Protozoan Parasites of Cage and Aviary Birds : A Review of Epidemiology, Pathogenesis, Diagnosis , Treatment and Clinical Significance in Australia

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SUMMARY

This article provides an overview of those protozoal parasites found in cage and aviary birds that may be considered as significant pathogens. The organisms covered include *Atoxoplasma*, Coccidial protozoa, *Cochlosoma, Cryptosporidium, Giardia, Haemoproteus, Hexamita, Leucocytozoon, Plasmodium, Sarcocystis, Toxoplasma,* and *Trichomonas.*

Each species is considered with regards to general epidemiology and pathogenesis (including life cycle, transmission, host range, and immunity), diagnosis (including clinical syndromes, antemortem and postmortem diagnosis), treatment and the significance of this parasite with regards to the avian population within Australia. Appendices containing diagnostic diagrams and drug information are provided.

In addition , I have attempted to provide a ready reference to this group of parasites with regards to the clinical situation in this country.

1.0 - INTRODUCTION

Protozoan parasites form a complex and often misunderstood group of pathogenic and potentially pathogenic organisms. To add to this confusion, much less research has been carried out to help understand the epidemiology and significance of those parasites that don't occur in commercial poultry compared to those that do. As a result of this, there is a paucity of information available to the general avian practitioner that may be presented with one of these species. Parasitic populations are adaptable and thus clinical syndromes seen within one city, state or country may not be the same as that seen in another. This is a phenomenon readily seen when comparing syndromes seen in Australia compared to the United States and Europe. This feature is commonly seen in avian protozoal parasites and it is the aim of this review to outline the known features of each parasite and then relate them to the different clinical syndromes seen overseas and here in Australia.

2.0 - PROTOZOAL PARASITE SPECIES

2.1 - Atoxoplasmosis

2.1.1 - Epidemiology and Pathogenesis

Atoxoplasma (formerly *Lankasterella*) infects many avian species but causes clinical disease only in Passerines. It is very host specific and strains infecting one species are unable to infect hybrids between this species and another (Greenwood 1989) due to loss of specificity. Young are more severely affected than adults. Although it is commonly thought of as a haemoparasite in peripheral blood cells, its primary target site in passerines is the intestine . It has a direct life cycle but there is potential for arthropods to acts as intermediate hosts by ingesting its sporulated oocysts in the environment (Doneley 1996). Following ingestion of the oocyst by the bird, it excysts in the small intestine as sporozoites. The sporozoites penetrate intestinal epithelial cells and lymphoid-

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macrophage system and this enables them to spread to parenchymal organs where they undergo shizogony. The target parencymal organs are the lungs, liver, spleen, pancreas and pericardium. Gametogony occurs only in the intestine. The oocysts are shed in the faeces intermittently for 5-8 months and are viable in the environment for several months (Doneley 1996). Within the body, *Atoxoplasma* incites a primarily mononuclear cell response with multiple inflammatory foci forming following the rupture of shizogonous stages from parenchymal organs and release of oocysts from intestinal cells (Patton 1993). Vogelnest (1991a) considers *Atoxoplasma* to be nothing more than a tissue invading systemic form of *Isospora* however this is disputed by Patton (1993) who differentiates the two by comparing their oocyst length : width ratios (*Isospora* 1.13 versus *Atoxoplasma* 1.05). Macwhirter (1994) further differentiates the two based on prepatent period, patency, and duration of infection. From a clinical point of view the argument is purely academic and has no bearing on the syndromes described. It is not known if individuals can develop immune tolerance to this parasite.

2.1.2 - Diagnosis

Clinically , *Atoxoplasma* produces diarrhoea, non-specific illness and death. The liver is dark, enlarged and is visible ventral to the sternum as a dark patch from which it receives its common name of “black spot” (Macwhirter 1994). Clinical signs are most evident at weaning (Greenwood 1989). Adults may act as asymptomatic carriers (Page and Haddad 1995).

Faecal examination may reveal oocysts that appear very similar to Isosporan oocysts.but these may be absent in birds showing clinical signs as it is the systemic shizonts and not the intestinal gamonts that are creating the clinical signs (Vogelnest 1991a) . A fine needle aspirate biopsy of the liver may reveal *Atoxoplasma* organisms when stained with Giemsa (Van der Heyden 1996).Only 20% of known positively infected birds will show a positive result if a simple blood smear is relied upon for diagnosis (Vogelnest 1991a).

Buffy coat smears stained with Romanowsky’s stain will identify pale staining, round to oval, intracytoplasmic inclusions that indent the host nucleus in mononuclear leucocytes (Vogelnest 1990a). Using Giemsa stain, the same inclusion bodies will stain reddish (Greiner and Ritchie 1994).

On post mortem, distension of the bowel loop, liver and spleen are evident. Multiple necrotic foci are evident on the surface of the liver and spleen (Patton 1993, Greiner and Ritchie 1994).

Impressions smears of liver and spleen stained with Wright’s will demonstrate organisms (Patton 1993).

2.1.3 - Treatment and Control

Therapeutics include primaquine (1mg/kg body weight) repeated weekly for 2-3 weeks will eliminate the tissue stage of *Atoxoplasma* whilst sulphachlorpyridazine (50 g/ litre water) for 5 days each week until after molting will suppress faecal oocyst shedding. Environmental control requires decreased exposure to droppings, a dry , clean floor, reduction in stocking density and treatment of concurrent disease (Doneley 1996).

2.1.4 - Significance in Australia

Atoxoplasma has been isolated in Australia from a number of native species, introduced species (Sparrows (*Passer domesticus*)) as well as from canaries (*Serinus canarius*) and captive finches. The potential for infection in urban areas where these species overlap exists. Difficulties in determining whether the infection is due to *Atoxoplasma* or *Isospora* may result in misdiagnosis with a bias for the better known parasite (*Isospora*).

2.2 - Avian Malaria - *Plasmodium*

2.2.1 - Epidemiology and Pathogenesis

Plasmodium is a worldwide species that infects many species of birds and mammals. The life cycle is complicated and involves a sexual stage and sporogony within an invertebrate host and an asexual stage in the vertebrate host. The invertebrate hosts for birds are *Culex* and *Aedes* mosquitoes (Campbell 1995). Infective sporozoites in the mosquito saliva are inoculated whilst the mosquito feeds. A shizogony stage occurs in

reticuloendothelial cells of the liver and spleen and the bone marrow (Pre-erythrocytic shizogony). A second shizogony stage may occur if merozoites produced by the first stage invade other reticuloendothelial cells. Merozoites are released to invade erythrocytes where they develop into shizonts and undergo several stages of shizogony within the erythrocytes, breaking out between each stage and destroying the erythrocyte. At some stage the merozoites are trigger to form sexual stages (microgamonts (male) and macrogamonts (female)) which are ingested by the mosquito whilst feeding (Vogelnest 1990b). Clinical infection is relatively rare but has been most recorded in passerines, waterfowl and penguins. Within the passerines, Canaries (*Serinus canarius)* and Blue Faced Parrot Finches (*Erythura trichroa*) appear particularly susceptible. Sporadic outbreaks may occur with increases in mosquito numbers (Campbell 1995, Vogelnest 1990b). Clinical signs are related to endothelial damage by shizogony in the organs and the erythrocytic damage by releasing stages. Clinical signs may therefore occur before an erythrocytic parasite is evident (Van der Heyden 1996). Each *Plasmodium* species has a limited host spectrum but they are not tightly host specific (Vogelnest 1990b). Many asymptomatic carriers occur and carry parasitaemias generally below 0.1% of erythrocytes. Clinical disease occurs more often in newly introduced, naive birds rather than as relapses in chronically infected birds (Van der Heyden 1996). Direct blood to blood transfer between birds can spread infection without the need for the intermediate host due to transfer of shizonts in erythrocytes (Tudor 1991, Greiner and Ritchie 1994). Non-pathogenic strains can occur in the same avian species that are susceptible to pathogenic strains (Rae 1995). Controlled exposure may allow development of latent infections and a state of immunity (Vogelnest 1990b).

2.2.2 - Diagnosis

Infected birds may show no clinical signs if infected by a non-pathogenic species. Pathogenic forms may cause sudden death, respiratory distress, pedal oedema, pale skin and breast tissue, fluffed up appearance, weakness, swelling of the eyelids (due to a competitive pantothenic acid deficiency), bright green urates, pigmented liver visible through the skin, anorexia, vomiting, haemoglobinuria terminally and haemolytic anaemia (Rae 1995, Vogelnest 1990b, Tudor 1991, Petrak 1969).

Post mortem examination will reveal marked splenomegaly, hepatomegaly, splenic infarcts, subcutaneous haemorrhage, pigmented liver, lung and spleen, pericardial effusion and pulmonary oedema (Van der Heyden 1996, Marx 1987, Petrak 1969, Vogelnest 1990b).
Haematology and biochemistry reveal (apart from the parasites), normocytic, normochromic haemolytic anaemia, leukocytosis, lymphocytosis, low PCV (2-5%) and an elevated AST (Van der Heyden 1996,

Air-dried blood smears can be stained with Wright's, Diff Quik, Giemsa or Romanowsky's and should be examined under oil (Tudor 1991, Vogelnest 1990b). Many erythrocytes will be observed to contain thin walled sacs within the cytoplasm that are irregular in shape and contain stippled aggregations (Tudor 1991) (see Appendix III). The parasites in the erythrocytes are pigmented (as with Haemoproteus) but only *Plasmodium* has a multinucleated shizont stage within the erythrocyte (Vogelnest 1990a). Gamonts contain fine, dark golden-brown malarial pigment (Vogelnest 1990b) and may be round and displace the nucleus or elongated and not displacing the nucleus. Macrogametocytes stain a deeper blue than microgametocytes. Organisms can also be found in thrombocytes, leucocytes and endothelial cells (Campbell 1995).

Impression smears of the liver, lung, heart blood, bone marrow or spleen may be stained with Diff Quik to examine immature erythrocytes containing *Plasmodium* shizonts and gamonts and other extraerythrocytic schizonts in other reticuloendothelial origin cells. Affected erythrocytes are often poorly haemoglobinised. (Vogelnest 1990b).

Histologically, liver sinusoids may be distended with immature erythrocytes containing pigment, shizonts and gamonts and Kupffer cells with quantities of pigment (Vogelnest 1990b).

2.2.3 - Treatment and Control

Human anti-malarial drugs form the basis of avian malarial treatment. Some therapeutics used include sulphadoxine 5mg and pyrimethamine 25mg (per 2.5ml) at 0.5 ml IM (for a 30 g Finch) once and repeated if hepatomegaly still evident one month later (Vogelnest 1990b), Quinacrine 7.5 mg/kg daily for 7-10 days and pyrimethamine 0.3 mg/kg (Doneley 1996). Chloroquine 500mg and primaquine 75mg have been used at 1 tablet in 15 ml water to form stock solution which is then diluted 1ml into 480 ml water to yield 0.07mg/ml chloroquine (10mg/kg) and 0.01 mg/ ml primaquine (1mg/kg) when used in seed eating passerines once weekly as a treatment and a preventative (Joseph 1992).

Additional treatment may include careful nursing, blood tranfusion, fluid therapy, steroids, antibiotics, avoidance of stress and other diseases, and maintenance of a mosquito proof enclosure for susceptible species in endemic areas (Doneley 1996, Van der Heyden 1996, Vogelnest 1990b).

2.2.4 - Significance in Australia

Significant outbreaks of avian malaria have been recognised in wild and captive birds in Australia. It should be considered as an important differential in those susceptible passerine species when associated with sudden death, anaemia and dyspnoea. Plasmodium may be less likely to be diagnosed due to reduced use of haematolgy amongst general avian practitioners.

2.3 - Coccidial Parasites - *Eimeria* and *Isospora*

2.3.1 - Epidemiology and Pathogenesis

These are the two principal two genera of coccidial parasites producing the clinical syndrome known as coccidiosis in avian species.

Eimeria species have a monoxenous life cycle in which oocysts that mature or sporulate in the environment are ingested by the avian host and the sporozoites are released in the avian gut by digestion. *Isospora*, alternatively can be a monoxenous life cycle or a heteroxenous life cycle. In the latter, the sporozoites released from the oocysts are ingested by an invertebrate host and form zooites within this invertebrate host. The avian host is infected by ingestion of the invertebrate and the zooites encapsulated within the invertebrate musculature (Page and Haddad 1995). The usual method of spread is via a faecal-oral route.

Although these organisms are found throughout all avian species, there is a significant host specificity with little cross infection recorded (Doneley 1996). *Eimeria* species are primarily found in galliformes and columbiformes and less so in psittacines.. *Isospora* species are primarily found in passerines, psittacines and piciformes but can also be found in struthioformes, falconiformes, galliformes, coraciformes, charadriformes and strigiformes (Page and Haddad 1995, Greiner and Ritchie 1994) . These parasites are mainly distributed throughout the intestine but extraintestinal infections can occur in the kidneys, bile ducts and respiratory tract (Page and Haddad 1995). Extraintestinal infection occurs as a result of infection of the mononuclear phagocyte system after intestinal epithelial penetration and transportation to the other tissues. Multiple shizogonous multiplications result in rapid amplification of pathogenic organism numbers (Helman and Jensen 1984). The intestinal cells are destroyed by the exit of excysting sporozoites primarily in the tips of the intestinal villi and again by the merozoites at each shizogony stage. This result in villous atrophy (Page and Haddad 1995), epithelial ulceration and epithelial erosions. This predisposes the animal to malabsorption, blood loss anaemia, hypoproteinaemia, exudative enteritis, and colitis (Patton 1993). Central nervous system signs may also become evident and are attributable to hypoglycaemia, electrolyte imbalances and debilitation (Greve 1996).

Most severe infections are associated with the very young or in immunocompromised animals (Page and Haddad 1995). Subclinical infection is common and the level of damage is dependent on the number of parasites multiplying at each infective site and the level of host immunity (Patton 1993). Potent pathogenic species may also exist as subclinical infections. The presence of another disease with or without debilitation or a combination of one or more subclinical infections may result in a clinical syndrome developing (Greve 1996).

Immunity is developed to infection following exposure to small levels of infective sporulated oocysts. Constant exposure maintains and reinforces this immunity. If management of birds excludes this protective exposure then immunity will be lost. Age resistance is therefore relative to previous exposure levels. Local tissue immunity does exist but is only protective for the species stimulating the immune reaction and present at the time and offers no cross immunity (Greve 1996, Tudor 1991).

2.3.2 - Diagnosis

Clinically, the syndrome of coccidiosis is well recognised with diarrhoea (with or without blood or mucous) as the primary clinical sign. Weight loss, dehydration, depression, ataxia, incoordination and sudden death are common sequelae (Cannon 1997). A soiled vent and swollen abdomen (due to distended intestines) may also be seen (Greve 1996). The diarrhoea may be mild to severe and will range from bloody and greenish to watery and brown.

Visceral coccidiosis may not be able to be discerned by clinical signs alone. Antemortem diagnosis is best achieved by faecal examination for characteristic oocysts. Oocysts are round, oval or pear shaped and 20-35 um in diameter depending on species. In sporulated oocysts, Eimeria will have four sporocysts with two sporozoites each whilst Isospora will have two sporocysts each with four sporozoites (Page and Haddad 1993, Greve 1996) but is not always sporulated when shed (Patton 1993) (see Appendix I). Clinical signs will begin as intestinal epithelium is damaged and this may occur before oocyst production begins (i.e. during the asexual stages) (Hooimeijer , Peek and Vertommen 1993, Patton 1993). Hooimeijer et al (1993) showed that budgerigars infected with Eimeria dunsingi would show faecal changes for days post infection but oocyst were not evident until seven days post infection.

Oocysts are commonly shed intermittently (Page and Haddad 1995) and false negative may be the result. False positives may occur due to the examination of the oocysts of non-pathogenic species (Greve 1996). Extraintestinal coccidiosis will also produce faecal oocysts as renal oocyts are present in the urates and respiratory oocysts are coughed up and then swallowed in sputum (Page and Haddad 1993). Faecal oocysts can be examined on fresh wet mounts, standard faecal flotation or with Sheathers sugar flotation (500 g sugar, 320 ml water and 6.5 g Phenol Crystals) (Greiner and Ritchie 1994).

Post mortem examination often reveals ballooned small intestine that has erythematous mucosa and haemmorhages or it may appear normal. Impression smears of the intestine at several sites (as each coccidial species has its own target site) will reveal numerous oocyst and the larger (50 um) round, single celled coccidial organisms (Greve1996). Finches with visceral coccidiosis will present at post mortem with hepatomegaly with pinpoint and coalescing tan to yellow lesions, marked splenomegaly, and swelling of the kidneys. Impression smears of organs particularly the kidneys will often reveal numerous oocysts. Histopathologically, there is necrosis and granulomatous inflammation in the liver, spleen, kidneys and lungs with the liver and spleen being the most severe. In all inflammatory areas, macrophage cytoplasm will contain variable numbers of oval to crescent shaped organisms (Helman and Jensen 1984).

2.3.3 - Treatment and Control

Therapeutically, coccidia may be targeted with either a coccidiostatic drug or a coccidiocidal drug. Coccidiostatic drugs competitively inhibit coccidial growth and thus inhibit clinical infection whilst still allowing immunity to develop. They are only effective in conjunction with thorough cleaning to prevent reinfection with removal of the drug. Coccidiostats are commonly present in commercial poultry diets or can be provided as in-water medication.

The usefulness of these drugs is often strain related and will reduce with prolonged usage. Drugs still in current use include amprolium plus ethopabate, sulphaquinoxaline, dinitolmide and nitrofurazone. Coccidiocidal drugs will destroy coccidial organisms and will thus remove any further source of immunological stimulation making these animals prone to more severe reinfections. Some coccidiocides include toltrazuril. diclazuril, narasin, lasolacid sodium, nicarbazin, salinomycin and maduramicin ,however many of these are available only as poultry feed premixes (Doneley 1996, Anon 1995, Ritchie and Harrison 1994, Cannon 1996).

Strategic anticoccidial treatments aim to remove excessive burdens of coccidial organisms at periods where they are most likely to cause clinical disease e.g. pre-breeding, after fledging and after heavy rain) .

Environmental control aims to remove oocysts from the environment and remove the environmental factors that favour oocyst survival (warmth, moisture). This requires a regularly cleaned, dry environment with minimal insect pests, reduction of stocking density and provision of generally sanitary feeding, drinking and living areas (Doneley 1996). A compromise must be reached between eradication of the parasite and development of immunity.

2.3.4 - Significance in Australia

Coccidial infections are common in Australia particularly amongst finches and quail but less so in psittacines except those housed in earthen floored aviaries. The trend towards suspended aviary use in this country may lead to better coccidial control but will surely also lead to reduced development of immunity and subsequent increased risk of severe infection.

The recent introduction of efficient and safe coccidiocidal drugs such as toltrazuril have significantly improved parasite reduction and improved survival of infected birds.

2.4 - Cochlosomiasis

2.4.1 - Epidemiology and Pathogenesis

This flagellate protozoan is a parasite primarily of finches, waterbirds and rarely psittacines and inhabiting the small intestine, large intestine, caeca and cloaca. It has a simple, but incompletely understood life cycle that is assumed to involve simple binary fission. *Cochlosoma* is superficially similar to *Giardia* with the presence of a sucking disc. Histologically, the parasite causes no visible lesions throughout the gut but it is regularly apparent on the mucosal surface (Filippich and O'Donoghue 1995). It would seem possible that *Cochlosoma* may act to cause a mechanically obstructive, malabsorptive state by attachment to the gastrointestinal surface (as is seen with *Giardia*). It can survive in environmental sites if there is adequate moisture such as damp soil and water receptacles but the length of survival time outside of the host is uncertain (Clipsham 1995, Doneley 1996, Filippich and O'Donoghue 1995). The organism is transmitted between adults via contaminated feed or water and between adults and young via feeding or faecal ingestion (Doneley 1996).

This is usually considered as a pathogen of young birds but adults may also be clinically affected. Other adults are commonly affected by this parasite without showing clinical signs suggesting a level of inherent immunity (Filippich and O;Donoghue 1995). Species such as the Bengalese Finch *(Lonchura domestica*) are often considered as carrier species and are often implicated as spreading this parasite to fostered chicks of other species (Greiner and Ritchie 1994).

2.4.2 - Diagnosis

Cochlosoma infected adult finches show debility, dehydration and pass whole seeds in their droppings (Greiner and Ritchie1994). Carrier adults have been described as having bulkier and moister droppings (Filippich and O'Donoghue 1995), a feature that would enhance exposure to other birds and environmental survival. I have also observed a syndrome of chronic facial alopecia in chronically infected Gouldian finches *(Erythrura gouldiae*) that resolves following treatment of this parasite. Infected chicks are debilitated, have shrivelled skin, are dehydrated, and following fledging often have yellow stained feathers, are fluffed up, have molting problems and moist bulky droppings. Most chicks are affected between 10 days and 12 weeks of age when infected (Filippich and O'Donoghue 1995, Greiner and Ritchie 1994, Greve 1996). Infection is often only suspected when fledging rates are dramatically reduced by chicks dying in the nest (D.Brown pers. obs.).

Identification of the organism requires fresh and warm faeces or post mortem gut mucosal smears to be used as fresh wet smears (Clipsham 1995, Greve 1996, Greiner and Ritchie 1994). Organisms are also consistently present on rectal or cloacal mucosa (Filippich and O;Donoghue 1995). This organism is easily overlooked if refrigerated post mortem specimens are provided as the parasite is no longer motile and difficult to visualise. It is better to examine unrefrigerated but fresh specimens where available (D.Brown pers.obs.).
The organism is a typical flagellate with 6 anterior flagella and a ventral sucker. Its movement is erratic and jerky

with horizontal rotation but no spiralling (Clipsham 1995) (see Appendix II).

Post mortem examination reveals the intestine filled with a yellow suspension or whole seeds (Greiner and Ritchie 1994).

Some important differential diagnoses in the Australian avian population include Candidiasis and *Acuaria* infestation.

2.4.3 - Treatment and Control

Therapeutic treatment of this parasite is relatively simple using either ronidazole or metronidazole. There is significant anecdotal reference to toxicities in finches with the use of metronidazole in finches. Studies by Filippich and O;Donoghue (!995) showed that this drug was safe and effective at doses of 29mg - 179 mg/kg body weight dosed orally by crop gavage for three days and doses of 40 mg - 2000 mg/litre of drinking water for 3 days. Most birds in this study ceased passing organisms within 24 - 48 hours of treatment and it is suggested that this is a suitable treatment for asymptomatic carriers . Ronidazole at 60 mg / litre drinking water for 7 days was also highly effective and safe. Dimetridazole at 100 mg/litre water has been used (Greiner and Ritchie 1994) but may be a greater toxicity risk than the above drugs. Carnidazole and metronidazole have been used for resolution of clinical infections in psittacines (Clipsham 1995).

Prevention is often aimed at treating carrier birds and reducing asymptomatic infections especially in foster parents. The utilisation of fostering for the breeding of finches in Australia is uncommonly used but infections are still commonly found. This suggests that classical carrier species (Gouldian Finches (*Erythura gouldiae*) and Bengalese Finches (*Lonchura domestica*)) may not be as important in the introduction of this parasite into a collection. Control should be aimed at prevention of parasite entry via adequate quarantine and prophylactic treatment, strict hygiene and maintenance of a dry clean aviary. If fostering is used, then foster parents should be prophylactically treated prior to breeding season.

2.4.4 - Significance in Australia

Cochlosomiasis is an emerging disease in Australia with diagnoses being more commonly found in Finches than previously. This involves several new species of finch in which *Cochlosoma* has not previously been described (Filippich and O'Donoghue 1995), and a suggestion that this disease may be endemic in some pet shop situations. Its previous absence from regular diagnosis may be due to its clinical similarity to *Acuaria* infection in finches which has usually been considered as a common clinical syndrome that is difficult to eradicate and thus continued losses are expected by aviculturist despite treatment. Introduction of improved anthelmintics for *Acuaria* control may make this disease more likely as a differential for debilitated finches passing whole seed in their droppings. As yet it has not emerged as a syndrome in psittacines in this country.

2.5 - Cryptosporidiosis

2.5.1 - Epidemiology and Pathogenesis

Cryptosporidium sp .has a direct life cycle similar to that of the coccidial organisms with asexual and sexual stages with the host resulting in the shedding of infective oocysts. The organism occurs in an intracellular but extracytoplasmic coccidian with a predelection for surface epithelial cells (apical portion) of gastro-intestinal, respiratory and genito-urinary systems (Patton 1993, Goodwin and Davis 1993). The organisms appear free in the lumen and attach to the microvilli of the epithelial villi cells. They "acquire" epithelial cell membrane to form a "parasitophorous vacuole" and thus become enclosed by host cell membrane but outside of the host cell cytoplasm. It is theorised that this disruption of normal cell continuity creates a degree of malabsorption plus stimulating crypt cell hyperplasia causing diarrhoea. In extraintestinal locations, the organism acts similarly, causing focal cellular disturbance, destruction and loss of epithelial cells (Radostits, Blood and Gay 1994).

As the oocyst is immediately infective it is possible for the host to auto-infect with its own oocyst without requiring external oocyst passage (Page and Haddad 1995). *Cryptosporidium* is not host specific within bird

species but birds are not susceptible to mammalian Cryptosporidia (Doneley 1996).*Cryptosporidium* in healthy animals is usually self limiting and is considered as a secondary pathogen only (Doneley 1996, Clyde and Patton 1996). This parasite is mainly transmitted through contaminated water or feed but may also be introduced by inhalation (Page and Haddad 1995, Goodwin and Davis 1993). As a considerable number of oocysts can be passed by a single bird and are infective immediately, basic sanitation can be an important factor (McKeon 1995).

The parasite has a particular affinity for immunocompromised individuals such as juveniles or animals with concomitant diseases (Page and Haddad 1995) such as Psittacine Beak and Feather disease, Polyomavirus (Clyde and Patton 1996) and salmonellosis (Greve 1996). Apparent subclinical infection without oocyst shedding or much reduced shedding is common

(Patton 1993) and this allows perpetuation of infection within aviaries (Clubb 1997).
There is no evidence that immunity is developed, only that infection rates increase when birds become less immune competent.

2.5.2 - Diagnosis

Clinical signs are dependent on the site of infection. Generally , infected birds present with depression, anorexia, cachexia, dehydration, coughing, dyspnoea, diarrhoea but infection has also been associated with rhinitis, conjunctivitis, bronchopneumonia, air sacculitis and nephritis (Greiner and Ritchie 1994, Clubb 1997, Doneley 1996, Patton 1993, Goodwin and Davis 1993).

Oocysts may be evident in the faeces of infected birds but they are small (4um), potentially low in number, are often confused with yeast and may be absent from the droppings of birds without gastrointestinal infection (Page and Haddad 1995, Clubb 1997). Oocyst identification may be improved by sugar flotation of pooled samples or centrifugation of dilute faeces in high salt solution. The oocysts float in a slightly higher plane that coccidial oocyst on a simple wet mount examination (Patton 1993, Clubb 1997 , Greiner and Ritchie 1994) and have a slight pink glow under light microscope (Clyde and Patton 1996). Commercial immunofluorescent tests for mammalian Cryptosporidial cysts are unsuitable for avian use whilst ELISA tests were suitable in fixed, unfixed, fresh, refrigerated or frozen faecal samples (Mohan 1993). Modified acid- fast (stains oocysts pink on a blue background) and Indirect Fluorescent Antibody tests (IFA) are also useful for diagnosis (Page and Haddad 1995). Faecal smears can also be stained with Giemsa, Carbolfuschin and Periodic Acid Schiff (Greiner and Ritchie 1994).

Histopathology of gastrointestinal tissue must be provided fresh as rapid autolysis yields false positives (Clubb 1997). Fresh tissues may be collected into 2-5% aqueous potassium dichromate and will withstand refrigeration for nine months under these conditions (Goodwin and Davis 1993).

At post mortem, findings are related to site of infection. Respiratory infections can be sampled via transtracheal washes or touch imprints. Grossly, only excessive mucous is evident and this is due to displacement of respiratory epithelium over the primary and secondary bronchi, thus interfering with pulmonary clearance via the mucociliary elevator. The epithelium lining the the secondary bronchi may be hyperplastic in response (Goodwin and Davis 1993. Greiner and Ritchie 1994). The gastrointestinal tract presents as a dilated intestine containing thick, yellowish fluid.

Histopathology reveals multifocal infiltrates of lymphocytes and plasma cells in the lamina propria of the small intestine associated with villous atrophy, blunting and villous fusion (Patton 1993, Clubb 1997, Greiner and Ritchie 1994). Proventricular infection as seen in finches produces cuboidal metaplasia of the proventricular glandular epithelium (Greiner and Ritchie 1994). Renal infection shows grossly enlarged, pale firm kidneys that are uniform in appearance. The renal tissue shows the lumem of the kidney tubules containing sloughed epithelial cells and heterophils and regeneration and hyperplasia of the tubular epithelium. The interstitial tissue is infiltrated by plasma cells, lymphocytes, histiocytes, and heterophils (Gardner and Imes 1984).

2.5.3 - Treatment and Control

Treatment for Cryptosporidiosis is generally unrewarding. Some success has been found using the aminoglycoside, paromamycin sulphate at suggested doses of 165mg/kg b.i.d. for 5 days orally and 100 mg/kg mixed with soft food supplement for 5 days. It has been successful in reducing morbidity and mortality and produces repeatable negative antigen tests (Clubb 1997, Clyde and Patton 1996).

Another drug that has showed repeatable positive results for clearing Cryptosporidial infections in cats is spiramycin (J. Rand pers. comm). This is now available in a tablet form here in Australia in combination with metronidazole (Stomorgyl 2 tablets (Rhone-Merieux-Cyanamid Websters) - spiramycin 150000 iu and metronidazole 25mg) at a dose rate of 1 tablet per 2 Kg body weight. It would be interesting to trial this drug for its efficacy in avian Cryptosporidiosis.

Control of infection is otherwise hinged on strict sanitation to remove infective oocysts. Disinfectants of use include formaldehyde, 5% ammonia and heating to 65 ^{o}C for 30 minutes.

2.5.4 - Significance in Australia.

Cryptosporidiosis has not emerged as a significant disease in this country but this may be because it has been misdiagnosed or overlooked. It has been reported in ostrich (*Struthio camelus*) chicks in association with the so-called “Ostrich Fading Syndrome” (Doneley 1995). Commercialisation and importation of psittacines in Australia may encourage the development of pathogenic strains in this country.

2.6 - Giardiasis

2.6.1 - Epidemiology and Pathogenesis

Giardia is a simple binucleate flagellate with a large ventral sucking disc. It has a simple life cycle involving longitudinal binary fission in the small intestine , migration to the large intestine and formation of cysts that are shed in the faeces (Petrak 1969). Gastro-intestinal signs are due to massive attachment to the villi of the proximal gut by sucking discs of the parasites causing a competitive malabsorptive state involving fats, vitamins and proteins (Clyde and Patton 1996, Clipsham 1995). It is found predominantly in psittacines, but rarely in amazons, conures, cockatoos or macaws (Fudge 1985). Transmission is via faecal-oral route. *Giardia* species found in mammals are not pathogenic to avian species (Doneley 1996).

Immunity to infection is short lived only (Gallagher, Gartrell and Upcroft 1995). Infected animals are commonly nutritionally marginal, overcrowded or unhygenically kept (Doneley 1996). Outbreaks commonly follow introductions and stressors such as molting, incubation, and poor ventilation (Greve 1996).

2.6.2 - Diagnosis

Giardiasis has a number of sydromes associated with it. The major syndrome is one of chronic, recurrent diarrhoea that is mucoid, discolored and foul smelling combined with weight loss, vomiting, lethargy and anorexia (Petrak 1969, Clipsham 1995). Another syndrome that may be seen independently or in combination with gastrointestinal signs is one of excessive feather grooming, oily greasy feathers, feather picking and screaming, whole seeds in droppings, dry skin, pruritus, and bleeding feather quills. This latter syndrome is the result of malabsorption of fat soluble vitamins, riboflavin, essential fats, and proteins and it is suggested that also an allergic reaction to *Giardia* may be involved (Cannon 1996, Fudge 1985). A third syndrome of shifting leg lameness and rough feathering is associated with white muscle disease due to inability to absorb vitamin E (Clipsham 1995).

Microscopic evaluation of infection requires a refined technique to avoid false negatives. A single negative sample is not considered as diagnostic. A lightly concentrated, fresh smear taken from faeces less than ten minutes old should be examined under at least 20 high dry fields within three minutes of making the smear.

Giardial cysts are often confused with yeasts or urate crystals and are often overlooked. *Giardia* trophozoites have a characteristic gliding, "falling leaf" motion (see Appendix II) and two nucleii "eye spots" (Clyde and Patton 1996, Clipsham 1995).

The trophozoites are delicate organisms and if the substage diaphragm is nearly closed this will maximise contrast (Greve 1996). Standard sugar/salt flotation will distort cysts and destroy trophozoites. Cyst concentration and visualisation may be improved by Zinc Sulphate flotation or by gauze straining the sample and centrifuging the liquid at 1200 rpm for 10 minutes. The use of faecal ELISA is controversial and it is suggested that three zinc flotations will give better results (Clipsham 1995). Faecal trichrome staining of sample collected into polyvinyl alcohol or 5% formalin can be used to highlight trophozoites. Trophozoites will appear blue-green, nuclear material, erythrocytes and bacteria appear purple/ red and eggs and larvae red (Fudge 1985). Airdried faecal smears can be stained by flooding with carbol fuschin (from an acid fast kit) for 60 seconds, then rinse and air dry. The trophozoites and cysts will stain deep red (Dalhausen 1993).

Post mortem of infected adults shows the small intestine typically dilated and filled with a milky broth-like contents but normal mucosa.Infected chicks will have a distended crop as well (Greve 1996). A circulating eosinophilia or hyperproteinaemia may be seen but are not consistent (Greve1996).

2.6.3 - Treatment and Control

Therapeutic treatment alone is insufficient to control Giardiasis. Therapies include metronidazole (orally, in water or injectable), carnidazole, fenbendazole (50 mg/kg x 3 days), albendazole (10 mg/kg x 5 days). The nitroimidazoles may cause secondary Candidiasis by killing off competitive anaerobic gut flora (Clipsham 1995, Fudge 1985). Metronidazole will relieve pruritus within hours of administration . Improved nutrition, reduced stress, vitamin A, vitamin D3, vitamin B5 and vitamin E can all be utilised during treatment.(Fudge 1985). Relapses can be due to incomplete treatments, resistance, or environmental reservoirs. Sanitation is an important adjunct to treatment and limiting faecal access and the use of quaternary ammonia disinfectants to inactivate cysts is essential (Doneley 1996, Clyde and Patton 1996).

2.6.4 - Significance in Australia

Giardiasis in Australia , unlike in the United States is rarely diagnosed. Only one reported case in psittacines exists (Gallagher et al 1995). Considerable anecdotal evidence suggests that metronidazole-responsive dermatopathies do exist but these have never been positively associated with *Giardia*.

2.7 - *Haemoproteus*

2.7.1 - Epidemiology and Pathogenesis

Haemoproteus is the most commonly observed of the haemoparasites. The life cycle involves transmission by the bite of a *Culicoides* mosquito or a Hippoboscid fly. The life cycle is essentially the same as for *Plasmodium* but only exo-erythrocytic shizogony occurs and this takes place in the endothelial cells primarily of the liver, lungs and spleen. The gametocytes only are found in the erythrocytes (Rae 1995, Doneley 1996). The liver acts as a reservoir for the swimming merozoites prior to entry into erythrocytes (Tudor 1991). They may also be found in leukocytic and extraerythrocytic sites in heavily parasitised animals (Van der Heyden 1996). Haemoproteus is largely apathogenic but high parasitaemias of apathogenis strains can cause clinical problems if stressed or immunosuppressed (Greiner and Ritchie 1994). The exception is in pigeons, quail and seed eating passerines which may show high morbidities and mortalities. Infections in captive psittacines are rare except imported birds that acquire infection in their country of origin (Rae 1995). Deaths in Australia have occurred with aberrant infections involving massive skeletal myonecrosis due to production of megaloshizonts in Currawongs (*Strepera graculina*) and shizont formation in pulmonary capillary endothelial cells causing pulmonary infarcts in psittacines . Host specificity does occur but mainly at the Family level. For example, *Haemoproteus columbae* may infect 45 species of pigeon with the Bleeding Heart pigeon *(Gallicolumba luzonica*) being most severely affected (Tudor 1991, Rae 1995). Asymptomatic birds become carriers for life. A level of immunity is developed with repeated exposure (Tudor 1991).

2.7.2 - Diagnosis

Clinically infected birds show signs of anaemia, anorexia, depression and loss of feather gloss.

Diagnosis is best made by peripheral blood smears to examine pigmented gametocytes in erythrocytes. The gamont typically fills >50% of the cytoplasm and partially encircles the nucleus but rarely displaces the nucleus (see Appendix III). The pigment granules are coarse and appear yellow-brown when stained with Romanowsky's stain. Microgametocytes stain pale blue or pink and contain pigment granules in spherical masses whilst macrogametocytes stain blue and contain diffuse pigment granules. Diff Quik stain can also be used for identification of this organism (Rae 1995, Vogelnest 1991a, Van der Heyden 1996). Tudor (1991) suggests that at least 50 fields must be examined before a bird can be classified as negative for this parasite. Merozoites can be observed free in the blood by using an unstained, warmed, hanging drop slide. Parasitaemias involving >50 % of erythrocytes may be found in clinically normal birds (Vogelnest 1991a)

Post mortem specimens may show ventricular enlargement, hepatomegaly, splenomegaly, pulmonary oedema, myonecrosis or pulmonary infarcts (Vogelnest 1991a, Tudor 1991, Rae 1995). Impression smears of liver, lung, spleen and muscle may show mature shizonts in endothelial cells with the cells enlarged and containing numerous multinucleate bodies known as cytomeres (Rae 1995).

Biochemistry may show an increase in AST (Vogelnest 1991a).

2.7.3 - Treatment and Control

Treatment for *Haemoproteus* is not often attempted as it is considered as an incidental finding in many species. Therapy that has been used includes the use of chloroquine and primaquine to suppress erythrocytic stages but not merozoites, quinacrine daily for 3 months for both stages or ivermectin at a dose of 10 drops of 10mg/ml liquid orally but the clinical effect is strain dependent (Tudor 1991).

Control aims to reduce the level of insect vectors and the level of stress in infected birds (Tudor 1991).
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2.7.4 - Significance in Australia

Fatal clinical cases in captive psittacine birds (coastal Queensland) and wild birds in Australia suggests that we should be looking for this disease in cases of acute death with anaemia. Both vector species are common, particularly in the northern states and susceptible avian species are held in these areas. Peripheral blood smears should be examined in instances of sudden death in pigeons particularly Bleeding Heart pigeons (*Gallicolumba luzonica*).

2.8 - Hexamitiasis

2.8.1 - Epidemiology and Pathogenesis

Hexamita is an inhabitant of the duodenum and large intestine particularly caecum, caecal tonsil, and Bursa of Fabricius (Greve 1996, Clipsham 1995). This flagellate has a life cycle similar to that of Giardia with reproduction by binary fission and a cystic stage that is shed to the environment. Incubation periods have been recorded as 4-7 days and as few organisms are needed to establish infection, multiplication can be rapid (Tudor 1991). The organism is usually found in largest numbers in the cypts of Lieberkuhn where their presence induces an epithelial proliferation and mild inflammatory reaction. This results in a hypersecretory and malabsorptive state (Hooimeijer 1995). Classically it is recorded as a parasite of poultry, but outbreaks have also been recorded in psittacines (King Parrots (*Alisterus scapularis*), Scarlet Chested parrots (*Neophema splendida*), Cockatiels (*Nymphicus hollandicus*)) and pigeons (McKeon 1995, Greve 1996, Greiner and Ritchie 1994). Transmission is via the faecal oral route. Faecal cysts are infective for several weeks in moist conditions and warmth also favours survival (McKeon 1995, Tudor 1991). Adults will often harbour the organism in their large intestine and will shed organisms that then infect the small intestine of juveniles (or immunocompromised) which will show clinical signs. Age related resistance to clinical signs may develop (Greve 1996, Tudor 1991).

2.8.2 - Diagnosis

The clinical syndrome most often recognised is weight loss, unthriftiness, catarrhal enteritis, and foamy diarrhoea (often chronic) (Greiner and Ritchie 1994, Greve 1996, Harper 1991). In pigeons, a loss of flight performance is recorded as well as posture change, slowed crop emptying and a preference for small seeds (Harper 1991, Hooimeijer 1995). Infected birds remain fairly bright and maintain an appetite and vocalisations until shortly before death. Diagnosis should be based on wet smear examination of very fresh faeces or cloacal scrapings (Greve 1996, Tudor 1991).

Standard faecal concentration techniques prevent identification of *Hexamita* organisms or cysts (Hooimeijer 1995). *Hexamita* swims in a rapid , smooth, linear fashion and can be difficult to maintain in the field of view (Greiner and Ritchie, Tudor 1991) (see Appendix II).

Post mortem examination reveals duodenal dilation, catarrhal enteritis, foamy and watery diarrhoea, mucous covered faeces, and lack of intestinal tone (Harper 1991, Tudor 1991, Greve 1996). Deep intestinal scrapings may reveal trophozoites (Hooimeijer 1995) but these are often absent as they rapidly degenerate with autolysis (McKeon 1995).

As suggested above, histopathology suggests that *Hexamita* does not produce a true enteritis as organisms are found only in ther lumen and crypt surface of the duodenum (Greve 1996, Hooimeijer 1995). The diarrhoea may be more correctly thought of as a malabsorptive or hypersecretory in nature.

2.8.3 - Treatment and Control

Treatment is similar to that suggested for *Trichomonas*, with carnidazole being drug of choice (20 mg/kg body weight once) in combination with ronidazole in water for 7 days (Greve 1996, Clipsham 1995). Other drugs suggested include chlortetracycline and oxytetracycline plus furazolodine but efficacies are not known (Clipsham 1995). Environmental control is based on hygiene. Susceptibility of *Hexamita* to disinfectants is not known.

2.8.4 - Significance in Australia

Hexamita has been recorded in wild psittacines in Australia (Clipsham 1995) as well as being associated with a syndrome of fatal enteritis in King Parrots (*Alisterus scapularis*) . Otherwise, it is rarely diagnosed in Australia. It is probably more important to recognise that it can easily be misdiagnosed as *Giardia* due to its similar appearance.

2.9 - Leucocytozoonosis

2.9.1 - Epidemiology and Pathogenesis

Leucocytozoon has a world wide distribution with the exception of South America, Central America and the Carribean islands where insect vector species do not occur (Rae 1995). The natural intermediate hosts are Simulid flies or *Culicoides* mosquitoes (Tudor 1991). They have highest natural infections in waterfowl, nestling passerines, raptors, corvids, honeyeaters, and gamebirds but aberrant pathogenic infections have also been recorded in 10 genera of common psittacines and in the canary (*Serinus canarius*) (Vogelnest 1990a, Greiner and Ritchie 1994) . The life cycle begins with sporogony in the insect host and sporozoites migrate to the salivary glands of the insect to be deposited at feeding. The entering sporozoites undergo shizogony in endothelial cells, hepatocytes and cells of the heart, kidney, slpeen and brain resulting in cellular destruction. Megaloshizonts may form in macrophages and lymphoid cells in various organs. Development of unpigmented gamonts in erythrocytes occurs after this stage only (Rae 1995). The prepatent period from entry to the appearance of erythrocytic gametocytes is 4 -9 days. The gamonts within erythrocytes produce anti-erythrocytic factors that cause intravascular haemolysis and anemia (Greiner and Ritchie 1994). Aberrant infections occur when reticuloendothelial cells deposit merozoites in parenchymatous organs and megaloshizonts form without forming gametocytes in circulating erythrocytes (Rae 1995, Page, Greiner and Schmidt 1987). Walker and Garnham (1972) suggest that psittacine erythrocytes are naturally immune to parasite invasion.

2.9.2 - Diagnosis

Clinical signs of *Leucocytozoon* infections include visible hepatomegaly, dehydration, anorexia, depression., haemoglobinuria , haemolyic anaemia and acute death (Rae 1995, Greiner and Ritchie 1994).

Peripheral blood smears will allow definitive identification of erythrocytic forms. Two types of gamonts are recognised. The first is an elongate form with the host cell nucleus pushed to the side and the cytoplasm with long tapering tails. This form does not occur in Australia.

The second form is round with an eccentically located host cell nucleus and reduced to absent cytoplasmic tails. The host cell is usually distorted beyond recognition (Vogelnest 1990a, Van der Heyden 1996) (see Appendix III). Remember that these will be absent from aberrant infection.

Post mortem specimens have hepatomegaly with rounded edges and multiple pale tan, red and black raised areas, splenomegaly, pulmonary congestion, pericardial effusion (gelato-sanguinous), thickened and haemorrhagic pericardial sac, myositis and myodegeneration of skeletal and cardiac muscle, epicardial haemorrhage, thickened miliary nodules and multiple haemorrhages of the gizzard and heart muscle, and free thoracic and coelomic blood (Walker and Garnham 1972, Page, Greiner and Schmidt 1987, Fowler and Forbes 1972).
Histpathology reveals phagocytosed merozoites in parenchymatous organs, hepatocytes slightly swollen and vacuolated, and megaloshizonts in a “Bamboo shoot” pattern. Merozoites produce a gram negative staining reaction (Walker and Garnham 1972, Page, Greiner and Schmidt 1987).

2.9.3 - Treatment and Control

Coccidiostatic drugs, sulphaquinoxaline and pyrimethamine have been used as an attempt at prevention and treatment of this parasite but success has not been described (Rae 1995). Control should target the avoidance of vectors and their breeding sites (running water) (Doneley 1996).

2.9.4 - Significance in Australia

Most *Leucocytozoon* infections recorded in Australia have been incidental findings in wild birds. Those psittacine birds that have succumbed to aberrant infections were mainly native Australian species and therefore there is potential for infection if both the host, correct parasite species and vector species occur together.

2.10 - Sarcocystosis

2.10.1 - Epidemiology and Pathogenesis

Sarcocystis utilises a predatory animal as its definitive host and prey animal as its intermediate host. Classically, the American opossum (*Didelphis virginianum*) has been the definitive host with various native American bird species acting as intermediate hosts. When the opossum comes in contact with a naive intermediate host population (captive birds), clinical disease has been recorded . An infective oocyst is shed in the faeces of the predator and is ingested by the prey species either directly or via an arthropod transport host. The sporozoites are are released in the small intestine and invade the hosts tissues. A first stage shizogony occurs in the endothelial cells of arterioles and a second stage shizogony occurs in the endothelial cells of cappilaries or venules within organs. If the host survives cellular damage associated with these shizogonous stages then the meronts produced will form muscle cysts containing bradyzoites.

Consumption of this host by a predator will release the bradyzoites under the influence of proteolytic enzymes in the predators intestine. These invade the predators intestine and produce oocysts (Doneley 1996, Clubb et al 1986). Transport hosts such as cockroaches will feed readily on animal faeces (Hillyer et al 1991). Sarcocysts start to develop approximately 2 weeks after oocyst ingestion and induce little pathology except a slight interstitial myositis. All muscle tissues develop cysts at the same time but cardiac and pectoral muscle cysts degenerate after about 2 months (Bicknese 1993). Male psittacines are seen to become affected more often but usually their mates will succumb at a later date (Hillyer et al 1991).

The most susceptible hosts are naive hosts that originate from areas where *Sarcocystosis* is less prevalent in the wild state. This corresponds with the species of Old World psittacines and columbiformes (Bicknese 1993). Ten species of Australian psittacine have been diagnosed with Sarcocystosis infections in American collections (Hillyer et al 1991). Within Australia, several species of predatory owl, falcon and Elapid snakes have been recorded to produce Sarcocystis oocysts and many species of Australian bird (including psittacines and passerines) have been found to harbor sarcocysts. Definitive hosts within Australia could potentially be fulfilled by predatory birds, snakes and potentially Dasyurid marsupials (Munday et al 1979).

2.10.2 - Diagnosis

Numerous clinical syndromes have been associated with *Sarcocystis* infection. The pulmonary form is the most common and results from the initial endothelial shizogonies. Clinical signs usually include peracute death, or if observed just prior to death, severe dyspnoea, yellow urates, depression, watery fluid or fresh blood from the mouth and neurological abnormalities may be seen (Hillyer et al 1991, Clubb et al 1986, Page et al 1992). The muscular form is a response to cyst formation and can show acute onset weakness of wings and legs , ataxia, mild to moderate wasting and sudden death due to myocarditis induced mechanical failure of the heart (Page et al 1987, Bicknese 1993). The encephalitic form is as a direct result of cyst formation within the brain and produces posterior or unilateral paresis, intention tremor and head tilt . Haematology and biochemistry in all forms show an increased AST (>1900 U/l), increased ALT (>1900 U/l), increased CPK (>3200U/l), increased LDH (>3500 U/l), increased WCC (> 20000) and an increased uric acid (~15mg/dl) (Bicknese 1993, Page et al 1992).
Radiographically there may be a homogenous increase in lung density and mild to moderate increase in the size of the liver, spleen and kidney (Page et al 1992).
On post mortem, specimens showed signs consistent with a septicaemia such as pulmonary oedema, splenomegaly, hepatomegaly with mottling and hyperaemia, minimal weight loss, and gross myopathy but bacterial cultures are persistently negative (Clubb et al 1986, Hillyer et al 1991). Muscle cysts may be visible as small, yellow-white areas 4mm long and <0.5mm wide in the muscle of the leg and pectoral region and may be seen through the intact skin. Cardiac cysts are microscopic only. Blood smears may show crescent shaped “spores” resulting from cysts rupture as the blood was taken (Petrak 1969). If the suspected definitive host is available, oocysts may be extracted from the intestinal submucosa by administration of a hypotonic solution to lyse intestinal cells and release oocysts (Hillyer et al 1991). Lung impressions are consistently positive for free shizonts. They appear as light blue with a dark purple central or subcentral nucleus in a background of monocytes and heterophils when stained with modified Wright’s stain. (Hillyer et al 1991) .They may also be identified from impression smears of lung, liver and spleen using Wright’s-Giemsa with which meronts appear as extracellular, fusiform, light blue with a dark purple nucleus (Bicknese 1993).

Histopathology shows shizonts in lung capillaries, severe interstitial and alveolar serofibrinous and lymphocytic-histiocytic effusion, Reticuloendothelial cell hyperplasia, myocardial degeneration, myocardial vacuolation and myocardial haemorrhage, severe portal inflammation and severe interstitial inflammation of the kidneys (Clubb et al 1986, Page et al 1982, Hillyer et al 1991)..

2.10.3 - Treatment and Control

Therapy for treatment of *Sarcocystis* has been unrewarding with few successful treatments being achieved. Pyrimethamine (25mg tablets) at 0.5 mg/kg body weight per os b.i.d. (25mg tablet in 21 ml water and 4 ml KY Jelly) and trimethoprim- sulphadiazine at 30 mg/kg for 30 days has been successful. Success is monitored by reduction in serum liver and muscle enzymes and lack of cysts in serial muscle biopsies. Relapses may occur and are monitored in the same manner (Doneley 1996, Page et al 1992).

Environmental control should concentrate on avoiding access to predators faeces or transport hosts carrying the oocyst.

2.10.4 - Significance in Australia

Sarcocystosis has not been described as a clinical entity in captive birds in Australia but it may potentially exist as we have both the intermediate host species and a number of definitive host species. Over 40 wild bird species in Australia have been found to contain sarcocysts with an average incidence in one study of 12.6 % . Shedding of oocysts by potential definitive host species was seen at an incidence of 28 - 40 % (Munday et al 1979). This indicates the potential for infection of captive birds in Australia. The species of *Sarcocystis* in Australia may not cause the pathogenic pulmonary effects of those seen in the United States. Zoological collections here in Australia may be more at risk with both definitive and intermediate hosts living on the same premises as suitable transport hosts.

2.11 - Toxoplasmosis

2.11.1 - Epidemiology and Pathogenesis

Toxoplasma gondii affects all species of birds and mammals. The cat is the definitive host and the source of all infections, directly or indirectly. Cats shed infectious oocysts in thei faeces. Birds act as intermediate hosts following ingestion of the oocyst or an invertebrate harbouring the oocyst. Birds either harbor actively multiplying tachyzoites and/or bradyzoites in resting cysts. The life cycle is completed if the bird is eaten by a cat. It is the multiplying tachyzoites that most commonly cause clinical signs in birds. The tissue cysts become a problem if they are located in a position causing pathology (Patton 1995). Free tissue forms can potentially be passed in crop milk thus infecting pigeons in the nest (Tudor 1991). Stress of transportation may reactivate latent infections (Macwhirter 1994). Antibodies may limit circulating blood organisms. Less virulent forms will encyst more readily as immunity increases (Tudor 1991).

2.11.2 - Diagnosis

Clinical signs associated with Toxoplasmosis are more often associated with passerines but also with a number of psittacine species. Affected birds may present with acute death, anorexia, prostration, weight loss, diarrhoea, dyspnoea, crusty lesions around eyes, white lesions in vitreous humour, bilateral cataracts, collapsed eyeballs, conjunctivitis, blepharitis, ocular atrophy, chorioretinitis, circling, head twitching, blindness, nystagmus and ataxia.
Canaries (*Serinus canaria*) and closely related finch species are more likely associated with ocular and neurological signs (Howerth et al 1991, Parenti et al 1986, Vickers et al 1992, Lindsay et al 1995)

Antemortem diagnosis is complicated in birds. Most surveys of *Toxoplasma* underestimate the parasites avian prevalence (Orosz, Mullins and Patton 1992). This is because of unpredictable seronegativity in the presence of infection based on mammalian tests particularly the Sabin-Feldman Dye Test). Most birds do not fix the first part of complement therefore testing should begin with undiluted serum to compensate for incompatabilities between sera complement of birds and mammals (Frenkel 1981). Pigeons are the exception and the dye test works well in these birds (Tudor 1991). Alternatively, avian tissue may be passaged through mice first and the mice diagnosed by seroconversion or parasite identification. (Frenkel 1981).

The modified agglutination test (MAT) with formalin fixed tachyzoites as antigen is sensitive and specific for detecting specific T.gondii antibodies (IgG). Agglutination titres of greater than twenty indicate infection. Unfortunately it is relatively unavailable, even in the United States. The commercially available latex agglutination test is not sensitive enough to be reliable for avian use. ELISA tests are sensitive but must be developed for each individual species. If a toxoplasma titre is recognised, it should be assumed to have an existing infection or prexeisting latent cysts. A four-fold increase indicates an active infection (Patton 1995). Gross pathology of infected birds may reveal hepatomegaly, pulmonary consolidation, muscular atrophy, myositis, serous and fibrinous serositis, splenomegaly, haemmorrhagic pneumonia, renal congestion, frank haemmorhage and catarrhal enteritis (Howerth et al 1991, Parenti et al 1986).

Histopathology is complex. It may include lesions associated with the presence of tachyzoites or immunological reactions to the presence of cysts. Cardiac changes include multifocal necrotising myocarditis, fragmentation of muscle fibres, mononuclear infiltration, and non-purulent pericarditis (Howerth et al 1991, Parenti et al

1986). Visceral organ changes include interstitial pneumonia with multifocal necrosis and vasculitis , disseminated massive pulmonary haemmorhage, multifocal necrotizing periacinar hepatitis, hepatic necrobiosis, splenic necrobiosis and renal necrobiosis. Ocular changes include keratitis, corneal ulceration, corneal perforation, non-purulent granulomatous inflammation of the vascular tunic, retinal atrophy, non-suppurative inflammation of choroid, retinal detachment, and demyelination of optic nerve. Brain lesions include mononuclear perivascular cuffing away from the sites of cysts, local and disseminated chronic inflammation around tachyzoites , scattered foci of non-suppurative meningoencephalitis with gliosis, and patchy increased prominence of cerebral blood vessels associated with hypertrophy of vascular endothelium and thickening of connective tissue walls (Vickers et al 1992, Howerth et al 1991, Parenti et al 1986)

2.11.3 - Treatment and Control

Therapy for toxoplasmosis requires early treatment to be successful. Some successful therapies include pyrimethamine (40 mg per litre water) for six days, trimethoprim (0.08g/ml) and sulphadiazine.(0.4 g/ml) for 14 days or pyrimethamine (0.5 mg/kg) (Lindsay et al 1995, Parenti et al 1986, Doneley 1996). Clindamycin has been used successfully to treat toxoplasmosis in cats (Doneley - pers. comm.), humans and wombats (*Vombatus ursinus*) (Booth 1994). Its efficacy against avian toxoplasmosis is worthy of further study. Control involves restricting access of birds to cat faeces in feed or water or to transport hosts that may have contacted cat faeces. *Toxoplasma* is inactivated with strong iodine, ammonia, dry heat (70^{o}C) and boiling water.

2.11.4 - Significance in Australia

Toxoplasmosis in cat is found in relatively levels throughout most of Australias cities. The potential for exposure of birds is therfore significant. Identified cases of Toxoplasmosis in birds may be reduced due to diagnostic difficulties with available serological testing.

2.12 - Trichomoniasis

2.12.1 - Epidemiology and Pathogenesis

Trichomonas sp. is a flagellate protozoan found in the gastrointestinal tract from the pharynx and crop to the level of the proventriculus and occasionally in the small intestine (Doneley 1996). Extraintestinal parasitism has been recorded with lesions in the liver, orbit, brain, pancreas, sinuses (Narcisi, Sevoian and Honiberg 1991), heart, air sacs (Ramsay , Drew, and Johnson 1990) and tracheal bifurcation (Garner and Sturtevant 1992). It is an obligate parasite, surviving poorly in the environment. Reproduction in by simple longitudinal binary fission and the mature infectious form is known as a trophozoite.(McKeon 1995). *Trichomonas* is potentially pathogenic to all bird species but some species are more susceptible to infection (Wood, Chin and Barr 1987). Different avian species are susceptible to different *Trichomonas* species and strains and this is evident in the different sites of infection seen. Columbiformes, falconiformes and psittacines are commonly infected but passerines and galliformes may be infected if in close proximity to other infected bird types (Greiner and Ritchie 1994, Wood et al 1987)).

The parasite invades the epithelial mucosal cells causing a massive inflammatory response primarily involving heterophils (Tudor 1991). The extent of the lesions is dependent on the strain of the organism with velogenic strains causing diptheritic membranes with moist centres and deep tissue lesions and associated with high mortality, mesogenic strains cause caseated abscesses of the upper gastrointestinal tract and oropharynx and lower mortality, and lentogenic strains which may show visible lesions. The deep tissue lesions (e.g. liver) may occur under conditions of stress, overcrowding and poor sanitation (Clipsham 1995). These occur as a result of infiltration of the organism into ulcerative lesions and into the systemic circulation where they are circulated (Narcisi et al 1991).

Transmission is primarily via freshly contaminated food or water or infection may be transfered directly to neonates by parental feeding or to mating partners during courtship feeding (McKeon 1995, Clyde and Patton 1996). The existence of this organism in a primarily pre-proventricular site renders faecal contamination less important than oral mucosal contamination (McKeon 1995).

Immunity appears to be both age and strain related. recovery from sublethal strains appears to enable protection from more virulent strains. Persistent low grade exposure will also develop an immunotolerance to this parasite. It is not known if immunity is lost with loss of exposure to the paarsite. Plasma from immunotolerant pigeons will protect other pigeons from more virulent strains (Tudor 1991).

2.12.2 - Diagnosis

Trichomoniasis has been recognised clinically for centuries. It is commonly referred to as Canker in pigeons and as Frounce in birds of prey. In adult and young pigeons, the common clinical presentation is a depressed, lethargic, inappetant bird with stringy, grey, turbid mucous in the oral cavity, a putrid oral odour and variable, circumscribed, caseous and diptheritic lesions on the oral mucosal surfaces (Tudor 1991, D.Brown pers. obs). In psittacines and other birds, the syndrome may be one of retching, vomiting, rapid head flicking, nasal discharge, respiratory distress, frequent nibbling, stained and wet facial and crown feathering, weight loss, repeated molting, reduced breeding results, reduced fertility, reduced chick growth, acute death, caseous oral masses, green diarrhoea and asphyxiation (McKeon 1995, Ramsay et al 1990, Baker 1986, Clyde and Patton 1996, Greiner and Ritchie 1994) .

Diagnosis is by examination of oral or crop fluids or caseous lesions for the presence of trophozoites. The sample for a wet examination should be as fresh as possible and should be warmed for examination. Refrigerated specimens may yield false negatives as recovery of organisms at 5-10 ^{o}C is less than 25% after 2 days and presumably considerably less at lower temperatures (McKeon 1995, Petrak 1969). False negatives in fresh crop samples may also occur due to the parasite being intracellular and not free in the crop lumen (Baker 1986). Standard flotation techniques will destroy Trichomonads (Greiner 1989). The organism appears as circular to oval, motile , tumbling organism moving in a jerky fashion by the use of its four flagella (Vogelnest 1991b) (see Appendix II). Stained dried smears or impressions of crop samples or post mortem material may be useful for delayed diagnosis. Wrights, Iron Haematoxylin, Bodia Silver impregnation and Schoudin's staining may be used for trophozoite identification (Clipsham 1995).

Haematology shows no significant white cell changes but a tendency for hypochromia (Henderson, Gulland and Hawkey 1988).

Post mortem examination classically reveals yellow nodular deposits on the mucosal surface of the oropharynx, oesophagus and crop with ulceration of the mucosal surface under these lesions. A clear, watery to viscous, grey mucous is present in variable amounts over the lesion and surrounding lumenal spaces (Ramsay et al 1990, Baker 1986). Lesions in pigeons appear to be primarily of diptheritic strains whilst Budgerigars (*Melopsittacus undulatus*) showed primarily proliferative and inflammatory changes with a sex predelection for females (Baker 1986). Caecal *Trichomonas* occurs in gallinaceous birds with weight loss and foamy yellow droppings being seen predominantly in 2-3 week old birds. The caeca is filled with frothy yellow-brown faeces on post mortem (Clyde and Patton 1996).

Histopathology of simple cases reveals proliferative tissue containing numerous Trichomonad parasites, mucosal necrosis, mixed cellular infiltrates and ulcers with massive infiltrates of heterophils and histiocytes. The mucosae is greatly thickened and folded with large cells throughout and much ballooning near the fold tips. The mucosae is thickened from its normal 8-10 cells thick to 20-30 cells thick. Apical cells contain abundant cytoplasm and indistinct cell walls. Squames are rare. Parasites will be found in the lumen, in high numbers in the folds and intracellularly (Ramsay et al 1990, Baker 1986).

In cases of extra-intestinal *Trichomonas* due to highly virulent strains, histopathology in pigeons shows vascular congestion, epithelial sloughing, submucosal oedema and perivascular cuffing as early as four days post infection. By seven days post infection, a decrease in abdominal fat, hepatomegaly, slpenomegaly, fatty degeneration are evident. Eight days post infection reveals Trichomonads in necrotic hepatocytes (delineated by a wall of leukocytes, mainly granulocytes and occasional giant cells), fibrinous exudate over the liver and heart, follicular degeneration of the ovary and evidence of decreased spermatogenesis (Narcisi et al 1991). Important differential diagnoses to consider are hypovitaminosis A, avian pox virus lesions, salmonellosis, candidiasis (McKeon 1995) and oesophageal capillariasis (D.Brown pers. obs)

2.12.3 - Treatment and Control

The most important feature of treatment of Trichomonad infections is the variability of response to drugs shown by different strains with different resistance features. The primary drug type used is the group known as Nitroimidazoles containing the drugs metronidazole, carnidazole, dimetridazole and ronidazole. All of these drugs have been shown to be susceptible to failure due to resistant strains. The problem lies is the reliance on in vitro established dose rates which have often proven to lack cytocidal efficacy in vivo. Resistance to one drug (particularly carnidazole) may allow the parasite cross resistance against other drugs. Resistance to these drugs is only demonstrated by the parasite when in an oxygenated environment (Franssen and Lumeij 1992). Changes in environmental oxygen status within the avian patient by changes in pH or diffusion gradients may provide a means of targeting these resistant strains. Multiple resistances have been determined in Australia particularly in pigeons It has been suggested that ronidazole at 2-3 times the recommended dose be used in these situations (Doneley 1996, D.Brown pers. obs.). Preventative treatment on a regular basis by laypersons may enhance development of resistance especially if dosages are not followed closely. Franssen et al (1992) suggests that the only adult treatment that should be justified is in severe illness and during incubation to reduce resistance problems. Racing pigeons harboring resistant strains will show reduced performance and owners will often demand regular treatments to improve performance. It is often found that these flocks are harboring multiple subclinical infections that reduce immunocompetence and hence allow regular relapses of infection. Relapses are also common following cessation of treatment in psittacines (Clyde and Patton 1996). Treatment is best carried out by direct oral administration of the drug but in a flock situation , in-water medication may be the only option available. In-water medication has the disadvantages of poor palatability, variable water intakes, settling out, species intake differences, variable weather and toxicities (Clipsham 1995).Advanced cases with large necrotic foci are generally difficult to treat and have a guarded to poor prognosis (Greiner and Ritchie 1994) Strategic treatment may be implemented before breeding, weaning and before racing (Doneley 1996).

Supportive treatment involves treatment of secondary bacterial infections, supportive feeding, and removal of diptheritic plaques (Doneley 1996). Additional control methods may involve culling of repeatedly affected birds, evaluation of the presence of lentogenic strains to competitively inhibit velogenic/mesogenic strains, improved sanitation, quarantine, hyperimmune plasma and strategic treatment implementation (Doneley 1996, Clipsham 1995, Tudor 1991).

2.12.4 - Significance in Australia

Unlike the situation in the United States, *Trichomonas* is a very common pathogen of pigeons, psittacines (particularly the Budgerigar (*Melopsittacus undulatus*)) and to a lesser extent passerines. This is commonly seen as a flock problem and multiple resistance problems are faced with therapeutic treatment of some strains. It must be differentiated from Candidiasis which is also relatively common an a regular sequelae of prolonged antimicrobial therapy in these problem flocks.

DISCUSSION

The preceding review of the significant protozoal parasite species occurring in Australia reveals the paucity of information available on some aspects of avian protozoology. Considerable effort is still required to determine important facts regarding safe and efficacious therapeutic regimes and antemortem diagnoses. Clinicians may easily play a role in trialing of potential therapeutics and presenting the results of these trials to others. It appears possible that the majority of failed diagnoses in the avian species are as a result of not looking rather than poor access to diagnostics. Avian practitioners need to retrain themselves to use simple ,but often forgotten, diagnostic tools such as blood smear evaluation and the use of staining techniques for specific determinations of parasites. Use of specific protocol for identification of certain species, such as *Giardia*, may reduce the incidence of false negative diagnosis in cases where clinical signs and response to treatment are suggestive that the pathogen is in fact present. Further investigation using these techniques may further elucidate the existence or otherwise of protozoal diseases that have a significant potential to exist in this country. Extrapolation of therapeutics and diagnostics from other species provides an important source of information especially with emerging diseases that are seen in both birds and animals such as *Cryptosporidia.* Improved maintenance of avian collections and implementation of more preventative medicine programs has reduced the incidence of some previously common diseases (e.g. nematode infestations) and this may be leading to increased prevalence

and diagnosis of previously less important differentials such as cochlosomiasis.

Differences seen between Australian protozoal syndromes and those seen in other countries are, at the very least, complex. *Trichomonas* and *Giardia* appear to replace each other geographically in terms of pathogenic prevalence. Why two cosmopolitan parasite species behave so differently in different avian populations is uncertain, but worthy of further study.

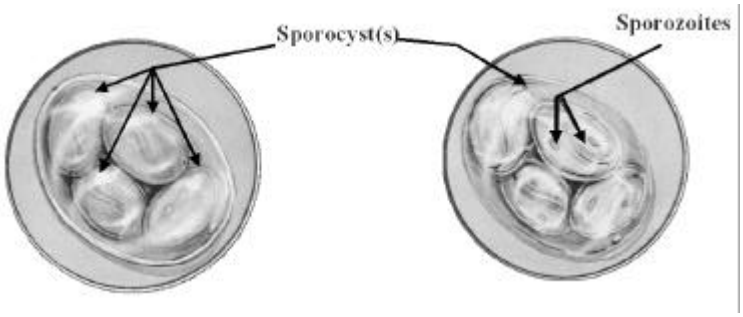
The potential risks for fatal outbreaks in captive specimens from parasites found in endemic native populations has been demonstrated in the United States with *Sarcocystis*. This, and other protozoal infections occur in our wild bird populations but are yet to be diagnosed as captive clinical syndromes. Interactions between veterinarians and wildlife rehabilitators in this country may be needed to develop an idea of the true prevalence of clinical casaes in these wild populations and the likely sources of pathogens for captive avians.

This review has outlined some of the major protozoal problems currently existing or emerging in this country. Simply making avian practitioners aware of these parasites should increase the likelihood of them being diagnosed when present. There is still a lot to learn about many of these paarsites and there interactions with their avian hosts. It is the responsibility of avian veterinarians to make a concerted effort to find out more through experience, trial and error.

CONCLUSION

Several species of protozoal parasites occur in Australia. Many of these form a significant proportion of some of the most commonly occurring pathogenic conditions seen in Australian avian veterinary practice. The importance of some of these parasites are however in complete contrast to the importance of these parasites in other countries. The result of this is that literature from other countries may not be valid for Australian conditions. This review has brought together available information and applied it to the syndromes seen under Australian conditions.

Appendix I - Coccidial Identification



Eimeria spp

GENUS	SPOROCYSTS	SPOROZOITES
Eimeria	4	2
Isospora	2	4
Wenyonella	4	4
Tyzzeria	-	8
Cryptosporidium	-	4

Appendix II - Flagellate Identification

CHARACTERISTIC MOTION OF PATHOGENIC FLAGELLATES

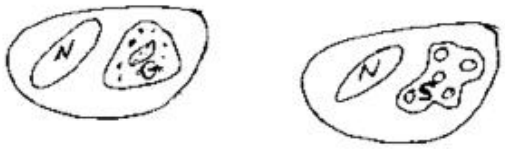
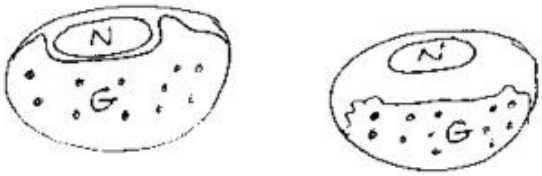
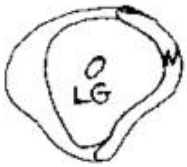
A - *Trichomonas* -Tumbling, jerky motion

B - *Giardia* - Smooth, gliding, "falling leaf"motion

C - *Hexamita* - Direct, fast, erratic motion

D - *Cochlosoma* - Direct, fast, with horizontal rotation

Appendix III - Haemoparasite Identification

A - *Plasmodium* gamont (G) and shizont (S) in avian erythrocyte	
B - Two forms of *Haemoproteus* gamont (G) in avian erythrocyte	
C- *Leucocytozoon* gamont (LG) compressing host cell nucleus (N) in avian erythrocyte	

(From Vogelnest 1990a)

Appendix IV - Drugs Mentioned in the Text

DRUG NAME	TRADE NAME	MANUFACTURER
Albendazole	Valbazen Rycoben Proftril	Smithkline-Beecham Young's Animal Health Smithkline-Beecham
Amprolium and Ethopabate	Coccivet Amprolmix Plus	Vetafarm MSD-Agvet
Carnidazole	Spartrix	Boehringer-Ingleheim
Chloroquine Phosphate	Chlorquin	Fisons
Chloroquine + Primaquine	Aralen	Fisons
Chlortetracycline	Aureomycin Tricon	Cyanamid Apex
Clindamycin	Antirobe	Upjohn
Diclazuril	Clinacox	Smithkline-Beecham
Dimetridazole	Emtryl Soluble	May and Baker
Dinitolmide	DOT Premix	Rhone-Poulenc
Fenbendazole	Panacur	Hoescht Animal Health
Furazolidine	Neftin	Pfizer
Ivermectin	Ivomec	MSD Agvet
Lasolacid Sodium	Monteban Aratec	Elanco Roche
Maduromycin	Cygro	Cyanamid
Metronidazole	Flagyl-S	Rhone Poulenc
Narasin	Maxiban	Elanco
Nicarbazin	Carbigran 250	Elanco
Nitrofurazone	Furacin	Smithkline-Beecham
Oxytetracycline	Oxymav -B Terramycin Engemycin	Mavlab Pfizer Intervet
Paromamycin Sulphate	Humatin	Parke Davis

Appendix IV - Drugs Mentioned in the Text (continued)

DRUG NAME	TRADE NAME	MANUFACTURER
Primaquine Phosphate	**Primaquine**	**Sinofe-Winthrop**
Pyrimethamine	**Daraprim**	**Burroughs Welcome**
Pyrimethamine + Sulphonamide	**Maloprim**	**Burroughs Welcome**
Quinacrine	**Atabrine**	**Winthrop**
Ronidazole	**Ronivet-S**	**Vetafarm**
Salinomycin	**Coxistac**	**Pfizer**
Spiramycin + Metronidazole	**Stomorgyl**	**Rhone-Merieux-Cyanamid**
Sulphachlorpyridazine	**Vetisulid**	**Solvay**
Sulphadoxine + Pyrimethamine	**Fansidar**	**Roche**
Sulphaquinoxaline	**Sulphaquin**	**Inca-Flight**
Toltrazuril	**Baycox**	**Bayer**
Trimethoprim + Sulphadiazine	**Tribrissen** **Trimazol**	**Coopers** **Ilium**

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