

Disseminated coccidiosis in green turtles Fact Sheet March 2024

Key points

- Coccidiosis in green turtles (*Chelonia mydas*) is a neurological and gastrointestinal disease caused by coccidian parasites morphologically similar to *Caryospora cheloniae*.
- Outbreaks in Australian green turtles, although sporadic, can result in mass mortality.
- The first outbreak in wild green turtles occurred in 1991 and affected large subadult animals in Moreton Bay, south-east Qld. Since then, sporadic outbreaks have been reported in Qld and NSW.
- Outbreaks appear to be related to drought conditions and subsequent algal blooms.

Aetiology

Caryospora cheloniae was thought to be the causative agent of systemic coccidiosis in green turtles and is a coccidian protozoan in the Phylum Apicomplexa, Family *Eimeriidae*. This species is included in the genus *Caryospora* on the basis of sporocyst morphology; its endogenous development and structure could be quite different from other *Caryospora* spp. ^[1]. Recent molecular work suggests the protozoa identified in coccidiosis outbreaks in green turtles do not all fall within one species, but are morphologically similar to *Caryospora cheloniae* ^[2-4]. As a result, the coccidia that cause disease in green turtles are often reported as *'Caryospora*-like' organisms (CLOs) ^[5].

The CLOs causing disease in green turtles are grouped by genotype. Genotypes 1 and 2 were first identified in turtles in Qld, followed by genotypes 3 and 4 in NSW ^[2, 3]. Genotypes 1, 3 and 4 are typically recovered from brain, gastrointestinal tract and lung tissue, and genotype 2 is found in the kidney and thyroid. Several other *Caryospora*-like genotypes have been described in green turtles from the USA ^[5].

One Health implications

Wildlife and the environment: outbreaks of coccidiosis in green turtles appear to be related to drought and algal blooms and have the potential to cause significant mortality events. Green turtles are an IUCN-listed endangered species ^[6] and are important bioengineers, members of marine food webs (both as predators and prey) and hosts. Their continued population decline is a significant concern for the health of many marine ecosystems ^[7].

Domestic animals: infection with *C. cheloniae*-like organisms has only been recorded in sea turtles and is not known to infect domestic animals.

Humans: infection with *C. cheloniae*-like organisms has not been reported in humans and is not considered zoonotic.

Natural hosts

Green turtles are the only known natural host for parasites resembling *C. cheloniae*. The life cycle has not been elucidated ^[8]. Cases of morphologically similar CLOs have also been reported in loggerhead turtles (*Caretta caretta*) and Kemp's ridley turtles (*Lepidochelys kempii*) ^[5].

World distribution and occurrences in Australia

Coccidiosis in green turtles was first described from an epidemic amongst aquaculture-reared hatchlings in the Cayman Islands ^[9, 10]. Since this initial outbreak, coccidiosis has been reported in wild green turtles in North and South America ^[2, 5, 11].

There have been three major outbreaks of coccidiosis in Australian green turtles, in 1991, 2002 and 2014 ^[2]. The disease has been reported in stranded individuals along the east coast of Australia from Sydney, NSW to the south of K'gari (Fraser Is) in Qld, with most cases concentrated around Moreton Bay and Port Stephens. Despite initial concerns, no evidence of the disease has been confirmed in WA ^[7]. Australian outbreaks tend to occur around areas of agricultural, industrial, and urban waste runoff.

Epidemiology

Infectious *Eimeriidae* oocysts, such as *Caryospora* spp., are transmitted via the faecal-oral route, either directly or indirectly ^[12]. The ability of CLOs to persist in the environment seems to have allowed them to disperse across oceans ^[5]. Coccidiosis in free-living green turtles results in both sporadic mortalities and epidemics.

Environmental conditions and anthropogenic factors are thought to contribute to the frequency and severity of coccidiosis in green turtles ^[2]. The original 1973 epidemic of coccidiosis in aquaculture-reared green turtle hatchlings in the Cayman Islands was attributed to poor hygiene ^[9]. The disease appears to be endemic in the Moreton Bay region of south-east Qld, where degraded catchment areas are subjected to algal blooms. However, the factors making this population more prone to coccidiosis outbreaks than others are not well understood ^[8].

In Australian outbreaks, larger subadult turtles are mainly affected, perhaps due to their favouring different habitats, possibly exposing them to different environmental pathogens ^[8]. Outbreaks in Australia have occurred during periods of El Niño droughts and negative Southern Oscillation Index, from September to February. Rainfall, atmospheric temperature and sea surface temperature do not appear to be factors in coccidiosis outbreaks ^[2]. The concentration of cases in areas subjected to anthropogenic toxins or algal blooms and adverse environmental conditions may contribute to turtles' susceptibility to infection or persistence of oocysts in the environment. Turtles may be feeding on plants closer to the root, increasing their contact with high oocyst concentrations in the substrate ^[2].

Clinical signs

Green turtles with coccidiosis can present with both acute and chronic forms of disease. Signs of acute disease include:

- diarrhoea
- neurological signs, including head tilt, circling in the water and on land, and nystagmus
- weakness, severe depression
- dehydration, as indicated by sunken eyes and concavity of the plastron. These signs mimic emaciation, but acutely affected turtles are generally in good nutritional condition.

Turtles which survive acute infection, or are kept alive in captivity, can develop chronic manifestations, which include:

- intestinal tympany and obstipation; affected turtles may be abnormally buoyant, or list to one side
- continuing neurological disease
- emaciation.

Infection with *C. cheloniae* may be subclinical in green turtles. The pathogen was found in 22% of turtles in south-east Qld that died of other causes. Small numbers of oocysts were detected in faeces, without intestinal or neurological disease ^[8].

Diagnosis

The disease is diagnosed by the presence of oocysts in faecal samples and GIT mucosal scrapings, histopathology or PCR ^[3]. The presence of the organism in gut or faecal samples may not indicate disease. Confirmation of a disease state is through histopathology, with supportive PCR results. Molecular testing is required to determine pathogen genotype.

Laboratory diagnostic specimens and procedures

Faecal examination: faecal samples or scrapings of intestinal mucosa should be examined under light microscopy ^[12]. Faecal floatation can be used to detect the presence of coccidial oocysts which are typically present in large numbers and have a distinctive ellipsoidal shape. Microscopy of mucosal scrapings can be used to detect coccidial schizonts, gamonts or oocysts. Excysted sporozoites form characteristic 8-pointed "stars" ^[13].

Histopathology: key organs to target for histopathological examination include brain, kidney, thyroid, gastrointestinal tract (small and large intestine), lung and spleen. Infection may also be present in heart, liver, pancreas and adrenal glands. Diagnosis is based on the presence of coccidia in tissue samples collected at post mortem (from organs listed above).

PCR: tissues should be stored in 70% ethanol at 4°C for molecular analysis ^[3]. Blood samples, from the cervical dorsal sinus (ante mortem) or from the heart (post mortem), can also be used and should be collected into anticoagulant (lithium heparin or EDTA) ^[12]. A real-time Taqman PCR assay can be used to detect the presence of any *Caryospora* spp. molecular material. Histologically negative tissues may be PCR positive ^[2]. See Table 1 for different coccidia genotypes and the organs in which they are most commonly found.

Coccidia genotype	PCR-positive tissues
1	Brain, GIT, lung
2	Kidney, thyroid
3	Brain, GIT, lung, spleen, kidney
4	Brain, GIT, lung, spleen

Table 1. Organs from which tissue samples returned PCR-positive identification of different coccidiagenotypes. Adapted from Ban de Gouvea Pedroso et al. 2020 [2].

Pathology

Gross lesions: findings include a mucoid to severely necrotising enteritis, generally involving the entire length of the intestine (Gordon *et al.* 1993; Gordon 2005). Most turtles are in good nutritional condition, suggesting relatively acute disease. Occasionally, pinpoint white foci can be visualised in thyroid, kidney and brain. Sparse petechial haemorrhages may be present in the kidney. Reddening or cloudiness of the meninges may also be observed ^[14].

Histology: coccidial infection is present in the intestine and a range of other tissues ^[2]. In some animals, infections appear to be confined to the intestine. Coccidial gamonts are found in the intestinal epithelium, whereas schizonts are found in intestinal epithelium and lamina propria, as well as a number of extra-intestinal sites, including brain, thyroid gland, kidney, adrenal gland and urinary bladder. Heavily infected intestinal epithelium may undergo necrosis, and is accompanied by a variable inflammatory response. Disseminated infection is associated with inflammation in a number of tissues, especially brain, where it can result in multifocal, necrotising meningoencephalitis. Schizonts may be present in some tissues without an accompanying inflammatory response.

Differential diagnoses

Diarrhoea, if present, is a very specific indicator of coccidiosis, but could theoretically occur with any cause of enteritis. Differential diagnoses of neurological disease in green turtles include spirorchid fluke infection ^[15], bacterial meningoencephalitis (*ARWH case reports 3402/1, 3402/22*), head trauma and environmental stressors (toxins and algal blooms) ^[16].

There are many causes of debility and emaciation in green turtles, including spirorchid fluke infection, foreign body ingestion, constipation, bacterial and fungal respiratory disease.

Treatment

The prognosis for green turtles diagnosed with clinical coccidiosis is poor. Severely affected animals are typically euthanased ^[2]. However, treatment options do exist, the most common being the use of triazine anticoccidials (ponazuril or toltrazuril) which are reportedly effective at reducing clinical disease by blocking intracellular pathogen propagation ^[4, 17]. While ponazuril was effective in managing clinical signs, treated individuals still had mild enteritis and remained PCR-positive for CLOS ^[4].

Prevention and control

Strict hygiene procedures should be observed when handling or caring for sick and injured marine turtles, both to reduce the risk of introducing secondary infection, and to limit the spread of pathogens such as *C. cheloniae* to other animals. However, detailed control methods for *Caryospora* spp. are not well described and coccidian oocysts are known to be resistant to many forms of chemical and physical disinfection ^[18].

Turtles with subclinical coccidiosis pose a great risk of infection to their conspecifics. It is recommended that captive populations be monitored regularly for the presence of CLOs through regular faecal microscopic examinations^[5].

Research

Further research in the following areas is required is required to improve our understanding of coccidiosis in Australian green turtles:

- disease epidemiology, particularly the parasitic life cycle of CLOs, specific transmission routes and environmental pathogen reservoirs.
- host and environmental factors influencing the severity of disease experienced by infected turtles.
- targeted treatment and control regimes for both wild and captive turtles.
- taxonomic classification and specific diagnosis of pathogen species and genotype in order to better inform treatment and control decisions.
- confirming the full host range of CLOs in Australia.

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 <u>https://wildlifehealthaustralia.com.au/Our-Work/Surveillance</u> and <u>https://wildlifehealthaustralia.com.au/Our-Work/Surveillance/eWHIS-Wildlife-Health-Information-System</u>.

There is no targeted surveillance program for disseminated coccidiosis in green turtles caused by CLOs. We encourage those with laboratory confirmed cases of this condition in native Australian or feral animals to submit this information to the national system for consideration for inclusion in the national database. Please contact us at <u>admin@wildlifehealthaustralia.com.au</u>.

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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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References and other information

- 1. Upton S and Sundermann C (1990) Caryospora: Biology. In 'Coccidiosis of Man and Domestic Animals.' (Ed P. Long). (CRC Press: Boca Raton, FL)
- 2. Ban de Gouvea Pedroso S, Phalen DN et al. (2020) Coccidiosis in green turtles (*Chelonia mydas*) in Australia: pathogenesis, spatial and temporal distribution, and climate-related determinants of disease outbreaks. *Journal of Wildlife Diseases*, **56**(2): 359-371
- 3. Chapman PA, Owen H et al. (2016) Molecular characterization of coccidia associated with an epizootic in green sea turtles (*Chelonia mydas*) in South East Queensland, Australia. *PLoS One*, **11**(2): e0149962
- 4. Falconnier N, Warshaw M et al. (2023) Systemic *Caryospora*-like coccidiosis in a clutch of hatchling redeared slider turtles (*Trachemys scripta elegans*). *Veterinary Pathology*: 03009858231179129
- Stacy BA, Chapman PA et al. (2019) *Caryospora*-like coccidia infecting green turtles (*Chelonia mydas*): an emerging disease with evidence of interoceanic dissemination. *Frontiers in Veterinary Science*, 6: 372
- Seminoff JA (2023) Chelonia mydas: IUCN Red List of Threatened Species. 13/01/2024]; Available from: <u>https://dx.doi.org/10.2305/IUCN.UK.2023-1.RLTS.T4615A247654386.en</u>
- 7. Young EJ (2022) Health and disease status of sea turtles in Western Australia. PhD thesis School of Veterinary Medicine, Murdoch University: Perth
- 8. Gordon AN (2005) A necropsy-based study of green turtles (*Chelonia mydas*) in south-east Queensland. PhD thesis School of Veterinary Science, University of Queensland: Brisbane, Qld
- 9. Rebell G, Rywlin A et al. (1974) Coccidiosis in the green turtle in mariculture (*Chelonia mydas*). In 'Proceedings of the annual meeting-World Mariculture Society'. Wiley Online Library
- 10. Leibovitz L, Rebell G et al. (1978) *Caryospora cheloniae* sp. n.: a coccidial pathogen of mariculturereared green sea turtles (*Chelonia mydas mydas*). *Journal of Wildlife Diseases*, **14**(2): 269-275
- 11. Conde B, Alvarado MC et al. (2019) First report of coccidiosis in green turtles (*Chelonia mydas*) from the Gulf of Venezuela. *Caldasia*, **41**(2): 278-288
- 12. Chapman P (2017) Diversity, impacts and diagnosis of pathogenic parasites in sea turtles from Queensland, Australia. PhD thesis, University of Queensland: Brisbane, Qld
- 13. Gordon A, Kelly W et al. (1993) Epizootic mortality of free-living green turtles, *Chelonia mydas*, due to coccidiosis. *Journal of Wildlife Diseases*, **29**(3): 490-494
- 14. Ladds P (2009) Protozoal disease in reptiles. In 'Pathology of Australian Native Wildlife.' pp. 261-271. (CSIRO: Collingwood)
- 15. Gordon A, Kelly W et al. (1998) Lesions caused by cardiovascular flukes (Digenea: Spirorchidae) in stranded green turtles (*Chelonia mydas*). *Veterinary Pathology*, **35**(1): 21-30
- 16. Mashkour N, Jones K et al. (2020) Disease risk analysis in sea turtles: A baseline study to inform conservation efforts. *PloS ONE*, **15**(10): e0230760
- 17. Jacobson ER, Stacy NI et al. (2021) Pharmacokinetics of ponazuril after administration of a single oral dose to green turtles (*Chelonia mydas*). *Veterinary Quarterly*, **41**(1): 323-331

18. Daugschies A, Bangoura B et al. (2013) Inactivation of exogenous endoparasite stages by chemical disinfectants: current state and perspectives. *Parasitology Research*, **112**: 917-932

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