

AN ACUTE FATAL ILLNESS IN OLD WORLD PSITTACINE BIRDS  
ASSOCIATED WITH SARCOCYSTIS FALCATULA OF OPOSSUMS.

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An acute fatal illness was observed in an outdoor breeding collection of psittacine birds in southern Florida. Protozoal meronts were found in the lungs of affected birds. An opossum infected with *Sarcocystis falcatula* was trapped on a farm where the disease had occurred. Feces from this opossum were fed to cockatoos reproducing the disease.

In this report we describe the sarcocystis infection in psittacine birds including clinical signs, species susceptibility, gross and microscopic post mortem lesions, clinical pathology, and transmission.

#### HISTORY

During a four year period an acute fatal illness was observed in a breeding collection of psittacine birds. The collection consisted primarily of new world psittacine species however deaths were predominately in old world species. Intensive attempts to uncover bacterial, fungal or viral pathogens were inconclusive. Histopathologic examination revealed protozoal schizonts (meronts) in the lungs. The initial identification of the organism as *Toxoplasma* was ruled out on morphologic grounds, inability to subinoculate, and absence of antibody in mice fed tissues from affected birds. An intensive search for the identity and source of the organism was undertaken. A second outbreak which occurred at another psittacine facility approximately 12 miles away was also studied.

Heaviest losses were usually in the winter, (especially in February) at one farm, but mostly late fall at the second. Deaths occurred sporadically year round. Many of the affected birds had

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The life cycle of what was originally *Sarcocystis debonei* was reproduced in the laboratory by Box et al. Because the organism was found to infect species of passeriform, psittaciform and columbiform birds it was synonymized with *S. falcatula*, Styles 1893. Naturally infected grackles were fed to opossums from which sporocysts were recovered and fed back to grackles resulting in the

*Sarcocystis* are coccidia which have an obligatory two host life cycle, alternating between predator and prey hosts. The predator, in this case opossums, serve as the definitive host. The prey, grackles (*Cassidix mexicanus*, *Quiscalus quiscula*) or cowbirds (*Molothrus ater*), serve as the intermediate hosts. 2,6

## LITERATURE REVIEW

The disease has been diagnosed in birds from at least five separate premises in Dade, Broward and Palm Beach Counties, in Florida. One author (JKF) has observed similar protozoa in tissues of psittacines from southern California. Another author (DLG) has observed similar cases in birds from Texas.

An opossum (*Didelphis virginiana*) was trapped at the second facility and found to be shedding *sarcocystis* sporocysts. The acute fatal illness was reproduced when feces from this opossum were fed to cockatoos. Cockatoos were proven to be possible carriers when feces from the infected opossum were fed to cockatoos, which were subsequently fed to cockatoos, which died of the disease on days 10 and 14 post inoculation. Infected opossums were also found on the first and third premises where the syndrome had occurred.

Tentative identification of *Sarcocystis* sp. was made from the appearance of meronts in lung tissue by electron microscopic examination. Attempts to reproduce the life cycle of other similar protozoal species were unsuccessful. A subsequent search for the carrier host shedding sporocysts, and the means of transmission, included sampling water sources, feed and utensils, soil, wild birds, mammals including rodents, and reptiles, for the presence of oocysts. Numerous attempts to reproduce the disease in the laboratory by direct feeding or inoculation of tissues from affected birds to other birds or mammals were unsuccessful.

Large as opossums. were stored in closed areas which were inaccessible to mammals as flights where opossums had no access to food supplies. All feeds which were elevated off the ground on poles or in wire enclosed year, but adults were rarely affected. Most cases occurred in cages observed in 1 to 2 % of the nestlings of new world species each residence at the second facility. The acute fatal illness was also illness. One greater sulphur crested cockatoo died after 38 years been in residence at these facilities for many years without



development of muscle cysts. 1,2,3,4,5,6,7

Spindle shaped muscle cysts from infected birds, measuring up to 0.5 by 9.0 mm, contain bradyzoites which measure 6.5 by 2.1  $\mu$ m. Bradyzoites are released from the cysts by proteolytic enzymes in the opossum's small intestine. These bradyzoites penetrate the lamina propria where sexual reproduction takes place producing oocysts, measuring 10.7 x 13.9  $\mu$ m, which sporulate in the lamina propria. Infectious sporocysts which measure 11-12  $\mu$ m by 7-8  $\mu$ m, are shed in the feces in small numbers over an extended period of time. 7

Sporocysts are ingested by the intermediate host (bird) with a possible mechanical carrier such as cockroaches. Sporozoites are released from sporocysts in the intestine of the bird and invade many tissues. Schizonts then pass through two asexual replicative phases in blood vessels. The first stage in endothelial cells of arterioles and the second in endothelial cells of capillaries or venules in most organs of the intermediate host, releasing meronts which develop into typical muscle cysts. 7,9

Mammalian hosts of *Sarcocystis* species studied to date are generally limited. In laboratory experiments which included cats, rats and a dog, only the opossum was found to be a suitable definitive host for *S. falcatula*. Opossums that ate infected cowbirds and grackles had prepatent periods of 5 to 10 days and remained patent for at least 90 to 105 days. 3,4

Ernst et al. mentioned the presence of feathers of a brown headed cowbird in the intestinal contents of an opossum indicating that birds are a part of its diet. 11

Box et al. further investigated the intermediate host spectrum of *S. falcatula* by feeding sporocysts to birds of four orders including psittaciformes (budgerigars - *Melopsittacus undulatus*), Passeriformes (canaries - *Serinus canarius* and zebra finches - *Poephilia guttata*), Galliformes (chickens - *Gallus gallus* and guinea fowl - *Numida meleagris*), and Columbiformes (pigeons - *Columba livia*). Budgerigars and pigeons were susceptible to the acute fatal illness. Of 13 inoculated budgerigars only one bird lived past the period of lung merogony (during the second week post inoculation) and it had muscle cysts at necropsy 12 weeks post inoculation. In pigeons, 4 of 5 birds also exhibited the acute fatal illness while canaries, zebra finches, chickens and guinea fowl survived the lung merogony stage and developed muscle cysts. 7

Pulmonary lesions in budgerigars were studied by Smith et al. Early schizony of *S. falcatula* occurs in the endothelia of pulmonary capillaries, then venules and veins and produces blood outflow obstruction by stenosis or occlusion of vessels by endothelial hypertrophy, meronts and endophlebitis. The subsequent edema is associated with displacement of the myelinoid surfactant layer and retraction and degeneration of squamous pneumocytes. 16



Todd et. al. reported muscle cysts of *Sarcocystis* in a halfmoon conure (*Aratinga canicularis*) as well as citing reports of similar cases in *Forpus passerinus*, *Aratinga auricapillus*, *Ara ararauna* and *Brotozeris jugularis*, all new world psittacine species. 13

Mechanical transport of *Sarcocystis* by cockroaches and flies was also shown in laboratories as well as an eagerness to consume the feces of carnivores. 13, 15

The range of the opossum extends over most of the continental United States with the exception of the Rocky Mountains, desert southwest, and extreme northern areas. The Brown-headed Cowbird ranges over the entire continental U.S. and the Common grackle ranges over the continental U.S. east of the Rocky Mountains. 8,14

## CLINICAL SIGNS

Pulmonary sarcocystosis is a peracute disease and many birds show no signs of illness until immediately prior to death. Many birds were found dead after being observed as normal just hours before. Clear fluid often runs from the mouth when a dead bird is lifted. Birds are typically in good condition with no weight loss or other indication of longstanding disease. Males are affected more often than females. Often the mate will die within a few days, however many birds have survived after the death of it's mate.

Fig

In those birds found ill prior to death clinical signs include severe dyspnea, yellow pigmented urates, and depression. Birds showed elevated serum liver enzymes including LDH (Lactate Dehydrogenase) values of 1199 to 4355 iu/l and AST (Aspartate Aminotransferase) values of 612 to 3082 iu/l. Other serum chemistry values were usually within normal ranges. Birds surviving the initial pulmonary sarcocystosis would typically die within a few days to 2 weeks following the initial illness.

## POST MORTEM FINDINGS

Visible gross lesions resemble those of septicemia and include hepatomegaly and splenomegaly. The lungs are congested and edematous and appear deep reddish in color. Birds usually show no muscle wasting. Bacterial cultures of liver, spleen, lung and heart blood are typically negative for bacterial growth.

Microscopic lung lesions include congestion, edema, diffuse interstitial pneumonia, mononuclear cell infiltration and reticuloendothelial cell hyperplasia. Protozal meronts are found in the capillary endothelium, usually in small numbers. Meronts measure about 2 x 7 um and from a few up 40 meronts may be found in a single section, often obstructing capillaries. Meronts can also be found in reticuloendothelial cells of the spleen as well as in other organs including liver, proventriculus, and pancreatic islets.

F



By light microscopy definitive identification is not possible, but by electron microscopy Isospora and Toxoplasma are found in parasitophorous vacuoles where as *S. falcatula* is in the cytoplasm of affected cells and is not associated with a vacuole. Toxoplasma gondii in asexual form is found in pseudocysts in the cytoplasm of cells throughout tissues. Isospora serini is usually found in macrophages. Sarcocystis meronts are found in capillary endothelial cells.

#### DIAGNOSIS, TREATMENT AND PROPHYLAXIS

No definitive antemortem diagnostic tests have been found. Diagnosis can be made only on post mortem examination.

Treatment of presumed affected birds has been difficult. The use of diuretics, antihistamines, corticosteroids, Trimethoprim & sulphadiazine or Trimethoprim & sulphamethoxazole combinations, and oxygen in treatment of affected birds was not clearly successful. Birds which survived the initial pulmonary edema invariably succumbed to hepatic failure. Addition of amprolium, or sulpha drugs to the feed or water for extended periods of time have shown little effect however it may have prevented illness or death while given. No untreated controls were used.

#### PSITTACINE SPECIES SUSCEPTABILITY

Old world psittacines, especially cockatoos, cockatiels, and african parrots are most commonly affected with the acute fatal illness. Table #1. New World psittacine species appear to be more resistant to the disease as adults but young birds were often affected. Table #2 At the first psittacine facility mortality rates of 1-2% are expected in conures which are removed from the nest for hand feeding at 5 to 7 days of age, while death of mature birds is extremely rare. Table #3

Rarely are all chicks in a clutch affected. In one clutch of military macaws one chick died acutely at approximately 18 days of age, and a second died at 21 days of age after a brief illness. The third bird in the clutch was never ill.

An adult blue & gold macaw (*Ara ararauna*) which died 4 months after arrival at the first premises had sarcocystis cysts in skeletal and cardiac muscle. Although the bird exhibited pulmonary and splenic congestion no meronts were observed in these tissues.

#### SEARCH FOR AN EXPERIMENTAL REPRODUCTION

Toxoplasmosis was initially ruled out as the causative agent by feeding the lungs of affected birds to 6 mice. These mice failed to develop antibody to Toxoplasma gondii and remained free of



*clinical signs of disease for a two month period post inoculation.*

Toxoplasmosis titers were 1:32 (diagnostic titer 1:256) in 8 of 9 birds tested. All seronegative birds were surviving mates of birds which died of sarcocystosis. One male Cayman Island Amazon (*Amazona leucocephala*) which survived what was believed to be sarcocystosis had a toxoplasmosis titer of 1:256. A feral cat trapped on the same premises also had a titer of 1:256 but was not shedding oocysts.

*Isospora serini* was then suspected as a possible causative agent. Wild birds were collected around the breeding facility including, blue jays, red winged black birds, sparrows, and pigeons. Any oocysts found in these birds were sporulated in 2% dichromate solution and identified. Those identified as *Isospora* sp. (found only in redwinged blackbirds) species were fed to cockatiels. These cockatiels remained asymptomatic for a two month period post inoculation.

Many wild and captive reptile species occurring on the farm were likewise sampled. Sporocysts resembling sarcocysts were observed in Bahama brown anoles, spiny tail iguanas and red footed tortoises. However, when these organisms were fed to cockatiels no adverse effects were observed for a two month period. Water sources, feed sources and storage areas, soil samples and feeding utensils were repeatedly sampled and no oocysts or sporocysts were found. Rodents were trapped and checked repeatedly for the presence of oocysts. On 2 occasions oocysts were found in mouse feces and fed to cockatiels with no adverse effects. Extracts of lungs from birds which died of the disease were injected into cockatiels, budgerigars, and cockatoos subcutaneously and intraperitoneally. Cockroaches were captured on both premises and fed to cockatiels again with no ill effects.

Road killed cats, raccons, and foxes were collected and sampled for sporocysts. Fecal samples from 22 dogs residing at the first premises were likewise free of sporocysts resembling sarcocysts.

An opossum was trapped at the second facility in which the disease occurred and was found to be shedding sarcocysts sporocysts. The feces from this opossum were offered to fasted american cockroaches (*Periplaneta americana*) which had been captured in a building which did not contain animals. The cockroaches eagerly consumed the feces and were subsequently liquified in a blender and fed by gavage to one Moluccan cockatoo (*Cacatua moluccensis*) and one lesser sulphur crested cockatoo (*Cacatua sulphurea*). The birds died on the 10th and 14th days post inoculation and meronts were found in the lungs. A contact control bird in the same cage exhibited no signs of illness during this time and for a subsequent two month period. Cockroaches which were not fed feces were liquified and fed to the contact control cockatoo and 15 cockatiels with no ill effects observed over a two month period.

Wild cockatoos rarely eat in the presence of humans. In order to determine if cockatoos would eat cockroaches, live cockroaches with the legs removed to prevent escape were added to the feed dishes of cockatoos. On three occasions five cockroaches were added



to the feed in a cage of five cockatoos. On all three occasions some cockroaches were missing on subsequent examination of the dishes and cage area. On the last trial a cockatoo was filmed eating a cockroach by video camera.

## DISCUSSION

*Sarcocystis* sporocysts shed by opossums, probably as *S. falcatula*, present a hazard to psittacine birds housed outdoors in areas of the country where both host species exist. The supplementation of psittacine diets with dog food as well as the easy accessibility of other feeds may attract opossums to feed in these areas at night. Opossums are nocturnal and can easily hide during the day in rubbish piles, heavy vegetation or under outbuildings making their presence on a farm difficult to detect. Since sporocysts of *S. falcatula* are shed over a prolonged period of time a single infected opossum, visiting a farm regularly, could seed the farm with infectious sporocysts which parrots might ingest resulting in sporadic losses. While opossums may not have direct access to birds or their feed supplies transmission can be accomplished by mechanical carriers, in this case cockroaches which eat opossum feces. While it was proven that cockatoos will eat cockroaches, even in the presence of adequate food supplies, it is also possible that cockroaches could directly contaminate feed with their feces.

It is postulated that the relative resistance to the acute fatal illness by new world psittacines is due to evolution of these species in an environment containing the parasite. Old world species which evolved in an environment free of the host species are more highly susceptible to the acute fatal illness. The increased incidence of the disease in winter may be related to a massive influx of migratory grackles and cowbirds at that time.

Control of this disease by the use of drugs is impractical. Control efforts must be aimed at elimination of the carrier animals. In southern coastal areas of the United States control of cockroaches in heavily planted outdoor areas is difficult if not impossible. Limiting access to psittacine breeding or holding areas by opossums is more practical.

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TABLE #1

SPECIES IN WHICH ACUTE FATAL ILLNESS WAS OBSERVED IN ADULT BIRDS;

OLD WORLD SPECIES;

Gang Gang Cockatoo*	( <i>Cacatua fimbriatum</i> )
Leadbetters cockatoo*	( <i>Cacatua leadbeateri</i> )
Greater Sulphur Crested Cockatoo*	( <i>Cacatua galerita galerita</i> )
Medium Sulphur Crested Cockatoo*	( <i>Cacatua galerita eleonora</i> )
Triton Cockatoo*	( <i>Cacatua galerita tritoni</i> )
Lesser Sulphur Crested*	( <i>Cacatua sulphurea</i> )
Moluccan Cockatoo*	( <i>Cacatua moluccensis</i> )
Umbrella Cockatoo*	( <i>Cacatua alba</i> )
Eclectus parrot*	( <i>Eclectus roratus</i> )
African Grey Parrot*	( <i>Psittacus erithacus</i> )
Cockatiel*	( <i>Nymphicus hollandicus</i> )
Alexandrine	( <i>Psittacula eupatria</i> )
Moustache parakeet	( <i>Psittacula alexandri</i> )
Indian ringneck parakeet	( <i>Psittacula krameri</i> )
Great Bill Parrot*	( <i>Tanygnathus megalorhynchos</i> )
Peach Faced Lovebird	( <i>Agapornis roseicollis</i> )

NEW WORLD SPECIES;

Yellow Faced Amazon*	( <i>Amazona xanthops</i> )
Thickbilled Parrot*	( <i>Rhynchopsitta pachyrhyncha</i> )
Pacific parrotlet	( <i>Forpus coelestis</i> )

\* Confirmed by histopathologic examination



TABLE #2

NEW WORLD SPECIES IN WHICH ACUTE FATAL ILLNESS HAS OCCURED IN NESTLINGS;

Military Macaw*	(Ara Militararis)
White Crowned Pionus*	(Pionus senilis)
Sun Conures*	(Aratinga solstitialis)
Peach Fronted Conures	(Aratinga aurea)
Slenderbill conure*	(Enicognathus leptorhynchus)
Blue winged parrotlets	(Forpus xanthopterygius)

\* Confirmed by histopathologic examination

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TABLE #3

SPECIES ON FIRST PREMISES WHICH HAVE BEEN EXPOSED TO SARCOCYSTIS WITH NO ASSOCIATED DEATH OR ILLNESS

OLD WORLD SPECIES;

Citron Crested Cockatoo	Cacatua sulphurea citrionocristata
Vasa Parrot	Coracopsis vasa

NEW WORLD SPECIES;

Hyacinth Macaw	Anodorhynchus hyacinthinus
Scarlet Macaw	Ara macao
Buffons Macaw	Ara ambigua
Greenwing Macaw	Ara chloroptera
Caninde Macaw	Ara caninde
Red Fronted Macaw	Ara rubrogenys
Severe Macaw	Ara severa
Yellow Collared Macaw	Ara auricollis
Red Bellied Macaw	Ara manilata
Nobles Macaw	Ara nobilis
Tucuman Amazon	Amazona tucumana
Yellow Billed Amazon	Amazona collaria
Hispanolian Amazon	Amazona ventralis
Spectacled Amazon	Amazona albifrons
Lilac crowned Amazon	Amazona finschi
Red Lored Amazon	Amazona autumnalis
Yellow Faced Amazon	Amazona xanthops
Blue Fronted Amazon	Amazona aestiva
Yellow Naped Amazon	Amazona ochrocephala auropalliata
Double Yellow Head	Amazona oratrix oratrix
Yellow Crowned Amazon	Amazona oratrix ochrocephala
Blue Crowned Amazon	Amazona farinosa guatemala
Bronze Winged Pionus	Pionus chalcopterus
Coral Billed Pionus	Pionus sordidus
Maxmillian	Pionus maximiliani
Dusky Pionus	Pionus fuscus



Table #3 (continued)

Blue Headed Pionus	Pionus menstruus
Black Headed Caique	Pionites melanocephala
White Belly Caique	Pionites leucogaster
Hawkhead Parrot	Derophtus accipitrinus
Queen of Bavaria	Aratinga guaroua
Blue Crowned Conure	Aratinga acuticaudata
Orange Throated Conure	Aratinga holochlora rubitorquis
Finsch's Conure	Aratinga finschi
Red Masked Conure	Aratinga erythrogenys
Golden Capped Conure	Aratinga auricapilla
Jenday Conure	Aratinga janday
Olive Throated Conure	Aratinga nana
Nanday Conure	Nandayus nenday
Green Cheeked Conure	Pyrrhura molinae
Black Capped Conure	Pyrrhura rupicola
Painted Conure	Pyrrhura picta
Austral Conure	Enicognathus ferrugineus
Grey Cheeked Parakeet	Brotogeris pyrrhopterus
Tui Parakeet	Brotogeris sanctithoma