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Sunshine virus in Australian pythons

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ABSTRACT

Sunshine virus is a recently discovered novel paramyxovirus that is associated with illness in snakes. It does not phylogenetically cluster within either of the two currently accepted paramyxoviral subfamilies. It is therefore only distantly related to the only other known genus of reptilian paramyxoviruses, *Ferlavirus*, which clusters within the *Paramyxovirinae* subfamily. Clinical and diagnostic aspects associated with Sunshine virus are as yet undescribed. The objective of this paper was to report the clinical presentation, virus isolation, PCR testing and pathology associated with Sunshine virus infection. Clinical records and samples from naturally occurring cases were obtained from two captive snake collections and the archives of a veterinary diagnostic laboratory. The clinical signs that are associated with Sunshine virus infection are localised to the neurorespiratory systems or are non-specific (e.g. lethargy, inappetence). Out of 15 snakes that were infected with Sunshine virus (detected in any organ by either virus isolation or PCR), the virus was isolated from four out of ten (4/10) sampled brains, 3/10 sampled lungs and 2/7 pooled samples of kidney and liver. In these same 15 snakes, PCR was able to successfully detect Sunshine virus in fresh-frozen brain (11/11), kidney (7/8), lung (8/11) and liver (5/8); and various formalin-fixed paraffin-embedded tissues (7/8). During a natural outbreak of Sunshine virus in a collection of 32 snakes, the virus could be detected in five out of 39 combined oral-cloacal swabs that were collected from 23 of these snakes over a 105 day period. All snakes that were infected with Sunshine virus were negative for reovirus and ferlavirus by PCR. Snakes infected with Sunshine virus reliably exhibited hindbrain white matter spongiosis and gliosis with extension to the surrounding grey matter and neuronal necrosis evident in severe cases. Five out of eight infected snakes also exhibited mild bronchointerstitial pneumonia. Infection with Sunshine virus should be considered by veterinarians investigating disease outbreaks in snakes, particularly those that are associated with neurorespiratory disease.

1. Introduction

A range of pathogenic viruses have been detected in snakes throughout the world and for overviews of these viruses and their associated diseases, the interested reader is directed to the excellent reviews by Wellehan and Johnson (2005), Jacobson (2007) and Marschang (2011). Of the viruses that have been reported in snakes, paramyxoviruses are particularly important since disease outbreaks of significant morbidity and mortality have been detected in Europe, USA and Brazil (Folsch and Leloup, 1976; Jacobson et al., 1992; Kolesnikovas et al., 2006). Prior to the discovery of Sunshine virus, all phylogenetically characterised reptilian paramyxoviruses had clustered within the recently accepted paramyxoviral genus, *Ferlavirus* (Marschang et al., 2009; ICTV, 2012). Australian native species (e.g. *Morelia* sp.) are present in herpetological collections all over the world, and have been described as

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being afflicted with as yet poorly described, possibly paramyxoviral neurological disease (Boyer et al., 2000; Jacobson, 2005). In contrast to the rapidly expanding knowledge of snake virology that exists elsewhere in the world, snake virology in Australia remains in its infancy.

For many years, Australian snakes, especially pythons from eastern Australia, have presented to veterinarians with neurological and/or respiratory disease (Rose et al., 2005). The diagnostic tests available to these practitioners to investigate infectious aetiologies have been limited and the cause of disease for many animals has remained elusive. Information is limited to only a few reports containing limited information.

Reovirus particles have been identified by electron microscopy in the brains of Australian snakes with neurological dysfunction (Rose et al., 2005).

In 1998, Carlisle-Nowak et al. reported on inclusion body disease (IBD) in two Australian pythons. Diagnosis was based on clinical signs and histopathological findings that were consistent with IBD.

Evidence for the presence of ferlaviruses in Australia is tenuous. Serology from some captive snakes has been positive for ferlaviruses but the details of testing are not provided (Rose et al., 2005). Histopathology consistent with ferlaviruses has been briefly described in snakes within Australia (Sullivan, 2005). However, there are no reports concerning the isolation, visualisation by electron microscopy, or molecular detection by PCR, of ferlavirus in an Australian snake.

In 2008, an outbreak of neurorespiratory disease occurred in a collection of 70 native Australian python species from the Sunshine Coast of Queensland, Australia (approximately 100 km north of Brisbane). The entire collection was euthanased and samples from 17 of these snakes were opportunistically retrieved by the attending veterinarian for virus isolation. A syncytium-forming virus was isolated using viper heart cells (VH2) but a range of PCR primers for the detection of ferlaviruses (genus-specific), paramyxoviruses (family- and subfamily-specific) and reoviruses failed to identify this isolate (Hyndman et al., 2012). Biochemical testing of this isolate provided largely equivocal results due to the low viral titre ($TCID_{50} = 10^{2.75}\,mL^{-1}$). As such, any haemagglutinating and/or neuraminidase activity of this isolate could not be determined. Illumina® high-throughput sequencing revealed this new virus to be a novel paramyxovirus (GenBank accession number: JN192445) that was named Sunshine virus after the geographical origin of this first isolate. Phylogenetic analysis supported the assignment of Sunshine virus as a member of the family *Paramyxoviridae* but as being distinct from the two existing subfamilies: *Paramyxovirinae* and *Pneumovirinae*. The divergence between the attachment protein sequences of Sunshine virus and other paramyxoviruses, did not allow the nature of the Sunshine virus attachment protein (H, HN or G) to be determined by molecular methods.

This report expands the knowledge of Australian and international snake virology by describing the clinical signs, gross pathology, histological findings and the results of PCR testing associated with Sunshine virus infection.

2. Materials and methods

2.1. Sample collection

Samples that were analysed in this investigation came from three sources: two Australian captive collections and the archives of an Australian veterinary diagnostic laboratory.

2.2. Collection 1

In 2008, all the snakes (70 native Australian pythons from the genera *Aspidites*, *Morelia* and *Antaresia*) in a private collection were humanely euthanased in response to an outbreak of neurorespiratory disease. During the outbreak, but before destocking, two snakes died and a further 14 were displaying signs of neurorespiratory disease (further historical details of the events leading up to destocking are presented in Supplementary Fig. 1). In total, freshly frozen samples from 17 livers, kidneys and lungs, 16 brains and 13 serum samples were collected from 17 snakes and submitted to Murdoch University for virus isolation and PCR testing. Snakes were selected for sample collection based on clinical signs and/or which snakes they had been in direct contact with.

2.3. Collection 2

In 2011, sporadic cases of neurological and other non-specific signs of disease occurred in a collection of 32 snakes (20 Australian pythons from the genera *Morelia* and *Antaresia*; four exotic boas; and eight Australian elapids). Cloacal and oral swabs were opportunistically sampled on multiple occasions from 23 of these snakes. In addition, from one snake that was euthanased, fresh samples of brain, liver, kidney and lung were collected. Samples were submitted to Murdoch University for PCR testing.

None of the eight venomous snakes from this collection (*Pseudechis*, the black snakes; *Oxyuranus*, the taipans; *Acanthophis*, the death adders; and *Notechis*, the tiger snakes) were showing overt signs of ill-health and for safety reasons, these snakes were not sampled.

For combined oral-cloacal swabs, a cotton-tipped applicator was pre-moistened in isotonic saline (or Hartmann's solution) and then the inside of the mouth (especially the glottis) and the cloaca were swabbed. Oral-only and cloacal-only swabs were also taken from a subset of the snakes. All swab tips were broken off into sterile containers, submerged in isotonic saline (or Hartmann's solution) and then sent to Murdoch University for PCR testing. For all snakes that were PCR tested for Sunshine virus using swab samples, the combined oral-cloacal swab was tested first. If a snake tested positive, and individual swabs were available, the individual swabs were then tested to determine whether the oral-only and/or the cloacal-only swabs were positive.

2.4. Veterinary Diagnostic Laboratory

Archives of the Berrimah Veterinary Laboratories (BVL, Northern Territory, Australia) were searched for snake