# Nipah Encephalitis – An Update

**Sherrini Bazir Ahmad, MRCP, Chong Tin Tan, FRCP**

Neurology, Department of Medicine, University of Malaya, Kuala Lumpur, Malaysia.

**SUMMARY**

**Between September 1998 to May 1999, Malaysia and Singapore were hit by an outbreak of fatal encephalitis caused by a novel virus from the paramyxovirus family. This virus was subsequently named as Nipah virus, after the Sungei Nipah village in Negeri Sembilan, where the virus was first isolated. The means of transmission was thought to be from bats-to-pigs and subsequently pigs-to-human. Since 2001, almost yearly outbreak of Nipah encephalitis has been reported from Bangladesh and West Bengal, India. These outbreaks were characterized by direct bats-to-human, and human-to-human spread of infection. Nipah virus shares many similar characteristics to Hendra virus, first isolated in an outbreak of respiratory illness involving horses in Australia in 1994. Because of their homology, a new genus called Henipavirus (Hendra + Nipah) was introduced. Henipavirus infection is a human disease manifesting most often as acute encephalitis (which may be relapsing or late-onset) or pneumonia, with a high mortality rate. Pteropus bats act as reservoir for the virus, which subsequently lead to human spread. Transmission may be from consumption of food contaminated by bats secretion, contact with infected animals, or human-to-human spread. With wide geographical distribution of Pteropus bats, Henipavirus infection has become an important emerging human infection with worldwide implication.**

**Key words:** *Nipah Encephalitis, Nipah Virus, Hendra Virus, Japanese Encephalitis*

## INTRODUCTION

It has been nearly fourteen years since the severe outbreak of *Nipah* encephalitis, the fatal viral encephalitis that initially affected a substantial number of people from several pig-farming villages in Malaysia and abattoir workers in Singapore. In Malaysia, 265 cases of *Nipah* encephalitis and 105 deaths were estimated from September 1998 to May 1999[1], with highest death rate reported from Bukit Pelanduk district of *Negeri Sembilan* state[2]. The initial outbreak was first thought to be another endemic of *Japanese encephalitis* (JE), a flavivirus transmitted to human via *Culex* mosquitoes and is known to have caused major porcine-associated outbreaks in Malaysia in 1974 (*Langkawi*), 1988 (*Penang*) and 1992 (*Serian* district of *Sarawak*). However, epidemiological features were unlike JE[3]. Electron microscopy, immunohistochemistry and immunofluorescence study of the cerebrospinal fluid (CSF) from several affected patients later identified a newly isolated strain of syncytium-forming virus that has features of the *Paramyxoviridae* family and shares close similarities to the *Hendra virus* (HeV)[3]. Viral genomic sequencing subsequently identified that this novel virus is distinct from Hendra and was named *Nipah virus* (NiV), after the *Sungei Nipah* village, where the virus was first isolated[1,4].

## EPIDEMIOLOGY

The outbreak initially involved pig-farming villages in *Ipoh*, a town in *Perak*, which subsequently spread to the southern part of Peninsular Malaysia in *Selangor* and *Negeri Sembilan* states, including the *Sikamat* and *Bukit Pelanduk* villages[5]. JE was initially suspected to be the causative agent for this fatal outbreak in 1998 because of the apparent detection of JE antibodies in some patients and temporal history of exposure to infected pigs. However, certain clinical and epidemiologic features of this outbreak seem to be atypical to JE i.e. most patients were adult males rather than children, clustering of cases within members in the same household, which suggests an infection of high attack rate (as opposed to the JE virus which caused symptomatic encephalitis in 1 in 300 of those infected); and the fact that many patients have been previously immunized against JE[2]. Furthermore, the effort of clearing the affected areas from JE virus-bearing mosquitoes failed to cease the rising numbers of infected patients.

Figure 1 demonstrated the geographical distribution of pig farming villages affected by the outbreak in *Negeri Sembilan*. No cases were reported from the Malay villages in *Bukit Pelanduk* despite the close proximity with the adjacent Chinese farms with clustering of the encephalitis infection. There was also no reported case of NiV in *Sungai Pelek*, which is a village north of *Bukit Pelanduk,* across the *Sepang River*, which also has pig farms, within the reach of mosquito flight. This suggested that close contact with infected pigs is required to develop the infection. The Muslims from the Malay villages are prohibited from having any close contacts with pigs due to their religious belief. Pig-to-pig and pig-to-human transmissions were thought to be from direct contact with the pigs' excretions including urine, saliva, pharyngeal and tracheal secretions, except for two cases of transmission from infected dogs[5-8]. Two affected patients were involved in repairing pig cage and supervising the pig culling operation[5].

All these epidemiological features make mosquito-borne JE unlikely and this was confirmed when Chua and his colleagues from the University of Malaya discovered the new virus and named it as *Nipah virus*[1].

## HUMAN-TO-HUMAN TRANSMISSION

The isolation of virus from urine and tracheal secretions from affected patients in Malaysia suggested that human-to-human transmission is possible[9-10]. Serum IgG antibodies to NiV were isolated from 3 health-care workers. One of the cases was a staff nurse who also had MRI changes similar to those seen in acute NiV. She cared for the infected patients, but had no previous contact with pigs. She remained asymptomatic despite the positive serology and MRI changes. These show that human-to-human transmission of infection did occur during the Malaysian outbreak. However, because of the early practice of barrier nursing, it was not common[9,11,12].

*Corresponding Author: sherrini@ummc.edu.my*

## FROM MALAYSIA TO SINGAPORE

The spread of the epidemic was thought to be due to the massive 'fire sales' and movements of infected pigs from Perak to other neighbouring states in the country [Figure 2], including *Selangor* and *Negeri Sembilan*. The outbreak subsequently spread to involve the abattoir workers in *Singapore* due to the international export of pigs from the affected areas[7,13]. The outbreak was successfully contained in Peninsular Malaysia after a nation-wide surveillance of pig farms and the mass culling of sick pigs[5]. Pigs from Malaysia were banned from being imported into Singapore and all abattoirs in the country were closed temporarily[14].

## NIPAH VIRUS

Nipah virus is a member of *Paramyxoviridae* and is now classified as genus *Henipavirus* (Hendra + Nipah), due to very high genomic resemblance between these two viruses. In cell culture, the viral nucleocapsids have the typical 'herringbone' appearance with negative staining, characteristic for paramyxovirus[15]. It is the largest paramyxoviral genome described so far with a total length of 18, 246 nucleotides, only 12 nucleotides longer than *Hendra*[4]. There is a high degree of nucleotide homology in the various genes of HeV and NiV that exceeds 70%, and a high amino acid identity of more than 80% in most genes[16].

## CLINICAL FEATURES

All symptomatic patients from Malaysia had neurological features at presentation and none had primary respiratory disease. However, 2 of the 11 cases in Singapore presented with pneumonia without encephalitis[7]. Like any other viral encephalitis, prodromal symptoms of sore throat, myalgia, fever, headache, vomiting and altered mental status are common. Three main medical centres in Malaysia have published reviews on their clinical experience in managing this potentially fatal illness[17-19]. They were the *University Malaya Medical Centre, Kuala Lumpur General Hospital* and *Seremban Hospital*. A number of severely infected patients were also transferred from *Seremban Hospital to Kuala Lumpur General Hospital*, with the later skewed towards more severe patients [Table 1]. Febrile encephalitis seems to be the main clinical feature in infected NiV patients. Prodromal symptoms include fever, headache, nausea, vomiting, dizziness, lethargy, non-productive cough and myalgia. In a review by Goh KJ *et al*, 97% (91of 94 patients) had fever and more than half presented with headache. Close to half of (55%) patients had reduced level of consciousness with majority showing signs of brainstem dysfunction such as abnormal vestibule-ocular reflex, pinpoint pupils with variable reactivity and vasomotor dysautonomia i.e. hypertension, and tachycardia. All the seizures occurred in patients with reduced level of consciousness with almost all having generalized tonic-clonic attacks. Interestingly, about one third of their patients had segmental myoclonus, usually associated with more severe infection. The myoclonus characteristically involved the diaphragm and anterior neck muscles; but was also seen in other parts including arms, legs, and facial muscles[17]. The focal myoclonus is not time-locked to any focal discharges on the EEG findings, suggesting that the myoclonus is most likely to be brainstem or spinal cord in origin[20]. Absent/reduced tendon reflexes and hypotonia, tachycardia and hypertension were the other common signs particularly in those with more severe disease. Other reported signs include cerebellar dysfunction and bilateral postural tremors of arms[17].

Tan *et al* reported high infection rate in the household of infected farms in which 33% of them were affected by the disease[5], with 8-11% of the household members had subclinical disease[5,6]. It is thought that exposure to infected pigs correlates to the development of symptomatic disease[5]. About one sixth (16%) of patients with asymptomatic NiV infection may have abnormal cerebral MR imaging[11]. The MRI abnormalities were similar to that of acute Nipah encephalitis but less numerous. These patients developed antibody to *Nipah* virus (IgG) without any symptoms. It is an important aspect of the disease as some of these patients went on to develop late-onset encephalitis[21].

Table II summarises the mortality rate of patients with *Nipah encephalitis*, based on data from three different hospitals in Malaysia[17-19]. As shown, the overall mortality was close to 39%. The mortality in patients from the Kuala Lumpur General Hospital was higher probably because the Centre had patients with more severe disease. All patients, except one, were in a comatose state requiring ventilator support[18]. Evidence of severe brain-stem involvement, segmental myoclonus, seizures, and areflexia was associated with high mortality[17]. Chong *et al* also found that diabetic patients had increased mortality by 123% ($p<0.001$) and it is speculated that immunoparesis might be the reason to this observation[22].

## LABORATORY TESTING

Table II summarises the laboratory findings of patients with Nipah encephalitis, based on data from three different hospitals in Malaysia1[7-19]. Thrombocytopenia has been frequently reported[7,17-19] and present in 30-60% of patients, whilst leukopenia in 11-60% [23]. Abnormal liver functions with raised alanine and aspartate aminotransferase were present in 33-61% and 42-60% of patients respectively[23]. Lower platelet count and higher liver enzyme were associated with higher mortality. They were thought to be nonspecific changes in very ill patients[17]. Cerebrospinal fluid analysis from patients in Malaysia mostly showed normal glucose with raised white cell counts and protein[24]. These changes are non-specific and can be seen in any other CNS viral infections. Nevertheless, 25% of symptomatic patients had normal CSF findings[17-19].

Anti-*Nipah virus* IgM and IgG antibody can be detected in both serum and CSF of infected patients. Antibodies can be tested using IgM-capture enzyme-linked immunosorbent assay (ELISA) technique. This technique initially utilizes Hendra virus antigen to detect antibodies against Nipah virus, as both of these viruses share very close structural similarities. The IgM antibodies were obtained using *Hendra virus*-infected γ-irradiated Vero E6 cells and anti-*Hendra* hyperimmune mouse ascitic fluid antibody. The IgG antibodies were detected using indirect IgG ELISA assay[23].

The rate of detecting *anti-Nipah* IgM is the highest on day 12 of illness with a sensitivity of 100%[25]. In a study of 176 patients from *Seremban Hospital* and *University Malaya Medical Centre*, the antibody was positive in 44-50% of patients at day one of illness and increased to 60-71% by day 4. The sensitivity for detecting anti-Nipah IgG is 100% by day 25-26 of illness[25]. The rate of IgG detection is relatively low in the first week of illness (7-29%). The assay was subsequently switched to *Nipah virus*-infected Vero E6 cells and there was no significant difference in the sensitivity and specificity of the assay between the two antigens used. Other methods used to support viral detection include plaque reduction neutralisation and RT-PCR assay to detect viral RNA[26]. NiV grow well in Vero cells, but require BSL4 laboratory. Positive viral isolation from CSF is associated with high mortality, similar to the cases seen in JE[27]. Interestingly, the presence of CSF IgM does not have protective effect in disease severity and mortality[28].

Close to all (97.%) of electroencephalograph (EEG) carried out in the acute phase of encephalitis were abnormal. The most common abnormality was continuous diffuse slow slowing with or without focal discharges (87.5%). The degree of slowing correlated with severity of disease. Independent bitemporal periodic complexes were associated with 100% mortality[24].

## RADIOLOGICAL AND HISTOPATHOLOGICAL FINDINGS

Abnormal chest radiographs were reported in 6-24% cases in Malaysia[17,19]. In contrary, 3 out of 11 patients in Singapore presented with primary lung disease and abnormal chest radiographs[7].

Magnetic resonance imaging (MRI) of the brain was a very useful diagnostic tool for diagnosing Nipah encephalitis with 100% sensitivity among the Malaysian patients 29. Patients with acute encephalitis showed small, disseminated discrete hyperintense lesions in the subcortical and deep white matter, to a lesser extent, the gray matter, best seen on fluid attenuated inversion recovery (FLAIR) images. These changes are thought to be due to small vessels vasculitis and thrombosis, which resulted in focal disseminated areas of ischaemia and microinfarctions. However, there is poor correlation between disease severity and outcome with changes on MRI brain. These MRI changes also differ from the typical MRI brain seen in JE and *Herpes Simplex Virus (HSV) encephalitis*. In JE, the usual MRI changes are high signal intensity areas on T2-weighted and low signal intensity on T1-weighted sequences seen classically in bilateral thalami with or without haemorrhagic changes. These lesions can also be seen in the white matter, brainstem and basal ganglia[30]. MRI changes in HSV encephalitis classically involve oedematous changes and confluent high signal areas on T2-weighted sequence in the temporal lobe and limbic system[31].

Pathologically, the lung, heart and kidney were affected but the brain is the most severely affected organ[2]. Histopathological findings from post-mortem examination of the brain tissues include syncytial giant cell formation, vasculitis, and viral inclusions. There were also perivascular cuffing, parenchymal inflammation and neuronophagia[32]. Neuronal damage occurred via two possible mechanisms. Firstly, the formation of multinucleated syncytium in the endothelium of the blood vessels caused inflammation and vasculitis, which subsequently resulted in vascular thrombosis and occlusion leading to cerebral micro-infarction/ischaemia. Secondly, the findings of viral inclusions support possible direct viral cytolysis of parenchymal cells[24]. These findings are not specific for NiV encephalitis per se and can be seen with other encephalitides. However, the presence of syncytial multinucleated endothelial cells is exclusive for *Nipah* and *Hendra* virus encephalitis[32]. The vasculitic changes on autopsy correspond to the discrete small hyperintense lesions found on MRI brain of these patients. Vasculitis changes are also seen in other organs, the heart, lung and spleen, indicating widespread systemic involvement 32.

## LONG-TERM NEUROLOGICAL AND FUNCTIONAL OUTCOME

Neurological and neuropsychiatric sequelae may persist years after the initial presentation and[33]. Of the survivors of the acute infection, 21% (14/64) of those from UMMC and 19% of those from *Seremban* Hospital had persistent cognitive impairment and residual neurological deficits including cerebellar signs, tetraparesis, cranial nerve palsies and peripheral nerve lesions[17,19]. The survivors of the patients from Kuala Lumpur Hospital did poorly, with 6 out of 7 patients having significant neurological deficits. However, the patients in Hospital Kuala Lumpur had more severe illness 18. Long-term neurological assessment and serological pattern done on 39 NiV infection survivors, 10 years after the Malaysian outbreak, showed that fatigue (31%) and daytime somnolence (26%) were the common persistent clinical features. About a fifth (21%) had focal neurological deficits. Of those with previous encephalitis, 38% of had a significant disability on the Modified Rankin scale. All patients were tested negative for IgM antibodies and positive for IgG[34].

## TREATMENT

To date, the most important mode of treatment of acute *Nipah* encephalitis remains supportive, using mechanical ventilation for patients with respiratory failure, anticonvulsants for seizures, and management of secondary infection and rehabilitation[17]. Empirical treatment with ribavirin, a broad-spectrum antiviral against both DNA and RNA that can cross blood-brain barrier, was tried during the outbreak in Malaysia. In an open label trial of 140 patients treated with the drug, there was a 36% reduction in mortality with more survivors without residual neurological deficits. The latter however was not statistically significant[35]. The number was too small to allow comparison between the efficacies of oral vs. intravenous preparation of Ribavirin. Ribavirin has been shown to inhibit NiV replication in vitro[35]. However, more studies are needed to understand the pathogenesis of *Nipah* encephalitis, both at the biological and molecular level so that a more targeted therapy can be developed. The current animal models have shown that Ribavirin may delay *Nipah* virus disease and death but has no therapeutic effect against *Hendra* virus infection in hamsters[36].

Chloroquine, an antimalarial drug, also has been tried on animal studies with no therapeutic benefit. Similarly, the potential development of vaccine using NiV glycoproteins as the immunological target is still at an experimental level. At present, research are being done to look at therapeutic options of using human monoclonal antibody that may potentially inhibit glycoprotein-mediated entry of these viruses into cells[39] and applying RNA interference to inhibit *Henipavirus* replication in vitro[37].

## RELAPSED AND LATE-ONSET NIPAH ENCEPHALITIS

One of the unique and interesting feature of NiV infection is the development of relapsed and late-onset encephalitis, which may occur months or years after the acute illness[21,33,38].The longest delay in the onset of late-onset encephalitis is 11 years[39]. Relapsed encephalitis occurs after the recovery from acute encephalitis and late-onset encephalitis occurs after asymptomatic or mild non-encephalitic infection. It is believed that the relapsed and late-onset encephalitis represent the same disease process, the only difference being that in the later, the initial infection is not severe enough to cause neurological manifestation[21]. Tan *et al* reported a prevalence rate of relapsed encephalitis of 9%, and late-onset encephalitis of 5%[21,38].

The relapsed and late-onset encephalitis are usually of acute onset. The common clinical features are fever, headache, seizures and focal neurological signs with CSF pleocytosis[21]. As compared to the acute encephalitis, more of the patients with relapsed and late-onset had seizures. Their MRI brain showed areas of confluent cortical involvement, rather than the small, disseminated discrete hyper intense lesions seen in acute cases[29]. The EEG shows more focal slowing and sharp waves discharges, corresponding to the predominance of focal MRI and clinical lesions[21,29,38]. Relapsed and late-onset *Nipah* encephalitis has lower mortality rate than patients with acute encephalitis (18% vs. 40%)[1,17,19]. This could be explained by the minimal brainstem involvement in the relapsed and late-onset encephalitis patients[21].

Immunohistochemistry of the autopsy tissue suggests that the relapsed and late-onset cases are due to recurrent attacks from the persistent virus that remained dormant in the brain, and became reactivated by some unknown triggering factors[21]. Even though small vessels vasculitis associated with thrombosis and vascular occlusion were found in brain autopsy of patients with acute Nipah encephalitis, this was not demonstrated in relapsed cases[32,38]. However, viral inclusions and larger parenchymal lesions were more abundantly found in relapsed cases with focal encephalitis but no evidence of peri-venous demyelination[38]. These lesions would correlate with the confluent lesions seen on MRI brain of the patients[32,38]. Interestingly, though the viral antigen can be demonstrated by the positive immunolocalization, the CSF culture failed to isolate the virus. This suggests that the Nipah virus, like the measles virus in subacute sclerosing pan encephalitis, could have undergone mutations, resulting in the failure of viral morphogenesis at the cell membrane[17,21].

## BATS AS RESERVOIR

Malaysia is a home for at least 13 species of fruit bats (2 species of flying foxes) and more than 60 species of insectivorous bats. Fruit bats or flying foxes of the family *Pteropodidae (Pteropus vampyrus and Pteropus hypomelanus)* had been identified as the natural reservoir for NiV in Malaysia[40]. *Pteropus hypomelanus* was thought to have infected the pigs in Malaysia from their saliva through half-eaten fruits[6,40]. NiV has also been isolated from the half-eaten fruits and the urine of these roosting bats from *Tioman Island*[41]. These partially eaten fruits may have been dropped or thrown into pigsties and subsequently infected the pigs that consumed the contaminated fruits.

The infected pigs act as amplifying hosts and contributed to pig-to-pig and pig-to-human transmission of the virus[40]. The reason for virus spill-over from bats to pigs and subsequently to human were attributed to multiple factors including encroachment of bats into cultivated fruit orchards in West Coast of Peninsular Malaysia following deforestation and reduction in wildlife habitat; severe haze, prolonged El Nino-related drought resulting in reduction in availability of flowering and fruiting forest trees for foraging by the bats, and poor pig farming practice[42].

## OUTBREAKS IN BANGLADESH AND INDIA

No further outbreak was reported in Malaysia and Singapore after the mass culling of pigs[43]. However, the global public health community was again alerted when cases of Nipah encephalitis were reported in Bangladesh. There was recurrent *Nipah* encephalitis in Bangladesh almost annually since 2001 affecting close to 200 patients with 70% mortality. The latest outbreak was reported between January and March this year, resulting in 17 total deaths[44-46]. Two Indian outbreaks were reported in the neighbouring Siliguri in 2001 and Nadia District in West Bengal in 2007[46-49].

In contrast to the viral strains found in Malaysia, Cambodia and Thailand, the NiV isolated from the outbreak in Bangladesh showed some differences in their nucleotide sequences, suggestive of a different strain that might have co-evolved within the local natural reservoirs[50]. Studies from the outbreaks between 2001-2010 showed that the bat-to-human transmission in Bangladesh was associated with different pathways. Firstly, transmission occurred via drinking raw date palm sap contaminated with the virus from urine or saliva of these fruit bats[51,52] and subsequently led to human-to-human spread via respiratory droplets or bodily fluids. Close to half (62/ 122) of the cases identified in Bangladesh between 2001 and 2007 involved human-to-human transmission[53]. The other possible pathway is through animal-to-human transmission from contacts with secretions from infected pigs, cows and goats[16,48].

*Nipah* encephalitis was first reported in Siliguri, West Bengal, India, in 2001. It involved 66 patients, 75% had hospital exposure; i.e. they were hospital staffs, those who attended or visited patients in the hospital. The mortality rate was 74%. No cause was initially identified. Retrospective analysis of patients' samples (serum and urine) for *Nipah virus* was subsequently carried out and 9 out of 18 patients tested positive for IgM and IgG antibodies for NiV[46]. Studies to detect intermediate host was not carried out but human-to-human transmission on the ward was thought to be due to nasocomial infections, inadequate barrier-nursing and spreading of the virus via respiratory secretions and urine from infected patients. Sequencing analysis of this virus reveal close similarities to the strains in Bangladesh, as opposed to the ones in Malaysia[46]. This finding supports the environmental and geographical influence on the genetic evolution of this virus.

## EPIDEMIOLOGY AND CLINICAL FEATURES IN INDIA AND BANGLADESH COMPARED TO MALAYSIA AND SINGAPORE

Table III illustrates the difference in epidemiologic and clinical features of NiV encephalitis outbreaks between Malaysia and Singapore, versus Bangladesh and India[54,55]. As shown, the Malaysian and Singapore outbreak did not recur since 1999, but there was almost yearly outbreak in Bangladesh since 2001. The outbreak involved mainly adults in Malaysia and Singapore, but all ages in Bangladesh and India. The mode of spread was from bats-to-pigs, and pigs-to-human in Malaysia and Singapore; whereas in Bangladesh and India, transmission mainly occurs via bats-to-human through consumption of contaminated date palm juice, and human-to-human spread.

As for the clinical features, respiratory illness was not a prominent feature in the Malaysian patients. However, 3 of 11 affected patients in Singapore presented with atypical pneumonia with abnormal chest radiographs. One of them later developed encephalitis[7]. On the other hand, half to two thirds of the patients from the Bangladesh and Indian outbreak had respiratory symptoms, with chest radiographs of some patients showing changes consistent with acute respiratory distress syndrome. The prominent respiratory involvement and the relative lack of implementation of the infectious control practices probably underlie the human-to-human transmission in the Bangladesh and Indian outbreak.

Segmental myoclonus was a prominent feature in acute Nipah encephalitis patients in Malaysia. However, this was not seen in cases from Bangladesh and India. The typical changes in the MRI brain of the Malaysian patients were disseminated high-signal intensity lesions. Despite lack of MRI brain facilities in Bangladesh, the neuroimaging findings of 4 patients from Rajbari district differ from that of Malaysian patients, in which confluent high signal lesions involving both gray and white matter was the prominent features[56].

In Bangladesh, Sejvar *et al* conducted a study on 22 patients who survived NiV illness between 2005 and 2006, to assess their neurological and functional outcome based on questionnaire, neurological examinations and MRI brain imaging. Close to a third of patients (32%) had persistent neurologic and cognitive dysfunction. Almost all had disabling chronic fatigue syndrome and more than half had behavioural and neuropsychiatric changes, similar to the long-term findings on survivors in Malaysia and Singapore[33,34]. The behavioural and neuropsychiatric disturbances manifested as violent outbursts,

**Table I: Summary of clinical presentations in Nipah encephalitis patients treated in three major hospitals in Malaysia[17-19].**

| Clinical symptoms | Nipah Virus* | UMMC Goh *et al.*[17] | HKL Sim *et al.*[18] | Seremban Hospital Chong *et al.*[19] |
|---|---|---|---|---|
| | N=215 | N=94 | N=18 | N=103 |
| Age (mean)/ range in years | 37 (4-75) | 37 (13-68) | 37 (14-64) | 38 (4-75) |
| Sex (M/F) | M>F | 4.5:1 | 11:7 | 7.5:1 |
| Mean Incubation period** (days) | 14 days or less | N=94 Several days to 8 weeks (92% =<14 days) | N=6 13 (2-30) | N=49 10+/- 8.7 (1-32) |
| Fever | 95.4% (205) | 91 | 17 | 97 |
| Headache | 74.9% (161) | 61 | 12 | 88 |
| Dizziness | 37.7% (81) | 34 | 8 | 39 |
| Reduced level of consciousness/ altered mental status | 71.6% (154) | 52 | 17 | 85 |
| Vomiting | 31.6% (68) | 25 | 7 | 36 |
| Myalgia | 28.8% (62) | 11 | 4 | 47 |
| Chills and Rigors | 47.1% (57) | N/A | 6 | 51 |
| Diarrhoea | 18.2% (22) | N/A | 1 | 21 |
| Sore throat | 20.4% (21) | N/A | N/A | 21 |
| Cough | 20.9% (45) | 13 | 3 | 29 |
| Arthralgia | 6.6% (8) | N/A | 1 | 7 |
| Hyporeflexia | 60.5% (130) | 53 | 18 | 59 |
| Segmental Myoclonus | 49.3% (106) | 30 | 17 | 59 |
| Brainstem Dysfunction | | | | |
| *-hypertension* | 43.3% (93) | 36 | 5 | 52 |
| *-tachycardia* | 42.3% (91) | 37 | 1 | 53 |
| *-abnormal pupils* | 52.1% (49) | 49 | N/A | N/A |
| *-abnormal VOR**** | 38.3% (36) | 36 | N/A | N/A |
| *-profused/ segmental sweating* | 26.2% (27) | N/A | N/A | 27 |
| *-Nystagmus* | 20% (43) | 15 | 3 | 25 |
| Ptosis | 17.7% (38) | 4 | 4 | 30 |
| Meningism | 19.1% (41) | 26 | 3 | 12 |
| Seizures | 23.2% (26) | 22 | 4 | N/A |
| Limb weakness | 15.3% (33) | 10 | 8 | 15 |
| Hypotonia | 39.6% (78) | 53 | N/A | 25 |

** Cumulative data collected from case series of patients treated at University Malaya Medical Centre (UMMC), Kuala Lumpur Hospital (HKL), and Seremban Hospital (SH)*

** Incubation period = interval between last contact with pig and the first onset of clinical symptoms

All patients had direct contact or were in close proximity to pigs

*** VOR: Vestibulo-ocular reflex (Doll's eyes reflex)

**Table II: Summary of laboratory findings, treatment and mortality in Nipah encephalitis patients treated in Malaysia[17-19].**

| | Nipah Virus* | UMMC Goh *et al.*[17] | HKL Sim *et al.*[18] | Seremban Hospital Chong *et al.*[19] |
|---|---|---|---|---|
| | N=215 | N=94 | N=18 | N=103 |
| Positive sera Hendra IgM serology (N) | 79% (191) | 71% (83) | 100% (17) | 81% (91) |
| Positive CSF Hendra IgM serology (N) | 38% (124) | 31% (83) | 30% (10) | 58% (31) |
| Abnormal CSF (N) *– raised protein, raised white cell count* | 78% (161) | 75% (92) | 78% (15) | 83% (54) |
| Number of patients treated with Ribavirin | 71.6% (154) | 73 | 14 | 67 |
| Death | 39% (83) | 32% (30) | 61% (11) | 41% (42) |

**Table III: Differences in epidemiologic and clinical features of Nipah virus encephalitis outbreaks in Malaysia, Singapore, India and Bangladesh. (Modified from: Chang LY, Tan CT. *Nipah Virus Infection*. Jackson AC ed: Viral Infections of the Nervous System. Springer Basel, 2013, 317-336.)[54].**

| | Malaysia-Singapore | Bangladesh-India |
|---|---|---|
| Age and occupation | Mainly adult pig farm workers | Adults, children and healthcare workers |
| Spread | Bats-to-pigs, pigs-to-human<br>Human-to-human occasional | Direct bats-to-human infection by consumption of date palm juice and fruits contaminated by bats. Also reported possibility of bats-to-cows, bats-to-pigs<br>Human-to-human spread important |
| Respiratory involvement | 14-29%; 2 out of 11 patients in Singapore present with pneumonia without encephalitis | Cough (62%), respiratory difficulty (69%); chest radiographs with acute respiratory distress syndrome in some patients |
| Encephalitis | Segmental myoclonus seen in 32-54% | Segmental myoclonus not reported |
| MR Imaging | Disseminated small high-signal intensity lesion hallmark of MR imaging | Confluent high-signal brain lesion in limited MR imaging |
| Relapsed and late-onset encephalitis | About 10% | Delayed onset neurological abnormalities in 4 out of 22 patients in a follow-up study |
| Mortality | 32-41% | 73% |

**Table IV: The detection of Nipah virus in various species of bats around the world. (Reproduced with permission from Chong *et al. Nipah virus and bats*. Neurology Asia 2009, 14;73-76.)[63].**

| Location | Bat Species | Evidence of Infection |
|---|---|---|
| East coast, Australia | *Pteropus conspicillatus, P. alecto, P. scapulatus, P. poliocephalus* | Serology |
| Papua New Guinea | *Dobsonia moluccense, P. neohibernicus, D. andersoni, P. capistratus, P. hypomelanus, P. admiralitatum* | Serology |
| West coast, Peninsular Malaysia | *Cynopterus brachyotis, Eonycteris spelaea, P. hypomelanus, P. vampyrus, Scotophilus kuhlii* | Serology (ELISA) and serum neutralizing test |
| Tioman Island, East coast, Peninsular Malaysia | *Pteropus hypomelanus* | Virus culture, gene sequencing |
| Bangladesh | *P. giganteus* | Serology |
| Thailand | *Pteropus hypomelanus, P. lylei, P. vampyrus, Hipposideros larvatus* | Serology (ELISA) and RT-PCR |
| Cambodia | *Pteropus lylei* | Serology (ELISA), seroneutralising test and PCR |
| Sumatra, Java, Indonesia | *Pteropus vampyrus* | Serology (ELISA), virus neutralizing test |
| Yunan and Hainan Island, China | *Myotis sp., Rousettus leschenaultia,* | Serology (ELISA) and serum neutralizing, PCR |
| India | *Pteropus giganteus* | Serology (ELISA) and serum neutralizing test |
| Ghana | *Eidolon helvum, Epomophorus gambianus, Hypsingathus monstrosus* | Serology (Luminex multiplexed binding assay) |
| Madagascar | *Eidolon dupreanum, Pteropus rufus* | Serology (ELISA), serum neutralizing test |

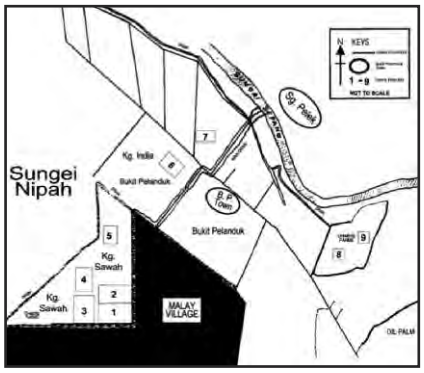


**Fig. 1:** Map showing geographical distribution of affected pig farming villages (numbered 1-9) in Bukit Pelanduk. (Reproduced with permission from: Tan *et al. Epidemiological aspects of Nipah Virus infection*. Neurol J Southeast Asia 1999, 4:77-81)[5]


**Fig. 2:** Map of peninsular Malaysia showing spread of Nipah encephalitis outbreak among neighbouring states. (Reproduced with permission from Sim *et al. Nipah Encephalitis: A report of 18 patients from Kuala Lumpur Hospital*. Neurol J Southeast Asia 2002, 7: 13-18)[18].

irritability, frequent nightmares, personality change and depression[57].

Relapsed and late-onset encephalitis is another unique feature of the NiV infection among the Malaysian patients. The long-term outcome study by Sejvar *et al.* from Bangladesh also showed that 4 out of 22 patients had delayed neurological symptoms manifesting as oculomotor palsy and cervical dystonia.

There were therefore significant differences in the epidemiologic and clinical features of NiV infection in the Malaysian and Singapore outbreaks, as compared to the subsequent Bangladesh and Indian outbreaks. These differences are likely to be due to local socio-cultural and economic factors, such as the consumption of date palm juice in Bangladesh, as well as genetic variation of NiV.

## HENDRA VIRUS

As previously stated earlier in this review, Nipah and Hendra viruses are two new zoonotic viruses that have emerged in recent years. Both are from the paramyxoviridae family and shares many similar characteristics. Because of their homology, a new genus called *Henipavirus* (Hendra + Nipah) was created for these two viruses.

Hendra virus was first isolated in an outbreak of acute respiratory illness involving horses in Australia in 1994. A horse trainer and stable handler were also infected, manifesting with respiratory illness from which the horse trainer died. A second human death occurred in 1995, where a farmer who had contact with ill horses about a year earlier died from encephalitis. Another two deaths involving veterinary workers occurred in the Hendra virus outbreaks in July 2008 and July 2009, also in Australia. Up until 2011, there have been 14 outbreaks of Hendra virus infection, all involving horses, 5 of these involving subsequent horse-to-human transmission, with 4 deaths among a total of 7 human cases[58].

Thus, *Hendra virus* is able to cause respiratory and encephalitic illness in humans who have close contact with infected horses. The predisposition to affect brain and lung is comparable to NiV infection. Similarly, there is also acute encephalitis and delayed neurological manifestation, with high mortality rate. The reservoir of *Hendra virus* is also the *Pteropus* genus of fruit bats, but the infection is transmitted through sick horses[59-61].

Brain necropsy of the 2 fatal cases of acute and relapsing *Hendra virus* encephalitis suggest that the pathology and pathogenesis are similar to *Nipah virus* with neuronal infection and micro-infarction/vasculitis in the brain. There is also widespread vasculitis involving lung, kidney and other major organs[62].

## THE WORK ON BATS WORLD WIDE AND ITS IMPLICATION

To date, the outbreak of NiV and Hendra virus infections in Malaysia, Bangladesh, and Australia have been attributed to Pteropus bats as the reservoir. Human becomes infected directly from the bats by consuming contaminated food such as date palm juice in Bangladesh, or indirectly via contact with sick animals such as pigs or horses. The distribution and evidence of Henipavirus or related virus is therefore crucial in predicting future outbreak. In Malaysia, other than *Pteropus* vampyrus and *Pteropus hypomelanus*, Yob *et al* also found serum-neutralizing antibodies to NiV from 5 out of 14 different species of bats sampled from other states in Peninsular Malaysia[40]. However, these neutralizing antibodies titres were much lower when compared to the anti-*Hendra* virus titres isolated from the flying foxes in Australia.

*Pteropus* bats live in the tropics and subtropics of Asia, Australia, islands off East Africa and some oceanic islands in both the Indian and Pacific Oceans. Other than Malaysia, neutralizing antibodies to NiV has also been identified in *Pteropus* bats in Cambodia, Thailand, India, Bangladesh and Madagascar, with *Pteropus Giganteus* being abundantly found in Bangladesh[63]. The isolation of *Nipah virus* from different species of bats around the world (Table 4) raised the question of whether these bats posses further threat to human and whether the same virus can potentially infect other species of bats in different parts of the world[63,64]. More work needs to be done in trying to determine factors that could potentially influence the transmission and pathogenic potentials of this virus, especially in humans living in close proximity to these bat colonies.

## WHAT HAVE WE LEARNT?

The emergence of this new paramyxovirus has cost us not only many lives, but also a great socio-economic burden. The initial positive serology testing for JE had possibly delayed the identification and treatment of NiV. In fact, a proportion of patient's sera that were tested positive were subsequently found to be of false positive[65]. Pig farmers treated with JE vaccines returned to the hospitals, being infected by this fatal virus, which had cost them not only their lives, but also the lives of their family members. This experience has thought us the importance of recognizing new outbreaks and searching for

novel diseases, especially in this globalized world with ease of travelling, and also when there are atypical features to the clinical presentation. In his recounting of the discovery of Nipah virus, Chua concluded *"epidemiology and clinical knowledge opened the mind to the possibility of other virus than JE during the 1998/99 Malaysian viral encephalitis outbreak. Viral culture led to the discovery of the novel infective agent which was not possible with serology and RT-PCR"*[65].

**ACKNOWLEDGEMENT**

We would like to sincerely thank the Director-General of Health, Malaysia for his permission to publish this paper.

**REFERENCES**

1. Chua KB, Bellini WJ, Rota PA, Harcourt BH, Tamin A, Lam SK, Ksiazek TG, Rollin PE, Zaki SR, Shieh W, Goldsmith CS, Gubler DJ, Roehrig JT, Eaton B, Gould AR, Olson J, Field H, Daniels P, Ling AE, Peters CJ, Anderson LJ, Mahy BW, Nipah virus: a recently emergent deadly paramyxovirus. Science, 2000. 288: p. 1432-1435.
2. Chua KB, Goh KJ, Wong KT, Kamarulzaman A, Tan PS, Ksiazek TG, Zaki SR, Paul G, Lam SK, Tan CT, Fatal encephalitis due to Nipah virus among pig-farmers in Malaysia. Lancet, 1999. 354: p. 1257-1259.
3. Solomon T, Nguyen MD, Rachel Kneen, Mary Gainsborough, David W Vaughn, Vo Thi Khanh, Japanese Encephalitis. J Neurol Neurosurg Psychiatry, 2000. 68: p. 405-415.
4. Harcourt B, Tamin A, Ksiazek TG, Rollin PE, Anderson LJ, Bellini WJ, Rota PA, Molecular characterisation of Nipah virus, a new emergent paramyxovirus. Virology, 2000. 271: p. 334-339.
5. Tan KS, Tan CT, Goh KJ, Epidemiological aspects of Nipah virus infection. Neurol J Southeast Asia, 1999. 4: p. 77-81.
6. Parashar UD, Sun LM, Ong F, Mounts AW, *et al*, Case-control study of risk factors for human infection with a new zoonotic paramyxovirus, Nipah virus, during 1998-1999 outbreak of severe encephalitis in Malaysia. J Infect Dis, 2000. 181: p. 1755-1759.
7. Paton NI, Leo YS, Zaki SR, *et al*, Outbreak of Nipah-virus infection among abattoir workers in Singapore. Lancet, 1999. 354(9186): p. 1253-1256.
8. Mohd Nor MN, Gan CH Ong BL, Nipah virus infection in pigs in peninsular Malaysia. Rev Sci Tech Off Int Epiz, 2000. 19(1): p. 160-165.
9. Tan CT, Tan KS, Nasocomial transmissibility of Nipah virus. J Infect Dis, 2001. 184(10): p. 1367.
10. Chua KB, Lam SK, Goh KJ, Hooi PS, *et al*, The presence of Nipah virus in respiratory secretions and urine of patients during an outbreak of Nipah virus encephalitis in Malaysia. J Infect Dis 2001. 42: p. 40-43.
11. Tan KS, Ahmad S, Tan CT, *et al*, Patients with asymptomatic Nipah virus infection may have abnormal cerebral MR imaging. Neurol J Southeast Asia, 2000. 5(69-73).
12. Mounts AW, Kaur H, Parashar UD, *et al*, A cohort study of health care workers to assess nasocomial transmissibility of Nipah virus, Malaysia. J Infect Dis, 1999. 183: p. 810-813.
13. Lee KE, Umapathi T, Tan CB, *et al*, Neurological manifestatios of Nipah virus encephalitis, a novel paramyxovirus. Ann Neurol 1999. 46(428-432).
14. Chew MH, Arguin PM, Shay DK, Goh KT, *et al*, Risk factors for Nipah virus infection among abattoir workers in Singapore J Infect Dis, 2000. 181(1760-1763).
15. Hyatt A, Zaki SR, Goldsmith CS, Wise TG, Hengstberger SG, Ultrastructure of Hendra virus and Nipah virus within cultured cells and host animals Microbes Infect, 2001. 3: p. 297-306.
16. ICDDRB outbreaks of encephalitis due to Nipah/Hendra-like viruses, Western Bagladesh. Health and Science Bulletin, 2003. 1(5): p. 1-6.
17. Goh KJ, Tan CT, Chew NK, Tan PSK, *et al*, Clinical features of Nipah Encephalitis among pig farmers in Malaysia. N Engl J Med 2000. 342: p. 1229-1235.
18. Sim BF, Jusoh R, Chang CC, Khalid R, Nipah encephalitis: A report of 18 patients from Kuala Lumpur Hospital. Neurol J Southeast Asia, 2002. 7(13-18).
19. Chong HT, Kunjapan SR, Thayaparan T, *et al*, Nipah encephalitis outbreak in Malaysia, clinical features in patients from Seremban. Neurol J Southeast Asia, 2000. 5(61-67).
20. Chew NK, Goh KJ, Tan CT, Ahmad Sarji S, Wong KT, Electroencephalography in acute Nipah encephalitis. Neurol J Southeast Asia, 1999. 4(45-51).
21. Tan CT, Goh KJ, Wong KT, *et al*, Relapsed and late-onset Nipah encephalitis. Ann Neurol, 2002. 51(703-708).
22. Chong HT, Tan CT, Goh KJ, *et al*, Occupational exposure, age, diabetes mellitus and outcome of acute Nipah encephalitis Neurol J Southeast Asia, 2001. 6(7-11).
23. Wong KT, Shieh WJ, Zaki SR, Tan CT, Nipah virus infection, an emerging paramyxoviral zoonosis Springer Semin Immunopathol, 2002. 24: p. 215-228.
24. Tan CT, Chua KB, Wong KT, Nipah virus - A review ASM Science Journal 2000. 3(1): p. 91-96.
25. Ramasundram V, Tan CT, Chua KB, Chong HT, Goh KJ, *et al*, Kinetics of IgM and IgG seroconversion in Nipah virus infection. Neurol J Southeast Asia, 2000. 5: p. 23-28.
26. Daniels P, Ksiazek T, Eaton B, Laboratory diagnosis of Nipah and Hendra virus infections Microbes Infect, 2001. 3: p. 289-295.
27. Chua KB, Lam SK, Tan CT, *et al*, High mortality in Nipah Encephalitis is associated with the presence of virus in cerebrospinal fluid Ann Neurol, 2000. 48(5): p. 802-805.
28. Ramasundram V, Tan CT, Chong HT, Goh KJ, *et al,* Presence of CSF IgM do not have protective efefct in Nipah Encephalitis Neurol J Southeast Asia, 1999. 4: p. 73-76.
29. Ahmad S, Abdullah BJ, Goh KJ, Tan CT, Wong KT, MR imaging features of Nipah encephalitis. AJR, 2000. 175: p. 437-442.
30. Shoji H, Kida H, Hino H, 1994. J Neuroimaging Magnetic resonance imaging findings in Japanese encephalitis: white matter lesions. 4: p. 206-211.
31. Hansman Whiteman ML, Bowen BC, Donovan Post MJ, Bell MD, Intracranial infection, in Magnetic resonance imaging of the brain and spine, A. SW, Editor. 1996, Lippincott-Raven Philadelphia. p. 707-772.
32. Wong KT, Shieh WJ, *et al,* Pathology and pathogenesis of an emerging paramyxoviral zoonosis. Am J Pathol, 2002. 161(6): p. 2153-2167.
33. Ng BY, Lim CC, Yeoh A, Lee WL, Neuropsychiatric sequelae of Nipah virus encephalitis J Neuropsychiatry Clin Neurosci, 2004. 6(4): p. 500-504.
34. Siva SR, Chong ST, Tan CT, Ten year clincal and serological outcomes of Nipah virus infection Neurol J Southeast Asia, 2009. 14: p. 53-58.
35. Chong HT, Kamarulzaman A, Tan CT, Goh KJ, *et al*, Treatment of acute Nipah encephalitis with Ribavirin Ann Neurol, 2001. 49: p. 810-813.
36. Steffen DL, Xu K, Nikolov DB, Broder CC, Henipavirus mediated menraine fusion, virus entry and targeted therapeutics. Viruses 2012. 4(2): p. 280-309.
37. Mungali BA, Schopman NC, Lambeth LS, Doran TJ, Inhibition of Henipavirus infection by RNA interference. Antiviral Res, 2008. 80(3): p. 324-331.
38. Chong HT, Tan CT, Relapsed and late-onset Nipah encephalitis, a report of three cases. Neurol J Southeast Asia, 2003. 8(109-112).
39. Abdullah S, Chang LY, Rahmat K, Goh KJ, Tan CT, Late-onset Nipah virus encephalitis 11 years after the initial outbreak: A case report. Neurol J Southeast Asia, 2012. 17(1): p. 71-74.
40. Yob JM, Field H, Rashdi AM, Morrisy C, *et al*, Nipah virus infection in bats (order Chiroptera) in Peninsular Malaysia. Emerg Infect Dis, 2001. 7: p. 439-441.
41. Chua KB, et al, Isolation of Nipah virus from Malaysian Island flying-foxes. Microbes Infect, 2002. 4: p. 145-151.
42. Chua KB, Chua BH, Wang CW, Anthropogenic deforestation, El Nino and the emergence of Nipah virus in Malaysia. Malaysian J Pathol, 2002. 24(1): p. 15-21.
43. Chua KB, Nipah virus outbreak in Malaysia. J Clin Virol, 2003. 26(265-275).
44. Hossain MJ, *et al*, Clinical presentation of Nipah virus infection in Bangladesh. Emerg Infect Dis, 2004. 10(12): p. 2082-2087.
45. ICDDRB, Outbreaks of Nipah virus in Rajbari and Manikgonj. Health and Science Bulletin, 2008. 6(1): p. 12-13.
46. Chadha MS, *et al*, Nipah virus-associated encephalitis outbreak, Seliguli, India. Emerg Infect Dis, 2006. 12: p. 235-240.
47. Arankalle VA, Bandyopadhyay BT, Ramdasi AY, Jadi R, Patil DR, Rahman M, et al, Genomic characterisation of Nipah virus, West Bengal, India. Emerg Infect Dis, 2011. 17: p. 907-909.
48. Hsu VP, Hossain MJ, Parashar UD, *et al*, Nipah virus encephalitis re-emergence, Bangladesh Emerg Infect Dis, 2004. 10: p. 2082-2087.

49. Harit AK, Ichpujani RL, Gupta S, *et al,* Nipah/Hendra virus outbreak in Siliguri, West Bengal, India in 2001. Indian J Med Res, 2006. 123(553-560).
50. Harcourt BH, Lowe L, Tamin A, Liu X, Bankamp B, et al, Genetic characterisation of Nipah virus, Bangladesh. Emerg Infect Dis, 2004. 11: p. 1594-1597.
51. Rahman MA, *et al*, Date palm sap linked to Nipah virus outbreak in Bangladesh, 2008. Vector Borne Zoonotic Dis, 2012. 12: p. 65-72.
52. Montgomery JM, *et al*, Risk factors for Nipah virus encephalitis in Bangladesh. Emerg Infect Dis, 2008. 10: p. 1526-1532.
53. Luby SP, Hossain J, Gurley E, *et al*, Recurrent zoonotic transmission of Nipah virus into Humans, Bangladesh, 2001-2007. Emerg Infect Dis, 2009. 15(8): p. 1229-1235.
54. Chang LY, Tan CT, Nipah virus infection in Viral infections of the Nervous System, J. AC, Editor. 2013, Springer Basel: Canada. p. 317-336.
55. Chong HT, Hossain MJ, Tan CT, Difference in epidemiologic and clinical features of Nipah virus encephalitis between Malaysian and Bangladesh outbreaks. Neurol J Southeast Asia, 2008. 13: p. 23-26.
56. Quddus R, *et al* A report of 4 patients with Nipah encephalitis from Rajbari district, Bangladesh in the January 2004 outbreak. Neurol J Southeast Asia, 2004. 9: p. 33-37.
57. Sejvar JJ, Hossain MJ, Saha SK, *et al*, Long-term neurological and functional outcome in Nipah virus infection. Ann Neurol, 2007. 62(3): p. 235-242.
58. O'Sulivan JD, Allworth AM, Paterson DL, *et al,* Fatal encephalitis due to novel paramyxovirus transmitted from horses. Lancet, 1997. 349: p. 93-95.
59. Field H., Hendra virus infection risks. Neurol J Southeast Asia, 2009. 14: p. 77-78.
60. Playford EG, McCall B *et al,* Human Hendra virus encephalitis associated with equine outbreak, Australia 2008. Emerg Infect Dis, 2010. 16(2): p. 219-223.
61. Field H, Young P, *et al,* The natural history of Hendra and Nipah viruses Microbes Infect, 2001. 3: p. 307-314.
62. Wong KT, Robertson B, Ong BB, Chong JW, *et al,* Human Hendra virus infection causes acute and relapsing encephalitis. Neuropathology and Applied Neurobiology, 2009. 35: p. 296-305.
63. Chong HT, Abdullah S, Tan CT, Nipah virus and bats. Neurol J Southeast Asia, 2009. 14: p. 73-76.
64. Lehlé C, Razafitrimo G, *et al*, Henipavirus and Tioman virus antibodies in Pteropodid Bats, Madagascar. Emerg Infect Dis, 2007. 13(1): p. 159-161.
65. Chua KB, The discovery of Nipah Virus: A personal account. Neurol J Southeast Asia, 2004. 9: p. 59-63.