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78) AUSTRALIAN BAT LYSSAVIRUS: OBSERVATIONS OF NATURAL AND EXPERIMENTAL INFECTION IN BATS

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Australian bat lyssavirus (ABLV) is a rabies-like virus that occurs as two virus variants (1) pteropid-ABLV, which affects the four common species of flying fox (*Pteropus alecto. P. poliocephalus, P. scapulatus and P. conspicillatus* and (2) ybst-ABLV that occurs in the insectivorous Yellow-bellied sheathtailed bat (YBST bat, *Saccolaimus flaviventris*). Both variants were first detected in 1996 and each variant has subsequently been diagnosed as the cause of single cases of fatal human encephalitis.

Between June 1996 and March 2002, surveillance of 1143 bats for ABLV by fluorescent antibody tests on fresh brain impression smears indicated that while ABLV is rare in whole bat populations (95% confidence <1%), it is common amongst sick, injured or orphaned *P. alecto*, *P. poliocephalus*, *P. scapulatus* and YBST bats. Multivariate analysis of data relating to wild megachiroptera (n=893) indicated that the prevalence of ABLV was associated with three factors: species, age and health status (clinical characterisation). No regional, temporal or seasonal association was found by analyses of epidemiological or phylogenetic (viral nucleoprotein sequence) data. ABLV was most prevalent in YBST bats (5 of 7, 71%) and *P. scapulatus* (21 of 124, 17%), common in *P. alecto* and *P. poliocephalus* (37 of 474, 8% and 8 of 175, 5% respectively) and least common in *P. conspiculatus* (1 of 95, 1%). One of 17 flying foxes not identified to species and none of 8 other fruit bats were ABLV-positive. It was significantly more common in sexually mature adults (>3 years) rather than juvenile flying foxes. The prevalence of ABLV was significantly higher (21%) amongst bats submitted with clinical signs suggesting central nervous system (CNS) disease (e.g. paralysis, paresis, overt aggression, seizures, cranial nerve deficits) than those with non-CNS signs (2%) and absent amongst clinically normal bats. Statistical analysis indicated that certain combinations of the three risk factors of species, health status and age gave rise to very high predicted prevalences for ABLV, e.g. the predicated prevalence of ABLV amongst adult *P. alecto* and *P. scapulatus* showing signs of CNS disease (of which 72 were submitted) were 35% and 59% respectively.

The range of clinical signs associated with naturally occurring ABLV were similar to those of rabies and included a most common ‘paretic’ form (unable to fly, progressive weakness) and a less common ‘furious’ form characterised by overt aggression towards other animals, humans and objects, including flying from trees to ‘attack’ humans and dogs. The incubation periods of only four natural cases of ABLV are known. Two bats developed clinical signs in captivity 36 to 57 days or 30 days after exposure to ABLV, and the two human patients became unwell 4 to 6 weeks and 27 months after being bitten by bats. The clinical duration of naturally occurring ABLV in bats is rarely observed in full. While most bats died or were humanely killed within 36 hours of being found, ten bats survived at least 3 (n=5), 5, 6 (n=2), 8 or 9 days while ill, during which time wildlife carers were at prolonged and repeated risk of exposure to ABLV.

It is clear that ABLV-infected bats commonly come into contact with humans and other animals. Consequently there is an ongoing risk of ABLV transmission to humans, their pets and wildlife. It should be noted that while YBST bats, flying foxes and humans are the only species in which ABLV infection has been diagnosed, the numbers of bats of other species, other wildlife and domestic animals that have been tested is too small to indicate an absence of ABLV in other animals. The use of animal rabies vaccines in Australia is restricted, limiting the management options for domestic animals. In the absence of any available or proposed mode of vaccine delivery for wild bats, there is no prospect of applying the effective wildlife vaccine strategies that have reduced the prevalence of terrestrial rabies in Europe and America. Consequently Australian ABLV management protocols rely on avoiding contact with bats, testing bats that bite or scratch people and pre- and post-exposure rabies virus vaccination of humans exposed to bats.

Experimental infection of 10 wild-caught *P. poliocephalus* with inocula prepared from the submandibular and sublingual salivary glands of a naturally infected *P. alecto* containing $10^{5.2}$ to $10^{5.5}$ $MICDED_{50}$ of pteropid-ABLV, resulted in clinical disease in 7 of 10 bats. Infection with ABLV was confirmed by FAT, immunoperoxidase staining of formalin fixed tissue, virus isolation in mice, and TaqMan PCR assay. The incubation periods were comparatively short (10 to 19 days), with clinical signs lasting 1 to 4 days prior to euthanasia or death. Five of the 7 cases were overtly aggressive towards humans, other bats and objects. One died during a seizure, while otherwise apparently well, approximately 1 hour after an uneventful recovery from anaesthesia, and the last became ataxic and moved incessantly and purposelessly about the cage. None developed the 'classic' calm progressive paresis described in most naturally occurring cases. The reasons for this are unclear.

Of the three that remained well until the completion of the trial on days 80 and 82, two showed no evidence of seroconversion in either rabies virus- or ABLV-based rapid fluorescent antibody tests (RFFITs). One developed a strong, apparently protective, neutralizing response between days 7 and 35 suggesting subclinical resolution of experimental ABLV infection. This response was most evident in pteropid- and ybst-ABLV-RFFITs rather than rabies-RFFITs and subsequent pteropid-ABLV-based testing of pre-inoculation serum (which had appeared seronegative in rabies-RFFITs) indicated a low but apparently biologically significant pre-existing (naturally acquired) ABLV titre. The apparent failure of cross-reactive rabies-RFFITs to detect low but functionally significant ABLV titres has implications for the interpretation of all serological studies for ABLV that use cross-reactive rabies virus rather than ABLV-based assays. All three 'survivors' were negative for pteropid-ABLV by FAT, immunoperoxidase staining of formalin fixed tissue, and virus isolation in mice.

In summary, ABLV has never been detected in a clinically well bat, is common in sick injured or orphaned bats (5-10%) and very common (20-30%) in bats showing signs of CNS disease. Other causes of CNS disease in wild flying foxes include spinal and head injuries, neuro-angiostrongylosis (involving *Angiostrongylus cantonensis,* a nematode) congenital hydrocephalus, bacterial meningoencephalitis and tick paralysis (*Ixodes holocyclus*). Mass spectrometry of formalin fixed livers showed no evidence of plumbism (lead poisoning) in bats with undiagnosed CNS signs or clinically well urban bats caught in Brisbane (n=50).Veterinarians and carers need to consider the possibility of ABLV infection for bats and potentially other animals in their care. In particular those with CNS signs should be submitted for ABLV testing.