ORIGINAL ARTICLE

Screening for Nipah Virus Infection in West Kalimantan Province, Indonesia

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Impacts

- Nipah virus is an emergent zoonotic pathogen whose natural host is Old World fruit bats, primarily those of the genus *Pteropus*.
- Serological evidence Nipah virus was detected in 19% of the 84 Large Flying-foxes (*Pteropus vampyrus*) from West Kalimantan, Borneo.
- No evidence of Nipah virus exposure was detected in 610 pigs from West Kalimantan, Borneo.

Keywords:
Nipah virus; *Henipavirus; Pteropus;* fruit bat; Kalimantan; Borneo

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Summary

Compared to other viruses, research on Nipah virus has been limited in Indonesia because attributable disease outbreaks have not been reported. However, Nipah virus is a zoonotic Biosafety Level 4 (BSL4) agent, so strategic monitoring is prudent. Farmer interviews and a serologic survey of 610 pig sera and 99 bat sera from West Kalimantan province were conducted. Farmers reported no recent or historic encephalitic or respiratory disease in themselves, their families, workers or pigs. The survey found no evidence of exposure to Nipah virus in pigs. In contrast, 19% of the 84 *Pteropus vampyrus* bat sera reacted in the ELISA, but none of 15 *Cynopterus brachyotis* bats reacted.

Introduction

Zoonotic pathogens are a major cause of emerging and re-emerging infections of humans (Woolhouse and Gowtage-Sequeria, 2005). Since the mid-1990s, lethal zoonotic pathogens, including Hendra and Nipah viruses, Menangle virus, Japanese encephalitis virus and Australian bat lyssavirus (Mackenzie et al., 2001; Reynes et al., 2005), have caused disease in a number of countries in Asia and Australasia. Nipah virus is of particular concern in Indonesia as it potentially threatens the Indonesian pig industry and human health, as graphically illustrated by the serious disease outbreak in adjacent Malaysia in 1999. In 2007, the Indonesian national pig herd was estimated at 6.8 million head, and an estimated 597 000 tonnes of pig meat were produced for human consumption (Food and Agriculture Organisation FAO, 2009).

Nipah virus disease is caused by Nipah virus (Family *Paramyxoviridae*, Genus *Henipavirus*). Nipah virus is an emerging zoonotic pathogen causing severe febrile encephalitis in humans (Chua et al., 2000). Fruit bats (Order *Chiroptera*, Family *Pteropodidae*) act as a reservoir host (Field et al., 2001), and pigs act as an amplifier host, transmitting the virus to other susceptible animals and humans (Nor et al., 2000). The fruit bats *Pteropus vampyrus* and *Pteropus hypomelanus* were shown to play an important role in the Nipah outbreak in pigs and humans in Malaysia in 1999 (Chua et al., 2000; Johara et al., 2001).

There are two apparent clinical presentations associated with Nipah virus infection – encephalitic and respiratory.

In animals, the respiratory type is more common (Hooper et al., 2001), while in humans, neurological disorder is the more common presentation (Wong et al. 2002). In Malaysia, Nipah virus killed 105 people, and more than a million pigs were culled to control the human epidemic and eradicate infection in the pig population (Chua et al., 2000; Nor et al., 2000). In addition to Malaysia, Nipah has also been reported in humans in Singapore (Chew et al., 2000; Chua et al., 2000), Bangladesh (Hsu et al., 2004) and India (Chadha et al., 2006). Serological studies in several Asian countries (including Malaysia, Bangladesh, Cambodia and Thailand) have found antibodies against Nipah virus in fruit bats (Daniels et al., 2007). Notably, illness or death in bats due to Nipah infection has not been reported (Daniels et al., 2007; Field et al., 2007; Middleton et al., 2007). Human-to-human transmission, not evident in Malaysia or Singapore, has been reported in Bangladesh (ICDDRB 2003; Hsu et al., 2004; ICDDRB, 2004a,b; WHO 2004; ICDDRB 2005).

In Indonesia, Nipah virus disease has not been reported in humans, bats or pigs, but encephalitic disease in humans does occur (Woeryadi and Soeroso, 1989; Kari et al., 2006). Thus, reports of Nipah virus infections in neighbouring countries, and the occurrence of human encephalitic disease in West Kalimantan (attributed to Japanese encephalitis virus) prompted surveillance for Nipah virus in West Kalimantan.

Serological surveys for Nipah virus have previously been conducted on pig farms in different areas in Indonesia (Sendow et al., 2008). This study reports testing for Nipah virus infection in pigs and bats in West Kalimantan Province, Borneo.

Materials and Methods

The study had three components: farmer interviews, a bat serological survey and a pig serological survey. Traditional small-holder farms in West Kalimantan province usually have fewer than 10 pigs and commercial pig farms typically have <500 pigs. All production is consumed locally or distributed within the immediate region. In small-holder farms, pigs are penned near to houses, usually in the backyard, close to fish ponds. Farmers may also have other species such as chickens, cattle or swallows (for nests). Reports of clinical cases of encephalitis and respiratory disease in pigs and humans were sought by interview. A non-random purposive sample of the owners of 75 farms and one abattoir was obtained. Those enrolled were interviewed regarding their own health, their family's health, the health of their employed workers and pig health records.

A non-random opportunistic sample of bat sera was collected from bats from a bat seller in Pontianak District (Fig. 1). The bats were reportedly from Pontianak District and Singkawang district (Table 1). A non-random convenience sample of pig sera was collected from pigs at abattoirs and pig farms (small-holder and commercial) from six districts (Table 2).

Fig. 1. Large flying-foxes (*Pteropus vampyrus*) in a cage for sale for human consumption in West Kalimantan Province.

Table 1. Nipah antibody detection in bats by ELISA test

				Results	
Sample location	Species	Gender	Sample size	Reactor (%)	Non-reactor (%)
Pontianak district	*Pteropus vampyrus*	M	53	12 (23)	41 (77)
		F	31	4 (13)	27 (87)
		Total	84	16 (19)	68 (81)
Singkawang district	*Cynopterus brachyotis*	M	11	–	11 (100)
		F	4	–	4 (100)
		Total	15		15 (100)

Table 2. Nipah antibody detection in pigs by ELISA

			Results	
Sample location	Gender	Sample size	Reactor (%)	Non-reactor (%)
Pontianak district	M	18	–	18 (100)
	F	68	–	68 (100)
Landak district		64	–	64 (100)
Sanggau district		74	–	74 (100)
Singkawang district		162	–	162 (100)
Sambas district	M	8	–	8 (100)
	F	92	–	92 (100)
Bengkayang district	M	28	–	28 (100)
	F	96	–	96 (100)
Total		610		610 (100)

All sera were tested by indirect Nipah virus ELISA following the procedure according to Anon (2004). The ELISA has a sensitivity of >70% and a specificity of 98.5% (Daniels et al., 2001). Testing was carried out at the Balitvet laboratory, Bogor, Indonesia, using reagents (including inactivated antigen) provided by the CSIRO Australian Animal Health Laboratory, Geelong, Australia (AAHL). Bat sera were subsequently forwarded to AAHL for further testing by serum neutralization (SN) test (Daniels et al., 2001).

Results

A total of 99 bat sera, comprising 84 *P. vampyrus* and 15 *Cynopterus brachyotis* sera, were tested by ELISA (Table 1). There were 16 *P. vampyrus* reactors (19%) and zero *C. brachyotis* reactors. Eighty-four sera (82 *P. vampyrus* and two *C. brachyotis*) were then sent to AAHL for SN tests (the balance had insufficient volume). Of the 82 *P. vampyrus*, five were assessed at AAHL as having insufficient volume for SN, and a further four sera produced toxic reaction at low dilutions, precluding a definitive result. Of the remaining 73 *P. vampyrus* sera, 30 (41%) neutralized Nipah virus. Neither of the 2 *C. brachyotis* sera neutralized Nipah virus. All sera that reacted on ELISA test gave a positive result in the SN test, with titres from 1 : 5 to 1 : 80. Fourteen sera that did not react on ELISA gave positive results on SN test, with titres from 1 : 5 to 1 : 20.

In the parallel pig survey, 610 pig sera were collected from six districts in West Kalimantan province (Table 2). Statistically, a sample of this size provides a 99% probability of detecting an antibody prevalence of <1%, notwithstanding the 75% test sensitivity (Daniels et al., 2001). The results showed that none of the 610 pigs had antibodies to Nipah virus. This finding is consistent with those of Sendow et al. (2008), where antibodies were not detected in pig populations from other parts of Indonesia (North Sumatra, West Sumatra, Riau, Jakarta and North Sulawesi).

Pig farmer interviews recorded no recent or historic observations of neurological and respiratory disease syndromes in pigs, in either small-holder farms or commercial farms. None of the farmers, workers and families interviewed reported encephalitis or serious respiratory disease. Coughing and sore throat, followed by rapid recovery, were commonly reported.

Discussion

Our detection of antibodies to Nipah virus in *P. vampyrus* (a widespread and common species in Indonesia) is consistent with findings in this, and other *Pteropus* species, across much of their global range. Seropositivity has been reported in Malaysia [*P. vampyrus* 17% and *P. hypomelanus* 30% (Johara et al., 2001)], Thailand [*Pteropus* spp. 9% (Wacharapluesadee et al., 2005)], India [*P. giganteus* 54% (Epstein et al., 2008)] Cambodia [*P. lylei* 12% (Reynes et al., 2005)] and Bangladesh [*P. giganteus* 5% (Hsu et al., 2004)]. This growing list reinforces the contention that the *Pteropus* genus of fruit bat is indeed a major reservoir of Nipah virus and suggests the possibility that related henipaviruses await discovery in bats in regions where *Pteropus* bats and related species remain unsurveyed. The absence of Nipah virus antibodies in the small sample of *C. brachyotis* in this study (95% CI 0–23%) is not inconsistent with findings of Johara et al. (Johara et al., 2001), who reported a 4% neutralizing antibody prevalence in *C. brachyotis* in Malaysia (95% CI 0–12%).

Lack of apparent clinical disease was reported following experimental infection of fruit bats with Hendra virus (Williamson et al., 1998, 2000), and supports the contention that *Pteropus* species and henipaviruses are well adapted and may have co-evolved (Field et al., 2001, 2007). The mechanism of Nipah virus transmission from fruit bats to other species is uncertain, although Chua et al. (Chua et al., 2002) isolated virus from fruit bat urine, and from fruit partly eaten by bats. Experimental studies support urine as a medium of virus transmission (Daniels et al., 2007).

The discordance between ELISA and SN results of the bat sera has several plausible explanations: the ELISA detects only IgG-class antibody, thus sera of recently infected bats (with circulating IgM only) would not react in the ELISA (the low neutralizing titres are consistent with this explanation); the SN may be detecting cross-neutralization from unidentified related henipaviruses that are not reacting in the ELISA (the low neutralizing titres are also consistent with this explanation); the ELISA test sensitivity is sub-optimal.

The absence of encephalitic cases in pigs and humans gives additional support for the negative serologic findings in pigs and provides a high level of confidence that the West Kalimantan pig population is free of Nipah virus infection. In the traditional small-holder farms in West Kalimantan, pigs roam freely and graze near the house during the day, and return to their pens in the afternoon. Growing fruit trees on small-holder farms is common. Those highly favoured by fruit bats (mango, durian, rambutan, duku/langsat, guava) were not present on the pig farms sampled. Plausibly, their presence on pig farms may increase the risk of transmission of Nipah virus from bats to pigs. On commercial farms, sanitation and management were generally good, with apparent limited likelihood of bat–pig transmission.

In Bangladesh, recent reports indicate that Nipah virus has transmitted from bats to humans, in contrast to previously recognized human cases in Malaysia and Singapore, where pigs were the source of human infection. While strain variation might account for this (Hsu et al., 2004), it is also plausible that identified human risk behaviours such as date palm juice collection and consumption may be unique to Bangladesh (Luby et al., 2006). Further, proximity of bat roosts to human habitation differs; in Bangladesh, bat roosts are commonly found in towns and villages, close to human housing, whereas in Indonesia, bats tend to roost in the forest far from human aggregations. Thus, the likelihood of contact between fruit bats and humans or pigs is more limited. Nonetheless, fruit bats are hunted and eaten in many parts of Southeast Asia and hence bat-human contact does occur. Also, given the evident strain variation in Bangladesh, nucleotide sequence of the virus(es) circulating in Indonesian bats is urgently needed to allow phylogenetic comparisons, and to ensure that screening tests being used in surveillance are sensitive and robust.

Notwithstanding the absence of *P. vampyrus* roost and feeding trees from surveyed pig farms, the potential for (albeit less frequent) contact between bats and pigs still exists. Wild boar (*Sus scrofa*) and bearded pigs (*Sus barbatus*) occur in forest areas of Borneo, although potential for contact between fruit bats and wild pigs has not been investigated. *P. vampyrus* and related species are nomadic and capable of covering large distances in their foraging movements. Fruit bats can fly at a speed of 25–30 km/h (sustainable for several hours) and frequently forage over distances of 60–70 km per night (Hall and Richards, 2000). Recent studies using satellite telemetry in Australia and Asia, including Indonesia (Breed et al., 2006) showed that *Pteropus* species can cover hundreds of kilometres in a period of weeks. One interviewed farmer reported that around 20 bats flew over the farm per evening during the major fruit season, while outside the fruiting season, fewer than eight bats were observed per evening. As the primary driver for fruit bat movements is available food resources (Hall and Richards, 2000), increased migration to areas where trees are fruiting is logical. If these trees are at pig farms, potentially infectious body fluids have an increased probability of contact with pigs, and a Nipah outbreak could plausibly ensue. As natural food resources become more scarce and fragmented, we can expect changing movement patterns with increased fruit bat activity around areas of human habitation (seeking horticultural crops). It is plausible that changed infection dynamics will accompany these changed population dynamics, and with that, the potential for increased risk of spillover events.

Conclusion

This study adds to the current body of knowledge of the prevalence and distribution of Nipah virus. Our results indicate that neither clinical signs of Nipah virus infection nor seroconversion has occurred in the pig populations sampled, and combined with previous negative findings in pigs in other parts of Indonesia, support the absence of Nipah virus infection in the Indonesian pig population. In contrast, the moderate seroprevalence identified in *P. vampyrus* in this study, along with our previous positive findings in fruit bats from other parts of Indonesia (Sumatra, Java, Sulawesi) (Sendow et al., 2008) confirms that Nipah virus infection is endemic in *P. vampyrus* in Indonesia. These findings, together with the extensive geographic occurrence of Nipah virus infection in *P. vampyrus* and other species across Asia, mean that on-going surveillance is required to detect indicative changes in infection dynamics in fruit bats, or the early introduction of infection to the pig population. Consideration should also be given to the implementation of preventive biosecurity measures to minimize the probability of bat-to-pig transmission and the risk of pig-to-pig transmission should spillover occur. These measures might include excluding potential fruit bat food trees or roost trees from pig farms, monitoring the transport of livestock and pigs from potential risk areas, and quarantine procedures for pigs being imported from risk areas.

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