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# Prevalence and pathology of helminths of ciconiiform birds from the Brazilian swamplands

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**Abstract**

The prevalence of helminths recovered from 108 birds representing eight species of Ciconiiformes from the Brazilian west-central region are presented. The digeneans *Ascocotyle* (*Phagicola*) *longa*, *Clinostomum marginatum*, *Cotylotretus grandis*, *Ithyoclinostomum dimorphum*, the nematodes *Contracaecum multipapillatum*, *Desmidocercella ardeae*, *Eustrongylides ignotus*, and the cestode *Valipora mutabilis* were identified. *Contracaecum multipapillatum* was the most prevalent species and *E. ignotus* the most pathogenic. Gross lesions due to infections with *C. multipapillatum* were characterized by ulcerative processes and hyperemia of the mucosa whereas those caused by *E. ignotus* consisted of perforations of the gastric mucosa and fibrotic tubular lesions in the gastric serosa. Histopathological examinations revealed necrosis and mixed leucocyte infiltrations and discrete compression of the mucosa in *C. multipapillatum* infections. Destruction of the mucosa and submucosa with the presence of fibrous capsules were observed in *E. ignotus* infections. Reports of accidental human infections, with severe clinical signs induced by these parasites, indicate the necessity of a proper evaluation of the pathogenicity of helminths of aquatic birds.

## Introduction

The Ciconiiformes show a close relationship with the aquatic environment, feeding almost exclusively on fish and crustaceans. Individuals of this group are of great epidemiological relevance in the spreading of pathogens in different geographical areas due to the migratory habits of some species (Corn *et al.*, 1993; Spalding & Forrester, 1993a; Spalding *et al.*, 1993; Sick, 2001).

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Helminth species of the genera *Eustrongylides* (Jäegerskiöld, 1909), *Contracaecum* (Railliet & Henry, 1912) and *Ascocotyle* (Looss, 1899) play an important role in studies of endoparasites infecting aquatic birds, in view of their zoonotic potential, with frequent reports of human infections caused by the ingestion of raw or poorly cooked fish meat. Fish act as intermediate hosts in the life cycle of these helminths, the pathogenicity of which can be different and more evident in mammals than in birds (Buendia, 1997).

Reports of accidental human infections, with severe clinical signs induced by these parasites, indicate the necessity of the proper evaluation of the pathogenicity of

helminths of aquatic birds by means of experimental infections in laboratory mammals with larvae recovered from fishes (Guerin *et al.*, 1982; Shirazian *et al.*, 1984; Conroy & Perez, 1985; Armas de Conroy, 1986; Barros & Amato, 1995a,b, 1996; Cancrini *et al.*, 1997).

The aims of the present study are to investigate the prevalences of helminths of ciconiiform birds and the pathology associated with the nematodes *Contracaecum multipapillatum* and *Eustrongylides ignotus* infecting these hosts.

## Materials and methods

One hundred and eight specimens of ciconiiform birds representing eight species from the Pantanal do Mato Grosso (Barão de Melgaço county, State of Mato Grosso, Brazil) and Sorocaba, State of São Paulo, Brazil (23°30′S, 47°30′W) were investigated for helminths. The Pantanal is a prairie area subject to periodical inundations and is part of the Paraguay river basin, with about 140,000 $km^2$, located in South America, between 14°00′–22°00′S and 53°00′–66°00′W. The chosen localities for the recovery of helminths were the Zoological Gardens of Cuiabá, State of Mato Grosso, Brazil and Sorocaba since both are strategic arrival areas for these birds. Cuiabá Zoo, close to the Pantanal area, receives from September to November, a great number of birds collected from the nests. Sorocaba Zoo maintains a reserve in which, at the same period of the year, large flocks of white-necked herons and black-crowned night-herons nest.

Birds were killed either because they presented compound fractures followed by infection or died due to other aetiologies at the veterinary facilities in the Cuiabá and Sorocaba Zoological Gardens. Birds had not been held in capitivity before they showed signs of illness and, even then, they were kept in the zoo for short periods of time. Necropsies of the birds, which were performed from 1997 to 1999 in Cuiabá and from 2000 to 2001 in Sorocaba, were in accordance with the methodology described by Latimer & Rakich (1994) and the Brazilian Code of Ethics for Animals (APA, 1989). The development of the present investigation has been authorized by the Committee of Ethics for the Use of Animals (CEUA/Fiocruz), no. P0093-01.

Nine specimens of *Ardea cocoi* L., 1766, three of *Cochlearius cochlearius* (L., 1766), six of *Ajaia ajaja* (L., 1758), 42 of *Ardea alba* [ = *Casmerodius albus* (Gmelin, 1789)], 35 of *Nycticorax nycticorax* (Gmelin, 1789), two of *Butorides striatus* (L., 1758), one of *Jabiru mycteria* (Lichtenstein, 1819) and ten of *Mycteria americana* L., 1758, were examined. Samples of the parasitized stomachs and intestines were removed and immediately fixed in formalin. The material was then routinely processed (Luna, 1968) for paraffin embedding. Sections (5 $\mu$m thick) were stained with haematoxylin and eosin. Photomicrographs were obtained in a Zeiss Axiophot brightfield microscope and in a Leica stereoscopic microscope.

Helminths were collected in a 0.85% NaCl solution and were processed for study in accordance with an adaptation of the methodology described by Amato *et al.* (1991).

Table 1. Data on Brazilian ciconiiform hosts and helminths obtained in Sorocaba, São Paulo and in the Pantanal do Mato Grosso, Mato Grosso, between 1997 and 2001.

| Birds | No. examined | Helminths | Prevalence (%) | Absolute values | Site of infection |
|---|---|---|---|---|---|
| *Ardea cocoi* | | | | | |
| (White-necked heron) | 09 | *Clinostomum marginatum* | 66.7 | 06 | OC |
| | | *Ithyoclinostomum dimorphum* | 44.4 | 04 | OE |
| | | *Contracaecum multipapillatum* | 33.3 | 03 | ST, SI |
| | | *Eustrongylides ignotus* | 77.8 | 07 | ST |
| | | *Ascocotyle (Phagicola) longa* | 11.1 | 01 | SI |
| *Cochlearius cochlearius* | 03 | *Contracaecum multipapillatum* | 100 | 03 | ST |
| (Boat-billed heron) | | | | | |
| *Ajaia ajaja* (Roseate spoonbill) | 06 | *Cotylotretus grandis* | 83.3 | 05 | OC, SI |
| *Ardea alba* (Great egret) | 42 | *Clinostomum marginatum* | 16.7 | 07 | OC, OE |
| | | *Ithyoclinostomum dimorphum* | 26.2 | 11 | OE |
| | | *Eustrongylides ignotus* | 71.4 | 30 | ST |
| | | *Contracaecum multipapillatum* | 71.4 | 30 | ST, SI |
| | | *Valipora mutabilis* | 7.1 | 03 | SI |
| | | *Desmidocercella ardeae* | 2.4 | 01 | OC |
| *Nycticorax nycticorax* | 35 | *Clinostomum marginatum* | 20 | 07 | OC, OE |
| (Black-crowned night-heron) | | *Eustrongylides ignotus* | 42.9 | 15 | ST |
| | | *Contracaecum multipapillatum* | 54.3 | 19 | ST |
| | | *Ithyoclinostomum dimorphum* | 5.7 | 02 | OE |
| | | *Valipora mutabilis* | 5.7 | 02 | SI |
| *Butorides striatus* (Striated heron) | 02 | *Contracaecum multipapillatum* | 50 | 01 | ST |
| *Jabiru mycteria* (Jabiru) | 01 | *Contracaecum multipapillatum* | na | 01 | ST |
| *Mycteria americana* | 10 | *Eustrongylides ignotus* | 30 | 03 | ST |
| (American wood-ibis) | | *Contracaecum multipapillatum* | 20 | 02 | ST |

OC, oral cavity; OE, oesophagus; ST, stomach; SI, small intestine; na, not applicable.

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Classification of the helminths follows Yamaguti (1971), Khalil *et al.* (1994), Vicente *et al.* (1995), Measures (1988) and Scholz (1999); identification and common names of hosts are in accordance with Schauensee (1970) and Sick (2001). The terms prevalence and infrapopulation follow Bush *et al.* (1997). Representative specimens of the identified helminth species were deposited in the Helminthological Collection of the Oswaldo Cruz Institute (CHIOC), under numbers 33896, 34412, 34662, 34666, 34674 (wet material), 34522 a-b, 34584, 34585 a-b, 34712, 34770 (whole mounts).

## Results

Ninety of 108 birds (83.3%) examined were positive for at least one helminth species (table 1). The helminth species recovered were *Clinostomum marginatum* (Rudolphi, 1819) Braun, 1899, *Desmidocercella ardeae* (Nawrotzky, 1914) Yorke & Maplestone, 1926, *Valipora mutabilis* Linton, 1927, *Ithyoclinostomum dimorphum* (Diesing, 1850) Witenberg, 1926, *Ascocotyle (Phagicola) longa* Ransom, 1920, *Cotylotretus grandis* (Rudolphi, 1819) Odhner, 1902, *Contracaecum multipapillatum* (Drasche, 1882) Baylis, 1920 and *Eustrongylides ignotus* Jägerskiöld, 1909.

In the 59 birds of different species infected with *C. multipapillatum*, gross lesions were present, the intensity of which varied with worm burdens. The lesions showed elevated margins and hyperemic centres, varying in size and located mainly in the posterior part of the stomach. Worms were loosely attached to the mucosa (fig. 1a) and on occasional penetration of the mucosa, there was necrosis with mixed leucocyte infiltrations surrounding the parasites (fig. 1b). In most cases, worms were observed in the lumen of the stomach (fig. 1c).

Thirty-one of the birds were infected with *Eustrongylides ignotus*, always with significant gross lesions. Infections with *E. ignotus* resulted in perforations of the gastric mucosa forming whitish circular lesions, 0.5 cm diameter, with elevated margins, distributed throughout the mucosa (fig. 2a). Hyperaemia and haemorrhagic gastritis were present. The nematodes perforated the gastric wall into the serosa, forming whitish and firm sinuous tubular lesions, 0.3–0.5 cm wide (fig. 2b,c). Adult worms were found with their anterior portions directed towards the gastric lumen indicating that the lesions reached the gastric mucosa (fig. 2d). In the more severe cases, adult and larval *E. ignotus* had migrated into the body cavity of birds, to form haemorrhagic lesions with thickening of the serosa associated with perforation of the liver, intestines and lungs (fig. 2e). The gastric mucosa and submucosa showed necrotic foci surrounded by an inflammatory infiltration with a predominance of eosinophils. Lesions in the gastric serosa contained fibrotic capsules surrounding *E. ignotus* (fig. 2f,g).

## Discussion

Environmental changes due to anthropogenic action intensify the injuries in the wildlife hosts and the latter can act as bioindicators of these changes (Friend, 1981).

Spalding *et al.* (1993) reported that fish more exposed to pollutants were those with heavier worm burdens of *E. ignotus*. Infections in naturally infected birds are not characterized by specific symptoms and diagnosis is always difficult; nevertheless, the authors referred to a greater number of cases of regurgitation in tricoloured

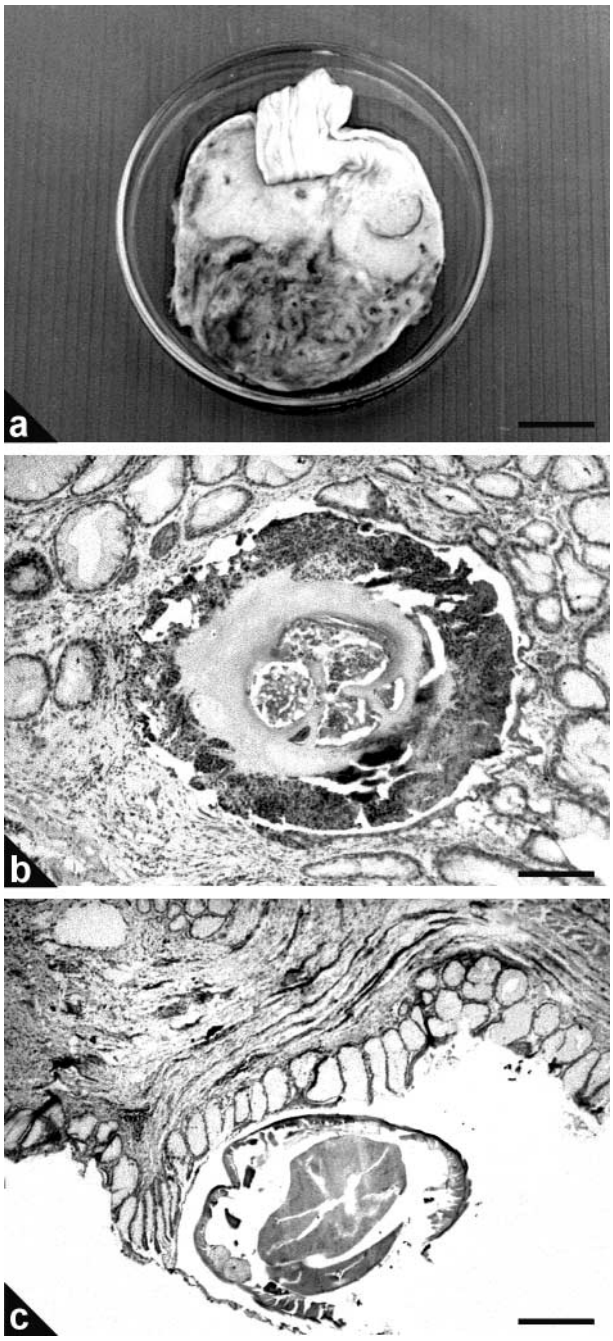


Fig. 1. (a) Proventriculus of *Ardea alba*, infected with *Contracaecum multipapillatum*, showing gross lesions. Bar = 8 mm. (b) Cross-section of the gastric mucosa of *Nycticorax nycticorax*, infected with *C. multipapillatum*, with parasite debris in a necrotic area, surrounded by a mixed leucocyte infiltrate and fibrosis. Bar = 0.1 mm. (c) Cross-section of the gastric mucosa of *Nycticorax nycticorax*, with *C. multipapillatum* in the lumen of the stomach. Bar = 0.1 mm.

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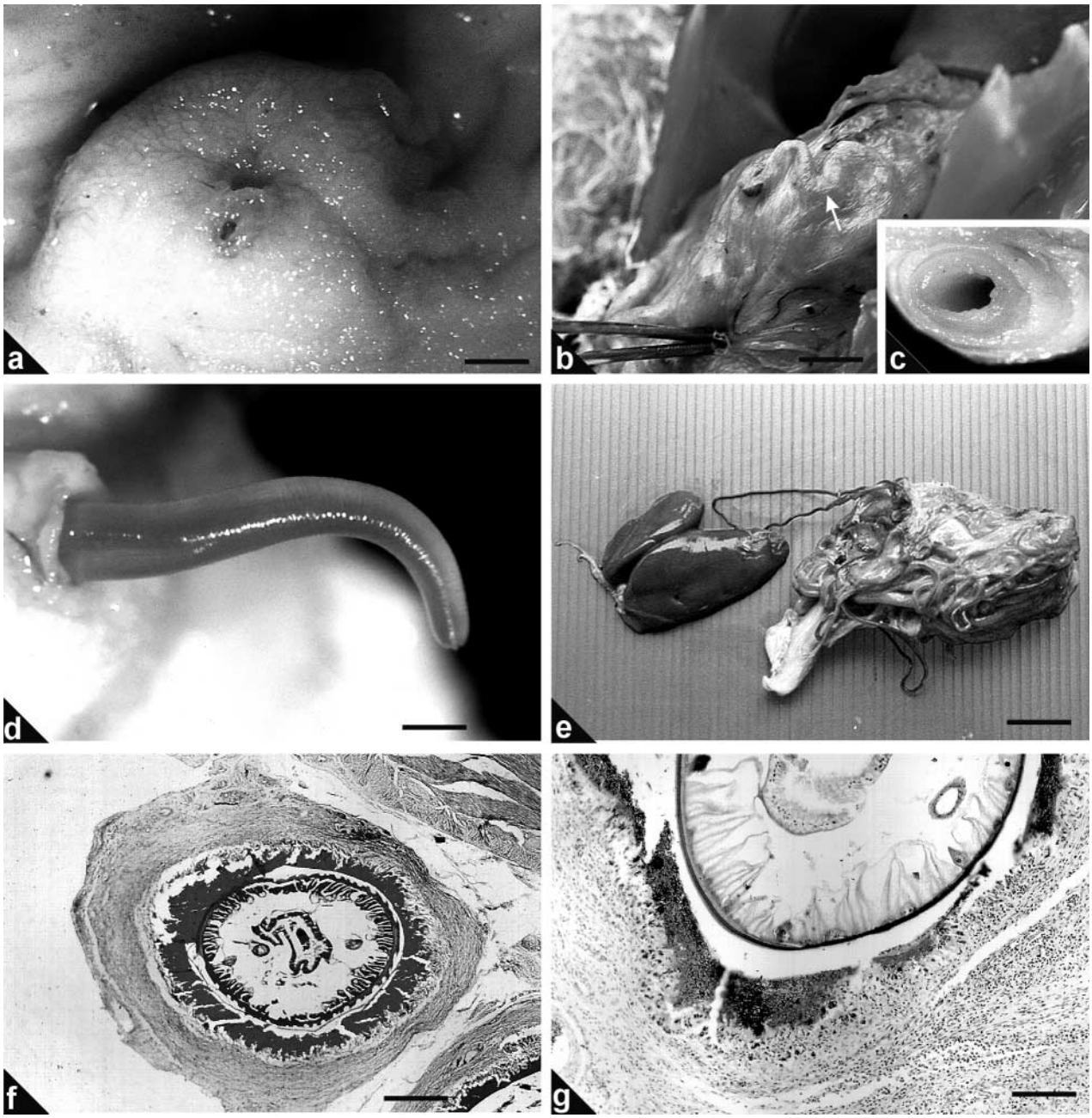


Fig. 2. (a) Fistular lesion of the gastric mucosa in *Casmerodius albus* infected with *Eustrongylides ignotus*. Bar = 0.5 mm. (b) Sinuous tubular lesion (arrow) caused by adult worms of *E. ignotus*. Bar = 0.1 mm. (c) Cross-section of a tubular lesion in detail. Bar = 0.8 mm. (d) Anterior end of an adult *E. ignotus* in the gastric mucosa of *Nycticorax nycticorax*. Bar = 0.8 mm. (e) Intestinal haemorrhagic lesions and liver perforations in *C. albus*, infected with *E. ignotus*. Bar = 8 mm. (f) Cross-section of the gastric serosa of *N. nycticorax* with *E. ignotus*, showing the worm surrounded by necrotic tissue and a fibrous capsule. Bar = 0.1 mm. (g) Cross-section of the stomach of *N. nycticorax*, infected with *E. ignotus*, showing the worm surrounded by necrotic and granular tissue. Bar = 0.1 mm.

herons experimentally infected with larvae of *E. ignotus*, when compared to uninfected birds of the control group.

In the present study, as birds were not maintained for long periods in the zoo, being killed shortly after their arrival, it was not possible to relate mortality rates with parasitism. Spalding *et al.* (1993) report a 2% prevalence of *E. ignotus* in the great egret, *Ardea alba* (=*Casmerodius albus*) whereas this nematode species was present in 71.4% of the specimens of the same host in the swamplands of Brazil. Although data on the populations of *A. alba* in the Pantanal do Mato Grosso are not available, this high prevalence does suggest that further studies should focus on avian conservation in this important ecosystem together with the control of pollution sources.

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Helminths from ciconiiform birds and fish from the Pantanal do Mato Grosso were previously studied by Rego & Eiras (1988) and Schäffer *et al.* (1990) on the basis of material deposited in the CHIOC. *Eustrongylides ignotus* was analysed in tissue samples, and lesions in the gastric wall and liver of black-crowned night-herons and white-necked herons were observed. These results are in agreement with those of the present study and the most common cases involve gastritis and fibrotic tubular lesions caused by the migration of *E. ignotus* from the gastric mucosa to the serosa and often to the liver.

The present findings are also in accordance with the data of Spalding & Forrester (1993b) that refer to natural and experimental infections of ciconiiforms with *E. ignotus* in the USA, when larvae perforated the ventriculus in 3 to 5 h, causing haemorrhaging and bacterial peritonitis which, in certain cases, progressed to a fibrous peritonitis with extensive adhesions.

Coprological screening is not always indicated in the detection of the presence of avian helminths. In the case of *C. multipapillatum*, the worms are trapped by the tubular fibrotic lesions which, by maintaining the parasites in contact with the gastric lumen, where oviposition occurs, can alter the results of the faecal examination for eggs.

Spalding (1990) proposed a method, i.e. abdominal palpation, for detecting fibrotic lesions caused by the migration of larval *Eustrongylides* sp. in newborn birds. During the present study, the employment of this technique was not adequate since we dealt almost only with adult birds, and their more developed abdominal musculature and large amount of fatty tissue can interfere in the efficacy of this diagnostic method. Nevertheless, for the few young birds examined, the technique proved to be useful and the results were confirmed during post-mortem examination.

The present data on the pathology induced by *Contracaecum multipapillatum* in birds are the first to be reported so far.

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