Fatal encephalitis due to Nipah virus among pig-farmers in Malaysia

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Summary

Background Between February and April, 1999, an outbreak of viral encephalitis occurred among pig-farmers in Malaysia. We report findings for the first three patients who died.

Methods Samples of tissue were taken at necropsy. Blood and cerebrospinal-fluid (CSF) samples taken before death were cultured for viruses, and tested for antibodies to viruses.

Findings The three pig-farmers presented with fever, headache, and altered level of consciousness. Myoclonus was present in two patients. There were signs of brainstem dysfunction with hypertension and tachycardia. Rapid deterioration led to irreversible hypotension and death. A virus causing syncytial formation of vero cells was cultured from the CSF of two patients after 5 days; the virus stained positively with antibodies against Hendra virus by indirect immunofluorescence. IgM capture ELISA showed that all three patients had IgM antibodies in CSF against Hendra viral antigens. Necropsy showed widespread microinfarction in the central nervous system and other organs resulting from vasculitis-induced thrombosis. There was no clinical evidence of pulmonary involvement. Inclusion bodies likely to be of viral origin were noted in neurons near vasculitic blood vessels.

Interpretation The causative agent was a previously undescribed paramyxovirus related to the Hendra virus. Close contact with infected pigs may be the source of the viral transmission. Clinically and epidemiologically the infection is distinct from infection by the Hendra virus. We propose that this Hendra-like virus was the cause of the outbreak of encephalitis in Malaysia.

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Introduction

Between February and April, 1999, there was a severe outbreak of viral encephalitis among pig-farmers in the Bukit Pelandok area in Negri Sembilan state, Malaysia, that affected more than 200 individuals. 91 patients from the outbreak area were admitted to the University of Malaya Medical Centre, Kuala Lumpur, and there were 28 deaths. Because this outbreak occurred among people in close contact with pigs, the differential diagnoses included 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 high disease attack rate (as opposed to the Japanese-encephalitis virus which causes symptomatic encephalitis in one in 300 of those infected); a high proportion of patients in direct contact with pigs as opposed to other individuals living in the same neighbourhood (evidence 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.[1]

We report on the first three fatal cases seen at our hospital. All three patients were farmers with a history of direct contact with pigs.

Case reports

Patient 1

This 51-year-old man was admitted to hospital with fever (2 days since onset), acute confusion (1 day), and pain associated with myoclonus in the left arm. He had received two doses of Japanese-encephalitis vaccine, the second dose more than a month before onset of illness. On admission, his temperature was 38°C and he was disoriented. The patient's pupils were reactive, and doll's eye reflex was present. There was no focal limb weakness and no meningism. Examination of the heart and lungs showed no abnormalities, and chest radiographs were normal. Brain computed tomography (CT) scan was normal. Cerebrospinal fluid (CSF) examination showed a pressure of 13 cm water, no white blood cells, glucose concentration of 46 mmol/L, and protein concentration of 0·6 g/L. The next day his condition deteriorated; there were signs of deepening coma and flaccid tetraparesis and he needed mechanical ventilation. Electroencephalography (EEG) showed continuous diffuse slow waves with bitemporal independent sharp waves. Intravenous aciclovir was started empirically. Persistent tachycardia and high blood pressure (maximum 230/122 mm Hg) were noted despite sedation with intravenous midazolam and propofol infusion. He deteriorated further and on day 6 had pinpoint unreactive pupils and no doll's eye reflex. The patient developed hypotension and bradycardia and died.

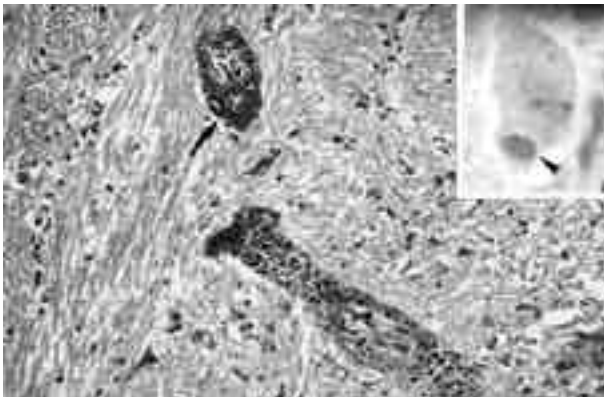

Figure 1: **Section of the infarcted pons (patient 1) showing two small blood vessels with vasculitis and thrombosis (arrow)**
Haematoxylin and eosin; reduced by 35% from ×50. Inset shows a neuron in the medulla with an inclusion body (arrow head), which was found close to an area of vasculitis; reduced by 35% from ×200.

Patient 2

This 34-year-old man was admitted with fever (3 days since onset) drowsiness, and lethargy, but without headache. He had received three doses of Japanese-encephalitis vaccine, the last being 3 weeks before onset of illness. A month before his illness, some of the patient's pigs became ill and died suddenly. On admission he was oriented but drowsy, and his temperature was 38°C. Examination of the lungs showed no abnormalities, and chest radiography was normal. Blood pressure ranged from 130/70 mm Hg to 170/95 mm Hg with a heart rate of 100–160 beats per min. There were no focal neurological deficits and no neck stiffness. The patient refused lumbar puncture. He remained febrile and became comatose 2 days after admission, only responding to painful stimuli with facial grimacing. He developed jerking of the abdominal wall and right leg; this feature suggested segmental myoclonus. The patient was intubated and ventilated. EEG showed continuous diffuse slow waves with intermittent bitemporal sharp waves. The next day the doll's eye reflex was absent but his pupils remained reactive. He did not improve, and on day 7 developed hypotension (unresponsive to fluid and inopressor therapy) and died. A CSF sample was obtained at necropsy.

Patient 3

This 52-year-old man was admitted after having headache and nausea for 14 days, and fever and chills for 7 days. He had received two doses of Japanese-encephalitis vaccine, the second 1 month before admission to hospital. At presentation he was alert and conscious but had horizontal nystagmus to the left. No other focal neurological abnormalities were noted. Examination of the lungs showed no abnormalities, and the chest radiographs were normal. Blood pressure ranged from 122/76 mm Hg to 200/70 mm Hg with a heart rate range of 100–127 beats per min. CSF examination showed a pressure of 16 cm water, white blood cell count of 720 per μL (88% lymphocytes), glucose concentration of 4·0 mmol/L, and protein 1·75 g/L. 2 days later his consciousness deteriorated and mechanical ventilation was started. On the next day he had no doll's eye reflex, although his pupils remained reactive. EEG showed continuous diffuse slow waves. He did not improve and died 5 days after admission.

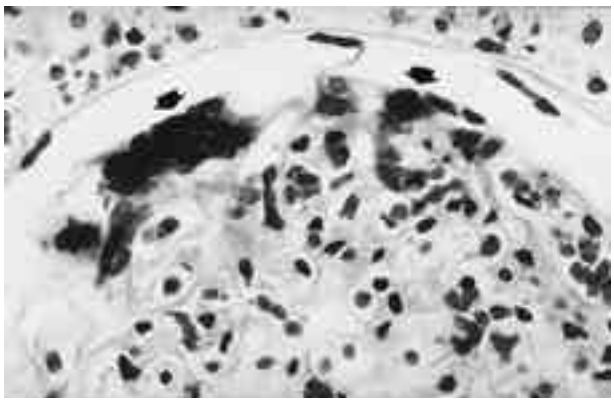

Figure 2: **Kidney section showing syncytial formation at edge of glomerulus (arrows)**
Haematoxylin and eosin; reduced by 35% from ×200.

Findings

Histopathology

Samples from all three patients showed similar histological findings of endothelial damage and vasculitis (mainly in arterioles, capillaries, and venules, although these features were also seen in some large muscular arteries). The brain was the most severely affected organ, but other organs including the lung, heart, and kidney were also affected. Vasculitic vessels were characterised by vessel-wall necrosis, thrombosis, and inflammatory-cell infiltration of neutrophils and mononuclear cells (figure 1). Syncytial-cell formation was seen in the endothelium of affected blood vessels in the brain and lung, and in the Bowman's capsule of the glomerulus (figure 2). Zones of microinfarction and ischaemia were commonly found around or adjacent to vasculitic blood vessels.

In the brain, many neurons adjacent to vasculitic vessels had eosinophilic cytoplasmic and nuclear viral inclusions as seen with other paramyxovirus infections. Neuronophagia and microglial-nodule formation were noted in focal areas. Perivascular cuffing and meningitis were generally mild. There was no evidence of perivenous demyelination. The distribution of vasculitis and zones of microinfarction and ischaemia suggested a random process, equally affecting the grey and white matter of the cerebrum, basal ganglion, cerebellum, brainstem, and spinal cord. Overall, the main cause of death was widespread focal infarction of the brain and possibly direct viral infection of the neurons.

Virology

A virus causing rapid syncytial formation on vero-cell cultures (ATCC, CCL81) was isolated from the CSF of patients 1 and 3 on day 5 of inoculation (figure 3). The infected vero cells in patient 1 gave a strong positive reaction with antibodies to Hendra virus by indirect immunofluorescence assay with hyperimmune mouse ascitic fluid. The infected cells did not stain by indirect immunofluorescence assay with specific monoclonal antibodies against respiratory viruses (adenovirus, influenza A, influenza B, parainfluenza 1–3, respiratory syncytial virus, and measles virus), enteroviruses (coxsackievirus A and B, echovirus, and enterovirus 70 and 71), human herpesviruses 1, 2, and 5, and Japanese-encephalitis virus, and other flaviviruses. The result indicates that the virus is related to Hendra virus.[2]

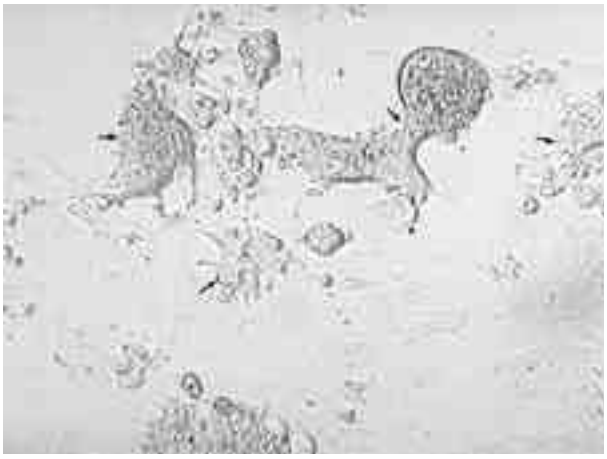

Figure 3: **Monolayer vero-cell culture infected with virus from CSF of patient 1, showing formation of giant syncytial multinucleated cells (arrows)**
Reduced by 35% from ×100.

Preliminary nucleotide-sequence studies show that this virus, now called Nipah virus, is a new paramyxovirus related to but distinct from Hendra virus.[3]

We did not attempt to isolate the virus from the CSF of patient 2 because of concerns about laboratory-acquired infection. At some stage of the illness, all three patients had IgM antibodies against Hendra viral antigens by IgM capture ELISA in CSF (table). In patient 3, the appearance of specific IgM against the virus in the serum preceded the IgM response in the CSF. IgM antibodies against the Japanese-encephalitis virus were not detected in the serum or CSF of any of the three patients.

Discussion

The virus implicated in this outbreak is related to but distinct from the Hendra virus. Clinically and epidemiologically the disease in our three patients differed from the previously described Hendra-virus infection.[4–6] Hendra virus was transmitted from horses, and two of the three reported patients with Hendra infection had respiratory involvement, with only one patient showing a severe meningoencephalitis. In our patients, however, the infection involved direct contact with pigs and predominant central-nervous-system disease with no clinical or radiological evidence of pulmonary involvement.

The features of the brain in this illness are distinct from other disorders in that there is clear histological evidence of necrotising vasculitis and syncytial formation in all cases, which is not found in postinfectious encephalomyelitis, including measles, or in many primary viral encephalitides, including measles. However, there are some similarities to Hendra virus encephalitis.[6]

Our patients had widespread central-nervous-system disease due to severe vasculitis of mainly small blood vessels that resulted in endothelial damage. This damage in turn caused thrombosis and ischaemia or infarction in areas supplied by these vessels. We believe that the neurological dysfunction seen in these patients was the result of widespread focal ischaemia or infarction in the brain and direct neuronal infection, suggested by the presence of paramyxoviral inclusion bodies. Lung, kidney, and heart involvement were also noted but were not severe enough to be clinically significant.

Date sample collected	Hendra IgM in serum	Hendra IgM in CSF	Virus cultured from CSF*
Patient 1 (onset of fever Feb 24)			
Feb 27	+	NA	+
March 1	+	+	+
Patient 2 (onset of fever March 3)			
March 11	−	NA	NA
March 15	NA	+	ND
Patient 3 (onset of fever Feb 22)			
March 8	+	−	+
March 9	+	NA	NA
March 11	NA	+	ND

*Positive culture characterised by syncytial cytopathic effect.
NA=not available; ND=not done.

Hendra IgM antibodies and results of viral culture

The incubation period of the illness in our patients was short (up to a month; patient 2 reported that some of his pigs were ill and many died about 1 month before his illness). The main presentation was fever with headache followed by a rapid deterioration in consciousness. Segmental myoclonus was seen in two of three patients. No generalised or focal seizures were seen. A notable feature was hypertension associated with tachycardia, which suggested dysfunction of the medullary vasomotor centre. Brainstem signs occurred with deepening coma, impaired doll's eye reflex, and pupillary abnormalities, and were followed rapidly by death.

The Nipah virus described here may be the agent responsible for the encephalitis outbreak in Malaysia.

Contributors

Kaw Bing Chua, Khean Jin Goh, Kum Thong Wong, Adeeba Kamarulzaman, Patrick Tan, and Chong Tin Tan wrote the paper. Kaw Bing Chua and Sai Kit Lam were responsible for the isolation of the virus. Chong Tin Tan, Khean Jin Goh, Adeeba Kamarulzaman, and Patrick Tan managed the patients. George Paul and Kum Thong Wong did the necropsies. Kum Thong Wong and Sherif Zaki described the histopathology. Thomas Ksiazek was responsible for the serological tests and initial viral work at the Centers for Disease Control and Prevention, Atlanta, Georgia. Chong Tin Tan was the senior investigator and coordinated the work.

Acknowledgments

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