Cryptosporidiosis in Finches

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Cryptosporidium is a coccidian parasite classified within the phylum Apicomplexa, order Eucoccidiorida, suborder Eimeriorina, and family Cryptosporidiidae (Fayer et al, 1997). It infects epithelial surfaces, especially those along the gut of many vertebrate animals. It has been associated with outbreaks of diarrhoea in calves, lambs, kids, piglets, foals, young dogs and cats, turkey poults and man.

In avian species, respiratory or more commonly intestinal disease is usually manifest. Species reported to be infected include turkeys, chickens, ducks, geese, quail, pheasants, peacocks, parrots, finches & various wild birds. It has also been found in ostrich chicks (Gajadhar, 1994). In commercial poultry flocks, outbreaks of severe disease with variable mortalities have been noted. Young & immunocompromised birds appear to be more susceptible to clinical infection.

In birds, it is currently accepted that there are 2 species of *Cryptosporidium* found (Fayer et al, 1997). *C. baileyi* has been found to infect chickens (*Gallus gallus*) whereas *C. meleagridis* has been isolated from turkeys (*Meleagris gallopavo)* and an Indian ring-necked parrot (*Psittacula krameri)* (Morgan et al, 2000). In most other avian species infected with cryptosporidium, the species of cryptosporidium has not been determined. In this report the presence of a potentially new species of cryptosporidium in finches with a unique site of infection will be presented.

Case Report: A Red-faced Pytilia finch (*Pytilia hypogrammica)* from an aviary in Victoria was presented with a two month history of inactivity, fluffed plumage, perching for long periods with its head held under its wing but responsive to stimuli. When the owner placed the bird into a heated cage on its own, its plumage tightened and the bird appeared to be more active. However, once placed in the aviary, it regressed to its former clinical signs.

Upon presentation to the Kingston Veterinary Clinic the bird appeared to be in fair body condition. Its droppings contained partially digested seeds, but the urate & urine components appeared to be grossly normal. Faecal smears and faecal floatation did not reveal any evidence of parasitism. Feacal Gram stains contained a moderate number of mixed Gram positive organisms, with only occasional Gram negative rods evident (<5%). There was no evidence of megabacteria, yeasts or other fungal elements.

It was decided to euthanise the bird which was done via inhalation of neat halothane. The coelomic cavity was opened & the entire bird was fixed in 10% buffered formal saline and sent for histopathological analysis to Murdoch University.

Histopathological findings included necrosis and hyperplasia of proventricular glandular epithelial cells associated with a myriad of cryptosporidia attached to the surface of glandular epithelial cells. DNA sequence and phylogenetic analysis of the *Cryptosporidium* involved at the 18S r RNA and HSP-70 loci revealed a distinct species of *Cryptosporidium* which could be loosely grouped with

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C. serpentis and *C.muris* (Morgan, submitted for publication). At the HSP-70 locus, it shared 94% similarity with *C. serpentis* and 93% similarity with *C. muris*, but only shared 71-87% similarity with other species of *Cryptosporidium.*. At the 18S r RNA locus, it shared 96% similarity with *C. serpentis* and 95-96% similarity with *C. andersoni* and *C.muris*. This isolate shared 89-93 % similarity with other *Cryptosporidium* species. This isolate also shared 99% similarity with other finch isolates.

Discussion:

Typically, cryptosporidial parasites infect the epithelium of the intestinal tract. *C. parvum*, the species isolated from most mammals including man, is predominately found in the small intestine, although in heavy burdens both the stomach and large intestine may be infected. In naturally infected turkeys, *C. meleagridis* has also been isolated predominately from the small intestine, with numbers falling away in the colonic and cloacal mucosae (Pavlasek, 1994). This is not surprising, given that phylogenetic studies of gene protein nucleotide sequences indicate that *C. parvum* and *C. meleagridis* may belong to the same subgroup or clade (Xiao et al, 1999; Sulaiman et al, 2000) or in fact be variants of the same species (Champliaud et al, 1998; Sreter and Varga, 2000).

In finches it appears that *Cryptosporidium* has a predeliction for the proventriculus and not the intestine as it does with other species. A previous report of cryptosporidiosis in a Diamond Firetail Finch (*Emblema guttata)* by Blagburn et al (1990) also demonstrated invasion of the surface, ductal & glandular epithelium by cryptosporidial organisms. No other organisms were detected. This bird had died following an acute episode of diarrhoea. It has also been reported in Bronze-winged Mannikin finches (*Lonchura cucullata)* (Lindsay et al, 1991), solely affecting the proventriculus; in canaries (Macwhirter, 1994) and in the kidneys of a Black-throated Finch (*Poephila cincta*) (Gardiner et al, 1984).

This affinity for the proventricular glandular epithelial cells is also shared by *C. muris* which infects the gastric glands of laboratory rodents and several other mammalian species, but not humans (Tzipori, 1983). A report of chronic *Cryptosporidium* infections in Australian elapid snakes also resulted in hypertrophic gastritis characterised by mucosal thickening with cystic dilation of gastric glands, moderate oedema and fibrosis of the lamina propria, and a mild to moderate patchy infiltration of inflammatory cells (Carmel et al, 1993). Gastric cryptosporidiosis was also found in a dead emaciated frilled lizard (*Chlamydosaurus kingi*) in the Northern Territory, Australia. (Oros et al, 1998).

In addition, investigations carried out by Morgan et al (submitted for publication) showed that the isolates of *Cryptosporidium* derived from finches showed distinct genetic differences at two loci (HSP-70 and 18S r RNA) from other species of *Cryptosoridium*, whilst maintaining homology amongst themselves.

The unique affinity for infecting the proventriculus together with the distinct genetic differences suggest that the species of *Cryptosporidium* infecting finches may be a new valid species, distinct from others described in the literature to date.

The life cycle of *C. parvum* and *C.* meleagridis begins with the ingestion of the sporulated oocyst, the resistant stage found in the environment (Fayer et al, 1997). The ingested oocyst contains 4 sporozoites which leave the oocyst and penetrate individual epithelial cells. The parasite resides intracellularly on the lumenal surface of the cells. Merogony and schizogony occurs resulting in the formation of 8 merozoites within the meront. These “Type I” meronts rupture open releasing free merozoites which penetrate new cells where they undergo merogony to form more meronts. This self perpetuating cycle has the potential for Type I meronts to arise continuously.

It is thought that some Type I meronts are somehow triggered into forming a second type of meront, the Type II meront. This contains only 4 merozoites, which go on to form the sexual stages. Some Type II merozoites enter cells, enlarge and form macrogametocytes. Others enter cells and undergo multiple fission where they form microgametocytes, each containing 16 non-flagellated microgametes. These microgametes rupture from the microgametocyte and penetrate the macrogametes, forming a zygote. A resistant oocyst wall is then formed around the zygote, meiosis occurs, and 4 sporozoites are formed. These sporozoite-containing oocysts are passed in the faeces and into the environment.

Interestingly, about 20% of oocysts fail to form an oocyst wall and have only a series of membranes surrounding the developing sporozoites. The sporozoites produced by these "thin-walled oocysts" can excyst whilst still in the gut and infect new cells, thus providing a second route of autoinfection.

Each generation of *Cryptosporidium* can develop in as little as 12-24 hours. Oocysts can be shed in the environment for 6-12 days in immunocompetent humans but may be prolonged in immunosuppressed patients.

In the cases seen in finches oocysts have not been identified in the droppings of clinically affected finches. The owner of the Red-faced Aurora finch has had other species of finches show similar clinical signs which upon histopathological examination have revealed proventricular cryptosporidiosis. These include Red-cheeked Cordon Bleus (*Uraeginthus bengalus)*, Black-headed Yellow Siskin (*Cardeulis magellanica*). The problems all appear to originate with birds kept in one particular aviary, but not all birds kept in this aviary over a two year period have gone on to show clinical signs of the disease. Cryptosporidial organisms can be detected on histopathological sections where they appear PAS- positive. Faecal smears stained with Giemsa may show up the organisms if present. Monoclonal antibody, enzyme immunoassay (EIA) and direct and indirect immunofluorescent antibody (FA) tests have been developed but these have been directed toward identifying *C. parvum* isolates from humans (Graczyk et al, 1996). Their effectiveness in identifying other species of *Cryptosporidium* have not been evaluated fully.

The role of this organism in disease has traditionally been linked to underlying immunosuppression. It is thus commonly associated with other disease entities, classically with human AIDS patients and also in young animals. However, its role as a primary pathogenic agent cannot be truly ruled out. Blagburn et al (1990) considered it to be the primary cause of death in the Diamond Firetail Finch described above whilst Lindsay et al (1991) consider it to be a primary disease in commercially raised chickens, turkeys and bobwhite quail. Antemortem diagnosis of this disease is often difficult due to the poor shedding or misidentification of the oocysts.

Treatment of this disease is difficult. A healthy immune system is of paramount importance in eliminating this organism from infected hosts. Therefore, identification and treatment of any underlying diseases, optimal housing, feeding and general management practices and minimising stress are all important.

In human AIDS patients were secondary cryptosporidiosis is a common problem , antiretroviral therapies such as indinavir and zidovudine have resulted in elimination of the parasite from the gastrointestinal tract (Blagburn et al, 1997). Azithromycin & roxithromycin have also succeded in reducing burdens or eliminating the infection in some cases, as have letrazuril, nitazoxanide and paromomycin which are unavailable in Australia at present. Tony Gestier (pers com) noted rapid clinical response in chronically ill young black cockatoos which tested positive to cryptosporidiosis on a monoclonal antibody test. These birds were given paromomycin at 100 mg/Kg daily diluted in dextrose by crop gavage for 5-7 days, after which they thrived, gained body weight and were weaned without subsequent problems.

Management procedures such as preventing contact with faeces and infected substrate and maintaining strict hygiene are all essential. Ionophores appear to be effective against the parasite and may be the disinfectants of choice for environmental control.

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