Journal of the Association of Avian Veterinarians Australasian Committee Ltd.
Annual Conference Proceedings Melbourne Victoria 2019 27: 19-23.

Review of Isosporosis in Passerine Birds

Matthew Gosbell BVSc MANZCVS (Avian Health)

Melbourne Bird Veterinary Clinic
1 George Street
Scorsby Vic 3179

Abstract

Isosporosis is a significant disease in captive and wild passerine species. *Isospora* spp. belong to the Apicomplexa phylum and form part of the Eimeriidae family of parasites and have been described in several different animals. *Isospora* spp. are considered widespread, however since its first discovery there have been several reclassifications of *Isospora* spp. This has continued to be a source of confusion especially with *Isospora* spp. that invade the macrophage system of passerine birds. Species of *Isospora* with extra intestinal stages causing atoxoplasmosis have previously been recorded as *Lankasterella* spp., and, more commonly, *Atoxoplasma* spp. Coccidiosis is the disease caused by *Isospora,* however several genera of protozoa also cause coccidiosis. Reference to *Isospora* as coccidia or reference to previous classifications of *Isospora* leads to confusion about the parasite. Our understanding on the impact of this parasite in Australia is minimal, yet it is considered endemic, since many prevalence studies around the world suggest a significant incidence in wild and captive collections of passerine species. The parasite has a complex life cycle with an intestinal phase, and it may include a systemic phase, often referred to as atoxoplasmosis. Systemic and intestinal isosporosis can cause significant losses in collections of birds including rare and endangered species in zoological collections. Although isosporosis has been treated successfully with anticoccidial treatments, none have been described to treat systemic isosporosis.

Description of Classification

The phylum Apicomplexa is a large group of parasitic protists, all species being obligatory parasites. All vertebrates and invertebrates host at least one species belonging to this phylum (Votypka *et al*., 2017). They are host-specific and pathogenic to their host. Apicomplexa comprise five principal working groups: gregarines, haemogregarines, coccidia, hematozoans (malarial parasites), and piroplasms. *Isospora* is a monoxenous coccidian, a genus of the Eimeriidae family with several hundred described species found mostly in birds and reptiles (Votypka *et al*., 2017). It is most commonly described in passerine species with approximately 500 species reported (Madani *et al*., 2018). Different species can be differentiated by the structure of their sporulated oocyst stage. *Isospora* species are defined by the production of sporulated oocysts that have two sporocysts each with four sporozoites (Schrenzel *et al*., 2005) (Figures 1a and 1b). The size, shape, colour, texture and type of internal contents of the oocysts are important features for identification (Lindsay *et al*., 1997). It is considered important to use morphological studies as well as molecular studies (at least on multiple genes,

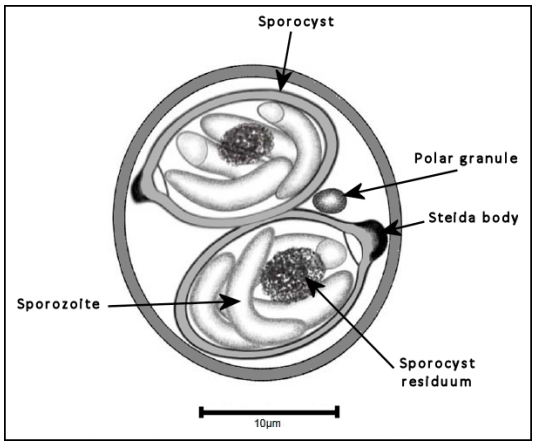

Figure 1a. Line drawing of sporulated *Isospora vanriperorum* oocyst. From Lopez *et al.* (2007)

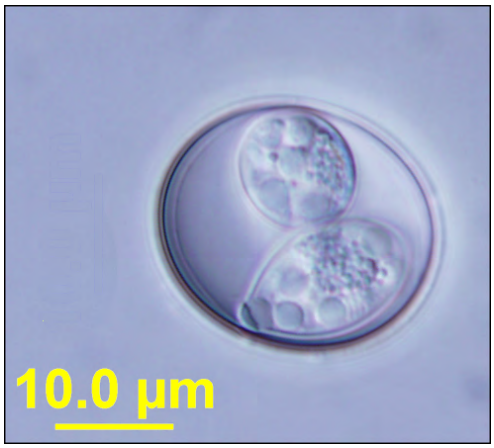

Figure 1b. Sporulated Isospora oocyst from endangered Rengent honeyeater (*Xanthomyza phrygia*). From Morin-Adeline *et al*. (2010)

intranuclear and non-nuclear) to distinguish between closely related species of *Isospora* (Berto *et al*., 2011).

Description of *Isospora* Life Cycle (Figure 2)

The life cycle includes both a sexual and asexual multiplication in the parasitised host. The sexual phase resulting in the formation of environmentally tolerant oocysts (Box, 1977) (Figure 2). In the case of *Isospora* spp., oocysts are ingested by the host, and the sporozoites are released into the intestinal contents. The gliding sporozoite has a short time to find a host cell, which it penetrates via the apical complex initiating infection. The organelles of the apical complex then undergo resorption, and the sporozoite transforms into an oval meront that starts to mature. Once a critical size is reached, the meront divides into merozoites which have a similar structure to sporozoites. After release by the rupture of the cell, merozoites rely on energy sources that last a short time, allowing them to find and invade a new host cell leading to a new generation of meronts and merozoites. Some merozoites are predetermined to become female macrogametocytes, the rest become male microgametocytes (gamogony). The sexual phase (sporogony) occurs with the fusion of the small flagellated microgamete with the large non-motile macrogamete. Further cell division with the oocyst results in a characteristic oocyst with two sporocysts containing four sporozoites (Votypka *et al*., 2017). The asexual (merogony) phase can occur in intestinal cells and lymphoid-macrophage cells. Merozoites in lymphocytes are disseminated within the blood stream to the viscera where asexual merogony may continue. However, gametogenesis has only been noted to occur in the intestinal epithelial cells (Quiroga *et al*., 2000; Adkesson *et al*., 2005; Cushing *et al*., 2011). Systemic disease resulting from the dissemination of infected lymphocytes around the body is known as systemic isosporosis (atoxoplasmosis). It has been previously thought that *Atoxoplasma* was a separate species causing systemic disease but *Atoxoplasma* are asexual stages of *Isospora* in the lymphoid-macrophage cells and is now considered the junior objective synonym of *Isospora* (Box, 1970: Oliveira *et al*., 2018).

Oocytes are released into the environment in the droppings where they sporulate. Only sporulated oocysts are then infective to the next host. It has been shown in species of *Isospora* that host species release the oocysts in the late afternoon where they quickly sporulate to become infective. This phenomenon appears to be a survival mechanism as ultraviolet radiation and warmth increase the desiccation and reduce the infectivity of the oocyst (Martinaud *et al*., 2009).

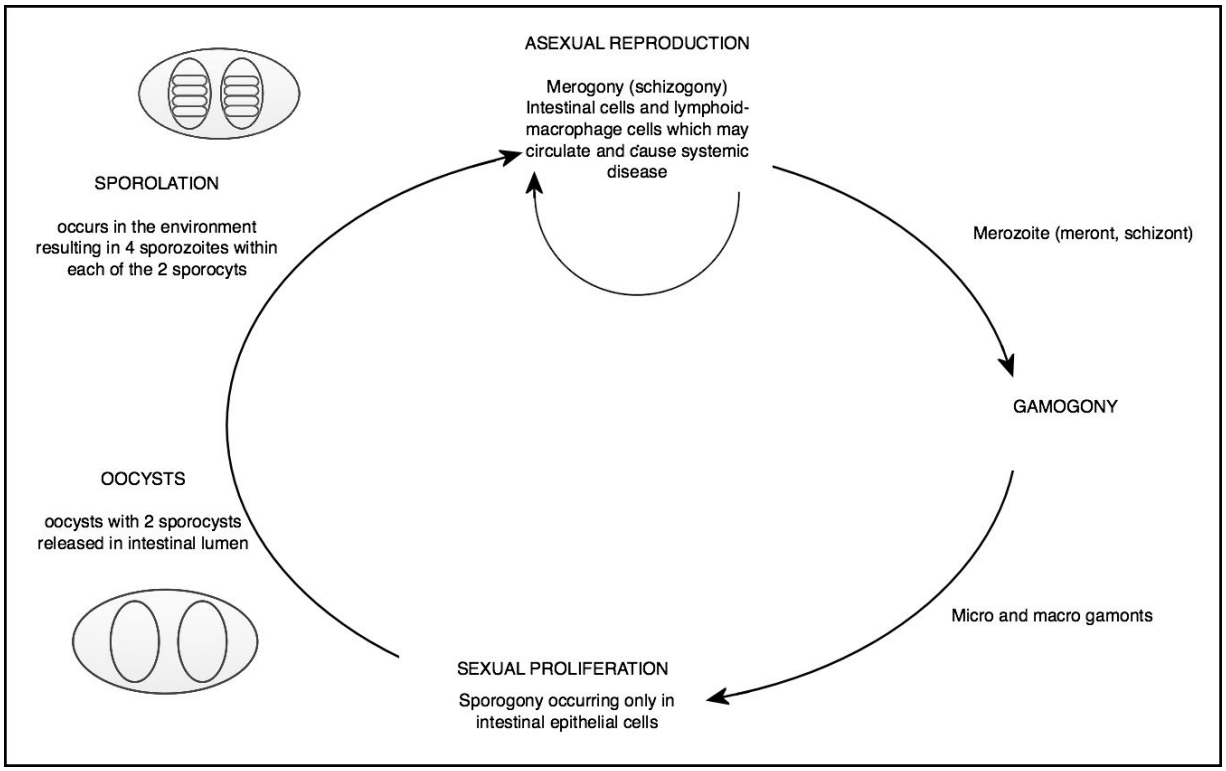

Figure 2. Schematic drawing of the life cycle of *Isospora* spp. in passerine birds.

Pathophysiology of the Disease

Invasion of an apicomplexan into the host cell is multifaceted. It consists of four phases, primary contact without orientation, attachment followed by apical reorientation, induction of the parasitophorous vacuole and translocation of the parasite into the vacuole. The apical end of the parasite attaches to the host cell, the connection is established through sequential secretion from secretory organelles of the parasite. Secretions continue to allow the parasite to enter the host cell enclosed within a cavity of invaginated host cell membrane, thus protecting the parasite from the host immune system. Nutrients are exchanged through the membrane where the parasite continues to develop and undergo merogony (Votypka *et al*., 2017)

Systemic Isosporosis is considered endemic in free-ranging birds (Cushing *et al*., 2011). Infection in captive birds resulting in significant mortality may be exacerbated by the stresses of captivity and poor sanitation, resulting in increased shedding and contamination of the environment (Cushing *et al*., 2011). Clinical signs of systemic isosporosis remain non-specific and include weight loss, reduction of pectoral musculature and diarrhoea. Histopathological descriptions describe an influx of inflammatory cells in the intestinal architecture, close examination of these cells often revealing *Isospora* merozoites in their cytoplasms (Cushing *et al*., 2011). Systemic Isosoporosis, has been associated with infection of mononuclear cells, necrosis of the liver and spleen, and infiltration of the intestines and multiple visceral organs with inflammatory cells (Cushing *et al*., 2011).

Clinical diagnosis

Demonstration of oocysts in fecal samples is the method of choice for detection of *Isospora spp*.. Faecal flotation fluids are often used when examining samples including sugar solution, zinc sulfate or saturated sodium chloride (Lindsay *et al*., 1997). As some hosts have been shown to release oocysts in the late afternoon, samples from this time are best to examine.

Systemic disease can be difficult to confirm in the live bird. The parasite may be found in peripheral blood however parasitaemia in the monocytes of peripheral blood is inconsistent (Quiroga *et al*., 2000). Demonstration of the parasite using tissues samples from dead birds is diagnostic for intestinal and systemic forms of the disease. Intestinal, liver, spleen and lung samples fixed in 10% formalin and prepared for examination histologically are most likely to demonstrate intestinal and systemic infection (Toshihiro et al,. 2017).

Confirmation of Isospora and species identification requires a combination of characterization of the sporulated oocyst and molecular studies including DNA sequencing (Berto *et al*., 2011).

Treatment and Control Options

Management of the environment as well as pharmacologic treatments are vital for controlling Isosporosis. Compared to bacteria, *Coccidia* spp. including *Isospora* have diverged more recently from animals (Wellenhan, 2016). Antimicrobials target the differences between the pathogen and host. Fewer differences between coccidian and animal cells mean fewer options for anticoccidial drug therapy (Wellenhan, 2016). Medications commonly used include Toltrazuril (a triazine anticoccidial) and Ponazuril which are thought to act on the apicoplast, the affected organism developing vacuoles and degenerating (Wellehan, 2016). Diclazuril and sulfadiazine-trimethoprim have also been effective at eliminating *Isospora* oocsyt shedding in the common Mynah (*Acridotheres tristis*) (Madani *et al*., 2018). Oocysts are stable in the environment and may remain viable for prolonged periods (Partington *et al*., 1989, Lindsay *et al*., 1997). Herbal extracts, commercial recommended dose of Dettol, TH4, Phenol, VirkonS®, and Diclazuril 20% have no effect on the sporulation of chicken *Eimeria* oocysts. Sodium hypochlorite had a significant effect on sporulation, with 70% ethanol and 10% formalin showing 100% sporulation inhibition (Sahar et al., 2018). Peroxide-based disinfectants also seem to have some effect (Wellenhan, 2016). Strict hygiene helps to prevent reinfection, including moving birds to a clean dry enclosure, or even suspended enclosures. Removing exposure to contaminated faeces, drying of the parasite (reducing coccidial persistence) and ultraviolet irradiation is also helpful (Wellehan, 2016). Steam cleaning has also been effective at killing sporulated and unsporulated oocysts (Lindsay *et al*., 1997). Warmth and UV radiation can cause desiccation of the oocyst reducing its infectivity; however, oocysts in water are protected from this phenomenon (Connelly *et al*., 2007)).

Attempts to treat systemic isosporosis have been unrewarding, and birds that survive the infection may remain carriers and pass the disease onto their young. As well as taking hygiene measures, treating breeding adults may reduce shedding of the oocysts and decrease transmission risk to the offspring (Adkesson *et al*., 2005).

Further Direction for Research

Improved classification

The classification and phylogenetic understanding of *Isospora* has been confused, especially in relation to systemic infection, Atoxoplasmosis is still often documented or inferred as being caused by *Atoxoplasma* spp rather than *Isospora* spp. Molecular characterisation of *Isospora* spp. has assisted in improving classification and understanding of the virulence of some species. Full characterisation by whole genome sequencing will assist in defining the pathogenicity of various species. This may lead to a greater understanding of how the disease may

be better prevented or managed.

Prevalence studies

Understanding the distribution of this disease in Australia and the identification of species that may be more common and pathogenic across species of passerine birds may help to appreciate the importance of the disease in wild and captive passerine birds.

Clearer understanding of the systemic disease

Votypka *et al*. (2017) suggested that *Isospora* spp. can attach to, and invade, almost any cells. Further studies are required to understand if all species of *Isospora* have the potential to become systemic and under what circumstances this may occur.

New medications

Isosopora is a coccidial organism belonging to the Emeridae family. Chemotherapeutic agents are effective across this family. In chickens, *Eimeria* sp cause significant disease and is considered one of the most costly diseases of the poultry industry (McDougald and Fitz-Coy, 2013). Resistance to chemotherapeutic agents is common (Wellehan, 2016). Chemotherapeutic medications for *Isospora* only seem to be effective for the gastrointestinal phase of the disease. Therefore, only supportive care can be offered to systemically affected animals. Further research into treatment options of systemic disease will help reduce the impact of this disease in captive-bred species and endangered species.

References

Adkesson, M.J., Zdziarski, J.M., Little, S.E. (2005). Atoxoplasmosis in Tanagers. *Journal of Zoo and Wildlife Medicine* **36**(2): 265-272.

Berto, B.P., Flausino, W., McIntosh, D., Teixeira-Filho, W.L, Lopes, C.W (2011). Coccidia of New World passerine birds (Aves: Passeriformes): a review of *Eimeria* Schneider, 1875 and *Isospora* Schneider, 1881 (Apicomplexa: Eimeriidae). *Systematic Parasitology* **80**(3): 159-204.

Box, E.D. (1970). *Atoxoplasma* associated with an Isosporan oocyst in canaries. *Protozool* **17**(3): 391-396

Box, E.D. (1977). Life cycles of two isospora species in the canary, Serinus canarius Linnaeus. *Protozool* **24**(1): 57-67

Connelly S.J., Wolyniak E.A., Williamson C.E., Jellison K.L. (2007). Artifical UV-B and solar radiation reduce in vitro infectivity of the human pathogen Cryptosporidium parvum. *Environ. Sci. Technol.* **41,** 7101-7106.

Cushing, T.L., Schat, K.A., States, S.L., Grodio, J.L., O'Connell, P.H., Buckles, E.L., (2011). Characterization of the host response in systemic isosporosis (Atoxoplasmosis) in a colony of captive american goldfinches (*Spinus tristis*) and house sparrows (*Passer domesticus*). *Veterinary Pathology* **48**(5): 985-992.

Lindsay, D.S., Dubey, J.P., Blagburn, B.L. (1997). Biology of Isospora spp.. from humans, nonhuman primates, and domestic animals. *Clinical Microbiology Reviews* **10**(1): 19-34.

Lopez, B. Do B., Berto, B.P., Massad, F.V., Lopes, C.W.G. (2007). *Isospora vanriperorum* Levine, 1982 (Apicomplexa: Eimeriidae) in the green-winged saltator, *Saltator similis* (Passeriformes: Cardinalinae) in Southeastern Brazil. *Revista Brasileira de Parasitologia Veterinária* **16**(4):211-214.

Madani, S.A., Arabkhazaeli, F., Shakeri, E., Nabian, S. (2018). Molecular and morphological description of Isospora sp. from the common mynah (*Acridotheres tristis*) and a preliminary survey of two anticoccidial drugs in natural infection. *Avian Pathology* **47**(2): 206-212.

Martinaud, G., Billaudelle, M., Moreau, J. (2009). Circadian variation in shedding of the oocysts of *Isospora turdi* (Apicomplexa) in blackbirds (*Turdus merula*): An adaptative trait against desiccation and ultraviolet radiation. *International Journal for Parasitology* **39**(6): 735-739.

McDougald, L.R., Fitz-Coy, S.H. (2013). Coccidiosis. In: *Diseases of Poultry*. Swayne, D.E., Glisson, J.R. and McDougald, L.R. (eds). [electronic resource], Wiley-Blackwell. pp. 2120-2148
'

Morin-Adeline, V., Vogelnest, L., Dhand, N.K., Shiels, M., Angus, W., Slapeta, J. (2011). Afternoon shedding of a new species of Isospora (Apicomplexa) in the endangered Regnet Honeyeater (*Xanthomyza Phrygia*). *Parasitology* **138**, 713-724.

Oliveira, A.R., Souza, T.D., Mol, J.P.S., Flecher, M.C., Hiura, E., Santos, R.L. (2018). Pathological and molecular characterization of systemic isosporosis (atoxoplasmosis) in captive green-winged saltator (*Saltator similis*). *Veterinary Parasitology* **255**: 98-101.

Partington, C.J., Gardiner, C.H., Fritz, D., Phillips, L.G., Montali, R.J. (1989). Atoxoplasmosis in Bali Mynahs (*Leucopsar rothschildi*). *Journal of Zoo and Wildlife Medicine* **20**(3): 328-335.

Quiroga, M.I., Alema, N., Vazquez, S., Nieto, J.M. (2000). Diagnosis of Atoxoplasmosis in a Canary (*Serinus canarius*) by histopathologic and ultrastructural Examination. *Avian Diseases* **44**(2): 465-469.

Sahar, G.M., Waleed, A.M., Shawky, A.M. (2018). In vitro activity of natural and chemical products on sporulation of Eimeria species oocysts of chickens. *Veterinary Parasitology* **251**: 12-16

Schrenzel, M.D., Mallouf, G.A., Gaffney, P.M., Tokarz, D., Keener, L.L., McClure, D., Griffey, S., McAloose, D., Rideout, D.A. (2005). Molecular Characterization of Isosporoid Coccidia (*Isospora* and *Atoxoplasma* Spp..) in Passerine Birds. *The Journal of Parasitology* **91**(3): 635-647.

Toshihiro, T., Atsushi, K., Shun, S., Rie, K., Kazunori, I. (2017). Isospora lunaris n. sp. (Apicomplexia: Eimeriidae) from the domestic Java Sparrow in Japan. *Parasitology International* **66: 100-105**

Votypka, J., Modry, D., Obornik, M., Slapeta, J. and Lukes, J. (2017). Apicomplexa. In: *Handbook of the protists*. Archibald, J.M., Simpson, A.J.B., Slamovits, C.H. (eds)., Springer: pp. 567- 624.

Wellehan, J.F.X Jr.(2016). Coccidial diseases of birds. In: *Current therapy in Avian Medicine and Surgery*. Speer, B.L (ed). Elsevier, St. Louis MO: pp. 73-77.