persistent and painful erection [Lat. priapus, God of procreation]. It is not associated with sexual stimulation. It is an important complication of sickle cell disease. By adulthood, 90% of males with sickle cell anaemia will have had a least one episode of priapism. The blood that flows into the corpora cavernosa of the penis has difficulty leaving the organ due to venous thrombosis. Because of acidification and hypoxia, sickling of red blood cells increases still further. If priapism persists longer than 4 hours, surgery is definitely required. Persistent priapism (>24 hours) results in fibrosis and impotence. As an initial treatment the patient can be made to go up and down stairs in order to divert blood flow to the leg muscles (the "steal mechanism" principle) or have external compression of the perineum applied, perhaps with ice. General measures such as hydration, (exchange) transfusion and analgesics are necessary. Aspiration and irrigation of the corpus cavernosum with or without saline irrigation is necessary in an episode of priapism lasting more than four hours. The alpha-adrenergic agonist phenylephrine can be injected in the corpora cavernosa, causing blood to leave the corpora cavernosa due to smooth muscle contraction in the penile arteries.

## Diagnosis

### *Laboratory*

A sickling test can be carried out in field laboratories (Emmel's test). In this, a drop of blood is placed on a glass slide. This is covered with a coverslip and the edges are sealed with some vaseline (to prevent contact with the air). As time goes by and the oxygen in the blood falls further (due to the metabolism of the cells) the red blood cells will sickle. This test can be accelerated by adding a drop of sodium metabisulphite to the blood.



Red blood cells of a homozygote sickle cell anaemia patient undergo dramatic change in shape when oxygen is excluded from their environment (Emmel test). Copyright ITM

### **Heterozygotes**

Since normal and mutated beta chains are produced equally rapidly, it may be expected that heterozygotes would have  $\pm 50\%$  Hb S and  $\pm 50\%$  of Hb A. However, because alpha chains bind more easily to the normal beta chains than the mutated forms, there is a relative excess of mutated beta chains in the tetramers. The excess mutated chains are then destroyed. As a result, most heterozygotes have about 35% Hb S rather than 50%, and about 65% Hb A. The diagnosis of sickle cell trait is established by haemoglobin electrophoresis. If a non-transfused patient with sickle cell disease would have e.g. 65% HbS and about 30% HbA, especially if Hb A2 would be elevated, the suspicion of Hb S/beta+thalassemia would be strong.

## Homozygotes

There is severe anaemia (usually Hb 6-9 g%) with considerable reticulocytosis. A blood smear of a homozygote shows many sickle cells, in contrast to that of a heterozygote. The diagnosis can be confirmed by haemoglobin electrophoresis. On electrophoresis it can be seen that most of the haemoglobin consists of Hb S (often more than 80%); the remainder consists of Hb F and Hb A<sub>2</sub>. Of course, no Hb A can be found. There is often thrombocytosis and leucocytosis.

## Treatment

### General

Apart from bone marrow transplantation, there is no curative therapy. Hematopoietic stem cell therapy and gene therapy remain possibilities for the future. The suffering of children can be reduced.

It is possible to stimulate the induction of haemoglobin F by medication. In contrast with haemoglobin A<sub>2</sub> (alpha<sub>2</sub> delta<sub>2</sub>), a minor haemoglobin which is uniformly distributed in all adult red cells, haemoglobin F is found (in normal people) in 0,2 to 7 percent of the adult red cells, and in those cells, it constitutes 14 to 28 percent of the total haemoglobin. They are called "F"cells. Hb F contains gamma chains instead of beta chains (structure alpha<sub>2</sub> gamma<sub>2</sub>). Hb F has a greater affinity for oxygen than Hb A. This helps the fetus to draw oxygen from the mother's blood. Hb F inhibits the polymerization of deoxy-Hb S. This inhibits the sickling of red blood cells. After birth, the neonate still has more than 50% Hb F in his blood.

This explains why very young children are free of sickling crises. After birth the genes for the gamma chains are less active because they become methylated. This is reversible however.

Hydroxyurea is a mainstay in the treatment of sickle cell anaemia. Hydroxyurea (Hydrea®) is a cytostatic drug which was long used in patients with polycythemia vera or chronic myeloid leukaemia, to counter hyperleukocytosis. Another important effect of hydroxyurea is production of nitric oxide (NO), a vasodilator. The anti-sickling activity results from induction of haemoglobin F through activation of a specific promoter for the haemoglobin gamma-chain gene. There is also a reduced expression of adhesion molecules (e.g. VCAM-1, L-selectin), as a result of which red blood cells and neutrophils adhere less easily to the vascular endothelium. Hydroxyurea can be used in prevention (not in an acute crisis). Hydroxyurea reduces the frequency and the severity of the attacks (up to 40% decrease in mortality).

### Maintenance treatment

1. Folic acid. The patients have a greater need for this vitamin due to the high demands of the red bone marrow. It is important to check vitamin B<sub>12</sub> status, in order not to mask a cyanocobalamin deficiency.

2. Penicillin prophylaxis is given to reduce the number of infectious episodes. Today vaccination with pneumococcal vaccine lessens the importance, but prophylactic penicillin is still recommended to all children with sickle cell diseases till the age of 5 years. Penicillin V 125 mg orally twice daily till the age of 3 years is increased to 250 mg twice daily until the age of 5.

3. Zinc. Up to 20% of sickle cell anaemia patients have persistent leg wounds. Many patients tend to be zinc deficient, possible via excessive renal excretion due renal damage secondary to repeated infarctions in the hypertonic renal medulla. Zinc is a trace element needed for certain enzymes, including some metalloproteases which are important in wound healing. Zinc deficiency makes wounds heal more slowly. Zinc sulphate or zinc acetate per mouth (e.g. 30 mg per day) can help here, but is often given as part of multivitamin supplements (without iron). It is important to mention that prolonged treatment with hydroxyurea can lead to slower healing of leg ulcers.

#### *Additional important measures*

1. Immunizations are a cornerstone to prevent infections in sickle cell disease: routine childhood vaccinations are recommended including vaccination against *Streptococcus pneumoniae*, *Neisseria meningitidis*, *Haemophilus influenzae* B and hepatitis B. Yearly influenza vaccination is advised.

2. Antibiotics. Every patient must have at home a stand-by broad-spectrum antibiotic such as coamoxiclav. Azitromycin can be used in case of penicillin-allergy. They should take this at the first signs of infection. The reason for having the antibiotic at home is that patients often live a long way from a hospital and might lose lots of precious time before they are seen by a medical doctor.

3. Malaria prevention is absolutely indicated in an endemic area as infection with this parasite can be fatal.

4. Preventive transfusions are a double-edged sword and are not given routinely. With transfusions the haemoglobin level can be kept at a higher level, which reduces the consequences of anaemia. This also reduces the concentration of Hb S in the blood, thus reducing the risk of complications.

However, repeated transfusions gradually cause severe transfusion reactions. It is best always to give blood that is low in leukocytes. Since in the long term there will be sensitization to the minor blood groups, the red blood cells in later transfusions will be destroyed very quickly. Slow iron poisoning also occurs, damaging the heart, the liver and some endocrine organs (pancreas, testis). Iron chelation therapy is indicated. In the case of acute aplastic crisis (triggered by infection with parvovirus B19) and splenic sequestration crisis, transfusions are essential. They are also important in acute chest syndrome. Exchange transfusions are also a therapeutic option.

Hydroxyurea (= hydroxycarbamide, Hydrea®). Usually 500 mg three times daily is given in order to raise the level of haemoglobin F to above 15%. Regular checking of the number of white blood cells is indicated (it is a cytostatic drug). Another side-effect is slower healing of leg ulcers. Pregnancy is a contra-indication. Since the introduction of hydroxyurea in treatment, the quality of life for many patients has improved dramatically. Since the drug is cheap, it is not outside the means of many thirdworld families and hospitals.

Heredity should be explained to the parents so that they have the correct information in order to decide whether or not to have another child. If both parents are carriers, the probability of a normal child (Hb A) is 25%, the probability of a healthy heterozygous child is 50% and the probability of a homozygous Hb S child is 25%.

### *Management of ARDS in sickle cell crisis*

The mainstay of treatment for patients with ARDS is supportive care and mechanical ventilation.

Although the ventilator can be lifesaving, it can be a source of further lung injury. A crucial intervention in the acute chest syndrome is reduction of the percentage of haemoglobin S in the patient's blood. One can use "normal" transfusions, but this also increases blood volume and viscosity. Red-cell exchange transfusion avoids this complication. The aim is to reduce the percentage haemoglobin S to well below 30%. The final (desperate) measure is the use of extracorporeal membrane oxygenation (ECMO).

### *What should be done in the event of a sickle cell crisis?*

Antibiotics, transfusions (normal or exchange transfusion), oxygen, pain control with paracetamol/codeine, ibuprofen or morphine analogues are all part of sickle cell crisis management. Sufficient fluid should be administered because the kidneys have difficulty in producing concentrated urine. Often 3 to 4 litres a day are given (adults) if possible orally, otherwise IV. Severe acidosis is best corrected quickly with bicarbonate, although no spectacular results can be expected. In the case of rib or tissue infarctions, and also in chest disorders, it is important that the patient is urged to breathe deeply (10 maximum inspirations) at regular intervals e.g. every two hours. This prevents atelectasis. The polymerizing of Hb S is promoted strongly by dehydration. The higher the salt concentration in the blood, the more quickly the cells sickle. Patients with a sickle cell crisis often have a hypercoagulable state, thus thromboembolism prophylaxis is essential during hospitalization. This can be done with LMWH's or unfractionated heparin.

### *What happens if an operation is carried out?*

Many homozygous sickle cell patients have to undergo surgery due to complications of their illness (mainly cholecystectomy or orthopaedic surgery) or for other reasons. Perioperative complications are common in patients with sickle cell anaemia. During anaesthesia, the operation itself and in the postoperative phase hypoxia must be avoided. Perioperative hypoxia, tissue hypoperfusion and acidosis can trigger vaso-occlusive crises and cause organ dysfunction (mainly acute chest syndrome and pain crises). Pre-operatively (exchange-)transfusion can be given.

### *Pregnancy and prenatal care*

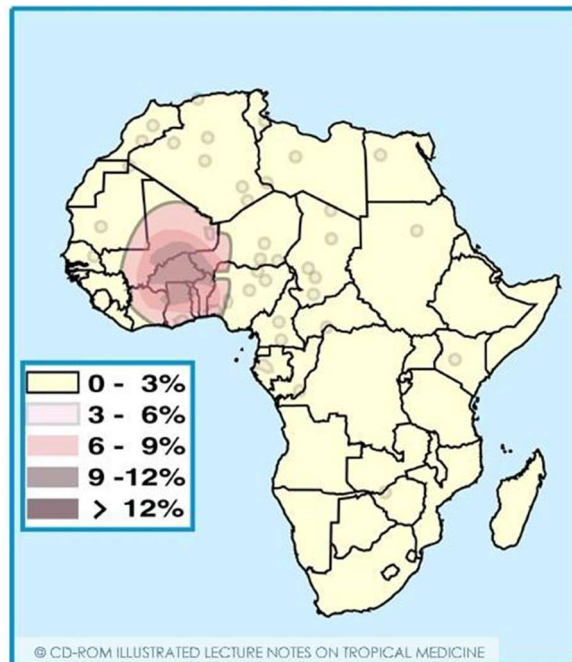
Pregnant homozygous sickle cell anaemia patients are rare in Africa. In the absence of medical care, mortality for mother and neonate can be as high as 20% and 50% respectively. The most common complications during pregnancy for women with sickle cell disease are hypertension and preeclampsia (14%). It has been suggested that maternal anaemia and placental ischemia may play a role, as slow placental circulation and a high degree of oxygen-extraction promote sickling. A high percentage of the pregnancies result in preterm deliveries (27%) and infants small for gestational age (21%). It is best to keep the mother's haemoglobin level above 10 gram %, although there is controversy about the use of prophylactic transfusions. It seems logical to reserve transfusions for complications, rather than use them routinely. Hydroxyurea is contra-indicated in pregnancy as it is teratogenic. However, in a small number of cases where hydroxyurea was taken throughout pregnancy, no fetal malformations occurred. During labour and delivery the mother should receive oxygen and should be well hydrated.

### *Bone marrow transplantation*

For patients with severe symptoms, especially severe neurological symptoms or complications, an argument could be made for early bone marrow transplantation if a HLA-identical sibling donor were available. The principal complication of allogeneic stem-cell transplantation (the transplantation of grafts from genetically different donors) is graft-versus-host disease (GVHD), which can occur despite aggressive immunosuppressive prophylaxis, even when the donor is a so-called "perfectly matched" (syn. HLA-identical) sibling. The few patients, mostly children, with sickle cell disease who have undergone bone marrow transplantation after a myeloablative conditioning regimen have become asymptomatic despite incomplete replacement of their marrow with donor cells (mixed chimerism).

## Other haemoglobinopathies

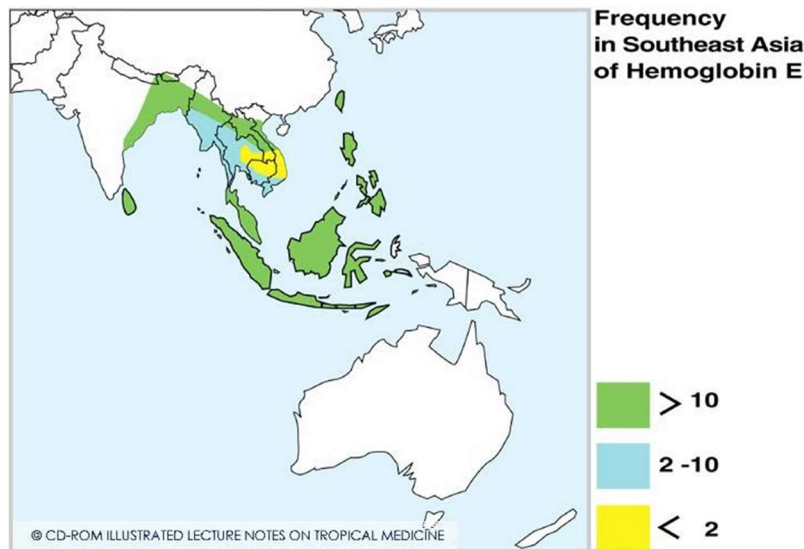
### Haemoglobin C



Map, distribution of haemoglobin C. Copyright ITM

Hb C is another haemoglobin variant that is common in West Africa. Here the 6th amino acid of the beta chain (glutamic acid, negative charge) is not replaced by a valine but by a lysine (positive charge, basic amino acid), i.e.: (beta6 Glu -> Lys) mutation. Sickling does not occur. Haemoglobin C has no protective effect on *P. falciparum* infection. The heterozygote state for Hb C is clinically silent. By electrophoretic analysis, 30-40% of the haemoglobin is Hb C and 50-60% is Hb A. People who are homozygous for Hb C (Hb CC) display mild chronic haemolysis, mild to moderate anaemia and mild splenomegaly. On electrophoresis Hb A is absent. There is often microcytosis and there are many target cells and some spherocytes. Cholelithiasis is common. There are rarely major complications. Treatment is not necessary.

## Haemoglobin E



Map, distribution of haemoglobin E in Southeast Asia. Copyright ITM

Three splice site mutations are known to occur in exon 1 of the beta globin gene. These mutations result in three different abnormal haemoglobins: Malay, E, and Knossos. Haemoglobin E is a very common abnormal haemoglobin in Southeast Asia and India. The mutation GAG to AAG which leads to haemoglobin E, creates an alternate splice site competing with the normal splice site. This results in abnormal haemoglobin production and mild thalassemia in the homozygous state, with a mild microcytic anaemia with a haemoglobin usually above 10 g%. Clinically the affected persons are not ill, although a mild splenomegaly can develop. Electrophoresis reveals approximately 90% Hb E with varying amounts of Hb F.

The heterozygote has a haemoglobin of about 12 g% with microcytosis and an electrophoretic pattern showing Hb E plus Hb A<sub>2</sub> of 20 to 30%. On standard alkaline electrophoresis haemoglobin E comigrates with Hb A<sub>2</sub>.

When Hb E trait combines with a beta<sup>0</sup> thalassemia mutation, a severe transfusion-dependent (EBeta<sup>0</sup>) anaemia will ensue. EBeta<sup>0</sup> thalassemia patients who undergo splenectomy may stop being dependent on transfusions.

## Glucose-6-phosphate dehydrogenase deficiency

### General

In 1926 some people who had been given primaquine (an antimalarial) developed dark urine and haemolytic anaemia. The mechanism was not understood until 30 years later. Adult red blood cells have neither mitochondria nor a nucleus. The cells have no Krebs cycle and meet their energy requirements by glycolysis, an anaerobic process (Embden-Meyerhof chain). This is a very inefficient way of producing ATP, but in this way the erythrocytes do not use the oxygen they transport and the cells are effective carriers of oxygen. By glycolysis a molecule of glucose supplies two ATP molecules and two NADH molecules. By a side-reaction, 2,3 diphosphoglycerate is also produced, a substance that has an important effect on the release of oxygen (see oxygen dissociation curve).

Another metabolic pathway in the cytosol of the red blood cell is the hexose monophosphate shunt (also called the pentose phosphate shunt). The first enzyme in this latter chain is G6PD. The hexose monophosphate chain provides two molecules of NADPH per molecule of glucose. It is the only source of NADPH in the red blood cell.

The normal enzyme is called "type B".

About 20% of Black people in Africa have "type A+". This variant is functionally normal, but has a different electrophoretic pattern. "Type A-" has the same electrophoretic characteristics as "type A+", but has lesser activity. This form is common in Central Africa. People with "type A-" are normally not anaemic. Enzymes with little or moderate activity rarely cause clinically serious problems.

Another important variant is the "Mediterranean type" and is virtually totally inactive. The less active the enzyme, the easier it is for the red blood cell to be damaged by certain chemical substances. Enzymes with very little or no activity are common in people in the Mediterranean basin.

### Clinical aspects

People with a very low G6PD activity can lead a normal life. In some situations, problems can arise. A crisis begins acutely and symptoms worsen in the course of a week. Jaundice, renal pain, haemoglobinuria and mild splenomegaly occur. Newborn children with G6PD deficiency are at greater risk of kernicterus and phototherapy is sometimes necessary. In many people the haemolysis is self-limiting, even if primaquine, for example, is continued to be administered. Circumstances that can trigger symptoms include:

**The neonatal period (neonatal jaundice).** Severe kernicterus due to G6PD-deficiency-related haemolysis is an avoidable cause of mental retardation. It is possible that the icterus is due to haemolysis combined with impairment of the liver function in these neonates.

A short list of drugs and chemicals that should be avoided by persons with G6PD deficiency includes: primaquine, methylene blue, niridazole, nitrofurantoin, sulfacetamide, sulfamethoxazole, sulfanilamide, sulfapyridine, phenylhydrazine, uropyrine. The administration of such medication is followed, after a 1 or 2-day delay, by falling haemoglobin



concentration. Heinz bodies (denatured haemoglobin adherent to the RBC membrane), appear in the early stages of drug administration and disappear as haemolysis progresses.

In sub-Saharan Africa there are few clinically relevant problems, but in the Mediterranean basin severe, even life threatening reactions are more common. Serious infections involving acidosis can cause acute haemolysis. The mechanism by which this occurs is not clear, but leukocytes might damage erythrocytes in their environment by releasing active oxygen species during phagocytosis (cfr production of H<sub>2</sub>O<sub>2</sub> by neutrophils and macrophages).

**Favism.** In the case of severe deficiency, serious haemolysis can occur if fava beans ["*Vicia fava*", favism] are eaten or the pollen of the plants are inhaled. The symptoms occur quite quickly after aerogenic exposure but only develop after 5 to 24 hours after eating fava beans. Divicine and isouramil are oxidants present in this plant and they are normally reduced and inactivated by reduced glutathione. Our knowledge of favism is however incomplete. There is no absolute correlation between G6PD activity and the clinical symptoms. Other factors undoubtedly also play a part. People with "type A-" do not suffer from favism.

Transfusions. Normal red blood cells keep their G6PD activity if they are stored for transfusion. The small amount of activity that G6PD-deficient cells still have, will decrease as time goes by. If this type of blood is transfused into a person who is already ill and who may be receiving potentially haemolysing medication, haemolysis of the transfused blood can occur quickly.

## Diagnosis



G6PD-deficiency. Heinz bodies are visible in the erythrocytes and consist of denatured haemoglobin.

The morphology of the red blood cells is normal between crises. During a crisis inclusions can be detected in red blood cells (Heinz bodies) by means of a supravital dye such as crystal violet. Heinz bodies are formed by denatured, damaged haemoglobin. Cells with these inclusions are quickly removed via the spleen. Detection of Heinz bodies is an insensitive test for G6PD deficiency. G6PD activity can be measured directly in a well-equipped laboratory. The simplest quantitative assay measures the reduction of NADP to NADPH in the presence of glucose-6-P and haemolysate. It is important to know that a test might give misleadingly high results if performed in less than 2 weeks after a haemolytic episode. Older erythrocytes have less enzyme activity and will be eliminated first after a haemolytic crisis. If the test is then performed on the remaining younger red cells (which have a higher enzyme activity), the activity of the enzyme is overestimated. In a blood smear stained with May-Grünwald Giemsa; Heinz bodies cannot be detected, but one can recognise 'bite cells' (keratocytes, blister cells) and dense erythrocytes with irregular outline. In normal people, the activity of G6PD is reduced by half over 120 days (the normal life span of an erythrocyte). It is therefore mainly

the older cell population that is affected. This also explains why most clinical episodes are self-limiting (usually about 25% of the cells are haemolysed). It is precisely because of this limited haemolysis that people with, for example, leprosy, can often continue to take dapsone. Reticulocytosis increases after a few days.

For didactic examples of the more unusual blood smears (including G6PD-pathology), see: <http://content.nejm.org/cgi/content/full/353/5/498>

#### *G6PD-deficiency methaemoglobinemia and methylene blue*

In haemoglobin, iron in haem is present as Fe<sup>2+</sup>. If iron in haem becomes oxidised to Fe<sup>3+</sup> it is called methemoglobin. This cannot carry oxygen. Normally, the unpronounceable enzyme NADH-dependent cytochrome b5 methemoglobin reductase will reduce methemoglobin to haemoglobin. This is a rather slow process. When methemoglobinemia occurs, one would usually administer methylene blue. However, after administration methylene blue first has to be reduced in the body to its active metabolite leukomethylene blue. It is the leukomethylene blue which will convert Fe<sup>3+</sup> in haem into Fe<sup>2+</sup>. The conversion of methylene blue to leukomethylene blue is catalysed by NADPH methemoglobin reductase, a reaction requiring NADPH. Because there is an important NADPH-deficit in G6PD-deficient red blood cells, this conversion will not take place, and treatment with methylene blue will not work. What is more, administration of methylene blue in case of important methemoglobinemia is dangerous in case of G6PD-deficiency, because it will increase haemolysis. Methylene blue is an oxidant which will increase the anaemia and the hypoxemia. If one cannot wait for spontaneous improvement, blood transfusion and oxygen administration are warranted.

#### G6PD deficiency, hereditary transmission

The activity level of the G6PD enzyme is genetically determined. The G6PD gene is located on the X chromosome (a man has XY and a woman has XX). A man with a defective gene (hemizygote) and a woman with 2 defective genes (homozygote) are affected. A woman with just 1 mutant gene (heterozygote) is a carrier, but normally does not display any symptoms. She may well pass the defective gene on to her child. Because in women 1 of the 2 X chromosomes is inactivated in each nucleated cell (Lyons hypothesis), a heterozygous woman has 2 populations of erythroblasts and therefore also 2 populations of red blood cells: a normal population and a deficient population.

Heterozygous women with a high percentage of deficient cells may become symptomatic. Normal women are therefore genetic chimaeras: some cells contain an active paternal X-chromosome and others contain an active maternal X-chromosome. There are no cells in which both chromosomes are active. Note of course that early precursor cells contain DNA but erythrocytes themselves have no nucleus, and therefore contain no chromosomes or even DNA.

#### *Oxidative stress*

Oxidative stress is defined as an imbalance between free-radical production and antioxidant protection. There are many varieties, but important ones include the hydroxyl radical (<sup>•</sup>OH),

hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) and the superoxide radical O<sub>2</sub><sup>•-</sup>, with the ° symbol indicating an unpaired electron. To give a ballpark idea, it is estimated that an average adult human forms 1.7 kilograms of superoxide each year. Each cell in our body produces about 50 hydroxyl radicals each second, one of the most reactive species which exists. It basically reacts instantly with any other molecule, be it fat, protein or DNA which it encounters, thereby damaging it. If there would be no defence against free-radicals, cellular damage would advance at a very fast pace. The cellular defence against free-radicals include antioxidants such as vitamin C and E, glutathione and catalase.

### *Hexose monophosphate shunt*

In order to have enough reduced glutathione, a supply of NADPH is needed. The first reaction in the hexose monophosphate shunt produces NADPH. This reaction is catalyzed by the enzyme G6PD. In very general terms it can be said that the hexose monophosphate shunt (= pentose phosphate chain), has two main functions:

-the production of ribose, a component of nucleotides, for e.g. DNA and ATP. In summary, this pathway transforms glucose-6-phosphate into ribose-5-phosphate. However the erythrocyte has no nucleus nor ribosomes, there is no need for ribose synthesis in these cells.

-the generation of reducing power in the form of NADPH. The pentose phosphate chain reduces NADP<sup>+</sup> to NADPH. By oxidising NADPH to NADP<sup>+</sup> again, other substances are reduced via a redox reaction. NADPH is an important electron donor (= reducing capacity). The main function of NADPH is to reduce oxidised substances such as glutathione and to allow reductive biosyntheses to take place.

The NADPH/NADP ratio controls the rate of reaction in an autoregulatory manner. In a quiescent state, this ratio is very high and G6PD is nearly completely inhibited. When NADPH is oxidized, as when glutathione is reduced in the glutathione reductase reaction, NADPH is converted to NADP<sup>+</sup> and G6PD becomes active, reconverting NADP<sup>+</sup> to NADPH.

### *Red blood cells and NADPH*

Why does G6PD deficiency seem to affect red blood cells especially? Erythrocytes do not have mitochondria therefore red blood cells do not have a back-up system for NADPH production. They have no alternative source of NADPH, as opposed to other cells which have mitochondria. Acetyl-CoA in the mitochondria (entry point for the Krebs cycle) cannot pass through the mitochondrial membrane by itself. If it is bound to citrate it can pass through the membrane. In cells with mitochondria some citrate bound to acetyl-CoA shifts from the mitochondrial matrix to the cytosol, after which the compound is divided again. The citrate therefore acts as a carrier. Citrate is then converted to oxaloacetate and then to malate. Afterwards (malate + NADP<sup>+</sup>) is converted to (pyruvate + CO<sub>2</sub> + NADPH). As a result, even if the hexose monophosphate shunt is functioning poorly, cells with mitochondria can still produce NADPH. The effects of G6PD deficiency are therefore most apparent in the red blood cells (cells without mitochondria).

### *Glutathione*

Why do we need glutathione? Haemoglobin and many other biological molecules contain many sulphur groups (SH groups =sulfhydryl groups). These are necessary for the molecule to function properly. If these are oxidized, haemoglobin can no longer function as it should.

Glutathione is a tripeptide containing cysteine as the second amino acid. This amino acid has a SH group. The reduced glutathione (i.e. with a SH group), converts non-functional, oxidized cysteine disulphide groups (S-S) in other molecules such as haemoglobin into functional SH groups via the enzyme glutathione peroxidase. In this process glutathione itself is oxidized (two glutathione molecules are then bound by a disulphide bridge). Glutathione also reacts with hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) and corrosive organic peroxides. In this way it has an important protective role as an anti-oxidant. If the G6PD enzyme is deficient, no NADPH is formed, neither is any protective reducing glutathione formed and haemoglobin molecules and red blood cell membrane molecules that contain SH groups may be permanently damaged by oxidizing substances. The non-functional, denatured haemoglobin is precipitated in the form of Heinz bodies and the resulting damage to the membrane then leads to haemolysis resulting in moderate, but acute anaemia.

**Note on *P. vivax* eradication:** In countries attempting to eliminate *P. vivax* infection, the existence of G6PD deficiency is driving the development of a simple, user-friendly point-of-care test for its detection. Today, primaquine and tafenoquine are the only drugs capable to eliminate the hypnozoites in *P. vivax* infections. However, both drugs can provoke a severe haemolytic crisis in a person with G6PD deficiency. Therefore, testing for G6PD deficiency is imperative before these drugs can be administered safely.

## Beta thalassemia

### General

In addition to the Mediterranean basin the disease also occurs in Africa, the Middle East, India and Myanmar, Southeast Asia including southern China, Malaysia and Indonesia. There are indications that the high frequency of heterozygous beta thalassemia carriers in the tropics can be explained by a relative protection against the fatal *P. falciparum* malaria (compare with sickle cell trait and G6PD deficiency), but this is controversial.

Embryonal Hb	Fetal Hb	Adult Hb
Hb Gower 1: $\zeta_2\epsilon_2$ Hb Gower 2: $\alpha_2\epsilon_2$ Hb Portland: $\zeta_2\gamma_2$	Hb F: $\alpha_2\gamma_2$	Hb A: $\alpha_2\beta_2$ Hb A <sub>2</sub> : $\alpha_2\delta_2$

An average normal adult has Hb A 97%, Hb A<sub>2</sub> 2%, Hb F 1%.

About 150 different mutations have been reported in people with beta thalassemia. About 20 mutations are responsible for 80% of the beta thalassemsias. Some are simple nucleotide substitutions, with missense or nonsense consequences, multiple substitutions, deletions with frameshifts or abnormalities in the promoter. Sometimes something goes wrong with the splicing of mRNA. Within each geographic population there are unique mutations. Individuals who have beta thalassemia major are usually homozygous for one of the common mutations, or heterozygous for one of the common mutations and one of the geographically-unique mutations. All result in reduced synthesis of beta globin chains (beta<sup>+</sup>-thalassemia) or the absence of synthesis of beta globin chains (beta<sup>0</sup>-thalassaemia). Clinically mild forms of beta thalassemia are called thalassemia intermedia, whereas minor forms are non-symptomatic. If the production of both beta and delta chains is diminished, there is delta-beta-thalassemia (a consequence of gene fusion). The imbalance in globin chain synthesis (there are more alpha chains than beta chains) leads to precipitation of alpha chains in the red cell (= inclusion bodies or  $\alpha$ -hemichromes), which leads to premature destruction of the cell in the bone marrow or the peripheral blood.

### Clinical aspects

#### *Beta thalassemia minor*

The heterozygous condition is known as beta thalassemia minor. One beta gene is defective, the other is normal. Fewer beta globin chains than normal are produced but the healthy gene largely compensates for this. There is a typical microcytosis but rarely anaemia. This form is often found by chance and can wrongly be regarded as an iron deficiency.

There is a diagnostic problem for patients suspected to be double heterozygous for Hb E and betathalassemia. Hb E and Hb A<sub>2</sub> cannot be distinguished in alkaline gel, but diffuse differently in an acid gel.

But Hb A and E cannot be distinguished in acid gel. This occurs mainly in people from Southeast Asian origin.

## Beta thalassemia major



Beta-thalassemia major. Skull bossing due to expansion of the diploë (red bone marrow in skull). See also “hair-on-ends” aspect by Rx



Beta-thalassemia minor with microcytes and target cells

The homozygous or doubly heterozygous condition is much more serious. The severity depends on which mutation(s) causes or cause the disorder and how many beta globin chains can still be produced.

There is therefore a spectrum of clinical severity: thalassemia major or thalassemia intermedia. Patients with thalassemia major are by definition transfusion dependent. The affected infants are normal at first. Newborns still have Hb F, which is not affected in this condition. By the age of 6 to 9 months, the children develop faulty erythropoiesis with anaemia and hypertrophy of the bone marrow, spleen and liver with hepatosplenomegaly. In severe beta thalassemia, erythropoiesis can increase up to 10-fold. The relative excess of alpha globin chains interferes with the normal maturation of the cells in the bone marrow. Ineffective erythropoiesis occurs. There is pronounced haemolysis with considerable splenomegaly. Enlargement of the liver always occurs. Sometimes there are gallstones (bilirubin stones due to the haemolysis). The red bone marrow increases in volume, with swelling of the diploë in the cranial bones, osteopenia and a lowering of the fracture threshold and often microfractures around the main joints. The diploë is the central layer of spongy bone between the two layers of compact bone of the flat cranial bones. The face is often deformed somewhat due to cranial bossing and hypertrophy of the maxillae resulting in a mongoloid appearance. Bone marrow expansion can lead to compression of the spinal cord.

Extramedullary haematopoiesis can occur, not only in spleen and liver, but also in the posterior mediastinum and even kidneys, leading to local masses which can resemble lymphoma.

There is haemolytic anaemia; microcytosis with normoblasts in the peripheral blood and an increase in the minor haemoglobins (Hb F, Hb A<sub>2</sub>). Children can survive only with regular blood transfusions and folic acid supplements. Iron overload and infections due to the repeated transfusions are a very real risk. The abnormal accumulation of iron results in dilated cardiomyopathy, endocrine disorders (destruction of the pituitary gland and hypogonadism with impaired sexual development) diabetes, liver disease (often together with hepatitis B and C). Later, restrictive lung disease and pulmonary hypertension can occur (pulmonary hypertension tends to occur in all chronic severe haemolytic diseases).

### Laboratory

Severe haemolytic anaemia is present, which is accompanied by microcytosis (low MCV), target cells, a high RBC count with a relatively low reticulocyte count considering the severity of anaemia. The high RBC count is a compensation for the low amount of normal Hb in each red blood cell (contrary in iron deficiency where the marrow cannot produce as many RBCs). The Mentzer index is helpful in differentiating iron deficiency anaemia from thalassemia: it is the quotient of the MCV (in fl) divided by the red blood cell count (in millions per  $\mu$ l). If the Mentzer index is less than 13, thalassemia is more likely, if the result is greater than 13, iron-deficiency is more plausible.

On hemoglobinophoresis, a higher level of HbA<sub>2</sub> ( $\alpha_2\delta_2$ ) is usually found in beta thalassemia patients: the excess alpha globin chains bind to the delta globin chains. Diagnostic confirmation by globin gene testing will be rarely available in the tropics.

### Prevention

A child born of two heterozygous parents has a 25% probability of being homozygous. There are screening programmes for detecting carriers in Italy, Sardinia, Cyprus and Greece. These are based on MCV and the concentration of Hb A<sub>2</sub>. Prenatal diagnosis can be carried out with various techniques (e.g. villous chorion sampling carried out in weeks 9-13).

### Therapy

Non-transfused thalassemia intermedia patients are encouraged to avoid high-iron and iron supplemented foods and are encouraged to drink tea with meals, which decreases iron absorption.

Folic acid is usually given. With beta thalassemia major there is a great need for transfusion. Because of the repeated transfusions, iron overload occurs after a number of years (the time varies). Iron chelation is carried out with deferoxamine (Desferal).

Bone marrow transplantation can be carried out as curative therapy and at present is the only definite treatment. Of course, the bone marrow of an identical twin cannot be used but that of a HLA-DR matched relative can be used.

## Alpha thalassemia

Alpha genes can be lost through deletion or inactivated by point mutations. If insufficient alpha chains are produced, the condition is known as alpha thalassemia. This condition is very frequent in Asia (from India to China, including Southeast Asia). The disease also occurs in Africa. Since 4 genes code for alpha chains, there are a number of possibilities:

**All 4 alpha genes functional:** normal. Genetic alpha alpha/alpha alpha

**Only 3 alpha genes functional:** silent carrier with no symptoms or signs (thalassemia minima). Genetic alpha-/alpha alpha

**Only 2 alpha genes functional:** silent carrier, often microcytosis (alpha thalassemia minor or alpha thalassemia trait). Genetic alpha alpha/-- (= alpha<sup>0</sup> thalassemia) or alpha-/alpha- (= alpha<sup>+</sup> thalassemia).

The two genes can either occur on the same chromosome (cis-type) or on each of the pairs (trans-type). Cis-type alpha<sup>0</sup> thalassemia trait tends to be found in individuals of Asian descent, while trans-type alpha<sup>+</sup> tends to run in individuals of African descent. Expert laboratory tests help to distinguish between these two conditions, which is important. If a mother is a carrier of alpha<sup>0</sup> thalassemia, her pregnancy is at risk for Bart's hydrops fetalis syndrome (worst case scenario), while the worst possible outcome of a pregnancy of a mother with alpha<sup>+</sup> thalassemia is a much milder condition, haemoglobin H disease.

**Only 1 alpha gene functional:** excess of beta globin chains. Genetic alpha-/--. The excess beta chains form tetramers and are deposited:  $\beta_4$  (haemoglobin H). Haemoglobin H is not stable and thermally labile. It contains two reactive SH groups per beta chain. The beta chains in Hb A have only one SH group. This may explain the susceptibility of Hb H to oxidation. The red blood cell inclusions (Heinz bodies =  $\beta$ -hemichromes) can be seen readily with brilliant cresyl blue staining (the same dye as for reticulocytes). The patient is anaemic and there is splenomegaly.

**No alpha genes functional:** the excess of gamma chains leads to the depositing of tetramers composed of four gamma chains: gamma<sub>4</sub> (Barts haemoglobin). Without the alpha globin chains, there can be no fetal or adult haemoglobin which means the red blood cells cannot carry oxygen efficiently throughout the body. Hydrops fetalis with stillbirth is the result. There is an increased risk of toxemia of pregnancy and of post-partum haemorrhage (hypertrophy of the placenta). The only haemoglobins found in these infants are: Hb Portland (delta<sub>2</sub>gamma<sub>2</sub>), Hb H ( $\beta_4$ ), and Hb Bart's (gamma<sub>4</sub>), and no Hb A, Hb A<sub>2</sub> or Hb F. Electrophoresis of fetal haemoglobins shows about 80% Barts haemoglobin and about 20% Portland haemoglobin (normally only present in the embryo in the first trimester).

### Onyalai

#### General

Onyalai is a rather mysterious disease, which only seems to occur in central southern Africa (southern Angola and northern Namibia; Kavango and Ovambo territories). Onyalai means "blood blister" in the language of the Kimbundu, an Angolan tribe.



Onyalai is a disease of unknown aetiology. Defective nutrition may be the cause. One hypothesis is that a toxin, possibly acting as a hapten, is responsible for this form of thrombocytopenia. The possible etiological role of mycotoxins from contaminated millet, sorghum and/or maize requires further investigation.

### **Clinical aspects**

The disease differs clinically, epidemiologically and immunologically from immune (previously idiopathic) thrombocytopenic purpura (ITP) It is an acute disease, characterized by the formation of haemorrhagic vesicles and blisters on the palatal and buccal mucous membranes, together with severe thrombocytopenia. This acquired form of thrombocytopenic purpura can lead to haematuria and melena. Epistaxis, petechiae and ecchymoses are common, as are subconjunctival bleeding and menorrhagia. Haemorrhage from ruptured bullae, epistaxis or gastrointestinal bleeding can be severe and may cause shock and even death.

### **Treatment**

Transfusion of blood and of platelets can be lifesaving. High dose intravenous gammaglobulin may be followed by a rise in the platelet count and cessation of haemorrhage but in general this treatment is disappointing (and expensive). Splenectomy can be considered for patients with severe uncontrollable bleeding, although splenectomy does not always control the disease.

# Micronutrient deficiencies

## Introduction

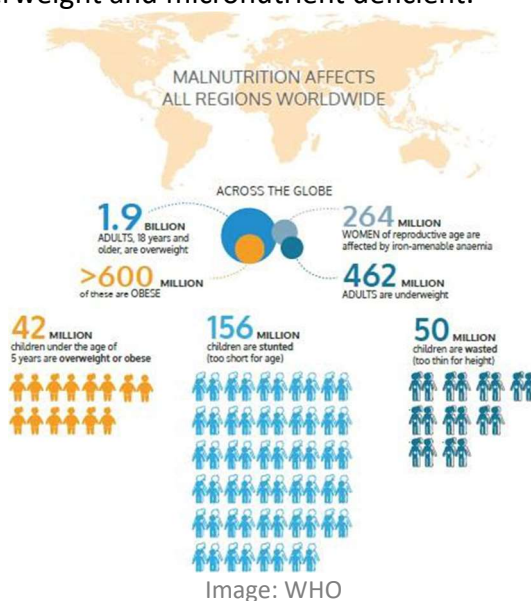
Malnutrition refers to deficiencies, excesses or imbalances in a person’s intake of energy and/or nutrients. Malnutrition covers 2 broad groups of conditions. One is ‘undernutrition’— which includes stunting (low height for age), wasting (low weight for height), underweight (low weight for age) and micronutrient deficiencies or insufficiencies (a lack of important vitamins and minerals). The other is overweight, obesity and diet-related noncommunicable diseases (such as heart disease, stroke, diabetes and cancer).

WHO defines malnutrition as follows: Malnutrition refers to a number of diseases, each with a specific cause related to one or more nutrients (e.g. protein, iodine or iron) and each characterized by cellular imbalance between the supply of nutrients and energy on the one hand, and the body’s demand for them to ensure growth, maintenance, and specific functions, on the other.

## Consequences of malnutrition

Malnutrition affects people in every country. Around 1.9 billion adults worldwide are overweight, while 462 million are underweight. An estimated 41 million children under the age of 5 years are overweight or obese, while some 159 million are stunted and 50 million are wasted. Adding to this burden are the 528 million or 29% of women of reproductive age around the world affected by anaemia, for which approximately half would be amenable to iron supplementation.

Many families cannot afford or access enough nutritious foods like fresh fruit and vegetables, legumes, meat and milk, while foods and drinks high in fat, sugar and salt are cheaper and more readily available, leading to a rapid rise in the number of children and adults who are overweight and obese, in poor as well as rich countries. It is quite common to find undernutrition and overweight within the same community, household or even individual – it is possible to be both overweight and micronutrient deficient.



### *Protein-energy malnutrition*

Undernutrition is sometimes used as a synonym of protein–energy malnutrition (PEM). While other include both micronutrient deficiencies and protein energy malnutrition in its definition. The term "severe malnutrition" or "severe undernutrition" is often used to refer specifically to PEM. PEM is often associated with micronutrient deficiency. Two forms of PEM are kwashiorkor and marasmus, and they commonly coexist.

#### **Kwashiorkor**

Kwashiorkor is mainly caused by inadequate protein intake. The main symptoms are oedema, wasting, liver enlargement, hypoalbuminemia, steatosis, and possibly depigmentation of skin and hair.

Kwashiorkor is further identified by swelling of the belly, which is deceiving of actual nutritional status.

The term means 'displaced child' and is derived from a Ghana language of West Africa, means "the sickness the older one gets when the next baby is born," as this is when the older child is deprived of breast feeding and weaned to a diet composed largely of carbohydrates.

#### **Marasmus**

Marasmus ('to waste away') is caused by an inadequate intake of protein and energy. The main symptoms are severe wasting, leaving little or no oedema, minimal subcutaneous fat, severe muscle wasting, and non-normal serum albumin levels. Marasmus can result from a sustained diet of inadequate energy and protein, and the metabolism adapts to prolong survival. It is traditionally seen in famine, significant food restriction, or more severe cases of anorexia. Conditions are characterized by extreme wasting of the muscles and a gaunt expression.

#### **Undernutrition, hunger**

Undernutrition encompasses stunted growth (stunting), wasting, and deficiencies of essential vitamins and minerals (collectively referred to as micronutrients). The term hunger, which describes a feeling of discomfort from not eating, has been used to describe undernutrition, especially in reference to food insecurity.

### **Micronutrients**

Micronutrients are essential elements required by organisms in small quantities throughout life to orchestrate a range of physiological functions to maintain health. Micronutrient requirements differ between organisms; for example, humans and other animals require numerous vitamins and dietary minerals, whereas plants require specific minerals. For human nutrition, micronutrient requirements are in amounts generally less than 100 milligrams per day, whereas macronutrients (carbohydrate, protein and fat) are required in gram quantities daily.

The minerals for humans and other animals include 13 elements that originate from Earth's soil and are not synthesized by living organisms, such as calcium and iron. Plants are the primary origin of nutrients for humans and animals and some micronutrients may be available in low levels and deficiencies can occur when dietary intake is insufficient, as occurs in **malnutrition**.

Trace minerals	Vitamins	Essential fatty acids	Essential amino acids
Boron	Vitam B complex <ul style="list-style-type: none"> <li>- Vitamin B1 (thiamine)</li> <li>- Vitamin B2 (riboflavin)</li> <li>- Vitamin B3 (niacin)</li> <li>- Vitamin B5 (panthothenic acid)</li> <li>- Vitamin B6 group (pyridoxine, pyridoxal-5-phosphate, pyridoxamine)</li> <li>- Vitamin B7 (biotin)</li> <li>- Vitamin B8 (ergadenylic acid)</li> <li>- Vitamin B9 (folic acid)</li> <li>- Vitamin B12 (cyanocobalamin)</li> <li>- Choline</li> </ul>	Alpha-linolenic acid	Histidine
Cobalt	Vitamin A (retinol, retinal, retinoic acid and provitamin A carotenoids (mainly beta carotene))	Lenolenic acid	Isoleucine
Chlorine	Vitamin C (ascorbid acid)		Leucine
Chromium	Vitamin D (ergocalciferol, cholecalciferol)		Lysine
Copper	Vitamin E (tocopherol)		Methionine
Iodine	Vitamin K (phylloquinone, menaquinone complices)		Phenylalanine
Iron	Carotenoids (alpha carotene, beta carotene, cryptoxanthin, lutein, lycopene, zeaxanthin)		Threonine
Lithium			Tryptothan
Manganese			valine
Molybdenum			
Selenium			
Sodium			
Zinc			

Table: Essential Micronutrients

There are 4 essential nutrients: essential mineral (nutrient)s, vitamins, essential fatty acids, and essential amino acids. An alternative method of classifying nutrients as either type I or type II. This classification is based on the way in which the body responds to a nutrient deficiency. A type I response is characterised by specific physical signs of deficiency as a result of a reduced tissue concentration of the nutrient. For example, if the diet is deficient in a type I nutrient such as iron, there is an initial consumption of body stores followed by clinical signs characteristic of iron deficiency. The concentration of iron in the tissues is markedly reduced, but there is no effect on growth or body weight. In contrast, a type II response is characterised by reduced growth rate or weight loss in the absence of specific deficiency signs. For example, if the diet is deficient in a type II nutrient like zinc, growth stops, followed by weight loss. Protein and energy (derived from carbohydrates and fat) are classified as type II nutrients.

Type I nutrients	Type II nutrients
Iodine	Sodium
Iron	Potassium
Folic Acid	Zinc
Calcium	Magnesium
Selenium	Nitrogen
Copper	Sulphur
Manganese	Phosphorous
All vitamins	Water
	Essential amino acids
	Energy (carbohydrates, fats)

Table: Type I and type II nutrients

The type I and II classification is important because it emphasises that poor growth is not caused solely by protein-energy malnutrition but can result from other nutrient deficiencies which may not be recognised and so appropriately treated. Furthermore, it demonstrates the importance of a wide range of nutrients in causing poor growth or weight loss, and therefore the need for a nutritionally balanced diet.

In much of the developed world, such micronutrient deficiencies are rare; this is due to (1) an adequate supply of food and (2) the addition of vitamins and minerals to common foods (fortification).

Micronutrient deficiencies are widespread in developing countries and affect approximately 2 billion people worldwide which is equivalent to more than one-third of the total world population. The most common deficiencies are due to lack of iron (anaemia), vitamin A (xerophthalmia) and iodine (goitre and cretinism). Outbreaks of deficiency disorders, which are rarely seen in normal circumstances, have also occurred in emergencies among populations entirely dependent on food aid. These include deficiencies of vitamin C (scurvy), niacin (pellagra) and thiamine (beri beri). The general ration provided in emergencies by agencies like WFP and ICRC are frequently lacking in some essential micronutrients, which means that populations always require other foods (or in some cases micronutrient supplements) to complement the rations. Donor agencies can assist populations to maximise their intake of micronutrient-rich foods by adopting a number of different strategies which, in preferred order, include: promoting the production of vegetables and fruit; providing fresh food items in the ration; adding a food to the ration which is rich in a particular vitamin or mineral; providing fortified foods; and supporting the distribution of nutrient supplements.

### *Vitamins*

Vitamins have a special place in the history of medicine. At the end of the 19th century, it was thought that infectious diseases could explain most of the illnesses of mankind. It took a while to show that nutritional deficiencies were responsible for certain ailments, instead of a particular infection. The study of thiamine deficiency earned its author the Nobel Prize (Eijkman 1929). The research connected with vitamin C was likewise awarded this prestigious prize (Haworth and Szent-Gyorgyi, 1937).

A vitamin is an organic molecule (or related set of molecules) which is an essential micronutrient — that is, a substance which an organism needs in small quantities for the proper functioning of its metabolism but cannot synthesize, either at all or in sufficient quantities and therefore must obtain through its diet.

Vitamins can fulfil different biochemical functions. Some function as regulators of cell and tissue growth and differentiation (e.g. vitamin A), other serve as cofactors/coenzymes (B complex). Vitamin D and vitamin E/C serve as hormone-like regulators of mineral metabolism and antioxidants.

The name vitamin refers to “vital amine” (amine of life), even though not all vitamins (in particular vitamin A) have an amine components. As the word was already ubiquitous by the time it was shown that not all vitamins are amines, the final “e” was dropped to deemphasize the “amine” reference.

Humans must consume vitamins periodically but with differing schedules, to avoid deficiency. Body stores for different vitamins vary widely; vitamins A, D, and B12 are stored in significant amounts, mainly in the liver, and an adult's diet may be deficient in vitamins A and D for many months and B12 in some cases for years, before developing a deficiency condition. However vitamin B3 (niacin and niacinamide) is not stored in significant amounts, so stores may last only a couple of weeks. For vitamin C, the first symptoms of scurvy in experimental studies of complete vitamin C deprivation in humans have varied widely, from a month to more than six months, depending on previous dietary history that determined body stores.

A primary vitamin deficiency occurs when an organism does not get enough of the vitamin in its food. A secondary deficiency may be due to an underlying disorder that prevents or limits the absorption or use of the vitamin, due to a "lifestyle factor", such as smoking, excessive alcohol consumption, or the use of medications that interfere with the absorption or use of the vitamin. People who eat a varied diet are unlikely to develop a severe primary vitamin deficiency. In contrast, restrictive diets have the potential to cause prolonged vitamin deficits, which may result in often painful and potentially deadly diseases. Well-known human vitamin deficiencies involve vitamin A deficiency, thiamine (beriberi), niacin (pellagra), vitamin C (scurvy), and vitamin D (rickets). These specific deficiencies will be discussed as well as iodine deficiency disorder. The description of other micronutrient deficiencies is beyond the scope of these lecture notes.

## Vitamin A deficiency

### Summary

- Vitamin A deficiency (VAD) can be caused by insufficient intake through food or by increased need in case of infection
- Leading cause of preventable childhood blindness
- Causes xerophthalmia: dryness of conjunctiva and cornea, Bitot spots, keratomalacia and night blindness
- Is associated with excess mortality
- Treatment with large and repeated doses, lower doses in pregnancy
- Prevention can be achieved with diet change, periodic supplementation and fortification

### Epidemiology

Vitamin A deficiency (VAD) or hypovitaminosis A is a shortage of vitamin A in blood and tissues. It is the leading cause of preventable childhood blindness and is related with child mortality. VAD affects about one-third of children under five worldwide and claims the lives of more than 500,000 children annually, mainly in Southeast Asia and Africa. An estimated 250,000 to 500,000 children go blind each year due to vitamin A deficiency and half of them die within a year of becoming blind. VAD prevalence is high among pregnant women in many developing countries and contributes to maternal mortality. VAD affects the immune system and infectious diseases such as measles have higher fatality rates. Even subclinical deficiency can be a problem as it may increase child's risk of developing respiratory and diarrhoeal infections, decrease growth rate (stunting), slow bone development and decrease likelihood of survival from serious illness. Periodic, high-dose vitamin A supplementation is a proven, low-cost intervention which has been shown to reduce all-cause mortality by 12 to 24 percent. Globally, around 65% of all children aged 6 to 59 months received two doses of vitamin A, fully protecting them against VAD.

However between 2015 and 2016 vitamin A supplementation coverage dropped by more than half in countries with the highest under-five mortality rates, the countries where it is needed most. This caused an increase of children aged 6 to 59 months left unprotected from 19 to 62 million. Two-thirds of at risk countries have no VAD data of use data that are > 10 years old, challenging vitamin A supplementation programs.

### Vitamin A metabolism and pathophysiology

The term vitamin A should be used as the generic descriptor for retinoids exhibiting the qualitative biological activity of retinol. The main molecular structure contains a cyclic part and a non-cyclic chain with 5 double bonds in the all-trans position. A functional group is found at the end of the non-cyclic part which can be an alcohol (retinol), an aldehyde (retinaldehyde), a palmitate (retinopalmitate), etc.

The term *provitamin A carotenoid* should be used as the generic descriptor for all carotenoids exhibiting qualitatively the biological activity of beta-carotene.

Vitamin A is fat soluble and is absorbed in the gut in the chylomicron fraction and then transported via the lymphatics, to the liver. The availability of fats in the intestine will influence the fraction of the available vitamin that will be absorbed. Vitamin A (retinol) is ingested as

either retinyl esters or carotenoids and metabolized to active compounds such as 11-*cis*-retinal, which is important for vision, and all-*trans*-retinoic acid, which is the primary mediator of biological actions of vitamin A. Once stored in the liver as retinylpalmitate it will be transported to the target organs bound to a protein, the retinol binding protein (RBP). Zinc and an adequate intake of proteins are required for normal production of RBP. Transthyretin (TTR= transports thyroxine and retinol) is a transport protein in the serum and cerebrospinal fluid that carries the thyroid hormone thyroxine (T4) and retinol-binding protein bound to retinol. The liver secretes transthyretin into the blood, and the choroid plexus secretes TTR into the cerebrospinal fluid. If retinol is not needed, it is instead stored in liver stellate cells in the form of retinyl esters.

Rhodopsin, the light-sensitive pigment in rods of the eye, is formed when 11-*cis*-retinal combines with the protein opsin. Absorption of light energy causes rhodopsin to decompose by a series of photochemical reactions to all-*trans*-retinal and opsin. As this occurs, a visual signal is transmitted to the central nervous system. Night blindness is an early symptom of vitamin A deficiency. In night blindness, the small amount of light at night does not elicit an adequate response because the amounts of 11-*cis*-retinal and rhodopsin that can be formed are depressed. Another important function of vitamin A is regulation of growth and differentiation of cells. In the absence of vitamin A: 1) proper stem cell differentiation does not occur; 2) growth and development of embryos are altered; 3) epithelial cellular development with ciliary function is deficient, and the barrier to infection is decreased; 4) cells involved in innate and acquired immune function are decreased; 5) xerophthalmia develops because of abnormalities in corneal and conjunctiva development; 6) normal bone growth and tooth development do not occur, contributing to stunting.

#### *Vitamin A in skin creams*

Companies that produce skin creams often juggle with terms as '*Pro-retinol A*',... The creams contain pro-retinols (precursors to retinols) that break down to retinol on exposure to the skin. Vitamin A itself is what does all the work. As well as being the precursor to retinal, it is also a chemical messenger, one function of which is to instruct cells to begin multiplying more uniformly, and to produce more elastin and collagen, two protein building materials essential in healthy, young-looking skin cells.

### Causes of vitamin A deficiency

Both an insufficient input and an increased need can result in the deficiency. Insufficient intake is seen when following food items are lacking the diet:

- Animal sources of vit A: milk - butter - fish oils - liver - meat - egg yolk
- Vegetables: green leafy vegetables – carrots
- Fruits: mango – papaya
- Oils : palm oil

Infections of the gut, malabsorption, worm infestations and particularly giardiasis that provokes steatorrhea decrease vitamin A absorption. Infections can increase vitamin A demands dramatically. Some investigators even calculated the increase during infections in the order of 3000 IU per day. Particularly children with measles are very likely to develop a very fast progressing keratomalacia.



### Recommended daily intake

Adult:	750 µg
Pregnancy:	750 µg
Breastfeeding:	1200 µg
Children:	
< 1 yr:	300 µg
1-4 yr:	250 µg
4-6 yr:	300 µg
7-9 yr:	400 µg
10-12 yr:	575 µg
13-15 yr:	725 µg

Note: 1 IU = 0,3 mcg retinol

There is a very strong association of vitamin A deficiency with malnutrition (PEM). Both are diseases of the poorer people of the population and of the deprived. They will have an overall lower food intake but particularly of meats and milk products, sources rich in vitamin A and of oils and fats, which are necessary for the vitamin A absorption. These children will also have more frequent infections, increasing their demands and interfering with the absorption at the level of the gut. Once their serum protein levels decrease like in severe malnutrition the necessary enzymes for absorption and transportation to the target organs will diminish further aggravating the deficiency.

### Clinical aspects

VAD is an important contributing factor in mortality which is still very high in the majority of the third world countries. This can for a large extend be explained by the role vitamin A has in maintaining the immunological response and the differentiation and maintenance of epithelial surfaces, like the skin, bronchi, gut and genito-urinal tract, which are more prone to invasion by bacteria in a vitamin deficiency state. A higher frequency of diarrhoea, ARTI (acute respiratory tract infection) and otitis media have been noted. These effects are present well before there are overt clinical signs at the level of the eye.

### *Xerophthalmia*

Although xerophthalmia literally means (xeros= dry ; ophthalmos = eye) dryness of the eye and is used as such by the ophthalmologists, it is used in a broader sense in the public health context of vitamin A deficiency. Here it means all lesions, internal and external, attributable to the deficit of vitamin A: dryness of conjunctiva and cornea, Bitot spots, keratomalacia and night blindness. Xeroderma is another expression of xerosis.

The natural course of the disease progresses from night blindness to dryness of the cornea, sometimes with Bitot spots, to keratomalacia, although many children will not pass through this sequence. In a community where children have eye signs, there will be many other children who are vitamin A deficient but who have completely normal eyes and vision. Children with eye signs due to VAD are only the 'tip of the iceberg' explaining why community approaches to control VAD are important. Some eye signs reflect long-standing VAD, whereas

other eye signs reflect severe, acute, sudden-onset VAD. A child who is vitamin A deficient, but who does not have eye signs, may develop immediately corneal ulcers as a result of infections or diarrhoea. Children with any of the eye signs of VAD are at high risk of dying.

Grade of xerophthalmia		Peak age group (years)	Type of deficiency	Risk of death
XN	Night blindness	2–6; adult women	Long standing. Not blinding	+
X1A	Conjunctival xerosis	3–6	Long standing. Not blinding	+
X1B	Bitot’s spot	3–6	Long standing. Not blinding	+
X2	Corneal xerosis	1–4	Acute deficiency. Can be blinding	++
X3A	Corneal ulcer/ <1/3 cornea	1–4	Severe acute deficiency. Blinding	+++
X3B	Corneal ulcer/keratomalacia ≥1/3	1–4	Severe acute deficiency. Blinding	++++
XS	Corneal scarring (from X3)	>2	Consequence of corneal ulceration	+/-
XF	Xerophthalmic fundus	Adults	Long standing. Not blinding. Rare	-

Table: WHO classification of vitamin A deficiency and the age groups most affected

### Night blindness

- Nyctalopia or night blindness is not always perceived because it is a subjective sign; on the one hand and because its perception is very much influenced by the availability of electricity on the other hand. The child has an inability to see in poor lighting conditions like those which prevail at the end of the day when the evening is setting. A longer adaptation of vision to the dark is needed, like when one is getting from a light to a darker environment. Children will usually not complain and mothers should be asked if they stumble over objects in the house in the evening or that their children can't find the parents anymore in the house in the evening. The child might become less active and may be fearful of moving around. Night blindness is quantifiable through a dark adaptation test, but it is difficult to evaluate objectively in children.

#### *Historical note: Xerophthalmia and Vitamin A*

The Eber’s Papyrus describes night blindness in ancient Egypt. Physicians treated the condition by squeezing the “juices” of a grilled lamb’s liver into the eyes of afflicted patients. In 1971, George Wolff speculated that these topically applied “drops,” rich in retinol, probably drained into the lachrymal sac, where they were absorbed into the systemic circulation and thereby reached the retinal cells. Perhaps that was the case, but Alfred Sommer observed the treatment of a young boy in rural Indonesia that was described in exactly the same fashion, but provided a more direct explanation for the way in which “liver juices,” applied topically, could reach the back of the eye. At the conclusion of the ceremony, after juice from a goat liver had been squeezed onto the boy’s eyes, the traditional healer fed the child the remaining liver! The healer did not consider eating the liver part of the treatment; he fed the child the liver so as not to waste precious food.

Modern concepts of xerophthalmia date from the early 1800s, when dogs that were “starved” on sugar and distilled water developed perforating corneal ulcers resembling those in “ill-nourished infants”. One hundred years elapsed before investigators realized

that these changes were caused by lack of a specific nutrient “fat soluble A”, present in the lipid fraction

of milk, eggs, butter and cod-liver oil, and -as provitamin A carotenoids- in dark-green leafy vegetables and certain coloured fruits. Bloc -studying the growth and development of children in a Danish orphanage, noted that vitamin A-deficient children were far more likely to develop urinary tract infections, grew less and were less likely to develop xerophthalmia, and that vitamin A treatment cured the condition. By 1928, Green and Mellanby dubbed vitamin A the “anti-infective factor”.

In 1932 Ellison administered daily vitamin A to one-half of the cases of measles admitted to the Grove fever hospital outside London. Those given vitamin A had only half the case-fatality rate of those restricted to standard therapy. Vitamin A was finally crystallized in 1937.

### **Conjunctival- and corneal xerosis**

VAD causes squamous metaplasia and keratinization in the eye. Conjunctival xerosis can be difficult to detect. One can see a slight wrinkling of the conjunctiva. In corneal xerosis glands in the conjunctiva no longer function normally, leading to loss of tears and mucous with an increased risk for infections. The light reflex of the cornea loses its well-defined appearance and becomes mottled and hazy. The cornea becomes dry, less translucent and more opaque.

**Bitot spots** are painless, triangular, whitish, pearly coloured spots, usually found on the lateral side of the conjunctiva, which are pathognomonic for VAD. They consist of keratin accumulations, often intermixed with an overgrowth of *Corynebacterium xerosis*, which result from epithelial (squamous) metaplasia: the conjunctival cells become more like skin than a mucous membrane. The white foamy deposits can be wiped away partially, but they don't disappear completely, even when the deficiency is reversed.

### **Corneal ulcer and keratomalacia**

If the acute VAD is not treated promptly, the cornea can become ulcerated and melt away. The liquefaction necrosis of the cornea varies from small ulcerations to softening and rupture of the cornea, with resulting loss of anterior chamber fluid and collapse of the eye. Keratomalacia indicates that more than one-third of the cornea is affected. In just a few days the cornea can be completely destroyed and secondary infection is common. As long as there is no superinfection, there is no pain or redness. The end result is corneal scarring, staphylomas (bulging of a badly damaged cornea) or phthisis bulbi (a shrivelled up eye). Children with keratomalacia are often malnourished, but previously healthy appearing children can develop keratomalacia following measles infection or diarrhoea. It is important to screen young children from the same family and community.

## **Diagnosis**

### *Clinical*

In low resource settings the diagnosis of individual patients is usually made clinically. Fundus examination can be useful to detect xerophthalmic fundus, which is more present in adults. Small white spots are found on the retina. This moderate form of VAD (night blindness,

conjunctival dryness) will disappear after 2-4 days of treatment without leaving any lesions or sequelae.

### *Plasma levels and Hepatic reserves*

The problem with measuring plasma retinol levels is that they only change after a prolonged period of vitamin deficit, due to the buffering action of the liver. Their use is limited to research evaluations of vitamin A deficiency and of very little practical use in real life situations. Hepatic reserves can be determined with a liver biopsy, which is only done on an experimental and research basis. The reserves can be estimated: after administration of a small dose of retinol (1.800 IU) the plasma retinol levels are measured again and compared with the retinol concentrations before the administration. If the concentration increases by more than 20 % then this indicates reserves are low.

#### **Plasma retinol**

>= 30 mcg/100 ml	Normal
30-20 mcg/100 ml	Mild deficiency
20- 10 mcg/100 ml	Associated with night blindness, Bitot spots Moderate deficiency
< 10 mcg/100 ml	Severe deficiency

### *Impression cytology*

Impression cytology is a technique to detect the degree of metaplasia of the conjunctiva. The lack of differentiation and the decrease or absence of goblet cells is looked for. It is not a routine diagnostic test.

### *Vital staining*

Vital staining detects the degree of conjunctival metaplasia by putting dye (Lissamon green or Bengal rose) on the conjunctiva. This method lacks specificity.

## **Treatment**

The presence of clinical signs of vitamin a deficiency should be considered an emergency. The most urgent are those infants with corneal signs. Large and repeated doses are therefore given. Associated illnesses should always be treated.

In an endemic zone, all children with PEM and measles need vitamin A treatment.

#### **Treatment dosage**

<b>Children &lt; 1 yr</b>	<b>Children &gt; 1 yr and adults except pregnant women</b>
100.000 IU immediately	200.000 IU immediately
100.000 IU after 24 hrs	200.000 IU after 24 hrs

100.000 IU after 14 days	200.000 IU after 14 days
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Below one year or below 8 kg the dose is half of the dose delivered in the vitamin A high dosage capsules. These contain 6 drops; to administer throw away three drops and give the remainder.

Although teratogenic in animals, a clear correlation between ingestion of large doses of vitamin A and congenital malformations has not been established. As a precautionary measure, pregnant women should not receive large doses of vitamin A due to the possible teratogenic effect. Smaller doses up to 10.000 IU per day are safe. A total dose of 200.000 IU should be aimed at. Lactating women should receive 200.000 IU in the first month postpartum. One month after delivery again smaller doses up to 10.000 IU per day are preferred. This because one month after delivery there is the possibility of recurrent pregnancy.

Xerophthalmia is treated with topical antibiotics and padding of the eye. Topical steroids should be used with caution. Corneal grafting and conjunctival reconstruction using a flap are out of scope for most settings where VAD is prevalent.

## Prevention

"Appropriateness" is a basic premise for vitamin A intervention. Two conditions dictate whether a program, designed to prevent vitamin A deficiency, is appropriate:

1. A substantial segment of the population is "at risk" of developing clinical or biochemical vitamin A deficiency of sufficient severity to be considered of Public Health importance.
2. The problem is serious enough to warrant the diversion of scarce resources toward a program to control vitamin A deficiency versus other preventable diseases or community projects within the country.

Currently vitamin A prophylaxis is approached through one of the three major intervention strategies:

1. A change in diet directed toward achieving a continuous intake of vitamin A rich foods.
2. Administration of a single, large dose of vitamin A administered on a periodic basis.
3. Fortification of an appropriate dietary vehicle with vitamin A.

## Change in dietary intake

Different strategies have been applied to increase dietary intake. Promotion of breastfeeding is effective in entirely breastfed children, provided the mother has adequate daily intakes of vitamin A.

They have a lower prevalence of mild and severe xerophthalmia during early childhood. Nutritional education, kitchen gardening programs, larger scale agricultural programs and income generating programs are possibilities to achieve a higher vitamin A intake. Diet adaptation is the most sustainable solution and avoids the risk of hypervitaminosis. This

approach de-medicalizes a food related condition. Challenges can be the availability of vitamin A rich products. These products must also be culturally accepted and land suitable.

#### *Distribution of large dose vitamin A capsules*

Large doses of 200.000 IU are distributed at regular intervals, most frequently every six months. The seasonal distribution approach is used to protect children in the higher prevalence seasons, reduces cost while maintaining the same impact. There are three possible delivery strategies: the 'medical' or 'therapeutic' approach, which offers treatment to children who present to a health facility with an illness episode. They will be given a dose of vitamin A according to a set of pre-set criteria of high risk of developing vitamin A deficiency. The 'targeted' distribution covers groups within the larger general target population; e.g., residents of a high prevalence neighbourhood, those attending mother and child health clinics, etc. The 'universal' distribution in which all pre-school children and not pregnant lactating mothers in a prescribed region are dosed at prescribed intervals by single or multipurpose workers in the community.

#### *Vitamin A fortification*

Fortification of Mono-sodium Glutamate in the Philippines and of sugar in Guatemala has been highly successful. Other possible vehicles are wheat and milk. Vitamin A is light- and heat sensitive so it must be protected from light and stored in a cooler environment. The success of this type of program depends on the identification of a suitable vehicle, which has to be consumed by all and particularly the population at risk, and in a continuous and constant fashion. Fluctuations between people and in time should be as small as possible. The cost of the program on a national scale is usually high enough to raise the question as to who is going to bear it; the government, the industry or the consumer. Disagreement over this last point has led to the discontinuation of some fortification programs.

#### *Vitamin A toxicity*

##### **Acute hypervitaminosis**

Ingestion of large dose can give rise to transient signs and symptoms of toxicity, which are self-limiting and completely reversible. No deaths have been reported after the ingestion of the doses used in treatment and prevention. Intracranial pressure rises giving rise causing headaches and a bulging fontanel in young children. Nausea, vomiting, dizziness, headaches have been described in adults. Desquamation of the skin, bone pains and hair loss can occur in the following days.

##### **Chronic hypervitaminosis**

Ingestion of large doses on a regular basis can lead to hepatitis, cirrhosis, hair loss, dry scaling skin, hyperpigmentation, hyperostosis and bone pains, hepato-splenomegaly and anaemia. It is therefore recommended not to exceed a daily intake of 3000 µg (10.000 IU) in children and 7500 µg (20.000 IU) in adults. Why does the liver get damaged in chronic hypervitaminosis

A? The liver gets a double blood supply: arterial via the arteria hepatica and venous via the portal vein. The blood vessels branch until they form a capillary-like network, the so-called liver sinusoids. These vessels are rather different from ordinary capillaries and containing large fenestrations. They do not rest upon a basal membrane but are surrounded by

reticuline fibers. The sinusoids contain, apart from vascular endothelial cells, also Kupffer cells, monocyte-derived phagocytes. Outside the sinusoids is the space of Disse (German anatomist, Joseph Disse; 1852–1912). This space contains the microvilli of hepatocytes as well as Ito cells (syn. stellate cells; Japanese physician Toshio Ito: 1904–1991). Ito cells store fat and fat soluble vitamins, like vitamin A. Excessive intake of vitamin A leads to pathologically enlarged Ito cells. When damaged, Ito cells can change into an activated state. These are responsible for secreting collagen scar tissue. This leads to fibrosis, cirrhosis and portal hypertension.

## Rickets

### Summary

- Vitamin D in food: sequentially converted in the skin (sunlight), liver and kidneys
- Calcitriol needed for mineralization of osteoid and calcium uptake in the intestine
- Deficiency in children (epiphyseal plate still open) leads to rickets
- Deficiency in adults (epiphyseal plate closed) leads to osteomalacia
- Irregularly frayed, wide, cup-shaped distal ulna and radius, rachitic rosary, hypocalcaemia
- Pseudofractures, bone deformities, gait disturbance
- Rapid recovery after deficiency correction, except if end-organ resistance
- Do not confuse rickets with rickettsiosis

### *Rickets: Historical Note*

For centuries, rickets - despite being common - was a mysterious disease. In 1650, Francis Glisson, a Cambridge physician published in Latin a treatise on rickets titled "De Rachitide." Glisson's treatise addresses the clinical features of rickets in a scientific tone, but lapses into medieval mysticism while discussing the aetiology of rickets. Glisson ascribed the aetiology of rickets to "cold distemper, that is moist and consisting of penury or paucity of and stupefaction of sprits." Despite his affirmation of mysticism in the cause of rickets, Glisson was convinced that rickets was neither contagious nor heritable.

Glisson's suggested treatments for rickets included: cauterization, incisions to draw out bad humours, blistering and ligature of soft wool around the limb to retard the return of blood. For correction of bony deformities, Glisson proposed splinting and artificial suspension of the affected infant: "The artificial suspension of the body is performed by the help of an instrument cunningly made with swathing bands, first crossing the breast and coming under the armpits, then about the head and under the chin, then receiving the hands by two handles, so that it is a pleasure to see the child hanging pendulous in the air, and moved to and from by the spectators. This kind of exercise is thought to be many ways conducive in this affect, for it helped to restore the crooked bones, to erect the bended joints, and to lengthen the short stature of the body."

After Glisson's discoveries, no advances were made in the study of rickets for 2 centuries. At the turn of the 20th century, rickets was rampant among the underprivileged infants residing in industrialized cities of North in the United States and several polluted cities in Europe. In 1919, Edward Mellanby, an English physician, conducted the earliest definitive experimental study exploring the role of diet in the aetiology and treatment of rickets. Puppies between 5 and 8 weeks of age were exposed to 1 of 4 natural diets. All 4 diets were rachitogenic after a variable period of exposure. Rickets was severe and developed easily in dogs that grew well on the rachitic diets. Neither yeast (antineuritic vitamin) nor orange juice (antiscorbutic vitamin) hindered the development of rickets. Various foods were added to the rachitic diets and their effect on development of rickets was studied. Foods rich in fat-soluble vitamin A (cod-liver oil, butter, and whole milk) were able to prevent rickets. Mellanby postulated, "It therefore seems probable that the cause of rickets is a diminished intake of an antirachitic factor which is either fat-soluble A, or has a somewhat similar distribution to fat-soluble A. Mellanby's work clearly established the role of diet in the cause of rickets."



McCollum was now confronted with same question faced by Mellanby, whether fat-soluble A was anti-rachitic by itself or if there was another substance with specific anti-rachitic function with similar distribution as fat-soluble A. McCollum and Mellanby were aware of F. G. Hopkins' report that oxidation destroyed fat-soluble A. Mellanby found oxidized butter fat had lost its anti-rachitic effect, but similarly treated cod-liver oil still retained its protective action against the development of rickets. Mellanby stated "this difference can be explained by the fact that cod-liver oil contains greater quantity of antirachitic vitamin than butter, or that the destructive change takes longer time, or whether some other explanation must be sought.

McCollum and his coworkers were soon able to explain the preservation of anti-rachitic function in oxidized cod-liver oil. Unlike Mellanby, they chose to explore the anti-xerophthalmic and anti-rachitic functions of oxidized butter fat and oxidized cod-liver oil. They chose "diet 3143," which was adequately restricted with regard to fat-soluble A to cause severe rickets but still able to prevent the onset of xerophthalmia, to induce rickets in rats. Using the "line test," the anti-rachitic potency of several fish liver oils, vegetable oils, and butter fat were tested. Oxidized cod-liver oil had lost its anti-xerophthalmic function, but still retained its calcium-depositing properties. Untreated coconut oil had no anti-xerophthalmic property, but had minimal anti-rachitic function. McCollum and his coworkers concluded that the anti-rachitic substance found in certain fats was distinct from fat-soluble vitamin A and its "specific property was to regulate the metabolism of the bones." In the sequence of discovery of vitamins, the newly discovered antirachitic substance was the fourth; hence it was called vitamin D.

In 1890, addressing the aetiology of rickets, Palm studied the relationship between incidence of rickets and its geographical distribution and concluded that rickets was caused by lack of exposure to sunlight. Palm was able to point out that, despite a superior diet and relatively better sanitary condition, infants residing in Britain were more at risk for rickets than infants living in the tropics. Exposure to plenty of sunshine, which was the norm for infants residing in the tropics, was responsible for their protection against rickets. Palm recommended "systematic use of sun-baths as a preventive and therapeutic measure in rickets."

The bridging of the knowledge that photosynthesized vitamin D and vitamin D in cod-liver oil were similar was responsible for the eventual conquest of rickets. By the 1930s, the use of cod-liver oil in the treatment and prevention of rickets became common place. The eventual public health prevention initiative of fortification of milk with vitamin D led to eradication of rickets.

## Vitamin D metabolism and calcium homeostasis

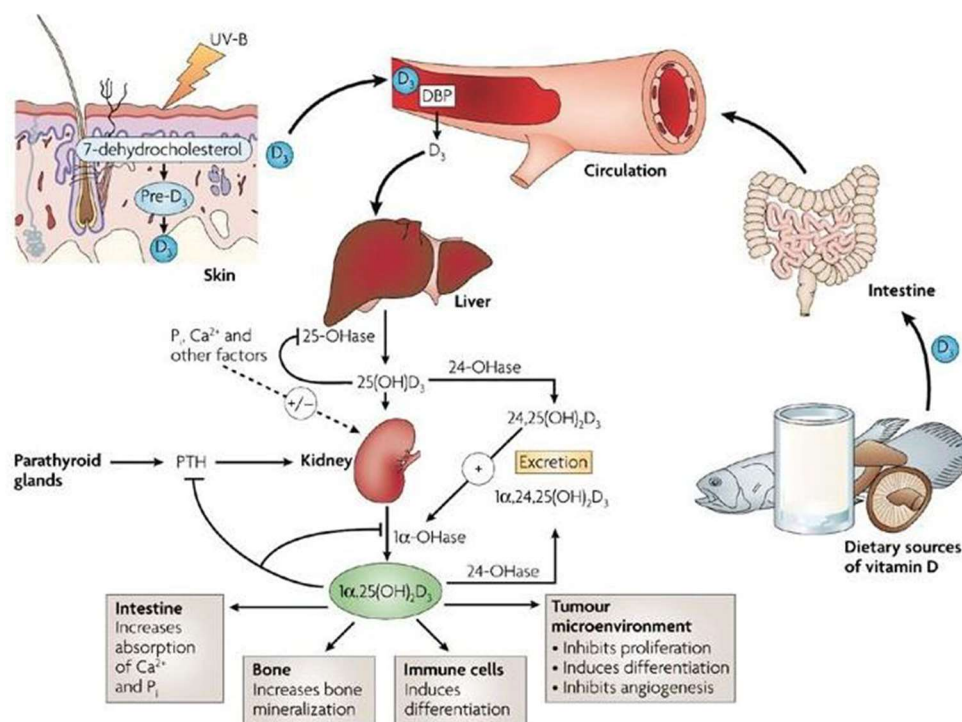


Figure: Vitamin D Metabolism (source: Nature Reviews Cancers 7, 684-700\_2007)

Vitamin D is present in food as a fat-soluble provitamin. Vitamin D is regarded as a sterol, although the B ring of the molecular steroid skeleton is open. A photochemical conversion and two hydroxylations take place in the body before the final form is reached. The absorption of vitamin D is determined by the fat content of the food; by the proper functioning of the pancreas (lipase) and by the presence of sufficient bile. After absorption in the intestine, the provitamin is first transported to the skin, where a photochemical conversion takes place via ultraviolet light. Vitamin D<sub>3</sub> (= cholecalciferol = calciol) is formed. This compound can also be produced via UVB radiation from an endogenous precursor, 7dehydrocholesterol or pre-vitamin D<sub>3</sub>. Sunlight breaks the B ring of the cholesterol structure to form pre-D<sub>3</sub>. Pre-D<sub>3</sub> then undergoes a thermal induced rearrangement to form D<sub>3</sub>. Continued irradiation of pre-D<sub>3</sub> leads to the reversible formation of lumisterol and tachysterol (isomers of pre-vitamin D<sub>3</sub>) which can revert back to pre-D<sub>3</sub> in the dark. Vitamin D<sub>3</sub> is subsequently bound to a carrier protein and transported to the liver, where an initial hydroxylation takes place with the formation of 25-OH-D<sub>3</sub>. In the kidneys, 25-OH-D<sub>3</sub> (calciol) is further hydroxylated to the metabolically much more active form 1,25-(OH)<sub>2</sub>-D<sub>3</sub> (calcitriol). A similar hydroxylation takes place in the placenta. Extra-renal synthesis of 1,25-(OH)<sub>2</sub>-D<sub>3</sub> may occur in pathological conditions, such as sarcoidosis and other granulomatous disorders.

It is important to maintain calcium concentrations at a constant level to preserve a normal neurological function, muscular contractility and bone mass. In the extracellular compartment calcium is always in equilibrium with phosphate. Their product has to remain constant, otherwise the calcium-phosphate complexes will precipitate. If calcium increases, phosphate will decrease. The biggest reservoir of those two minerals is the bone. When the calcium concentration drops, the parathyroid glands secrete the parathyroid hormone PTH. This stimulates the production of 1,25(OH)<sub>2</sub>D. The receptor of 1,25(OH)<sub>2</sub>-D<sub>3</sub> is located in the

cytoplasm of the cell. After binding, the complex migrates to the cell nucleus where (as a transcription factor) it mediates the expression of various genes. As a result of this (1) the active absorption of calcium in the intestine is stimulated, (2) the loss of calcium through the kidneys is decreased (resorption is stimulated) with increase in phosphate excretion and (3) bone cells (osteoclasts) are stimulated to resorb bone minerals and release calcium in the extra-cellular compartment. As a result of this the calcium concentration will rise and the secretion of PTH decrease.

### Rickets, causes

Rickets and osteomalacia develop when there is insufficient vitamin D, when its metabolism is disturbed or when the tissues are resistant to its activity (e.g. mutation of the vitamin D receptor). By following the metabolic chain that leads to the active  $1,25\text{-(OH)}_2\text{-D}_3$  the various causes of osteomalacia/rickets can be visualized. For instance, the food may contain too few precursors. If there is insufficient fat in the diet, or there is insufficient bile and the fat is not absorbed (steatorrhoea), a deficiency of fat-soluble vitamins (ADEK) will occur. Prolonged treatment with cholestyramine is a risk factor. Insufficient exposure to sunlight is also an aetiological possibility. Dark-skinned people residing for a long time in the northern hemisphere are a high-risk group. This also applies to those who wear protective clothing and people who spend most of their time indoors (elderly people and Islamic women and children are high-risk groups). For instance, rickets/osteomalacia is not uncommon in Indian and Pakistani immigrants in Britain. A lack of direct sunlight and calcium (chelation of calcium by the phytates in their traditional diet and low intake of milk) contributes to the problem. There are several diseases that may be associated with vitamin D deficiency, such as chronic renal failure (lack of  $1\text{-}25\text{-(OH)}_2\text{-D}_3$  and hyperparathyroidism), hypoparathyroidism, genetic diseases such as hereditary hypophosphataemia, or vitamin D-resistant rickets.

Clinical nutrition and bone disease	
<b>Vitamin D</b>	Rickets, osteomalacia
<b>Vitamin C</b>	Scurvy
<b>Copper</b>	Fractures (in premature infants with parenteral nutrition)
<b>Calcium</b>	Osteoporosis

Vitamin D content of food ( $\mu\text{g}/100\text{ g}$ )	
<b>Cereals</b>	
Grain, flours, starches	0
<b>Milk &amp; milk products</b>	
Cow's milk	0.01-0.03
Human milk	0.04
Dried milk	0.21
Cream	0.1-0.28
Cheese	0.03-0.5
Yoghurt	Trace-0.04
<b>Eggs</b>	
Whole	1.75
Yolk	4.94
<b>Fats and oils</b>	
Butter	0.76
Cod liver oil	210
Margarines and spreads*	5.8-8.00
<b>Meat &amp; meat products</b>	
Beef, lamb, pork, veal	Trace
Poultry, game	Trace
Liver	0.2-1.1
<b>Fish and fish products</b>	
White fish	Trace
Fatty fish	Trace-25
Crustacea & molluscs	Trace
<b>Vegetables</b>	
	0
* Added during production (Vitamin D <sub>2</sub> ).	
Source : Holland et al 1991	

Table: Vitamin D content in foods

## Pathophysiology

Osteomalacia refers to a disorder in which there is abnormal bone mineralization and the ratio of mineral to matrix is diminished due to an excess of unmineralized osteoid. This in contrast to osteoporosis where there is a reduction in quantity of bone mass per unit of volume. Osteomalacia in children is known as rickets, and because of this, use of the term "osteomalacia" is often restricted to the milder, adult form of the disease.

Crystallization of minerals in osteoid requires adequate concentrations of ionized calcium and phosphate. Vitamin D influences these levels after its dihydroxylation into calcitriol (hepatic position 25 and renal position 1). When concentrations are too low crystallization does not proceed normally.

Vitamin D disrupts mineralization because it normally regulates and enhances the absorption of calcium in the intestine. A lack of vitamin D causes plasma calcium concentrations to fall. Low plasma calcium levels stimulate parathyroid hormone (PTH). PTH raises calcium concentration but also increases renal clearance of phosphate. When phosphate decreases below a critical level, mineralization cannot proceed normally. On top of this, hypophosphatemia causes a disturbed apoptosis of chondrocytes, leading to an excess of unmineralized osteoid.

Rickets in the strict sense of the term is a disease caused by any interference with the process of enchondral bone formation (calcium phosphate deposition in cartilaginous bone), the cascade of events normally taking place in the epiphyseal growth plates and resulting in gain in length of long bones. In children, the abnormalities are clearest in the areas of most active growth, i.e. the epiphyses. In chronic deficiency there is resorption of trabecular and cortical bone, which is not compensated by mineralization of osteoid. Adequate treatment with vitamin D causes a rapid reversal of this situation.

Normal enchondral bone formation is resumed. In adults, the changes are similar but are not limited to the extremities of the long bones. As a consequence, the skeleton will be affected in its two main functions as the mechanical support for the other organs and the major reservoir of calcium to serve a large array of physiologic functions.

### Clinical aspects

The clinical picture is one of bone deformities ranging from mild signs to very distinctive bone deformities. Clinical and radiological bone lesions predominate in the areas of rapid bone growth, namely the long bone epiphyses and the costochondral junctions. Thus the clinical manifestations are most striking at the time of greatest velocity. The maximum frequency of signs is usually found between 4-12 months with most of the signs seen in children below 18 months. Bone changes, visible on X-rays, precede clinical signs, becoming evident in the 3rd or 4th month of life (more common 6-9 months) sometimes even at birth if the mother is severely vitamin D deficient. Bone changes in rickets are most evident at the distal ends of the radius and ulna. The bony ends lose their sharp, clear outline. They are cup-shaped and show a spotty or frayed outline. Later, the distance between the ends of the radius and ulna and the metacarpal bones appears to be increased because the noncalcified ends are invisible on the X-ray. This increase in the width of the epiphyseal cartilages can also be seen at the distal extremities of the tibia and fibula ("Erlenmeyer deformity"). As healing begins, a thin white line of calcification appears at the epiphysis, becoming denser and thicker as calcification proceeds.

Kyphoscoliosis may develop and walking is delayed. Older children and adolescents experience walking as painful and in extreme cases develop bowlegs or knock-knees.

Maternal osteomalacia leads to changes in the bones of the foetus and even to tetany or seizures in the newborn (hypocalcaemia). Young infants with vitamin D deficiency are restless and sleep poorly. They have reduced mineralization of the skull (craniotabes = "wasting of the skull") and frontal bossing can be seen. On the thorax, palpable lumps develop at the costochondral junctions: costochondral beading (rachitic rosary). Harrison's groove, corresponding to the costal insertion of the diaphragm, may be present.

In adults, osteomalacia occurs particularly in the vertebrae, pelvis and legs. Fine lines appear in the cortex: ribbon-like areas of demineralization, the so-called pseudofractures or Looser's lines.

Histologically they consist of focal accumulations of non-calcified osteoid. Preferential localizations for pseudofractures are the lateral edge of the scapula, femur neck, medial femoral shaft, ribs and ramus pubis. Looser's lines are usually symmetrical, extending

perpendicularly to the cortex; are manifestly shorter than the diameter of the bone and display no callus formation. As the bones soften, body weight may cause bowing of the long bones, vertical shortening of the vertebrae and flattening of the pelvic bones, which narrows the pelvic outlet. This may subsequently cause difficulties in childbirth.

#### Rickets: clinical signs in babies

1. Aspecific restlessness and irritability
2. Head sweating
3. Skeletal signs (ricketsial thoracic rosary at 6-9 months of age). Disturbed bone maturation with wide epiphyseal plates and fraying of metaphysis. Frontal bossing and soft osseous borders of cranial vault (craniotabes) with or without widened fontanelles.
4. Delayed teething, enamel hypoplasia and numerous caries
5. Hypotonia: muscle flabby or muscle cramps (eventual seizures, tetany, laryngeal spasms)
6. Higher risk of upper respiratory tract infections due to muscle weakness and thoracic cage deformities
7. Anaemia (von Jaksch-Luzet syndrome) due to marrow space fibrosis. If severe, extramedullary production of red cells in liver and spleen can lead to hepatosplenomegaly

Human breast milk contains very little vitamin D (approx 25 IU per litre). Prolonged breast feeding by mothers who don't take extra vitamin D, followed by sudden switch to milk formula (containing lots of phosphate) can precipitate overt hungry bone syndrome, sometimes presenting with signs of acute or subacute hypocalcemia (e.g. convulsions).

### Diagnosis

In the blood there is approximately 500 times more 25-OH-D<sub>3</sub> present than 1,25-(OH)<sub>2</sub>-D<sub>3</sub> and the half-life of 25-OH-D<sub>3</sub> is 15-45 days, constituting a factual reservoir of the vitamin. As a consequence, serum level of 25(OH)D is the laboratory test ordered to indicate whether or not a person has vitamin D deficiency or insufficiency.

The half-life of 1,25-dihydroxyvitamin D is short (4 to 6 hours). The levels of this compound can remain normal (or even raised) even when a person may be vitamin D deficient, depending on the activity of the 1-alpha-hydroxylase that converts 25-hydrovitamin D to 1,25-dihydroxyvitamin D, which in turn depends on the current blood concentration of calcium, phosphate and parathyroid hormone.

Measuring the active form of vitamin D (1,25-dihydroxyvitamin D) lacks utility in the routine evaluation of suspected vitamin D deficiency.

In healthy people, normal levels are 25 to 40 ng/mL (62 to 100 nmol/L) for 25-OH-D<sub>3</sub> and 20 to 45 pg/mL (48 to 108 pmol/L) for 1,25-(OH)<sub>2</sub>-D<sub>3</sub>. In nutritional rickets and osteomalacia, 25-OH-D<sub>3</sub> levels are very low.

Hypophosphatemia and high serum alkaline phosphatase are characteristic. Calcium is low or normal, depending upon the effectiveness of parathormone (secondary hyperparathyroidism) in restoring serum calcium to normal.

It is also considered reasonable to treat at-risk persons with vitamin D supplementation without checking the level of 25(OH)D in the serum, as vitamin D toxicity is very rare.

### Differential diagnosis

A review of the patient's history may suggest nutritional problems. Rickets must be distinguished from infantile scurvy (cfr. scorbutic rosary), congenital syphilis (serologic tests) and from chondrodystrophy (large head, short extremities, thick bones; normal calcium, phosphate and alkaline phosphatase levels). Frontal bossing can be a sign of congenital lues, hemolytic anemia (thalassemia's, sickle cell disease), Hurler syndrome, achondroplasia). Yaws (= Pian = Framboesia) can give rise to sabre tibia.

Osteogenesis imperfecta, cretinism, congenital dislocation of the hip, hydrocephalus and poliomyelitis should be readily distinguishable. Tetany must be distinguished from convulsions due to other causes.

Vitamin D-resistant rickets may be caused by severe renal damage, as in chronic renal tubular acidosis (e.g. Fanconi's syndrome or X-linked hypophosphataemia). Osteomalacia must be distinguished from other causes of bone decalcification, such as hyperparathyroidism, senile or postmenopausal osteoporosis; osteoporosis of hyperthyroidism, steroid use, Cushing's syndrome and atrophy of disuse.

### Treatment

The World Health Organization defined an "International Unit" of vitamin D<sub>3</sub> as 0.025 micrograms (or one microgram = 40 IU).

Treatment usually consists of vitamin D<sub>2</sub>(ergocalciferol) or vitamin D<sub>3</sub> (cholecalciferol), in addition to dietary advice and sunlight exposure. While there is evidence that vitamin D<sub>3</sub> raises 25(OH)D blood levels more effectively than vitamin D<sub>2</sub>, other evidence indicates that D<sub>2</sub> and D<sub>3</sub> are equal for maintaining 25(OH)D status. Treating vitamin D deficiency depends on the severity of the deficit. An initial high-dosage treatment phase until the required serum levels are reached, is followed by the maintenance of the acquired levels. The lower the 25(OH)D serum concentration is before treatment, the higher is the dosage that is needed in order to quickly reach an acceptable serum level. The initial high-dosage treatment can be given on a daily (1000 IU for newborns, 1000 to 5000 IU for 1-12 months old infants and 5000 IU for patients older than 1 year) or weekly basis or can be given in form of one or several single doses orally or intramuscular (200,000 IUI), especially when there are concerns about compliance. Maintenance supplementation of 400 IU per day is recommended, with double

doses for premature infants, dark-skinned infants and children residing in areas of limited sun exposure.

It is important to make sure that the children are receiving enough calcium. A daily intake of 800 mg in infants and children, and 1 g in adults, is the required minimum during the first month of treatment. Milk and dairy products can easily supply this, but when this does not seem possible, calcium supplementation must be provided.

The first radiological signs of healing will appear after 2-4 months.

### Vitamin D intoxication

When accidental or intentional high doses of vitamin D are taken, the clinical picture is dominated by hypercalcaemia. The rate at which the symptoms develop depends upon the dose and duration of excess vitamin D intake. The first symptoms are anorexia, nausea, vomiting, polyuria, polydipsia and pruritus. Polyuria is secondary to a massive increase of urinary calcium excretion. Complications consist of metastatic calcifications (nephrocalcinosis!) and renal failure.

Patients sometimes complain of eye irritation. Physical examination may reveal a bandlike grey-white opacity across the corneal surface: band keratopathy. Treatment consists of stopping further administration of vitamin D and giving corticosteroids. Urinary acidification is recommended. Diuretics serve no useful purpose. Bisphosphonates such as pamidronate (an osteoclast inhibitor) may be used in extreme cases.

### Prevention

With the major source of vitamin D derived from the skin, exposure to sunlight is the best prevention. In high latitude countries, supplements or fortification may be needed. Human breast milk is deficient in vitamin D ( $1.0 \mu\text{g/L} = 40 \text{ IU/L}$ ), whereas fortified cow's milk contains ten times as much. Breastfed infants should therefore be given a supplement of vitamin D (400 IU/day) from birth to 6 months, at which time they are given a more diversified diet. Large doses of 200.000 IU (5 mg) can also be given every 3 months. This dose is not always well absorbed. The safest is to give daily small doses. Bottle feeds have already adjusted levels of vitamin D. Food fortification of margarine and cow's milk has eradicated rickets in Europe and the United States.

The elderly are a particular group at risk. Many older people stay indoors most of the time and get very little exposed to sunlight. They can develop demineralization of the bone with bone pains and fractures.



## Beriberi

### Summary

- Thiamine = vitamin B1, water-soluble, heat-labile
- Deficiency caused by lack of thiamine intake
- Deficiency caused by thiaminases
- Symptoms may develop acutely
- Dry beriberi: peripheral neuritis with paralysis and loss of sensation
- Wet beriberi: high-output heart failure
- Cerebral beriberi: ophthalmoplegia, mental confusion, ataxia
- Infantile beriberi: aphonia, areflexia and heart failure
- Diagnosis by empirical treatment

## Thiamine

Thiamine (Vitamin B1) is an essential micronutrient with dual co-enzymatic and non-co-enzymatic functions. It is involved in carbohydrate and branched-chain amino acid metabolism; as well as in the production of neurotransmitters, myelin, and nucleic acids. There is also evidence that thiamine plays a role in immune and anti-inflammatory processes and gene regulation. Thiamine is a water-soluble, heat-sensitive and very unstable vitamin which is present in many foods: meat, grain products, potatoes, beans, nuts and yeast. The richest sources are cereal grains and pulses. Green vegetables, fish, meat, fruit and milk all contain useful quantities. The refining of sugar, rice and grain products reduces the thiamine content. Whole grain rice requires more chewing and is heavier, but polishing of brown rice (removal of the dry outer layer) reduces the content of vitamin B1 to practically zero.

Thiamine resists temperatures up to 100°C, but it tends to be destroyed if heated further (e.g. if fried in a hot pan or cooked under pressure). It is often washed away with the cooking water, which can be avoided by preparing food with just the amount of water that will be absorbed in cooking, or by using water that is left over in soups or stews. Cassava contains only about the same low quantity as polished, highly milled rice. It is surprising that beriberi is not common among the many people in Africa, Asia and Latin America whose staple food is cassava, although underdiagnosis might play a role. Some nutrients contain thiaminases which have the ability to break down vitamin B1 in the food: raw fish, coffee and tea leaves. Certain plants, such as bracken (especially the young fern fiddleheads) contain thiaminases and are consequently toxic (cfr. the disease called "staggers" in horses eating these ferns).

This thiaminase is destroyed by cooking. The uptake of thiamine takes place in the proximal small intestine. A small amount is stored in muscle tissue. In Asian countries such as China, Indonesia, Japan, Malaysia, Myanmar, the Philippines and Thailand beriberi used to be a major cause of morbidity and mortality in those whose diet consisted mainly of rice. In contrast, people in many parts of the Indian subcontinent were relatively protected from beriberi because they consumed mainly parboiled rice, which conserves enough thiamine.

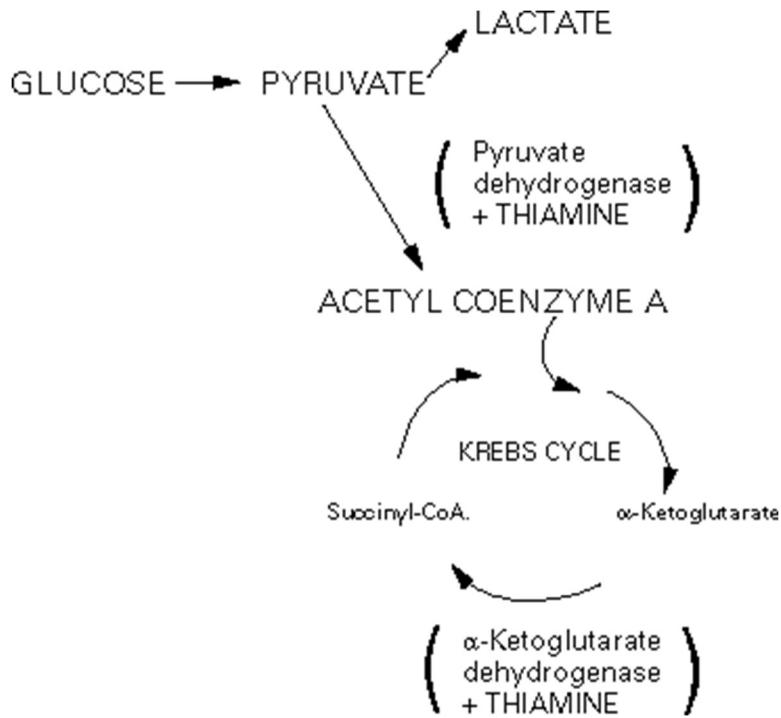


Fig: Thiamine is a co-enzyme in the conversion from pyruvate to acetyl-CoA and in the conversion of alphaketoglutarate to succinyl-CoA. Non availability of thiamine leads to lactic acidosis

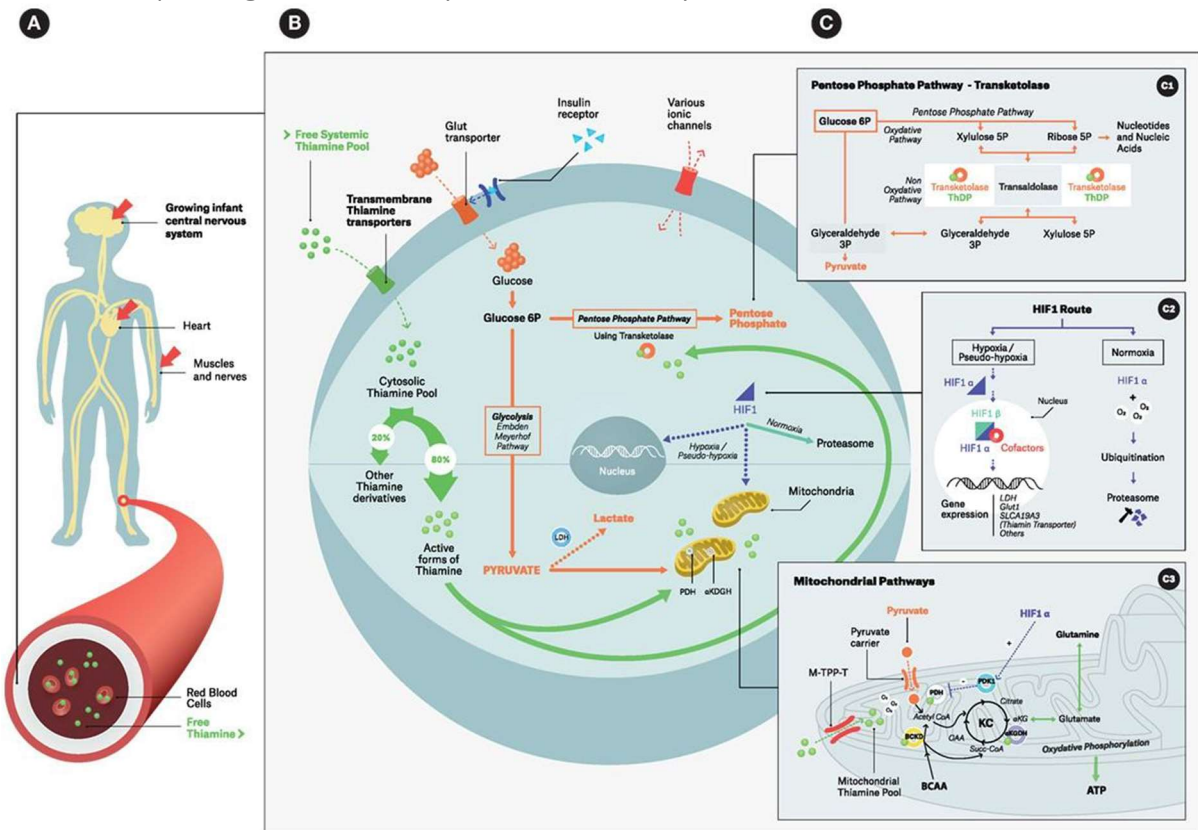


Fig: overview of thiamine intracellular action: focus on co-enzymatic functions. (A) Infant thiamine distribution with its three main target organs; (B) thiamine cellular metabolism with its main pools and thiamine-related metabolic pathways; (C) zooming boxes: (C1) – cytosolic pentose phosphate pathways using transketolase, (C2) – HIF-1 alternative routes, (C3) – mitochondrial events (source: *Frontiers in Nutrition* June 2016, Volume 3, Article 16)

Thiamine pyrophosphate (= thiamine diphosphate) is the co-enzyme of several enzymes of carbohydrate metabolism:

1. Pyruvate dehydrogenase, which is needed to convert (decarboxylate) pyruvate to acetyl CoA (Krebs cycle).
2. Transketolase: involved in the pentose phosphate pathway. This pathway generates NADPH which is essential for reductive biosynthesis, e.g. production of myeline.
3. 2-Oxo-glutarate dehydrogenase (= alpha ketoglutarate dehydrogenase), needed for the Krebs cycle (converting into succinyl-CoA via decarboxylation and production of NADH) and the synthesis of some neurotransmitters, e.g. GABA.

Thiamine is important for neural cell membranes and it has a modulating function in neuromuscular transmission.

### *Beriberi, historical overview*

At the end of the 16th century, the first reports emerged of a new disorder in the Far East. In Indonesia this disease was called beriberi. The etymology of the word is not clear. Dr. Jacobus Bontius reported that beriberi is similar to the local name for sheep, and was believed to refer to the peculiar gait of that animal. Together with Nicolaas Tulp from Holland (cf. the painting "The Anatomy Lesson of Dr. Tulp" by Rembrandt, 1632) he gave the first European description of the disease. In Hindi, the term 'bharbari' means swelling; in Arabic the term 'burh' means shortness of breath; and 'bahri' means marine. In Singhalese, 'bhayree' means weakness. Beriberi was found to be a ravaging disease which occurred with varying frequency. This was dramatically illustrated in 1883 when a training ship of the Japanese navy sailed to Hawaii via New Zealand and South America for over nine months. Of the crew of 376 sailors and officer cadets, 169 fell ill with beriberi and 25 died of the disease. On the recommendation of the Japanese medical officer Takaki, a different diet was used, with more meat, fish, barley and beans and less rice on a new voyage with the ship Tsukuba that was undertaken the following year. On this voyage there were only 14 cases of beriberi and no fatalities. Takaki observed that beriberi was common among low-ranking crew who were often provided free rice and thus ate little else, but not among crews of Western navies, nor among Japanese officers who consumed a more varied diet. These findings prompted the Japanese Navy to change its staple diet. More barley was used instead of rice, with a drastic reduction of beriberi as a result. However, the physicians of the time concluded that it was not the different diet that was responsible but the improved hygiene. During the Russian-Japanese war of 1904-1905, no fewer than 90,000 cases of beriberi were diagnosed in Japanese soldiers.

The disease occurred in communities that ate white rice, but not in all individuals and the disorder was also seen (to a lesser extent) outside rice-growing areas. The Dutch doctor Christian Eijkman, who won the Nobel Prize in 1929, was working in Indonesia and he used chickens as an animal model for beriberi. He noticed the symptoms of beriberi in some chickens used in his laboratory when their feed had been altered for a few months. During that time, chickens in the laboratory had been fed leftover rice from military rations, until a new cook refused to allow military rice to be fed to civilian animals. Rice was then purchased from another source, and the birds soon recovered. During the months that the chickens developed beriberi, the feed had been polished rice, and when the birds' diet was switched back to unpolished rice, the birds recovered in a few days.

Eijkman surmised that polished rice lacked a dietary component found in unpolished rice, and that beriberi was caused by depriving the body of this component, which he called "the anti-beriberi factor". Subsequently, Eijkman was able to prove that the disease was not caused by blood contamination, respiratory metabolism, perspiration, or seasonal or temperature variation. He suspected the disease was caused by an unknown bacteria. The Polish researcher Casimir Funk isolated the antiberiberi factor and established that it was an amine. He coined the term 'vitamin' for 'vital amine'. As a result of his discovery, research into deficiency diseases gained momentum. It wasn't until 1936, however, that the correct chemical structure of the antiberiberi factor was finally revealed. Funk was sure that more than one substance like Vitamin B1 existed, and in his 1912 article for the Journal of State Medicine, he proposed the existence of at least four vitamins: one preventing beriberi ("antiberiberi"); one preventing scurvy ("antiscorbutic"); one preventing pellagra ("antipellagric"); and one preventing rickets ("antirachitic").

### **Rice bran and thiamine**

Rice bran is a tiny covering membrane that entirely encloses brown rice. It comprises several thin layers. On the outside of the kernel is the fused testa-pericarp (seed coat and fruit wall) and immediately below is the aleurone layer, which is rich in fat and protein. This layer plays an important role in the germination of rice. When an intact grain of rice is exposed to a moist environment, the central core of the grain (embryo) absorbs water. As a consequence, the embryo secretes a plant hormone (gibberellin) that diffuses into the aleurone layer. This layer subsequently secretes amylase, which converts the starch in the endosperm ('the grain') into sugars that can then be absorbed by the embryo. The endosperm is rich in starch but poor in thiamine and other compounds. The embryo and the bran, on the other hand, are rich in proteins, fats and thiamine. The high oil content of bran makes it subject to rancidification, one of the reasons that it is often separated from the grain before storage or further processing. Bran is often heat-treated to increase its longevity. In white rice the bran and embryo have been removed, as a result of which the rice becomes rancid less quickly but is also deficient in thiamine. When brown rice is steeped in water and partly cooked (parboiled) before preparation, the thiamine in the aleurone layer is able to diffuse into the starchy endosperm. When the rice is then polished, the grain still contains some of the vitamin. This is why beriberi was absent in those regions where the people ate parboiled rice. Parboiling makes it easier to remove the husk but a lot of people don't like the rather musty taste that this treatment gives the rice. In most cultures this thin membrane is removed without parboiling by mechanical polishing, beating or shaking.

## Causes

Thiamine in the human body has a half-life of 18 days and is quickly exhausted, particularly when metabolic demands exceed intake. A biochemical deficiency can become apparent rather quickly, even after just 7 days. The course of the disease is usually somewhat slower. A daily intake of 1 mg of thiamine is sufficient for a moderately active man and 0.8 mg for a moderately active woman. Pregnant and lactating women may need more. FAO and WHO recommend an intake of 0.4 mg per 1 000 kcal for most persons. Deficiency may develop in alcoholics, elderly people, malabsorption, use of diuretics, prolonged administration of antacids, dialysis, folate deficiency, diets with a high content of refined grain products lacking

fruits and vegetables and ingestion of thiaminase-containing food. Refugees, victims of famine, prisoners and alcoholics are especially at risk for beriberi.

Because thiamine is involved in carbohydrate metabolism, a person whose main supply of energy comes from carbohydrates is more likely to develop signs of thiamine deficiency if their food intake is decreased. With a deficient diet, clinical complaints often develop in strong young males because they have a high glucose metabolism. Increased thiamine consumption may develop in seriously ill patients, hyperthyroidism, pregnancy, lactation and fever. Chronic malabsorption (chronic diarrhoea) leads to reduced uptake. Clinically particular attention should be paid when people are at risk of deficiency and are temporarily receiving no food (persistent vomiting, hyperemesis gravidarum). Especially when a glucose solution is administered quickly by intravenous injection and the metabolism suddenly has to cope with additional substrate, symptoms of acute deficiency may be induced. In practice such a situation can arise when a confused alcoholic with suspected hypoglycemia is admitted to hospital and a sudden deterioration of the clinical condition is observed after glucose administration.

In infants, refeeding syndrome is a potentially fatal complication of SAM management, especially when the introduction of food is too fast. Rapid initiation of nutritional rehabilitation also increases intracellular thiamine turnover which, on a background of pre-existing low whole body thiamine status, can precipitate the onset of true thiamine deficiency and may contribute to the mortality linked with refeeding syndrome.

### Clinical aspects

The energy used by the nervous system is derived entirely from carbohydrate, and a deficiency of thiamine blocks the final utilization of carbohydrate, leading to a shortage of energy and lesions of the nervous tissues and brain. Deficiency causes degeneration of peripheral nerves, the thalamus, mammillary bodies and the cerebellum. The cerebral blood flow is markedly reduced and vascular resistance is increased. The heart may become dilated, muscle fibers become swollen, fragmented and vacuolized with interstitial spaces dilated by fluid. Vasodilation occurs and can result in oedema in the feet and legs. Arteriovenous shunting of blood increases and eventually high-output heart failure may occur.

Deficiency signs may initially be very limited. Muscular cramps and paraesthesia may develop. Tiredness is already present but is often camouflaged: deficient patients often do normal activities with less movement. Anaesthesia over the shin is one of the first clinical signs. In more severe deficiencies, cardiovascular problems may develop (Wet beriberi). This concerns a high-output heart failure with peripheral pitting oedema, low peripheral resistance, warm extremities, full pulse, "pistol shot" heart tones, swollen neck veins, slight cyanosis and lactate acidosis. Quick deterioration with sudden death may occur. When neurological symptoms are prominent, this is called 'Dry beriberi'. This term indicates a mixed motor-sensory neuropathy with pain, paraesthesia, hyporeflexia and muscle atrophy.

Nocturnal muscular pain in the calves may develop. The symptoms are more pronounced in the legs than in the arms. Frequently the patient is unable to get up from the squatting position without assistance and wrist drop or drop foot can develop. Patients often succumb due to infectious complications (TB, decubitus) when they become bedridden.

Acute Wernicke's syndrome manifests by horizontal nystagmus, ophthalmoplegia with diplopia, fever (dysfunction of the hypothalamus), ataxia, confusion and coma. Frequently there are autonomous disorders, both sympathetic hyperactivity with tremor and agitation and hypoactivity with hypothermia and low blood pressure. Acute cerebellar ataxia may develop. During alcohol abstinence with simultaneous thiamine deficiency an acute delirium tremens may develop. Retrograde amnesia, confabulation, psychosis and learning difficulties are signs of Korsakoff's syndrome (psychosis). This develops in 80% of Wernicke patients.

Infantile beriberi is manifested by aphonia, areflexia and heart failure. Breast-fed babies of thiamine-deficient mothers - who often have no overt signs - become restless between 2 and 5 months of age, cry frequently (a loud piercing cry) and often refuse breastfeeding. They soon become debilitated and cry soundlessly. Soshin beriberi, a fulminant form of congestive heart failure with cyanosis and oedema; lactic acidosis is also documented in infants. Administration of thiamine IV results in very rapid recovery, often with noticeable improvement in less than 24 hours. Due to the non-specific presentation, thiamine deficiency is often overlooked or misdiagnosed as typhoid fever, sepsis, malaria, pneumonia or decompensated congenital cardiomyopathy in infants.

## Diagnosis and treatment

The diagnosis of thiamine deficiency is initially a clinical one.. A practical and easy test to determine the thiamine status does not exist. Since the vitamin is cheap and not toxic if suspicious of deficiency a trial of therapy is reasonable. A high level of clinical suspicion should be demonstrated in the following situations: suspicion of infantile beriberi; unexplained neurological signs, encephalitis, and cardiac failure; early clinical deterioration after initiation of feeds in malnutrition; sepsis (including in SAM); severe burns; major trauma; hypoxia; and unresponsive lactic acidosis. In acute situations a dose of 100 mg thiamine is administered IV. It is best to add 2 ml of a 50% magnesium sulphate solution, since magnesium is a cofactor for transketolase an associated hypomagnesaemia is frequently observed.

The clinical response in heart failure is usually very dramatic and fast. Improvement can already be observed just a few hours after administration. The patient is subsequently treated with 20 mg thiamine daily together with a multivitamin and efforts are made to eliminate the cause of the deficiency (diet, including avoidance of thiaminases, treatment of alcoholism, absorption problems, antiemetics, etc.). Central lesions usually do not fully recover. In the case of peripheral neural lesions, the degree of recuperation depends upon the duration and severity of the damage.

## Prevention

A balanced diet, sufficiently rich in vitamins, is essential. Food supplements may be given to high-risk groups. An unbalanced diet (e.g. based on polished rice) should be avoided. Lactating mothers in endemic regions should preferably take thiamine.

### *Thiamine deficiency in alcoholics*

Although classical beriberi is uncommon in industrialized countries, thiamine deficiency is by no means a rarity. It is prevalent in the alcoholic population worldwide. Alcoholism is an increasingly prevalent condition, and several clinical features previously believed to be due

to chronic alcoholic intoxication are now known to be the result of nutritional deficiencies. The most common of these conditions is probably alcoholic polyneuropathy, which has similarities to neuritic beriberi and is believed to result mainly from thiamine deficiency. Alcoholics who get much of their energy from alcoholic drinks often consume insufficient food and do not get adequate amounts of thiamine and other micronutrients. They may develop a peripheral neuritis, which can influence both the motor and the sensory systems, often affecting the legs more than the arms. The various manifestations include muscle wasting, abnormal reflexes, pain and paresthesia. These symptoms often respond to treatment with thiamine or B-complex vitamins taken orally.

Another condition resulting from thiamine deficiency in alcoholics is Wernicke-Korsakoff syndrome. Wernicke's disease is characterized by eye signs such as nystagmus (rapid involuntary oscillation of the eyeball), diplopia (double vision arising from unequal action of the eye muscles), paralysis of the external rectus (one of the muscles of the eyeball) and sometimes ophthalmoplegia (paralysis of the muscles of the eye due to lesions in the nuclei of cranial nerve III and VI). It is also characterized by ataxia (loss of coordination of body movements) and mental changes. Korsakoff's psychosis involves a loss of memory of the immediate past and often elaborate confabulation which tends to conceal the amnesia. Korsakoff syndrome (KS) is a late neuropsychiatric manifestation of Wernicke encephalopathy (WE). They are two different syndromes, each representing a different stage of the disease. Wernicke encephalopathy (WE) is an acute syndrome requiring emergent treatment to prevent death and neurologic morbidity. Korsakoff syndrome (KS) refers to a chronic neurologic condition that usually occurs as a consequence of WE. It is now generally agreed that any distinction between Wernicke's disease and Korsakoff's psychosis in the alcoholic patient may be artificial; Korsakoff's psychosis may be regarded as the psychotic component of Wernicke's disease. This view is supported by the fact that many patients who appear with ocular palsy, ataxia and confusion, and who survive, later show loss of memory and other signs of Korsakoff's psychosis. Similarly, psychiatric patients with Korsakoff's psychosis often show the stigmata of Wernicke's disease even years after the illness. Pathological evidence also indicates the unity of the two conditions.

That Wernicke-Korsakoff syndrome is caused by thiamine deficiency and not by chronic alcohol intoxication is shown by the fact that the condition responds to thiamine alone, even if the patient continues to consume alcohol. Of overriding importance in this syndrome is the rapid occurrence of irreversible brain damage; early recognition and treatment are therefore vital. A patient suspected of having the syndrome should immediately receive 500 mg of thiamine by injection (500 mg IV 3x/d for 3 days followed by 250 mg IV/IM per day for 4 days), even before a definitive diagnosis is made.

### **Prevention**

The prevention of Wernicke-Korsakoff syndrome calls for considerable public health ingenuity. Several possible measures have been suggested:

- the "immunization" of alcoholics with large doses of thiamine at regular intervals (the development of a suitable depot carrier to reduce the frequency of these injections would be very helpful);
- the fortification of alcoholic beverages with thiamine;

· a provision by public health authorities that thiamine-impregnated snacks be made available on bar counters.

The cost of any of these measures would almost certainly be less than the present enormous expenditure on institutional care of those who have suffered from Wernicke-Korsakoff syndrome.



## Pellagra

### Summary

- Disease caused by lack of vitamin PP (niacin) or tryptophan
- High risk if unbalanced maize based diet
- 3 D's clinical signs: dermatitis, diarrhea and dementia
- Treatment by nicotinamide supplements/vitamin B complex and a balanced diet

### Introduction

For many years, chiefly in regions where maize is the staple diet, a condition has been known which was characterized by cutaneous, mucosal and neurological abnormalities. This condition is known as pellagra. The disease derives its name from an old Italian description. It had been established that prisoners on a prolonged diet consisting solely of maize developed a skin problem. The etymology of the word is based on the Italian "pelle" (skin) and "agra" (rough). In the 18th century the inexpensive polenta, based on maize meal, was a staple of many rural regions of Italy. It was initially thought that the disease was caused by a fungal toxin in the food. In 1796, Dr Casper Casal, of Oviedo (Spain), described the disease mal de la rosa. The illustration in his work shows manifest skin lesions of the neck. Since that time, this symptom has been known as Casal's necklace.

### *Pellagra, historical note*

In the early 20th century, pellagra was a major problem among the poor Southerners of the USA. The work of the American scientist Joseph Goldberger represented a milestone in the history of epidemiology when he discovered that orphans whose diet consisted mainly of maize with molasses developed pellagra and that others (who had a more varied diet) were not affected by the disease. None of the staff ever contracted the disease (they had the first choice of the food). He injected himself and several volunteers with blood from pellagra patients. Not one of them developed the disease. Even eating faecal matter of the patients (!) was likewise unable to induce the disease in these intrepid volunteers, which was a strong argument against an infectious origin. After milk, eggs and meat were put on the menu of the orphanages, pellagra disappeared. A controlled experiment at a State Prison Farm in Mississippi manifestly demonstrated that pellagra only develops after living on an unbalanced diet. An animal model was developed using dogs that were fed on maize and subsequently developed so-called 'black-tongue'.

In 1937 Conrad A. Elvehjem an agricultural chemist at the University of Wisconsin, discovered that nicotinic acid cures black tongue. It was discovered that the disease has its origins in a deficiency of a compound present in small quantities in food. The compound was designated as vitamin PP (pellagra preventing factor). Sometimes the term vitamin B3 is used. The identification of pellagra as a deficiency disease was not evident. There were sometimes apparently contradictory data. Early in the 20th century, for instance, pellagra was rife in the maize-eating population of Romania. Paradoxically, however, their maize contained more niacin than the food of the indigent population of India, where pellagra did not occur. The explanation was only discovered later when it became clear that maize contained very little tryptophan and that much of the niacin in maize is present as a bound form called niacytin (which is not absorbed in the intestine). The reason why pellagra did not occur in the indigenous maize-eating population of Central America was found to be based on the fact that

they used alkali in the preparation of their maize meal, which released niacin from niacytin. They also had a more varied diet, which included a lot of beans (i.e. another food that contains niacin). It should be noted that white bread contains much less niacin than maize, but the niacin in maize is not fully available because it is in a bound form.

The highest prevalence in recent times has probably been in southern Africa, where conditions for some agricultural and industrial workers until 1994 were not unlike those in the southern United States between 1900 and 1920. A report from South Africa suggested that 50 percent of patients seen at a clinic in the Transvaal had some evidence of pellagra, and that the majority of adults admitted to the mental hospital in Pretoria had the disease. Pellagra regrettably has also been widely reported in refugee camps and in famine situations where maize has been the relief food and relief agencies have given too little attention to providing a balanced diet or adequate micronutrient intakes.

## Niacin

Niacin is also known as nicotinic acid, although the latter term is avoided in order not to evoke an association with tobacco and thus make people suspicious. The amide is likewise active (nicotinamide).

Niacin is absorbed from food in the stomach and small intestine. A small quantity of niacin is produced endogenously from tryptophan, an essential amino acid. Food that is rich in tryptophan and deficient in niacin will not give rise to clinically manifest deficiency. Alcoholics and people with hyperthyroidism are at higher risk of contracting pellagra. The conversion from tryptophan to niacin is more difficult in people with vitamin B2 (riboflavin) and B6 (pyridoxine) deficiency.

Niacin is required for adequate cellular function and metabolism as an essential component of nicotinamide adenine dinucleotide (NAD) and nicotinamide adenine dinucleotide phosphate (NADP).

NAD and NADP are the active forms of niacin and are coenzymes for many dehydrogenases that play an important role in glycolysis, protein and amino acid metabolism, pyruvate metabolism, pentose biosynthesis, generation of high-energy phosphate bonds, glycerol metabolism, and fatty acid metabolism. In case of deficiency, all sorts of cell functions become deranged. High energy requirements (brain) or high turnover rate (gut, skin) organs are most susceptible to deficiency.

## Aetiology

On average, a person needs approximately 20 mg niacin on a daily basis. Primary pellagra may be caused by niacin and/or tryptophan deficiency in the diet. A generally poor balance of amino acids in the diet could also give rise to pellagra. For instance, pellagra frequently affects people who eat sorghum (millet) as a staple food. This grain crop contains high concentrations of leucine. Although this grain contains adequate tryptophan, excessive concentrations of leucine interfere with tryptophan metabolism and subsequent niacin synthesis. Zein the main protein in maize (= corn) - which is the staple food in many parts of the world- contains very small amounts of tryptophan. Niacin in maize is chemically bound and is not absorbed in the

intestine unless the food is treated with alkalis as lime water. An example of the latter is the tortilla. Food products that contain large quantities of niacin are liver, kidney, groundnuts and yeast and, to a lesser extent, wheat and green vegetables. The bioavailability of niacin from meat, milk, beans and eggs is excellent.

Secondary deficiency may develop in persistent chronic diarrhoea with malabsorption, liver cirrhosis and alcoholism and in the event of prolonged parenteral nutrition being given without vitamin supplements. During treatment with isoniazid (INH) the drug is substituted for nicotinamide in the synthesis of NAD. The resulting molecule is inactive. In prolonged treatment with INH (tuberculosis) it is possible for iatrogenic induced pellagra to be provoked. On top of that, INH tends to bind to vitamin B<sub>6</sub> and reduce niacin synthesis, since B<sub>6</sub> (pyridoxine) is a required cofactor in the tryptophan-to-niacin reaction. There are also several situations where tryptophan metabolism is disrupted. For instance, pellagra may develop in carcinoid syndrome due to the conversion of tryptophan into serotonin (5hydroxytryptamine). Beware the cluster abdominal pain, diarrhoea, flushing, variable blood pressure, pulmonary valve stenosis and intermittent wheezing in carcinoid syndrome.

### Clinical aspects

Clinically, the disease is identified by the so-called classical three Ds: dermatitis, diarrhoea and dementia. Mucositis should also be added to these characteristic symptoms. The symptoms may develop alone or in combination. People suffering from pellagra usually appear poorly nourished with weakness and underweight.

Skin lesions occur symmetrically on areas of the skin exposed to sunlight, such as the face, the back of the hands, the neck, the forearms and exposed portions of the leg. Patients initially present with deepening of the pigmentation. The hyperpigmented areas lose the oily sheen of healthy skin and become dry, scaly and eventually cracked. There is usually a definite line of demarcation between these lesions and the healthy skin, and this line can be felt as the affected area is rough to the touch. The skin condition may remain static, heal or progress. If it progresses, desquamation commonly occurs; there may be deep cracking and fissuring and the skin becomes thick and rough; occasionally the skin may blister. The blisters contain a colourless exudate. In white subjects the skin lesions initially look like the erythema of sunburn. In both black and white patients, the lesions of pellagra produce burning sensations and pain when exposed to the direct rays of the sun, just as sunburn does in a person with pale skin.

The most conspicuous is a sharply defined symmetrical, desquamating rash in the neck (Casal's necklace) and on the forearms. A butterfly-shaped rash may appear on the face, which must be distinguished from skin abnormalities in SLE patients. Secondary infection may develop, including wound myiasis. Skin lesions may be associated with acute intertrigo with erythema, maceration and abrasion; superinfection may develop in the predilection areas (folds of the groin, genitals). Pellagra sometimes occurs without skin lesions (*pellagra sine pellagra*).



Casal's necklace



Pellagra dermatitis with hyperpigmentation, drying, cracking and fissuring of the skin

Mucositis develops in the mouth, vagina and urethra. A red tongue and stomatitis are characteristic of acute deficiency. The tip and edges of the tongue are the first to be affected. This is followed by a generalized painful, burning glossitis, with swelling of the tongue and hypersalivation. Lip and tongue ulcers may develop. The area around the parotid duct orifice may become necrotic (the area opposite the molar teeth). Deeper mucosae may be affected, with sore throat and oesophageal damage with dysphagia and abdominal pain. Some patients report loose stools but these complaints are not usually predominant. Caution: chronic malabsorption in itself may induce niacin deficiency. Gastrointestinal hyperemia, ulceration and proctitis may lead to bloody diarrhoea. When angular stomatitis is present this usually indicates an associated riboflavin deficiency (vitamin B2).

Neurological symptoms are due to an organic encephalopathy. Psychosis may occur with sleep and memory disorders, anxiety, agitation, rapid irritability, disorientation, confusion and confabulation (compare this with Wernicke-Korsakoff's syndrome in thiamine deficiency). Mania, delirium, paranoia and depression occur in later stages of the disease. At one time many pellagra patients were incarcerated in mental institutions. Muscular rigidity may develop together with a cogwheel phenomenon, hyperreflexia and a positive Babinski's sign. In the motor cortex, lysis of Betz's cells and to a lesser extent, lysis of Purkinje's cells are found. In the spinal cord, the posterior columns are chiefly affected (proprioception tracts; cfr vitamin B12 deficiency). In peripheral nerves there is myelin degeneration, but to what extent this overlaps with the findings in beriberi is unclear (nutritional deficiencies are often mixed). Post-mortem examination may reveal cardiac, adrenal gland, liver and spleen atrophy.

### *Dracula and Pellagra*

Dracula was not the first time a vampire appeared in literature, but it's truly the book that established vampires as a horror staple. The question is, where did the author Bram Stoker gain inspiration for the vampiric flaws and habits of Dracula? The origins may be surprising.

In 1735, pellagra was a newly recognized disease in Europe. In the 18th and 19th centuries, a big change to the European diet occurred – Corn. Corn is a crop that originated in the Americas, domesticated by Native Americans over the course of many generations. Corn could produce more calories per acre than traditional European staple crops, and corn cultivation slowly spread. However, corn is lacking in many vital nutrients. Where corn cultivation went, pellagra was soon to follow.

To societies with little medical knowledge, pellagra was a spooky illness indeed. People with pellagra (called pellagrins) developed a hypersensitivity to sunlight. Avoidance of sunlight is a classic vampire trait and one of the foremost symptoms of pellagra. The tongues of pellagrins became swollen and beefy red. Lips became red and cracked. The reddened mouth and

tongue might have led to suspicions of blood drinking. In *Dracula*, the count himself is described as having very red lips.

Mental problems also plagued pellagrins. The lack of niacin led to degradation of the neurons, causing dementia in sufferers.

Insomnia is a fairly common symptom of this, leading pellagrins to adopt the vampire-like habit of staying awake into the night. Increased levels of irritability and aggression occurred as well. Did this lead their neighbours to fear attack from redlipped people in the dead of night?

Death was the end result of pellagra for many unfortunate people in those times. After one person died from pellagra their family members might have appeared to be wasting away due to sustained supernatural attack. In traditional vampire folklore, the vampire returns night after night to slowly drain its victim of life. However, the real reason for entire families declining was the result of shared poor dietary conditions. If one family member died from pellagra, it was likely that the other family members were sickened as well.

When Bram Stoker researched for *Dracula*, he delved into the folklore of the communities most affected by pellagra. With this in mind, it doesn't seem like a coincidence that Stoker's description of vampires bears resemblance to the symptoms of pellagra. Vampire legends may have arisen as an explanation for a frightening illness that people back then encountered every day. So what's the best way to defeat a vampire? Maybe it's time to put away the crosses and holy water and instead feed the vampire some chicken and eggs.

### Diagnosis

When all symptoms and signs are present; the clinical diagnosis is simple. In most cases there are only a few symptoms present. Especially in non-endemic settings the linkage of the different symptoms can be very challenging contributing to an additional "D": delay in diagnosis. The diagnosis is confirmed by measuring serum niacin or the urinary excretion of N'-methylnicotinamide (NMN). NMN excretion of <0.8 mg/day suggests niacin deficiency.

Patients with pellagra also have increased urinary excretion of coproporphyrins. In clinical practice a successful trial of therapy will confirm the original diagnosis.

## Treatment

As there is seldom a deficiency of only one vitamin, treatment should include a polyvitamin preparation in addition to a balanced diet. The diet should contain at least 100 g per day of good protein (if possible, meat, fish, milk or eggs; if not, groundnuts, beans or other legumes) and should be high in energy (3 000 to 3 500 kcal per day). Specifically for pellagra, nicotinamide (precursor of niacin) is given as a supplement in a dose 300 mg daily using divided doses. If niacin itself were to be administered, the patient would complain of flushing, paresthesia and a burning sensation. If no oral supplement can be given (severe stomatitis, severe diarrhoea, uncooperative patient), 100 to 250 mg can be injected SC twice daily. In the acute phase the patient should avoid exposure to sunlight. Pellagra is often a very gratifying disease to treat. Violent, almost uncontrollable mental patients can become normal, rational, peaceful human beings within a few days of taking a few tablets of nicotinamide. In persons with severe skin lesions, a sore mouth and severe diarrhoea with frequent watery stools, dramatic improvements occur within 48 hours. The skin redness and pain on exposure to sunlight improves; pain in the mouth abates and eating becomes a pleasure for the patient; and most gratifying for the patient, the intractable diarrhoea disappears. Neurological improvement is rather slow.

## Prevention

A balanced diet is essential for prophylaxis and reliance on maize as the sole staple food should be discouraged. In some countries flour is systematically enriched with extra niacin. Niacin tablets should be administered in prisons and institution in areas where pellagra is endemic and to refugees in famine relief.

### *Nicotinic acid and hyperlipidemia*

Nicotinic acid has been in use as a lipid-lowering drug for several decades. It is effective in lowering low-density lipoprotein (LDL)-cholesterol, triglycerides, and lipoprotein (a), and in increasing high-density lipoprotein (HDL)-cholesterol. All these effects are pronounced, and at present greater increase of HDL-cholesterol cannot be obtained by any other drug. Patients with hypertriglyceridaemia/low HDL-cholesterol despite being treated with a statin, are the most suitable candidates for being treated with this drug. However, more recent studies have delivered disappointing results, leading to the conclusion that no further benefit is achieved by adding niacin to existing statin therapy in patients with high cardiovascular risk. Moreover, in these studies, several adverse effects of the treatment were observed and niacin for hyperlipidemias is not recommended anymore.

## Scurvy

### Summary

- Deficiency of ascorbic acid leads to poor quality collagen
- Hemorrhages and bone abnormalities dominate the clinical picture
- Barlow's disease (infantile scurvy) with periosteal hemorrhage)
- Rapid improvement with vitamin C tablets or fresh fruit and vegetables

### Introduction

Scurvy is a disease caused by lack of vitamin C. The condition was a common ailment aboard European seagoing ships in the early days of world exploration and was a serious problem on long voyages. In 1497, Vasco da Gama, in his epic trip from Portugal to India and back, lost no fewer than 100 of his original crew of 160 to scurvy. Magellan's expedition of circumnavigation of the world (1519-1521) lost 200 of his original crew of 218. Of the 110 crew members of Jacques Cartier's exploration of the St Lawrence river (Canada), 100 were affected during the winter of 1535-1536. A quarter died, the rest recovered with grounded cedar bark, a native Indian remedy. Aboard the ships there was a systematic lack of fresh fruit and vegetables.

In the nineteenth century, scurvy began to occur among infants receiving the newly introduced preserved milk instead of breastmilk or fresh cows' milk. The preserved milk contained adequate carbohydrate, fat, protein and minerals, but the heat used in its processing destroyed the vitamin C, so the infants got scurvy. Nowadays, scurvy only occurs in the event of an unbalanced diet with nutritional deficiency, as in some elderly people and alcoholics. Scurvy is sometimes seen in persistent problematical situations in the tropics (refugees, starvation), certainly in warm and dry regions where there is a lack of fresh fruit and vegetables. In a population living in stable conditions, scurvy is rare.

### *Ascorbic acid*

For a long time the origin of scurvy was a mystery. Before vitamin C was identified, however, a form of empirical treatment and prophylaxis had been discovered, but the nature of the compound that cured scurvy was not clear. A breakthrough came with the discovery that guinea pigs could develop scurvy. Guinea pigs, fruit-eating bats and higher primates (Old and New World monkeys, apes and humans) - unlike most mammals - are unable to synthesize ascorbic acid. Lower primates or prosimians, such as lemurs, lorises and tarsiers have active L-gulonolactone oxidase, and so make their own vitamin C.

Humans have an inactivating mutation in this enzyme, which leads to an afunctional pseudogene and therefore the inability to synthesize vitamin C. One could say that the entire human race has an inborn error of metabolism. When the defect in guinea pigs was discovered, scientists had an animal model and an in vivo assay for measuring the antiscorbutic activity of different food products.

It was demonstrated that drying, cooking and prolonged exposure to air destroyed the active ingredient. During his research at Cambridge University in 1928, the Hungarian biochemist Szent-Gyorgyi isolated vitamin C. He isolated the compound from lemons, oranges, cabbages and adrenal cortex. After his return to Hungary, he continued his work on paprikas, as befits

a good Hungarian. It turned out that paprikas are very rich in vitamin C. He received the Nobel Prize for Medicine in 1937. He initially proposed to name his crystalline sample "ignose", indicating its relationship to sugars while at the same time underlining his ignorance of its true nature. The editor of the Biochemical Journal where he wanted to publish his findings, did not like jokes and reprimanded him. A second suggestion "godnose" was judged to be equally unacceptable. Szent-Györgyi finally accepted the more prosaic "hexuronic acid", since the molecule had 6 carbons and was acidic. Haworth suggested the term "ascorbic acid," acknowledging the antiscorbutic nature of the compound.

Subsequently it became evident that vitamin C occurs in numerous food products. Vegetables such as broccoli and tomatoes, but also potatoes and citrus fruit have large concentrations of vitamin C. Sir Walter Norman Haworth discovered an efficient synthesis method for the preparation of vitamin C based on a carbohydrate precursor. Sir Norman Haworth and Paul Karrer (Switzerland) were jointly awarded the Nobel Prize for Chemistry for their work in 1937.

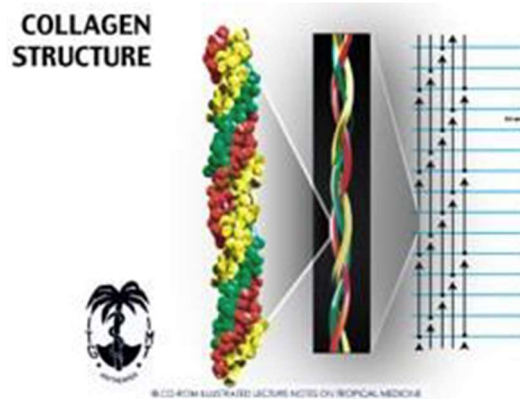
The name ascorbic acid refers to 'antiscorbutic' (from the Low German term for scurvy: schorbock). Vitamin C is essential for the production of mature collagen. It is a highly reducing compound and is capable of undergoing reversible oxidation. In consequence, it fulfils a role in redox reactions in the body. Vitamin C is the L-enantiomer of ascorbate; the D-enantiomer is not physiologically active. Vitamin C promotes the uptake of iron in the intestine and protects folic acid reductase. Vitamin C regenerates antioxidants such as vitamin E, flavonoids and glutathione. It plays a role in the synthesis of steroids and the production of carnitine.

### *Collagen*

Vitamin C is important in redox reactions. At least 8 different enzymes use vitamin C as a cofactor (maturation of collagen, production of several peptide hormones and neurotransmitters, synthesis of carnitine). Several symptoms of scurvy can be traced back to defective collagen. Collagen is the commonest protein in the animal kingdom. Large amounts of unusual amino acids are found in collagen: hydroxylysine and hydroxyproline. These are essential for the chemical stability of collagen. The conversion of proline into hydroxyproline is stimulated by the enzyme proline hydroxylase. For this purpose it uses a Fe<sup>2+</sup> ion, which is converted during the reaction into Fe<sup>3+</sup>. This inactivates the enzyme. Enzyme regeneration takes place by an interaction with ascorbate, in which vitamin C is converted into dehydroascorbic acid. For a better understanding of scurvy, we briefly sketch the normal production of the commonest form of collagen. Individual collagen polypeptide chains are synthesized on the ribosomes of the rough endoplasmic reticulum. The strands are released in the lumen of the endoplasmic reticulum as large precursor molecules, the so-called pro- $\alpha$  chains. Signal peptides are still present at front and rear. In the lumen, selected proline and lysine residues are hydroxylized to hydroxyproline and hydroxylysine. Every pro- $\alpha$  chain subsequently combines with two other chains to form a triple-strand helix via hydrogen bridges, the fibrillar procollagen. This is subsequently secreted. Procollagen is converted extracellularly into tropocollagen by enzymatic cleavage (with the exception of collagen IV in the basal lamina). Tropocollagen subsequently develops further into mature collagen.



Normal collagen is broken down slowly by extracellular collagenases. In scurvy, defective pro-alpha chains are formed (the formation of hydroxy-amino acids is disrupted). They do not form a triple helix and are quickly degraded. The consequences are first noticed first in the tissues where collagen turnover is fastest, such as blood vessels. Owing to the gradual loss of the existing collagen, the blood vessels become progressively fragile.



Collagen structure. This is disturbed in osteolathyism and in scurvy (vitamin C deficiency). Drawing by JP Wenseleers, copyright ITM.

## Aetiology

Primary deficiency is due to an unbalanced diet, i.e. a diet containing less than 10 mg vitamin C per day. There is little agreement on the minimal daily dose to avoid scurvy. Pregnancy, lactation, smoking, surgical procedures, thyrotoxicosis, burns and chronic inflammation increase the body's requirements up to 70-90 mg/day. In achlorhydria and chronic diarrhoea, less vitamin C is absorbed. Ascorbic acid is unstable in the presence of heat and prolonged cooking of food considerably reduces the quantity of active vitamin C. Scurvy is uncommon nowadays but outbreaks can be seen in refugee camps, during famines and occasionally in prisons.

## Clinical aspects

The highest concentrations of vitamin C are found in white blood cells, the lens and the brain. The total body pool of vitamin C is approximately 1500 mg. The excess is excreted. There is a turnover of 3% per day, which gives a half-life of approximately 18 days. This explains the latency period of 3 to 6 months for symptoms to occur after starting a diet without vitamin C.

Ascorbic acid is necessary for the proper formation and maintenance of intercellular material, particularly collagen. In simple terms, it is essential for producing part of the substance that binds cells together, as cement binds bricks together. In a person suffering from scurvy, the endothelial cells of the capillaries lack normal solidification. They are therefore fragile, and haemorrhages take place. Similarly, the dentine of the teeth and the osteoid tissue of the bone are improperly formed. The patient first complains of pronounced fatigue, general debility of slow onset, irritability, weight loss and vague myalgia and joint pain. Sometimes the first symptom is stiffness in the calves, due to local haemorrhages. Because of the pain in the legs, children may present with pseudoparalysis. In many cases they spontaneously adopt an analgic posture, with bent knees and hips: frog-leg posture as described by Thomas Barlow. This is usually seen in babies born prematurely when they reach about 612 months of age if

they have been fed deficient artificial food: Barlow's disease or infantile scurvy. Splinter haemorrhages beneath the fingernails may occur as in infective endocarditis. Haemorrhages around the eyes, ears, neck and on the roof of the mouth may occur. Spontaneous bleeding may occur anywhere in the body, including bleeding leading to palpable subperiosteal haemorrhages. Hyperkeratotic hair follicles and perifollicular petechiae (scorbutic purpura) are quasi pathognomonic. Corkscrew hairs is a typical scorbutic feature. The poor cell-binding also explains the poor scar formation and slow healing of wounds manifest in persons deficient in ascorbic acid. Old scars might break open. The gums become swollen, purple and spongy and bleed easily. Often there will be secondary infection. In advanced scurvy, teeth fall out spontaneously. Endochondral bone development ceases because osteoblasts no longer produce osteoid. A fibrous area is formed between diaphysis and epiphysis. The costochondral junctions enlarge. This is clinically palpable as a scorbutic rosary (not to be confused with rachitic rosary). Other symptoms include femoral neuropathy and oedema of the legs. Microcytic hypochromic anaemia may develop as vitamin C is needed to absorb iron. Sudden cardiac failure and death can occur in a patient with above mentioned symptoms, even if the person does not appear seriously ill.

### Differential diagnosis

Scorbutic rosary on the thorax and bone abnormalities must be distinguished from rachitic rosary (vitamin D deficiency). Scorbutic gingivitis must be distinguished from other causes such as candidiasis, herpes, trench mouth, syphilis, pemphigus and Behçet's syndrome. Scorbutic haemorrhages must be distinguished from other bleeding diatheses. Subperiosteal haemorrhage with periosteal elevation should be distinguished from congenital syphilis.

### Diagnosis

The vitamin C content in peripheral blood can be measured in specialized laboratories, although plasma vitamin C levels quickly normalize with enteral intake of ascorbic acid and do not reflect tissue levels. A level of less than 11  $\mu\text{mol/liter}$  is diagnostic for scurvy. Measurement in leukocytes – a storage pool for ascorbic acid – is more precise. A capillary fragility test will be positive. When this is measured using the sphygmomanometer, it is called the Hess capillary test. The regular haemostasis parameters (platelets, coagulation times) are normal. Findings on X-rays of the legs include a lucent transverse metaphyseal band with an adjacent dense sclerotic band, metaphyseal spurring and nonspecific evidence of diffuse osteopenia and cortical thinning. Radiographs may reveal periosteal fluid consistent with haemorrhage.

### Treatment

The treatment of scurvy consists of administering extra vitamin C (at least 100 mg three times daily for two weeks) and adjusting the daily diet with plenty of fresh fruit and vegetables. Clinical improvement may be expected within one to two weeks. Chronic gingivitis and extensive subcutaneous haemorrhages take longer to heal. Increased intake of vitamin C with meals can have a manifest effect on the absorption of iron. In many iron-deficient populations, increasing vitamin C intake will help reduce the incidence and severity of iron deficiency anaemia.

### *Treatment: historical note on James Lind and Captain Cook*

Various therapies were used in ancient times but as long as the cause remained unknown, no rational treatment could be suggested. Some people believed that certain plants could be used as a remedy for scurvy. For instance, *Cochlearia officinalis* (Family: Cruciferae) is known as common scurvy grass. Naval surgeon James Lind was on board the *Centurion*, a British gunship which had been put to sea in 1740 in order to give a hard time to the Spanish. After three years he had gained considerable experience with scurvy. In 1747, he conducted a kind of clinical trial ahead of its time. He had 12 patients with scurvy on board. They were divided into six groups and each group received a different treatment: (1) one glass of cider a day, (2) 25 drops of an elixir of vitriol three times a day, (3) two spoonfuls of vinegar three times a day, (4) half a pint of sea water three times a day, (5) a mixture of garlic, mustard, horseradish and balsam of Peru three times a day, (6) two oranges and a lemon each day. The two men who were given citrus fruit made a spectacular recovery. Cider also brought some improvement, although to a more limited extent. Lind published his findings. In July 1772, Captain Cook set out from Plymouth on board HMS *Resolution* on an expedition that was to last three years. He didn't lose a single member of the crew to scurvy. A paper that he presented on the prevention of scurvy won for Cook the Royal Society's Copley Gold Medal. He ordered the crew to eat sauerkraut twice a week and gave a malt potion and an orange and lemon to everyone who showed the first signs of scurvy. Furthermore he made sure that the ship was provisioned with fresh fruit and vegetables each time they made landfall. He also demanded strict hygiene on board, which was very unusual at the time. The Royal Navy implemented Captain Cook's regimen regarding hygiene and ordered that on voyages lasting longer than two weeks, everyone on board was to be given a spoonful of lemon juice and sugar each day. This mixture was incorrectly described as 'lime juice', and to this day, British sailors are known as 'Limeys'. Unfortunately, limes (*Citrus medica* var *acidum*) were sometimes used instead of lemons (*Citrus medica* var *limonum*). Limes contain much less vitamin C than lemons so that fatalities sometimes occurred and the use of lemon juice was regarded with suspicion. After 1860, only lemons were officially allowed for antiscorbutic use. The reason why scurvy was banished from the long-distance sailing ships of the Chinese Ming dynasty (1368-1644) was due to the fact that the crew were regularly given fresh, germinated soya beans to eat, as part of their traditional food. Unlike non-germinated seeds, these shoots are rich in vitamin C. The importance of the absence of scurvy is not to be underestimated, since the voyages of the Chinese admiral Zheng He (1421) led to world maps, which were obtained by the Portuguese crown and were a crucial element for the major discovery expeditions of Henry the Navigator, opening the world for the West, a fundamental turning point in history.

### Prevention

A sufficiently varied diet containing fruit and green vegetables will prevent the development of scurvy. Prolonged cooking of all food should be avoided. Vitamin C 60-100 mg/day PO provides protection against scurvy. Some people use vitamin C megadoses in the hope of preventing colds and other ailments. There is little evidence to support this but no definitive conclusion has yet been reached.

Vitamin C is metabolized to oxalate. When megadoses vitamin C are consumed on a daily basis, this might facilitate the formation of oxalate kidney stones but there is no consensus on

this. Excess ascorbate is normally excreted in the urine, but in patients with renal failure, it is retained and converted to insoluble oxalate and can accumulate in multiple organs.

## Iodine deficiency disorders

### Summary

- Iodine deficiency disorders (IDD) are most prevalent in mountainous, alluvial plains and areas far away from oceans due to low iodine intake
- About 2 billion people in the world have low iodine intake
- Cretinism is only the tip of the iceberg of IDD manifestations
- Iodine deficiency is the most frequent cause of avoidable mental retardation
- Goitrogenous factors like thiocyanate and selenium deficiency contribute to goiter formation
- Neurological cretinism is irreversible
- Myxoedematous cretinism can be reversed when treated early
- Prevalence of endemic goiter, urinary iodine concentrations, TSH dosage and prevalence of cretinism determine endemicity of IDD
- Salt, water or oil are used for iodine fortification

### Introduction

Iodine is an oligo-element that is present in the human body in a very small quantity (15 to 20 mg for adults). Its only known function is as essential element in the production/composition of the thyroid hormones T3 and T4. These hormones have a specific role in the metabolism of all cells of the organism and in the growth process of most organs, in particular the brain. In a situation of iodine shortage, thyroid hormone synthesis and availability is reduced, with numerous health consequences. In the past the deficiency was called “endemic goitre”, related to the most prominent sign of the deficiency “the goitre”, but the health problems due to iodine deficiency are far more important than goitre alone. It is now replaced by “iodine deficiency disorders” or “IDD”.

### Epidemiology

At present there are no exact figures on the prevalence iodine deficiency disorders available: in 1990 it has been estimated that among the 1572 million people in the world exposed to iodine deficiency (28.9 % of the world population), 11.2 million were affected by overt cretinism, the most extreme form of mental retardation due to the deficiency and that another 43 million people were affected by some degree of mental impairment. It therefore appeared that iodine deficiency was the leading cause of preventable mental retardation. A WHO report of 2007 concludes that global progress in controlling iodine deficiency has been made, but still 2 billion people (of which 266 million school-aged children) have insufficient iodine intake. This report warns that more than adequate or even excessive iodine intake in 34 countries.

Although present in 95 countries, the problems due to iodine deficiency occur most in mountainous regions: the mountain chains of the Himalayas, the Andes (where the neurological form is dominant), the mountainous regions of Vietnam, etc. Regions that are situated at a low level, far away from the oceans, like the central part of the African continent (where the myxoedematous form is dominant) and to a lesser degree the European continent, are also affected as well as the high plains of China and Australia. The groups with the highest risk for iodine deficiency are in order of importance the fetus, the newborn, the pregnant and

nursing woman, the young child. The prevalence increases with age until puberty, and is higher among women than among men.

The real problem of the iodine deficiency, from a public health point of view, is not goitre itself, but the mental retardation secondary to the thyroid deficiency that is present in fetal life and in the beginning of postnatal life. The socio-economic consequences (high number of disabled, learning difficulties in children, infant death in children with cretinism) are quite important and they are a real obstacle to the development.

## Aetiology

### 1. Low iodine intake

Several arguments confirm that iodine deficiency is the main cause of the observed problems: there is an inverse relation between the prevalence of goitre and the urinary excretion of iodine over 24 hours, used as an indicator of iodine intake. The correction of the iodine deficiency decreases the prevalence of endemic goitre, cretinism and of hypothyroidism.

Low iodine intake can be explained by 2 phenomena:

## Geography

A soil that is poor in iodine produces water and foods, poor in iodine. The ocean is the essential reservoir for iodine. The iodized ions are oxidized in elementary iodine on the surface of the water by the sunlight. The iodine is volatile and diffuses in the atmosphere and returns to the soil by rain. So it's brought along by rivers, running water and melting ice. The poorest soils in iodine are found in mountainous regions: these were covered by the glaciers of the Quaternary and because these melted the underlying iodine was swept away with the erosion. Most mountainous districts in the world have been or are still endemic goitre regions. The disease may be seen throughout the Andes, in the whole sweep of the Himalayas, in the Alps where iodide prophylaxis has not yet reached the entire population, in Greece and the Middle Eastern countries, in many foci in the People's Republic of China, and in the highlands of New Guinea. The iodine content of the drinking water is low, as is the quantity of iodide excreted each day by residents of these districts.

Non-mountainous regions, far away from the oceans can have poor iodine concentrations in their soils. Plants absorb iodine from the ground, plants are eaten by animals and plants and animals are eaten by humans, so the iodine concentration in food is often a good reflection of the distance from the sea.

Examples of iodine deficient low-land regions are the belt extending from the Cameroon grasslands across northern DRC and the Central African Republic to the borders of Uganda and Rwanda, Holland, Central Europe and the interior of Brazil.

Last, a wash-away effect in soils that are regularly flooded can be seen, like the alluvial plains in deltas of big rivers.

## Isolation

Food diversity and the mobility of the populations bring along a spontaneous reduction of the

prevalence of the endemic goitre. Isolation leads to poor food exchanges and diversification. The phenomenon of opening isolated regions, observed in the last decades, explains as much of the decrease in the prevalence of IDD as the iodination campaigns. It is also the reason for the observed spontaneous historical reduction of the prevalence of IDD in most countries.

## 2. *Goitrogenous factors*

The role of additional factors playing a role in the aetiology of IDD has been suspected because goitre exists in regions where the iodine intake is adequate. The additional role of goitrogens from food origin or in the environment has been looked into and has been proved in a number of regions in the world.

**Thiocyanates** inhibit the iodine pump and increase the renal clearance of iodide. They are derived from manioc, in a variable quantity that depends on the nature of the soil, the type of cassava that is cultivated, the way of preparation and consumption of cassava. DRC, Mozambique and Indonesia are countries where thiocyanate can be found. Thiocyanate is derived from intestinal breakdown of linamarin - a cyanogenic glycoside - from cassava and its conversion to thiocyanate by the liver. Thiocyanate is a competitive inhibitor of the Na/I symporter in thyroid follicular cells. A reciprocal relationship exists between iodide and thiocyanate in that increasing amounts of iodide protect increasingly against the thiocyanate derived from the cassava. It now seems well established that cassava may contribute to the severity of endemic goitre and probably the incidence of endemic cretinism, but there are many severe endemics where cassava is not eaten. In these regions, it is possible that other goitrogens in the local food may contribute to the effects of a prevailing iodine deficiency. Thiocyanate may cross the human placenta and affect the thyroid of the fetus.

**Thioureas** act on the level of the oxidation and metabolism of iodine in the thyroid.

## 3. *Selenium deficiency*

It has been shown that selenium deficiency may have profound effects on thyroid hormone metabolism and possibly also on the thyroid gland itself. In this situation the function of type I deiodinase (a selenoprotein) is impaired. Type I deiodinase plays a major role in T4 deiodination in peripheral tissues like kidney, liver and gut. It has been shown that when in an area of combined iodine and selenium deficiency, only selenium is supplemented, serum T4 decreases. This effect is explained by restoration of type I deiodinase activity leading to normalization of T4 deiodination and conversion to T3, while T4 synthesis remains impaired because of continued iodine deficiency. Selenium deficiency also leads to a reduction of the selenium containing enzyme glutathione peroxidase. Glutathione peroxidase detoxifies H<sub>2</sub>O<sub>2</sub> which is abundantly present in the thyroid gland as a substrate for the thyroperoxidase that catalyzes iodide oxidation and binding to thyroglobulin, and the oxidative coupling of iodotyrosines into iodothyronines. Reduced detoxification of H<sub>2</sub>O<sub>2</sub> may lead to thyroid cell death. Elevated H<sub>2</sub>O<sub>2</sub> levels in thyrocytes may be more toxic under situations of increased TSH stimulation such as is present in areas with severe iodine deficiency. Finally decreased availability of glutathione peroxidase impairs thyroid hormone synthesis in the thyroid gland, a fact that could also contribute to decreased T4 synthesis. Selenium deficiency certainly plays a role in the aetiology of the type of myxedematous endemic cretinism seen in Central Africa but does not by itself constitute a cause of endemic goitre. Extensive epidemiological data

collected in China indicated that all selenium-deficient areas were IDD-endemic areas. However, the reverse is not true: IDD can be very severe in many selenium-rich areas.

### *Iodine needs*

The physiologic needs are equal to the hormonal quantity of iodine that is produced every day. This means 50 to 100 µg/day for an adult. The quantity starts increasing in puberty certainly among women. Among the girls of 11 to 12 years a slight increase in the volume of the thyroid body is not rare (transitory hypertrophy).

RECOMMENDED INTAKE	µg/day	
0 - 6 months	35	8 µg/kg 5 µg/100ml of milk 7 µg/100 kcal
6 - 12 months	45	
1 - 10 years	60 – 100	
>= 11 years	100 - 115	
pregnancy – lactation	125 - 150	

Table: recommended daily intake of iodine (µg/day)

## Pathophysiology

### *Goitre*

Because of a deficiency of iodine, the synthesis of the thyroid hormones is reduced. A low level of thyroxin in the blood stimulates the hypophysis to free TSH. This results in a hyperplasia of the cells of the thyroid gland with increase in thyroid volume (goitre). This in turn makes a higher captivation of circulating iodine possible. If the normal production of thyroid hormones cannot be maintained, hypothyroidism appears.

However, efficient adaptation to iodine deficiency is possible in the absence of goitre as demonstrated in nongoitrous patients in endemic goitre areas such as New Guinea and the Congo. Moreover, adequate adaptation to iodine deficiency has been demonstrated in areas of severe iodine deficiency in the absence of endemic goitre. This clearly indicates that goitre is not required for achieving efficient adaptation to iodine deficiency. Rather in these conditions, efficient adaptation to iodine deficiency is possible thanks to a high iodide trapping capacity but with only a slight enlargement of the thyroid. At this stage, the characteristic hyperplastic picture includes abundant parenchyma, high follicular epithelium and rare colloid.

On the contrary in large goitres, the major part of the gland is occupied by extremely distended vesicles filled with colloid with a flattened epithelium. The mechanism responsible for the development of colloid goitre is not fully understood but it does not appear to be TSH hyperstimulation. It must be the consequence of an imbalance between thyroglobulin synthesis and hydrolysis, i.e. secretion. In these conditions, iodide is diluted while thyroglobulin is in excess, resulting in a lesser degree of iodization of thyroglobulin and consequently a decrease in iodothyronine synthesis and secretion. Hydrolysis of large amounts of poorly iodinated thyroglobulin will result in an important leak of iodide by the thyroid and enhanced urinary loss of iodide, further aggravating the state of iodine deficiency. Therefore, large colloid goitres in endemic iodine deficiency represent maladaptation instead of adaptation to iodine deficiency because they may produce a vicious cycle of iodine loss and defective thyroid hormones synthesis.



### *Iodine deficiency in the fetus*

The fetus and the new born are more sensitive than the adult to the effects of low levels of circulation thyroid hormone seen in iodine deficiency or goitrogenous substances. There is an immaturity of the adaptation mechanisms and iodine reserves are small. The period of growth, pregnancy and lactation increases the needs and make the individual more vulnerable.

The human brain develops during its fetal life until the end of the third life-year. Consequently an iodine and/or thyroid hormone deficiency during this critical period of life causes irreversible changes in the development of the brain. Iodine deficiency in the fetus is the result of iodine deficiency in the mother.

The consequence of iodine deficiency during pregnancy is impaired synthesis of thyroid hormones by the mother and the fetus. An insufficient supply of thyroid hormones to the developing brain may result in mental retardation.

The physiologic role of thyroid hormones can be defined as to insure the timed coordination of different developmental events through specific effects on the rate of cell differentiation and gene expression. Thyroid hormone action is exerted through the binding of T3 to nuclear receptors which regulate the expression of specific genes in different brain regions following a precise developing schedule during fetal and early postnatal life. The T3 which is bound to the nuclear receptors is primary dependent on its local intracellular production from T4 via type II deiodinase and not from circulating T3.

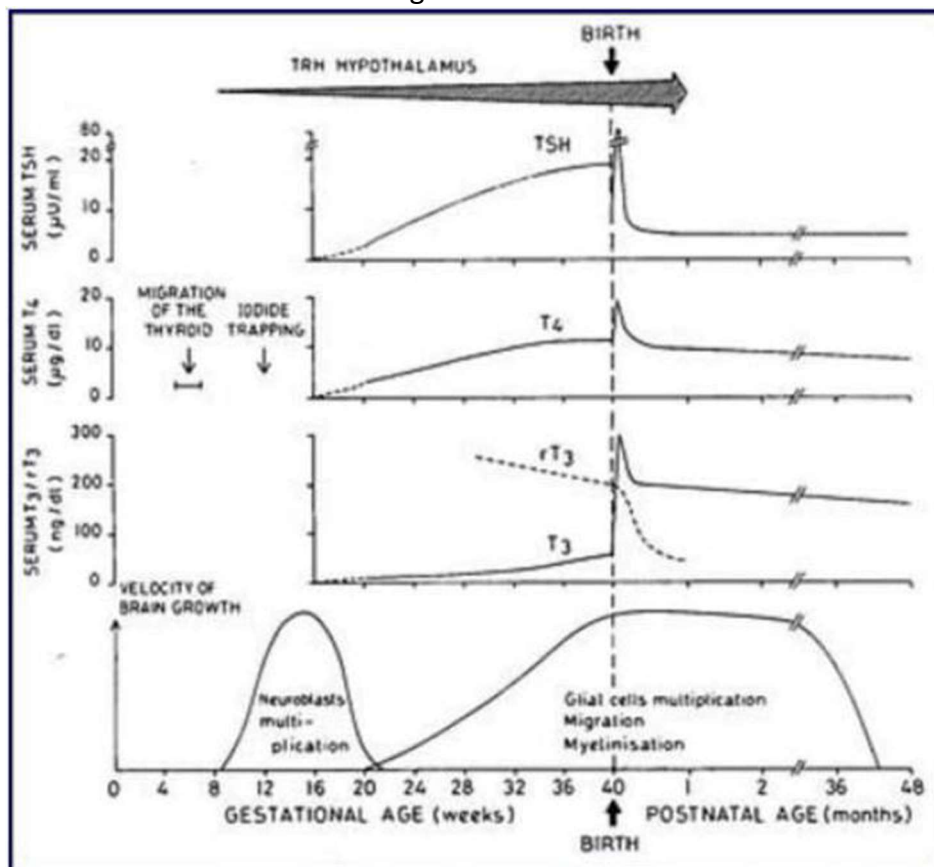


Figure: ontogenesis of thyroid function and regulation in humans during fetal and early postnatal life

Brain growth is characterized by two periods of maximal growth velocity. The first one occurs during the first and second trimesters between the third and the fifth months of gestation. This phase corresponds to neuronal multiplication, migration and organization. The second phase takes place from the third trimester onwards up to the second and third years postnatally. It corresponds to glial cell multiplication, migration and myelination. The first phase occurs before fetal thyroid has reached its functional capacity. It is now largely agreed that during this phase, the supply of thyroid hormones to the growing fetus is almost exclusively of maternal origin while during the second phase, the supply of thyroid hormones to the fetus is essentially of fetal origin. Thyroid hormones are transferred from mother to fetus both before and probably after the onset of fetal thyroid function, contrasting with the previous dogma that this transfer is minimal or does not exist. Nuclear T3 receptors and the amount of T3 bound to these receptors increase about six to tenfold between 10 and 16 weeks, also before the secretion of hormones by the fetal thyroid. This transfer is decreasing but persists during later gestation. Up to 30 % of serum T4 in cord blood at birth could be of maternal origin.

### Clinical aspects

The term Iodine Deficiency Disorders (IDD) refers to all the ill-effects of iodine deficiency in a population that can be prevented by insuring that the population has an adequate intake of iodine.

These effects are listed in in the table below.

<b>Fetus Abortions:</b>	Stillbirths Congenital anomalies Increased perinatal mortality Endemic cretinism
<b>Neonate Neonatal goitre:</b>	Neonatal hypothyroidism Endemic mental retardation Increased susceptibility of the thyroid gland to nuclear radiation
<b>Child and goitre</b>	Adolescent (subclinical) hypothyroidism Impaired mental function Retarded physical development Increased susceptibility of the thyroid gland to nuclear radiation
<b>Adult goitre with its complications</b>	Hypothyroidism Impaired mental function Spontaneous hyperthyroidism in the elderly Iodine-induced hyperthyroidism Increased susceptibility of the thyroid gland to nuclear radiation

The Spectrum of Iodine Deficiency Disorders, IDD, Adapted from Hetzel, Laurberg et al.; Stanbury et al.

### *Goitre*

Goitre is an increase in thyroid volume of four to five times that can cause aesthetic problems or compression of the oesophagus and trachea. Goitre can be associated with hypothyroidism, but also Iod-Basedow (not to be confused with Basedow's disease which is the same as Graves' disease) hyperthyroidism can occur in a patient with an endemic goitre due to iodine deficiency relocates to an iodine-abundant geographical area. Cancer is a rare complication of goitre.

### *Cretinism*

Cretinism exists in two extreme forms, but most presentations are intermediate forms. **Neurological** cretinism is secondary to a state of maternal and fetal hypothyroidism supervening in the beginning of fetal life. The child is euthyroid but presents with spastic diplegia (symmetrical paralysis), deafmuteness, strabismus and serious mental retardation. This condition is irreversible. **Myxedematous** cretinism is the long-term consequence of a permanent, earlier unknown hypothyroidism; it begins during the fetal or neonatal period if mothers are deprived of iodine during the later process of pregnancy. Myxedematous cretinism has a picture of hypothyroidism with important stature and variable mental retardation. This condition can still respond to thyroid hormone replacement therapy and early detection and treatment is crucial to safeguard the baby's prognosis.

The mental deficiency is the iceberg of which cretinism is only the top. Retardation of intellectual development was noted in up to 5% of the total population in an endemic zone. This makes iodine deficiency the most frequent cause of avoidable mental retardation. These people often have a clinically and biologically euthyroid aspect since the retardation is a consequence of a transient hypothyroidism during the critical phase of the cerebral development which resolved spontaneously.

### *Iodine deficiency in the neonate*

Miscarriages are more frequent in iodine deficient regions. An increased perinatal mortality due to iodine deficiency has been shown in DRC from the results of a controlled trial of iodized oil injections alternating with a control injection given in the latter half of pregnancy. There was a substantial fall in infant mortality with improved birth weight following the iodized oil injection. Low birth weight of any cause is generally associated with a higher rate of congenital anomalies and higher risk of death throughout childhood. Apart from mortality the importance of the state of thyroid function in the neonate relates to the fact that the brain of the human infant at birth has only reached about one third of its full size and continues to grow rapidly until the end of the second year. The frequency distribution of IQ in apparently normal children in such conditions is shifted towards low values as compared to matched controls who were not exposed to iodine deficiency during the critical period of brain development because of correction of the deficiency in the mothers before or during early gestation.

More globally, in a meta-analysis of studies on neuromotor and cognitive functions in conditions of moderate to severe iodine deficiency, iodine deficiency resulted in a loss of 13.5 IQ points at the level of the global population.

### *Iodine deficiency in the adult*

A high degree of apathy has been noted in populations living in severely iodine deficient areas. This may even affect domestic animals such as dogs. It is apparent that reduced mental function due to cerebral hypothyroidism is widely prevalent in iodine deficient communities with effects on their capacity for initiative and decision making. This indicates that iodine deficiency can be a major block to the human and social development of communities living in an iodine deficient environment and constitutes a major teratogen at the community level. In addition to this impact to brain and neurointellectual development, iodine deficiency at any period in life, including during adulthood, can induce the development of goitre with mechanical complications and/or thyroid insufficiency. Another consequence of longstanding iodine deficiency in the adult but also in children is the development of hyperthyroidism, especially in multinodular goitres with autonomous nodules. It is now accepted that hyperthyroidism is one of the disorders induced by iodine deficiency.

### Treatment

The prolonged administration of iodide or of T<sub>4</sub> reduces the volume of goitre. Surgical treatment is rarely indicated. Unfortunately, these individual treatments are frequently impossible to apply on the whole population because of the magnitude of the problem and of the lack of medical infrastructure.

The logical medical attitude is to focus all efforts on the prevention. The principle is simple: the prevention of iodine deficiency = a regular and stable iodine administration.

### Prevention

#### *Diagnosis of endemicity*

Several factors can be taken into consideration when determining and quantifying the endemicity of the problems related to iodine deficiency:

#### **1. Prevalence of endemic goitre**

Its determination is based on the percentage of people with a goitre in a specific population. During field inquiries, the best method consists in examining the whole population of the region. In case of difficulties, it is allowed to limit these inquiries to children from 6 to 12 years. By palpation, a thyroid is considered goitrous when each lateral lobe has a volume greater than the terminal phalanx of the thumbs of the subject being examined. However, palpation of goitre in areas of mild iodine deficiency has poor sensitivity and specificity. In such areas, measurement of thyroid volume by ultrasound is preferable.

Classification	Description
<b>Grade 0</b>	No palpable or visible goitre.
<b>Grade 1</b>	A goitre that is palpable but not visible when the neck is in the normal position (i.e. the thyroid is not visibly enlarged). Thyroid nodules in a thyroid which is otherwise not enlarged fall into this category.
<b>Grade 2</b>	A swelling in the neck that is visible when the neck is in a normal position and is consistent with an enlarged thyroid when the neck is palpated.

Revised classification of goitre according to WHO/UNICEF/ICCIDD

## 2. Dosage of urinary iodine

It is difficult to measure precisely the food iodine content. When in nutritional balance, the intake of iodine equals the urinary excretion of iodine. Urinary iodine excretion is a good marker of the very recent dietary intake of iodine and therefore is the index of choice for evaluating the degree of iodine deficiency and of its correction. Iodine concentrations in casual urine specimens of children or adults provide an adequate assessment of a population iodine nutrition, provided a sufficient number of specimens is collected. Twenty four hours samples are difficult to obtain and are not necessary.

Relating urinary iodine to creatinine is expensive and unnecessary. However the median urinary iodine is often misinterpreted. Individual iodine intakes and therefore a spot urinary iodine concentration are highly variable from day-to-day, and a common mistake is to assume that all subjects with a spot UI <100 µg/L are iodine deficient.

For epidemiological studies, the population distribution of urinary iodine is required rather than individual levels. Because the frequency distribution of urinary iodine is usually skewed towards elevated values, the median is considered instead of the mean as indicating the status of iodine nutrition.

Median urinary iodine (µg/l)	Iodine intake	Iodine nutrition
< 20	Insufficient	Severe iodine deficiency
20-49	Insufficient	Moderate iodine deficiency
50-99	Insufficient	Mild iodine deficiency
100-199	Adequate	Optimal
200-299	More than adequate	Risk of iodine-induced hyperthyroidism following introduction of iodized salt in susceptible groups
> 300	Excessive	Risk of adverse health consequences: iodine-induced hyperthyroidism, auto-immune thyroid diseases

Table: Epidemiological criteria for assessing iodine nutrition based on median urinary iodine concentrations in school-aged children

## 3. TSH dosage (thyroid stimulation hormone)

TSH level in the serum are elevated in cases of iodine deficiency. However difficulties are often

encountered in obtaining venous blood samples in populations due to apprehension about blood collection and operational difficulties. Therefore these measurements are not routinely recommended in routine assessment and monitoring. In spite of the difficulties in blood collection, it has to be kept in mind that the final objective of correction of iodine deficiency is not only to increase the access of the population to iodized salt and to normalize the urinary iodine concentration but mostly to normalize thyroid function tests. Elevated serum TSH, unless exceptional pathological situations, indicates an insufficiency in the saturation of the T3 receptor in the brain, whatever the level of serum thyroid hormones. Therefore, elevated serum TSH constitutes an indicator of the potential risk of iodine deficiency on brain development. Serum T4 and T3 are less specific indicators of iodine deficiency because they are modified usually only in conditions of at least moderate iodine deficiency. Moreover these levels are largely influenced by age and sex. Elevated serum T3 in spite of low serum T4 is considered as a protective mechanism to most parts of the body, except the brain, where T3 is produced locally and not derived from the circulating T3.

The use of whole blood from finger pricks spotted on filter paper cards can be used at least for the measurement of serum TSH as indicators of thyroid hyperstimulation. A frequency distribution of serum TSH in neonates shifted to high values is a particularly sensitive index of the risk of potential damage of the developing brain due to iodine deficiency. In normal conditions, less than 3 % of neonatal TSH are above the critical threshold of 5 mU/L whole blood. However because of technical and financial limitations the use of this variable has been recommended only in countries and areas where a program of systematic neonatal hypothyroid screening is already implemented.

#### 4. Prevalence of cretinism

The study of the prevalence of cretinism can be completed by a study of the light forms (deaf muteness) when necessary. The prevalence of the cretinism can be up to 10 % of the whole population in certain regions.

#### *Criteria on the intervention level*

An operational definition of endemicity based on the experiences and a consensus between the experts has been refined and allows identification of the need for interventions in a formal manner. A zone is arbitrarily defined as affected by endemic goitre when more than 10 % of the children between 6 to 12 years suffer from goitre.

Iodine Deficiency	Severe	Moderate	Mild
<b>Number of cases of goitre among school children</b>			
Visible goitre	> 50 %	20-49 %	10-19 %
Total goitre	> 10 %	5-9 %	1-5 %
Urinary iodine (median, µg/l)	< 20 %	20-49 %	50-99 %
Prevalence of cretinism	>1%	<1%	0%

Indicators of iodine status at population level

In case of suspicion of endemic disease a fast inquiry on the prevalence among school children from 6 to 12 years old will give a first approximation of the magnitude of the problem. The consultation of a specialist is recommended for the following stages which will consist in

refining the endemicity diagnosis and in deciding if an intervention is a good idea and what sort of intervention is needed.

## Intervention strategies

### 1. Iodized salt

The iodination of salt is one of the most simple, least expensive and most efficient measures, in nutrition as well as in public health. It was used for the first time in 1917 in the United States. Since then its efficiency has been recognized in several countries: Guatemala, Argentina, Brazil, and Switzerland. It is a simple technology with an ignorable risk for toxicity. Iodine is added to the salt under the form of potassium iodide or, in humid tropical regions, potassium iodate because of its increased stability. The proposed concentration varies between 1/25.000 and 1/100.000 in function of certain criteria like the consumption of salt by the population and the severity of the deficiency. The cost averages 0.20 US\$/person/year and the efficiency of the program depends on:

- the control and monitoring of the iodine quantity
- the resistance of the producers of salt
- the geographical distribution of the production sites
- the distribution in the risk zones
- the accessibility of the iodized salt and the by-passes

Iodized salt is considered as the most appropriate measure for iodine supplementation. The advantage of supplementing with iodized salt is that it is used by all sections of a community irrespective of social and economic status. It is consumed as a condiment at roughly the same level throughout the year. Its production is often confined to a few centres which means that processing can occur on a larger scale and with better controlled conditions. However this is often not the case in developing countries.

The packaging of the iodized salt is very important. Jute bags have been used extensively but in humid conditions salt absorbs moisture. The iodate dissolves and will drop out of the bag if it is porous with a heavy loss. This has been found to reach 75% over a period of nine months. To avoid this waterproofing is required, achieved by a polythene lining inside the jute bag or else a plastic bag. The additional cost of a plastic bag (50-80% more) would be justified by reduced losses and their resale value.

### 2. Iodination of water

Water is really a good means of transportation with a large distribution and it is easy to adjust. There are no negative effects and costs are moderate. It can be done by iodizing the water distribution system or wells with slow release capsules. As salt, it is a daily necessity and thus the iodization will reach the most vulnerable groups.

### 3. Iodized oil

An iodized oil supplementation program is necessary when other methods have been found ineffective or can be considered to be inapplicable. Iodized oil can be regarded as an emergency measure for the control of severe IDD until an effective iodinated salt program can be introduced. Spectacular and rapid effects of iodized oil in reducing goitre can be expected.

Iodized oil can be given in injections (Lipiodol®) or orally. Protection of an oral dose is around one year, that of an injection four to five years.

The possibility of linking up an iodized oil program with childhood vaccination and antenatal care has been considered in the past. Diversification and modification of food habits in endemic zones is another preventive measure, but is challenging as it often requires importation of sea food to remote areas.

### *Monitoring*

In the countries that have begun iodized salt programs, sustainability is a major focus. These programs are fragile and require a long-term commitment from governments. In several countries where iodine deficiency had been eliminated, salt iodization programs fell apart and iodine deficiency recurred.

The indicators used in monitoring and evaluating IDD control programs include:

- 1) Indicators to monitor and evaluate the salt iodization process (Process indicators)
- 2) Indicators to monitor the impact of salt iodization on the target populations (Impact indicators).

The impact indicators include in order of priority the determinations of urinary iodine, of the prevalence of goitre and of the serum levels of TSH and thyroid hormones. It is now considered that iodine deficiency has been eliminated from one particular country when the access to iodized salt at household level is at least 90 %, together with a median urinary iodine of at least 100 µg/L and with less than 20 % of the samples below 50 µg/L.

### *Side effects of iodine supplementation*

The effect of iodine on the thyroid gland is complex with a U shaped relation between iodine intake and risk of thyroid diseases as both low and high iodine intake are associated with an increased risk. It is stated that normal adults can tolerate up to about 1000 µg iodine/day without any side effects.

However this upper limit of normal is much lower in a population which was exposed to iodine deficiency in the past. The optimal level of iodine intake to prevent any thyroid disease may be a relatively narrow range around the recommended daily intake at 150 µg.

The possible side effects of iodine excess are as follows:

#### **1. Iodide goitre and iodine-induced hypothyroidism**

When the iodine intake is chronically high, as for example in coastal areas of Japan and China due to the chronic intake of seaweeds rich in iodine such as laminaria or in Eastern China because of the high iodine content of the drinking water from shallow wells, the prevalence of thyroid enlargement and goitre is high as compared to normal populations and the prevalence of subclinical hypothyroidism is elevated. The mechanisms behind this impairment of thyroid function are probably both iodine enhancement of thyroid autoimmunity and reversible inhibition of thyroid function by excess iodine (Wolff-Chaikoff effect) in susceptible subjects. However, this type of thyroid failure has not been observed after correction of iodine



deficiency, including in neonates after the administration of huge doses of iodized oil to their mothers during pregnancy.

## **2. Iodine-induced hyperthyroidism**

Iodine-induced hyperthyroidism (IIH) is the main complication of iodine prophylaxis. It has been reported in almost all iodine supplementation programs. Iodine deficiency increases thyrocytes proliferation and with the development of multifocal autonomous growth. These nodules become autonomous and can result in hyperthyroidism after iodine supplementation. A multicentre study conducted in seven African countries, including Zimbabwe and Congo showed that the occurrence of IIH in the last two countries was due to the sudden introduction of poorly monitored and excessively iodized salt in populations which had been severely iodine deficient for very long periods in the past.

The conclusion of the multicentre study was that the risk of IIH is related to a rapid increment of iodine intake resulting in a state of acute iodine overload.

It thus appears that IIH is one of the Iodine Deficiency Disorders. It appears to be largely unavoidable in the early phase of iodine supplementation. It affects principally the elderly with long lasting autonomous nodules. Its incidence reverts to normal or even below normal after one to ten years of iodine supplementation.

## **3. Iodine-induced thyroiditis**

Another possibility is the aggravation or even the induction of autoimmune thyroiditis by iodine supplementation. However, no large surveys have been performed which have analyzed the impact of large scale programs of iodine supplementation on the occurrence of clinically significant iodine-induced thyroiditis with public health consequences on thyroid function.

## **4. Thyroid cancer**

Although in animal studies the chronic stimulation of the thyroid by TSH is known to produce thyroid neoplasms, in humans correction of iodine deficiency rather decreases the risk of and morbidity from thyroid cancer.

## Konzo

### Summary

- Acute hypertonic paraparesis
- Cyanide intoxication caused by badly processed bitter cassava (manioc)
- Other factors such as deficiency of sulphur-containing amino acids seem to be important

### Definition

Konzo is characterised by an epidemic acute isolated and symmetrical hypertonic paraparesis, which is permanent but non-progressive. The condition is to date only known in poor regions of Africa. In the Yaka valley konzo means "bound legs", a good description of the hypertonic gait. This is the name used in Congo and is now the official term for this motor neuron disorder.

### Epidemiology

Two large epidemics have been reported, each of more than 1000 cases. The first was in the Bandundu region in Congo (1936-37) and the second in the Nampulla province of Mozambique (1981). Outbreaks that are related to households living in absolute poverty that have sustained themselves for weeks or months on insufficiently processed bitter cassava, have been reported from 6 countries: Congo (esp Bandundu region), Mozambique, Tanzania, the Central African Republic, Cameroon and Angola. The total number of reported cases up to 2009 was 6788, but most cases are never reported and there are estimates of 100,000 cases in DRC alone. The majority of cases of konzo occur in the dry season, chiefly during a long drought. Sporadic cases of konzo also occur. Children who are being breastfed are not affected. Familial clustering is common.

### Aetiology

The aetiology of konzo has not yet been fully clarified. At present a toxic/nutritional aetiology is assumed. There is an epidemiological connection between konzo and eating bitter cassava. Nevertheless, konzo only occurs in 1% of the cassava consuming population. Consumption of bitter cassava is a precondition, but not in itself sufficient to induce konzo. Cassava contains very little sulfur and shortage of sulfur-containing amino acids are probably contributory, since these are essential for the detoxification in the body of cyanide to thiocyanate, which is removed in the urine. People of the same ethnic group living only 5 km away from those with konzo might have a near zero konzo prevalence which is related to different protein intake through fish or bushmeat. It is possible that as yet unidentified components also play a role. Due to its clinical similarity to neuroleptism, a search for the neurotoxin beta-ODAP was performed but turned up negative. Epidemics coincide with periods of food shortage, drought, intense trading in cassava and war. These are circumstances in which people may be inclined to shorten the long preparation which bitter cassava requires. If shortcuts are taken to process the cassava quickly, large amounts of cyanogens may remain in the food. The disorder is regarded as a form of cyanide intoxication, although the final word on this has not yet been spoken.

### Cassava



Cassava originated in South America and was first cultivated by the Maya in Yucatán. It was introduced in Africa by Portuguese traders from Brazil in the 16th Century and around the same era it arrived in Asia with Portuguese and Spanish ships. There are various species, all belonging to the Euphorbiaceae: *Manihot esculenta*, *M. aipi* and *M. utilissima*. Over the last 400 years, the plant has become a staple for millions of Africans, especially those in areas with marginal land where few other crops survive. Cassava is known by several names in tropical and subtropical countries: manioc, yuca, mandioca, Brazilian arrowroot. It is named tapioca when it is dried to a powdery extract. Food items such as the gelatinous porridge “fufu” in West- and Central Africa and the bammy of Jamaica come from cassava. Cassava is a woody shrub and is extensively cultivated as an annual crop for its edible starchy tuberous root, a major source of carbohydrates. The young leaves and shoots may be eaten as vegetables (“saka saka”). Cassava is the third-largest source of food carbohydrates in the tropics, after rice and maize. It is a major staple food in the developing world, providing a basic diet for over half a billion people. It is very drought-tolerant and grows on marginal soils where other crops do not grow well. It is usually harvested after 18 months. Cassava roots are poor in protein, but the leaves are a good source of protein rich in lysine. The cassava roots, when they are still attached to the stalk, remain good for many months if stored under the earth. Once harvested deterioration begins quite quickly. There is an unwanted conversion of starch to sugar and a number of enzymatic reactions occur which cause discoloration of the product and reduces its value. Bacterial and fungal deterioration also occur. Drying the roots to a moisture content of less than 14% prolongs their storage life considerably.

Apart from its nutritional value, cassava has several other uses: alcoholic beverages made from cassava have distinct local names (cauim and tiquira (Brazil), kasiri (Guyana, Suriname), impala (Mozambique), masato (Peruvian Amazonia chicha), parakari or kari (Guyana), nihamanchi (South America) also known as nijimanche (Ecuador and Peru), ö döi (chicha de yuca, Ngäbe-Bugle, Panama), sakurá (Brazil, Suriname), tarul ko jaarh (Darjeeling, Sikkim, India)); ethanol biofuel made from cassava is increasingly used in China; cassava serves as a good roughage source for ruminants such as cattle and manioc starch diluted in water can be sprayed over clothing before ironing to stiffen collars. It was claimed that cassava has anti-cancer activity but a report from the American Cancer Society states that “there is no convincing scientific evidence that cassava or tapioca is effective in preventing or treating cancer”. Nigeria is the world's largest producer of cassava producing 57 million tons or 21% of the world total, while Thailand is the largest exporter of dried cassava.

There are "sweet" and "bitter" varieties, indicating the absence or presence of toxic cyanogenic glucoside levels, respectively. In particular the bitter form survives well under dry conditions. Bitter cassava produces up to 1 g/kg of cyanide, especially during prolonged dry seasons. This is 50 times more than the sweet variety. The more toxic varieties of cassava are a fall-back resource (a "food security crop") in times of famine or food insecurity in some places. Farmers often prefer the bitter varieties because they deter pests and animals. If large amounts of bitter cassava are eaten for long periods, without special precautionary measures being taken to remove the toxin from the plant, and if there is a deficiency in sulphurcontaining amino acids Konzo results.

## Pathophysiology

The capacity to produce toxic hydrogen cyanide is present in more than 2000 plant species, classified into over 100 plant families. In all cases the HCN is not stored as such in the cells. The plant produces complex molecules, generally glucosides (e.g. amygdalin) but also some lipids. From these, HCN can enzymatically be released. The enzyme that accelerates this reaction is physically separated from the cyanogenic substance. If the plant is crushed and its structural integrity is threatened, the enzyme comes into contact with the cyanogenic substance and the reaction can then take place. It can be assumed that the cyanide is intended to protect the plant from damage.

In cassava, above mentioned process is mirrored as follows. The bitter varieties contain large amounts of the two cyanogenic glucosides linamarin and lotaustralin, in a ratio of 10 to 1. Linamarin is found in vacuoles in the cytoplasm. The concentrations are highest in the peel. Linamarase, the enzyme which breaks down linamarin, is found in the cell wall. When the cells burst (accidental crushing of the plant, being eaten by insects or during processing), the linamarin comes into contact with linamarase. This enzyme splits linamarin into glucose and acetone cyanohydrin. The latter spontaneously releases acetone and HCN. This reaction may be accelerated by the cassava enzyme hydroxynitril lyase. Once HCN has been produced, it spreads in the air as gas (boiling point of HCN =25.7°C).

Cyanides are rapidly acting toxic substances. Cyanide (CN<sup>-</sup>) inhibits cellular respiration by binding to the trivalent iron (Fe<sup>3+</sup>) of cytochrome oxidase, a component of the mitochondrial electron transport chain. This impairs the energy-generating function of the mitochondria, leading to cell death.

Cyanide (CN<sup>-</sup>) is normally converted in humans to the less toxic thiocyanate (SCN<sup>-</sup>) by the enzyme rhodanase (also written as rhodanese). This is a mitochondrial enzyme which is widely present throughout the human body, with the highest concentrations in the liver and kidneys. Thiocyanate is the chief metabolite of cyanide. Thiocyanate itself has a goitrogenic effect if there is a shortage of iodine in the diet. The body uses sulphur-containing amino acids to render cyanide harmless. If the diet is deficient in sulphur, cyanide will be converted to cyanate (OCN<sup>-</sup>), which induces neurodegenerative disease in both animals and humans. The cells which are most affected are Betz' cells in the motor cortex.

## Clinical aspects

Konzo begins abruptly, without prodromal signs. In 90% of cases the onset of symptoms takes less than one day. The initial symptoms are described as tremor, cramps, a heavy feeling and/or weakness in the legs, a tendency to fall down and difficulty remaining upright. There is a visible hypertonic gait when walking or running. Occasionally there will be lower back pain, blurred vision, speech difficulties and/or paraesthesia of the legs, but they disappear within a month. During the first two days the majority of patients have general muscular weakness and are confined to bed. Hypertonicity is present from day one. Flaccid paralysis of the limbs does not occur. Since this is an upper motor neuron disorder, very brisk reflexes are found in the legs and Babinski's sign is present. Pronounced clonus occurs, or may be triggered by physical examination, e.g. dorsiflexion at the ankle joint. Later there is a slight partial

improvement. Finally the affected person develops a stable hypertonic paraparesis, which persists for the remainder of life, or might improve a little. After onset the neurological signs remain constant or improve minimally if no further cyanide is ingested, unlike for example HTLV-1 infection in which further deterioration takes place. Some sufferers will later have a second attack with deterioration of their condition, possibly with dysarthria, abnormalities of eye movement, hypertonicity of the arms.



Konzo, symmetrical spastic paraparesis; ©Studio Leyssen 14; winner 'Best Medical picture 2017, the Lancet'

## Differential diagnosis

Lathyrism is a neurological disease caused by eating large quantities of the Lathyrus grain that has high concentrations of the neurotoxin  $\beta$ -oxalyl-L- $\alpha,\beta$ -diaminopropionic acid (ODAP). It causes paralysis due to upper motor neuron damage. It is mainly seen in Bangladesh, India, Nepal and Ethiopia. Tropical spastic paraparesis has symptoms similar to konzo, but the onset is much slower. Polio can be easily distinguished as it provokes an asymmetrical flaccid paralysis.

Chronic, low-level cyanide exposure can lead to the tropical ataxic neuropathy (TAN) that manifests with polyneuropathy, ataxic gait, optic atrophy and sensory deafness. It was first described by Osuntokun among the Ijebu speaking Yorubas in south western Nigeria in 1968. Till today TAN remains an enigmatic disease with no effective treatment. The exact pathogenesis remains unresolved, and several factors have been proposed including malnutrition, vitamin B deficiencies, malabsorption, poor protein consumption, chronic cyanide and nitrile toxicity, with a strong geospatial endemic prevalence in areas of cassava cultivation.

### *Motor neuron disease*

The term "motor neuron disease" includes disorders in which (1) both the upper and the lower motor neurons are affected (amyotrophic lateral sclerosis), (2) disorders in which only the lower motor neurons are abnormal (spinal muscular atrophies, post-poliomyelitis, Guillain-Barré syndrome, botulism, trauma) and (3) disorders of exclusively the upper motor neurons (neurolethyrism, konzo, hereditary spastic paraplegia, primary lateral sclerosis, stroke, multiple sclerosis, cerebral palsy, trauma).

Symptoms of upper motor neuron disease (= lesion above the anterior horn cell of the spinal cord or the motor nuclei of cranial nerves): muscle weakness, spasticity, clasp-knife response, Babinski sign present, increased deep tendon reflexes. Symptoms of lower motor neuron diseases (= lesion in nerves distal from the anterior horn of the spinal cord or lesion in fibres from the cranial motor nuclei to the muscles): muscle paresis or paralysis, fasciculations, hypotonia, hyporeflexia, muscle wasting.

## Diagnosis

The following criteria are used for the diagnosis of konzo:

1. A visible symmetric hypertonic gait when walking or running
2. The onset of the disease takes less than one week and then remains stable
3. Bilateral brisk knee and Achilles tendon reflexes without signs of vertebral lesions
4. Eating bitter cassava and no consumption of grass peas (*Lathyrus sativus*)

Urinary concentrations of thiocyanate and linamarin are elevated. The patient is HTLV-1 negative.

## Treatment

There is no known etiological treatment for konzo. Treatment with sodium thiosulphate ( $\text{Na}_2\text{S}_2\text{O}_3$ ), a cyanide antidote, gave disappointing results. A good and varied diet, high dose multivitamins and physical rehabilitation with walking aids are advised. Since the sufferers have no cognitive defects, affected children should be encouraged to continue their education. Some children have been operated with an elongation of the Achilles tendon which improved the position of the foot but the long term outcome remains uncertain.

## Prevention

Konzo is not a large public health problem when Africa is regarded as a whole. It is, however, a real problem in the communities affected and of course for the individual patient. The message should be that (1) konzo is not infectious in order to avoid sufferers becoming socially isolated, (2) cassava should be processed correctly without missing out any steps (shortcuts in processing are to be avoided), (3) a varied diet is important. Including maize (corn) flour when making porridge, or including other sulphurcontaining food product, such as onions in the diet, is advised; but food habits take a long time to change. The tubers can be made safe by correct processing. As a first step the cells should be burst in order to bring the linamarin into contact with the endogenous glucosidase. In a second step (drying or heating) cyanohydrin is converted to hydrogen cyanide which then evaporates (this is faster at a higher temperature). One of the following precautionary measures should be taken when preparing cassava:

- Fermenting by immersion in water, followed by drying in the sun or cooking, (sufficient time necessary, usually 3 days or longer if the water is cold)
- Grating and fermenting of fresh pulp followed by drying with heat (3 days needed).
- Direct drying of the roots in the sun (less effective)

# Snakes

## Summary

- Not all snakes are venomous
- Often dry bites by venomous snakes
- Vipers: primarily haemorrhages and necrosis
- Elapids: primarily paralysis and necrosis.
- No arterial tourniquet.
- Pressure-immobilisation technique during transport (neurotoxic snakes)
- Antivenom if symptoms of envenomation
- Neostigmine + Atropine if paralysis
- Potential side effects of antivenom (anaphylaxis, serum sickness)

## Description

There are around 2700 snake species, including around 375 venomous snakes with medical relevance. Of the latter, around 200 are potentially lethal. The biotopes vary greatly: from the arctic circle to the equator, and from sea level to 5000 m in elevation. Venomous snakes are not found in Chile, Madagascar, New Zealand, Hawaii and New Caledonia. In Belgium there are a very small number of indigenous vipers (*Vipera berus* = common European adder), ringed snakes (*Natrix natrix* or grass snake) and smooth snakes (*Coronella austriaca*). The last two are not venomous.

It is estimated that at least 421,000 envenomings and 20,000 deaths (figures may be as high as 1,841,000 and 94,000 resp.) occur annually worldwide. The highest burden of snakebites is in South Asia, Southeast Asia and sub-Saharan Africa. People most at risk are agricultural workers and children. One of the most frequently bitten people are drunken young men harassing a snake.

The majority of snake-bite victims seek traditional treatment and may die at home unrecorded. The amount of disability (permanent sequelae due to snakebites) is unknown and underreported. Although it is more common in rural areas, snakes can be present in town areas (e.g. in India). Snake bites have been recognized as a neglected disease by WHO.



*Crotalus atrox*, the Western diamondback rattlesnake. Photo Protherics, used with permission



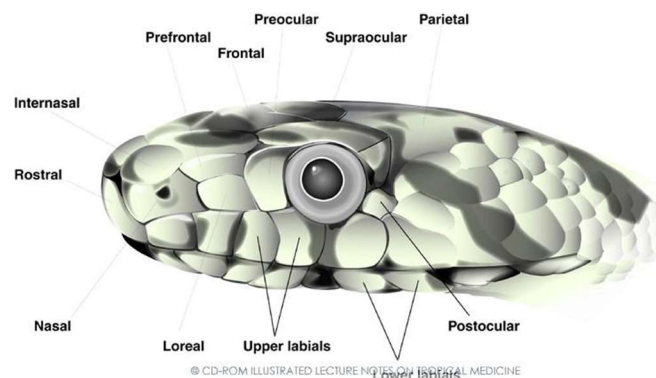
Juvenile *Elaphe situla* snake (Leopard snake), not venomous. This escaped (?) specimen was found in the middle of a main street in Antwerp, Belgium. Illegal breeding of protected species is common. Copyright ITM

### *Biology of snakes*

Snakes are quasi-cylindrical reptiles without limbs. They move using a concertina movement, rectilinear, curvilinear, via "sidewinding" or by a combination of these methods. There are even 5 species of "flying" (gliding is a better word) snakes. In snakes, the left lung is atrophic, except in boas. The right lung can have an extension in the throat, which is important for the animal because there is airway compression when it swallows large prey. In general, the length of the lung is about one-half of the total body length, although in seasnakes the lung is longer. In reptiles, the nostrils come out in the mouth cavity (there is no palate to separate the mouth cavity from a nasal cavity). They can breathe through their mouth if it is empty. A full mouth blocks respiration. They can tolerate apnoea for a fairly long time, because as poikilothermic animals they have a rather low oxygen demand. By exhaling quickly some snakes can produce a hissing noise (cf. the puff adder).

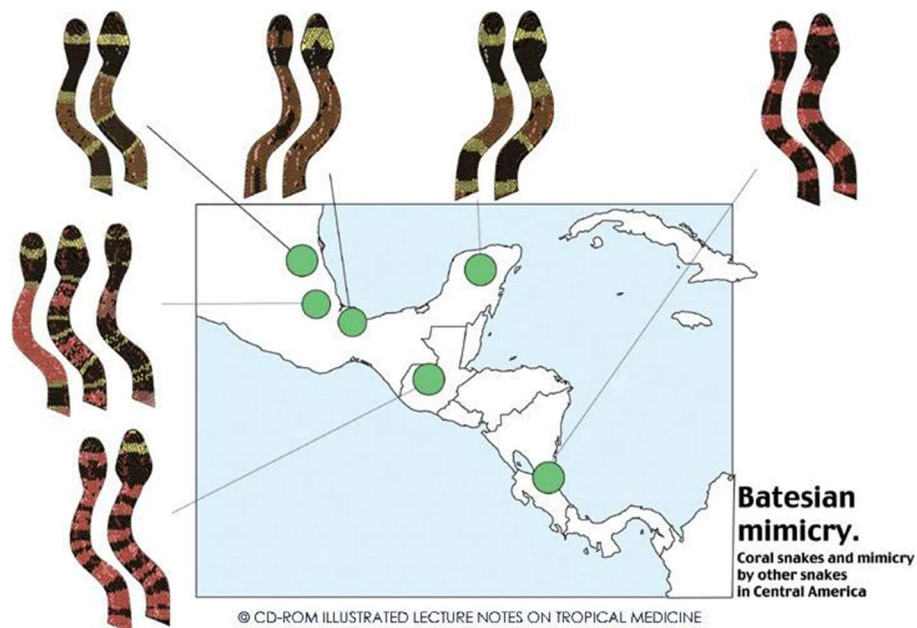
### *Description, scales and colour*

**SNAKE: terminology head scales**



The scales on the head of a snake are rather constant within a species and are used for taxonomic identification. Adapted from "The Encyclopedia of Snakes" by Chris Mattison





Examples of Batesian colour and shape mimicry in Central American snakes. The left snake in each pair and the outermost snakes of the triple cluster are dangerous *Micrurus* species. The snakes on the right side and the central of the 3 snakes are nonvenomous *Pliocercus* sp. The similarity is striking. Drawing adapted by ITM, from original.

Stripes and/or spots can act as a camouflage, breaking up the visual outline against the surroundings. A harmless snake can imitate a venomous one when both live in the same environment, i.e. Batesian mimicry (1861, Henry Walter Bates, English naturalist). In this way predators avoid the snake, if they have learned earlier that an animal with such coloration is dangerous.

#### *Description, heat sensors and Jacobson's organ*

Most snakes have poor hearing and limited visual acuity. By contrast, in the roof of their mouth they possess an extremely sensitive organ, known as a Jacobson's organ. It consists of two openings lined with sensory cells. The animal flicks out its forked tongue and brings it back into the mouth, inserting the tips into the two openings of Jacobson's organ. The tongue brings molecules from the environment into the organ. In this way the snake can sense its environment. Snakes are very good at perceiving vibrations, e.g. of the ground. Some people use this as a means of prevention, by regularly beating a stick on the ground in front of them when they walk in an area with venomous snakes.

#### *Description, food and body heat*

All snakes are carnivorous. Because they do not have to continually maintain their body at a constant temperature, their food intake requirement is a good deal lower than that of warm-blooded animals. Because chronic "constipation" is most pronounced among sit-and-wait predators - animals for which body weight is of great importance - some people assume that these snakes make good use of the extra weight (3 to 22% of their body weight is faecal material). These animals lie still on the ground and use their heavy intestine as a counterweight in order to be able to strike more quickly with the mouth.

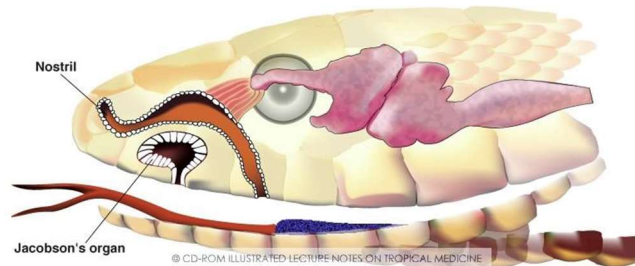
Since the environment of the snake is so important for the animal, it is not unusual for a snake to lie at night on a path or road, where the temperature is somewhat higher than in

nearby vegetation. Obviously this increases the chances of an accidental bite being suffered by a night time walker. In order to conserve heat, they can roll themselves up (small surface/weight ratio).

This is also important to limit transcutaneous loss of water. In cold regions snakes can hibernate, individually or in a group.

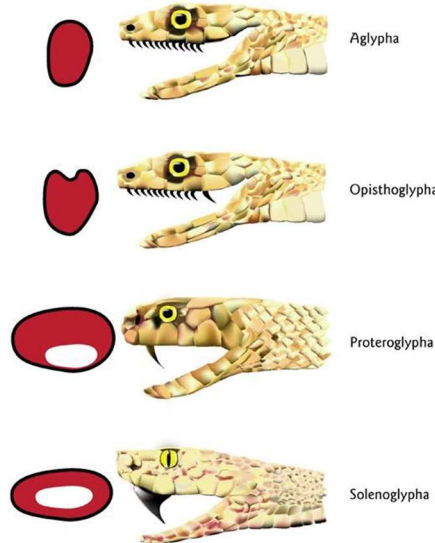
Many snakes have a limited territory. After having bitten somebody, a snake can generally be found within a rather small radius around the site of the incident, even after several hours.

### Description, venom gland



Jacobson's organ in the roof of the mouth of a snake is covered with chemosensory cells. The tongue will bring chemicals from the environment in contact with the epithelium. Nerves connect the organ to the olfactory lobes of the brain.

#### FANGS



Snakes. The structure of the fangs differs between taxonomic groups. Copyright ITM

Colubrids have a modified salivary gland (Duvernoy's gland), which discharges near the fangs at the rear of the mouth. The venom is slowly introduced into the prey via capillary action. Therefore, in order to get sufficient venom into the tissues, a long contact period is necessary. However, this occurs only exceptionally in humans. This explains why most bites by colubrids are harmless. This also explains why occasionally envenomations are described by snakes that traditionally are regarded as non-venomous. In elapids and vipers, in contrast the venom glands consist of the uppermost labial salivary glands. They can be actively emptied by the musculus constrictor glandulae, so that the animals can actively and very quickly inject venom, or even spit venom (several meters).

*Description: jaws, fangs and teeth*

The left and right sides of the jaws can move independently of one another. This makes it possible to swallow large prey, yet the animals cannot chew. Snakes have no sternum, so that a large ingested prey does not constitute a mechanical obstacle when it is being swallowed (some prey have a diameter which is greater than the resting diameter of the snake).

In snakes, the teeth are not so firmly attached to the top/inner side of the jawbones (so-called "pleurodont dentition"). This makes it possible for the teeth to be easily replaced throughout a snake's lifetime. The teeth break off easily. This influences the biting behaviour. Thus vipers bite, inject venom and release again in rapid succession, because a struggling prey could cause injury or break the teeth.

A temporomandibular joint is a purely mammalian characteristic that is not found in snakes. In snakes, the joint between lower and upper jaw is formed by the os articulare at the bottom and the os quadratum (quadrate bone) at the top.

### Infections transferred via snakes



Pentastomiasis. *Armillifer armillatus* causes porocephalosis in humans. This tongue worm normally infects snakes. Copyright ITM



C-shaped calcifications due to infection with *Armillifer armillatus*, a tongue worm. Pentastomiasis is also known as porocephalosis. Photo ITM

Pythons can be infested by tongue worms (Pentastomida) such as *Armillifer armillatus* in Africa or *A. moniliformis* in Asia. These parasites live in the lungs of the reptiles. The eggs in the snake's sputum can infect humans, e.g. through contamination of drinking water or when a snake is prepared as food.

Porocephalosis (syn. pentastomiasis) is the result. In general, infection leads to asymptomatic crescent-shaped calcifications in the abdomen. Living parasites are rarely found elsewhere (e.g. subconjunctival). *Gnathostomiasis* (infection with the nematode *Gnathostoma spinigerum*) can also follow consumption of undercooked snake meat. A larva migrans syndrome or a very serious eosinophilic meningo-encephalitis can then develop. *Spirometra* sp. can be transferred via snakes (also via frogs) and cause sparganosis, whereby the immature cestode can be found in the eye. These worms can survive for up to nine years in humans.

## Taxonomy

### Introduction

The classification is important because a certain correlation exists between snake family and pathology. This correlation is not absolute. Studying the fangs in the mouth of a dead snake which has been brought in can help determine the treatment. However, it is better to be cautious when doing this (the bite reflex can continue for over 1 hour after death even after decapitation). It can be useful to have on hand a number of photos or a poster illustrating most of the snakes in the surrounding area. On the basis of these pictures, a patient can sometimes indicate which animal has bitten him or her.

**Table 1: Examples of venomous snakes**

<b>Snake family</b>	<b>Species - some examples</b>
<p><b><u>Elapidae</u></b></p> <p>A large and diverse Family of exclusively venomous snakes, covering all continents (except Antarctica) and several major oceans, these snakes have well developed fangs towards the front of the mouth, which can deliver often highly potent venom, produced in paired venom glands.</p>	<p>Cobra's, including spitting cobra's (<i>Naja</i>), coral snakes (<i>Micrurus</i>, <i>Micruroides</i>)</p> <p>Kraits (<i>Bungarus</i>)</p> <p>Mamba's (<i>Dendroaspis</i>) → <i>D.polylepis</i></p> <p>Sea snakes</p>
<p><b><u>Viperidae</u></b></p> <p>A large and diverse Family of exclusively venomous snakes, covering most continents (except Australia and New Guinea, Antarctica), with a highly evolved fang structure. The fangs are at the front of the mouth, attached to a mobile maxilla, enabling the fang to fold away against the roof of the mouth, thus permitting longer fangs compared to head size.</p>	<p>Subfamily Viperinae "Old World" and Subfamily Crotalinae (Pit Vipers)</p> <p>Russel's Viper</p> <p>Puff viper, Gabon Viper, rhinoceros -horned viper (<i>Bitis</i>)</p> <p>Bush Viper (<i>Atheris</i>)</p> <p>Echis carinatus</p>
<p><b><u>Colubridae</u></b></p> <p>This is the largest Family of snakes, generally considered non-venomous and distributed globally. However, a few species have evolved fangs towards the back of the mouth, which deliver venom from venom glands.</p>	<p>Dispholidus typus (Boomslang) – sub-Saharan Africa</p> <p>Thelotornis (Twig Snake)</p>
<p><b><u>Atractaspididae</u></b></p> <p>A small Family of exclusively venomous snakes, found only in Africa and the Middle East, characterised by their side-striking</p>	<p>Atractaspis microlepidota (Burrowing Asp)</p>

fangs and unique venom components (sarafatoxins), only a few species of which appear able to significantly envenom humans.	
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### *Taxonomy, vipers (Viperidae)*

Vipers and pit vipers have very long hollow fangs in the front of the mouth. When the mouth is closed the fangs lie folded up against the roof of the mouth. Vipers are slow, heavy snakes and are generally "sit-and-wait" predators. They move flat over the ground. One does not expect vipers to be present among tree branches for example. Venomous European vipers have vertical pupils. Non-venomous snakes in Europe have round pupils.

### *Daboia russelli*

Russell's viper (*Daboia russelli* = *Vipera russelli* = "tic-polonga") is one of the most dangerous Asian snakes. This nocturnal animal is often lethargic and will avoid dense jungle. It can hiss loudly through its large nostrils. There are 5 subspecies, which is important because antivenom from one country is often not effective on the local subspecies in another country. The symptomatology too will depend on the subspecies: pituitary haemorrhages and chemosis in Burma and southern India, anticholinesteraseresistant neurotoxicity in India and Sri Lanka; haemorrhages with all subspecies.

### *Bitis arietans*

The puff adder (*Bitis arietans*, la vipère heurtante) gives rise to considerable problems in Africa. They can strike very quickly.

### *Bitis nasicornis*



*Bitis nasicornis* belongs to the vipers. The exact function of the horns on the snout of this snake is not clear.

Copyright ITM

### *Vipera berus* (Common viper or adder)

*Vipera berus*, the common European adder or common European viper, is a venomous viper species that is extremely widespread and can be found throughout most of Western Europe and as far as East Asia. Known by a host of common names including common adder and common viper, adders have been the subject of much folklore in Britain and other European countries. They are not regarded as especially dangerous; the snake is not aggressive and usually bites only when alarmed or disturbed.

Bites can be very painful, but are seldom fatal.



Vipera Berus distribution in Europe

## Bitis gabonica



*Bitis gabonica* is also known as the Gabon viper. Most animals have typical markings. Copyright ITM

## Echis carinatus complex

The saw-scaled vipers are among the most important venomous snakes in the world, it is estimated that they are responsible for 50% of the global mortality caused by snakes.

### *Taxonomy, pit vipers (Crotalidae)*

The pit vipers or Crotalidae get their name from the presence of two pits at the front of the head, about halfway between the eyes and the nostrils. These contain infrared sensors with which the animal can better locate its prey.

## Agkistrodon sp.



Pit viper: *Agkistrodon piscivorus*, also known as Mocassin. Courtesy of Protherics

## Crotalus sp.



Pit viper: North American rattlesnake, *Crotalus scutulatus*. Image courtesy of Protherics (producers of CroFab antidote).



*Crotalus atrox*, the Western diamondback rattle snake. Photo Protherics, used with permission

Rattlesnakes belong to the genus *Crotalus* and *Sistrurus*. When a rattlesnake administers a venomous bite to a human being, it injects 25-75% of its venom. It takes on average 3 weeks for the venom supply to be entirely replenished. Rattlesnakes have a typical tail structure. The rattle is used when the snake feels threatened. In this situation, the snake will raise its head and front part of the body, as well as the rattle and hold the body in an S-shape, ready to strike. The North American *Crotalus cerastes* is also called the "sidewinder", referring to the way it moves. There are several desert snakes which demonstrate this behaviour.

### *Taxonomy, burrowing vipers or Atractaspididae*

These animals (mole vipers or burrowing vipers) were earlier classified among the Viperidae, but currently form a separate family with over 50 species. They are primarily found in Africa. They are rather small animals, although some individuals can be as long as 1 meter. They live primarily underground. Bites are rare, but can have serious consequences. The hollow fangs can be moved sideways, even without opening the mouth. The venom of *Atractaspis engaddensis* contains an extremely powerful cardiotoxin, the so-called "sarafotoxin", a word deriving from the Hebrew "Saraf 'En Gedi" (saraf meaning 'snake', En Gedi refers to an oasis in the Judea desert in Israel).

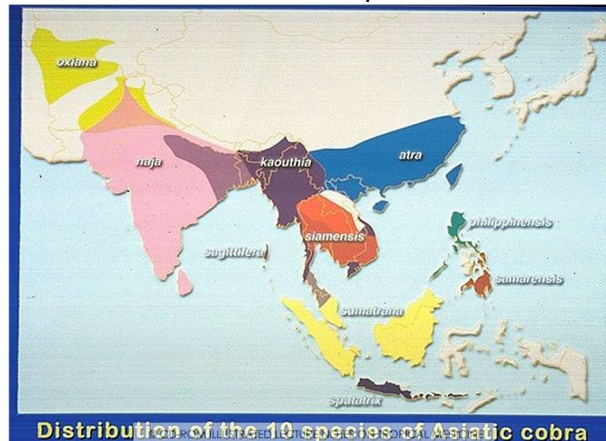
### *Taxonomy, Elapidae*

This family includes the cobras, mambas, kraits and coral snakes (Gr. Elaps: snake). The venom

produces primarily local necrosis and paralysis. Elapids have moderately short, immobile fangs on the maxillae, at the front of the mouth. They cannot be folded backwards as in vipers. Often these snakes have small teeth behind the fangs and sometimes there is a small diastema.

### Cobras

A cobra often raises its head and neck when it is threatened. The animals are characterised by the typical "hood", the widening of the neck caused by spreading its cervical ribs when threatened. The king cobra (*Ophiophagus hannah*) is a very large Asian elapid, which eats other snakes. Some African and Asian cobras can spit venom.



Distribution of the 10 species of Asiatic cobra  
Geographical distribution of asiatic cobras (*Naja* sp.). Copyright ITM

### Coral snakes

Elapids also live in the New World: the coral snakes. They often have a beautiful colour pattern. A mnemonic device for the colour bands in North America: "red on yellow, kill a fellow; red on black, venom lack". This phrase does not work in other geographical areas.

### Bungarus sp. : Kraits

Often the animals are distinctly passive during the day. At night, however they are active and they sometimes enter houses and bite. People with krait bites generally experience remarkably little local pain.

### Dendroaspis sp. : Mambas

Mambas are only found in sub-Saharan Africa. These venomous snakes are notorious. They belong to the genus *Dendroaspis*: *D. polylepis* (black mamba), *D. viridis* (Western green mamba), *D. angusticeps* (Eastern green mamba) and *D. jamesoni* (Jameson's mamba).

### Australian elapids

The fauna of Australia is complex, and it differs in many ways from the fauna on other continents. The medical relevant Australian snakes belong to the elapids.

### Taxonomy, sea snakes or Hydrophiidae

The taxonomical classification is controversial, but these animals can be classified among the Elapidae or be grouped in their own family. Taxonomically they are broken down into the



Hydrophiinae (real sea snakes) and the Laticaudinae (sea kraits). In some taxonomic diagrams these groups get the status of family: Hydrophiidae and Laticaudae.

### *Taxonomy, Colubridae*

The name derives from the Latin "coluber", which means snake. Only a few are genuinely dangerous.

They have short small fangs on the maxillae at the back of the mouth so that they have to open their mouth very wide (170 to 180°) to inject venom. They also require a long contact period to introduce enough venom into the bite wound. Colubrids are often kept as pets. , e.g. *Elaphe* sp. (rat snakes) or *Lampropeltis* sp. (king snakes, milk snakes). The boomslang (*Dispholidus typhus*) in southern Africa is another dangerous colubrid, yet bites by this animal are quite exceptional. Haemorrhages are the most obvious symptom after a bite by a boomslang.

### *Taxonomy, Boidae*

The Boidae include boas and pythons. Constrictor snakes such as the anaconda, boas and pythons are not venomous. Boas are viviparous snakes from the New World and pythons are oviparous snakes from the Old World. Because they must be able to hold their body in small-diameter loops, they have short vertebrae. When they are wrapped around their prey, what makes them so deadly is not that they squeeze so hard, but rather that they can very effectively resist attempts to stretch. Every time the unfortunate prey exhales, the snake contracts a little bit more, and prevents the prey from inhaling.

After this has been repeated a few times, the prey simply suffocates.

## Distribution

As far as native venomous snakes are concerned, only vipers are found in Europe.

In Africa there are elapids, vipers and colubrids.

The most important snakes in America are the pit vipers and several coral snakes.

In Asia, all families are represented (but not all genera).

A number of elapids live in Australia.

Problems with venomous sea snakes are limited to coastal areas of Asia and Australia.

Imported exotic pet snakes can be responsible for bites, especially in affluent countries.

### *Viper populations in Belgium*

There are three isolated wild viper populations in Belgium: the largest one in Brecht (Groot Schietveld), and much smaller populations in Kalmthoutse Heide and in the Visbeekvallei (Lille).

## Distribution of the most important snakes

It is useful to have an idea of which major venomous snakes can be found where.

In **Southeast Asia** Russell's viper (*Daboia russelli*), *Echis carinatus*, the habus and the Malayan pit viper (*Calloselasma rhodostoma*) are the most important.

In **Africa** the saw-scaled vipers (*Echis carinatus complex*), the puff viper (*Bitis arietans*) and to a lesser extent cobras and mambas are important.

In **South and Central America** the cascabel (*Crotalus durissusterrificus*), jararaca (*Bothropsjararaca*) and fer-de-lance (*Bothrops atrox*) are the most important venomous snakes. Bites by the notorious bushmaster (*Lachesis muta*) are actually quite rare.

In **North America** the various rattlesnakes (*Crotalus sp.* and *Sistrurus sp.*) are the most important, with *Crotalus atrox* (Western diamondback) heading the list. Mocassins (*Agkistrodon sp.*) and coral snakes (*Micrurus* and *Micruroides*) are statistically less important.

**Coastal areas in Southeast Asia and Northern Australia:** sea snakes such as *Pelamis, Laticauda sp, Enhydrina sp.*

**Australia:** Brown snake (*Pseudonaja sp*), black snake (*Pseudoechis*), death adder (*Acantophis*), Taipan (*Oxyuranus*), tiger snake (*Notechis*).

**The five medically most important snakes in the world are:**

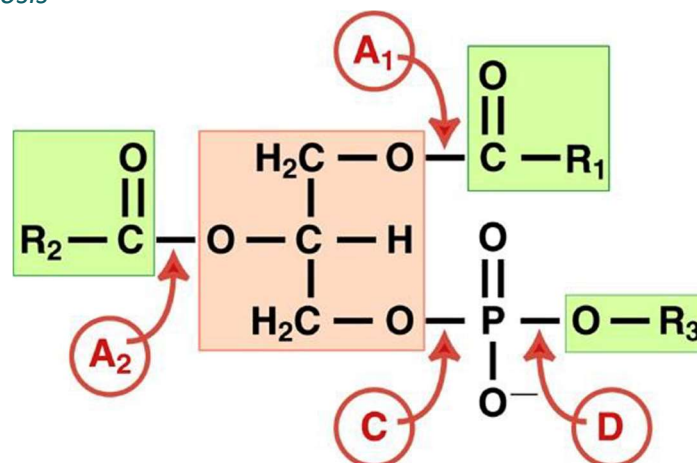
- Echis carinatus complex*
- Bitis arietans*
- Daboia russelli*
- Calloselasma rhodostoma*
- Bothrops atrox*

**Snake venom**

*Snake venom, composition*

Snake venom is a complex mixture of enzymes, toxins and all sorts of smaller molecules. The most important components are the substances with a cytotoxic effect, neurotoxins and the factors leading to bleeding tendency. Some toxins have multiple effects.

*Snake venom, necrosis*



Mode of action of phospholipase A1, A2, C and D on cell membrane phospholipids. Several snake venom components have phospholipase A2 activity. Copyright ITM

Enzymes which help the snake to digest its prey are often cytotoxic for man. Proteolytic enzymes have a trypsin-like activity. Hyaluronidase splits acidic mucopolysaccharides and promotes the distribution of venom in the extracellular matrix of connective tissue. Snake

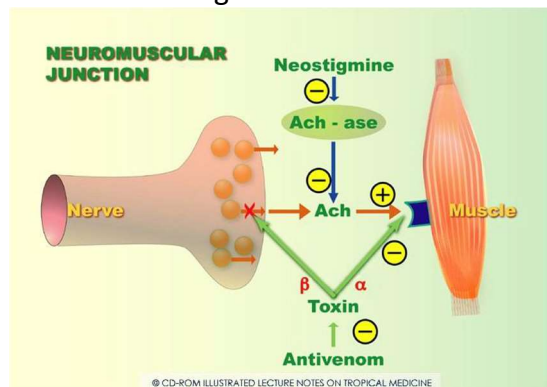
venom often contains various phospholipases A2. These are esterolytic enzymes which break down membrane phospholipids. This causes cellular membrane damage ("lyso", lysis: destroy). Certain venom components have phospholipase C activity. In humans, all these enzymes cause oedema, blister formation and local tissue necrosis.

Myotoxins are present in sea snakes and Australian elapids, as well as in *Bothrops*, *Crotalus*, *Naja* and certain colubrids (*Philodryas* sp). They bind to potassium or calcium channels on muscle membranes and provoke massive rhabdomyolysis.

### Snake venom, paralysis

Neurotoxins are divided into several subgroups. The venom of all elapids contains alpha-neurotoxins.

They act on the post-synaptic nicotinic acetylcholine receptors of the motoric end-plate. With regard to their activity on the neuromuscular junction, the alpha-neurotoxins can be compared with curare or with the autoantibodies in myasthenia gravis. They block the stimulus transmission from nerve cell to muscle and cause paralysis. The postsynaptic effects are reversible with antivenom and neostigmin.



Neurotoxic snake venom with presynaptic and postsynaptic components. Neuromuscular junction with acetylcholine and inhibition of acetylcholinesterase by neostigmine. Copyright ITM

A second subgroup are the presynaptic beta-neurotoxins. They inhibit recycling of acetylcholine and augment the action of the presynaptic alpha-neurotoxins. Presynaptic neurotoxins inhibit the fusion of the vesicles containing acetylcholine, with the nerve's membrane of the neuromuscular junction. Neostigmin will not be effective in these cases.

### Snake venom, blood coagulation

Some components of certain snake venom interfere with blood coagulation. The diversity is staggering. It seems that nearly every step of the coagulation cascade, as well as the fibrinolysis mechanism can be activated or inhibited by one or other component in snake venom.

### Clinical aspects

Bites by venomous snakes are not always accompanied by venom injection and symptoms of envenomation (so-called "dry bites"). Dry bites occur in 50 to 80% of bites. The interval between bite and possible death can vary greatly. In general it can be said that death comes most quickly after cobra bites and most slowly after viper bites. A 24-hours observation period after a snake bite without envenomation symptoms is recommended before a patient can be discharged with clear advice about alarm signs that require readmission.

Inappropriate pre-hospital treatment, such as prolonged arterial tourniquet, incisions at the bite site and sustained aspiration by suction pumps; can cause major complications. Clinical effects of venomous snake bites include vomiting, pain at the bite site and anxiety. This anxiety can lead to dizziness, sweating, shortness of breath or hyperventilation (not to be confused with neurotoxicity). Further, there are a number of specific problems:

### *Local cytotoxicity*



The patient was bitten by *Bothrops atrox*, a venomous South American pit viper. Extensive skin necrosis for which skin grafts are needed. Copyright Alexander von Humboldt Institute, Peru.



Necrosis of the lower leg after a viper bite. Copyright ITM

Local cytotoxicity is characterised by local swelling and blister formation. Later necrosis can develop which can be promoted by arterial thrombosis, inappropriate tourniquet use and local excess pressure in the tissues. A compartment syndrome is probable if the tissue pressure amounts to  $>30$  to  $40$  mm Hg. This is rare. Prophylactic fasciotomy is not recommended. Local necrosis is primarily encountered with vipers, pit vipers and some elapids. Wound infections are not unusual and can aggravate local necrosis. Sometimes fangs or teeth break off and remain in the wound. Most tissue destruction develops in the first 3 days. Chronic ulceration, osteomyelitis or arthritis can follow a snakebite.

### *Cardiovascular toxicity*

Cardiovascular toxicity can occur with viper bites. Hypotension can result from vasodilatation, extravasation, haemorrhages and direct myocardial toxicity. Venom-induced shock leads to a combination of hypotension, lactic acidosis, haemoconcentration and hypoproteinemia. The venom of mole vipers includes so-called "sarafotoxins", peptides which strongly resemble mammalian endothelins and provoke profound vasoconstriction, including the coronary arteries. On the other hand, vasodilatation can occur due to ACE inhibition. Historically, the first angiotensin-converting enzyme inhibitor was discovered in the venom of a South American venomous snake, *Bothrops jararaca*. This formed the basis for developing captopril, the prototype of a very important class of drugs (Lasker Award 1999). The effect of some

components of certain snake venom is comparable to an overdose of captopril, with serious hypotension as a consequence.

### *Haemostasis disturbances*

Haemostasis disturbances are primarily seen with vipers, pit vipers, Australian elapids and colubrids.

The haemorrhagic tendency manifests itself as minor subcutaneous haemorrhages, bleeding gums, epistaxis, haematemesis, melena and/or bleeding from venipuncture sites. Haemorrhages in the adrenal gland and pituitary gland are found with bites by the Russell's viper. This last symptom can be compared with Sheehan's syndrome (post-partum pituitary necrosis). An acute Addison crisis can follow; which must be treated with steroids. Panhypopituitarism, secondary hypogonadism and diabetes insipidus can be late consequences.

### *Neurotoxic effects*

Neurotoxic effects are a characteristic of elapids and sea snakes. The venom of the rare "berg adder" (*Bitis atropos* in South Africa and Zimbabwe) is also neurotoxic, which is highly exceptional for a viper.

Gradually ptosis develops, with vision impairment and eye muscle paralysis (ophthalmoplegia) and mydriasis. Afterwards hoarseness, dysphagia and pharyngeal paralysis develop producing drooling of saliva. The patient can sometimes have difficulty sticking out his or her tongue. Weakening of the neck muscles means the patient can appear to have a "broken-neck symptom". When the patient is drawn up by the hands from a supine position to 45°, the head hangs backwards if there is neck muscle paralysis. Ultimately the patient develops respiratory paralysis.

Neurotoxicity must be distinguished from the symptoms caused by anxiety. Some people who believe that they have been bitten by a snake (even if this is not the case), will hyperventilate, resulting in perioral or diffuse paresthesiae or rigidity and tetany of the hands (decrease of the free plasma Ca<sup>++</sup> concentration due to respiratory alkalosis). Others experience dizziness or syncopal tendencies including vasovagal syncope. A few people will become agitated, possibly with a series of bizarre complaints.

### *Muscle toxicity*

Severe muscle pains and myoglobinuria develop. Cardiac arrhythmia can occur as a result of hyperkalaemia. Hyperkalaemia results as a result of rhabdomyolysis with the additional consequence of acute renal failure.

### *Renal toxicity*

Kidney toxicity is often multifactorial. Hypotension/shock, diffuse intravascular coagulation with intrarenal micro-thrombi, myoglobinuria and haemoglobinuria are major causes of kidney damage.

Myoglobinuria as a result of rhabdomyolysis can cause acute tubular necrosis. Myoglobin is filtered through the glomeruli and causes renal vasoconstriction and tubular injury. The urine

is dark and will test positive for blood. Massive haemolysis causes a similar picture. Another cause of renal failure is immune complex nephritis following administration of antiserum.

### Eye lesions

Eye lesions can occur when a snake spits venom in the eyes (spitting cobras). The snake can spit its venom over distances of up to 3 meters. Burning pain, itching, oedema and eyelid spasms develop. In more than 50 % of cases there are corneal erosions, sometimes leading to blindness. After rinsing copiously with a non-irritating liquid, a local anaesthetic can be given to stop the pain and the blepharospasms. Afterwards an eye ointment containing antibiotics is applied. In case of bites by Burmese Russell's vipers, chemosis can develop (conjunctival oedema), sometimes combined with subconjunctival haemorrhages. Chemosis reflects a generalised increase in vascular permeability and has a poor prognosis. Due to this increased capillary permeability, periorbital oedema, facial oedema and serous effusions can develop.

### Clinic: rule of thumb

<b>Local necrosis</b>	vipers, pit vipers, elapids
<b>Paralysis</b>	elapids and sea snakes
<b>Haemorrhages</b>	vipers, pit vipers, colubrids, Australian elapids

However, there are exceptions to this rule of thumb:

- e.g. *Naja nigricollis* (black-necked cobra): only haemotoxic
- e.g. *Crotalus durissus terrificus*: primarily neurotoxic
- e.g. *Bitis atropos* ("berg adder"): primarily neurotoxic

### Prognosis after snakebite - example

#### Chance of envenomation symptoms

Rattlesnake bite	80%
Sea snake bite	20%
Russell's viper bite	50%
Malayan pit viper	50%

#### Mortality

<i>Crotalus durissus terr.</i>	75% if untreated; 12% with antiserum
<i>Echis carinatus</i>	20% if untreated; 3% with antiserum
<i>Dendroaspis polylepis</i>	almost 100% lethal if untreated

#### Local necrosis



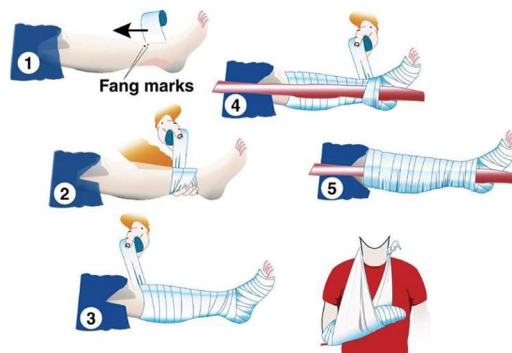
<i>Echis carinatus</i>	9%
<i>Bitis arietans</i> (puff viper)	36%
<i>Naja nigricollis</i> (cobra)	71%

**Interval between snakebite and death**

<i>Naja naja</i> (cobra)	8h	(1/4-60h)
<i>Crotalus</i> species (rattlesnakes)	16h	(2h-26h)
<i>Bungarus caeruleus</i> (Indian or common Krait)	18h	(3h-63h)
<i>Vipera berus</i> (European viper)	34h	(6h-60h)
<i>Vipera (Daboia) russelli</i> (Russell’s viper)	40h	(1/4h-9 d)
<i>Calloselasma rhodostoma</i> (Malayan pit viper)	60h	(5h-10 d)
<i>Echis carinatus</i> (saw-scaled viper)	5d	(1-41 d)

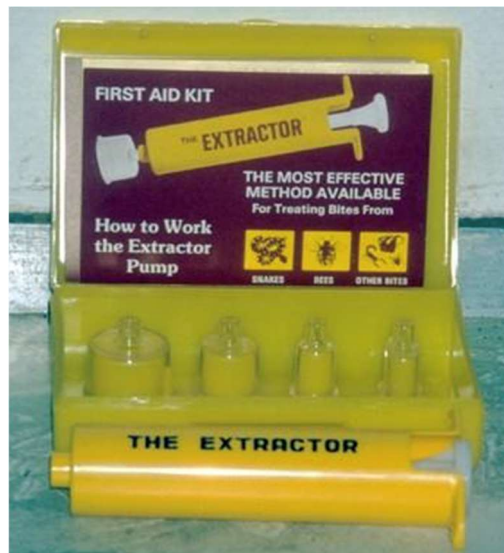
**Treatment**

*Initial*



Australian compression and immobilization technique used in neurotoxic snake bites. A large (minimum 15 cm) elastic bandage is used. Peripheral arterial pulsations should remain present. The optimum pressure under the bandage lies probably between 55 and 70 mm Hg. Adapted from Wilderness Medicine 4rd edition, Mosby.





Device for the extraction of snake venom shortly after a bite, by applying underpressure over the bite site (inverted syringe). This technique is controversial. Copyright ITM, Dr Van den Enden

Victims are often afraid of dying. This anxiety must be reduced which is best done by showing a professional approach. The bitten body part should be immobilised, ideally with a splint as for a broken limb. Immobilisation reduces absorption of the venom, which delays systemic effects. A tight elastic bandage is wrapped around the bitten limb (slower lymph flow). It is important to use a large (15 cm) elastic bandage, tight enough to impair spread of venom, but not too tight in order to avoid interfering with oxygenation. If a bite by a cytotoxic snake is involved, this might be contraindicated, as necrosis could increase locally. For immobilisation the elastic bandage and the **splint** are of equal importance.

They must be applied as soon as possible. A tourniquet is not useful and can aggravate the injuries through ischaemia. The sudden removal of a tourniquet in the case of cobra bites can cause an acute worsening of the symptoms (situation e.g. soon after arrival in the hospital). Dangerous procedures such as incision, sustained suction pumps on the skin, amputation of a finger, prolonged tourniquet, etc. should be avoided. The commercial "Extractor" device consists of a syringe and a vacuum cup. If used within three minutes after the bite, it can remove up to 2-30% of the venom (the device remains on the site for 30 minutes). However, the negative pressure of almost 1 atmosphere also causes massive oedema. Whether there is a clinical benefit is not established (it might be counterproductive).

Quickly sucking out (< 3 minutes after the bite) the bite wound can remove up to 50% of the venom, but the usefulness of this has not been demonstrated. With eye injuries, immediate and copious rinsing with any non-irritating liquid is indicated. If possible and if this can be done without danger, it is best to bring the dead snake along for identification (note carefully: the bite reflex continues long after death, even after decapitation!). Attempting to kill the snake is dangerous and could lead to further bites.

Correct species identification is often difficult, but it is of course important to have an idea of the family to which the animal belongs.



### *Treatment upon arrival in hospital*

A **plasma expander** and **corticosteroids** such as methylprednisolone must be available. Antivenom is given as indicated (see below). In case of vomiting an anti-emetic can be administered. **Adrenaline** (adult 0.5 ml of 0.1 % SC or IM ; for a child 0.01 ml/kg) can be used against angioedema. Endotracheal intubation may be required. If shock and inadequate response to 1 to 2 litres of IV-Ringer or 0.9% saline solution (adult dose), IV albumin is administered. Albumin remains in the bloodstream longer. No salicylate derivatives (aspirin) should be used for painkilling, due to the risk of haemorrhage. **Tetanus vaccination** must not be overlooked. Take blood for full blood count and cross matching (check for thrombocytopenia, spherocytosis, schistocytes, anaemia). Coagulation parameters must be determined if possible. In under-equipped labs it is often impossible to perform conventional coagulation tests. Yet it is essential to determine whether there are blood coagulation problems. For this 2 ml of blood is taken in a dry clean glass tube. Normally blood coagulates and forms a clot within 15 minutes. If the blood has still not clotted after 20 minutes, then there is a haemotoxin present. This simple test can be repeated. If there are coagulation problems, antivenom should be given, if needed followed by or simultaneously with cryoprecipitate or fresh frozen plasma. The thrombocytopenia which often develops is sometimes not corrected by antivenom.

### *Treatment if respiratory paralysis*

In case of respiratory paralysis, the patient must be artificially ventilated. On average this lasts 1 to 4 days if no antiserum is given, but longer periods of paralysis do occur. Neostigmine -an acetylcholinesterase inhibitor - ensures that more neurotransmitter is present, so more stimulus transmission can take place. In this way, neostigmine reduces the effect of certain types of neurotoxins (cobra, mamba). For an adult 0,02 mg/kg and for a child 0,04 mg/kg is injected IM. Afterwards a neostigmine maintenance dose can be infused. Unpleasant side effects (diarrhoea, intestinal cramps, excessive salivation, sweating) are attributable to stimulation of the parasympathetic nervous system (muscarinic receptors). In order to prevent this, the anticholinergic atropine as antidote (0.6 mg IV every 4 hours) is also given. Atropine is a competitive inhibitor of the muscarinic receptors with constipation, dry mouth and mydriasis as side effects.

### *Treatment of hyperkalaemia*

Hyperkalaemia occurs primarily in sea snake bites with severe rhabdomyolysis (see above). In case of cardiac arrhythmia, 10 ml 10% calcium gluconate IV can save a life. This does not reduce the kalaemia, but counters the effects of potassium on the heart. Treatment is coupled with 250-500 ml of a 10% glucose-infusion together with 10-20 units of fast-acting insulin. Sodium bicarbonate can also be given. Salbutamol or albuterol (b<sub>2</sub>-agonists) can be administered via inhalation to lower the kalaemia, since they also cause a potassium shift to intracellular. In case of persistent hyperkalaemia, peritoneal or haemodialysis is necessary.

### **Hyperkalaemia - treatment**

Calcium gluconate

Insulin + glucose

NaHCO<sub>3</sub>

beta<sub>2</sub>-agonist, salbutamol

Kayexalate (no clear data on efficacy)

## Dialysis

### *Treatment with antivenom*



Stock of antisera in the Antwerp Zoo. Snake antivenom. In 2007, FAV-Afrique was available via this Zoo.  
Copyright ITM

### *Antivenom, to whom?*

To whom should antivenom (antiserum) be administered? The presence of "fang marks" – wounds caused by the fangs - is not per se an indication since dry bites also leave "fang marks". Many bites from nonvenomous or mildly venomous species result in discrete local tissue swelling. When the offending snake cannot be identified, giving antivenom for this situation will result in many patients receiving antivenom unnecessarily. Antivenom is administered to patients with local symptoms of envenomation, such as progressive important swelling, intense pain in and around the bite wound, haemorrhages which are difficult to stop, blister formation and/or when there are signs of systemic effects of the venom (muscle paralysis, blurred vision, difficulty in speaking, diffuse haemorrhages, respiratory problems, pulmonary oedema, shock, prolonged coagulation times). Antivenom is still useful up to more than one week after the bite. It is never too late to administer antivenom if there are symptoms of envenomation.

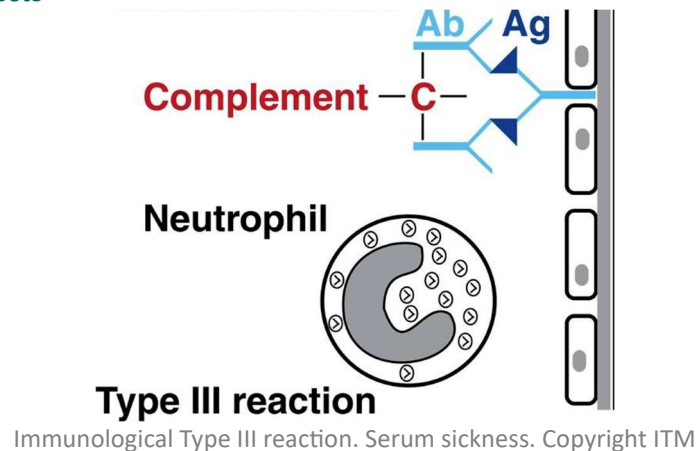
### *Antivenom, dose*

The initial dose of antivenom to be administered to a victim is subject of debate. In clinical practice, the severity of the symptoms will determine the amount of antivenom given to a specific patient. For example: one vial of any Indian polyvalent antivenom represents only 4.5 or 6 mg of total neutralising capacity, depending upon the offending snake species. Each vial neutralises a minimum of 6 mg of *Naja naja* venom and *Daboia russelli* venom and 4.5 mg of *Bungarus caeruleus* venom and *Echis carinatus* venom. But a Russell viper injects an average of 63 mg of venom in a bite. If one would give 2 vials as loading dose, one can expect to neutralise about 20% of the venom. In this case, the loading dose should be around 10 vials. Even "low-dose" strategies recommend a minimum of 6 vials as a starting dose. The objective of additional antivenom is to neutralise any circulating unbound venom that was not neutralised by the initial dose. In hemotoxic bites, the dose is repeated if coagulation is not restored after 6 hours. The liver requires 6 hours to restore clotting factors. Additional antivenom before this period is potentially unnecessary. In case of neurotoxic bites, the antivenom can be repeated after 1 or 2 hours if the patient has not improved or if his condition is worsening. True reversibility of neurotoxic envenomation (detaching tissue-bound post-synaptic neurotoxins) is only possible within the first 1 or 2 hours. After that period, the role of antivenom is to neutralise unbound venom. Patients paralysed due to destruction of the presynaptic nerve terminals will respond much less to antivenom.

The treatment with antivenom is effective for problems of blood coagulation, shock and specific neurotoxicity. For other problems (nephrotoxicity, local necrosis and some paralyses)

the effect is a great deal less spectacular. Note: the same dose of antivenom is required in children, as the amount of venom injected is the same as in adults.

### Antivenom side effects



Antivenom which is prepared from horse serum, contains foreign proteins and frequently produces side effects. Anaphylaxis (IgE-mediated type I reaction), anaphylactoid reactions (not IgE-mediated, but via complement activation through protein aggregates in the antivenom) and serum sickness (immune complex or type III reaction) can develop. Anaphylaxis risk is higher in a patient who has previously been treated before with antivenom (e.g. a snake hobbyist bitten on different occasions).

Soon after administration,  $\pm 20\%$  of the patients develop itching, urticaria, fever, cough, tachycardia, nausea and/or vomiting. Sometimes there are quite serious bronchospasms. Antihistamines do not reduce the incidence or seriousness of these symptoms, in contrast to a low dose of adrenaline (0.25 ml SC of a 1/1000 solution). Most clinicians currently advocate no routine prophylaxis, but will have adrenaline, corticosteroids and antihistamines drawn up, so they are ready to treat an early reaction.

Serum sickness as a result of immune complexes develops in 30 to 90% of the patients. It manifests itself after 5 to 24 days (average 7 days). The frequency depends on the dose of antivenom administered. Fever, itching, joint pain and periarticular swelling, lymphadenopathy, mononeuritis multiplex and immune complex nephritis with albuminuria characterise this disorder. If serum sickness develops, steroids are given for 5 days.

### Examples of antivenom:

**CroFab**<sup>®</sup> (= earlier CroTAB<sup>®</sup>, Protherics Inc.) was approved in October 2000 by the American FDA. The product includes Fab fragments against 4 North American venomous snakes: *Crotalus atrox* (Western Diamondback rattlesnake), *Crotalus adamanteus* (Eastern Diamondback rattlesnake), *Crotalus scutulatus* (Mojave rattlesnake) and *Agkistrodon piscivorus* (Cottonmouth). This antiserum covers via cross-protection virtually all pit vipers in North America and several in Central America.

**ViperaTAB**<sup>®</sup> is a monovalent antiserum that is used for bites by *Vipera berus*.

**ViperFav®** (Aventis Pasteur Merieux) is a polyvalent, yet narrow-spectrum F(ab')<sub>2</sub> antivenom against *Vipera berus*, *V. ammodytes* and *V. aspis*.

### *Venom detection kit*

In Australia there has existed for many years a detection kit to identify venom and determine the snake species (Commonwealth Serum Laboratories). This is based on a two-step enzyme immunoassay in which the wells in the ELISA plate are coated with antibodies against the various types of snake venom. Using a swab some venom is taken from the bite wound (in a person or a pet) and identified. This makes it possible to use specific antivenom. However, this method still has to be further developed for other parts of the world. A positive "venom detection kit" result per se is no indication for antivenom. The results must always be interpreted in the clinical setting.

### *Monitoring antivenom therapy*

When an adequate quantity of antivenom has been given, the following response can be expected:

1. The patient rapidly feels better.
2. Gum bleeding stops within 15 to 30 minutes.
3. The coagulation test (20' test) normalises within 3-9 hours, but the clinical haemorrhages stop much earlier.
4. The blood pressure normalises within an hour. Cardiac arrhythmias disappear.
5. Neurotoxic effects begin to disappear within 30 minutes; complete recovery takes much longer. Bites by kraits and sea snakes (presynaptic venom) improve slowly.
6. Active haemolysis and rhabdomyolysis stop within several hours. Urine afterwards returns to its normal colour.

### **Indications to repeat antivenom administration:**

1. Persistence or recurrence of non-coagulability after 6 hours or new bleeding after 1-2 hours.
2. Worsening neurotoxic or cardiovascular signs after 1-2 hours.

### *Treatment of complications*

Supportive therapy is necessary (fluid balance, analgesics, transfusion). Blood pressure, pulse, respiration, muscle functions, central venous pressure, urine production, blood coagulation and circumference of the bitten body part (leg, arm) must be monitored. Wound infections including tetanus must be prevented and combated.

In case of compartment syndrome, fasciotomy should only be considered in extreme cases (tissue pressure >40 mmHg). It often does more harm than good. With local necrosis, operative intervention is necessary (wound debridement, skin grafts, amputation). Deep abscesses can develop and must be drained. After the acute episode scars are likely. Skin grafts might be needed. A Volkmann's ischaemic contracture of the forearm can occur and requires intensive physiotherapy to regain some function.

Kidney failure can sometimes make (peritoneal) dialysis necessary. Strict fluid balance monitoring should be introduced in order to avoid any overload. With heavy myoglobinuria or haemoglobinuria an infusion of mannitol (200 ml of 20% over 20') may be given and

alkalinisation of the urine is advised. An adequate hydration of the patient must be maintained. Muscle rest is obligatory if rhabdomyolysis is suspected.

### *Shock*

Shock can be the result of anaphylaxis, direct vasodilatation due to the venom, cardiotoxicity with or without arrhythmia, hypovolaemia (fluid shift to extravascular and/or internal/external bleeding), respiratory failure, acute Addison crisis or sepsis. Plasma expanders under continuous control of the central venous pressure (watch carefully for pulmonary oedema), dopamine and steroids may be necessary.

### *Errors in evaluation/treatment of snakebite*

1. Not thinking of a venomous snake bite when confronted with a swollen ecchymotic limb
2. Cryotherapy and/or incision of the wound
3. Insufficient immobilisation of a bitten limb
4. Not looking for fang marks
5. Not keeping in mind that envenomation can change over the course of time, with clinical deterioration as a result
6. Only giving vasopressors to support the blood pressure, without giving IV fluid
7. Forgetting to check coagulation repeatedly
8. Delaying antivenom treatment if signs of envenomation are present, or thinking that it is too late to give antivenom
9. Administering a too low dose of antivenom
10. Not having adrenaline ready on stand-by
11. Applying an arterial tourniquet for a prolonged period
12. Performing a fasciotomy when not needed

### *Prevention*

It is very rare for a snake to be spontaneously aggressive. Snakes tend to note the presence of a person through detection of vibrations. If given the chance they generally flee as a person approaches. Never attempt to corner a snake. Many bites occur when people are attempting to kill the animals. The risk of a snakebite increases if the victim is drunk, reckless or imprudent. However, people can accidentally tread on a snake on a path at night or in a field. More than 50% of venomous snake bites are on the feet or lower legs. Wearing sturdy, high-topped footwear in areas with increased risk is recommended.

Some snakes follow their prey (generally small rodents) all the way into houses, and can bite a sleeping victim if they are surprised. Control of rats and mice around houses is not only beneficial in itself; but also reduces the number of snakes attracted to the area. The grass around the house must be kept short. There are specific high risk environments and professions. This encouraged the development of various experimental vaccines. Naturally they do not protect against the bite itself, but are designed to reduce mortality and morbidity. Sleeping under a bednet protects against snakebites, especially in these areas where snakes tend to enter houses when looking for their prey.

To the question whether people routinely need to carry preventive antivenom when travelling in remote areas, the answer is "no". The chance of incurring a venomous snake bite with envenomation is low. Furthermore antivenom is not a harmless product, it is expensive and

must be stored in specific conditions. Taking a couple of elastic bandages along is recommended. These can also be used for other purposes.

### Antisera: useful information

- MAVIN (Münich Antivenom Index) available via: <http://www.toxinfo.org/antivenoms/>
- [http://www.who.int/bloodproducts/snake\\_antivenoms/en/](http://www.who.int/bloodproducts/snake_antivenoms/en/)
- For Belgium: Antigif centre Brussels: tel 070.245.245. Usually antivenom against European vipers (Viperfav) should be available.

# Scorpions

## General

Today there are around 1400 species of scorpions, although estimates of the exact number vary widely. All scorpions are venomous, but only a minority twenty five or so are potentially lethal for humans. Scorpions do not bite. Stings by scorpions are fairly common. Every year, more than 1 million cases of scorpion envenomation are reported worldwide. However most clinical reports emphasize the serious cases and systematically overestimate the danger these creatures pose. In endemic areas, people don't go to a doctor for minor stings. Fatal stings are essentially limited to Mexico, Brazil, Trinidad, northern Africa, South Africa, the Middle East and India. It is primarily children and patients suffering from a respiratory and/or cardiovascular condition who run a high risk of complications.

### *Biology*

Scorpions are the most primitive members of all Arachnida. Their sting is used to kill prey, for defence against aggressors and in some species it also has a role in the courtship display. Some scorpion species are long (*Hadogenes troglodytes* up to 21 cm), others are heavy (*Pandinus imperator*, also called the Emperor scorpion; *Heterometrus sp.*), while others are small (*Microtityus waeringi*, adult 12 mm). All scorpions are exclusively carnivorous. A scorpion first grasps its prey (generally insects) with the pedipalps. If the prey is not immediately overpowered, they sting it by bending the tail forwards over the body. The venom is actively injected. The scorpion releases gastrointestinal juices over the prey in order to liquefy it and later suck it up. They consume only the body fluids and liquefied tissues of their prey. A meal can last several hours. Some species are cannibalistic.

Because many scorpions live in dry environments, they have become adapted to minimize loss of water. This is made possible in part by a watertight cuticle based on chitin. This has unusual optical characteristics. Scorpions fluoresce with a greenish colour under long-wave UV light. This makes them easy to spot at night with the aid of a UV lamp. The reason for this fluorescence is unclear.

## Taxonomy

### *Taxonomy, families*

**Buthidae:** virtually all medically important species belong to this family. However, it includes nearly one-half of all scorpion species (around 600 known species). Buthidae have a triangular central plate, whilst the other families have a pentagonal sternum.

**Bothriuridae:** unimportant

**Chactidae:** unimportant

**Diplocentridae:** unimportant, except for *Nebo hierichonticus*

**Scorpionidae:** unimportant, except for *Hemiscorpion lepturus*

**Vaejovidae:** of limited importance. *Vaejovus sp.* and *Hadrurus sp.* can cause painful stings; but the effect is always local and limited.



*Tityus serrulatus*. Yellow scorpion endemic in parts of Latin America. Copyright ITM



Scorpion. *Parabuthus granulatus*, from Namibia. With special thanks to Prof Verdonck, Kortrijk.

## Distribution

Virtually all lethal species belong to the Buthidae family. These animals are found primarily but not exclusively in dry areas. In the Buthidae family, the medically important and dangerous genera have the following distribution:

<i>Androctonus:</i>	from Morocco and Senegal eastwards to India
<i>Buthus:</i>	Mediterranean, Middle East and East Africa
<i>Hottentotta:</i>	Northern Africa and the Middle East
<i>Leiurus:</i>	East Africa and the Middle East
<i>Parabuthus:</i>	from Sudan to South Africa
<i>Mesobuthus:</i>	India, Southern and Central Asia
<i>Tityus:</i>	South America

The most important species are:

1. *Buthotus tamulus*
2. *Leiurus quinquestriatus*
3. *Androctonus crassicauda* (and *A. australis*)
4. *Tityus serrulatus*
5. *Centruroides suffusus*

## Scorpion venom

Scorpion venom is a mixture of various active substances, but generally the neurotoxins are the most important. The neurotoxins are small proteins. Alpha neurotoxins inhibit the closing of sodium channels, without interfering with the opening potential. They lead to a strong membrane depolarization and hence, neuronal excitation. In a second phase loss of



excitability is possible. Beta neurotoxins open Na<sup>+</sup>-channels. Sodium is primarily an extracellular ion, and is necessary inter alia for maintaining an electrical voltage difference across the cell membrane. When the Na<sup>+</sup>-channels open, sodium flows into the cell which depolarises the membrane. The nerves fire non-stop. The clinical effects of alpha and beta neurotoxins are similar. There follows a massive release of neurotransmitters, both acetylcholine and noradrenaline from nerve endings and adrenaline from the adrenal medulla. The main part is formed by the catecholamines, thus sympathetic effects usually outgo the parasympathetic effects.

Scorpion venom also contains serotonin, which contributes to local pain.

## Clinical aspects

### General

Most scorpion venoms contain little or no cytotoxic enzymes, so that a sting produces little local tissue damage. An exception to this is the cytotoxic venom of *Hemiscorpius lepturus*, a scorpion from Iran. Their stings are characterized by erythema and purpuric and bullous lesions that resolve, but in about 20% of cases there is delayed localized necrosis. Companion features include nausea, vomiting, fever, minor autonomic effects, direct haemolysis with haemoglobinuria and acute kidney injury that might necessitate dialysis. Bites by *Loxosceles* spiders can mimic a similar clinical syndrome.

There are four factors which play a role in defining eventual symptoms: the quantity of venom introduced and its toxicity as well as the size and medical condition of the victim. Many scorpions without medical interest have venom that can kill a mouse, but when introduced into human beings only produces symptoms analogous to those of a bee sting. If there is an allergy to this venom, anaphylaxis can follow and death can result from a sting of even a "harmless" scorpion. Stings without injection of venom do occur ("dry" stings).

### Local effects

Rapidly developing pain at the site of the sting is characteristic. Swelling and local redness are often limited but can be quite serious. Local necrosis is exceptional. Local paraesthesias can occur. Several South African scorpions can squirt venom up to one metre away. If this comes into contact with the cornea, chemical keratitis with burning pain develops.

### Systemic effects

There are 3 main mechanisms of action: adrenergic (sympathic) excess, cholinergic (parasympathic) excess and neuromuscular excitation. The adrenergic effect results in tachycardia and hypertension as well as mydriasis, agitation, seizures and myocarditis. The cholinergic effects are bradycardia and hypotension, vomiting, transpiration, salivation, lacrimation, miosis and bronchial spasms with excess secretions. The neuromuscular excitation can lead to oculomotor abnormalities, visual disturbances, muscle spasms and eventually paralysis. Complications are cardiac arrhythmias, myocardial depression with pulmonary oedema, hypotension and shock. Death can be caused by respiratory failure or by coma and multiple-organ due to shock.

Symptoms can either develop quickly, within ten minutes, or - more rarely - slowly, after only 24 hours. More and more, doctors use a clinical gradation:

Degree 1 : local effects only

Degree 2 : autonomic excitation, agitation and anxiety

degree 3: pulmonary oedema, hypotension and cardiogenic shock, severe neuromuscular excitation

Degree 4: multi-organ failure, coma, seizures, end-organ failure secondary to hypotension

Evolution from degree 1 to degree 4 can occur very quickly (sometimes within half an hour). Generalized fatigue with muscle stiffness and weakness, anxiety and restlessness are frequent. The tendon reflexes are hyperactive. Sometimes fasciculations, tremors and/or clonus develop. Other complication from scorpion stings include pancreatitis, rhabdomyolysis, diffuse intravascular coagulation and priapism.

Children are generally very restless, with crying and shouting, agitation, shaking, twisting and swinging of their limbs. The child cannot sit still. Mortality depends largely on age. Children, the elderly and people with a serious pre-existing medical condition have a substantially higher risk of death than adults.

## Diagnosis

The diagnosis is essentially clinical. Due to the quick and intense local pain, the scorpion is often noticed. Yet the typical victim is someone who is usually stung at night in the foot, outdoors, possibly when he/she has moved a stone or some wood. People are also stung in the morning when they try to put on a shoe in which a scorpion is hiding. Occasionally in North Africa men are stung in the genitalia when they urinate against an object while squatting. The laboratory often shows leukocytosis, hyperglycaemia and a transient increase of the pancreatic and cardiac enzymes. The ECG can display temporary ischemic deviations. Investigations should focus on potential complications of scorpion envenomation.

### **Scorpion stings, differential diagnosis:**

1. Spider bite by *Latrodectus mactans* (black widow). The bite of the female spider produces little local reaction, contrary to bites by *Loxosceles reclusa*, yet is characterized by marked abdominal muscle rigidity, pain and excessive sweating. Dysphagia, sialorrhea, vision impairment and generalized hyperesthesia are generally absent with these spider bites. In South America, bites by Phoneutria spiders need to be considered.
2. Overdoses of neuroleptics, anticholinergics or tricyclic antidepressants. Generally these products do not produce excessive salivation and there is no local pain.
3. Organophosphate poisoning causing inhibition of acetylcholinesterase, leading to a buildup of acetylcholine. These insecticides also cause agitation, restlessness, muscle weakness, muscle fasciculations, hypersalivation, diarrhoea, miosis, transpiration, tachycardia and respiratory difficulties. However there is no pain.
4. Tetanus, botulism, diphtheria, meningitis and encephalitis can cause similar symptoms.
5. Neurotoxic snakebite.
6. Thyroid storm, carcinoid or pheochromocytoma.

## Treatment

Patients with systemic symptoms must be hospitalized for 24-48 hours, preferably in an intensive care unit. Cardiac arrhythmia, hypertension and respiratory problems must be monitored. The airways must be kept open. Administration of oxygen and artificial respiration can be necessary.

Pain relief with powerful analgesia is often required. Local application of ice can reduce the pain, but may not be tolerated due to hyperaesthesia of the skin. Opiates should be avoided because of the danger of respiratory depression. Sometimes simple infiltration of the sting site with 2% xylocaine (i.e. lidocaine without adrenalin) can reduce the pain. The general management is aimed at neutralising the effects of the overstimulation of the autonomous nervous system. Hypertension is counteracted by giving the vasodilator prazosin (alpha-blocker, Minipress®) or nitroglycerin if there is pulmonary oedema. Atropine is sometimes used as a parasympatholytic agent, but can aggravate orthosympathic symptoms, so usually it is preserved for bradycardia associated with hypotension. In case of neuromuscular incoordination or convulsions diazepam IV should be given. Inotropes (e.g. dobutamine) and diuretics (furosemide) are indicated if there is heart failure. Hyperthermia requires cooling and salicylates.

Most cases improve without antiserum within 9-30 hours (except for pain and paresthesias). In the event of severe envenomation, death frequently occurs within 6 hours after the sting. When systemic symptoms or autonomic excitations is present, IV antiserum is recommended. It should be diluted and administered in an IV infusion over 20-30 minutes. The dose is not age-dependent. Antivenom is expensive and guidelines often suggest and that its use should be restricted to cases of severe envenomation. However, once severe envenomation has developed, the administration of antivenom may be less effective, since its primary therapeutic action is to bind toxins; it does not reverse established pathophysiological injury, such as excess levels of catecholamine, pulmonary oedema, and shock. Antisera are good for neutralizing neuromuscular effects but have little effect on pain or paraesthesias. A type III hypersensitivity reaction can develop after administering the antiserum (horse, donkey or goat serum; see treatment of snakebites). Type I reaction (anaphylaxis) is exceptional. Tetanus vaccination must be checked.

### Antitoxin:

USA and Mexico: anti-*Centruroides*

South America: anti-*Tityus*

South Africa: anti-*Parabuthus*

Maghreb: anti-*Androctonus* and anti-*Buthus*

Egypt and Israel: anti-*Leiurus* (also active against *Androctonus crassidauda*)

India: anti-*Mesobuthus*

## Prevention

Firstly reduction of the places of shelter and the food supply of the scorpions. Insecticides are only effective in the sense that they eliminate the prey of scorpions. There is often insufficient contact between the body of the scorpion and the insecticides to be directly toxic. Natural enemies of the scorpions include cats and solpugids (alias camel spider). Of course, a house cat is no guarantee that there will be no scorpions in or around a house. It is recommended to clear away junk, loose wood etc (fewer hiding places). The same applies for certain types of vegetation (e.g. *Opuntia* cactus hedges in northern Africa). Cracks and crevices in and around the house must be sealed. In endemic areas it is best to always shake out shoes before putting them on and to examine clothes and blankets before using them. It is prudent to also check the toilet before use. At night, scorpions can easily be detected with an UV-light.

# Spiders

## Summary

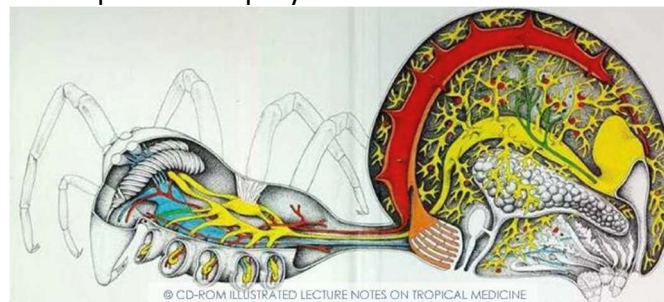
- *Latrodectus* (black widows): neurotoxic. Acute abdominal pain. Limited local signs
- *Loxosceles* (violin spiders): cytotoxic. Local pain, skin necrosis, rarely systemic symptoms
- Mygalomorph spiders: fine urticarial hairs. Itching and eye damage. Respiratory problems
- Funnel-web spiders: Neurotoxic and local pain
- *Phoneutria* (banana spiders): local pain and systemic reactions

## General

The subphylum of the Chelicerata includes 4 classes: Euryptera (extinct), Pycnogonida (sea spiders), Merostomata (horseshoe crabs) and Arachnida. The latter includes animals such as spiders, scorpions, mites and ticks. The name "Chelicerata" refers to the modified mouth parts (chelicerae). Spiders form the order of the Araneida (id. Araneae) within the Arachnida. The number of spider species is estimated to be 30,000 to 34,000. There are only a small number of spiders which are potentially harmful for human beings. The reason for this is that most species simply have too little venom or their fangs are too short to penetrate human skin. The venom of some spiders is only active against their natural prey and has no effect on human beings. Finally for some species the probability of spider-human contact is very low. Mortality as a result of spider bites is very low compared to snakebites, although there is a moderate morbidity (globally > 10,000 each year).

Some spiders are large. The record is held by *Theraphosa leblondi*, the Goliath bird spider from Guyana. A giant specimen had a body length of 10 cm, a leg span of 26 cm and fangs of 25 mm. Some species have a limited geographical range (e.g. *Atrax robustus* in a limited part of Australia, *Phoneutria nigriventer* in Brazil), yet others are quasi cosmopolitan. There have been repeated instances of spiders, originally endemic in one area accidentally being introduced into another area where they had not been naturally present. In 2003, there was a notorious incident where *Latrodectus mactans hasselti* (black widow) was accidentally introduced in Hasselt, Belgium. Knowledge about spider bites is limited and incomplete, given that bites are often not noticed immediately (including that of the black widow) or because the spider was not identified.

Spiders produce various types of silk for different purposes, such as for the web, winding around prey or eggs, "droplines" to make possible a hasty exit, for dissemination ("balloon riding" via a filament that is carried along by the wind), as a nuptial gift. Fibroin and sericin are produced in separate silk glands. Not all spiders produce webs. Some hunting spiders rely on their vision and speed to capture their prey.



Spider anatomy. Adapted from the original.

## Venom glands and venom

Most spiders have venom glands. The venom glands lie either in the chelicerae or at the front of the cephalothorax. The venom duct leads to the fangs, which are located at the end of the chelicerae. The venom is injected into the natural prey but is also used to defend against predators. After having killed the prey, the spider releases digestive juices over it and afterwards sucks up the liquefied mush. Spider venom has various purposes. Spiders which hunt, such as *Loxosceles* and *Phoneutria*, have neurotoxic / proteolytic toxins in their venom. Spiders which make webs generally have weaker venom, except for *Latrodectus*. Some bird spiders have urticarial hairs which, when lost by the animal, can irritate skin, conjunctivae and the mucous membrane of the mouth. Similar irritating hairs are also found in other animals, such as some caterpillars (e.g. procession caterpillar) and various adult butterflies.

## Species, clinical and treatment

### *Loxosceles*

*L. reclusa* (violin spider, brown recluse spider in North America) and *L. laeta* (South America) are the most familiar and notorious. They have a beige-brown colour with a dark spot in the form of an upsidedown violin dorsally on the cephalothorax (the "violin" points to the rear of the abdomen). There are three pairs of eyes (dyads), one in front and the others on the sides. In nature they can be found under stones, logs, etc., but the animals also often enter houses and thrive in this environment (they are "synanthropic"). They are often found there in large numbers. In South America *L. laeta* is known as the "araña de los rincones" ["rincón" = corner], which refers to this peridomestic character. In so far as their psychology is understood, spiders only bite when they feel threatened. They live 1 to 3 years.



*Loxosceles* spider bite. The venom can provoke skin necrosis. Copyright Alexander von Humboldt Institute, Peru

The venom of *Loxosceles* sp. is primarily cytolytic and haemolytic. Clinically, a bite results in initial pain followed by mild skin irritation. After 6 hours or so the pain intensifies. There are local vasospasms and ischemia develops. An itching oedema with a red halo and purple centre develops. A central bulla can form. This can evolve into a necrotic wound which nevertheless remains limited to the skin. A bite by *Loxosceles reclusa* can produce significant skin wounds, with necrosis and tissue loss. Underlying muscle tissue is not affected. A deep scar can remain. Occasionally there are systemic reactions with haemolysis and anaemia, clotting disorders, kidney failure and, in exceptional cases death. The patient can develop chills and fever, macular rash, joint pain, nausea and vomiting. Treatment is essentially symptomatic. Tetanus prophylaxis must not be overlooked when local necrosis is present. When a patient is seen soon after a bite, dapsone 50-100 mg twice per day can be given, in order to limit the necrosis. It is best if G6PD-deficiency is excluded first. The efficiency of dapsone is not clear however.

The wound should not be debrided too quickly (wait up to 2 weeks). Sometimes a skin graft may be necessary. When kidney failure or clotting disorders occur, the patient should be hospitalised. Anti *Loxosceles* antiserum exists, but is usually not available.

### *Latrodectus*



*Latrodectus hasselti*. Australian black widow spider. Notice the red mark on the abdomen. Photo by Aart Noordam with special thanks to Arabel.



*Latrodectus mactans*. North American black widow spider. A red mark, sometimes diabolo-shaped, can be seen on her round ventral abdomen. Photo by Gilbert Loos with special thanks to Arabel.

These spiders, also known as black widows, have a very wide geographical distribution. The common English name refers to the habit of the female to eat the male after inseminating her. There are no natural wild populations of black widows in central and northern Eurasia (yet), although accidental introduction of exotic species occasionally happens. E.g. introduction in Belgium was first seen in 1967 in Tervuren, in 1999 *L. hasselti* was found in Bree (near Hasselt, of all places! What's in a name?) and another introduction (*L. mactans*) was detected in August 2009 in Antwerp. Specialists recognize *Latrodectus mactans* (North American black widow), *L. hasselti* (Australian black widow) and *L. m. tredecimguttatus* (Southern Europe, including Italy; South America; spiders carry several ("13") pigment spots). At least 3 other species exist. *L. mactans* often bears a typical red hourglass-shaped spot on the abdomen. Only bites by female black widows are potentially dangerous. The fangs of the male are too small to penetrate human skin. Australia has probably the highest rate of latrodectism per capita in the world. Unlike loxoscelism, neurotoxicity plays a central role with latrodectism. The initial bite feels like a pinprick, but direct local tissue damage at the bite site is generally absent or insignificant. The venom contains several substances, the most important of which is alpha-latrodectin (= alphalatrotoxin), a neurotoxin that triggers an increased release of neurotransmitter at nerve endings. This results in a presynaptic depletion of neurotransmitter vesicles. Acetylcholine, noradrenaline, dopamine, glutamate and enkephalin systems are all sensitive to the toxin. This release followed by depletion of acetylcholine at neuromuscular junctions leads to fasciculations and muscle spasms, followed by flaccid paralysis and risk of respiratory arrest.

Sometimes painful muscle rigidity (primarily abdominal -"pseudo-appendicitis"- but without the specific focal tenderness or rebound pain) can occur. Other symptoms are hypertension, sweating, tremor, headache, malaise, vomiting, fever, general weakness, patchy muscle paralysis (sometimes with ptosis), excessive salivation, photophobia and priapism. Mortality in published series varies from 5 to 12% but these numbers are likely to be an overestimate due to case selection. Pets such as cats can easily be killed by female black widows.

Mild cases with limited signs and symptoms tend to resolve spontaneously over hours to days. The pain however can be very intense. If pain predominates, analgesics (including opiates) may be necessary and sometimes are insufficient. In more severe cases (usually within 12 hours, but sometimes up to days after the bite), benzodiazepines (diazepam) can be used against muscle spasms and to reduce the abdominal pain. Hypertension which does not improve after pain control sometimes requires the vasodilator IV nitroprusside or nifedipine. Antivenom can be given in serious cases, including priapism, but is rarely available. Full recovery might take several weeks. Respiratory or cardiac support is very rarely needed. Neostigmine for counteracting acetylcholine depletion has been proposed but never been proven to help.

#### *Atrax and other funnel-web spiders*

In Australia there are 35 species of funnel-web spiders. They have a funnel-shaped web, often with triplines to detect their prey. *Atrax* species are large, aggressive spiders. The most notorious is the Australian *Atrax robustus* or Sydney funnel-web spider. The venom of the spider includes a neurotoxin with potentially fatal consequences for humans. The fangs can be up to 5 mm long and can penetrate a fingernail. The spider makes its web under stones, logs, hedges, near vegetation and fences. Clusters of up to 150 animals have been found. With this species, the bite of the male spider is much more serious than that of a female spider and is justifiably highly feared. The venom includes the small protein robustoxin, a unique presynaptic neurotoxin. The venom interferes with the release of the neurotransmitters noradrenaline and acetylcholine at the level of the motor and autonomous nerves.

A bite by *Atrax* is followed by intense pain. This is probably due to the mechanical damage and the acidic pH (4.5) of the venom. No dermal necrosis results. In serious cases there follows a rapidly progressive neuromotor syndrome which can be fatal within two hours. Initially there is local piloerection and muscle fasciculation. These symptoms become generalised with tingling sensations around the mouth and tongue and lip spasms. Within a half hour they are followed by marked hypertension, tachycardia, 2nd degree AV block, hyperthermia, haemoconcentration and coma with increased intracranial pressure. Copious sweating, excessive salivation, lacrimation, diarrhoea and muscle cramps follow. Asphyxia can lead to death. If the patient survives, there follows hypotension and intermittent apnoea after one or two hours, possibly with pulmonary oedema. This too can be fatal. Muscle enzymes (CK) can rise substantially. Children are at higher risk than adults.

Shortly after a bite it is recommended that a splint and a lymphatic tourniquet ("pressure immobilisation technique") should be applied. In this way the systemic resorption of the venom is slowed down and the patient can be transferred to a hospital. Antitoxin has been available in Australia since 1981 (Funnel Web Spider Antivenom, CSL, Australia). Oxygen,



mechanical respiration with PEEP, atropine (against bronchorrhea and excessive salivation) and short-acting antihypertensives should be used depending on the symptoms. Since acute stomach dilatation can develop, nasogastric aspiration must be performed.

### *Phoneutria*

These aggressive South American spiders are nocturnal hunters which do not weave webs. They often hide in bunches of bananas, hence their popular name. *Phoneutria* is unusually aggressive and tends to bite several times at the same site in quick repetition.

The venom is a complex mixture with several neurotoxic components. It acts on both the peripheral and the central nervous system. A bite is followed by sharp pain and then by tachycardia, hypertension, hypothermia, profuse sweating, excessive salivation, nausea and vomiting, priapism and dizziness.

Death can occur within 6 hours and is generally attributable to respiratory arrest. A polyvalent antiserum (anti-*Loxosceles* and anti-*Phoneutria*) can be given through local infiltration and IV administration.

### *Lycosa*



Spider. *Lycosa tarantula*. Copyright ITM

Wolf spiders belong to the Lycosidae family. They are nocturnal hunters which generally do not spin webs. Earlier it was believed that they hunted in groups, hence the popular name. Wolf spiders include species with a moderately cytotoxic venom. The best known is the European tarantula (*Lycosa tarantula*). Its bite was earlier deemed to provoke tarantism in the victim, a syndrome characterised by stupor and a wish to dance. Possibly this refers to the consequences of *Latrodectus* bites, rather than *Lycosa* itself, since the latter only causes local pain, swelling and erythema. Tetanus vaccination, painkillers and local disinfection suffice for bites by these animals.

### *Bird spiders and their relatives*

"Bird spiders" belong to several genera. Some people keep them as "pets" although several species are protected under the CITES convention (Convention on International Trade in Endangered Species). Several American genera of bird spiders have fine urticarial hairs. The density amounts to 10,000 fine hairs/mm<sup>2</sup>. When the animals feel threatened they rub with their legs over their back, detaching the fine urticarial hairs which are armed with small barbs. These can be released by the thousands and cause persistent itching when they come into contact with the skin and work their way under the surface. Penetration into the cornea or

inhalation can lead to serious consequences. When handling these animals, wear gloves and safety glasses, avoid rubbing your eyes and wash carefully after manual contact. Bites by these spiders cause local pain and swelling, whether or not followed by lymphangitis. The treatment is symptomatic. Beware of the fact that urticarial hairs are also present on exuvia (shed skin), even when preserved in alcohol!

### *Chiracanthium*

*Chiracanthium* species are notorious for their annoyingly painful bites. A bite is followed within 30 minutes by local itching and redness. Sometimes this can evolve to local necrosis. Systemic effects include (rather rare) nausea and abdominal pain, as well as headache. The treatment is symptomatic. Besides black widows and wolf spiders, the only European spiders which should really be handled carefully are *Chiracanthium* species (Clubionidae) and the water spider *Argyroneta aquatica*.



Residual erythema after *Chiracanthium* spider bite. Copyright ITM

## Pre-travel consultation

In the last decade, a major increase in international travel has been seen. Each year, more than a billion international tourist arrivals are counted. 15-65% of those experience some sickness and 5-15% need to seek medical care. About 1/100.000 travelers die during their journey. Of travel related deaths 50% are due to accidents (car, drowning, ...), 40% due to a cardiovascular event, 9% secondary to neoplasms or other underlying diseases and only 1% due to an infectious cause.

A pre-travel consultation is a perfect example of preventive medicine. It is more than delivering vaccinations and prescribing antimalarial pills. The aim of a pre-travel consultation is to inform the traveler about potential health risks while travelling and to discuss possible interventions (like vaccinations, antimalarial pills).

Taking a glance at the above figures, the importance of safe travel vehicles with seat-belts cannot be underestimated: this advice might be much more cost-effective than any pre-travel vaccine given. Making sure that people with underlying conditions travel in their best possible health-status is also of major importance.

### Possible health risks depend on several factors:

- The traveler: pre-existing conditions, risk behavior, vulnerable travelers like the elderly,
- pregnant and young children
- Destination
- Type of travel: expat, backpacking, cruise...
- Special activities: diving, high altitude, biking...

### Each pre-travel consultation should include:

1. Discussion about **pre-existing conditions**: is the traveler fit enough for this travel? What about travel insurance? Especially in case of vulnerable travelers, the risks of traveling should be discussed. In any case, it is always a shared decision, but sometimes, the traveler may want to postpone the travel or to change the destination after being informed. E.g. people who have a contra-indication for yellow fever vaccine, pregnant women may not go to a country with an ongoing Zika virus outbreak.
2. **Prevention of travel related infectious diseases**
  - **Malaria risk**: is there malaria, which precautions to take? Only mosquito bite prevention or also prophylactic antimalarial drugs? Continuous malaria pills or only during shorter periods (“on demand”) when entering a higher risk region (e.g. South-East Asia)? No preventive measure protects 100%, so in case of fever up to 3 months after return, malaria needs to be ruled out.
  - Risk for **other diseases transmitted by mosquitos**? Think of dengue, chikungunya, zika, Japanese encephalitis...?
  - **Travelers diarrhea**: how to prevent, what to do when diarrhea occurs, whether or not to prescribe antibiotics, when and how to take them?
  - **Sexual transmittable diseases**: always to be discussed when people travel without their partner. Take condoms! Even though it is generally not “planned”, many travelers have sex occasionally while traveling.

### 3. Vaccinations:

#### Mandatory vaccines?

- Yellow fever: this vaccine is subject of the International Health Regulations. Proof of vaccination - written down in the "yellow vaccination card" - can be a requirement for entry
- in some countries. Since 1/7/2016 the vaccination is lifelong valid, however for some people the duration of protection might not be lifelong (eg HIV infected patients, pregnant women, young children, ...), but there are no international accepted criteria about these "exceptions".
- Meningitis ACW135Y (pilgrimage to Mekka)
- Polio in Afghanistan and Pakistan if staying longer than 4 weeks

#### Are standard vaccinations updated?

- tetanus-diphtheria-whooping cough;
- measles
- hepatitis B when risk behavior or social volunteer work or medical sector
- What about flu vaccination in vulnerable travelers?

#### Travel related vaccines:

- Hepatitis A,
- Japanese encephalitis
- Tick borne encephalitis
- BCG: seldom necessary, but sometimes mandatory in young children of expats (in e.g French Lycee or American Lycee). In some European countries, this vaccine is not available
- anymore.

#### References:

Guidelines: Guidelines can change in each country; the Belgian Guidelines can be found on the website of Institute of Tropical Medicine: [www.itg.be](http://www.itg.be).

ALWAYS USE THE LATEST UPDATE!!! ESPECIALLY YELLOW FEVER RECOMMENDATIONS AND RECOMMENDATIONS ABOUT MALARIA CHANGE!

#### International guidelines:

WHO guidelines of travel medicine: <http://www.who.int/ith/en/>

CDC yellow book (cave malaria guidelines are very "American" and in Europe we prescribe usually less frequently anti-malarial pills):

<http://wwwnc.cdc.gov/travel/yellowbook/2016/table-of-contents>

Polio recommendations: <http://www.polioeradication.org/>

#### General information

Travel medicine: Travel medicine 3rd edition, Keystone



General, information about vaccines: Vaccines, 6th edition, Plotkins

Info about travel equipment, survival: Field guide to wilderness medicine, P S Auerbach, 4th edition

Ongoing epidemics: Promed: <http://www.promedmail.org/>, CDC, ECDC