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Research Note

Occurrence of endoparasites in Ramphastidae (Aves: Piciformes) in São Paulo Zoo

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Summary

Ramphastidae (Aves: Piciformes) are animals recognized for their exuberant colours and long bill, with distribution range from south Mexico to north Argentina. They are important seed dispersers eating little vertebrates eventually. When in captivity, animals usually live in limited spaces, with high density and near by species that do not share a evolution history, facilitating the occurrence of infectious diseases for what they may not have a competent immune system against, including the parasitic ones. This study analyzed the endoparasites that occur in captive Ramphastidae at São Paulo Zoo in the period January 2009 to September 2011. Seven species of toucans and toucanets had parasitological results positive for Trichuridae nematodes, *Eimeria* sp., *Giardia* sp., non sporulated coccidian oocysts and unidentified nematode eggs, and microfilarie was the only hemoparasite found.

Keywords: Ramphastidae; captive; endoparasites; Trichuridae; coccidian; *Giardia* sp.

Introduction

Zoos are places that maintain a great number of animals, since the smallest invertebrate to large mammals; all of them have its importance in education and conservation in modern zoos. The role of these institutions in conservation have increased significantly in the last decades, and, nowadays, many zoos develop *ex-situ* and *in-situ* projects with financial and technical support. Some of them are considered as a genetic reservoir for many threatened species (WAZA, 2005).

Captive environment also offers some specials conditions to animals when compared to wildlife, such as enough food available through the year and for all individuals, protection against predators and veterinary care (Mukhin *et al.*, 2016). On the other hand, when in captivity, animals may live in a highly populated space, which can favour the transmission of infectious diseases and parasites. The most common parasites found in zoos have direct life

cycle, especially nematodes and protozoans (Panayotova-Penchewa, 2013). Many diseases have been studied in wildlife, with zoonoses being the preferred ones by researchers, but the ecology and diversity of parasites are being left in second plan (Smith *et al.*, 2009; Thompson *et al.*, 2010). Parasites is part of biodiversity and has its importance in communities which they belong to, helping to control population through influencing directly in births and longevity of infected animals and mediating hosts interactions (Wobeser, 2008, Smith *et al.*, 2009; Thompson *et al.*, 2010).

Toucans and toucanets (Piciformes: Ramphastidae) are known for their long beak and feather's exuberant colours (Short & Horne, 2002). Ramphastidae has five different genus, *Aulacorhynchus*, *Andigena*, *Selenidera*, *Pteroglossus* and *Ramphastos* and 78 subspecies (Short & Horne, 2002). International Union for Conservation of Nature (IUCN, 2016) classifies many of these species with some level of threat: *Aulacorhynchus huallagae* and *P. bitorquatus* are classified as endangered and *R. culminatos*, *R. tucannus* and

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R. vitellinus as vulnerable (IUCN, 2016). They can be found from south Mexico to north Argentina, including Andean region, usually in closed forests, with exception of *R. toco* which prefers open areas such as Brazilian Savannah (Short & Horne, 2002). These animals are considered ecologically important seed dispersers due to the fact they can fly through long distances and for eating a huge variety of fruits in its diet (Pizo *et al.*, 2008; França *et al.*, 2009). Besides that, some species, as *R. toco*, was already reported eating eggs and nestlings of other bird species, such as *Anodorhynchus hyacinthinus* (Pizo *et al.*, 2008), a normal behaviour specially during reproductive season.

There are few studies of hemoparasites infecting Ramphastidae (Bennett & Borrero, 1976; Young *et al.*, 1993; Valkiūnas *et al.*, 2004; Chagas *et al.*, 2016). The presence of *Plasmodium pinotti* and *P. huffi* was already reported in toucans and toucanets (Valkiūnas, 2005). Different filarioid nematodes such as the genus *Pelecitus* sp., *Splendidofilaria* sp., *Desseffilaria* sp., *Eulindana* sp. and other unidentified microfilaries were also reported (Young *et al.*, 1993; Barlett, 2008). Gastrointestinal parasites have been described in other Brazilian zoos such as *Heterakis* sp., *Capillaria* sp., Strongyloidea and coccidian (Upton *et al.*, 1984; Freitas *et al.*, 2002; Dubey *et al.*, 2004; Yabsley, 2008b; Dubey *et al.*, 2009). Trichuridae nematodes could be really common in Ramphastidae and many species were already described, like *Baruscapillaria obsignata* (commonly found in captive animals) and *Capillaria venusta* (Pinto *et al.*, 1996; Freitas *et al.*, 2002; Cubas, 2006; Yabsley, 2008a). Ramphastidae are also known to carry *Giardia* sp. in asymptomatic infections and acting as reservoir for this parasite (Cornelissen & Ritchie, 1994). This group was already reported to have one of the highest prevalence of parasites among different bird species in other zoos (Corredor *et al.*, 2013).

This study seeks to investigate the presence of parasites in a captive Ramphastidae population, determining the occurrence of hemoparasites and enteroparasites in all species studied and propose a preventive parasite control in captive facilities.

Material and Methods

The study site is São Paulo Zoo Foundation, located in a small fragment of Atlantic Forest in the metropolitan region of São Paulo

city that harbours the Ipiranga Stream, which forms a lake that shelters several wild species of birds including migratory species. Sampled animals, excepted two, lived in captivity, with water *ad libitum* and food offered twice a day, such as fruits and ration. Species sampled were *Ramphastos toco*, *Ramphastos tucannus*, *Ramphastos vitellinus*, *Ramphastos dicolorus*, *Pteroglossus aracari*, *Pteroglossus bailloni* and *Selenidera maculirostris*.

Results for hemoparasites were evaluated between January 2009 and September 2011. Samples were processed through two different techniques, direct blood smears as recommended by Godfrey *et al.* (1987) and Knott modified technique (Foreyt, 2001). Parasites were identified according to available literature (Foreyt, 2001; Valkiūnas, 2005; Atkinson *et al.* 2008). For hemoparasites, the species sampled are shown as follow, and in brackets the number of birds: *R. toco* (4), *R. tucannus* (5), *R. vitellinus* (3), *R. dicolorus* (7), *P. aracari* (4), *P. bailloni* (2) and *S. maculirostris* (9). Two were free living birds: *R. vitellinus* and *R. dicolorus*.

Results for enteroparasites were obtained during the period of January 2010 to September 2011. All samples were processed through three different methods: direct analysis, flotation with saturated sodium chlorite solution and Hoffman, Pons e Janer for sedimentation (Foreyt, 2001; Hendrix & Robinson, 2006). Parasites were identified according to available literature (Foreyt, 2001; Hendrix & Robinson, 2006; Atkinson *et al.* 2008). For enteroparasites, the species sampled are shown as follow and in brackets the number of birds: *R. toco* (4), *R. tucannus* (4), *R. vitellinus* (2), *R. dicolorus* (5), *P. aracari* (4), *P. bailloni* (1) and *S. maculirostris* (9).

Results

Hemoparasites

Between January/2009 and September/2011, 103 blood samples were screened for hemoparasites in 34 individuals from seven species; eighteen were females, fourteen were males and two were undetermined. All the results of hemoparasites are shown in Table 1. The only hemoparasite found were microfilarie, present in eight samples (7.8 %) from five individuals (14.7 %). Species infected were: *R. vitellinus* (1), *R. dicolorus* (2), *P. bailloni* (1) and *S. maculirostris* (1). Individuals positive were two males, one female and one of undetermined genus. Morphological differences were found

Table 1. Prevalence of hemoparasites found in captive Ramphastidae.

	Individuals (sample number)	Prevalence
<i>Ramphastos toco</i>	4 (15)	0.0 %
<i>Ramphastos tucannus</i>	5 (17)	0.0 %
<i>Ramphastos vitellinus</i>	3 (12)	33.3 %
<i>Ramphastos dicolorus</i>	7 (21)	9.5 %
<i>Pteroglossus aracari</i>	4 (11)	0.0 %
<i>Bailloni bailloni</i>	2 (4)	25.0 %
<i>Selenidera maculirostris</i>	9 (23)	4.3 %

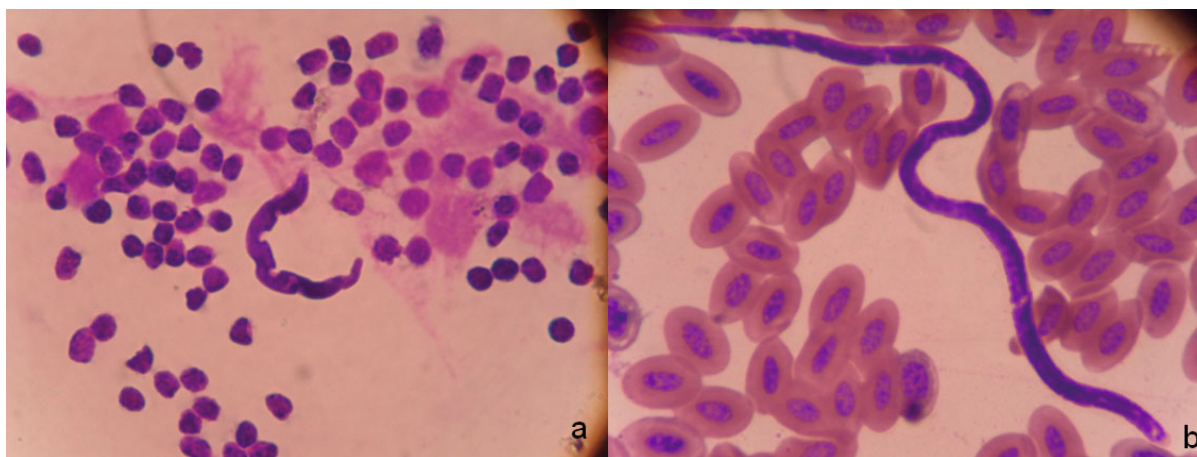


Fig. 1. Microfilarie found in *Ramphastos vitellinus* (a) and in *Selenidera maculirostris* (b). Rosenfeld stain. x400

between the microfilariae in *R. vitellinus* and the other positive bird species (Fig. 1), but no micrometrical tests were made to confirm the parasite species. Only one free living bird, *R. dicolorus*, were positive for microfilaria.

Enteroparasites

Results for enteroparasites were analyzed for the period of January/2010 to September/2011; 104 samples from 29 animals were screened. Some animals are allocated with more than one individual in the same enclosure, which not allowed individual analysis of each animal and, consequently, the results are presented by samples and not by individuals. All the results of enteroparasites are shown in Table 2.

The species with the majority number of samples was *S. maculirostris* representing 23.1 % of total, followed by *R. toco* (20.2 %), *R. dicolorus* (19.2 %) and *R. tucannus* (15.4 %). Three other species were less frequently sampled: *P. aracari* (11.5 %), *P. bailloni* (5.8 %) and *R. vitellinus* (4.8 %).

A total of 41 (39.4 %) samples were positive for some kind of parasite. The species with the majority number of positive samples was *R. tucannus* (75 %) followed by *R. vitellinus* (60 %). Other species

had less than a half of samples positive: *P. aracari* (41.6 %), *R. dicolorus* (35 %), *S. maculirostris* (33.3 %) and *R. toco* (28.6 %). The following nematodes and protozoan were found in this study: Trichuridae eggs, unidentified nematode eggs, *Giardia* sp., *Eimeria* sp. and non sporulated coccidian oocysts (Fig. 2). Single infections represented 93.3 % of positive samples, and samples with multiple infections represented 6.7 %. More than a half (60.6 %) of the samples were negative, and all species sampled had a negative result at some point during this study. It is worth highlighting that *Pteroglossus bailloni* did not have any infection (Table 2). Concerning single infections, Trichuridae eggs, unidentified nematode eggs and *Giardia* sp. were found in these conditions, with a frequency of 23.1 %, 1 % and 3.8 % respectively. Trichuridae eggs were found in all studied species, exception for *P. bailloni*, and *R. tucannus* was the most frequently infected with 11 positive samples. *Giardia* sp. was present only in *P. aracari* and *S. maculirostris* in a total of nine samples (8.7 %). A single infection with an unidentified nematode in just one sample was found only in *R. dicolorus* (1 %).

Multiple infections were seen in *R. toco*, *R. tucannus*, *R. dicolorus* and *S. maculirostris*. In total, 5.9 % had the association of two

Table 2. Prevalence of enteroparasites infections in Ramphastidae.

Parasites	Trichuridae			<i>Giardia</i> sp.		Unidentified Nematode		<i>Eimeria</i> sp.		Non sporulated coccidia	
Host	N	N+	P%	N+	P%	N+	P%	N+	P%	N+	P%
<i>Ramphastos toco</i>	21	6	28.6	-	-	1	5.8	2	9.5	-	-
<i>Ramphastos tucanus</i>	16	12	75.0	-	-	-	-	-	-	1	6.3
<i>Ramphastos vitellinus</i>	5	3	60.0	-	-	-	-	-	-	-	-
<i>Ramphastos dicolorus</i>	20	6	30.0	-	-	2	1.0	-	-	-	-
<i>Pteroglossus aracari</i>	12	1	8.3	4	33.3	-	-	-	-	-	-
<i>Pteroglossus bailloni</i>	6	-	-	-	-	-	-	-	-	-	-
<i>Selenidera maculirostris</i>	24	3	12.5	6	25	-	-	-	-	-	-
Total	104	31	29.8	10	9.6	3	3.9	2	1.9	1	1.0

N: number of investigated samples; N+: number of positive samples; P%: prevalence of infections

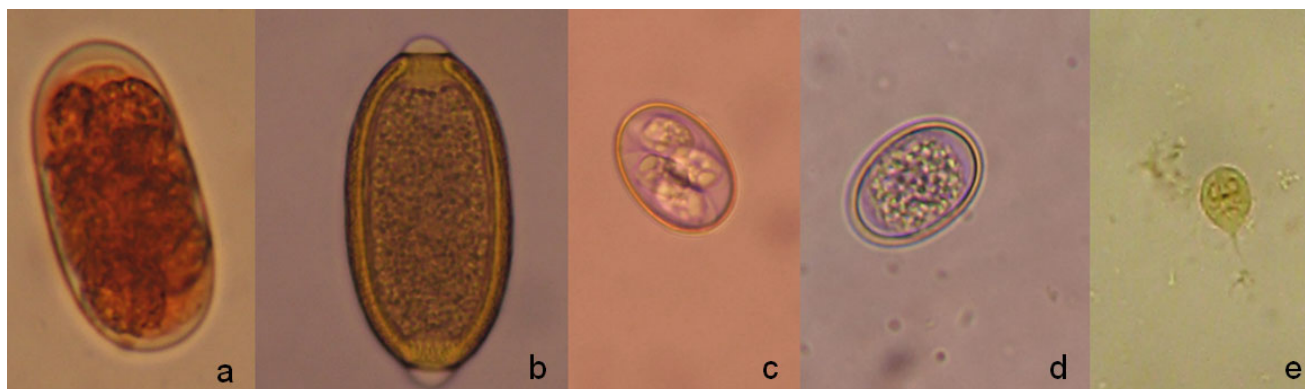


Fig. 2. Endoparasites found in Ramphastidae. (a) unidentified nematode eggs, (b) Trichuridae eggs, (c) *Eimeria* sp., (d) non sporulated coccidian oocysts, (e) *Giardia* sp. x400

parasites: Trichuridae/coccidian oocysts, Trichuridae/*Eimeria* sp., Trichuridae/unidentified nematode, Trichuridae/*Giardia* sp. Trichuridae eggs were present in all of them and only one had the association of three different parasites: Trichuridae, *Eimeria* sp. and an unidentified nematode.

Discussion

For hemoparasites, microfilaries with morphological distinct characters suggesting two different species were found in some animals, but no morphological or molecular identification was made to confirm this. Filarioid nematodes are difficult to identify (Barlett, 2008), for this, it is necessary to detect the adult nematode, usually found during necropsy after carefully examining the carcass.

During the period of this study, the animals were submitted to laboratorial exams twice a year for preventive medicine evaluation, and if any other veterinary care was necessary. The periodicity of the exams poses difficulties to diagnose the presence of microfilaria, because some species have a reproductive senescence and microfilarie are released by the adult and can be detected in laboratorial exams only during a short period. This characteristic is common for microfilaria species that lives in some body locations that a big number of parasites could favours inflammatory reactions and kills adult parasites. On the other hand, some species have fragilized adults, microfilarie can live for a long period, but adults die very soon after releasing the microfilarie (Barlett, 2008). As detected in this study, microfilarie are commonly found in Ramphastidae, as previously reported (Pinto *et al.* 1996; Young *et al.*, 1993; Pinto *et al.* 2003; Tantalean & Chavez, 2004), such as *Pelecitus* sp., *Splendidofilaria* sp., *Dessetfilaria* sp., *Eulindana* sp. were already reported in these birds (Young *et al.*, 1993; Barlett, 2008). The fact that one free-living bird was positive for microfilarie, could indicate that management of captive Ramphastidae would be a challenge, because there is no program to monitor this parasites in free-living birds. One possible solution for minimizing hemoparasites transmission would be some vector control in the park area and its surrounds.

Other hemoparasites was not found in this study. *Plasmodium* sp. has been found in *R. vitellinus* (Cornelissen & Ritchie, 1994; Woodworth-Lynas *et al.*, 1989), but it was not detected in any of the animals in this study. One free-living individual of *R. dicolorus* had been analysed for hemosporidian parasites, but it was negative (Chagas *et al.*, 2016).

Trichuridae was the most frequent parasite found in the coproparasitological exams, especially in the genus *Ramphastos*. They are commonly called as "capilarids" and are reported to infect birds of different groups (Yabsley, 2008a). Caution should be taken when identifying these parasites at genus level when only faeces are used in diagnosis, the eggs are all similar and to confirm the genus and even the species, it is recommended to use adults collected during necropsy (Yabsley, 2008a). This reinforces the caution when using commonly terms, since different species can have different impacts in their hosts.

In this study, nematode eggs found were not identified. They were present in 3 % of the samples, with 1 % in single infection in *S. maculirostris* and 2 % multiple infections found in *R. toco* and *R. dicolorus*. There are reports of the presence of *Ascaridia* sp. and *Trichostrongylus* sp. (Freitas *et al.*, 2002), but distinguishing between nematode eggs that infect birds only using morphological features of the eggs found in faeces could be a problem, they could be separated using their size, but this is not that easy, because this procedure needs to be run for experienced technicians (Fedynich, 2008).

São Paulo Zoo does not maintain any threatened Ramphastidae species, but has *R. vitellinus* classified by IUCN as vulnerable, which was positive for Trichuridae nematodes. Some studies demonstrated that the presence of nematodes infecting birds could minimize the availability of carotenoids in blood, which can compromise the feather colours and even reproductive success, which is one of the critical point analyzed by the females when they are choosing a mate (Martinez-Padilla *et al.*, 2007). Parasitological exams should be performed frequently in all captive animals, considering they can easily spread to other species that lives in a close enclosure, and special attention should be given to important species that belongs to conservation programs.

Giardia sp. was predominant in toucanets at São Paulo Zoo: *P. aracari* (3.8 %) and *S. maculirostris* in single infections (4.8 %) and in mixed infections with Trichuridae nematodes (1 %). *Giardia* sp. is a common protozoan in Ramphastidae, and asymptomatic hosts can act as a reservoir of this parasite (Cornelissen & Ritchie, 1994), spreading the cysts in the environment.

Among coccidian parasites, non sporulated coccidian oocysts and Trichuridae nematode eggs were found in 1 % of samples. *Eimeria* sp. was found in 3.9 % of samples, and it was present only in mixed infections with Trichuridae nematodes (2.9 %) and with Trichuridae nematodes and unidentified nematode (1 %). *R. toco* and *R. tucannus* were the only two species positive for this parasite. Coccidian is considered a common parasite in Ramphastidae, rarely causing any clinical symptom, unless animals are kept in high density enclosures and with poor hygiene (Cornelissen & Ritchie, 1994). *Isospora* was shown to be a typical parasite in Piciformes, but it was not found in our study, possibly due to the analysis of only one family that belongs to the Piciformes order, which comprehends many other Families and species. Lainson (1994) says that some *Eimeria* species could complete their life cycle in host species that are taxonomically close to each other, as shown in his work, which found *E. vitellini*, a coccidian known to infect *R. vitellinus* and two other *Ramphastos* species. This is very common in captive environment, including at São Paulo Zoo, where all individuals that belongs to these family lives in the same area in the park.

Some zoos in Latin America reported a high prevalence of parasites in Ramphastidae, with the predominance of coccidian (Corredor *et al.*, 2013), but our results registered the prevalence found of Trichuridae nematodes and *Giardia* sp.

Conclusion

Parasites found in this study are commonly reported for Ramphastidae, but the prevalence of Trichuridae nematodes in toucans and *Giardia* sp. in toucanets is an important information for preventive medicine and for elaboration of management protocols for these animals.

The presence of parasites in captive animals could represent a big problem for institutions, considering birds live in a limited space and in high densities, comparing to wild, facilitating the permanence of eggs and cysts in the environment, especially in cases of direct life cycle parasites, as we found. Free-living animals can act as natural reservoir for many of these parasites.

The energy that animals accumulate are essential in reproductive periods and if they need to spend this energy fighting against parasites or repairing the damage they cause, other physiological functions, as reproduction, can be compromised. This fact reinforces the need for implementation of preventive protocols that include periodic exams and prophylactic vermifugation.

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