Wildlife Health Australia

Cryptosporidium infection in wild reptiles in Australia

Fact sheet

Introductory statement

Cryptosporidium spp. are protozoal parasites which were first confirmed to infect reptiles in 1977 (Brownstein et al. 1977). Since then they have been identified as a cause of predominantly gastrointestinal disease in a wide range of reptile species, mostly snakes and lizards. Prevalence of *Cryptosporidium* spp. infection in wild Australian reptiles is not known.

Aetiology

Cryptosporidium spp. (Apicomplexa: Cryptosporidiidae) are small, 4-8 µm protozoa that infect the gastrointestinal and occasionally, respiratory and biliary tract of a wide variety of vertebrates, including humans (Upton et al. 1989). Currently over 30 *Cryptosporidium* species have been recognised (Šlapeta 2017).The two most common species that infect reptiles are *Cryptosporidium serpentis* and *C. saurophilum* [synonymous with *C. varanii* (Pavlasek and Ryan 2008)], both of which affect snakes and lizards (Xiao et al. 2004). A third species, *C. ducismarci*, which was identified in chelonians with intestinal disease, was recently proposed (Traversa 2010). *Cryptosporidium parvum* and *C. muris* are mammalian cryptosporidia that may be found in reptiles after consuming infected prey. They are not infective for reptiles but represent a potential zoonotic risk (Zahedi et al. 2016).

Natural hosts

The genus *Cryptosporidium* has been identified in numerous hosts, including mammals, birds, reptiles and fish (O'Donoghue 1995). The first confirmed reptilian cases of *Cryptosporidium* sp. infection were reported in 1977 in 14 snakes from three genera and four species (Brownstein et al. 1977). Since then, *Cryptosporidium* infections have been recorded in over 57 reptile species including 40 snake species, 15 lizard species and two species of tortoise (O'Donoghue 1995). There are also reports of infection in a single captive Nile crocodile (*Crocodylus niloticus*) in Egypt (Jacobson 2007) and wild green turtles (*Chelonia mydas*) in Hawaii (Graczyk et al. 1997).

World distribution

Widely distributed (Japan, Africa, North America, South America, Europe, Australia) (Upton et al. 1989; Carmel and Groves 1993; Xiao et al. 2004; Alves et al. 2005; Kuroki et al. 2008).

An extensive survey of 528 wild and captive reptiles from Namibia, Madagascar, Peru and the US found a *3%* prevalence of infection with *Cryptosporidium* (Upton et al. 1989).

Occurrences in Australia

Cryptosporidium spp. infection occurs in captive reptiles in Australia (McKenzie et al. 1978; Carmel and Groves 1993; Xiao et al. 2004; Ladds 2009). Infection has been reported in a range of native snake species, in the captive setting (see Surveillance, management and statistics).

There is a single published case of *Cryptosporidium* infection in a wild reptile, a frilled lizard (*Chlamydosaurus kingii*) that was found dead in the Northern Territory (Oros et al. 1998).

Epidemiology

The mode of transmission in reptiles is by the faecal-oral route, by direct contact between animals, or through contact with contaminated objects (Cranfield et al. 1999; Graczyk and Cranfield 2000). Once ingested, the parasite undergoes asexual and sexual reproduction within the host cell cytoplasm along the mucosal brush border of the stomach or intestine. The oocysts sporulate *in situ*, resulting in continuous self-infection (Rose 2007). The prepatent period is 2-14 days (O'Donoghue 1995).

Only a small number of *Cryptosporidium*-exposed reptiles become clinically ill. Reptiles with concurrent disease are more likely to develop clinical cryptosporidiosis (Cranfield et al. 1999).

A chronic carrier state exists in reptiles. *Cryptosporidium* spp. infections do not appear to be self-limiting and chronic shedding of organisms occurs (Cranfield et al. 1999; Graczyk and Cranfield 2000).

Unlike mammals and birds, the disease appears to affect animals of all ages (Cranfield et al. 1999).

Once clinical signs of cryptosporidiosis occur, snakes usually die or are euthanased for humane reasons (Carmel and Groves 1993).

Clinical signs

Acute cases in infected snakes show regurgitation and diarrhoea. Chronic cases show regurgitation, anorexia and weight loss. Regurgitation occurs one to three days after feeding. There may be a firm mid-body swelling associated with hypertrophic gastritis (Brownstein et al. 1977; Szabo and Moore 1984; Carmel and Groves 1993). The clinical course is protracted and almost always fatal (Brownstein et al. 1977; Boylan et al. 1985; Carmel and Groves 1993).

Clinical signs in lizards and chelonians include anorexia, diarrhoea, weight loss and lethargy (Cranfield et al. 1999).

Diagnosis

Diagnosis is based on clinical signs, direct examination of faecal or regurgitated material, histopathology, direct fluorescence antibody testing and PCR (Cranfield et al. 1999; Šlapeta 2017; Šlapeta et al. 2018).

Clinically affected reptiles tend to shed oocysts in the faeces intermittently (Carmel and Groves 1993; Cranfield et al. 1999). Repeated faecal testing is required in suspect cases.

Clinical pathology

- Direct faecal examination. Direct and concentrated faecal smears are used to identify *Cryptosporidium* oocysts by light microscopy at a 400x magnification after modified Ziehl-Neelsen staining (Henriksen and Pohlenz 1981; Alves et al. 2005). The sensitivity of faecal staining tests is increased through serial testing and centrifugation techniques that concentrate the oocysts (Rose 2007).
- DNA sequencing. Species and genotype determined by nested PCR (Alves et al. 2005; Šlapeta 2017).
- Histopathological analysis (Kuroki et al. 2008). Tissue fixed in 10% buffered neutral formalin, dehydrated, and embedded in paraffin. Histological sections are cut and stained with a modified acid fast stain for examination by light microscopy (Cranfield et al. 1999).
- Direct fluorescence antibody testing for *Cryptosporidium* of humans commonly recognises antigen on reptile parasites (Šlapeta et al. 2018).

Pathology

- Gross lesions - an increase in the diameter of the stomach with a decrease in luminal diameter occurs in snakes (Cranfield et al. 1999; Graczyk and Cranfield 2000). The gastric mucosa may be oedematous, with mucosal thickening and exaggeration of the normal longitudinal rugae to which copious amounts of mucous is adhered (Cranfield et al. 1999; Graczyk and Cranfield 2000; Kuroki et al. 2008). Mucosal petechiae, brush haemorrhages and focal necrosis are common (Brownstein et al. 1977). In cases of pathogenic enteritis, seen more commonly in lizards, inflammation of the bowel and loose contents are seen throughout the gastrointestinal tract (Cranfield et al. 1999; Graczyk and Cranfield 2000; Ladds 2009).
- Histology/ microbiology - in snakes, histological lesions vary with the severity of the disease but usually range from mild, with little architectural change and low numbers of organisms on the surface of the brush border, to severe, with loss of brush border, flattening of epithelial cells, and proliferation of gastric mucous cells (Cranfield et al. 1999; Graczyk and Cranfield 2000; Ladds 2009). Intestinal lesions in lizards, chelonians, and (rarely) snakes consist of heterophil, lymphocyte, and macrophage infiltration. More than 80% of the intestinal cells may harbour parasites (Cranfield et al. 1999; Graczyk and Cranfield 2000; Jacobson 2007).
  Cryptosporidia have occasionally been found in extra-intestinal locations. Renal cryptosporidiosis was reported in a green iguana (*Iguana iguana*) and Parson's chameleon (*Calumma parsonii cristifer*). *Cryptosporidium* was identified in a hyperplastic salivary gland in a green iguana. Pedunculated masses protruding into the oral cavity and ear canal of green iguanas have also been associated with *Cryptosporidium* infection (Jacobson 2007).

Differential diagnoses

- Regurgitation due to husbandry related issues such as low ambient temperature and overhandling,
- Neoplasia, metazoan parasitism, viral gastroenteritis and abscessation.

Laboratory diagnostic specimens

- Fresh faecal specimens stored in 2.5% potassium dichromate at 4°C until required.
- Tissues collected at post mortem from infected reptiles, fixed in 10% buffered formalin, embedded in paraffin blocks and the resulting sections stained with haematoxylin and eosin and modified acid-fast stain and examined by light microscopy.
- Tissue for scanning electron microscopy, fixed in 2.5% glutaraldehyde, post-fixed in 1% osmic acid, and dehydrated through a series of graded acetone solutions.

Laboratory procedures

Techniques for direct faecal examination for *Cryptosporidium* spp., *Cryptosporidium* oocyst isolation and purification and PCR based identification have been described (Rose 2007; Šlapeta 2017).

Treatment

There is no commercially available effective treatment for *Cryptosporidium* spp. infection in reptiles. Hyperimmune bovine colostrum has been used with qualified success (Graczyk et al. 1998; Graczyk et al. 1999). Paromomycin has also been used with mixed results (Gibbons 2014).

Prevention and control

Serious consideration should be given to euthanasia of infected snakes in the event of an outbreak for reasons of animal welfare, cost of control measures, supportive care and quarantine facilities, and the extensive reorganisation of husbandry procedures that may be required (Carmel and Groves 1993).

If appropriate precautions are instigated, together with a *Cryptosporidium* screening program and adequate quarantine procedures (eight weeks in this particular study), then cryptosporidiosis in a captive snake colony can be successfully contained (Carmel and Groves 1993).

Adoption of a number of simple control procedures can limit the spread of an outbreak of cryptosporidiosis. Scrubbing of contaminated surfaces and the prompt removal and appropriate disposal of contaminated wastes will remove reservoirs of parasites thereby reducing the risk of spread of infection. The provision of separate cleaning equipment for each enclosure decreases the risk of cross-transmission of cryptosporidiosis and of other pathogens (Carmel and Groves 1993).

The inherent resistance of *Cryptosporidium* oocysts to many disinfectants, including bleach and povidone-iodine, restricts the choice of disinfectant for cleaning enclosures (Angus et al. 1982; Campbell et al. 1982). Hydrogen peroxide, which is relatively non-toxic, is lethal to oocysts after exposure at room temperature for 10 minutes and is not inhibited by the presence of organic matter (Blewett 1989; Carmel and Groves 1993). Ammonia and formal saline solution are effective in eliminating oocyst infectivity after 18 hours contact at 4°C. Infectivity of oocysts is neutralised by exposure to moist heat between 45°C and 60°C for five to nine minutes (Cranfield et al. 1999).

Surveillance, management and statistics

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. Please contact admin@wildlifehealthaustralia.com.au.

There is one case of *Cryptosporidium* spp. infection (in a captive coastal taipan (*Oxyuranus scutellatus*) recorded in the National Wildlife Health Surveillance Database (www.wildlifehealthaustralia.com.au). The Australian Registry of Wildlife Pathology Database lists the cases in captive Australian species of reptiles (inland taipan, *O. microlepidotus,* tiger snake, *Notechis scutatus, c*ommon eastern brown snake, *Pseudonaja textilis*, red-bellied black snake, *Pseudechis porphyriacus,* black-headed python, *Aspidites melanocephalus*).

Research

More studies need to be undertaken on the incidence of *Cryptosporidium* spp. infection in free living reptiles in Australia.

Human health implications

According to current knowledge, reptile cryptosporidia do not infect humans or other mammals (Cranfield et al. 1999). However, a recent report found *C. serpentis* in dairy calves in China. The authors were able to infect mice but not rabbits or chickens with this particular isolate (Chen and Qiu 2012). *Cryptosporidium parvum* (usually obtained from infected prey items) can pass through the digestive tract of reptiles without causing illness but poses a significant zoonotic risk to humans (Cranfield et al. 1999).

Conclusions

Cryptosporidiosis in reptiles is a chronic, debilitating, and usually fatal disease. Only a small number of *Cryptosporidium*-exposed reptiles develop clinical disease (Cranfield et al. 1999). It appears that a contributing factor is concurrent disease. Surveillance of captive and free-living reptiles for *Cryptosporidium* spp. is important, as are strict quarantine procedures when dealing with suspect cases in both groups.

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To provide feedback on this fact sheet

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

Wildlife Health Australia would be very grateful for any feedback on this fact sheet. Please provide detailed comments or suggestions to admin@wildlifehealthaustralia.com.au. We would also like to hear from you if you have a particular area of expertise and would like to produce a fact sheet (or sheets) for the network (or update current sheets). A small amount of funding is available to facilitate this.

Disclaimer

reason of any person relying on information in this fact sheet. Persons should accordingly make and rely on their own assessments and enquiries to verify the accuracy of the information provided.

Wildlife Health Australia

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