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Mortality in Black Siskins (*Carduelis atrata*) with Systemic Coccidiosis

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ABSTRACT: Ninety-five (97%) of 98 black siskins (*Carduelis atrata*) died within 2 months of arrival in Italy from South America with the following clinical signs: rapid weight loss, breast muscle atrophy, congested and distended abdomen, diarrhea, and lethargy. Macroscopically we observed hepato-splenomegaly, pulmonary congestion, and thickening of the interstinal wall. Histologically, lymphomonocytic transmural enteritis, interstitial mononuclear cell infiltrates in the lungs and in the liver, as well as activation of splenic follicles were common features. Large numbers of protozoa belonging to *Isospora* sp. were observed in various stages of their life-cycle in the intestinal epithelium, and some zoites were found in the extra-intestinal cellular infiltrate as well. No viral or bacterial pathogens were found.

Key words: *Carduelis atrata*, black siskins, lymphomonocytic enteritis, coccidiosis, granulated intraepithelial lymphocytes, liver, lung.

Carduelis atrata (Lafresnaye and d'Orbigny, 1837) is a passeriform native to the Cordillera of the Andes which can be found at 4,800 meters above sea level in an area which ranges from southern Peru to northern Chile and western Argentina (Clement, 1993). Very little was known about diseases occurring in this species until recently, because of their increased importation by European breeders. These birds have a high mortality in captivity (DeBaseggio, 1995).

Two groups of black siskin (*Carduelis atrata*), consisting of 98 birds, were imported from Cordillera de Cochabamba, Bolivia, into Alessandria (northern Italy) and Pisa (central Italy) from August through December 1994. Upon arrival, all birds presented similar signs, including severe pectoral muscle atrophy and marked abdominal distension with hepatomegaly; the cloacas were swollen and congested with wet feces on the tail feathers. Ninety-five (97%) of the 98 birds died; 44 (46%) of 95 died 48 to 72 hr after arrival with clinical signs of diarrhea, lethargy, congested abdomen, and weight loss, and the other 51 (54%) birds died after a period of 3 to 4 wk with the same, but less severe, signs. Our objective was to identify the cause of this mortality.

The birds that survived the initial acute phase were placed in large cages (six to eight birds/cage) with a grid and removable floor and fed a seed mixture which contained seeds found in their area of origin. Medicated drinking water, with bacteriostatic or coccidiostatic substances containing sulfaquinossaline, furazolidone and menadione as active principles (Candiocidin®, Istituto Candioli, Beinasco, Torino, Italy) at 5 g/l of water for 4 days was given ad libitum to four groups while the other three groups received unmedicated water. Feces were collected twice daily from cage floors, and examined for coccidial oocysts using a saturated salt flotation solution and McMaster's chamber (Todd and Ernst, 1977).

Birds recently dead or euthanized by ether overdose) in the last stages of the disease were necropsied. Fresh smears of intestinal contents were made and examined for parasites, while the rest of the contents was dissolved in 2% potassium dichromate for the sporulation of oocysts. Samples of intestine, liver, pancreas, lungs, spleen, and heart were fixed in 10% buffered formalin, and embedded in paraffin; 5 μm sections were stained using hematoxylin and eosin, ferric-hematoxylin, toluidine blu, and periodic acid Schiffs (Pearse, 1985) for histological examination. In addition, portions of intestine, spleen, liver, and lungs were fixed in glutaraldehyde, post-fixed in 1% osmium te-

troxide, in phosphate buffered saline 0.1 M (PBS), and embedded in Epon/Araldite® (Fluka-Chemika-Biochemika, Buchs, Switzerland) for transmission electron microscopic examination (TEM). Blood smears, taken directly from the heart chambers of recently dead birds or the cephalic vein or heart of dying birds, were air-dried, and stained with May Grumwald giemsa (McManus and Mowry 1960). Smears from the spleen, liver, and lungs of dead birds were prepared similarly. For bacteriological examination, some of the intestinal contents was inoculated onto Desoxycholate Agar (Difco Laboratories, Detroit, Michigan, USA), and onto Campylobacter Kit Blaser (Difco Laboratories); some intestinal samples were enriched using Selenite Broth (Difco Laboratories) and then inoculated onto SS Agar (Difco Laboratories). For virological examination duplicate samples from dead black siskins were homogenized and diluted 1:5 with sterile physiological saline and centrifuged at 15.000 × G; the sediment was resuspended 1:5 in distilled water and negatively stained for electron microscopy and examined on a Philips (80 KV) transmission electron microscope (CAM-Bio, Milano, Italy). The sediment was also filtered and 100 µl inoculated onto a chicken fibroblast culture with media-growth EMEM (Bio-Whittaker, Walkersville, Maryland, USA) enriched with 10% of bovine fetal serum (CAM-Bio s.r.l., Milano, Italy) and 1% of penicillin, 1% of strepthomycin, and 1% of fungizone (mixture 100X) (Bio-Whittaker). The cultures were checked daily for signs of any cytopathic effect and processed for the TEM examination.

A severe *Isospora* sp. infection was found in all 95 *Carduelis atrata* examined. In fresh smears of intestinal contents, there was a heavy concentration of oocysts almost exclusively in the duodenal and jejunal tracts. Oocyst emission, measured with the McMaster method, was heaviest in the afternoon and evening and peaked in late afternoon.

All 95 *C. atrata* had similar lesions on necropsy. All birds had severe pectoral muscle atrophy, with a characteristic knife-blade apparence of the carena, and severe abdominal distension with hepatomegaly and intestinal loop distension (Fig. 1). The cloaca was swollen and congested with wet feces on the tail feathers, the duodenum and jejunum were discolored creamy white, with walls four to five times thicker than normal and dilated in some birds to a diameter of 4 mm. The liver was generally enlarged and, in many birds, was a yellowish-orange color. The spleen also appeared discolored and slightly enlarged in almost all black siskins. The lungs were generally congested and expanded, imbibed with exudate and, in two cases, presented a small area of about 1 mm in diameter in the apical portion which appeared to be a fungal granuloma upon microscopic examination. No other gross changes were seen, except for the kidneys, which were slightly discolored and swollen in about 10 birds. Blood smears showing mononuclear cells parasitized by zoites were present only in samples taken directly from the heart chambers, and with higher parasitemia in blood samples drawn in the morning. Parasites were not observed

FIGURE 1. Enlarged creamy-white gut of a black siskin. Scale bar in cm.

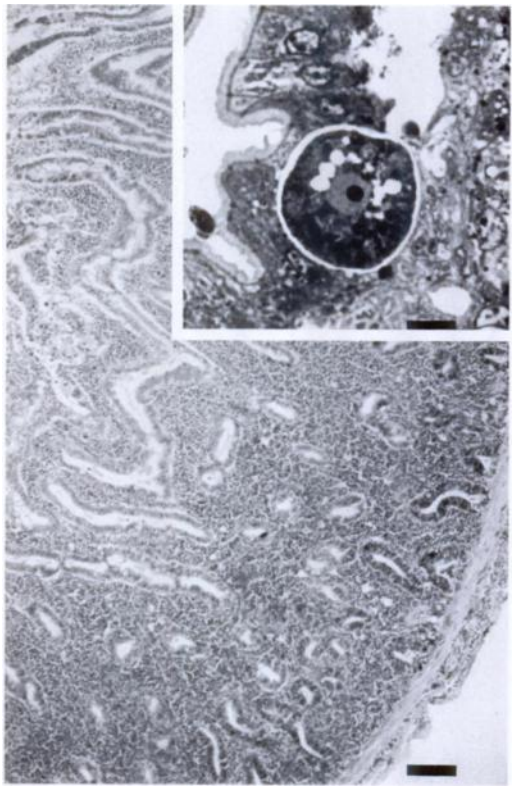

FIGURE 2. Heavy transmural lymphomonocytic enteritis characterizes histological aspect of the intestinal wall. H & E. Bar = 125 μm. Inset: *Isospora* sp. intraepithelial macrogametocyte in the duodenal villi is visible. Toluidine blue (semi-thin section). Bar = 8 μm.

in peripheral blood smears. Mononuclear cells containing parasites were also found in impression smears of liver, lungs and spleen. No bacterial and or viral organisms were isolated from cultures prepared with material collected on necropsy.

On histological examination of the intestines stained with hematoxylin and eosin, a transmural lympho-monocytic enteritis was observed (Fig. 2) which was readily apparent at low-power magnification; it had a mononuclear infiltrate which extended from the sub-epithelial area to the extra-intestinal serosa. At high-power (400×), the lamina propria and the submucosa were heavily damaged while crypts of Lieberkuhn were compressed by the infiltrate in some points to such an extent as to lose their central lumen and, in some cases, disrupted to the extent of disappearing altogether. Mononuclear cells invaded the muscularis, disrupting its structure and infiltrated the serosa of the adjacent mesentery. The duodenal and jejunal epithelium were badly damaged by parasites in various stages of their biological cycle, although epithelial necrosis was apparent only where there was a massive infection, particularly in the epithelium of the crypts in the duodenum where the protozoan was undergoing merogonic replication. The coccidial stages observed in the intestinal epithelium were macrogametocytes and oocytes, several schizonts, and scarce microgametocytes (Fig. 2). Areas of lymphomonocytic infiltrates were observable, in decreasing order, in the liver, lungs, heart, and pancreas. The spleen consistently had signs of activation of the lymphoid follicles with an overall cellular increase. The infiltrate in the liver was mainly around the portal triad and sometimes around the centrolobular vein (Fig. 3). A slight infiltrate along the hepatocyte corels was occasionally observed. About 45% of the livers examined had morphological changes ranging from torbid swelling to actual steatosis. The lungs also appeared markedly swollen along the interstitial septa due to intense mononuclear infiltration (Fig. 4). Fifteen birds presented a localization of the lymphoid infiltrate in sub-endocardial atria and, in two subjects, in the right ventricle as well (Fig. 5). One bird had periarterial infiltrates in the parenchyma of the pancreas.

The mononuclear infiltrate observed in the various organs examined was mainly composed of lymphocytes, a few plasma cells, and many cells with electrondense granules surrounded by a membrane and large mitochondria, and which seemed to correspond to granular lymphocytes, most easily distinguishable from macrophages. Several zoites were present in the cytoplasm of the granular cells and in macrophages and lymphocytes, particularly in the intestine, although very similar entities

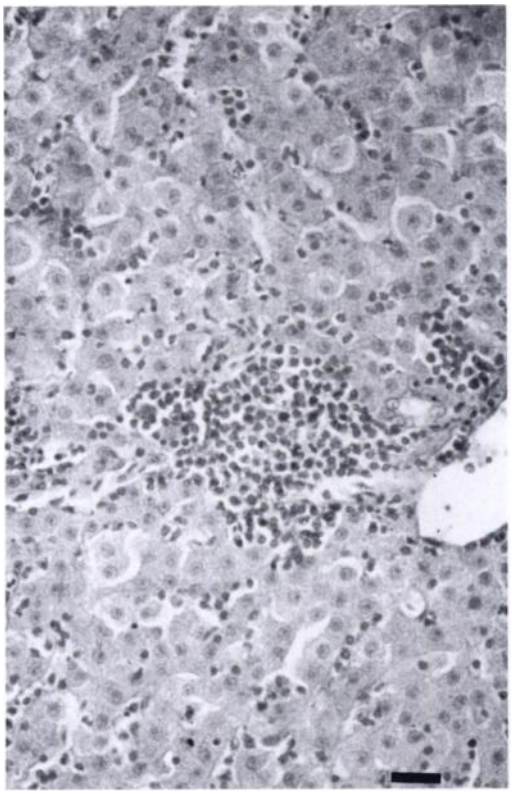

FIGURE 3. The microphotograph shows an evident lympho-monocytic infiltration localized near the central-lobular vein in the liver. H & E. Bar = 25 μm.

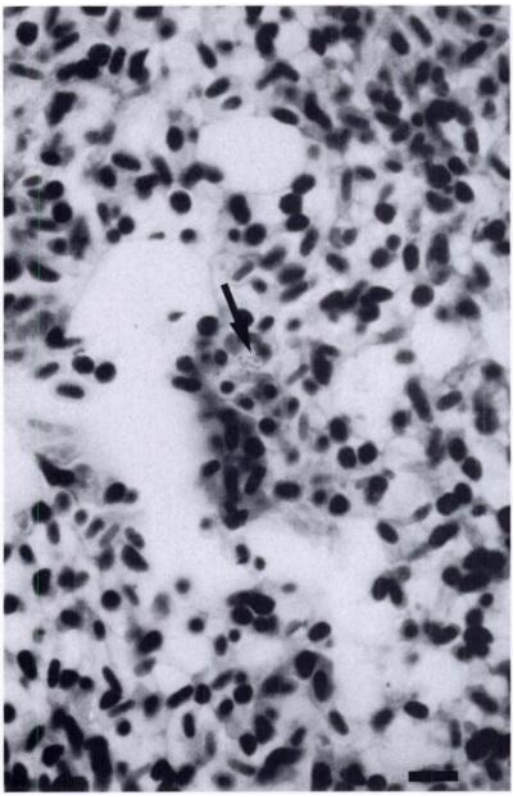

FIGURE 4. Diffuse mononuclear cell infiltration in the lung. A parasitized macrophage is visible (arrow). H & E. Bar = 10 μm.

were found, in low numbers, in the infiltrates of other organs.

Isospora sp. infections are very common in wild birds, (Middleton and Julian, 1983; Swayne et al., 1991). There are conflicting opinions as to the real pathogenicity of these protozoa, although most naturally-occurring infections probably are asymptomatic (Arnall and Keymer, 1976). In our case we dealt with 98 *C. atrata*, not a very well-known species, which had severe signs followed by death. It is possible that the stress brought on by their capture and the changes in food and climate that these birds undergo on their way to European and North American breeders may alter the delicate host-parasite equilibrium, exacerbating the infection and the pathology. In studies with *Carduelis tristis* (Middleton and Julian, 1983), *Vermifora ruficapilla* (Swayne et al., 1991) and *Hesperiphona vespertina* (Desser, 1980), others described the same clinical and pathological signs and lesions we found in *C. atrata*. The birds described in these other cases also shed large amounts of *Isospora* sp. oocyst in their feces. Our findings are similar in that we found a severe transmural lymphomonocytic enteritis like that described in *V. ruficapilla*, various protozoan stages in mononuclear cells as described in *H. vespertina*, and conspicious mononuclear infiltrates in various organs which were also found in *C. tristis*. In our case as well we believe that the histomorphologic changes found in the various organs of the 95 *C. atrata* necropsied may be the expression of a marked cell-mediated immune response to the parasite. The numerous mononuclear cells observed in the intestinal infiltrate had granular cyto-

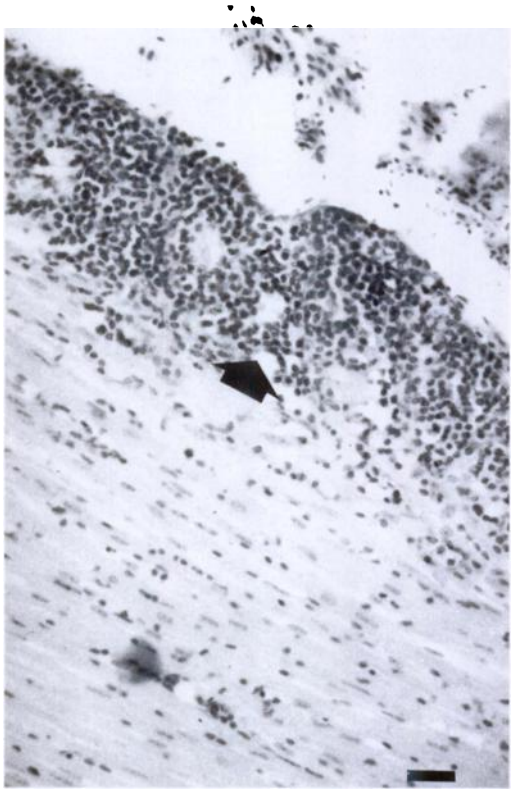

FIGURE 5. In the heart, foci of sub-endocardial mononuclear cell infiltration (arrow) are pointed out. H & E. Bar = 31.25 μm.

plasm. This finding corresponds to the intra-epithelial granular lymphocytes described by Lawn and Rose (1982), and Millard and Lawn (1982). This led us to attribute the pathological findings to an abnormal and inadequate cell-mediated response to the parasite. In fact, intraepithelial granular lymphocytes are non-B and non-T lymphocytes (Lillehoj and Trout, 1994), which are considered to be natural killer effectors of the intestine (Tagliabue et al., 1982) which, because of their intraepithelial location, are primarily involved in host defenses against invading parasites (Ferguson, 1977). These lymphocytes are also used, as are T CD8 lymphocytes and macrophages (Lillehoj and Trout, 1994), as a vehicle for the sporozoites of some coccidial species which undergo the schizogonic phases of their life-cycle in the epithelium of the crypts of Lieberkuhn (Fernando et al., 1983, 1987; Perry and Long, 1987). Yet, a similar histopathological situation, characterized by lymphomonocytic infiltrates associated with the presence of intracellular zoites, was described in *Grus americana* and in *Grus canadiensis* where *Eimeria reichenowi* and *Eimeria gruis* (Carpenter et al., 1980; Novilla et al., 1981) infections were characterized by disseminated visceral coccidiosis; lymphoproliferative changes, albeit to a lesser degree, are also found in other protozoan infections such as leishmaniasis (Keenan et al., 1984). The presence of zoites in granular lymphocytes and macrophages in the infiltrates of the intestines and other organs is evidence that the protozoan antigens may stimulate a cell-mediated response. In his description of a new species of *Isospora* sp., Desser (1980) stated that the massive replication of the parasite in mononuclear cells leads to marked mortality rates in parasitized hosts. We suspect that the intracellular zoites found in mononuclear cells of black siskins may represent extra-intestinal stages of *Isospora* sp. with an *Isospora serini*-like life-cycle (Box, 1977, 1981) which includes replication and developmental stages in the mononuclear cells of various organs and tissues.

Voucher specimens of slides containing the *Isospora* sp. are deposited in the Museum of the Veterinary School, University of Naples, Via F. Del Pino, 1-80137 Naples, Italy, with the repository number 469/3A, and in the Museum of the Veterinary School, University of Pisa, with the repository number 6243.

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