

Sunshinevirus in Australian snakes

Fact sheet

July 2020

Introductory statement

In 2008, an outbreak of neuro-respiratory disease occurred in a collection of Australian pythons in southeast Queensland, Australia. A novel virus was discovered during an investigation into this outbreak, at that time named Sunshine virus ^[1]. This was the first report of an infection by this novel virus in Australia or worldwide. The virus has since been detected in captive pythons from all Australian states and territories, except Western Australia. Following the formal classification of this virus, it is now known as sunshinevirus (one word, no capital letter).

Aetiology

Sunshinevirus was named after the origin of the first isolates – the Sunshine Coast of Queensland. It was originally identified as a paramyxovirus ^[1], however, formal reorganisation of the paramyxovirus family in 2016 resulted in the classification of sunshinevirus into the newly created family, *Sunviridae* ^[2]. Sunshinevirus is distantly related to the genus *Ferlavirus*, which contains all the known paramyxoviruses of reptiles¹.

Natural hosts

Most Australian python species commonly kept in private and zoological collections are known to be susceptible to sunshinevirus infection and it should be assumed, unless proven otherwise, that all Australian python species are susceptible. Sunshinevirus infection has also been detected in ball pythons (*Python regius*) and boa constrictors (*Boa constrictor*) [not Australian species]. This host range is likely to expand as further testing is performed. There is no information about age- or sex-linked predispositions to infection .

World distribution

Sunshinevirus has been detected in Germany in ball pythons ^[3] and in Thailand in boa constrictors ^[4]. There are no other published reports of sunshinevirus infection outside of Australia.

¹ See Wildlife Health Australia fact sheet: “Exotic - Ferlavirus (previously OPMV) in Australian reptiles”

Occurrences in Australia

Naturally acquired infections of sunshinevirus have been identified in captive pythons from all Australian states and territories, except Western Australia². To date, it has not been detected in wild Australian snakes (but only one small study on this exists).

Epidemiology

Infectious virus has been detected in oral-cloacal swabs and blood, and experimental infection was achieved by inoculation of virus into the trachea or the body cavity (intracoelomic) ^[5]. Therefore, potential routes of horizontal transmission include faecal-oral and aerosols, as well as inoculation directly into the circulatory system (perhaps by haematophagous ectoparasites). There is evidence that sunshinevirus can be transmitted vertically although this may be a self-limiting route of infection as all infected embryos were dead ^[6].

Shedding of the virus can occur for several months from asymptomatic individuals. This means that it should not be assumed that even extended quarantine periods (of many months) will reveal the clinical signs of disease in snakes infected with sunshinevirus. Serial sampling in quarantine is recommended to help prevent the entry of this virus into a collection. The initial outbreak in Queensland was associated with significant morbidity and some mortality. Experimentally inoculated pythons started to shed virus within days of inoculation, but did not show obvious clinical signs of disease for several months ^[5].

Clinical signs

Clinical signs appear to be related primarily to the central nervous and respiratory systems. A number of clinical signs have been reported:

- loss or reduction of righting response
- opisthotonos and torticollis
- spasticity
- tremors
- incoordination
- disorientation
- caudal flaccidity
- mouth gaping
- nasal discharge
- dermatitis
- non-specific signs such as anorexia, weight loss.

² A controlled infection study of carpet pythons with sunshinevirus was performed in Western Australia. At the time of writing, there were no known cases of sunshinevirus in a Western Australian python outside of this infection study.

Diagnosis

Diagnostic testing³

- PCR testing utilising a combined oral-cloacal swab is available through Dr Tim Hyndman at Murdoch University, Perth, Western Australia
 - Gross pathological findings are largely unremarkable and usually limited to mild or moderate pulmonary congestion and oedema
- Histopathology – most of the pathology is seen in the hindbrain and the cranial spinal cord. It is vital that the brain is submitted for examination. Be extremely careful to prevent artefact (e.g. crush artefact) in this very important but very fragile sample (see Sampling Protocol).

Histopathology

- mild to severe spongiosis of the white matter of the hindbrain and, in a minority of cases, the white matter tracts of the midbrain or the parenchyma of the cerebellum
- neuronal chromatolysis or necrosis can be evident in the hindbrain
- mild to marked gliosis, composed of both astrocytosis and microgliosis, generally accompanies the spongiosis
- severely affected areas contained necrotic cell debris and low numbers of gitter cells, primarily located in the meninges and surrounding parenchymal blood vessels ^[7]

Detailed information on the pathology of sunshinevirus infection is available from or Murdoch University (above) or the Australian Registry of Wildlife Health (www.arwh.org).

Differential diagnoses

Differential diagnoses include infectious agents and toxins that can cause respiratory or neurological signs, or chronic debilitation. For Australian pythons with neurological signs of disease, bornavirus is an important viral differential diagnosis. For cases with respiratory signs of disease, nidovirus and mycoplasma should be considered. Arenavirus (genus: *Reptarenavirus*) has been associated with neurological and respiratory signs of disease but in Australia, this virus appears to be uncommon and has required multiple sample types from multiple time points to diagnose.

It is very important to consider the possibility of mixed infections. It should not be assumed that the cause of disease is a single infectious agent.

Laboratory diagnostic specimens for PCR

- a combined oral-cloacal swab
- fresh samples of brain, lung, liver and kidney
- archival formalin-fixed paraffin-embedded tissues

³ This fact sheet should be a guide only. Individual institutions should make their own judgments regarding testing procedures using the latest information. Before testing, it is important that plans are in place to deal with the issue of positive animals.

- sample quality is often poor, so negative results are less informative than positive results.

Sampling protocol

- Combined oral-cloacal swab
 - moisten a cotton-tipped sterile swab with sterile saline. Rub the swab around the mouth paying particular attention to the glottis. If possible, advance the swab down into the trachea before sampling the rest of the mouth. Using the same swab, advance the swab through the vent and swab the cloaca and colon. Note that the opening to the colon is ventral.
 - break off the swab-tip into a ~5 ml sterile container (e.g. plain blood tube or yellow screw-capped tube) and add ~1-2 ml of sterile saline or Hartmann's solution. Do not use swabs with stainless steel shafts and do not use tubes with internal threads (i.e. where the lid screws into the inside of the tube).
- fresh tissue samples
 - small samples (~10 mm x 10 mm x 10 mm) of (in order of preference) brain, kidney, lung and liver can be collected into small sterile containers, and like the swabs, immersed in sterile saline. Pooling these samples is preferred to submitting them separately unless testing will be performed on individual tissue samples. If individual tissue samples will be tested, the samples should be collected into separate containers, with sterile instruments being used for each tissue sample. As an alternative to using multiple sets of sterile instruments, a pair of disposable scalpel blades can be used for each tissue: one blade "spears" the tissue, while the other is used to cut off a section of speared tissue. The two blades are then discarded, and two new sterile scalpel blades are used for the next tissue.

To sample the brain, use a fine-tipped set of bone cutters and very carefully cut around the caudal margin of the skull. The surrounding jaw bones will need to be cut. This will then allow the skull to be hinged forward (cranially) to reveal the brain. Remove and then freeze a ~10 mm x 10 mm x 10 mm section from one side of the brain for molecular testing. Fix the rest of the brain (while still in the skull) in formalin and submit the fixed brain (while still in the skull) to your pathologist.

Detailed information on laboratory diagnostic specimens required for diagnosis of sunshinevirus infection is available from the Australian Registry of Wildlife Health (www.arwh.org) or Murdoch University (above).

Laboratory procedures

PCR testing, gross pathology and histopathology.

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Treatment

There is no treatment.

The prognosis in infected animals is poor, however, the interval between infection and disease can be many months and in some cases, well over a year.

Prevention and control⁴

It is recommended that sampling in quarantine be performed to help prevent the entry of this disease into a collection. Persistent shedding of the virus for several months has been detected from infected (mostly asymptomatic) snakes in private collections and under experimental conditions. Because of the persistence of viral shedding, a negative or positive PCR result is likely to be a reliable indicator of an animal's infection status. However, if there have been recent opportunities for introduction of virus to a collection (e.g. new snakes brought in, or a snake loaned out for breeding has returned to the collection), then two samples, spaced at least six weeks apart, while an animal is in quarantine, may be required. In all situations, if an equivocal result is obtained, a second test at least six weeks after the first, is suggested.

Being an enveloped virus, it is expected that sunshinevirus is labile outside of the host and susceptible to commonly used cleaners and disinfectants. Detection of traces of viral RNA (as distinct from whole infectious virus) remaining in the environment from excreted materials such as faeces and urine have the potential to cause a misleading positive PCR result in an uninfected python introduced to such a contaminated environment. Disinfection with bleach can reduce viral RNA below the limits of detection by PCR. Household bleach diluted to 10% (equivalent to 0.4% hypochlorite), freshly made up, should be left on surfaces for 30 minutes. More heavily contaminated surfaces require two treatments. Surfaces must be rinsed afterwards to render them safe for animal contact.

If sunshinevirus is diagnosed in a collection, the measures taken to control the virus are influenced by the purpose and size of the collection:

- owners of private collections with only a few snakes are encouraged to “close” their collections by ceasing the movements of animals into and out of their collection. It is essential that hygiene is maintained fastidiously to minimise the spread of the virus between animals within the collection and to animals in other collections. Ideally, collections should only be “opened” when the threat of viral spread has ceased. For example, six months since the death of an infected animal and more than one negative test result on the surviving animals, would provide a level of confidence that the collection was free of the virus.
- owners of larger collections should balance the value of the individual animal against the value of the entire collection. Euthanasia of infected animals should be seriously considered. All other snakes should be tested for the presence of the virus. It is known that some infected

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animals are asymptomatic and so clinical signs should not be used a surrogate marker for infection. Two PCR-negative results should be obtained from each animal, evenly spaced at least six weeks apart, in the six months since the time of the most recent diagnosis or death (whichever comes last). The comments concerning “closing” collections mentioned above, equally apply to these larger collections.

Surveillance and management

Wildlife disease surveillance in Australia is coordinated by Wildlife Health Australia. The National Wildlife Health Information System (eWHIS) captures information from a variety of sources including Australian government agencies, zoo and wildlife parks, wildlife carers, universities and members of the public. Coordinators in each of Australia's States and Territories report monthly on significant wildlife cases identified in their jurisdictions. NOTE: access to information contained within the National Wildlife Health Information System dataset is by application. See the WHA website for more information: <https://wildlifehealthaustralia.com.au/ProgramsProjects/eWHIS-WildlifeHealthInformationSystem.aspx>

We are interested in hearing from anyone with information on this condition in Australia, including laboratory reports, historical datasets or survey results that could be added to the National Wildlife Health Information System. Negative data are also valuable. If you can help, please contact us at admin@wildlifehealthaustralia.com.au.

Only a single small study has tested wild Australian pythons for sunshinevirus. In this small study of Western Australian wild pythons, sunshinevirus was not detected (T Hyndman pers. com. July 2020). Whether wild Australian snakes (not just pythons) can become infected with sunshinevirus is unknown. Cases where there is suspicion of sunshinevirus in wild snakes should be reported as part of Australia's general wildlife health surveillance system.

Statistics

Limited information is available in the National Wildlife Health Surveillance Database (eWHIS – See www.wildlifehealthaustralia.com.au). We are interested in laboratory confirmed reports of sunshinevirus in wild Australian snakes. Please contact admin@wildlifehealthaustralia.com.au or your local WHA Wildlife Coordinator.

Research

Research is needed to clarify many aspects of this disease. Further investigations are required on the routes of natural infection of virus, and on the possibility of fomites (objects) being a means of horizontal transmission. Further investigations are also required to determine whether vertical transmission always results in non-viable embryos. PCR testing for sunshinevirus is currently being performed at Murdoch University, Perth.

Human health implications

There are no reports of sunshinevirus in humans.

Conclusions

Most Australian python species commonly kept in private and zoological collections are known to be susceptible to sunshinevirus infection. However, the status of Australia's wild snakes with respect to sunshinevirus is unknown. Further work in this area, including assessment of the risk to Australian wild snakes is recommended.

Acknowledgements

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References and other information

1. Hyndman TH, Marschang RE, Wellehan JFX, Jr., and Nicholls PK (2012) Isolation and molecular identification of Sunshine virus, a novel paramyxovirus found in Australian snakes. *Infection, Genetics and Evolution*, **12**(7): 1436-1446.
2. International Committee on the Taxonomy of Viruses. Virus Taxonomy: 2018b Release. Accessed 2018; July 2018:[]
3. Marschang RE, Stöhr AC, Aqrawi T, Hyndman TH, Plenz B, Blahak S, and Pees M (2013) First detection of Sunshine virus in pythons (*Python regius*) in Europe. *Proceedings of the Association of Reptilian and Amphibian Veterinarians Conference*: 15.
4. Kongmakee P (2014) Pathology and Molecular Diagnosis of Paramyxovirus Infection in Boidae and Pythonidae in Thailand. thesis Faculty of Veterinary Science, Chulalongkorn University: Bangkok, Thailand.
5. Wesson J, Shilton C, O'Dea M, Currie A, and Hyndman T (2019) Tips on sunshinevirus infection in pythons; Clinically important principles for diagnosis and management of sunshinevirus infection from an experimental infection study in Australian pythons in Australian Veterinary Association Annual Conference, 5-10 May: Perth
6. Hyndman TH and Johnson RSP (2015) Evidence for the vertical transmission of Sunshine virus. *Veterinary Microbiology*, **175**(2-4): 179-184.
7. Hyndman TH, Shilton CM, Doneley RJT, and Nicholls PK (2012) Sunshine virus in Australian pythons. *Veterinary Microbiology*, **161**(1-2): 77-87.

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