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Source: Journal of Wildlife Diseases, 32(1): 130-132

Published By: Wildlife Disease Association

URL: https://doi.org/10.7589/0090-3558-32.1.130

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An Unusual Coccidian Parasite Causing Pneumonia in a Northern Cardinal (*Cardinalis cardinalis*)

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ABSTRACT: In June 1993 an unusual coccidian parasite was identified in lung tissue from a northern cardinal (*Cardinalis cardinalis*) collected near Tucson, Arizona (USA), which died in respiratory distress. Histologically, there was evidence of severe, generalized interstitial pneumonia, associated with the parasite. Both asexual and sexual stages were seen. Schizonts, gamonts, and sporulated oocysts were seen in lung tissue. The parasite most closely resembled coccidia of the genus *Lankesterella*. This is the first report of such a coccidian parasite in the alveolar tissue of a cardinal.

Key words: Passeriformes, northern cardinal, Cardinalis cardinalis, pulmonary, parasite, protozoon, Lankesterella, Atoxoplasma.

In June 1993, four nestling cardinals (*Cardinalis cardinalis*) were collected for a songbird research project. The collection site was the Santa Rita Experimental Range (31°50′N, 110°51′W), located approximately 20 km southeast of Tucson, Arizona (USA).

Two of four newly hatched cardinals present in the nest appeared robust. A third nestling (bird A), the subject of this report, was undersized. A fourth nestling (bird B) was of medium size. Upon arrival at the research institution all four nestlings were housed together in an air conditioned room in a nest. At 16 days of age bird A was separated into an individual cage. At approximately 1 mo of age, cardinals were routinely placed, within their cages, in sound isolation chambers.

Prior to fledging, bird A was fed ground beef, boiled eggs, monkey chow, cat food, vitamins, and carrot baby food. After fledging, bird A also was provided hookbill seed diet, fresh fruit, corn, peas, lettuce, and meal worms. Water was provided ad libidum and changed daily.

At 44 days of age, 50 µl blood were collected from bird A via alar vein puncture. The packed cell volume (PCV) was 25%; normal is 22 to 35%; (Zinkl, 1986). At 46 days of age bird A developed anorexia, lethargy, and upper respiratory congestion with occasional sneezing. At 48 days of age bird A was found dead in its cage.

A necropsy was performed on bird A within 30 min of death. The lungs were perfused in situ with 10% neutral buffered formalin. Tissues, including liver, lung, heart, spleen, kidney, pancreas, small intestine, eyes, and brain were fixed in formalin. Five micrometer sections were cut from paraffin-embedded tissues and stained with hematoxylin and eosin (H&E). Mounted tissue sections of lung were also reacted to antibodies against Toxoplasma gondii, Neospora caninum (Lindsay and Dubey, 1989a), and Caryospora bigenetica (Dubey et al., 1990) in avidin-biotin immunohistochemical tests. Additional lung tissue was embedded in plastic and 3 µm sections cut and stained with H&E or periodic acid Schiff (PAS) (Jain, 1986).

Three days after the death of bird A, bird B became lethargic and anorexic, and died. A necropsy was similarly performed on this bird. Roughly 4 mo after the deaths of these birds, fecal samples were collected from the two remaining cardinals and examined for coccidial oocysts by double centrifugation flotation (Foreyt, 1986).

Histologically, bird A had generalized interstitial pneumonia characterized by

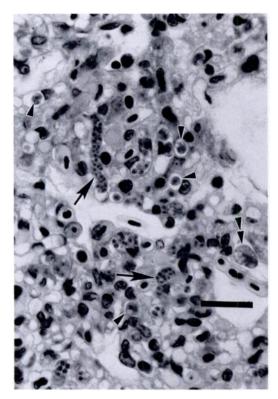


FIGURE 1. Cardinal lung with numerous uninucleate meronts (arrowheads), at least two multinucleate immature meronts (arrows), and an unsporulated oocyst (double arrowheads). H&E stain. Bar = 20 µm.

large numbers of parasites predominently within air capillaries and the interstitium. Tertiary bronchi were relatively spared. The interstitium contained small to moderate numbers of mononuclear inflammatory cells. Larger blood vessels were congested and had a marked increase in circulating leukocytes. There was no evidence of foreign material or aspiration pneumonia.

Both asexual and sexual stages of the parasite were seen. There were numerous immature and mature meronts (Fig. 1). The merozoites were approximately 5×2 μm and PAS-negative. Macrogamonts were difficult to distinguish from early meronts. Microgamonts were few and contained up to 16 microgametes (Fig. 2). Both unsporulated and sporulated oocysts

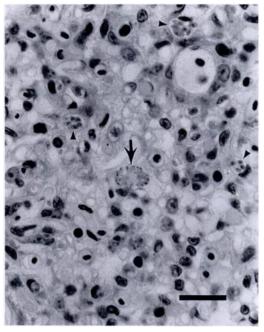


FIGURE 2. Cardinal lung in a 3- μ m plastic-embedded section, with a mature microgamont (arrow) with at least 13 mature microgametes arranged at the periphery of the microgamont, three mature schizonts (arrowheads), and several uninucleate meronts that are not in focus. H&E stain. Bar = 20 μ m.

were seen. Sporulated oocysts contained a prominent residuum and mature sporozoites. The sporozoites were approximately $10.0 \times 1.5~\mu m$, and contained eosinophilic bodies. The oocysts and sporozoites were PAS-positive and thus could be distinguished from the PAS-negative asexual stages. The number of sporozoites within oocysts was not determined. Organisms in the cardinal lungs did not react to antibodies against *T. gondii*, *N. caninum*, or *C. bigenetica* in avidin-biotin immunohistochemical tests.

Bird A had no evidence of aspiration pneumonia; thus the parasitic infection was the likely cause of death in this bird. Neither lesions nor parasites were identified in other tissues. Parasites were not seen in any of the tissues examined from bird B. The cause of death in that bird was undetermined. No oocysts were recovered from the fecal samples collected from the two remaining cardinals. These birds continued to appear healthy.

Little information is available on the protozoal parasites of cardinals. While *Haemoproteus mazzai* and *Trypanosoma everetti*-type parasites were found in blood smears from cardinals in Louisiana (USA) (Garvin et al., 1993), to the best of our knowledge, extraintestinal coccidian parasites have not been reported from cardinals.

The parasite found in the lungs of our cardinal was neither *T. gondii* or *N. caninum* because it divided by schizogony whereas *T. gondii* and *N. caninum* divide into two by endodyogeny (Lindsay and Dubey, 1989b). The parasite also was not a *Sarcocystis* spp. because asexual and sexual stages were found together whereas in *Sarcocystis* the asexual and sexual cycles occur in different hosts (Dubey et al., 1989). *Sarcocystis falcutula* is a common cause of protozoal pneumonia in some avian species (Dubey et al., 1989).

The pulmonary parasite in this report was not *Caryospora* spp. because it did not react with *Caryospora* spp. antibodies (Dubey et al., 1990). The parasite was not *Isospora* (*Atoxoplasma*) serini (Levine, 1982), the common cause of protozoal pneumonia in canaries, because the sexual cycle was found in the lungs whereas the sexual cycle of *I. serini* occurs in the intestine (Box, 1977; Levine, 1982).

The parasite in the cardinal closely resembles *Lankesterella* spp.-like organisms, which are parasites of reptiles. At present there is considerable confusion concerning the taxonomy of the genus *Lankesterella*

(Long, 1982); some authors consider it an isosporan parasite (Box, 1977).

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Received for publication 31 March 1995.