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# Experimental Nipah Virus Infection in Pteropid Bats (*Pteropus poliocephalus*)

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## Summary

Seventeen grey-headed fruit bats (*Pteropus poliocephalus*) were inoculated subcutaneously with an isolate of Nipah virus derived from a fatally infected human. A control group of eight guinea-pigs was inoculated intraperitoneally with the same isolate in order to confirm virulence. Three of eight infected guinea-pigs developed clinical signs 7–9 days post-inoculation. Infected fruit bats developed a subclinical infection characterized by the transient presence of virus within selected viscera, episodic viral excretion and seroconversion. A range of histopathological changes was observed within the tissues of infected bats. Nipah virus was excreted in bat urine while neutralizing antibody was present in serum. This intermittent, low-level excretion of Nipah virus in the urine of bats may be sufficient to sustain the net reproductive value of the virus in a species where there is regular urine contamination of the fur, mutual grooming, and where urine droplets are a feature of the environment.

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*Keywords:* Nipah virus; experimental infection; fruit bat; guinea-pig

## Introduction

In early 1999 a major epidemic of viral encephalitis in people, and febrile respiratory and neurological illness in pigs, occurred in the states of Perak, Negeri Sembilan and Selangor within Peninsular Malaysia. Clinical laboratory (Chua *et al.*, 1999, 2000) and experimental (Middleton *et al.*, 2002) investigations confirmed that the common aetiological agent was a new paramyxovirus, subsequently named Nipah virus after the home village of the pig farmer from whom the virus was first isolated (Nor and Ong, 2000). Nipah virus and the antigenically and genetically related Hendra virus (HeV) are currently the sole members of a new genus *Henipavirus* within the family *Paramyxoviridae*.

There is a high prevalence of neutralizing antibodies to HeV in the serum of free-living Australian pteropid bat species including *Pteropus poliocephalus* (Field *et al.*, 2001) and HeV has been isolated from wild *P. poliocephalus* and *P. alecto* (Halpin *et al.*, 2000). Similarly, *P. poliocephalus* has been shown to be susceptible to experimental infection by HeV virus (Williamson *et al.*, 1998). Accordingly, initial wildlife surveillance for a reservoir of Nipah virus in Malaysia was directed at the diverse bat fauna of that country. Neutralizing antibodies to Nipah virus were detected in five fruit bat species, including the flying foxes *P. hypomelanus* and *P. vampyrus* (Johara *et al.*, 2001). Nipah virus has subsequently been isolated from the urine of wild roosting bats (*Pteropus* species) (Chua *et al.*, 2002; Reynes *et al.*, 2005), further strengthening the hypothesis that pteropid bats are wildlife reservoirs for these viruses.

The aims of the study described below were to determine the susceptibility of an Australian pteropid bat species to infection by Nipah virus, and to assess the pathogenesis and possible routes of transmission of Nipah virus in that species.

## Materials and Methods

### *Animals*

Adult or juvenile captive-bred, grey-headed flying foxes (*P. poliocephalus*) were used in this study. Members of the original colony, which had been isolated from

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free-living bats for several years, had been negative on repeated testing for the presence of antibodies to Hendra virus. Most recently, the residual members of the group had been maintained at Biosafety Level 3 for over 1 year and were considered to be free of Hendra virus. Guinea-pigs have previously been shown to be susceptible to Nipah virus infection and disease (P.E. Rollin; personal communication) and were included in the study to provide confirmation of the virulence of the Nipah virus inoculum. Seventeen grey-headed flying foxes (ten males) weighing between 325 and 800 g and eight female guinea-pigs (500 g each) were used.

*Animal Accommodation, Handling and Biosafety*

The animal husbandry and study design were endorsed by the CSIRO AAHL Animal Ethics Committee. Animals were housed in a single room at Biosafety Level 4. Room temperature was maintained at 22 °C with 15 air changes per hour; humidity varied between 40% and 60%. Staff wore fully encapsulating suits with breathing apparatus while in the animal room.

Bats were housed either individually (bats 1–6) or in pairs (bats 7–17) in squeeze-bottom cages within visual and auditory contact of each other. Where bats were housed in pairs they were matched so that similar sized animals were housed together and, where possible, comprised a male and female animal. The bats were fed fresh fruit daily coated in powdered milk supplement. Guinea-pigs were housed in pairs in similar cages and were given plastic pipe to hide in and shredded paper to burrow into. They were fed lucerne pellets and green leafy vegetables daily. Each species was given water *ad lib*.

Animals were chemically immobilized prior to inoculation and specimen collection. A mixture of ketamine HCl (Ketamil®; Ilium, Smithfield, Australia; 5 mg/kg) and medetomidine (Domitor®; Novartis, Pendle Hill, Australia; 50 μg/kg) was administered by intramuscular injection to the bats and reversed by intramuscular atimepazole (Antisedan®; Novartis; 50% medetomidine dose). Guinea-pigs were given ketamine HCl (Ketamil®; Ilium; 25 mg/kg) and rompun (Xylazil®; Ilium; 5 mg/kg) by intramuscular injection.

*Virus*

The Nipah virus used in these studies was a non-plaque-purified, low passage (third passage in Vero cells) isolate of Nipah virus obtained from the central nervous system of a fatally infected human.

*Experimental Design*

The experiment was undertaken in two parts (Table 1). In part 1, six bats and four guinea-pigs were involved. The results from this study guided the design of part 2 of the experiment. In total, seventeen bats (B1–17) were challenged by subcutaneous inoculation with 50,000 $TCID_{50}$ ($5 \times 10^4$/ml) of Nipah virus. Eight guinea-pigs (GP 1–8) were used as controls, and were inoculated with the same inoculum by the intraperitoneal route.

Clinical signs were assessed daily in bats and guinea-pigs. Bodyweight and rectal temperature of the bats were collected when bats were immobilized and sampled. Briefly, in part 1 of the experiment bats 1–6 were sampled every second day. On day 22 or 23 post-inoculation (PI) surviving animals were immobilized as above and then killed by cardiac exsanguination for tissue collection for virology, histology and immunohistochemistry. In part 2 of the experiment bats 7–17 were sampled on days 3, 5, 7, 10, and 12 PI with two bats killed as described above on each sampling day. The remaining bat (bat 17) and surviving guinea-pigs were killed on day 14.

**Table 1**
**Experimental design and sample collection times**

| *Animal numbers* | *Procedures* | *Post-mortem examination* |
|---|---|---|
| *Part 1 of the experiment* | | |
| Bats 1–6 | Samples collected on alternate days, for 21 days | Days 22 and 23 |
| Guinea-pigs 1–4 | Killed for post-mortem examination if clinical signs observed | If guinea-pig remained clinically well, killed for post-mortem examination on day 22 or 23 |
| *Part 2 of the experiment* | | |
| Bats 7 and 8 | | Day 3 |
| Bats 9 and 10 | Samples collected on day 3 | Day 5 |
| Bats 11 and 12 | Samples collected on days 3 and 5 | Day 7 |
| Bats 13 and 14 | Samples collected on days 3, 5 and 7 | Day 10 |
| Bats 15 and 16 | Samples collected on days 3, 5, 7 and 10 | Day 12 |
| Bat 17 | Samples collected on days 3, 5, 7, 10 and 12 | Day 14 |
| Guinea-pigs 5–8 | Killed for post-mortem examination if clinical signs observed | If guinea-pig remained clinically well, killed for post-mortem examination on day 14 |

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