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Outbreak of Nipah encephalitis among pig-farm workers in Malaysia in 1998/1999: Was there any role for Japanese encephalitis?

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Abstract

A study to determine the possible role of Japanese encephalitis in the Nipah encephalitis outbreak among pig-farm workers in Malaysia in 1998/1999 was done. Two hundred and twelve patients who fulfilled the criteria of Nipah encephalitis from three epicentres during the outbreak were retrospectively analysed. Twenty-nine of these patients (14%) had positive IgM serology for Japanese encephalitis, and of these, 20 patients (9%) had positive IgM serology in the cerebrospinal fluid. The 29 patients with positive IgM Japanese encephalitis serology and 183 patients with negative serology were compared. There was no significant difference in the two groups for demographic, clinical features, laboratory findings and outcome. None of the 33 fatal cases who had post mortems showed positive immunohistochemical staining for Japanese encephalitis. There was thus no evidence that the encephalitis outbreak was due to either Nipah virus or Japanese encephalitis virus; or that a patient may have suffered encephalitis concurrently due to both viruses. There was also no evidence that a subclinical Japanese encephalitis infection have aggravated a patient with Nipah encephalitis. The positive Japanese encephalitis IgM serology probably reflected the endemic Japanese encephalitis infection, which was asymptomatic. The break down in blood brain barrier associated with disseminated microinfarction seen in Nipah encephalitis possibly contributed to the high rate of positive Japanese encephalitis IgM serology in the cerebrospinal fluid.

INTRODUCTION

From September 1998 to May 1999, an outbreak of fatal encephalitis occurred in Malaysia involving the workers associated with the pig farm industry. Of the 265 patients, 105 were fatal.[1-6] The outbreak was thought to have started in the state of Perak north of Kuala Lumpur in September 1998. It subsequently spread to the Bukit Pelanduk areas, south of Kuala Lumpur, the site which was previously the largest pig farming area in South East Asia, presumably by movement of infected pigs across state borders.[7] During the early part of the outbreak, the possibility was raised that the outbreak could be due to an unusual form of Japanese encephalitis, as the Japanese encephalitis IgM serology was positive in a substantial proportion of the patients, both in serum as well as cerebrospinal fluid. After discovery of the novel Nipah virus among some of the fatal cases[3] which suggest Nipah virus as the main culprit in the outbreak, the possibilities remained that the outbreak could be due to either of the two viruses, Nipah virus and Japanese encephalitis virus; or a patient may have suffered encephalitis concurrently from both viruses; or a subclinical Japanese encephalitis infection could have aggravated a patient with Nipah virus infection. The possible roles of Japanese encephalitis is reflected in the official reports of the outbreak from the various ministries of the government of Malaysia, which continues to label the outbreak as "Japanese/Nipah encephalitis".[8] This paper aims to address the significance of positive Japanese encephalitis IgM serology in these patients during the outbreak.

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METHODS

The medical records of the Nipah encephalitis patients who were admitted to the University Malaya Medical Centre, Seremban Hospital and Ipoh Hospital were examined retrospectively. Patients were defined to have Nipah encephalitis if they have clinical (fever, headache, altered sensorium, or focal neurologic signs), abnormal cerebrospinal fluid findings ($\geq$ 6 lymphocytes per cubic millimeter, or a protein level of at least 0.45 g per liter in patients under 50 years of age and a level of at least 0.55 g per liter in those who were 50 years of age or older) or characteristic findings on MRI of the brain, came from areas known to be involved in the outbreak, had had direct or close contact with pigs or other infected animals.[5,6] Incubation period was defined as the interval between the last contact with pig and the onset of clinical calculated. The first patient was admitted on 22nd November 1998. Lapsed day was calculated as the difference in the patient's date of admission from this date. Japanese encephalitis IgM was detected using enzyme-linked immunosorbent assay (ELISA). The test was done in Institute of Medical Research, Kuala Lumpur and University Malaya Medical Centre. The patients with positive Japanese encephalitis IgM serology were compared with those without in terms of their clinical manifestations, laboratory findings, short and long term outcomes. Univariate parametric variables were analyzed with ANOVA. Nominal non-parametric variables were analyzed with Chi square or Fisher exact test, ordinal non-parametric variables with Kruskal-Wallis statistics. Logistic regression analysis was used for multivariate analysis.

RESULTS

There were 212 patients who fulfilled the diagnostic criteria for Nipah encephalitis, which represents 80% of the total of 265 patients affected in the outbreak. Of these, 29 (14%) had positive Japanese encephalitis IgM serology; 9 had positive serum IgM serology only, 9 had positive cerebrospinal fluid IgM serology only, and 11 were positive for both serum and cerebrospinal fluid IgM serology. Thus, 20 patients (9%) had positive serology in the cerebrospinal fluid. The characteristics of these patients were listed in Table 1. There was no significant difference between the Japanese encephalitis seropositive and seronegative patients except that the lapsed days of the seropositive patients were significantly shorter than the seronegative, showing that most of the seropositive patients were seen during the earlier part of the outbreak. Figure 1 showed that during the first 40 days of the outbreak in Ipoh and Sikamat, the seropositivity rate for Japanese encephalitis was 70-100%. During the middle part of the outbreak when the Bukit Pelanduk areas were affected, the seropositivity dropped to 15-30%. The discovery of Nipah virus was made known around the third week of March in 1999. After that, the rate of positive Japanese encephalitis serology dropped further. After 19th March 1999, there were only one out of 103 patients with positive Japanese encephalitis IgM serology. When the serology of patients admitted before 7th March 1999 (less than 106 lapsed day) were compared with those admitted after that date, the positivity rate of Japanese encephalitis IgM serology was 40% versus 7.6% ($p<0.001$). Significantly higher proportion of patients with

Table 1: Patient characteristics according to Japanese encephalitis IgM serology

Parameter	JE* IgM serology positive (n=29)	JE* IgM serology negative (n=183)	p value
Age (means $\pm$ SD) in years	40 $\pm$ 12	38 $\pm$ 13	0.36
Proportion of male	93% (n=27)	85% (n=155)	0.39
Proportion of Chinese	79% (n=23)	70% (n=128)	0.42
Farm owners or workers	90% (n=26)	78% (n=143)	0.24
Diabetes mellitus	4% (n=1)	5% (n=9)	>0.99
Lapsed day**	80 $\pm$ 42 (n=29)	118 $\pm$ 22 (n=180)	<0.001
Ribavirin treatment	28% (n=8)	74% (n=132)	<0.001

*JE - Japanese encephalitis.

**Lapsed day – was the number of days the patient was admitted since the admission of the first patient on 22nd November 1998.

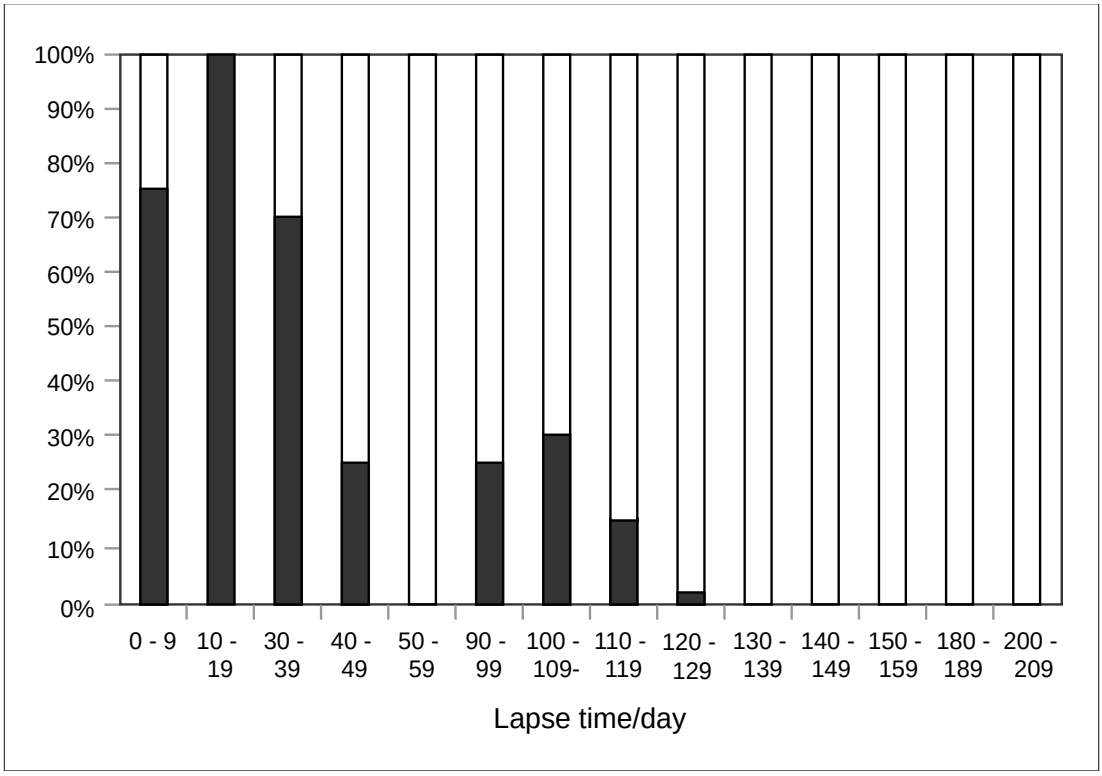

Figure 1: Time course during Nipah encephalitis outbreak and Japanese encephalitis IgM seropositivity

positive Japanese encephalitis IgM serology received Ribavirin treatment.

A comparison of clinical features in the seropositive and seronegative groups was listed in Table 2. As shown, the clinical features of the two groups of patients were similar, except that patients with positive Japanese encephalitis serology had poorer conscious state during admission and at nadir. They also had higher peak temperature. There was no significant difference in the proportion of patients being vaccinated against Japanese encephalitis in the two groups.

A comparison of laboratory findings between the seropositive and seronegative groups was listed in Table 3. As shown, the laboratory findings of the two groups of patients were similar. The outcomes of the two groups of patients were listed in Table 4. As shown, both groups had similar mortality, need for ventilation, length of hospital stay and rate of relapse. However the seropositive patients were more likely to have residual neurological deficits.

On logistic regression analysis, of the six statistically significant variables (Glasgow Coma Scale at admission and nadir, peak temperature, Ribavirin treatment, residual deficits and lapsed day) the only significant factor that was related to positive Japanese encephalitis IgM serology was the lapsed day ($p<0.001$). This suggested that seropositivity was not related to the severity of the disease, or the use of Ribavirin, but to lapsed day.

Post-mortem was performed in 33 of the deceased patients, and immunohistochemical (IHC) staining for Japanese Encephalitis and Nipah viruses was done in all. The characteristics of these patients are shown in Tables 5. As shown, there was no difference in the demographic features between patients undergoing post-mortem and the others. All but 4 patients were stained positive for the Nipah virus. Of the 4 patients with negative IHC staining, all had positive Nipah serology. These 4 patients died much later than those who stained positive for Nipah virus antigen (24.0 ± 10.3 days versus 7.8 ± 4.3 days, $p=0.002$). In fact, after 14 days of illness, only one of the post-mortem patients was stained positive for Nipah. None of the 33 patients had positive IHC staining for Japanese encephalitis virus. This included 11 patients with positive Japanese encephalitis IgM serology. Of these 11 patients, 10 patients had positive Nipah IHC staining, all had positive Nipah serology. Of the 29 patients

Table 2: Clinical features according to Japanese encephalitis serology

Parameter	JE* IgM serology positive	JE* IgM serology negative	p value
Mean incubation period in days	5.14 ± 5.88 (n=29)	8.65 ± 8.85 (n=127)	0.15
History of JE* vaccination	70.4% (n=19/27)	73.7% (n=98/133)	0.91
Fever	100% (n=29/29)	98% (n=171/174)	>0.99
Headache	86% (n=25/29)	77% (n=139/181)	0.36
Dizziness	31% (n=9/29)	38% (n=69/183)	0.63
Cough	17% (n=5/29)	21% (n=38/181)	0.83
Myalgia	45% (n=13/29)	28% (n=51/181)	0.11
Vomiting	45% (n=13/29)	31% (n=55/181)	0.18
Seizures	35% (n=10/29)	25%(n=45/183)	0.37
Brainstem involvement	59% (n=17/29)	55% (n=100/183)	0.84
Myoclonus	54% (n=13/28)	43% (n=76/179)	0.37
Admission systolic blood pressure in mmHg	134 ± 23 (n=29)	133 ± 20 (n=176)	0.73
Admission diastolic blood pressure in mmHg	82 ± 14 (n=29)	80 ± 14 (n=176)	0.53
Admission heart rate per minute	90 ± 17 (n=28)	86 ± 17 (n=176)	0.21
Admission mean GCS score**	14.5 (n=28)	15.0 (n=175)	0.026
Peak systolic blood pressure in mmHg	175 ± 24 (n=21)	166 ± 34 (n=175)	0.25
Peak diastolic blood pressure in mmHg	102 ± 11 (n=21)	94 ± 19 (n=175)	0.065
Peak heart rate per minute	126 ± 22 (n=21)	118 ± 30 (n=175)	0.28
Peak temperature in ^{o}C	39.9 ± 1.2 (n=28)	39.3 ± 1.2 (n=179)	0.02
Mean GCS[2] at nadir	6.0 (n=21)	9.0 (n=140)	0.021

*JE - Japanese encephalitis.
**GCS - Glasgow Coma Scale score, maximal of 15.

Table 3: Laboratory findings according to Japanese encephalitis serology

Parameter	JE* IgM serology positive	JE* IgM serology negative	p value
Abnormal Nipah serology	95% (n=18/19)	82% (n=126/154)	0.20
Trough platelets count in 10^9 /μl	150 ± 73 (n=29)	159 ± 72 (n=177)	0.55
Peak serum sugar in µmol/l	10.0 ± 6.0 (n=17)	11.2 ± 5.4 (n=97)	0.37
Aspartate transaminase in IU/l	73 ± 51 (n=14)	78 ± 92 (n=147)	0.46
Alanine transaminase in IU/l	97 ± 78 (n=14)	90 ± 94 (n=148)	0.57
Proportion with abnormal CSF**	73% (n=16/22)	79% (n=101/128)	0.58
CSF protein in g/l	2.7 ± 7.5 (n=22)	0.9 ± 0.8 (n=127)	0.66
CSF sugar in mmol/l	3.9 ± 1.4 (n=22)	4.5 ± 3.0 (n=125)	0.30
CSF white cell count /ml	105 ± 297 (n=20)	48 ± 129 (n=125)	0.35

*JE – Japanese encephalitis
**CSF - Cerebrospinal fluid, for the definition of abnormal CSF see text.

Table 4: Outcome according to Japanese encephalitis IgM Serology

Parameter	JE* IgM serology positive	JE* IgM serology negative	p value
Mortality	52% (n=29)	40% (n=183)	0.35
Proportion ventilated	69% (n=29)	56% (n=179)	0.29
Duration of hospital stay in days	17 ± 23 (n=29)	19 ± 39 (n=181)	0.78
Residual neurology deficits	43% (n=6/14)	16% (n=17/109)	0.024
Proportion with relapse	7.1% (n=1/14)	4.6% (n=5/109)	0.52

*JE – Japanese encephalitis

Table 5: Characteristics of patients with post-mortem

Parameter	Patients with post-mortem	Other patients	p value
Age in year	42 ± 15 (n=33)	38 ± 12 (n=179)	0.055
Proportion of male	91% (n=30/33)	85% (n=156/179)	0.59
Proportion of Chinese	70% (n=23/33)	72% (n=126/179)	>0.99
Farm owner or workers	88% (n=29/33)	78% (n=140/179)	0.30
Diabetes mellitus	6.1 (n=2/33)	4.7% (n=8/179)	0.66
Ribavirin treatment	6.4% (n=2/31)	77% (n=138/179)	<0.001
JE* serology positive	33% (n=11/33)	10% (n=18/179)	0.00097

*JE – Japanese encephalitis

who stained positive for Nipah virus on IHC, 10 (36%) had positive Japanese encephalitis IgM serology.

DISCUSSION

Although 14% of the patients in this encephalitis outbreak had positive Japanese encephalitis IgM serology, this study showed that there was no difference in the demographic and clinical features, laboratory findings and outcome between those who had positive Japanese encephalitis serology, and those whose serology were negative. Furthermore, none of the 33 fatal patients with post-mortem studies showed positive IHC staining for Japanese encephalitis. The latter included 11 patients who had positive IgM Japanese encephalitis serology. There was thus no evidence that the encephalitis outbreak was due to either of the two viruses, Japanese encephalitis and Nipah virus; or a patient may have suffered encephalitis concurrently from both viruses; or a subclinical Japanese encephalitis infection have aggravated a patient with Nipah encephalitis. On the other hand, all except 4 patients who had post-mortem examination had positive IHC staining for Nipah virus. The 4 patients whose IHC staining was negative had positive Nipah serology and died substantially later, suggesting that by the second week of illness, the Nipah virus would probably have been cleared by the immune system, as they were all tested positive for Nipah serology during their illness. It is likely that these patients succumbed to the complications of the illness. The positive staining in IHC for Nipah virus confirmed that the encephalitis outbreak was due to Nipah virus infection

The positive Japanese encephalitis IgM serology could be partly explained by a subclinical Japanese encephalitis endemic infection among the pig farm workers in Malaysia. A recent survey in East Malaysia showed that up to 10% of those involved in rearing pigs has positive Japanese encephalitis IgM serology (Jane Cardosa, University Malaysia Sarawak, personal communication). The Japanese encephalitis seropositivity rate of 14% in this cohort of patients was consistent with an endemic Japanese encephalitis infection. As only one in 300 persons infected by Japanese encephalitis suffers from clinical illness[9], the patients with positive Japanese encephalitis serology in our cohort were likely to be asymptomatic. As the outbreak was initially thought to be due to Japanese encephalitis, there were intensive measures to reduce the mosquito population. This could explain the much reduced positivity rate for Japanese encephalitis IgM

serology in the later part of the outbreak.

Pathologically, meningitis is usually mild in Nipah encephalitis. However, disseminated microinfarction secondary to vasculitis-induced thrombosis of the cerebral blood vessels is one of the main pathological findings in Nipah encephalitis.[3] There is also inflammation in the brain parenchyma adjacent to subarachnoid space.[10] The high rate of positive Japanese encephalitis cerebrospinal fluid IgM serology could thus possibly be due to the breakdown in blood brain barrier secondary to the brain parenchymal inflammation adjacent to the subarachnoid space.

As the outbreak was initially attributed to Japanese encephalitis infection, there was intensive effort to vaccinate the pig farm workers against the infection. However, IgM antibody response has been said to be seen in only small number of vaccinees even after booster immunization.[11] It thus would not significantly contribute to the high rate of IgM seropositivity in this outbreak. It is also not consistent with the drop of IgM positivity at the latter phase of the outbreak.

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