The dynamic landscape of bat borne zoonotic viruses in Australia

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This review discusses the history, epidemiology, diagnostics, clinical presentation in humans, as well as control and prevention measures, of the high-profile viruses Hendra virus (HeV) and Australian bat lyssavirus (ABLV). Since the discovery of HeV and ABLV in the 1990s, these viruses have only caused disease in areas where spill-over hosts, including humans, encounter the reservoir host.

Bats

Australia is home to over 90 species of bats, covering many different habitats. All but eight species belong to the suborder *Microchiroptera* (microbats). See Table 1 for a list of the eight species from the suborder *Megachiropteran* (megabats) found on mainland Australia, four of which belong to the genus *Pteropus* (commonly called flying foxes or fruit bats). Figure 1 provides a link to an interactive map showing flying fox camps in Australia.

The distribution of bats in Australia has changed over time. As their habitats are destroyed, many have been forced to adapt to life on the urban fringe. There are many successful flying fox camps in the heart of large and smaller cities across Australia – Brisbane, Sydney, Melbourne, Geelong and Cairns to name a few. In the past 10 years, we have seen the southern limit of the black flying fox (*Pteropus alecto*) distribution extend further south, and the south-western limit of the grey headed flying fox (*Pteropus poliocephalus*) distribution extend across into South Australia as well. By contrast, the very small footprint of the spectacled flying fox (*Pteropus conspicillatus*) in far north Queensland, is predicted to get even smaller over time[1]. The black flying fox will most likely fill this void. The ecological drivers behind these changes are complex but are highly likely to include loss of natural habitat, changes to food availability and warming climates.

Hendra virus

Since it was first described in Australia in 1994, HeV has caused horse and human illness and deaths. A high prevalence of neutralizing antibodies to HeV in bats of the genus *Pteropus*, and the isolation of Hendra virus from the same genus, confirmed flying foxes as reservoir hosts for this virus[2]. All four species of pteropus bats can be infected (Table 1). From recent work it appears that the risk of a spill-over event is greatest when either the black flying fox or the spectacled flying-fox is present[3]. The reservoir host appears to co-exist with this virus in complete harmony. The virus spreads easily amongst flying-foxes with the HeV seroprevalence in flying-fox colonies fluctuating over time and geography. The theory of viral co-evolution with chiropteran hosts has been previously suggested, and all field observations and experimental evidence to date supports this hypothesis[4]. Figure 1 provides a link to the results of Hendra virus research conducted in Australia, as well as information for horse owners.

Figure 2 compares the routes of transmission for HeV and ABLV and other closely related bat viruses which result in human infection. For HeV, horses are the main spill-over host and serve as amplifying hosts, capable of infecting humans. The disease in horses exhibits seasonality with more spill-over events occurring in winter. Since it was discovered in 1994, only 95 horses have died to date. Horses in paddocks where flying foxes either roost or come to feed, are at risk of exposure to infection. Infection in horses most likely occurs after close contact with bat urine and birthing material which contain sufficiently high titres of virus to infect a horse[15].

Extreme care must be taken in the handling of samples collected for HeV diagnostic testing. HeV is a Biosafety level four (BSL4) agent, in

Table 1. Megachiropteran bats, all belonging to the family *Pteropodidae*, found on mainland Australia. One common name for each is listed, noting that some have several common names. The last two columns highlight whether evidence of infection with HeV or ABLV has been found in that species.

Family *Pteropodidae*	Genus	Species	Common name(s) include	HeV	ABLV
Subfamily *Pteropodinae*	*Dobsonia*	*Dobsonia magna*	Bare-backed Fruit Bat		
	Pteropus	*Pteropus alecto*	Black Flying-fox	✓	✓
		Pteropus conspicillatus	Spectacled Flying-fox	✓	✓
		Pteropus poliocephalus	Grey-headed Flying-fox	✓	✓
		Pteropus scapulatus	Little Red Flying-fox	✓	✓
Subfamily *Macroglossinae*	*Macroglossus*	*Macroglossus minimus*	Lesser Long-tongued Fruit Bat		
	Syconyteris	*Syconycteris australis*	Queensland Blossom Bat		
Subfamily *Nyctimeninae*	*Nyctimene*	*Nyctimene robinsoni*	Queensland Tube-nosed Bat		

Figure 1. Useful resources for further information. Underlined headings and icons are hyperlinked. If hyperlinks are not available, the following URLs can be used: ABLV Bat Stats, https://wildlifehealthaustralia.com.au/ProgramsProjects/BatHealthFocusGroup.aspx; Flying fox camp census data, https://www.environment.gov.au/webgis-framework/apps/ffc-wide/ffc-wide.jsf; Horse owner information, https://www.business.qld.gov.au/industries/farms-fishing-forestry/agriculture/livestock/horses/hendra-virus/reducing-risk; National Hendra Virus Research, https://www.agrifutures.com.au/wp-content/uploads/publications/16-001.pdf; Bat FAQ, https://www.health.nsw.gov.au/environment/factsheets/Pages/flying-foxes-questions.aspx.

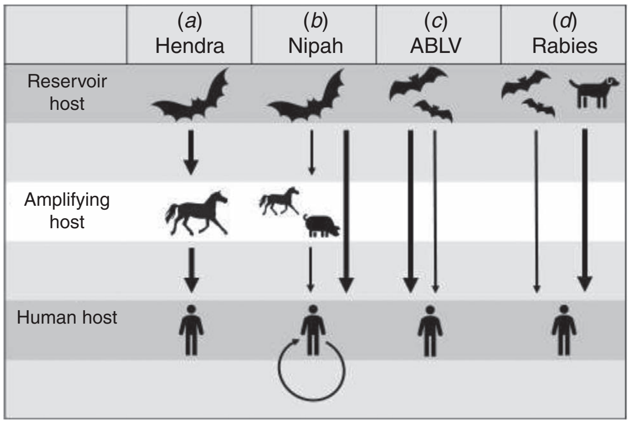

Figure 2. Known transmission pathways that result in human infection: the zoonotic transmission pathways of Hendra virus, Nipah virus, Australian bat lyssavirus and rabies virus: (a) Hendra virus: Pteropus bats are the reservoir host. Horses are the main spill-over host, and amplify the virus to very high titres, and succumb to clinical disease and death. From horses the virus can spread to humans if appropriate PPE and other precautions are not taken when handling infected horses and their secretions. Two apparently healthy dogs became infected after exposure to infected horses[5] and experimentally dogs have been shown to be susceptible but unlikely to spread the virus[6]. (b) Nipah virus: Pteropus bats are the reservoir host. Horses can be spill-over hosts and this was seen in one outbreak in the Philippines where humans became infected after eating infected horse meat[7]. The first outbreak of NiV in 1998 had pigs as the main spill-over amplifying hosts and humans involved in pig farming and pig slaughter in Malaysia and Singapore became infected from pigs[8]. In Bangladesh and India there have been almost annual outbreaks and most humans become infected by contact with Nipah virus contaminated date palm sap[9]. Human to human transmission is also seen[10]. While pteropus bats are suspected to be the source of human infection in the most recent outbreaks in Kerala, India, the source of exposure has not been identified[11]. (c) ABLV: Direct contact with infected bats has been the cause of all outbreaks to date, with only humans and horses presenting with clinical signs of infection. Horses have only been infected with a virus from microbats[12]. Two humans have been infected with virus from pteropus bats and one human has been infected from a microbat[13]. (d) Rabies: 99% of all human rabies infections arise from contact with rabid dogs[14]. Humans can also become infected directly from bats, or via another wildlife reservoir; however, these modes of transmission account for less than 1% of all human rabies cases.

recognition of its status as one of the most dangerous zoonotic agents. Safety precautions during field investigation and in the laboratory are of paramount importance. Blood collected in an EDTA tube, as well as tissue samples from lung, spleen and kidney can be tested by PCR, which is specific for Hendra virus. For the detection of antibodies to HeV in serum either a virus neutralisation test (VNT) or an ELISA can be conducted.

There have been seven known human cases, four of which were fatal. Clinical presentations ranged from self-limiting influenza-like illness, to severe pneumonia and encephalitis[16]. The typical incubation period in humans was 5–21 days, although one person experienced an initial aseptic meningitis, appeared to fully recover, but succumbed to severe encephalitis 13 months later. All human cases had high level exposure to infected horse secretions or tissues[16]. Human to human HeV transmission has not been described to date, unlike the closely related Nipah virus where human to human spread has been reported overseas[10].

In 2012 a vaccine was released for use in horses, to prevent infection with Hendra virus. This subunit vaccine based on the G glycoprotein of Hendra virus is very immunogenic and affords protection against HeV challenge in experimental infections[17]. Since the vaccine was released, no vaccinated horse has been diagnosed with Hendra virus infection. Vaccination of horses provides a public health and workplace health and safety benefit by reducing the risk of HeV transmission from horses to humans and other susceptible animals. Whenever HeV infection is suspected, even in vaccinated horses, appropriate biosecurity precautions, including personal protective equipment (PPE), should be used by all people in contact with sick horses.

ABLV

In 1996 a five-month-old female black flying fox was found under a fig tree in Wollongbar, NSW, unable to fly. From this bat, a virus with close serologic and genetic relationships to members of the *Lyssavirus* genus of the family *Rhabdoviridae* was isolated[18]. ABLV has since been found in all four flying fox species and in one species of microbat, the yellow-bellied sheath tailed bat[13]. It is assumed that all Australian bat species have the potential to carry and transmit ABLV. ABLV is transmitted to humans by bites or scratches from an infected bat.

No laboratory tests are currently available to diagnose ABLV in humans before the onset of clinical disease. In the early stages of disease, saliva and cerebrospinal fluid (CSF) can be tested by PCR. Antibody testing can also be performed on CSF. A positive serum antibody test is diagnostic of lyssavirus infection provided the person has never been immunised against rabies and may assist in the diagnosis of lyssavirus clinical disease. Any negative test on a symptomatic person is not definitive, as viral shedding in body secretions is intermittent and early tests may be negative for antibody. Therefore, repeat testing is often indicated.

For post mortem testing in humans and animals including bats, the standard diagnostic techniques include positive fluorescent antibody test (FAT) and PCR on fresh brain smears, and PCR from tissues.

ABLV infection has resulted in three human deaths, two adults and an eight-year-old child, in Queensland, Australia; 1996, 1998 and 2013[19]. Transmission from flying foxes and an insectivorous microbat were implicated, with all three cases displaying features of encephalitic (furious) rabies before their demise. The incubation period is thought to mirror rabies (usually 3–8 weeks, but potentially as short as a few days or as long as several years). Exposure through wounds close to the central nervous system on the head and neck or richly innervated areas like the fingers, carry an increased infection risk and may result in a shorter incubation period. In furious rabies, prodromal symptoms may precede sensorineural dysfunction, with progression to hyperactivity, aerophobia and/or hydrophobia, followed by convulsions[20]. The clinical course following symptom onset is usually rapid, almost invariably progressing to death within a few days.

Regarding prevention, the key strategy is for untrained and unvaccinated people to avoid handling bats. Public health authorities promote this message particularly during periods of high bat activity, including fruiting periods, and heat stress events when bats and especially pups drop to the ground. Prompt post-exposure vigorous wound cleaning, submission of the bat's brain for ABLV testing (where possible), rabies vaccination and administration of rabies immunoglobulin, are recommended following bat bites or scratches. Figure 1 provides a link to statistics on ABLV surveillance in Australia, as well as answers to questions about flying foxes and possible impacts on human health from NSW Department of Health.

Conclusions

ABLV and HeV can both cause an encephalitis syndrome in humans, sometimes with significant delay or recrudescence. Bats are the reservoirs of these viruses and may well be implicated in transmission of yet to be identified zoonotic pathogens. As the distribution of these reservoir hosts changes, so too does the risk of spill-over events that may involve humans.

Conflicts of interest

The authors declare no conflicts of interest.

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References

1. Martin, G. *et al*. (2018) Climate change could increase the geographic extent of Hendra virus spillover risk. *EcoHealth* **15**, 509–525. doi:10.1007/s10393-018-1322-9
2. Halpin, K. *et al*. (2000) Isolation of Hendra virus from pteropid bats: a natural reservoir of Hendra virus. *J. Gen. Virol.* **81**, 1927–1932. doi:10.1099/0022-1317-81-8-1927
3. Edson, D. *et al*. (2015) Routes of Hendra virus excretion in naturally-infected flying-foxes: implications for viral transmission and spillover risk. *PLoS One* **10**, e0140670. doi:10.1371/journal.pone.0140670
4. Halpin, K. *et al*. (2007) Emerging viruses: coming in on a wrinkled wing and a prayer. *Clin. Infect. Dis.* **44**, 711–717. doi:10.1086/511078
5. Kirkland, P.D. *et al*. (2015) Hendra virus infection in dog, Australia, 2013. *Emerg. Infect. Dis.* **21**, 2182–2185. doi:10.3201/eid2112.151324
6. Middleton, D.J. *et al*. (2017) Experimental Hendra virus infection of dogs: virus replication, shedding and potential for transmission. *Aust. Vet. J.* **95**, 10–18. doi:10.1111/avj.12552
7. Ching, P.K. *et al*. (2015) Outbreak of henipavirus infection, Philippines, 2014. *Emerg. Infect. Dis.* **21**, 328–331. doi:10.3201/eid2102.141433
8. Chua, K.B. *et al*. (2000) Nipah virus: a recently emergent deadly paramyxovirus. *Science* **288**, 1432–1435. doi:10.1126/science.288.5470.1432
9. Rahman, M.A. *et al*. (2012) Date palm sap linked to Nipah virus outbreak in Bangladesh, 2008. *Vector Borne Zoonotic Dis.* **12**, 65–72. doi:10.1089/vbz.2011.0656
10. Luby, S.P. *et al*. (2009) Recurrent zoonotic transmission of Nipah virus into humans, Bangladesh, 2001-2007. *Emerg. Infect. Dis.* **15**, 1229–1235. doi:10.3201/eid1508.081237
11. Yadav, P.D. *et al*. (2019) Nipah virus sequences from humans and bats during Nipah outbreak, Kerala, India, 2018. *Emerg. Infect. Dis.* **25**, 1003–1006. doi:10.3201/eid2505.181076
12. Annand, E.J. and Reid, P.A. (2014) Clinical review of two fatal equine cases of infection with the insectivorous bat strain of Australian bat lyssavirus. *Aust. Vet. J.* **92**, 324–332. doi:10.1111/avj.12227
13. Si, D. *et al*. (2016) Potential exposures to Australian bat lyssavirus notified in Queensland, Australia, 2009–2014. *PLoS Negl. Trop. Dis.* **10**, e0005227. doi:10.1371/journal.pntd.0005227
14. WHO (2013) Expert consultation on rabies second report. Geneva: WHO.
15. Halpin, K. *et al*. (2011) Pteropid bats are confirmed as the reservoir hosts of henipaviruses: a comprehensive experimental study of virus transmission. *Am. J. Trop. Med. Hyg.* **85**, 946–951. doi:10.4269/ajtmh.2011.10-0567
16. Playford, E.G. *et al*. (2010) Human Hendra virus encephalitis associated with equine outbreak, Australia, 2008. *Emerg. Infect. Dis.* **16**, 219–223. doi:10.3201/eid1602.090552
17. Middleton, D. *et al*. (2014) Hendra virus vaccine, a one health approach to protecting horse, human, and environmental health. *Emerg. Infect. Dis.* **20**, 372–379. doi:10.3201/eid2003.131159
18. Fraser, G.C. *et al*. (1996) Encephalitis caused by a Lyssavirus in fruit bats in Australia. *Emerg. Infect. Dis.* **2**(4), 327–331. doi:10.3201/eid0204.960408
19. Young, M.K. and McCall, B.J. (2010) Potential exposure to Australian bat lyssavirus in South East Queensland: what has changed in 12 years? *Commun. Dis. Intell.* **34**, 334–338.
20. Merritt, T. *et al*. (2018) Australian bat lyssavirus. *Aust. J. Gen. Pract* **47**, 93–96. doi:10.31128/AFP-08-17-4314

Biographies

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