# RISK FACTORS FOR NIPAH VIRUS TRANSMISSION, PORT DICKSON, NEGERI SEMBILAN, MALAYSIA: RESULTS FROM A HOSPITAL-BASED CASE-CONTROL STUDY

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**Abstract.** A hospital-based case-control study of viral encephalitis was carried out at Port Dickson Hospital, in the state of Negeri Sembilan, Malaysia. Between March and May 1999, 69 clinically diagnosed viral encephalitis cases and 31 controls were interviewed. Job histories on pig farming activities were assessed by a group of epidemiologists and veterinary surgeons. Results show that among clinical cases of viral encephalitis, 52 (75.4%) cases were diagnosed to have Nipah virus infection based on positive serology for antibodies to the cross-reacting Hendra virus antigen. The Nipah virus encephalitis was significantly associated with a history of working in pig farms ($p < 0.001$, OR = 196.0, 95% CI = 20.4 - 4741.6), history of contact with animals ($p < 0.001$, OR = 38.3, 95% CI = 8.2 - 209.0) and with history of direct contact with pigs ($p = 0.002$, OR = 34.4, 95% CI = 2.6 - 1,024.4). The Nipah virus infection was also significantly associated with history of feeding/cleaning pigs ($p < 0.001$, OR = 102, 95% CI = 11.9 - 2,271.5). These results provide evidence that involvement in pig farming activities is significantly associated with the risk of getting Nipah virus infection. They are potential risk factors for Nipah virus transmission in the major pig-producing area of Bukit Pelandok, Port Dickson Negeri Sembilan.

## INTRODUCTION

An outbreak of viral encephalitis, which resulted in over 100 human fatalities, began in late February and peaked around the middle of March 1999 in Bukit Pelandok, Port Dickson district, Negeri Sembilan state in Peninsular Malaysia. Bukit Pelandok is one of the largest pig-producing areas in the country. The outbreak primarily involved male adults with histories of direct contact with pigs. Their family members who also lived but did not work on the pig farms were not affected (Enserink, 1999). Early epidemiologic investigations suggest that the new virus is easily transmitted to humans, and spreads rapidly among pigs. By end of April 1999 most pig farms in Bukit Pelandok area were affected. It also seemed that the new virus only affects those who had been in close contact or directly involved in pig farming activities (CDC, 1999a,b; Enserink, 1999).

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Laboratory investigations of cases of encephalitis suggested of a previously unknown virus that replicated in pigs and readily spread to humans. The virus was initially isolated at the University Hospital in Kuala Lumpur in early March 1999. The virus was later identified as a new paramyxovirus closely related to the Hendra virus (formerly called equine morbillivirus) at the Centers for Disease Control and Prevention (CDC) in Atlanta, Georgia, USA. The Malaysian virus was subsequently named Nipah virus, named after the village *Sungai Nipah* (Nipah river) in Negeri Sembilan state, where a pig farm worker from which the first viral isolate came from had died.

Hendra virus was first recognized in 1994 after an outbreak of respiratory disease among horses and humans in Australia (Murray *et al*, 1995a,b; Selvey *et al*, 1995). To date, two outbreaks of Hendra virus have been reported, with three human cases and two deaths (Hooper *et al*, 1996; Murray *et al*, 1995a,b; O'Sullivan *et al*, 1997; Rogers *et al*, 1996; Selvey *et al*, 1995). Hendra virus is believed to spread through direct contact with body fluids of infected horses and has been isolated from both saliva and urine of horses

(McCormack *et al*, 1999; Williamson *et al*, 1998). This study identified potential risk factors for Nipah virus transmission in the district of Port Dickson, Negeri Sembilan state.

## MATERIALS AND METHODS

### Study population

The peak of the Nipah viral disease outbreak occurred during the middle of March and continued through May 1999 in the state of Negeri Sembilan. According to the Ministry of Health, Malaysia, most of the cases were reported from the Port Dickson district. Cases and controls for this study were chosen from this district. Most of the population were of Chinese ethnicity and involved in pig farming activities, particularly from the highly concentrated pig farming area of Bukit Pelandok, Negeri Sembilan state.

### Selection of cases

Cases were defined as persons with serological evidence of Nipah virus infection. Participants were recruited based on their residential addresses of Port Dickson, Negeri Sembilan, and who were hospitalized with a clinical diagnosis of viral encephalitis that were reported to the Ministry of Health, Malaysia, during the period from March through May 1999. Viral encephalitis patients who had been discharged from the hospital and could be located were recruited through home visits. Eligible encephalitis candidates whose serum specimen tested positive for Nipah virus antibody and who consented to participate in the study were included as cases. Clinical encephalitis patients whose specimens tested negative for Nipah virus antibody were excluded from the study.

### Selection of controls

Controls were patients of non-Muslim faith with residential addresses in Port Dickson and admitted to Port Dickson Hospital, Negeri Sembilan during the month of March through May 1999. Muslims were excluded because their religious beliefs and practices prohibit them from direct contact with pigs or consuming pork. Patients with clinical diagnoses of encephalitis, meningitis, pneumonia, and viral fever other than dengue, measles and chickenpox were excluded. Patients admitted to the obstetrics and gynecology wards were also excluded.

### Data collection

After obtaining consent, a standardized questionnaire was administered to study subjects by trained members of the investigation team between 1 March and 15 May 1999. An adult family member was interviewed for deceased cases and for cases who could not be personally interviewed due to the severity of their illness. Information was obtained about household, demographics and socioeconomic characteristics, illness, exposure to pigs and other animals, characteristics of the farm, and (Japanese encephalitis) vaccination history.

### Collection of specimens

A single specimen of cerebrospinal fluid (CSF) from case-patients and 10 ml of venous blood from cases and controls were obtained. These specimens were tested for presence of Nipah virus IgM and/or IgG antibodies. All specimens were tested at the special National Task Force Laboratory operated by the CDC at the University Hospital, Kuala Lumpur during the outbreak. Specimens were tested for IgM antibodies by using an IgM-capture antibody enzyme immunoassay (EIA) and for IgG antibodies by using an indirect EIA. Preliminary nucleotide sequence information indicated that the Nipah virus was closely related to Hendra virus, cross-reacting in a Hendra antigen ELISA (CDC, 1999a). Therefore, in this study, Hendra virus antigens, which cross react with Nipah virus antibodies, were used in the assays.

### Data management and analysis

Questionnaire and serological data were entered into databases. EPI-info version 6.04b (Centers for Disease Control and Prevention, Atlanta, George, USA) was used for analysis. We calculated odds ratio (OR), 95% confidence intervals (CI) and p-values by chi-square with Yates'correction or two-tailed Fisher's exact test, when appropriate.

## RESULTS

A total of 69 cases of viral encephalitis were identified. Of these, 52 (75.4 %) were serologically positive for Nipah virus. A total of 16 cases were excluded from the study either because their laboratory results were negative for Nipah virus (11 cases, 15.9 %) or their laboratory results were not available (six cases, 8.7 %) (Table 1). All 31 controls were negative for Nipah virus infection.

Table 1
Number of viral encephalitis cases included/excluded in the study of Nipah virus infection, Port Dickson, Negeri Sembilan, Malaysia, March-May 1999.

| Reasons for exclusion | No. | (%) |
|---|---|---|
| Serology negative (IgM/IgG) for Nipah virus | 11 | 15.9 |
| Serology results were not available (IgM/IgG) | 6 | 8.7 |
| Serology positive (IgM and /or IgG) for Nipah virus | 52 | 75.4 |
| Total | 69 | 100 |

Table 2
Age, sex, and race distribution of Nipah virus infection admitted to hospitals, diagnosed in March - May 1999, and controls, Port Dickson, Negeri Sembilan, Malaysia.

| | No. of cases | (%) | No. of controls | (%) |
|---|---|---|---|---|
| Age (years)[a] | | | | |
| < 20 | 3 | 5.8 | 4 | 12.9 |
| 20 - 29 | 7 | 13.5 | 6 | 19.4 |
| 30 - 39 | 16 | 30.8 | 3 | 9.7 |
| 40 - 49 | 16 | 30.8 | 6 | 19.4 |
| > 49 | 10 | 19.2 | 12 | 38.7 |
| Sex | | | | |
| Male | 44 | 84.6 | 19 | 61.3 |
| Female | 8 | 15.4 | 12 | 38.7 |
| Race | | | | |
| Chinese | 39 | 75.0 | 13 | 41.9 |
| Indian | 11 | 21.2 | 17 | 54.8 |
| Others | 2 | 3.8 | 1 | 3.2 |

[a]Mean age of cases and controls were 39.3 and 43.8 years respectively. The youngest and oldest were 14 and 77 years for cases and 15 and 78 years for controls respectively.

The majority of cases were males (84.6 %) and ages between 30 to 49 years (61.6 %), with mean age of 39.3 years. The youngest and the oldest cases were 14 and 77 years old respectively. Seventy-five percent of the cases were Chinese, followed by Indians (21.2 %) (Table 2).

Most of the cases were either pig farm owners and/ or pig farm workers (73.1 %). Three cases (5.8%) were lorry drivers transporting pigs. Others include housewife, students, carpenter, school teacher and construction worker. There was one pig farmer among the controls (Table 3).

The association between job history and risk of getting Nipah virus infection is shown in Table 4. The association between history of working in pig farms and infection was highly significant (OR = 196, 95 % CI: 20.4, 4,741.6, p < 0.001). Nipah virus infection was also significantly associated

Table 3
Distribution of occupation for Nipah virus infection (cases), March - May 1999, Port Dickson, Negeri Sembilan, Malaysia[a]

| Type of occupation | No. | (%) |
|---|---|---|
| Pig farm owner/pig farm worker | 38 | 73.1 |
| Lorry driver transporting pigs | 3 | 5.8 |
| Watchman for pig farm | 1 | 1.9 |
| Carpenter | 1 | 1.9 |
| School teacher | 1 | 1.9 |
| Student | 2 | 3.8 |
| Housewife | 1 | 1.9 |
| Unemployed | 1 | 1.9 |
| Laborer (construction) | 1 | 1.9 |
| Unknown | 4 | 7.7 |
| Total | 52 | 100 |

[a]Among the controls, there was only one pig farmer.

Table 4
Job history among cases and controls, Port Dickson, Negeri Sembilan, Malaysia, since 1st September 1999.

| Job history | No. of cases | No. of controls | Odds ratio (OR) | 95% CI confidence interval | p-value |
|---|---|---|---|---|---|
| History of working on pig farms: | | | | | |
| Yes | 42 | 1 | 196 | 20.4 - 4,741.6 | < 0.001 |
| No | 6 | 28 | 1[a] | | |
| History of contact with animals: | | | | | |
| Yes | 47 | 9 | 38.3 | 8.2 - 209 | < 0.001 |
| No | 3 | 22 | 1[a] | | |
| History of contact with pigs (direct/indirect): | | | | | |
| Yes | 47 | 1 | 470 | 39.5 - 13,762.6 | < 0.001 |
| No | 3 | 30 | 1[a] | | |
| History of direct contact with pigs: | | | | | |
| Yes | 43 | 1 | 34.4 | 2.6 - 1,024.4 | 0.002 |
| No | 5 | 4 | 1[a] | | |
| History of indirect contact with pigs: | | | | | |
| Yes | 8 | 2 | 0.5 | 0.1 - 6.6 | 0.6[b] |
| No | 32 | 4 | 1[a] | | |

[a]Reference category
[b]Fisher's exact test (2-tailed p-value)

Table 5
Pig farming activity among cases and controls, Port Dickson, Negeri Sembilan, Malaysia, since 1st September 1998.

| Activity | No. of cases | No. of controls | Odds ratio (OR) | 95% CI confidence interval | p-value |
|---|---|---|---|---|---|
| Feeding and cleaning pigs: | | | | | |
| Yes | 34 | 1 | 102 | 11.9 - 2,271.5 | < 0.001 |
| No | 10 | 30 | 1[a] | | |
| Giving medication and/or injection to pigs: | | | | | |
| Yes | 7 | 1 | 5.7 | 0.7 - 264.2 | 0.13[b] |
| No | 37 | 30 | 1[a] | | |

[a]Reference category
[b]Fisher's exact test (2 tailed p-value)

with a history of contact with animals (OR = 38.3, 95 % CI: 8.2, 209, p < 0.001), history of contact with pigs both directly or indirectly (OR = 470, 95 % CI: 39.5, 13,762.6, p < 0.001) and with history of direct contact with pigs (OR = 34.4, 95 % CI: 2.6, 1,024.4, p = 0.002). Infection was not significantly associated with history of indirect contact with pigs (p = 0.6).

The risk of acquiring infection with certain pig farming activities is shown in Table 5. Nipah virus infection is significantly associated with activities of feeding and cleaning pigs in the pig farms (OR = 102, 95 % CI: 11.9, 2,271.5, p < 0.001). Administering medication and/ or injecting pigs were not significantly associated with Nipah virus infection (p = 0.13).

## DISCUSSION

In the past four years, three newly described (emerging) zoonotic viral diseases have been re-

ported from Australia; two of these diseases are caused by the paramyxoviruses: Menangle and Hendra (formerly called equine morbillivirus, EMB), and the third is caused by Australian bat lyssavirus (Mackenzie, 1999). The Nipah virus which is also in the paramyxoviridae family is the fourth zoonotic viral disease in this group. While most paramyxoviruses are species specific, Hendra virus infects a variety of mammals, including horses, cats, and bats (Williamson *et al*, 1998). Several investigations are currently being carried out by the Malaysian Veterinary Department to determine whether Nipah virus also infects a variety of mammals.

An association between Nipah virus infection and pigs was suspected early in the outbreak because most patients were either pig farm owners or pig farm workers. The findings of this study confirm this association and clearly demonstrate that working on pig farms and contact with pigs were the most important source of Nipah virus infection for humans. Activities involving direct contact with pigs were most strongly associated with infection. A few lorry drivers transporting pigs to other pig farms and abattoirs were also infected with the virus. In addition, several others with history of indirect contact with pigs also became infected. This suggests that even minimal contact with pigs may result in infection.

The association between direct contact with pigs involving specific pig farming activities and Nipah virus infection, were demonstrated by further characterization of the activities. Activities involving direct contact with pigs such as feeding and cleaning pigs were strongly associated with risk of acquiring Nipah virus infection. We believe that these activities exposed pig farm workers to fluids or secretions of infected pigs and that these fluids or secretions were the source of infection. Early investigations suggested that both lung and kidney tissue of necropsied infected pigs have been shown to be Nipah virus antigen-positive (CDC, 1999a,b; Enserink, 1999), and contact with respiratory secretions and/ or urine of infected pigs are possible modes of transmission of Nipah virus. In addition, this study also shows that 15% of the cases became infected despite reporting history of indirect contact with pigs. This suggests that other sources of infection or modes of transmission of Nipah virus may be responsible for some cases.

It is important to note that bias might have been introduced and affected the findings of this study. Since at the beginning of the outbreak, it was hypothesized that pigs were the possible source of Nipah virus infection in humans, clinical encephalitis cases or their family members or other persons may have been aware and more likely to report contact with pigs. Similarly, the need to obtain information from family members or other persons rather than the cases might have led to inaccurate reporting of exposures. In order to reduce this possibility, other than cases themselves, their close family members where possible, were also asked to confirm the answers with regard to the exposure status or contact with pigs. Therefore, we believe that this appears unlikely to have a major impact on our results. However, we cannot find any significant association between administering medication and/ or giving injection to pigs and the risk of acquiring Nipah virus infection. This could be best explained by small size for the cases who were involved in the activities. This should be considered in future research.

In conclusion, this study provides evidence that close contact with pigs was the primary source of human Nipah virus infection in the outbreak. Working on pig farms and activities involving direct contact with pigs were strongly associated with risk of acquiring Nipah virus infection suggesting that contact with respiratory secretions and/ or urine of infected pigs are possible modes of transmission of Nipah virus. However not all cases had a history of direct contact with pigs and it is possible that other sources of infection or modes of transmission of Nipah virus may be responsible for a small percentage of cases. We believe that infected pigs are required to sustain transmission of Nipah virus to humans and therefore efforts to prevent and control outbreaks of this new zoonotic disease should focus on stopping transmission by eliminating infected pigs.

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