Reptarenavirus and inclusion body disease in Australian snakes

Fact Sheet

May 2024

Key points

- Boid inclusion body disease (IBD) is a disease of snakes (boas and pythons), caused by reptarenavirus, which occurs globally.
- Clinical signs are generally neurological and the disease is untreatable and is progressive and fatal once snakes show signs.
- Both inclusion body disease and reptarenavirus have been reported in Australian in captive snake populations but not in free-ranging snakes.
- Inclusion body disease should be included on the differential diagnosis list for any python or boa displaying neurological signs.
- Reptarenavirus has been detected in apparently healthy snakes, and it is unclear what factors cause IBD to develop following infection.

Aetiology

Boid inclusion body disease (IBD) in boas and pythons is caused by infection with *Reptarenavirus*, a viral genus within the family *Arenaviridae* [1]. They are enveloped single-stranded RNA viruses [2].

Inclusion body disease can cause significant disease in a range of snake species and has been reported since the 1970s [3-7]. A viral aetiology was confirmed in 2012 [4, 8, 9]. Prior to this, diagnosis was based on histological changes, including the typical formation of eosinophilic intracytoplasmic inclusion bodies in a range of epithelial cells (cells which line hollow organs such as those in the kidney, lungs, gastrointestinal tract and blood vessels).

One Health implications

Wildlife and the environment: IBD may cause deaths and increased management costs in captive snake populations (due to quarantine and diagnostic testing). If present in wild snake populations, IBD may threaten conservation and biodiversity [8, 10].

Domestic animals and humans: there is no evidence that *Reptarenavirus* can infect taxa other than snakes.

Natural hosts

Inclusion body disease can cause disease in a range of snake species but is most frequently reported as a disease of the family *Boidae* (boas and pythons) [3-7]. It has been detected mainly in captive populations. Diseases resembling IBD have been described in non-boids including corn snakes (*Pantherophis guttatus*), palm vipers (*Bothriechis marchi*) and an eastern kingsnake (*Lampropeltis getulus*) [11-14].

World distribution and occurrences in Australia

Reptarenavirus has been detected in snakes globally [4, 8, 9, 15]. It has been found in captive pythons throughout Australia, but not in wild snakes. In Costa Rica, *Reptarenavirus* has been reported in free-ranging constrictors, with studies suggesting both the disease (IBD) and the virus have been present in the wild since the 1980s [10].

Inclusion body disease has been reported in many countries including Germany, Belgium, Spain and South Africa [16-19]. Cases of IBD in Australia have been reported anecdotally, in pathology databases and in published literature, however all reported cases date to before the confirmation of a viral cause, and are based on typical histopathological changes [20-23]. No new cases have been reported since 2007.

Epidemiology

The first reports of inclusion body disease can be traced back to the 1970s, but the identification of an aetiological agent, reptarenavirus, did not occur until 2012 [4, 8, 9, 24].

The route of transmission of IBD is unknown but likely involves direct contact. Several routes have been suggested, including transmission by aerosol, by blood-feeding snake mites (*Ophionyssus natricis)*, bites during fighting and consumption of infected mice or bats [4, 25-27]. Mites are often present in snake collections with IBD or *Reptarenavirus*. The presence of the virus in free-ranging constrictors in Costa Rica suggests that constrictor species may play a role in circulating the disease, or may be an original source of the virus [10].

The incubation period and factors that drive the transition from infection to development of inclusion bodies in host cells and clinical disease remains unknown [5, 24]. Earlier research on IBD suggested the disease was always fatal, but since the detection of *Reptarenviruses* there have been instances where seemingly healthy snakes have tested positive for the virus but shown no signs of IBD [3, 4, 15, 18, 28, 29]. Studies in boas have demonstrated intranuclear inclusion bodies (indicative of the virus) and high viral loads without clinical signs over a two-year study period [30]. Other studies have shown development of disease over several years [31]. It is not known what percentage of snakes infected with *Reptarenaviruses* go on to develop signs of IBD [24, 32]. Coinfections with multiple reptarenavirus genotypes, and with other viruses, have been reported [33]. The genetic diversity of the *Reptarenaviruses* may influence development of disease. It has been suggested that the high genetic diversity of the virus may be the result of the high volume of global trade in boas and pythons [34]. It is thought that vertical transmission (passage of virus from mother to young) and

development of host immune tolerance to the virus may play a role in the transition of infection to disease [33].

There have been numerous reports of cancer in snakes with concurrent IBD, with inclusions seen either within the tumour, in epithelial organs or in the peripheral circulation [35-37]. The significance of these findings remain uncertain, however there is no current evidence that neoplasms are more likely in those infected with reptarenavirus [37].

Clinical signs

Inclusion body disease can present as a progressive and chronic disease in both pythons and boas, and is frequently fatal [3, 6]. Pythons generally succumb to illness faster than boas. Clinical signs include a range of neurological and systemic signs [3].

In **boas**, IBD is a slowly progressive disease with some individuals surviving for months or even years, with supportive care. Initial signs include regurgitation followed by anorexia, weight loss and lethargy. Terminally, snakes develop neurological signs such as a head tremor, incoordination, opisthotonus (hyperextension of the head and/or neck) and a loss of the righting reflex.

In **pythons**, neurological signs appear to be more pronounced and progress over a few weeks. Signs are similar to those seen terminally in boas and include a flaccid ascending paralysis. Regurgitation does not occur, but anorexia, weight loss and lethargy do.

Affected snakes commonly develop secondary diseases such as mouth rot, pneumonia and dermatitis, as well as other co-occurring conditions [32].

Diagnosis

Next generation sequencing and PCR are the most sensitive diagnostic for *Reptarenaviruses* [38]. PCR can be used to detect the virus in tissue samples, oral/cloacal swabs and blood [6]. Detection of virus in the live snake is possible by screening oesophageal swabs and whole blood, or tissue biopsies including liver and oesophageal tonsils [15, 30]. There may be a species differences in the most sensitive samples for virus detection, with oesophageal swabs preferred for boas, and blood preferred for pythons [6, 15, 29]. Detection of virus in the live snake allows early identification of positive animals to reduce transmission into a collection and possibly prior to development of a disease state.

Inclusion body disease should be included on the differential diagnosis list for any python or boa displaying neurological signs. Prior to molecular techniques, the diagnosis of IBD was confirmed by the presence of inclusion bodies in epithelial cells examined by microscopy of brain tissue, liver, blood smears and other internal organs including kidney, pancreas, gastrointestinal and lung [6, 24]. Microscopic examination of biopsies of the oesophageal tonsils, liver and kidney may be a diagnostic option for live patients, but may have limited sensitivity due to the number and distribution of inclusion bodies. Boas are more likely to have inclusions in these organs than pythons, which often have inclusions limited to their brain [6]. Inclusions in peripheral blood cells may be more readily detected in infected boas than pythons [31]. An absence of inclusions (on tissues or blood smears) does not exclude a diagnosis of reptarenavirus or IBD [7, 24]. There are

several other conditions which may lead to the formation of inclusions and could create false positive results [28]. Infection can occur without signs of disease, with many healthy animals testing positive for reptarenavirus [28, 29]. Individuals have also returned negative results on serial testing despite previous positive results [4, 15, 29]. This complicates the interpretation of a positive (or negative) result.

Laboratory diagnostic specimens and procedures

Heparinised and whole clotted blood should be collected from live snakes for haematology and PCR testing. The serum and blood cells can be frozen and stored for PCR testing. In dead snakes a complete range of tissues should be collected in formalin and retained as fresh frozen samples, especially brain tissue in pythons.

- PCR testing for reptarenavirus on serum and blood cells +/- oral/cloacal swab or tissues as available. PCR testing is available at Murdoch University, in Perth, WA through Dr Tim Hyndman
- Tissues should be stained with haematoxylin and eosin and checked microscopically for inclusion bodies
- Electron microscopy can be used to examine the inclusions, which are non-viral but contain an antigenically distinct protein [5].

Clinical pathology

Affected snakes often develop a marked leucocytosis early in the course of the disease, due to lymphocytosis. In advanced stages, lymphoid depletion may occur resulting in a marked leucopaenia. Inclusion bodies may be visible within circulating lymphocytes [32]. The primary pathological finding is the presence of eosinophilic cytoplasmic inclusion bodies in epithelial tissues with notable absence of inflammation [3, 16, 24]. The characteristic inclusions have been noted to occur in heterophils, lymphocytes and other leukocytes but are not present in all cases [3, 24].

Pathology

There are no gross lesions definitive for IBD or reptarenavirus infection.

In **boas**, eosinophilic intracytoplasmic inclusion bodies are found most commonly in mucosal epithelial cells adjacent to the oesophageal tonsils, lymphoid cells in the oesophageal tonsils, epithelial cells lining the gastrointestinal tract, renal tubular epithelial cells, pancreatic acinar cells and hepatocytes, but can be found in almost any epithelial organ [3, 24].

Pythons have intracytoplasmic inclusions within neurons in the central nervous system generally associated with a non-suppurative meningoencephalitis with perivascular cuffing and neuronal degeneration [3, 24].

Other lesions include exocrine pancreatic atrophy and necrosis, hepatocyte degeneration, renal tubular necrosis, and lymphoid depletion (more frequent in boas) [12].

Differential diagnoses

Because clinical signs of IBD are variable, at least initially, the list of differential diagnoses is long and includes bacterial infections, parasitic infections, other viral agents, trauma and exposure to toxins. Inclusion body disease and reptarenavirus should be included on a potential differential diagnosis list for any pythons or boids with neurological signs.

Treatment, prevention and control

Inclusion body disease can present as a progressive and chronic disease in both pythons and boas, and is frequently fatal [3, 6]. There is no treatment for IBD or reptarenavirus and mortality is 100% once clinical signs of IBD are apparent.

It is difficult to make recommendations regarding prevention and control as the mode of transmission and incubation period of reptarenavirus are unknown. Appropriate quarantine and biosecurity for snakes coming into a collection includes new arrival isolation and pre-export and quarantine screening. In addition, screening of existing individuals within a collection can help to ascertain the disease status of the population. Early identification of *Reptarenavirus*-positive individuals within a collection will help to reduce economic and population impacts. Snakes should only be sourced from collections with no history of IBD or *Reptarenavirus* infection. Good husbandry, including treatment and control of mite infections is important for prevention of IBD infection in a snake collection. As the virus is enveloped it should be inactivated by disinfectants such as sodium hypochlorite and chlorhexidine [32].

Research

Research is ongoing with the areas requiring the most clarification including transmission, pathogenesis and taxonomy. More research into the pathogenesis is required to determine what facilitates development of the IBD disease state following infection with *reptarenavirus* [15].

Surveillance and management

Wildlife Health Australia administers Australia's general wildlife health surveillance system, in partnership with government and non-government agencies. Wildlife health data is collected into a national database, the electronic Wildlife Health Information System (eWHIS). Information is reported by a variety of sources including government agencies, zoo based wildlife hospitals, sentinel veterinary clinics, universities, wildlife rehabilitators, and a range of other organisations and individuals. Targeted surveillance data is also collected by WHA. See the WHA website for more information: https://wildlifehealthaustralia.com.au/Our-Work/Surveillance and https://wildlifehealthaustralia.com.au/Our-Work/Surveillance/eWHIS-Wildlife-Health-Information-System. There is no targeted surveillance program for IBD.

Cases of IBD reported in the National Wildlife Health Surveillance Database are all from captive snakes and include Centralian carpet pythons (*Morelia bredli*), carpet pythons (*M. spilota variegata*), diamond pythons (*M. spilota spilota*), black-headed python (*Aspidites melanocephalus*), and green python (*Chondropython viridis*) from NSW and SA.

WHA 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. If you can help, please contact us at admin@wildlifehealthaustralia.com.au.

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Wildlife Health Australia recognises the Traditional Custodians of Country throughout Australia. We respectfully acknowledge Aboriginal and Torres Strait Islander peoples' continuing connection to land, sea, wildlife and community. We pay our respects to them and their cultures, and to their Elders past and present.

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