CONCISE COMMUNICATION

Case-Control Study of Risk Factors for Human Infection with a New Zoonotic Paramyxovirus, Nipah Virus, during a 1998–1999 Outbreak of Severe Encephalitis in Malaysia

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An outbreak of encephalitis affecting 265 patients (105 fatally) occurred during 1998–1999 in Malaysia and was linked to a new paramyxovirus, Nipah, that infected pigs, humans, dogs, and cats. Most patients were pig farmers. Clinically undetected Nipah infection was noted in 10 (6%) of 166 community-farm controls (persons from farms without reported encephalitis patients) and 20 (11%) of 178 case-farm controls (persons from farms with encephalitis patients). Case patients (persons with Nipah infection) were more likely than community-farm controls to report increased numbers of sick/dying pigs on the farm (59% vs. 24%, $P = .001$) and were more likely than case-farm controls to perform activities requiring direct contact with pigs (86% vs. 50%, $P = .005$). Only 8% of case patients reported no contact with pigs. The outbreak stopped after pigs in the affected areas were slaughtered and buried. Direct, close contact with pigs was the primary source of human Nipah infection, but other sources, such as infected dogs and cats, cannot be excluded.

From September 1998 through May 1999, 265 cases of encephalitis (105 fatal) were reported from 3 states—Perak, Negeri Sembilan, and Selangor—of Malaysia [1–3]. Most patients were pig farmers. Concurrently, an illness characterized by respiratory and neurological symptoms was observed in pigs on some farms with ill workers. In March 1999, laboratory studies indicated evidence of infection with Nipah virus, a new paramyxovirus, in both human patients and sick pigs. Nipah virus is most closely related to the Hendra virus, which has been associated with disease outbreaks among horses and humans in Australia [4–9]. Hendra virus appears to spread to humans through direct contact with body fluids of infected horses [10, 11]. We conducted case-control studies to characterize exposures associated with Nipah infection of humans during this outbreak.

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Methods

Study population. At the time this study was designed, in March 1999, most new cases were occurring in Negeri Sembilan. This state accounted for 231 (87%) of 265 reported patients with suspected Nipah encephalitis. Of the 224 patients in Negeri Sembilan, 97% resided in the Port Dickson district. Cases and controls for this study were chosen from Port Dickson.

Case patients. Case patients were defined as persons with serological evidence of Nipah infection. To identify candidates for inclusion as case patients, we recruited patients who were hospitalized with encephalitis at any time from January through April 1999. Encephalitis patients who were hospitalized during the study period were most easily accessible and were most likely to be recruited. Encephalitis patients who had been discharged from the hospital were recruited through house visits. Candidate encephalitis patients whose serum specimen(s) tested positive for Nipah antibody were included as case patients. In addition, persons selected as controls whose serum specimen(s) tested positive for Nipah antibody were reclassified as case patients.

Control subjects. Two sets of controls were selected: community-farm controls and case-farm controls. Community-farm controls were selected to identify characteristics of farms where human Nipah infection was detected; these controls were persons who either lived or worked on pig farms with no reported human encephalitis cases. They were selected from persons living in the temporary residential facilities assigned to persons from the affected

area who had been evacuated from their homes. Case-farm controls were selected to identify specific farming activities associated with Nipah infection of humans; these controls were persons who either lived or worked on pig farms with known cases of human Nipah infection. A serum specimen was obtained from all potential controls and was tested for Nipah antibody. Those who had detectable antibodies were reclassified as case patients, whereas seronegative persons were retained as controls for analysis.

Laboratory studies. Sera were tested for IgM and IgG antibodies by using an IgM–capture antibody EIA and an indirect EIA, respectively. Hendra virus antigens, which cross-react with Nipah antibodies, were used in the assays.

Data collection and analysis. Information was obtained regarding demographics, illness, pig farm characteristics, and specific farming activities. An adult family member was interviewed for children, deceased case patients, and case patients who were either comatose or required a ventilator. The association between Nipah infection and predictor variables was assessed by computing odds ratios (ORs) with 95% confidence intervals (CIs). Comparisons of exposures between case patients and case-farm controls were performed in a stratified analysis by forming a stratum for the case patient(s) and case-farm control(s) from each farm. Stratifying by farm allowed us to adjust for farm characteristics (e.g., illness in animals) that might confound the association between specific farming activities and infection.

Results

Case patients. Of the 224 encephalitis patients reported from Port Dickson, 109 (49%) were interviewed, and Nipah serology results were available for 101 of the 109 patients. Eighty (79%) of the 101 patients tested had detectable Nipah antibody and were included as cases, whereas the remaining 21 Nipah antibody–negative patients were excluded from all further analyses. Of the 21 excluded Nipah antibody–negative patients, 10 (~50%) patients had serum specimens obtained at least 1 week after onset of illness. In addition to the 80 Nipah antibody–positive encephalitis patients, 10 (6%) of 166 community-farm controls and 20 (11%) of 178 case-farm controls tested positive for Nipah antibody, yielding a total of 110 persons with Nipah antibody who were classified as case patients for further analyses.

The mean age of the case patients was 38 years (range, 9–76 years); 82 (75%) were male. Seventy-seven percent of case patients were Chinese, 20% were Indian, 1% were Indonesian, and 1% were Bidayuh; information on ethnicity was unavailable for 1 case patient. One hundred five (95%) case patients either lived or worked on a pig farm, and 101 (92%) reported either handling pigs or being within 1 m of a pig and coming into contact with pig urine or feces. Nine (8%) case patients had atypical epidemiologic features and reported limited or no contact with pigs. Three case patients reported that they neither lived nor worked on a pig farm and that they had no contact with pigs. Five case patients reported that they lived on a pig farm but had no contact with pigs. One case patient reported that he mixed feed for pigs on the farm but had no direct contact with pigs.

Comparison of case patients with community-farm controls. Case patients were compared with community-farm controls to identify characteristics of farms where Nipah infections of humans were detected. Of the 110 total case patients, 97 case patients who either lived or worked on a pig farm and had no other potential exposures to pigs outside the farm (e.g., transporting pigs in a truck or working in an abattoir) were included in this analysis (table 1). Case patients were similar to control subjects with regard to age (mean age of 37.8 vs. 37.8 years, respectively; $P = .99$) and sex but were significantly different in ethnicity and occupation. The difference in ethnicity and occupation most likely resulted from the process of selection of control subjects, because most foreign workers of non-Chinese ethnicity had left the area before this study was initiated and because pig farm workers were targeted for selection as controls.

No significant differences were observed between the prevalence of different animal species on farms of case patients and control subjects. However, case patients were significantly more likely than control subjects to report an increase in the number of sick or dying pigs, dogs, or chickens on the farm. The illness

Table 1. Characteristics of laboratory-confirmed Nipah virus case patients and community-farm control subjects in Malaysia during 1998–1999.

Variable	Case patients (n = 97)	Community-farm controls (n = 147)	OR	95% CI
Male	71/97 (73)	106/147 (72)	1.06	0.59–1.88
Ethnicity				
Chinese	78/97 (80)	137/147 (93)	0.30	0.14–0.66
Indian	19/97 (20)	10/147 (7)	1.00	—
Occupation[a]				
Pig farm owner/worker	86/97 (89)	142/147 (97)	0.28	0.10–0.77
Housewife	3/97 (3)	1/147 (1)	4.66	0.58–37.3
Student	9/97 (9)	10/147 (7)	1.40	0.55–3.58
Lived on pig farm	72/97 (74)	113/147 (77)	0.87	0.48–1.57
Worked on pig farm	91/97 (94)	147/147 (100)	—	—
Presence of illness among other animals on farm				
Dogs	77/89 (87)	106/139 (76)	2.00	0.98–4.09
Cats	57/89 (64)	86/139 (62)	1.10	0.63–1.91
Rats	71/89 (80)	107/139 (77)	1.18	0.62–2.26
Chickens	69/89 (78)	102/139 (73)	1.25	0.67–2.34
Bats	17/89 (19)	26/139 (19)	1.03	0.52–2.03
Increase in sick/dying animals on farm				
Pigs	54/92 (59)	34/140 (24)	4.43	2.55–7.70
Dogs	21/85 (25)	11/132 (8)	3.61	1.69–7.71
Cats	10/85 (12)	12/132 (9)	1.33	0.55–3.24
Rats	8/85 (9)	7/132 (5)	1.86	0.65–5.26
Chickens	9/85 (11)	3/132 (2)	5.09	1.50–17.3
Bats	0/83 (0)	0/132 (0)	—	—

NOTE. For this analysis, case patients were included only if they lived or worked on a pig farm and had no other potential exposures to pigs. Data are no./total population (%) unless otherwise noted. OR, odds ratio; CI, confidence interval.

[a] Occupations were not mutually exclusive.

in dogs and chickens was poorly described and included features similar to the illness in pigs, such as unsteady gait, loss of appetite, and frothing at the mouth. The association between infection and an increase in sick or dying pigs, dogs, or chickens did not differ significantly according to sex, ethnicity, occupation, or place of residence (on farm vs. outside farm). After adjusting for ethnicity, occupation, and illness among other farm animals, we found that infection was significantly associated with an increase in sick or dying pigs (OR, 5.52; 95% CI, 2.84–10.7) but not sick or dying dogs (OR, 1.89; 95% CI, 0.83–4.31) or chickens (OR, 1.07; 95% CI, 0.24–4.81).

Comparison of case patients with case-farm controls. Case patients were compared with case-farm controls to identify specific farming activities associated with Nipah infection of humans. Of the 110 total case patients, 48 patients, each of whom was matched with at least 1 control subject from the same farm (a total of 107 case-farm control subjects), were included in this analysis (table 2). Case patients were more likely than control subjects to be male and pig farm owners and were less likely to be housewives and students. Case patients were more likely than controls to work with pigs on the farm. Infection was not associated with direct contact with all pigs among persons who worked on the farm, but it was significantly associated with direct contact with pigs that appeared to be sick. Infection was not associated with performing activities that usually did not involve contact with pigs, such as cleaning pigpens and washing pigs with a hose. However, infection was associated with feeding pigs and with activities involving close contact with pigs, such as processing baby pigs (clipping tails, tagging ears, and giving iron medications), injecting or medicating pigs, assisting in pig breeding (collection of semen from boars, artificial insemination of sows), assisting in the birth of piglets, and handling dead pigs. When activities involving close contact with pigs were combined as a single variable, this exposure was strongly associated with infection (37 [86%] of 43 case patients vs. 38 [50%] of 76 control subjects; OR, 5.62; 95% CI, 2.07–15.3).

Discussion

An association between human Nipah infection and proximity to pigs was suspected early in the outbreak because most patients were male pig farmers and because viral isolates from sick pigs and from encephalitis patients showed identical nucleotide sequences. Our findings confirm this association and show that activities involving close contact with pigs, especially with sick pigs, were most strongly associated with infection. We suspect that these activities brought the workers into contact with body fluids or secretions of infected pigs and that these fluids or secretions were the source of infection. Both lung and kidney tissue taken from infected pigs at necropsy have been shown to be positive for the Nipah antigen [1–3], and contact with respiratory secretions or urine of infected pigs is a possible mode of transmission of Nipah virus. Although the risk of infection among persons on the farm who did not report performing activities involving close contact with pigs was substantially lower than that among those who did, it was not 0. Consequently, we have defined high-risk activities on farms with Nipah virus–infected pigs but have not determined which, if any, activities are completely safe.

The association between infection and the presence of sick

Table 2. Characteristics of laboratory-confirmed Nipah virus case patients and case-farm control subjects in Malaysia during 1998–1999.

Characteristic	Case patients (n = 48)	Case-farm controls (n = 107)	OR	95% CI
Male	36/48 (75)	55/107 (51)	3.29	1.44–7.53
Occupation				
Pig farm owner/worker	40/48 (83)	71/107 (66)	3.49	1.24–9.81
Housewife	2/48 (4)	10/107 (9)	0.32	0.07–1.42
Student	8/48 (17)	38/107 (36)	0.24	0.10–0.60
Living on farm	39/48 (81)	86/107 (80)	1.31	0.32–5.33
Working on farm	44/48 (92)	76/107 (71)	8.79	2.53–30.6
Contact with pigs on farm[a]	42/44 (95)	70/76 (92)	1.57	0.30–8.18
Contact with sick pigs on farm[a]	30/42 (71)	30/73 (41)	3.69	1.49–9.14
Specific activities on farm[a]				
Cleaning pigpens	42/44 (95)	70/76 (92)	1.48	0.24–9.15
Washing pigs	43/44 (98)	71/76 (93)	1.11	0.17–7.26
Feeding pigs	39/43 (91)	58/74 (78)	3.86	1.16–12.9
Processing baby pigs[b]	23/44 (52)	20/76 (26)	2.95	1.21–7.21
Assisting in breeding of pigs[c]	21/43 (49)	12/75 (16)	3.37	1.34–8.45
Assisting in the birth of pigs	22/44 (50)	13/73 (18)	4.42	1.66–11.8
Injecting or medicating pigs	29/44 (66)	21/75 (28)	3.10	1.47–6.56
Handling dead pigs	29/43 (67)	27/76 (36)	3.89	1.60–9.44

NOTE. Data are no./total population (%) unless otherwise noted. OR, odds ratio; CI, confidence interval.

[a] Analyses restricted to those who worked on the farm.

[b] Includes activities such as clipping tails, tagging ears, and giving iron medications.

[c] Includes activities such as artificial insemination of sows and collection of semen from boars.

or dying pigs on the farm is not unexpected, because Nipah infection in pigs is associated with an illness with respiratory and neurological symptoms. The finding that 41% of case patients reported no sickness in pigs is consistent with observations that workers from farms with sick pigs reported only a slight increase in sickness and deaths among pigs and that workers at an abattoir in Singapore noted no sickness in pigs slaughtered during the time pigs presumably transmitted the virus to workers [1, 2, 12]. The low rate of recognized sickness in pigs contrasts with the very high rate of Nipah antibody –positive pigs on farms with recently infected humans [13]. It is possible that most pigs have mild sickness resulting from infection. Studies of pigs experimentally infected with Nipah virus should allow us to better understand whether the risk of transmission increases with the presence of sickness and with increasing severity of sickness. In the meantime, sickness in pigs can be an indicator of infection but is not a reliable one; therefore, laboratory studies are needed to differentiate infected from uninfected pig herds.

The observation that 8% of case patients reported no direct contact with pigs suggests that other vectors may be associated with transmission of Nipah virus. The comparison of case patients with community-farm controls suggested an association between infection and the presence of sick or dying dogs and chickens. Nipah virus has been shown to infect dogs and cats [2, 14], but it is not yet known whether it infects chickens. It is possible that reports of increased numbers of sick or dying dogs and chickens represent an increased awareness of animal illnesses on those farms with a human case of Nipah infection and not the actual presence of Nipah infection in these animals. It would not be surprising if these animals become infected from pigs just as humans do, and thus the presence of other infected animals may be another indicator of infection among pigs and of the risk of pig-to-human transmission. It is also possible that dogs and chickens transmit infection directly to humans, but further laboratory and epidemiologic studies are needed to evaluate this possibility.

In conclusion, this study confirms that close contact with pigs, especially sick pigs, was the primary source of human Nipah infection during 1998–1999 in Malaysia. Activities involving direct contact with pigs were associated with the greatest risk of infection; however, not all case patients reported such exposures, and it is possible that other animals may be the source of some infections. The fact that the outbreak stopped after the culling of pigs in the outbreak-affected areas suggests that, even if other sources of infection exist, they are secondary to the presence of infected pigs and that pigs are required to sustain transmission. Efforts to prevent and control outbreaks of this new zoonotic infection should focus on preventing infection in pigs and restricting human contact with infected animals.

Nipah Encephalitis Outbreak Investigation Team

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