

Nipah Encephalitis Outbreak in Malaysia

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Abstract

Introduction: Between September 1998 and June 1999, there was a severe outbreak of viral encephalitis among the pig farm workers in Malaysia. **Methods:** This is a review of the published literature related to the outbreak with the focus on human diseases. **Results:** The encephalitis was caused by a newly discovered paramyxovirus related to Hendra virus, later named Nipah virus. There were 265 patients with acute encephalitis. The disease is thought to spread from pig to man through close contact. The risk of human-to-human spread is thought to be low. The disease affected mainly adult Chinese males, half of whom had affected family members. The disease presented mainly as acute encephalitis with a short incubation period of less than two weeks, with the main symptoms of fever, headache, and giddiness followed by coma. Distinctive clinical signs include segmental myoclonus, areflexia and hypotonia, hypertension, and tachycardia. Initial cerebrospinal fluid was abnormal in 75% of patients. Serology was helpful in confirming the diagnosis. Magnetic resonance imaging showed distinctive changes of multiple, discrete, and small high signal lesions, best seen with fluid-attenuated inversion recovery (FLAIR) sequences. Mortality was high at 40% and death was probably due to severe brainstem involvement. The main necropsy finding in acute encephalitis was that of disseminated microinfarction associated with vasculitis and direct neuronal involvement. Ribavirin was able to reduce the mortality by 36%. Relapse encephalitis was seen in 7.5% of those who recovered from acute encephalitis, and late-onset encephalitis in 3.4% of those with initial non-encephalitic or asymptomatic diseases. The mean interval between initial illness and the onset of the complication was 8.4 months. The relapse and late-onset encephalitis which manifested as focal encephalitis arose from recurrent infection. **Conclusion:** Nipah virus, a recently discovered paramyxovirus, causes a unique encephalitis with high mortality as well as relapse and late-onset encephalitis. The infection is mainly spread from pigs to man.

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Key words: Clinical manifestation, Malaysia, Nipah encephalitis, Pathology, Treatment

Introduction

From September 1998 to June 1999, there was an outbreak of viral encephalitis in several pig farming villages in Malaysia.¹⁻⁴ The outbreak, which started in Ulu Piah, Tambun and Ampang near Ipoh in the state of Perak, later involved Sikamat, Sungai Nipah, Kampong Sawah and Bukit Pelanduk areas in the state of Negri Sembilan, and Sepang and Sungei Buloh in the state of Selangor (Fig. 1). The outbreak subsequently spread to involve abattoir workers in Singapore.^{5,6} In Malaysia, more than 265 patients were affected nationwide with more than 105 mortalities.^{4,7} This review of the outbreak focusses on aspects related to human disease.

Differential Diagnosis from Japanese Encephalitis

Because the outbreak involved pig farm workers, the outbreak was initially thought to be due to Japanese encephalitis. Features that distinguished this outbreak from

Japanese encephalitis included: infection predominantly in adults rather than children; clustering of cases in members of the same household, which suggests an infection with a high disease attack rate (as opposed to Japanese encephalitis virus which causes symptomatic encephalitis in 1 in 300 of those infected); a high proportion of patients in direct contact with pigs as opposed to uninfected individuals living in the same neighbourhood, thus arguing against a mosquito-borne disease; a history of illness in the pigs belonging to affected farmers; and the fact that many patients have had previous immunisation against Japanese encephalitis. Isolation of a new paramyxovirus from cerebrospinal fluid specimens of several patients indicated that this was the aetiologic agent.^{3,4} The virus was subsequently named Nipah virus, after Kampung Sungai Nipah, where patients whose specimens yielded the first viral isolates lived. Viral genomic sequencing has now established Nipah virus as a new paramyxovirus closely

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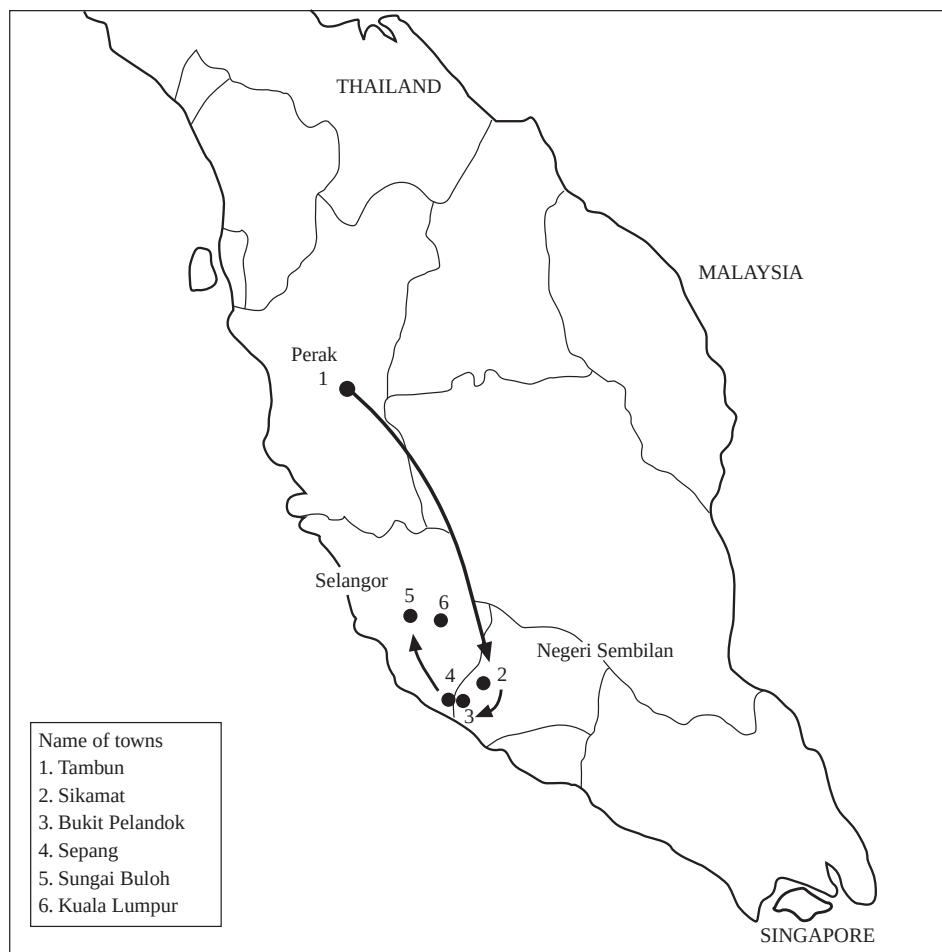


Fig. 1. Map of Malaysia showing spread of Nipah encephalitis outbreak among neighbouring states. (Source: Sim B F, Jusoh M R, Chang C C, Khalid R. Nipah encephalitis: report of 18 patients from Kuala Lumpur Hospital. *Neurol J Southeast Asia* 2002; 7:13-8. Reprinted with permission from the ASEAN Neurological Association.)

related to Hendra virus.^{4,8} Hendra virus caused disease among horses and affected 3 patients in Australia in 1994 and 1995.^{9,10} There is a high degree of nucleotide homology in the open reading frames of the various genes of Hendra virus and Nipah virus that exceeds 70%, and an amino acid identity of more than 80% in most genes.^{4,8}

Epidemiology

It has now been firmly established that close contact with pigs was responsible for viral transmission to humans.^{7,11,12} Case control studies of the local community and household members have shown that patients were more likely to perform activities requiring direct contact with pigs,⁷ involved with full-time pig farming,¹¹ and had contact with live pigs.¹² The case of a Muslim army major who supervised the pig culling operation, and who was in close contact but denied direct body contact with pigs, suggested that infection is possible via respiratory droplets at close range.¹¹ A case was also reported in which the patient was never in close proximity to pigs. However, the patient had personally

nursed two ill pet dogs, which subsequently died; so transmission was attributed to the infected dogs.¹¹

Transmission of Nipah virus to healthcare workers was thought to be generally low. In a large survey of 288 health workers, only 3 were found to have IgG antibodies but IgM and serum neutralisation tests were negative, and thus these cases were thought to be false positives.¹³ However, there is a report of a nurse who had previously cared for Nipah encephalitis patients and subsequently seroconverted but remained asymptomatic. The magnetic resonance imaging (MRI) of her brain showed multiple, discrete high signal lesions like those seen in acute Nipah virus encephalitis.^{14,15} Therefore, human-to-human transmission is possible as exemplified by this case, and the fact that virus could be isolated from patients' respiratory secretions and urine.¹⁶

Clinical Manifestations

Of the 94 patients with symptomatic Nipah virus infection seen in the University Malaya Medical Centre during the

outbreak, more than half of the patients had affected family members, suggesting a disease of high infection rate.¹⁷ In a study of 14 households with 110 members in the outbreak area, 27% had symptomatic Nipah infection, and another 8% had subclinical infection with seroconversion. This suggests a ratio of roughly 3:1 for symptomatic versus asymptomatic infection.¹¹

The main demographic features of 103 patients treated in Seremban Hospital were 88% males, 58% ethnic Chinese, mean age of 38 years, and 78% pig farmers or hired workers. This is reflective of the Malaysian pig-rearing industry, which consists mainly of Chinese-owned small family farms.¹⁸

Although the incubation period is difficult to determine with any degree of certainty, it was less than 2 weeks in 92% of patients.¹⁷ Chong and colleagues,¹⁸ from Seremban Hospital, estimated the mean incubation period to be 10 days. The clinical manifestation was that of an acute encephalitis with fever, headache, vomiting, and reduced level of consciousness.^{3,5,6,17,18} Distinctive clinical features were areflexia, hypotonia, prominent autonomic changes such as tachycardia and hypertension, and segmental myoclonus in 32% of patients which was characterised by focal, rhythmic jerking of muscles, commonly involving the diaphragm and anterior muscles of the neck.¹⁷

Respiratory tract involvement with cough was seen at presentation in 14% of patients.¹⁸ However, none of the patients from the University Malaya Medical Centre and Seremban Hospital^{17,18} had pneumonia attributed to Nipah virus, in contrast to Paton and colleagues⁵ who reported that 3 of their 11 patients from Singapore had atypical pneumonia with abnormal chest X-rays. There were some patients who had non-encephalitic infection with seroconversion and systemic symptoms, but no evidence of encephalitis. Of the 94 patients admitted to the University Malaya Medical Centre with Nipah virus infection, 91 had acute encephalitis, and 3 had non-encephalitic infection.¹⁷

The overall mortality of acute Nipah encephalitis was 40%.⁴ Severe brainstem involvement appeared to be associated with poor prognosis, such as tachycardia and abnormal doll's eye reflex,¹⁷ hypertension and high fever.¹⁸ Presence of virus in the cerebrospinal fluid, which suggested high viral replication, was also associated with high mortality.¹⁹ Concomitant diabetes mellitus, but not the level of exposure to sick animals, was also related to mortality, probably due to immunoparesis.²⁰

Laboratory, Radiologic and other Diagnostic Investigations

In the University Malaya Medical Centre patients, thrombocytopenia was a feature in 30% of patients while leukopenia was present in 11%. Alanine and aspartate

aminotransferases were elevated in 33% and 42% of patients, respectively, but blood urea, creatinine, and electrolyte levels remained normal in all patients.¹⁷ Similar findings were also seen in patients from Seremban Hospital.¹⁸ These changes were attributed to non-specific systemic changes in very ill patients.^{17,18}

Cerebrospinal fluid (CSF) examination was abnormal in 75% of patients with elevated protein levels or elevated white cell counts. Glucose levels were within normal limits.^{17,18} These features are non-specific, and may be found in many primary viral encephalitides.

IgM and IgG antibody detection in serum and CSF were critical to the diagnosis of Nipah virus infection. The antibody test utilised an IgM-capture enzyme-linked immunosorbent assay (ELISA) test while IgG antibodies were detected by an indirect IgG ELISA assay.^{1,3} Initially, before it was clearly identified and characterised, Nipah virus was suspected to be "Hendra-like" since cells infected with the Nipah virus reacted strongly by immunofluorescence to anti-Hendra virus antibodies. Subsequently, it was found that Nipah virus and Hendra virus have enough shared epitopes to enable Hendra virus antigens to be used in a prototype serologic test sensitive enough to detect Nipah virus antibodies.¹ The ELISA tests have a high specificity and is therefore useful as a screening test.²¹ The rate of positive IgM was 60% to 71% by day 4 and 100% by day 12 of illness. For IgG, it was 7% to 29% by day 1 and 100% by day 25–6 of illness.²²

Though the presence of cerebrospinal fluid IgM was useful in aiding diagnosis, it was not associated with mortality and severity of illness. Thus, it may not have a protective effect against the disease.²³ There was a high frequency of positive CSF IgM to Japanese encephalitis of up to 9% in acute Nipah encephalitis patients. This probably reflected the endemicity of Japanese encephalitis infection and appeared to play no part in the patient's illness.²⁴ The breakdown in blood brain barrier associated with disseminated vasculitis seen in Nipah encephalitis could possibly have contributed to the high rate of positive Japanese encephalitis CSF IgM in these patients.²⁴

Brain magnetic resonance imaging (MRI) proved to be a useful diagnostic aid in acute encephalitis.^{25,26} Typically, in acute Nipah virus encephalitis the brain MRI showed multiple, disseminated, small discrete hyperintense lesions best seen in the fluid-attenuated inversion recovery (FLAIR) sequence mainly in the subcortical and deep white matter, and occasionally in the cortex. The lesions, which measured about 2 to 7 mm in diameter, are likely to correspond to the microinfarctions noted in postmortem tissues. Similar changes were also seen in 16% of asymptomatic patients with Nipah virus infection showing that subclinical cerebral involvement is not uncommon even in these patients.¹⁴

The most common electroencephalography abnormality was continuous, diffuse, and symmetrical slowing with or without focal discharges. The degree of slowing correlated with severity of disease. Independent bitemporal, periodic complexes were common among those deeply comatose, and were associated with 100% mortality.²⁷

Treatment

Ribavirin, a broad-spectrum virustatic anti-viral agent, which showed varying degrees of efficacy against viruses such as respiratory syncytial virus, influenza and measles, was tried on an empirical basis in the patients. In an open label trial of 140 patients with 54 patients as controls (patients who refused treatment or otherwise not given the drug), there were 45 deaths (32%) in the ribavirin group versus 29 deaths (54%) in the control arm. This represented a reduction in mortality of 36%. Although this trial was disadvantaged by the use of historical controls, it does suggest that ribavirin may be useful in the treatment of acute Nipah encephalitis. There were no apparent serious side effects in this study.²⁸

Pathology and Pathogenesis of Acute Nipah Virus Infection

The available information on the pathological changes came from autopsies in various hospitals in Malaysia. A total of 31 fatal acute encephalitis cases were studied by light and electron microscopy, and immunohistochemistry.^{3,4,29} Macroscopically, the features were in general rather non-specific, although small lesions suggestive of necrosis may occasionally be observed.

The blood vessels appeared to be one of the early major targets of Nipah virus infection. Medium-sized to small blood vessels (arteries and veins) in major organs, including the brain, lung and kidney, were susceptible to infection. The earliest lesion seemed to be the formation of multi-nucleated syncytium in the endothelium. More commonly, vascular damage takes the form of endothelial ulceration with varying degrees of inflammation and fibrinoid necrosis. The inflammatory cells consisted of neutrophils, macrophages, and other cells found within the vascular wall. Vasculitis was frequently associated with thrombosis and vascular occlusion. Staining by immunohistochemistry often demonstrated the presence of viral antigen within the endothelium, endothelial syncytium, and tunica media. These findings confirmed that Nipah virus is able to infect blood vessels directly.

The brain, including the meninges, gray and white matter, showed widespread occurrence of vascular lesions, and is the organ most severely affected. The vasculitic vessels were seen with or without thrombosis and often adjacent to small areas of necrosis and ischaemia. The surviving

neurons in these areas may reveal eosinophilic, cytoplasmic, and or less frequently, nuclear paramyxoviral-type inclusions. Immunohistochemical staining confirmed that these viral inclusions contained Nipah viral antigens. Parenchymal inflammation consisting of perivascular cuffing and neuronophagia could be seen particularly in cases that died after more than a week of illness.

Outside the central nervous system, except perhaps in the liver, vasculitis and parenchymal lesions could be found in all the major organs including the lungs, kidneys and heart. After the central nervous system, the most severely affected organ is the lung in which focal fibrinoid necrosis of the lung parenchyma associated with vasculitis was encountered. In addition, there was inflammation associated with multi-nucleated giant cells in the alveolar spaces. Pathologic changes in the kidney were characterised mainly by vasculitis and glomerular lesions consisting of microthrombi, scattered inflammatory cells, and occasionally multi-nucleated syncytia. Occasionally, in the lymphoid organs, such as lymph node and spleen, multi-nucleated syncytia could be found. Viral antigen was detected in affected tissues.

Severe central nervous involvement explained why symptomatic patients usually presented with an acute encephalitic syndrome, in which a combination of ischaemia, microinfarction and direct neuronal infection resulted in neurologic manifestations. Other than immunohistochemical staining confirmation of Nipah viral antigens in neurons,²⁹ the contribution of direct neuronal infection to neurological manifestations is also evidenced by distinctive neurological signs such as segmental myoclonus,¹⁷ and the association between high mortality and the presence of virus in the CSF.¹⁹

Relapse and Late-onset Nipah Encephalitis

Most unexpectedly, a small number of patients suffered a second or even a third neurologic episode following what appeared to be complete recovery. These relapsed Nipah encephalitis patients constitute 7.5% of the total number of survivors.³⁰ Symptoms appeared after an average of 8.4 months following viral exposure. In addition, about 3.4% who were either asymptomatic or had only mild non-encephalitic illness initially also developed similar neurologic episodes (late-onset Nipah encephalitis) for the first time several months later. Clinical, radiologic and pathologic findings suggested that essentially relapse and late-onset Nipah encephalitis were the same disease process that was distinct from acute Nipah virus encephalitis. The common clinical features were fever, headache, seizures and focal neurological signs. There was an 18% mortality rate. MRI typically showed patchy areas of confluent cortical lesions.³⁰

Necropsy showed focal encephalitis in which the demonstration of neuronal viral antigen in brains suggested that relapsed and late-onset Nipah encephalitis were due to a recurrent infection, rather than post-infectious demyelination described following measles or other viral infections.³⁰ Relapsed Nipah encephalitis is probably analogous to the single human case of Hendra virus encephalitis, in which 13 months after meningitis associated with drowsiness, the patient developed fatal encephalitis.¹⁰ Hendra virus antigen was demonstrated in the brain tissues. Nonetheless, in both relapsed Nipah encephalitis and Hendra encephalitis, the respective viruses have not been isolated thus far.^{10,30}

The Bat as Reservoir Host: Implications for Future Outbreaks

There is now increasing evidence that the reservoir of Nipah virus is very likely the fruit bat (*Pteropus hypomelanus*). On Pulau Tioman, an island off the coast of peninsular Malaysia, the Nipah virus has been isolated from the urine of roosting bats.³¹ However, attempts to isolate virus or amplify viral sequences from bats caught in peninsular Malaysia nearer the epicentres of the outbreaks were unsuccessful.³² Nonetheless, serum neutralising antibodies were found in 4% to 31% of bat species, including those from Pulau Tioman. These may represent antibodies raised against the Nipah virus or other as yet unidentified virus, but cross-reaction to Hendra virus infection appeared to have been excluded.³²

If fruit bats were the reservoir host, it begs the question of how the Nipah virus came to be transmitted to pigs and other animals. One theory is that half-eaten fruits dropped off near pig farms may have enough viruses to infect an animal that subsequently ingests it. Indeed, viruses have been isolated from such fruits.³¹ Whatever the mode of transmission from bats to pigs, pigs played the main role of amplifying hosts for the virus. The close proximity of pigs in most Malaysian pig farms probably contributed significantly to pig-to-pig transmission. Preventive measures for further outbreaks would include frequent surveillance of the pig population for evidence of fresh infection and to avoid planting fruit trees, which may attract bats close to the pigsties.

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