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Nipah virus outbreak in Malaysia

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Abstract

Nipah virus, a novel paramyxovirus, closely related to Hendra virus emerged in northern part of Peninsular Malaysia in 1998. The virus caused an outbreak of severe febrile encephalitis in humans with a high mortality rate, whereas, in pigs, encephalitis and respiratory diseases but with a relatively low mortality rate. The outbreak subsequently spread to various regions of the country and Singapore in the south due to the movement of infected pigs. Nipah virus caused systemic infections in humans, pigs and other mammals. Histopathological and radiological findings were characteristic of the disease. Fruitbats of Pteropid species were identified as the natural reservoir hosts. Evidence suggested that climatic and anthropogenic driven ecological changes coupled with the location of piggeries in orchard and the design of pigsties allowed the spill-over of this novel paramyxovirus from its reservoir host into the domestic pigs and ultimately to humans and other animals.

Keywords: Nipah virus; Encephalitis; Outbreak; Malaysia

1. Introduction

The emergence of Nipah virus in Malaysia with its subsequent spread to Singapore came as a surprise to both countries and caught both relatively unprepared. In Malaysia, it caused tremendous human suffering especially among those involved in pig-farming, excessive economic loss and led to a near collapse of the local swine industry. The discovery of the novel virus played a major role and was a significant turning point in the control of this outbreak. The outbreak was swiftly brought under control with the assistance of international scientific partners. This article comprehensively reviews the chronological events of the outbreak, natural reservoir hosts of the virus, the epidemiological and clinical features of the disease and the possible events leading to its emergence which may have important bearing in the control and prevention of its potential re-emergence in this region.

2. Chronology of outbreak

Malaysia is situated in the heart of South East Asia. It is made up of Peninsular Malaysia and East Malaysia (Sabah and Sarawak). Peninsular Malaysia is bordered by Thailand in the north and Singapore in the south. Japanese Encephalitis (JE)

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is the important form of viral encephalitis affecting humans in Asia (Vaughn and Hoke, 1992) and it is also endemic in Malaysia with sporadic occurrence (Cardosa et al., 1995). Report from Ministry of Health, Malaysia showed that the total number of reported JE cases from 1989 to 1998 was 529, ranging from 9 to 91 cases per year with an annual average of 53. The number of deaths recorded for the same period was 35, ranging from 6 to 10 per year with an average of 3. Although there had been no significant increase in JE cases in the last few decades, three small outbreaks were reported; in 1974 in Pulau Langkawai, 1982 in Serian, Sarawak and 1988 in Penang (Cardosa et al., 1995).

In late September 1998, a cluster of patients associated with pig-farming in the suburb of Ipoh city within the Kinta district of Perak state in Peninsular Malaysia came down with acute febrile encephalitis that was associated with high mortality (CDC, 1999a,b; Chua et al., 1999; Uppal, 2000). The outbreak of febrile encephalitis in human was preceded by the occurrence of respiratory illness and encephalitis in pigs in the same district (Mohd Nor et al., 2000). At first, the illness in pigs was thought to be due to Classical Swine Fever. The deaths in humans were thought to be due to the JE virus as the initial investigations for the causes of acute encephalitis from 28 patients in the initial outbreak area by the Institute of Medical Research, Ministry of Health, were negative except for four serum samples that tested positive for JE-specific IgM (unpublished data). The four positive serological results were subsequently confirmed by the WHO Collaborating Center for Tropical Disease at the University of Nagasaki, Japan. The notion that the outbreak was due to JE virus was further propagated by the finding of the presence of JE-specific IgM in the sera derived from a number of patients who later came down with the illness (Chong et al., 2001c) and also the positive detection of JE nucleic acids in some of these patients' sera by reverse-transcriptase polymerase chain reaction carried out in the Arbovirus Unit of the Department of Medical Microbiology, University of Malaya (unpublished data). Various control measures based on the control of JE epidemic were taken proactively by the Ministry of Health to combat the outbreak in Kinta district and subsequently throughout the country.

By December 1998, the outbreak had spread to Sikimat, a town in Negeri Sembilan, and by February 1999, a similar disease in pigs and humans was recognized in Sungai Nipah village and Bukit Pelandok (the biggest pig-farming region) in the state of Negeri Sembilan (Chua et al., 1999). The spread of the outbreak was associated with the movement of pigs from Kinta district and between farms (CDC, 1999a,b; Mohd Nor et al., 2000). A month later, a cluster of 11 human cases of respiratory and encephalitic illness with one death was reported among abattoir workers in Singapore who handled pigs from outbreak regions in Peninsular Malaysia (Paton et al., 1999). The last locality to be involved in the spread of the outbreak was Sungai Buluh, Selangor, in the west central part of Peninsular Malaysia, with the last acute fatal case reported on 27th May 1999 (Fig. 1). In early March 1999, a novel paramyxovirus, Nipah virus (NiV), was isolated from the cerebrospinal fluid (CSF) of an encephalitic patient from Sungai Nipah village and subsequently identified as the aetiological agent responsible for the outbreak (Chua et al., 1999, 2000). The outbreak in Singapore ended when the importation of pigs from Malaysia was prohibited and the outbreak in Malaysia ceased with new control measures taken based on the discovery of the novel virus and the culling of over a million pigs (Chua et al., 2000). A total of 265 human cases of acute encephalitis including 105 deaths associated with the outbreak were recorded by the end of May 1999 (Chua et al., 2000; Parashar et al., 2000).

3. Natural reservoir host of Nipah virus

As with investigations in other emerging zoonotic diseases, the priorities for future prevention and control involved the identification of the natural reservoirs of Nipah virus. Surveillance for the reservoir host involving both domestic animals (dogs, cats, goats, chickens and fishes) and wildlife (wild boar, rodents, birds and bats) was

Fig. 1. Map of peninsular Malaysia showing the location of initial outbreak of Nipah virus and the temporal spread of the outbreak to other parts of the country and subsequently to Singapore.

undertaken with emphasis on fruit bats, based on the finding that fruit bats of Pteropid species were the reservoirs of Hendra virus (Young et al., 1996; Halpin et al., 2000), and the close phylogenetic relationship of Nipah virus to Hendra virus (Chua et al., 2000; Harcourt et al., 2000). Serological evidence indicated that domestic animals, notably dogs, cats, horses and goats were infected with the virus and it was believed that infected pigs were the source of infection for these species, and that all were effectively 'dead-end' hosts (Tan et al., 1999; Mohd Nor et al., 2000). No neutralizing antibody to Nipah virus was found in wildlife animals except for five species of bats (Mohd Yob et al., 2001). During the initial phase of surveillance, blood and tissue samples were collected from 324 bats, comprising 14 species, caught by mist-netting and shooting. Neutralizing antibodies to NiV were found mainly in Island flying-foxes (*Pteropus hypomelanus*) and Malayan flying foxes (*Pteropus vampyrus*) but no virus reactive with anti-Nipah virus antibodies was isolated (Mohd Yob et al., 2001). A novel approach was subsequently taken by collecting urine samples and swabs from fruits partially eaten by Island flying-foxes, *P. hypomelanus* and NiV was isolated from two pooled urine samples and a swab from partially eaten fruit (Chua et al., 2002b). Thus, the isolation of the virus from Island flying foxes corroborated the serological findings that fruitbats of Pteropid species were the natural reservoir hosts of the virus.

4. Epidemiological features of the outbreak

In humans, disease surveillance carried by the Ministry of Health from 29 September 1998 to December 1999, reported a total of 283 cases of viral encephalitis with 109 fatalities. The state of Negeri Sembilan reported the highest number of cases (231) and fatalities (86), followed by Perak with 28 cases and 15 deaths while Selangor had 24 cases and eight deaths. Other states did not report any human Nipah cases and deaths. The overall case fatalities rate for the outbreak was 38.5%

(109/283). The majority of patients were 25 years of age (86.2%). The largest number of cases (45, 15.9%) occurred in the 40–44 year old age group, followed by the 30–34 and 25–29 age groups with 39 (13.8%) and 37 (13.1%) cases, respectively. As expected for the local pig-farming industry that was mainly managed by Chinese population, more than half (69.6%) of the encephalitic cases were Chinese followed by Indians (17.3%), while Malays constituted only 2.1% of all cases. A large proportion of cases (231, 81.6%) occurred among males and the majority (70%) of cases were among those directly involved in pig-farming (pig farmers and workers). There was evidence that transmission of the virus to humans, was practically through close contact with infected pigs (Parashar et al., 2000; Amal et al., 2000; Premalatha et al., 2000; Chew et al., 2000; Sahani et al., 2001; Chan et al., 2002). Parashar et al. (2000) reported that there was significant association between Nipah virus infection and performing activities involving close contact with pigs, such as processing of baby pigs (clipping tails, tagging ears), administering injection or medication to pigs, assisting in the birth of piglets, assisting in pig breeding (collection of semen from boars and artificial insemination of sows), and handling of dead pigs. Human to human transmission was considered a very rare event (Mounts et al., 2001) though the Nipah virus could be isolated from saliva, urine, nasal and pharyngeal secretions of patients (Chua et al., 2001a). However, transmission of the virus from infected human to human and from infected dogs to human had also been documented (Tan et al., 2001, 1999).

The spread of the virus among pig farms within and between states of Peninsular Malaysia was due to movement of pigs (Mohd Nor et al., 2000). Evidence showed that farms that did not receive animals with suspected infection remained free from infection during the testing and surveillance program, although some of these farms were located fairly close to an infected farm. In the infected farm, the disease was observed to spread rapidly among pigs. Transmission between pigs in the same farm was attributed to direct contact with excretions and secretions such as urine, saliva, pharyngeal and lungs secretions. The possible mechanical transmission by dogs and cats, the repeated use of same needles or equipment without further sterilization after each use for health intervention and artificial insemination and sharing of boar semen within a farm were also implicated (Mohd Nor et al., 2000). Experimental transmission studies among pigs carried out in the Australian Animal Health Laboratory, Geelong, Australia, established that pigs could be infected by both oral and parental inoculation with the excretion of virus via oro-nasal routes. Infection was noted to spread quickly to the in-contact pigs (Middleton et al., 2002).

5. Clinical features of Nipah virus infection

Nipah virus caused a severe, rapidly progressive encephalitis that carried a high mortality rate (Goh et al., 2000; Chong et al., 2002). Based on the time interval between last exposure to pigs and subsequent onset of illness, the incubation period ranged from 4 days to 2 months with more than 90% of patients giving a history of 2 weeks or less. In Nipah virus, the rate of subclinical infection ranged from 8 to 15% (Tan et al., 1999; Parashar et al., 2000; Chew et al., 2000). Thus, the rate of symptomatic infection was high which is in contrast to JE, where only one out of 300 infected subjects had symptomatic encephalitis (Prasert, 1989). Unlike Hendra virus infection (Murray et al., 1995), the predominant presenting clinical features of acute illness due to Nipah virus related to involvement of the central nervous system (CNS), though a fair proportion of patients in Singapore presented symptoms involving respiratory system (Goh et al., 2000; Chong et al., 2002; Lee et al., 1999). The main presenting clinical features were fever, headache, dizziness and vomiting. More than 50% of the patients had a reduced level of consciousness and prominent brain-stem dysfunction. Distinctive clinical signs included segmental myoclonus, areflexia and hypotonia, hypertension, and tachycardia that suggested involvement of the brain-stem and the upper cervical spinal cord. Older patients, especially those having diabetes mellitus and those with severe brain-stem involvement as indicated by the presence of a

reduced level of consciousness, vomiting, abnormal doll's-eye reflex, abnormal pupils, hypertension, and tachycardia, carried a poorer prognosis (Goh et al., 2000; Chong et al., 2001b). Other poor prognostic factors were presence of segmental myoclonus, seizures and areflexia.

The main abnormalities in electroencephalographic (EEG) examinations were diffuse slow waves with focal sharp waves and continuous diffuse irregular slow waves (Chew et al., 1999; Goh et al., 2000). The EEG findings correlated with the severity of the disease and poor prognostic outcome. Those deeply comatose patients who subsequently succumbed to the infection had bilateral temporal periodic complexes of sharp and slow waves appearing every 1–2 s. There was no correlation between pattern of EEG and the presence of segmental myoclonus or focal lesions found in magnetic resonance (MR) imaging.

Thrombocytopenia and leucopenia were seen in less than 50% of patients and likewise, for mildly raised liver enzymes (Goh et al., 2000; Chong et al., 2000, 2002). Otherwise, blood urea, serum creatinine and electrolyte level were normal for all patients. In the acute phase of the illness, virus-specific antibodies were found to be positive in over seventy percent of serum samples of patients but in hardly a third of CSF samples (Ramasundrum et al., 2000). CSF examinations were abnormal as indicated by elevated white-blood cell counts, elevated protein level or both, in three quarters or more of the encephalitic patients. There was no correlation between abnormal CSF findings and the severity of the disease or fatality (Goh et al., 2000). However, the isolation of virus from the CSF was associated with high mortality (Chua et al., 2001b). In addition, positive virus isolation in the CSF occurred in the presence of specific IgM in some patients, indicating that the presence of CSF virus-specific IgM did not confer protection (Ramasundrum et al., 1999). Besides the CSF, the virus could also be isolated from the saliva, tracheal secretions, nasal secretions and urine samples of patients during the acute phase of the illness (Chua et al., 2001a).

The complications that occurred in severely ill patients included septicemia, bleeding from the gastrointestinal tract, renal impairment, haemothorax following the insertion of central venous line and rarely, pulmonary embolism and acute atrial fibrillation (Goh et al., 2000).

The direct cause of death was thought to be mainly due to the direct consequence of encephalitis especially when the brain-stem was involved except, one who died of a massive intracerebral hemorrhage after recovery from coma. Of the survivors, about three quarters recovered fully and a quarter had residual neurological deficits. All patients with normal levels of consciousness throughout the course of the acute illness recovered fully after a mean duration of illness of about 2 weeks, whereas, only about 15% with a reduced level of consciousness did so (Goh et al., 2000; Chong et al., 2002).

Treatments were mainly supportive. An antiviral agent, Ribavirin, was later used to treat these encephalitic patients in an open-label trial (Chong et al., 2001a). The trial results suggested that the drug was able to reduce the mortality of acute Nipah encephalitis with no associated serious side effect.

In a follow-up study conducted 24 months post-outbreak, 12 patients (7.5%) with acute encephalitis who had initially recovered developed recurrent neurological disease (relapse encephalitis) and ten patients (3.7%) who initially had acute non-encephalitis or aymptomatic infection presented with late-onset encephalitis (Tan et al., 2002). This delayed presentation of encephalitis was a feature similar to that seen in a case of fatal meningoencephalitis due to Hendra virus in Mackay, Qld, Australia, who developed the illness 13 months after the initial infection (O'Sullivan et al., 1997). Three patients had a second recurrence of neurological attack. The mean interval between the first recurrence and the time of initial infection was about 8 months and 15 days. The onset of relapse or late-onset encephalitis was acute in nature and the common presenting clinical features were fever, headache, seizures, and focal neurological signs. Four of the 22 relapse and late-onset patients succumbed to the illness. Nipah virus antigens were detected in the brain of the deceased but no perivenous demyelination was noted at autopsy. Late-onset and relapse encephalitis were attributed to the persistence of Nipah virus infec-

tion in the CNS although no virus was isolated from the CSF.

In pigs, mortality was low ranging from less than 15 to 5% but infection rate approaches 100% (Mohd Nor et al., 2000). The incubation period was estimated to be 7–14 days. Nipah virus caused illness in pigs with both CNS and respiratory manifestations though the clinical patterns of the disease varied according to the age of the pigs. Pigs less than 6 months old usually presented with an acute febrile illness with respiratory signs ranging from rapid and labored respiration to a harsh, non-productive cough and open mouth breathing with severe cough and haemoptysis occurred in severe cases. Neurological signs such as trembling, muscular twitching, spasm and myoclonus, hind-leg weakness with varying degrees of spastic or flaccid paresis and inco-ordinate gait may accompany respiratory signs in some affected pigs. In boars and sows, acute febrile illness with labored respiration, increased salivation and nasal discharge accompanied neurological signs such as agitation, head pressing or knocking, clamping of mouth, nystamus, tetanus-like spasm and seizures. Early abortion may occur in pregnant sows and sudden death may occur in both boars and sows.

6. Pathological changes associated with Nipah virus infection

Nipah virus caused systemic infections in both humans and pigs though clinical manifestations confined mainly to the respiratory and CNSs. Autopsy findings in humans showed that the brain was the most severely affected organ but other organs including the lungs, heart and kidneys were also involved (Chua et al., 1999; Goh et al., 2000; Wong, 2000). The affected organs were congested with petechial hemorrhage. Histological examinations showed vasculitis affecting the arterioles, venules and capillaries with endothelial damage, fibrinoid necrosis of vessel wall, hemorrhage and thrombosis. Giant syncytial-cell formation was seen in the endothelium of affected blood vessels in the brain, lungs and in the renal glomeruli. Zones of microinfarction and ischaemia were found around and adjacent to vasculitic blood vessels. In the brain, neurons adjacent to infected vessels showed degenerative changes with eosinophilic cytoplasmic and nuclear inclusions. Neuronophagia and microglial-nodule formations were present but perivascular cuffing was generally not a prominent feature. Immunohistochemical staining showed the presence of Nipah virus antigens in the endothelial cells of the affected vessels and the surrounding neurons and glial cells in the affected brain, bronchial epithelial cells and cells lining the lung alveoli.

In affected pigs, lungs showed varying degrees of consolidation and petechiae to ecchymotic hemorrhages (Mohd Nor et al., 2000). The bronchi and trachea were filled with frothy and in some cases blood stained fluids. The brain showed generalized congestion and oedema. A similar finding was noted in some affected kidneys. Histological changes of affected tissues were essentially the same as those seen in human cases except that immunohistological findings showed a high concentration of viral antigens in the endothelium of the affected blood vessels and bronchial epithelium of the lungs.

7. Radiological findings in Nipah virus infection

Though relatively few patients presented with respiratory symptoms, chest radiograph examination revealed abnormal findings in 6–10% of patients (Goh et al., 2000). However, a report in Singapore showed that eight out of the 11 patients studied had abnormal chest radiographs (Paton et al., 1999). The main abnormal finding was mild interstitial shadowing. In the acute phase of illness, the result of computed tomography examination of the brain was essentially normal (Goh et al., 2000). However, magnetic resonance imaging (MRI) of the affected brain showed the presence of widespread discrete, high signal intensity focal lesions that measured 2–7 mm and were disseminated throughout the brain but mainly in the subcortical and deep white matter and to a lesser extent in the gray matter (Ahmad Sarji et al., 2000; Lim et al., 2000, 2002). Some patients with asymptomatic Nipah virus infection also have similar abnormal cerebral MR images (Tan et

al., 2000, 2002). The lesions were attributed to widespread microinfarction due to underlying vasculitis and thrombosis of small blood vessels. This finding had not been described in other forms of encephalitis known to date. Unlike changes seen in the acute illness, the MRI findings in the relapse and late-onset cases showed diffuse confluent involvement of cortical gray matter mainly in the cerebral hemisphere (Goh et al., 2000; Ahmad Sarji et al., 2000; Tan et al., 2002).

8. Nipah virus

The Nipah virus is probably the easiest virus to isolate. It grew in all types of mammalian cells so far tested in the laboratory and produced syncytial cell formation (Chua et al., 1999, 2000). However, it did not grow in a number of insect cell-lines tested (unpublished data). The rate of growth and patterns of cytopathic effect (CPE) produced in all culture varied with the type of mammalian cells used. With Vero cells (ATCC, CCL-81), CPE could be easily visible by 5th–7th day post-inoculation of clinical samples. It caused full CPE in Vero cells by 24 h post-inoculation using supernatant culture fluid at high multiplicity of infection. It produced a high viral titre (10^8 infectious particles per ml) in Vero cells at full CPE (unpublished data).

Nipah virus shares many of its morphological features with other members of the *Paramyxoviridae* family, such as; inclusions of viral nucleocapsids within the cytoplasm, budding of the nucleocapsid at the plasma membrane and pleomorphic extracellular envelope virus particles filled with collection of tangled viral nucleocapsids (Goldsmith et al., 2000; Hyatt et al., 2001). In negative stain preparations, nucleocapsids show the typical 'herringbone' appearance that is characteristic for paramyxoviruses (Goldsmith et al., 2000; Chow et al., 2000).

The full-length genome sequence of Nipah virus has been determined for a few isolates (Harcourt et al., 2000, 2001; Chan et al., 2001). The genome comprises 18 246 nucleotides, that is, 12 nucleotides longer than that of the Hendra virus (HeV). Both NiV and HeV enjoy extra genome length in comparison with other members of the *Paramyxovirinae* subfamily (average about 15 500 nucleotides), though the lengths of their respective coding region of the genes are about equal except for the phosphoprotein gene. The excessive length of NiV and HeV genomes in comparison with that of other paramyxoviruses is mainly due to increased length of the 3′ non-coding region of all the genes except for the polymerase gene. Like HeV, the genome of NiV contains six genes flanked by a leader sequence at the 3′ end and a trailer sequence at the 5′ end. Both NiV and HeV have identical leader and trailer sequence lengths and hexamer-phasing position for all their genes including the P gene-editing site. They are also very closely related with respect to their genomic end sequences, gene start and stop signals, P gene-editing signals and deduced amino acid sequences of all their respective proteins (Chan et al., 2001). A new genus was created, named *Henipavirus* (Hendra + Nipah) to accommodate these two phylogenetically closely related viruses with HeV as the type species and NiV as the second member of the genus (Wang et al., 2000, 2001).

9. Virus emergence

With the outbreak under control and the establishment of fruitbats of Pteropid species as the natural reservoir hosts of the virus, it is essential to understand the underlying causal factors that resulted in its emergence so as to undertake necessary measures to prevent its re-emergence. It is impossible to ascertain retrospectively the definitive events and factors that led to its emergence, especially when the outbreak due to this novel virus was only realized nearly a year after its initial introduction into the swine population. However, available data and evidence suggest that a complex interplay of multiple factors led to the spillage of the virus from its natural reservoir host into the domestic pig population with subsequent spread to humans (Chua et al., 2002a). In a nutshell, over the last two decades, the forest habitat of these fruitbats (flying-foxes) in Southeast Asia has been substantially reduced by deforestation for pulpwood and industrial planta-

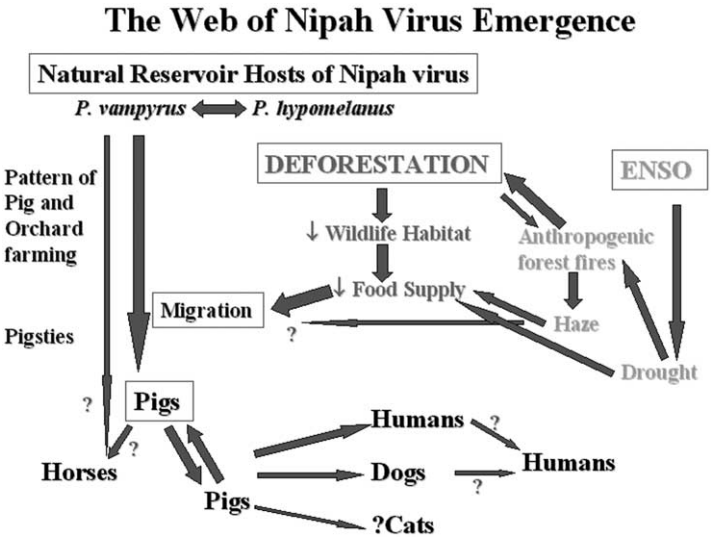

Fig. 2. A flow chart representing the proposed sequence of events leading to the spillover of Nipah from its reservoir hosts (*P. vampyrus*) into the swine population and subsequent transmission to other animal hosts including humans.

tion (Malingreau et al., 1985; Schweithelm and Glover, 1999). In 1997/1998, slash-and-burn deforestation resulted in the formation of a severe haze that blanketed much of Southeast Asia in the months directly preceding the Nipah virus disease outbreak (Schweithelm, 1998; Davies and Unam, 1999; Schweithelm and Glover, 1999). This was exacerbated by a drought driven by the severe 1997–1998 El Niño Southern Oscillation (ENSO) event (Glantz, 2001). This series of events led to an acute reduction in the availability of flowering and fruiting forest trees for foraging by flying-foxes in their already shrinking wildlife habitat (Tang et al., 1996; Mohd Shahwahid and Othman, 1999; Chua et al., 2002a). This culminated in an unprecedented encroachment of flying-foxes into cultivated fruit orchards in the initial outbreak area in the suburb of Ipoh, in 1997/1998. These anthropogenic events, coupled with the location of piggeries in orchards and the design of pigsties in the index farms allowed transmission of a novel paramyxovirus from its reservoir host to the domestic pig and ultimately to the human population and other domestic animals (Field et al., 2001; Chua et al., 2002a). A flow chart summarizing the proposed sequence of events leading to the spillover of Nipah virus from its resevoir hosts into the swine population and subsequent transmission to other animal hosts including humans is shown in Fig. 2. The available evidence suggested that the virus could have been introduced into the swine population in 1997 and the suggestion was supported by a later finding that six of the stored sera from patients with viral encephalitis admitted to Ipoh General Hospital in 1997 were found positive for Nipah virus IgG (unpublished data).

10. Future directions

The last human case that succumbed to acute infection of Nipah virus was seen on 27th May 1999. Since then, there had been no further reported cases of acute human infection. The declaration that Malaysia was free of Nipah virus in the livestock population was accepted by the Office International de Epizooties (OIE) in June 2001. An early sentiment was to develop a Nipah virus vaccine for the livestock. However, as is the case for other zoonotic infections, good surveillance and culling the infected animal population may be the most cost-effective and rapid measure for controlling the spread of the disease. In addition, with a better understanding of wild

animal reservoirs and reasons for its spilling over to domestic animals and subsequently to human hosts, necessary measures can be taken to prevent the re-emergence of the virus. Thus, it is more logical to develop a simpler, more sensitive and specific laboratory test for rapid diagnosis and surveillance (Daniels et al., 2001). To overcome the need for a neutralization test as the confirmatory test which requires biosafety level 4 facility, research is ongoing to develop a chimeric virus that expresses the fusion and attachment proteins of Nipah virus so that a surrogate neutralization test can be performed in either a level 2 or level 3 biosafety laboratory which is more readily available in this region.

The distribution of *P. vampyrus* and *P. hypomelanus* is widespread in South East Asia, extending from Myanmar, Thailand and the Indochina region to the Philippines, and south to include all of western Indonesia as far as Timor, Sumba and Suva (Medway, 1978). Steps need to be taken by each country in the region to strengthen the surveillance program for the possible re-emergence of this novel virus and other exotic infectious agents from this fruitbats such as the Tioman virus (Chua et al., 2001c).

On the other hand, more research need to be done to study the pathogenesis of this newly emerged and deadly virus with respect to its mechanism of entry into the host, virus receptor, neurotropism and tissues damage, viral persistence in the CNS and host immune response.

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