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Source: Journal of Wildlife Diseases, 27(4) : 615-620

Published By: Wildlife Disease Association

URL: https://doi.org/10.7589/0090-3558-27.4.615

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Journal of Wildlife Diseases, 27(4), 1991, pp. 615–620
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COCCIDIOSIS AS A CAUSE OF TRANSMURAL LYMPHOCYTIC ENTERITIS AND MORTALITY IN CAPTIVE NASHVILLE WARBLERS (*VERMIVORA RUFICAPILLA*)

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ABSTRACT: Transmural lymphocytic enteritis was diagnosed in thirteen Nashville warblers (*Vermivora ruficapilla*) during an epornitic with high mortality. In the intestinal lesions, asexual stages of coccidia were present within lymphocytes and asexual and sexual stages of coccidia were present within intestinal villar epithelium. Ultrastructurally, the infiltrating lymphocytes resembled granular ("intraepithelial") lymphocytes, a cell known to be important in the life cycle of some avian coccidia. Gross and histopathologic features of this enteritis resemble intestinal changes described for *Isospora/Atoxoplasma* spp. in other passeriformes and lymphoproliferative disease in goldfinches.

Key words: Nashville Warbler, *Vermivora ruficapilla*, lymphocytic enteritis, coccidiosis, lymphosarcoma.

INTRODUCTION

Mortality due to concurrent coccidiosis and lymphosarcoma has been described in captive passeriformes (Middleton and Julian, 1983). These authors concluded that the coccidial infection was secondary to the immunosuppressive effects of the lymphoid neoplasm and that it was unlikely that the coccidia had a primary role in lymphogenesis. The primary lymphocytic infiltration was intestinal, involving the duodenum and jejunum, but other viscera were involved. Some of the "neoplastic" lymphocytes in the intestine contained coccidial zoites. Other reports of coccidial infections and lymphosarcoma in passeriformes described the diseases as separate concurrent entities with no apparent relationship (Beach, 1962; Blackmore, 1966; Davis, 1971; Keymer, 1982).

In this report, transmural lymphocytic enteritis and concurrent intestinal coccidiosis were diagnosed in thirteen Nashville warblers (*Vermivora ruficapilla*) during the second year of a three year capture-and-release program. In each case of lymphocytic enteritis, the presence of coccidial zoites was documented either within submucosal lymphocytes or within the overlying epithelium. The lymphocytes, based on ultrastructural morphology, were characterized as granular lymphocytes (Lawn et al., 1988). In addition, while morphological evidence suggests that this lesion resembles intestinal lymphosarcoma, the changes observed are likely an exaggerated immunologic or inflammatory response to primary intestinal coccidial infection.

MATERIALS AND METHODS

The Nashville warblers (*Vermivora ruficapilla*) were trapped alive in north central Michigan (44°30′ to 44°40′N, 80°00′ to 84°30′W) during August of 1986, 1987 and 1988, transported to and housed at the Columbus Zoo (Powell, Ohio 43210, USA) and released consecutively in June of 1987, 1988 and 1989. This report will describe only the second year of the three year study and only the cases diagnosed as transmural lymphocytic enteritis.

The birds were housed by pairs or threes in 1.2 m × 2.4 m × 2.4 m indoor plywood pens maintained at 24 to 25 C. The flooring was smooth concrete covered with pine shavings. Feed and water were provided ad libitum. The diet was mealworms and dietary mash (Morning

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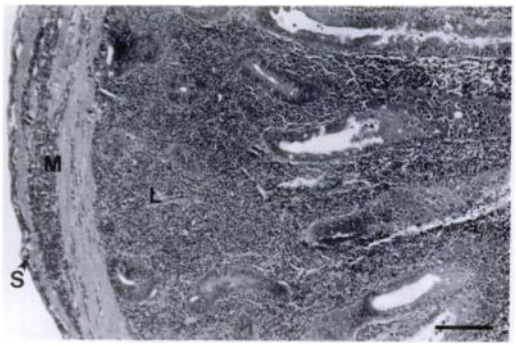


FIGURE 1. Photomicrograph of infiltrating round cells in the subserosa (S), tunica muscularis (M) and lamina propria (L) of the duodenum of a Nashville warbler. H&E. Bar = 100 μm.

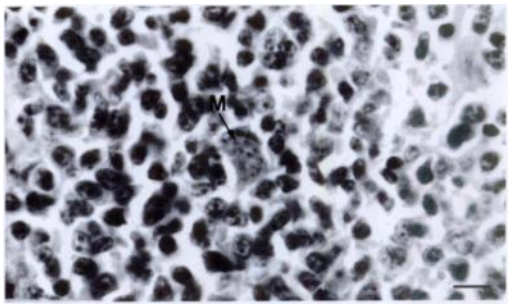


FIGURE 2. Photomicrograph of numerous subserosal lymphocytes of the duodenum of a Nashville warbler. Note the rare coccidial meront (M). H&E. Bar = 15 μm.

Chow, Columbus Zoo) supplemented with green vegetables and produce.

The clinically ill birds exhibited hunched posture, drooping of the wings, ruffled feathers and absence of singing. Twelve birds that died naturally, and one clinically ill bird that was euthanatized by cervical disarticulation, were necropsied and diagnosed with transmural lymphocytic enteritis. In addition, three clinically healthy birds from the wild were euthanatized in September of 1988 and necropsied for determination of "normal" histology of the intestines. Livers from six birds that died naturally were cultured aerobically on blood agar for bacteria. Virus isolation was attempted by the allantoic route of inoculation for the cloacal swab (three sequential passages) and the chorioallantoic membrane route for tissue homogenate preparations (1:10, weight:volume) of spleen, liver and duodenum (one passage) (Senne, 1989) obtained from the euthanatized clinically ill bird. Chorioallantoic fluids were checked for hemagglutinating agents, using the hemagglutination-inhibition test, and the chorioallantoic membranes were checked for plaque formations. All virus isolation attempts were conducted in 10-day-old specific-pathogen-free (SPF) chicken (*Gallus domesticus*) embryos (S-SPF/COFAL/MAREK'S/gs/chf-39; SPAFAS, Inc., Roanoke, Illinois 61561, USA).

Gastrointestinal tract, lung, kidney, liver, gonad, heart and spleen were fixed in 10% neutral buffered formalin solution, embedded in paraffin, sectioned at 5 μm and stained with hematoxylin and eosin stains. Tissue sections with histopathologic changes were stained with Grocott's fungal stain or Goodpasture's gram stain for bacteria or Giemsa stain. Duodenal samples from three birds were fixed in 4% glutaraldehyde, dehydrated, embedded in Medcast® (Ted Pella, Redding, California, 96099, USA), thin sectioned and examined in a Philips 300 transmission electron microscope. Minimal postmortem autolysis within duodenums from two of the three birds allowed quantitative differentiation of cell-types based upon ultrastructural detail. The percentages of specific infiltrating cells were determined by differentially counting 271 cells in four grid areas.

RESULTS

Grossly, the duodenums and jejunums had diffusely thickened walls and were discolored creamy white. Histologically, the intestinal walls were infiltrated by small round cells (Figs. 1, 2). The cellular infiltrates were most severe and usually diffuse in the lamina propria and submucosa. Fronds of round cells invaded the tunica muscularis and extended through to the subserosa and into the adjacent mesentery (Fig. 1). Exocytosis of small round cells across the surface epithelium was evident. Based on ultrastructure, most of the small round cells were a uniform population of lymphocytes (89%) but macrophages (5%), plasma cells (6%), Mott cells (<1%) and heterophils (<1%) were intermingled with the lymphoid cells (Fig. 3).

On light microscopy, the lymphocytes were 4.8 to 6.0 μm in diameter, had a single centrally located nucleus and a small rim of faintly basophilic cytoplasm (Fig. 2). The nuclei exhibited slight anisokaryosis, were usually vesicular and contained one or occasionally two large nucleoli. Mitotic figures were infrequent. On ultrastructural examination, the lymphoid cells

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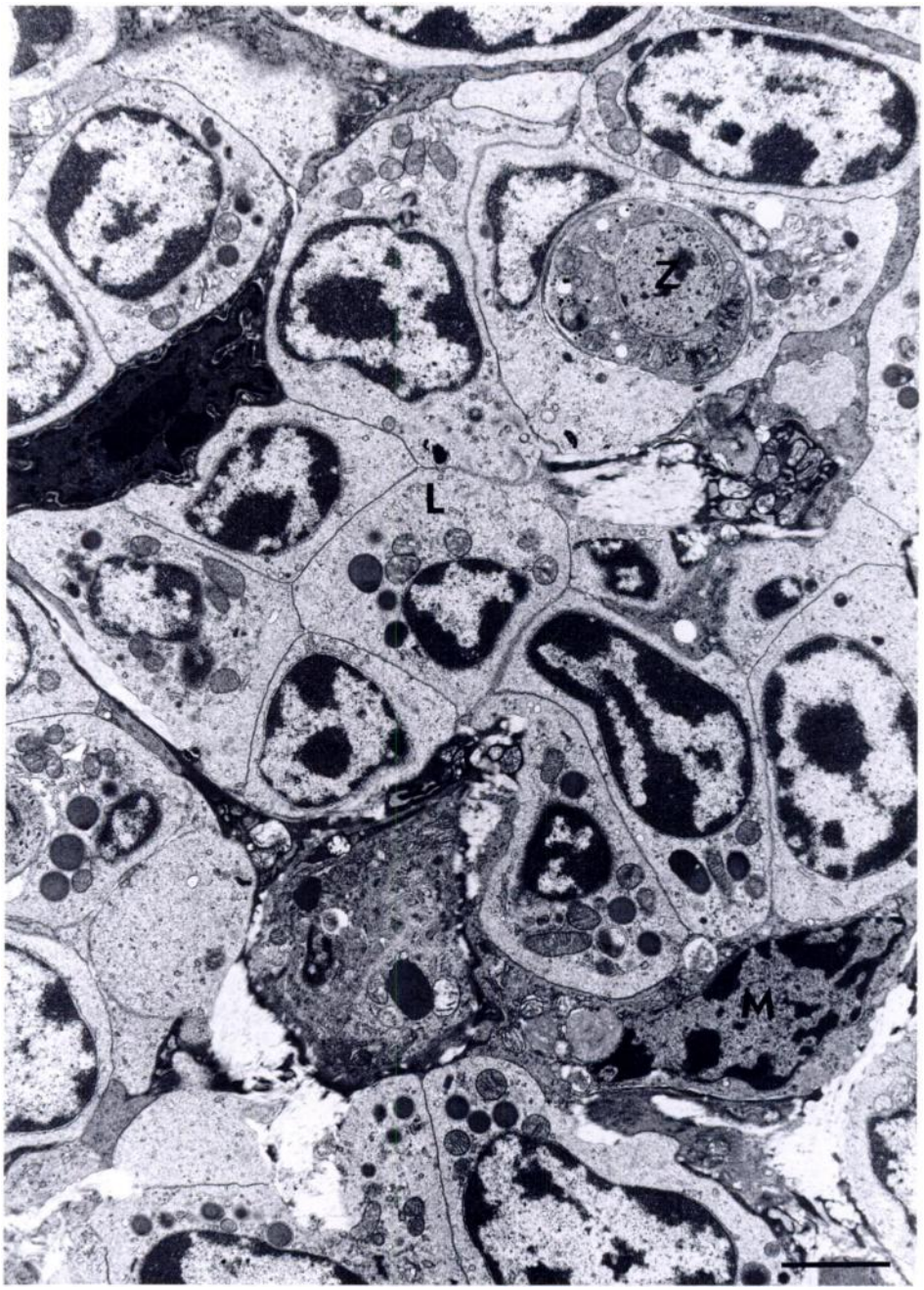


FIGURE 3. Electron photomicrograph of infiltration of jejunal lamina propria by granular lymphocytes (L) and macrophages (M) in a Nashville warbler. Note coccidial zoite (Z) within a lymphocyte. Bar = 1 μm.

containing one to six large ovoid mitochondria and occasional polyribosomes (Figs. 3, 4). The lymphocytes contained zero to eight membrane bound granules which were homogenously electron-dense, 650 to 930 *nm* in diameter and spherical (Figs. 3, 4). Intracytoplasmic coccidial zoites were identified within parasitophorous vacuoles of some lymphocytes (three of three cases) (Figs. 3, 4). However, the

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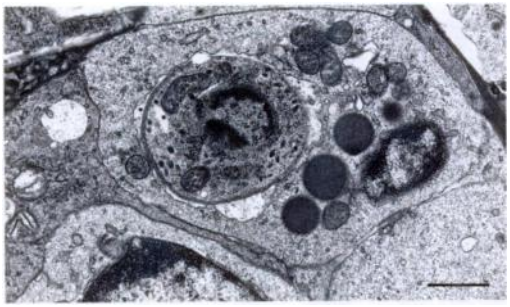


FIGURE 4. Electron photomicrograph of a granular lymphocyte containing a nucleus, a few mitochondria, golgi complex, three large electron dense granules and a coccidial zoite from the intestine of a Nashville warbler. Bar = 2 μm.

prevalence of parasitism in individual lymphocytes ranged from 0 to 5% of the lymphocytes depending on the grid area examined. The ileum, ventriculus, proventriculus, lung, kidney, liver, gonad, heart and spleen from all necropsied birds lacked the lymphocytic infiltrates. Nine of the thirteen birds had concurrent necrotizing pneumonia with associated fungal hyphae morphologically consistent with the class Zygomycetes.

The duodenal and jejunal mucosal epithelium was parasitized by coccidia, but necrosis of the parasitized intestinal epithelium was present only within intestines with severe coccidial infections. Coccidial stages identified within the intestinal epithelium, in decreasing order of incidence, included macrogamonts, oocysts, microgamonts and, rarely, schizonts. Within the intestinal epithelium, three birds had numerous coccidia, two birds had moderate numbers of coccidia and eight birds had occasional coccidia. In addition, single zoites (Figs. 3, 4) or rarely meronts (Fig. 2) were seen within granular lymphocytes of the transmural lymphocytic infiltrates. There was no association between the numbers of intestinal epithelial parasites and the severity of transmural lymphocytic infiltrates, but all cases of lymphocytic enteritis had similar numbers of coccidia within the infiltrating lymphocytes. All thirteen cases of lymphocytic enteritis had concurrent coccidial infections, but two cases of intestinal epithelial coccidiosis without lymphocytic enteritis were diagnosed during the same time period (data not shown).

Two of the three euthanatized, clinically healthy birds had low numbers of coccidia in the intestinal epithelial cells. However, all three birds lacked intestinal epithelial necrosis, lymphocytic enteritis and coccidia in the lamina propria.

Microbiologic cultures isolated single or combinations of various bacteria from individual birds and these bacteria included *Escherichia coli*, *Citrobacter* sp., *Acinetobacter* sp., *Proteus* sp., *Pseudomonas aeruginosa*, *Streptococcus* sp. (non-hemolytic), *Enterococcus* sp., *Klebsiella* sp., *Moraxella* sp., *Providencia rettgori*, *Morganella morganii* and *Enterobacter* sp. The intestines and visceral organs were negative for virus isolation.

DISCUSSION

Based on the disease prevalence, the clinical history and gross, histopathologic and ultrastructural data, the most appropriate diagnosis for the intestinal lesion was transmural lymphocytic enteritis. Ultrastructurally, the infiltrating lymphocytes resembled the granular lymphocytes identified in the intestine of the chicken, turkey (*Meleagris gallopavo gallopavo*) and Bobwhite quail (*Colinus virginianus*) (Al-Attar and Fernando, 1987; Lawn and Rose, 1982; Lawn et al., 1988; Millard and Lawn, 1982). Synonyms used for these lymphocytes included interepithelial lymphocytes, intraepithelial lymphocytes, granulated intraepithelial lymphocytes and globular leukocytes (Lawn et al., 1988).

Lymphoproliferative disease was diagnosed in three wild, captive-raised American goldfinches (*Carduelis tristis*) from western Ontario, Canada (Middleton and Julian, 1983). These goldfinches had severe transmural lymphocytic intestinal infiltrates and lymphocytic infiltrates in other visceral organs. The uniformity of the infiltrating lymphocyte population and the severity of the lymphoid infiltration could

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give credence to a diagnosis of lymphoproliferative disease. However, the presence of coccidial zoites in 10% of the intestinal granular lymphocytes raises the possibility that the diagnosis may have been an inflammatory disease; i.e., transmural lymphocytic enteritis and nonneoplastic lymphocytic infiltrates in other visceral organs. Passeriformes with systemic coccidiosis have had similar gross and histologic lesions in the intestines and visceral organs (Cooper et al., 1989; Helman et al., 1984). However, in the goldfinches, the presence or absence of coccidia in visceral organs was not described (Middleton and Julian, 1983).

By comparison to the goldfinches (Middleton and Julian, 1983), the Nashville warblers had similar transmural intestinal infiltrates of granular lymphocytes and had coccidial zoites within the infiltrating lymphocytes. However, the warblers lacked lymphocytic infiltrates in other visceral organs.

In the Nashville warblers, the intestinal coccidial infection was most likely responsible for this epizootic of transmural lymphocytic enteritis because (1) no consistent pathogenic bacterial agent or agents were cultured from viscera, (2) virus isolation was negative in chicken embryos after three passages, (3) no viral particles were identified on ultrastructural examination, (4) all cases of transmural lymphocytic enteritis had mild-to-severe coccidiasis, (5) the transmural lymphocytes were morphologically identical to non-neoplastic granular lymphocytes and (6) many of the granular lymphocytes contained intracytoplasmic coccidial zoites. Granular lymphocytes have been shown to play an important role in the pathogenesis of *Eimeria dispersa* in turkeys and Bobwhite quail (Millard and Lawn, 1982) and *E. tenella* and *E. necatrix* in chickens (Al-Attar and Fernando, 1987; Lawn and Rose, 1982). In addition, these coccidia have produced thickened intestinal walls because of infiltrating inflammatory cells, especially lymphocytes (Reid et al., 1984).

In the Nashville warblers, the coccidium probably represents an *Isospora* spp. Various *Isospora* spp. have been identified in several different warbler species from Europe and North America, but identification and classification of coccidia from Nashville warblers has not been performed (Davis, 1971). Furthermore, *Eimeria* spp. are rare in passeriformes (Davis, 1971). The presence of minimal-to-mild coccidial infection in the euthanatized clinically healthy Nashville warblers in this study suggests that coccidial infection is common in wild passeriformes. Stress-induced immunosuppression, such as associated with captivity, or increased density of oocysts in the captive environment may have made the coccidial infection of clinical significance during the second year of the capture-and-release program. Crowding and poor sanitation have been implicated in increasing the infection rate and clinical severity of *Isospora* sp. in house sparrows (Box, 1981). Alternatively, in our Nashville warblers, the presence of intestinal coccidial infection in two dead birds and in two clinically normal birds without the transmural lymphocytic enteritis could be supportive of multiple single or mixed coccidia infections in individual Nashville warblers. Multiple single and mixed *Isospora* sp. infections have been reported in other passeriformes (Box, 1977; van Riper and van Riper, 1987).

Intense lymphocytic infiltrates in the intestines and other visceral organs have been associated with systemic *Isospora* sp. infections of other passeriformes (Cooper et al., 1989; Desser, 1980; Helman et al., 1984). Asexual stages of *Isospora* sp. have been identified in fixed and circulating mononuclear phagocytes and lymphocytes from passeriformes (Cooper et al., 1989; van Riper and van Riper, 1987). The identification of disseminated coccidial asexual stages and intestinal coccidial sexual stages were responsible for the dual classification of these coccidia as *Atoxoplasma* sp. and *Isospora* sp., respectively. Our Nashville warblers lacked lymphocytic infiltrates and

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coccidia in lung, liver and spleen. Currently, evidence is lacking to suggest the coccidium from Nashville warblers can produce a systemic infection totally analogous to *Atoxoplasma/Isospora* spp. of other passeriformes (Box, 1977; Levine, 1982; van Riper and van Riper, 1987).

We suggest that the transmural lymphocytic enteritis was an exaggerated immunologic or inflammatory cellular response associated with the extraepithelial intestinal coccidium. Furthermore, the presence of meronts (Fig. 2) within mononuclear cells of the transmural lymphocytic enteritis suggests an extraepithelial asexual stage for this intestinal coccidium.

ACKNOWLEDGMENTS

The Nashville warblers capture-and-release program resulted from a cooperative project between The Ohio State University Department of Zoology, the Columbus Zoo and the United States Fish and Wildlife Service. R. F. Wack and G. Bates are thanked for providing veterinary clinical services. R. J. Julian, O. J. Fletcher, E. J. Mahaffey, C. Riddell, H. J. Barnes, P. C. Stromberg and S. E. Weisbrode are thanked for providing opinions on the intestinal histopathology and ultrastructural morphology.

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Received for publication 12 December 1990.

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