Epidemiol. Infect. (2002), **128**, 93–98. © 2002 Cambridge University Press
DOI: 10.1017/S0950268801006422 Printed in the United Kingdom

A survey of Nipah virus infection among various risk groups in Singapore

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(*Accepted 5 August 2001*)

SUMMARY

Following the Nipah virus (NV) outbreak in March 1999 in Singapore, a serological survey was undertaken to screen individuals potentially exposed to NV. Blood samples were tested for NV IgM, IgG and neutralizing antibodies. Twenty-two (1·5%) of 1469 people tested had antibodies suggesting NV infection. Although 12 of the 22 infected people (54·6%) were symptomatic, the remaining 10 (45·4%) were clinically well and had no past history of compatible pulmonary or neurological disease. Clinical and serological findings suggested three people had been infected with NV before the outbreak was recognized. All those who were infected were male abattoir workers. None of the people who had contact with horses, and no healthcare workers exposed to infected patients and their specimens had detectable antibodies. This study provides evidence that NV causes asymptomatic infection. All of the antibody positive individuals had direct contact with pigs and there was no evidence of human to human transmission.

INTRODUCTION

An outbreak of encephalitis caused by the recently discovered Nipah virus (NV) occurred in Singapore in March 1999 among abattoir workers, following a similar occurrence among Malaysian pig farmers in the preceding months [1]. Investigation of the epidemic in Malaysia had established the main risk of infection to be contact with pigs [1, 2]. Within 3 days of the presentation to hospital of the Singaporean index case, a programme was instituted to screen people potentially exposed to infected pigs for NV infection by serology [3]. Those screened included workers from the two Singapore abattoirs, meat inspectors, public butchers, zoo workers and the customs inspectors who had discovered the smuggled lorry-load of pork from Malaysia following the import ban on all Malaysian pigs on 19 March 1999.

The serological and genetic cross reactions of NV to another paramyxovirus, Hendra virus, had led the novel agent to be described as Hendra-like in the initial stages of the outbreak [1, 4]. Human infections with Hendra virus have only been reported in Australia and have been associated with infected horses [5, 6]. Like Hendra virus, NV is capable of infecting other animals, including horses, cats, dogs and probably bats [7]. Over 500 horses in Singapore were tested serologically for NV and were found to be negative (personal communication, Hilda Loh). Turf club workers and zoo keepers were similarly investigated and the results are included in this paper.

Although it was clear that close contact with infected pigs was a significant risk factor, whether

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there was human to human transmission of NV was less obvious. It was therefore necessary to determine if infection had occurred among medical and laboratory staff who had had contact with NV-infected patients and their specimens. Family contacts were excluded from the survey in response to concerns that their inclusion at that stage of the outbreak might trigger a panic among the public.

A total of 1469 people were investigated for serological evidence of NV infection. We report the results of the investigation.

METHODS

Blood was collected from both ill and well abattoir workers. Blood specimens were also obtained from meat inspectors and butchers, customs inspectors, personnel in contact with pigs and horses at the zoo, and turf club workers. Healthcare staff including doctors, nurses and physiotherapists, who had cared for the NV-infected patients as well as laboratory staff who had handled and processed specimens from NV patients were also bled. The first blood sample was collected within a month of the outbreak from all except the majority of the healthcare workers. Second and subsequent sera were obtained from the majority of pig- and horse-related workers.

Serum samples collected during the initial phase of the investigation were tested for NV antibodies at the Centers for Disease Control and Prevention (CDC), USA, using IgM capture enzyme immunoassay (ELISA) and indirect IgG ELISA with prototype Hendra virus antigens as described before [3]. When NV ELISA reagents became available, all the samples collected previously were retested with identical results. NV-specific reagents were used for subsequent specimens, comprising first serum samples from 201 healthcare workers not included in the early phase of testing and 149 follow-up samples, mainly of abattoir workers, that were collected after June 1999. These samples were tested at the Singapore General Hospital, Singapore, with reagents provided by the CDC. The assay was performed in accordance with the CDC protocol after training from the CDC scientists. The ELISA techniques used for haemorrhagic fever viruses [8] were modified by utilization of Hendra or NV antigens and immune serum. Briefly, serum IgM antibodies were captured onto 96-well microtitre plates coated with anti-human IgM, allowed to react with viral antigen, then detected by a specific hyperimmune mouse ascitic fluid and an appropriate enzyme conjugate and substrate. Each serum was tested in parallel with uninfected control antigen. IgG ELISAs were performed by coating microtitre plates with a gamma irradiated inactivated detergent extract of uninfected and Hendra or NV-infected Vero E6 cells. Dilutions of the sera were allowed to react with the coating antigen. Bound IgG was detected with an anti-human conjugate and 2,2′-azino-di-3-ethylbenzthiazoline-6-sulfonate (ABTS) was used as substrate. IgG and IgM ELISAs are specific for the Nipah–Hendra complex; no cross-reaction with any other virus including measles, respiratory syncytial virus, and parainfluenza virus types 1 and 3 has been shown [4]. The sensitivity of the tests varies with the status of the people tested. In acute febrile patients, IgM ELISA should be used, whilst a combination of IgM and IgG ELISAs is useful for the surveillance of exposed, asymptomatic individuals.

Specimens that gave questionable ELISA results were tested by the CDC using the neutralization test. The neutralizing antibody titre was recorded as the reciprocal of the highest dilution of the serum neutralizing cytopathic effect in 96 well microplates using Vero E6 cells and the prototype NV.

For this study, a person was considered to have had a recent infection with NV when IgM and IgG antibodies to Hendra or Nipah viruses were present. When virus-specific antibody of one class only was detectable, NV infection was likely when the serum contained specific neutralizing antibodies or, in case of death, when supported by positive immunohistochemistry for NV antigen or RT-polymerase chain reaction (RT–PCR) for NV RNA.

RESULTS

Of 1469 people tested, 572 submitted a single specimen and 897 submitted two or more specimens for NV serology. Of the people examined 521 (35·5%) were abattoir workers, 474 (32·3%) turf club workers, 228 (15·5%) healthcare workers, 127 (8·6%) pig and meat inspectors, 70 (4·8%) zoo workers, 25 (1·7%) laboratory workers, 12 (0·8%) public butchers, 7 (0·5%) recreational island staff workers and 5 (0·3%) customs inspectors (Table 1).

Altogether, 25 people had detectable ELISA antibodies to NV. Three of them, comprising 2 healthcare workers and 1 abattoir worker, had low optical densities in the IgG EIA only. All 3 were asymptomatic and the NV neutralization tests on their sera

https://doi.org/10.1017/S0950268801006422 Published online by Cambridge University Press

Table 1. *Categories of people and Nipah virus (NV) antibody status of those potentially exposed to NV*

	Animal exposure	No. tested (%)	No. NV antibody positive (%)
Abattoir workers	Pig	521 (35·5)	22 (100·0)
Turf club workers	Horse	474 (32·3)	0
Health-care workers	Human	228 (15·5)	0
Pig/meat inspectors	Pig	127 (8·6)	0
Zoo workers	Horse, pig	70 (4·8)	0
Laboratory workers	Human	25 (1·7)	0
Public butchers	Pig	12 (0·8)	0
Recreational island staff	Horse	7 (0·5)	0
Customs inspectors	Pig	5 (0·3)	0
Total		1469 (100·0)	22 (100·0)

Table 2. *Summary of serological results, clinical presentation and place of employment of Nipah virus (NV)-infected people*

Case	Sex	Age	NV IgM	NV IgG	NV neutralizing antibody	Symptoms	Place of work
1	M	64	+	+	n.d.	Well	Abattoir A & B
2	M	24	+	+	+	Encephalitis	Abattoir B
3	M	52	+	+	n.d.	Well	Abattoir B
4	M	53	+	+	n.d.	Encephalitis	Abattoir A
5	M	32	+	+	n.d.	Encephalitis	Abattoir A
6	M	44	+	+	n.d.	Well	Abattoir A
7*	M	47	+	−	n.d.	Encephalitis	Abattoir A
8	M	45	+	+	n.d.	Encephalitis	Abattoir A
9	M	32	−	+	+	Well	Abattoir B
10	M	62	+	+	n.d.	Well	Abattoir B
11	M	34	+	+	n.d.	Pneumonia	Abattoir A
12	M	40	+	+	n.d.	Encephalitis	Abattoir A
13	M	42	+	+	n.d.	Encephalitis	Abattoir A
14	M	43	+	+	n.d.	Well	Abattoir A
15	M	55	+	+	n.d.	Encephalitis	Abattoir A
16	M	27	+	+	n.d.	Pneumonia	Abattoir B
17	M	41	+	+	n.d.	Well	Abattoir B
18	M	24	+	+	n.d.	Encephalitis, pneumonia	Abattoir A
19	M	39	−	+	+	Well	Abattoir A
20	M	58	+	+	+	Well	Abattoir B
21	M	39	+	+	n.d.	Well	Abattoir A
22	M	65	+	+	n.d.	Encephalitis	Abattoir A

* Died. Brain, kidney, lung positive for NV by immunohistochemistry; brain also positive for NV by PCR
n.d., Not done.

were negative. These 3 people were not considered to be infected with the NV.

Of the remaining 22 people, 20 (90·9%) had NV IgM antibodies (Table 2). Thirteen (65%) of the 20 were concurrently positive for NV IgG, 6 developed IgG, detected on subsequent bleeds collected 4 days to 3 months after the first, and 1 died, before the appearance of detectable IgG, 3 days after admission to hospital for encephalitis. The postmortem brain of this individual was positive for NV antigen and RNA by immunohistochemistry and RT–PCR respectively, and the kidney and lung for NV antigen by immunohistochemistry.

Two abattoir workers (cases nos. 9 and 19), asymptomatic at the time of the outbreak, were negative for NV IgM but positive for IgG on their first bleeds 6 and 7 days respectively after the onset of the outbreak. Neutralizing antibodies at a titre of 40 were

https://doi.org/10.1017/S0950268801006422 Published online by Cambridge University Press

detectable in both. Case no. 9 remained asymptomatic 1 month later but was thereafter lost to follow-up whereas case no. 19 continued to be well when questioned 5 months later.

The sera from the 21 Hendra virus IgG positive individuals were also positive for NV IgG. No Hendra virus IgG negative sample, on the other hand, was positive for NV IgG. In addition, of 926 follow-up samples, except for 32 that were obtained from the infected cases which tested NV IgG positive, the remaining 894 sera that tested negative for NV IgG were obtained from individuals who were Hendra/Nipah virus IgG negative on their previous specimens. None of the people who had contact with horses nor the 25 laboratory workers had positive serology to Hendra or Nipah virus.

All the antibody positive individuals were male. They had worked in 1 or both of the 2 abattoirs in Singapore, 14 in abattoir A, 7 in abattoir B and 1 in both abattoirs. The age range of the seropositive men was 24–65 years.

Twelve out of the 22 infected individuals (54·6%) were symptomatic. The remaining 45·4% were clinically well when the serum specimens were taken and had no past history of either neurological or respiratory compatible disease. Nine ill cases presented with neurological symptoms, 2 with respiratory symptoms and 1 with both neurological and respiratory symptoms. Ten of the symptomatic persons worked in abattoir A and 2 in abattoir B. With regard to asymptomatic antibody positive individuals, 4 were employed in abattoir A, 5 in abattoir B and 1 in both abattoirs.

DISCUSSION

Beyond the 11 clinically ill NV cases in Singapore that were reported earlier [3], additional NV-infected individuals have been detected by serological testing, resulting in a total of 22 infected persons. Of these, 12 (54·6%) were symptomatic; 9 presented with encephalitis, 2 with pneumonia and 1 with both encephalitis and pneumonia. Our data also show that 10 abattoir workers (45·4%) who were well had subclinical NV infection as determined by the presence of specific antibodies. Other studies to date have not documented asymptomatic NV infection. Our finding suggests that inapparent infections are relatively common and this justifies the monitoring of subjects at risk and heightened awareness among medical personnel of this possible aetiology in encephalitic patients.

These data continue to support what has been previously reported, that contact with infected pigs is the major risk factor for NV infection. All 22 infected individuals had direct association with pigs. With the exception of one who worked in both abattoirs, the rest of the infected people worked in one or the other of the two abattoirs in Singapore, contrary to previous findings [3, 7] where infected individuals were reported to be employed in only one specific abattoir. It was puzzling at that time that no infected human case was found in abattoir B, to which were sent, at the start of the outbreak, 4 of 100 pigs imported from Malaysia which were subsequently found to be antibody positive [1, 7]. Our study shows that seven infected cases worked in abattoir B, suggesting that this slaughter house was also receiving pigs which were infected and shedding NV.

Disregarding the person who worked in both abattoirs, 10 out of the 14 (71·4%) infected workers from abattoir A were ill compared to only 2 of 7 (28·6%) from abattoir B. Could this difference in the proportion of ill to well workers between the two abattoirs be preliminary evidence for virus strain variation? This is unlikely as pigs from NV-infected areas in Malaysia were sent to both abattoirs. Possibly, the increased use in abattoir B of plastic face shields that commenced some months before the outbreak [7] had reduced the exposure of the workers to the virus and possibly, this lower viral load, being more efficiently handled by the immune system, had resulted less frequently in overt disease.

Although other categories of people who had pig-related activities were screened, no others were found with NV antibodies. Direct contact with pig urine and faeces has been found to be significantly associated with NV infection [7]. This suggests that without contact with live pigs, exposure to infectious porcine body excretions was less and therefore, the meat inspectors, public butchers and customs inspectors investigated in this study were at reduced risk for infection. There is support for this from a study of 28 pork sellers in Malaysia [9] which shows handling uncooked pork to be associated with a low risk of NV transmission. Although further larger studies are needed, if substantiated, it will be reassuring to the public who handle and consume pork.

Besides pigs, NV infection has been described in other animals including horses in Malaysia [7]. Although none of the people in our study who worked

with horses and other animals in Singapore had serological evidence of NV infection, this does not exclude the possibility of transmission of NV to man by animals other than the pig. It seems likely that in Singapore these animals were not infected in the first instance. It does suggest, however, that horses were not involved in this outbreak in Singapore.

Further, this study shows no evidence of transmission from patients to the healthcare staff. None of the 228 healthcare workers who managed the NV patients had NV antibodies. Although two of the healthcare workers had low NV IgG optical density values with ELISA, they had no detectable neutralizing antibodies. The lack of family contacts in our survey restricts the conclusion that can be drawn with respect to human to human transmission. Notwithstanding the need to avoid a panic, had there been a case of NV infection among family members, they would, doubtless, have been screened. However, since no family contact of the infected workers is known, thus far, to have presented with NV-like illness, it may perhaps be inferred that even if there was familial spread, it had not caused apparent infection although, in the light of our findings, subclinical infection cannot be excluded.

No NV infection was detected among the laboratory staff who processed the 2449 specimens of blood, cerebrospinal fluid and tissues of infected patients in a biosafety level 2 (P2) laboratory. Specific hazards for this group included growing the virus unknowingly from the cerebrospinal fluid of an encephalitic case and performing the necropsy of the fatal case of NV infection.

Based on IgG and IgM antibody findings, it appears that at least three people in Singapore had been infected with the NV prior to the recognized outbreak that began on 13 March 1999. Two asymptomatic abattoir workers had no detectable NV IgM but had virus-specific IgG on their first blood specimens collected on 19 and 20 March 1999. The results of these IgG findings were confirmed by neutralization tests. Second blood specimens obtained after 5 weeks in one case and after 5 months in another were similarly positive for IgG. The third person (case no. 2) was admitted to hospital in Singapore for encephalitis on 14 February 1999. A blood sample obtained during his illness was retrospectively tested and IgM, IgG and neutralizing antibodies to NV were detected. The history of his exposure to pigs, as an auctioneer's assistant who helped to drive pigs into the auction ring, was elicited only upon investigating his circumstances after positive NV serology. These findings suggest that infected pigs were imported into Singapore before March 1999, which is possible since the NV outbreak had commenced in Malaysia as early as September 1998 [10], and the imported infected pigs might not have been noticeably sick and might have escaped recognition [7].

Because of the genetic similarity between Hendra and Nipah viruses, some questions are raised: what implications does subclinical infection have? Follow up of these patients will probably provide the answers. Will NV reactivate to cause an SSPE-like relapse? The latter was described in an Australian man infected with Hendra virus, who recovered from meningitis to die a year later of progressive encephalitis [11]. Although no relapse has been reported so far among the 21 Singaporean and 157 Malaysian survivors [4] of NV infection, continued monitoring is necessary, and is undertaken in Singapore, to establish if there are sequelae to either clinical or subclinical NV infection. At the same time, medical personnel should be alert to the possibility of a NV infection, symptomatic or asymptomatic, among those who work with live pigs. A retrospective study of patients with unexplained viral encephalitis during the months preceding March 1999 for NV infection is also indicated if there had been a history of contact with pigs.

ACKNOWLEDGEMENTS

We thank Ms Seng Eng Hong, Ms Lim Siew Hoon and staff of the Virus Laboratory for their invaluable technical assistance, record keeping and data collection, as well as Debbie Cannon, Linda Pezzanite, Kathy Veilleux, Thomas Stevens and Marty Monroe for their help during the serological testing of the samples at the CDC, Atlanta. We also thank Drs Hilda Loh, Charlene Fernandez and Paul Ananth Tambyah for assistance in tracing the occupational history of a patient, and the staff at the Communicable Disease Centre for co-ordinating the screening exercise.

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