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Australian bat lyssavirus infection in two horses

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ABSTRACT

In May 2013, the first cases of Australian bat lyssavirus infections in domestic animals were identified in Australia. Two horses (filly-H1 and gelding-H2) were infected with the Yellow-bellied sheathtail bat (YBST) variant of Australian bat lyssavirus (ABLV). The horses presented with neurological signs, pyrexia and progressing ataxia. Intra-cytoplasmic inclusion bodies (Negri bodies) were detected in some Purkinje neurons in haematoxylin and eosin (H&E) stained sections from the brain of one of the two infected horses (H2) by histological examination. A morphological diagnosis of sub-acute moderate non-suppurative, predominantly angiocentric, meningo-encephalomyelitis of viral aetiology was made. The presumptive diagnosis of ABLV infection was confirmed by the positive testing of the affected brain tissue from (H2) in a range of laboratory tests including fluorescent antibody test (FAT) and real-time PCR targeting the nucleocapsid (N) gene. Retrospective testing of the oral swab from (H1) in the real-time PCR also returned a positive result. The FAT and immunohistochemistry (IHC) revealed an abundance of ABLV antigen throughout the examined brain sections. ABLV was isolated from the brain (H2) and oral swab/saliva (H1) in the neuroblastoma cell line (MNA). Alignment of the genome sequence revealed a 97.7% identity with the YBST ABLV strain.

1. Introduction

Australian bat lyssavirus (ABLV) belongs to the genus *Lyssavirus* of the family *Rhabdoviridae* within the order *Mononegavirales* (ICTV, http://www.ictvonline.org/virus-Taxonomy.asp). The genus is represented by rabies virus (RABV, genotype 1) and comprises Lagos bat virus (LBV, genotype 2), Mokola virus (MOKV, genotype 3), Duvenhage bat virus (DUVV, genotype 4), European bat lyssavirus 1 and 2 (EBLV-1 and 2, genotypes 5 and 6, respectively), Australian bat lyssavirus (ABLV, genotype 7), Irkut bat virus (IRKV, genotype 8), Aravan bat virus (genotype 9), Khujand bat virus (KHUV, genotype 10), West Caucasian bat virus (WCBV, genotype 11) and Shimoni bat virus

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(SHIBV, yet to be classified). Based on sequence analysis, ABLV is related to other bat lyssaviruses but it is most closely related to classical rabies virus (Gould et al., 1998). Hence, the cross-protection against ABLV infection provided by currently used rabies vaccines (Fooks, 2004). ABLV causes similar neurological symptoms to rabies in humans (Allworth et al., 1996; Hanna et al., 2000; Samaratunga et al., 1998). ABLV was first described in a black flying fox (*Pteropus alecto*) in 1996 by Fraser et al. (1996); however, retrospective testing of archival brain tissues indicated that there was an earlier case in 1995. This variant was also detected in the grey-headed flying fox (*Pteropus poliocephalus*), the spectacled flying fox (*Pteropus conspicillatus*) and the little red flying fox (*Pteropus scapulatus*). Another variant of ABLV was later isolated from a species of insectivorous microbat, the Yellow-bellied sheath tail bat (YBST) (*Saccolaimus flaviventris*) (Gould et al., 2002; Hooper et al., 1997). Therefore, there are two distinct variants of ABLV affecting frugivorous and insectivorous bats, respectively.

Spill-over of bat lyssavirus infection have been previously reported and bat lyssaviruses are known to infect humans, domestic animals and wildlife (Fooks et al., 2003; Müller et al., 2004; Ronsholt, 2002). In 2009, Dacheux et al. (2009) suspected bat-to-cat transmissions of EBLV on two occasions, when EBLV-1 was recovered from 2 domestic cats in France. Similarly, (Müller et al., 2004) reported a lyssavirus infection in a stone marten in Germany. Furthermore, Brookes et al. (2007) reproduced the disease experimentally in sheep, and Cliquet et al. (2009) compared the susceptibility of foxes to EBLV type 1 and 2 using intracranial and peripheral (intramuscular) inoculation methods and demonstrated a low spill-over chance of EBLV from bat to fox with EBLV-1 having a greater probability to do so than EBLV-2. In a series of experiments, Vos et al. (2004a,b) infected ferrets and foxes with EBLV-1, however they showed that these species were unlikely to transmit the virus to another host. In the United States of America, it has been shown that spill-over events have occurred and have resulted in the permanent adaptation of bat lyssavirus variants in raccoons, skunks, foxes, coyotes, mongooses, groundhogs and bobcats (Krebs et al., 2000). Similar events have been reported in Africa, particularly in Southern Africa, with adaptation of bat lyssavirus variants in mongooses, jackals and bat-eared foxes (Bengis et al., 2004). In South America, there have been reports of transmission of bat lyssavirus from wildlife, especially vampire bats (Favoretto et al., 2013).

According to the recommendations of the World Health Organization (WHO) and the World Organization for Animal Health (OIE), the fluorescent antibody test (FAT) is the method of choice for the identification of lyssavirus in the brain stem (thalamus, pons and medulla). Although FAT is the gold standard assay for the identification of lyssavirus in tissues, in recent years advances in molecular diagnostics have seen wide use of conventional and real-time RT-PCR for the diagnosis of lyssavirus infections (Banyard et al., 2009; Black et al., 2002; Echevarría et al., 2001; Foord et al., 2006; Smith et al., 2002; Vázquez-Morón et al., 2006; Wakeley et al., 2005).

Until recently, the only cases of lyssavirus infection recorded in Australia were in bats and humans. However, in May 2013 two horses were diagnosed with ABLV and are the first confirmed Australian field infections. We describe these 2 cases and discuss the relevance of these findings to the management of ABLV infections in domestic animals in Australia.

2. Materials and methods

2.1. Clinical presentation

2.1.1. Horse 1 (H1)

On May 5, 2013, a female yearling presented with pyrexia, subtle neurological signs and progressive ataxia. The attending equine practitioner submitted blood (EDTA), nasal, rectal and oral swabs to the Biosecurity Sciences Laboratory (BSL) for Hendra virus (HeV) testing. However, the horse deteriorated and it was euthanized, and no further samples were taken. Samples were tested the same day and were negative. As a differential diagnosis, the submitting veterinarian requested tests for flavivirus infection which were negative.

2.1.2. Horse 2 (H2)

Within four days of H1 becoming symptomatic, a male yearling from the same paddock presented with similar neurological signs. Samples were submitted for HeV, equine herpesvirus type 1 and flavivirus testing; all samples were negative. H2 deteriorated over the following 3 days and was euthanized. On advice of the duty pathologist, the veterinarian performed a necropsy and submitted samples preserved in 10% neutral buffered formalin and unpreserved samples from the central nervous system to BSL for testing. The preserved samples included small pieces of cerebral cortex, cerebellum, brain stem and spinal cord. The rest of the brain and cerebrospinal fluid were unpreserved. The unpreserved brain was stored at −20 °C after being sub-sampled for further testing soon after its delivery to the laboratory; therefore, further histological examination was not possible.

2.2. Histopathology

Central nervous system specimens including small portions of cerebral cortex, cerebellum, brain stem and cervical spinal cord from horse 2 were fixed in 10% neutral buffered formalin, and embedded in paraffin. Sections were cut at 4 μm thickness using a microtome (Microm HM325, Thermo), stained with haematoxylin and eosin (H&E) and examined.

2.3. Immunohistochemistry (IHC)

Additional sections for IHC were cut onto positively charged slides and placed in a 40 °C incubator to dry overnight. Following 10 min incubation in a 60 °C oven, the slides were dewaxed and brought to water.

Antigen retrieval was induced through heat treatment by placing the sections in EnVision Flex Target Retrieval Solution, High pH (Dako, Denmark) for 30 min at 97 °C