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Case Report

Ventricular Habronemiasis in Aviary Passerines

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A variety of Habronematidae parasites (order Spirurida) have been described as occasional parasites of avian species; however, reports on passerines are relatively uncommon. From 2007 to 2008, 11 passerine deaths at The North Carolina Zoological Park in Asheboro, NC, USA, were associated with ventricular habronemiasis, which was determined to be the cause of death or a major contributing factor in 10 of the 11 individuals. The number and species affected were 5 Red-billed Leiothrix (*Leiothrix lutea*), 2 Japanese White-eye (*Zosterops japonicus*), 2 Golden-headed Manakin (*Pipra erythrocephala*), 1 Blue-grey Tanager (*Thraupis episcopus*), and 1 Emerald Starling (*Coccycolius iris*). Affected animals displayed nonspecific clinical signs or were found dead. The ventricular nematodes were consistent in morphology with *Procyrnea* sp. Koilin fragmentation with secondary bacterial and fungal infections was the most frequently observed pathologic lesion. Secondary visceral amyloidosis, attributed to chronic inflammation associated with nematodiasis, was present in 4 individuals. An insect intermediate host is suspected but was not identified. Native passerine species within or around the aviary may be serving as sylvatic hosts.

1. Introduction

Habronemiasis is used to describe infection by any of the genera of the family Habronematidae within the order Spirurida. The most well-known Habronematidae parasites, *Draschia megastoma*, *Habronema muscae*, and *Habronema microstoma*, are of minimal clinical significance as equine stomach parasites [1]. However, many members of the family Habronematidae (*Odontospirura*, *Sicarius*, *Exsica*, *Procyrnea*, *Cyrnea*, and *Metacyrnea*) are found in birds [2].

In nonpasserines, a variety of habronemes have been described as occasional parasites found within the proventriculus, ventriculus, and/or intestine. Reports are predominantly from Africa, Asia, and Australia and include buzzard, eagle, egret, falcon, fowl, hawk, hummingbird, kite, kiwi, owl, parrot, pigeon, tinamou, vulture, and woodpecker species [3–22]. With the exception of a lethal case in a black-backed woodpecker (*Picoides arcticus*), no host morbidity, mortality, or significant pathology is reported with habronemiasis in nonpasserines.

Reports on habronemiasis in passerines appear to be much less common. *Habronema hyderabadensis* was found in the proventriculus and ventriculus of *Gracula* (*religiosa*) *intermedia* in India [11], while habronemes from the genera *Viguiera* and *Cyrnea* have been reported in the ventriculus of Australian passerines [12]. Neither described any clinical significance. A case report from Germany [23] describes a series of cases of ventricular habronemiasis causing morbidity and mortality in Red-billed Leiothrix (*Leiothrix lutea*) and Bali Myna (*Leucopsar rothschildi*). A survey of American robins (*Turdus migratorius*) appears to be the only published record of North American passerine habronemiasis. *Habronema* sp. were recovered from the esophagus and proventriculus of 6 adult birds (3 males, 3 females) out of 62 surveyed [24].

This report describes two outbreaks of ventricular habronemiasis occurring in 2007 and 2008, which resulted in

Table 1: Passerine deaths associated with habronemiasis at the North Carolina Zoological Park, Asheboro, NC, USA.

Date	Species	Sex	Age	Cause of death	Other/associated lesions
3/28/2007	*L. lutea*	M	10 yrs	Hepatic amyloidosis	Ventricular habronemiasis
6/18/2007	*L. lutea*	F	7 yrs	Hepatic amyloidosis	Ventricular habronemiasis with secondary bacterial ventriculitis; ovarian amyloidosis
6/19/2007	*T. episcopus*	F	2 yrs	Trauma	Ventricular habronemiasis with secondary bacterial ventriculitis
7/23/2007	*Z. japonicus*	M	7 yrs	Ventricular habronemiasis	Secondary fungal ventriculitis
7/23/2007	*P. erythrocephala*	N/A	13 days	Ventricular habronemiasis	Secondary bacterial and fungal ventriculitis
7/23/2007	*L. lutea*	F	2 yrs	Ventricular habronemiasis	Secondary bacterial and fungal ventriculitis
8/13/2007	*P. erythrocephala*	N/A	40 days	Ventricular habronemiasis	Secondary bacterial ventriculitis
10/5/2007	*L. lutea*	F	7 yrs	Ventricular habronemiasis	Secondary bacterial ventriculitis; hepatic amyloidosis
5/10/2008	*C. iris*	M	4 yrs	Ventricular habronemiasis	Secondary bacterial ventriculitis; hepatic, splenic, and renal amyloidosis
8/16/2008	*L. lutea*	M	11 yrs	Ventricular habronemiasis	Secondary bacterial and fungal ventriculitis
9/2/2008	*Z. japonicus*	F	8 yrs	Ventricular habronemiasis	Secondary bacterial and fungal ventriculitis; pulmonary aspergillosis; polyoma nephritis; and myocarditis

morbidity and mortality in passerines at The North Carolina Zoological Park, Asheboro, NC, USA.

2. Cases

2.1. Affected Animals. The R. J. Reynolds Forest Aviary at the North Carolina Zoological Park simulates tropical forest and displays approximately 36 species of exotic birds from Africa, Asia, Australia, Indonesia, and South America. The aviary sporadically houses native, rehabilitated, unreleasable passerine species. All birds complete at least a 30-day quarantine prior to introduction into the exhibit. While considered a closed system, native insects, reptiles, amphibians, and occasional birds have entered the habitat.

From March to October 2007 and May to September 2008, 11 passerine deaths were associated with habronemiasis. Five of the individuals were hatched at the North Carolina Zoological Park, while 6 were obtained from other institutions over time and had passed through quarantine. The number and species affected were 5 Red-billed Leiothrix (*Leiothrix lutea*), 2 Japanese White-eye (*Zosterops japonicus*), 2 Golden-headed Manakin (*Pipra erythrocephala*), 1 Blue-grey Tanager (*Thraupis episcopus*), and 1 Emerald Starling (*Coccycolius iris*). Gender and age of each individual are presented in Table 1.

2.2. Clinical Examination, Gross Pathology, and Microscopy. The deaths of 10 individuals were spontaneous: 8 were found dead, 1 individual died in-hand during examination, and 1 individual died en route to treatment. The eleventh individual was humanely euthanized because of declining quality of life.

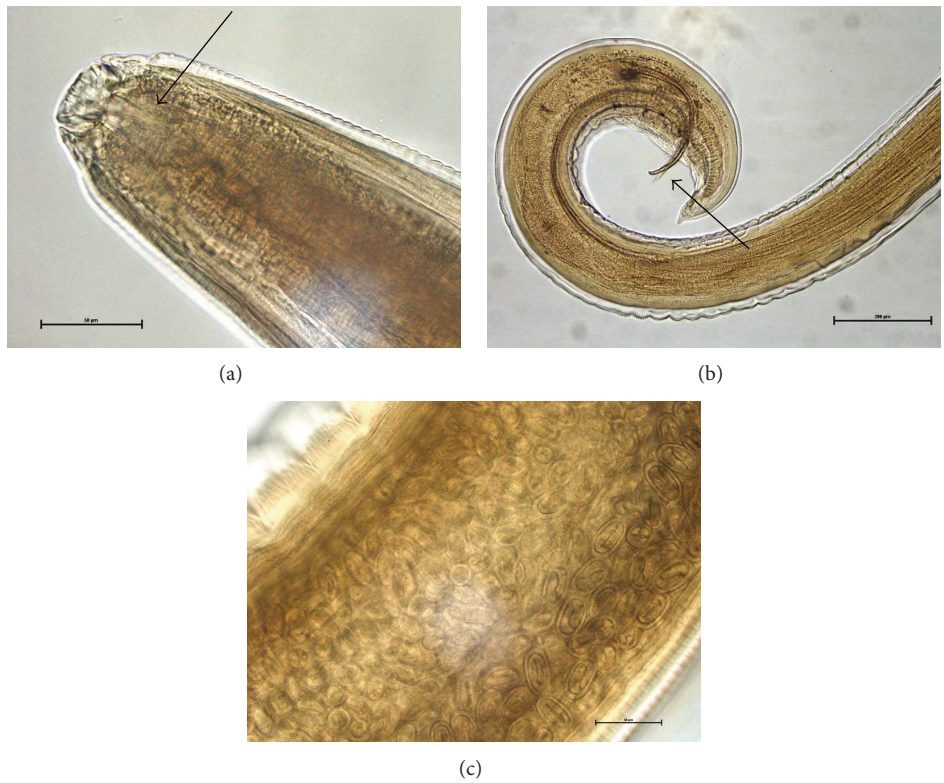
Before death, a majority of affected individuals ($n = 7$) displayed nonspecific clinical signs associated with illness in birds, such as depression or lethargy, perching low or on the ground, and/or a fluffed appearance. After death, 7 individuals (5 with other clinical signs) were noted to be in thin to emaciated body condition (body condition scores ranging from 1 to 2 out of 5 for the 6 individuals given a score).

All individuals were stored at 4°C until postmortem examination and necropsy by a veterinarian. On gross examination, 8 individuals were found to have visible nematodes associated with the koilin layer or within the lumen of the ventriculus or intestinal tract. Four individuals had gross hepatomegaly. Thickened, irregular, or friable koilin was noted in 3 individuals.

Nematodes were preserved in 70% ethanol for identification. Representative specimens were cleared and temporarily mounted in an alcohol and glycerine solution. Habronematidae taxonomy and identification follow those of Chabaud [2].

Whole nematode specimens recovered from the ventriculus of a *C. iris* were consistent in morphology with *Procyrnea* sp. As with Chabaud's [2] key, our specimen had weakly developed teeth near the anterior border of the pseudolabia. Median teeth and a cylindrical, chitinous buccal cavity (Figure 1(a)) were present. The male had a distinct copulatory spicule (Figure 1(b)) and a tail of the spirurid type with 9 preanal papillae, 4 postanal papillae, and a terminal group of papillae and phasmids. The uteri of females were filled with thick-shelled, larvated eggs (Figure 1(c)). Although the quality and number of specimens examined for identification were not adequate for confident speciation, our specimens were similar in appearance and measurement to *Procyrnea mansioni* [16, 23].

2.3. Histopathology. Samples of brain, lung, kidney, liver, and gastrointestinal tract were collected for histopathology from each individual. Spleen, heart, skeletal muscle, gonads, pancreas, and thyroid were collected from some individuals. The samples were fixed in 10% neutral buffered formalin. Fixed samples were embedded in paraffin wax; 5 μm serial sections were made and then stained with hematoxylin and eosin (H&E) stain. When applicable, additional staining



(a) (b) (c)

Figure 1: Whole mount of *Procyrnea* sp. nematodes from ventriculus of *C. iris*. (a) Anterior showing lips and cylindrical, chitinous buccal cavity (arrow). Scale bar = 50 μm. (b) Posterior aspect of male with copulatory spicule (arrow). Scale bar = 200 μm. (c) Uterus filled with thick-shelled, larvated eggs. Scale bar = 50 μm.

with acid fast, trichrome, Sirius red, periodic acid Schiff, Grocott's methenamine silver stain, or Congo red (pre- and post-acid digestion with 0.5% potassium permanganate and 0.3% sulfuric acid) was performed. Board-certified veterinary pathologists performed histopathologic examinations.

Histologically, *Procyrnea* sp. parasites were found between the koilin and mucosal layers of the ventriculus (Figure 2(a)) and occasionally within the lumen of the proventriculus, ventriculus, or intestinal tract. A thin cuticle, polymyarian, coelomyarian musculature, lateral cords, complete digestive tract, pseudocoelom, and a tripartite esophagus were identified on cross-section. Numerous oval, thick-shelled, embryonated eggs were present within the uterus (Figure 2(c)).

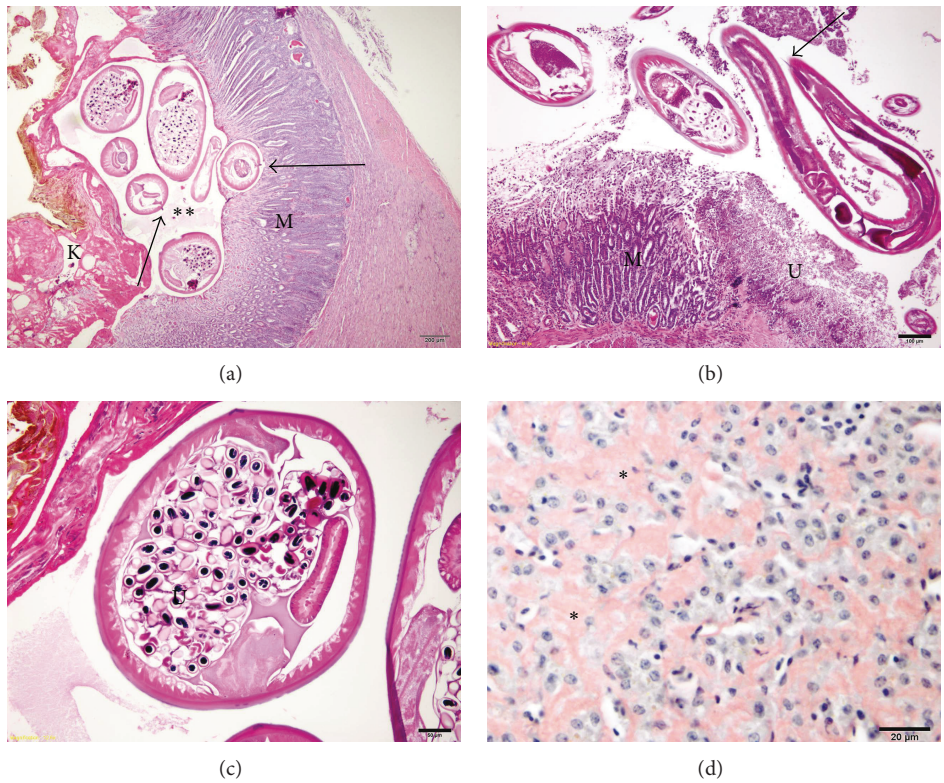
The associated koilin layer was frequently fragmented and admixed with aggregates of fibrin, necrotic cell debris, and inflammatory cells (Figure 2(b)). Ventriculitis was severe in 5 individuals, moderate in 2 individuals, and mild in 4 individuals. In 10 of the 11 affected individuals, secondary bacterial infections and/or fungal infections (consistent with *Candida albicans*) were identified. Mucosal ulceration was occasionally noted.

Four of the adult animals, including 3 of the 5 *L. lutea* and the single *C. iris*, also had visceral amyloidosis verified by Congo red staining (Figure 2(d)). Amyloidosis in the 3 *L. lutea* was moderate to severe, while the amyloidosis in the *C. iris* was mild. Amyloid deposits were most commonly found within hepatic sinusoids but were also identified in ovarian, splenic, and renal tissue. Loss of Congo red staining after acid digestion with 0.5% potassium permanganate and 0.3% sulfuric acid was consistent with reactive, serum amyloid-associated (SAA) secondary amyloidosis.

Severe ventricular habronemiasis was determined to be the cause of death in 8 of the 11 individuals (Table 1) and a major contributing factor in the death of 2 individuals with severe hepatic amyloidosis. A single individual died of trauma.

3. Discussion

Unlike the low pathogenicity often seen with equine gastric and nonpasserine avian habronemiasis, increased morbidity and mortality were associated with ventricular habronemiasis of passerines at the North Carolina Zoological Park. Ventricular habronemiasis in these individuals caused chronic ventriculitis and resulted in poor body condition and death. Disruption of the protective koilin layer of the ventriculus led to inflammation and increased susceptibility to secondary bacterial and fungal infections. Similar koilin disruption and large bacterial colonies were noted in the black-backed woodpecker [20], but no inflammatory reaction or change in the koilin layer was reported by Ehrsam et al. [23] for German cases of ventricular habronemiasis in *L. lutea* and *L. rothschildi*.



(a) (b)

(c) (d)

FIGURE 2: (a) Lower power view of ventriculus from *L. lutea*. Multiple cross-sections of *Procyrnea* sp. nematodes (asterisks) with lateral alae (arrows) are visible between the mucosa and fragmented koilin layer (H&E stain). M: mucosa; K: koilin layer. Scale bar = 200 μm. (b) Higher power view of the ventriculus from a more severely affected *Z. japonicus*. The koilin layer above the *Procyrnea* sp. nematodes is largely absent and there is regionally extensive mucosal ulceration which is covered with a mat of fungal hyphae admixed with fibrin and cell debris. The posterior spicule from a male nematode (arrow) is captured in longitudinal section (H&E stain). M: mucosa; U: ulceration. Scale bar = 100 μm. (c) Cross-section through a female *Procyrnea* sp. nematode from a *Z. japonicus* containing large numbers of larvated, thick-shelled eggs (H&E stain). U: uterus. Scale bar = 50 μm. (d) Large sinusoidal accumulations of brightly eosinophilic amyloid (asterisks) compress the adjacent hepatocytes in a liver from a *L. lutea* (Congo red stain). Scale bar = 20 μm.

Amyloidosis in birds usually occurs in the liver, spleen, and/or kidneys [25]. It is typically of the SAA type and is associated with chronic infectious disease [25]. Given the absence of other inflammatory lesions, amyloidosis was attributed to chronic ventriculitis caused by nematode infestation. The overrepresentation of *L. lutea* in cases of amyloidosis could denote species-specific variation in amyloid formation in response to chronic inflammation.

Antemortem direct fecal smears and sodium nitrate fecal flotations had been negative for spirurid nematodes. The eggs of the equine *Habronema* and *Draschia* spp. tend not to float well in fecal flotation solutions and are fragile, thus making them hard to find [1]. A combination filtration and centrifugation method is described by Ehrsam et al. [23] and could be attempted in the future.

Given the use of fly intermediates by equine habronememes, the clustering of cases during warm months, and the omnivorous habits of the species affected, an insect intermediate is suspected in this case. Maggots used in diets in the aviary were unlikely to be the intermediate as they are obtained from a commercial source and have been analyzed by a parasitologist and were negative for nematode larvae. Rehabilitated, unreleasable, native passerines introduced to the aviary undergo the same quarantine procedures as exotic aviary species prior to introduction to the general population including prophylactic deworming, typically with a single dose of ivermectin. However, while the aviary is technically a closed environment, native insects, reptiles, amphibians, and occasional birds still get into the exhibit. Efforts to identify an insect intermediate have been unsuccessful and collection and necropsy of native passerines found dead within the zoological park have not identified a sylvatic host. Reported intermediate hosts of *Procyrnea pileata* include pillbugs (*Armadillidium vulgare*) and earwigs (*Euborellia annulipes*) [20], while the German cockroach (*Blattella germanica*) has been experimentally infected with *Cyrnea colini* [23]. These insect species or close relatives could be foraged by birds within the aviary. If an intermediate host is identified, xenodiagnosis may be an effective antemortem diagnostic tool and biologic control using parasitoid wasps could be considered if the insect intermediate is *Musca* sp. [1, 26].

For companion and aviary birds, treatment with oral fenbendazole given daily for 3–5 days, pyrantel pamoate given orally and repeated after 14 days, or ivermectin has been successful at eliminating nematode infections [27]. Treatment with oral fenbendazole, repeated after 14 days, has been

reported to be an effective option in raptors [4]. Equine cases are typically treated with a single dose of ivermectin or moxidectin [1, 26, 28, 29].

After the initial diagnosis of habronemiasis, four of the affected *L. lutea* and the *C. iris* were treated symptomatically or prophylactically with either pyrantel pamoate (22 mg/kg by mouth repeated every 10 days for 2-3 doses) or fenbendazole (50 mg/kg by mouth every 24 hours for 1–6 days repeated at variable intervals). These treated birds died within 2 months after their last anthelminthic treatment. However, the small sample size, variation in dosing regimes, and lack of regular repeated dosing preclude any conclusions about anthelminthic efficacy or parasite resistance.

In future outbreaks, prophylactic deworming of all affected species with single doses of ivermectin or moxidectin during the summer months may prove to be more efficacious and practical for the free-flight aviary setting. Increased insect pest control and preventing entry of native avian species into the habitat may also help in the reduction or management of outbreaks.

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