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Original papers

Giardia spp., ten years of parasitological data in the biggest zoo of Latin America

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ABSTRACT. *Giardia* spp. (Diplomonadida: Hexamitidae) is an important and widely studied protozoan parasite with worldwide distribution. Nowadays have six described species, and the most important probably is *Giardia duodenalis* due to the zoonotical potential that some assemblages have. Many studies analysing samples from wild animals have detected *Giardia* in captive environment, including the zoonotic type. The aim of this study was to determine the prevalence of *Giardia* sp. in wild captive animals at São Paulo Zoo, using conventional parasitological techniques (direct smear, passive flotation with saturated sodium chloride solution and simple gravity sedimentation), from 2006 to 2016. In total, 7066 coprological exams were performed during this period with samples from mammals, birds, reptiles and amphibians. The prevalence of *Giardia* infections was of 1.5% (103/7066). Mammals had the higher prevalence of infections with 2% (77/3872), followed by birds with 1.1% (25/2186) and reptiles with only one positive sample (1/894). All samples from amphibians were negative. Representatives of thirteen families presented positive results for this parasite: Dromaidae, Phasianidae, Ramphastidae, Cervidae, Giraffidae, Canidae, Felidae, Herpestidae, Myrmecophagidae, Callithrichidae, Cebidae, Hylobatidae and Dipsadidae. This study presents the first report of *Giardia* sp. in *Pavo muticus* and *Brachyteles arachnoides*. Infections were prevalent in Cebidae and Ramphastidae species. The findings of this study highlight the importance of identifying which *Giardia* assemblage are involved in the infections and if they may have a zoonotic potential.

Key words: *Giardia*, wild animals, captive, coproparasitological exams

Introduction

Giardia spp. (Diplomonadida: Hexamitidae) is an important and widely studied protozoan parasite with worldwide distribution [1]. This protozoan is of interest because it has public health importance due to some species to be considered a zoonotic agent [1,2]. Previous research has established more than 40 species of *Giardia* [2], but nevertheless, nowadays, only six of them are recognized: *Giardia duodenalis* (syn. *G. intestinalis*; syn. *G. lamblia*) that are found in a wide range of domestic and wild animals, including humans; *Giardia agilis* from amphibians; *Giardia ardeae* and *Giardia psittaci* from birds; *Giardia microti* from muskrats and voles and *Giardia muris* from rodents [3].

Molecular studies have shown that *Giardia duodenalis* has eight different assemblages (named from A to H), presenting different host specificity, pathogenic and zoonotic potential [1,3–5]. Assemblages A and B are considered to harbour zoonotic potential being reported in humans, non-human primates, dogs, cats, rodents and in a high diversity of wild animals [3–6]. While assemblage C and D were reported in domestic and wild canids; assemblage E in livestock such as cattle, alpaca, goat, sheep and pig; assemblage F in cats; assemblage G in mice and rats [3–5] and assemblage H in seals [3,5].

This protozoan has direct life cycle, being encountered in small intestine (mainly in duodenum, jejunum, and upper ileum) [2]. It has two different life stages, cysts and trophozoites [2]. In general, cysts are found in formatted feces and trophozoites, in watery stools [2]. The direct life cycle and its resistance in the environment

facilitates the transmission in zoological collections, since animals live in limited spaces and, many times, high densities can be encountered [2,7].

Giardia infections can cause diarrhoea (acute or chronic), abdominal pain, dehydration and weight loss, but in some infections there are no clinical symptoms, depending on the species or *G. duodenalis* assemblage [8]. The only reliable way to diagnose these infections are through laboratory exams [8]. *Giardia* is a small parasite being easily mistaken with other structures present in faeces, the cyst is the life stage commonly found, which is small and can easily go unnoticed for untrained professionals [7,9]. When present, trophozoites have a characteristic way of swimming and can be seen easier than the cysts, but samples must be recently collected (up to 1 hour) and free of conservants, which kill them, for this observation, trophozoites can also be seen, but not moving after this time [personal communication]. Not all methods are indicated for both stages, and the diagnose technique must be chosen wisely, to avoid false-negative results [10].

Previous studies have highlighted the prevalence of *Giardia* sp. infections, its genotypes and zoonotic potential, mainly in domestic animals (dogs, cats and poultry) [9,11–16], despite of that, studies related to free-living and captive wild animals have increased in the last years [4,6,17–20]. In Brazil, wild animals were diagnosed with *Giardia* sp. in different localities across the country [21–27]. Presence of *Giardia* sp. were reported in other zoos around the world and in Brazil using different techniques [8,18,19,20,28–33].

The aim of this study was to determine the prevalence of *Giardia* sp. in wild captive animals in São Paulo Zoological Park Foundation, the biggest zoological park in South America, using conventional parasitological techniques and discuss the importance of detecting infections in wild captive animals.

Materials and Methods

São Paulo Zoological Park Foundation (FPZSP) was established in 1958, and, nowadays, is considered the biggest Zoo in South America. FPZSP maintains almost 3000 captivity animals, including mammals, birds, reptiles, amphibians and invertebrates. The park is in a remnant of Atlantic Rain Forest, in the biggest Brazilian megalopolis in São Paulo County. Due to this unique scenario, captive animals live closely to wild animals and feral cats.

FPZSP has a Clinical Analyses Laboratory that belongs to Applied Research Department, this laboratory process around 15000 exams/year, and parasitological exams corresponds to about 10% of them. In this study, the results of coproparasitological exams in the period of October 2006 to May 2016 were analysed and the positivity for *Giardia* sp. is reported here.

Due to group housing, many samples were collected and processed on pools of feces. In the laboratory, samples are processed using three different methods: direct smear; passive flotation using saturated sodium chloride solution and simple gravity sedimentation coloured with iodine solution to facilitated parasite identification [10,34]. A sample was considered positive when the presence of cysts and/or trophozoites of *Giardia* sp. were reported in any one of the techniques described above. When a sample had not enough volume for being processed using all three methods, they were prioritized as follow: direct smear, passive flotation and simple gravity sedimentation.

Results

During studied period, a total of 7066 fecal samples were processed in the laboratory, including mammals (3872), birds (2186), reptiles (894) and amphibians (114) (Table 1, S1 and S2). Samples from mammals correspond to 54.8%, representing 10 orders, 34 families and 107 species. Samples from birds correspond to 30.9% of total samples, and are represented by 23 orders, 41 families and 185 species. Samples from reptiles correspond to 12.7% and have representatives of four orders, 25 families and 76 species. Amphibian samples corresponds to 1.6% of total samples analysed, with represents of one order, nine families and 24 species, unfortunately it was not possible to determine the species of all sampled animals and 13 samples are reported here as frogs. In this study, almost all samples were processed using all three methods 95.2% (6724/7066), and a small portion using only direct smear and flotation techniques 4% (286/7066) and even less only using direct smear technique 0.7% (56/7066).

Total prevalence of infections during studied period was of 1.5% (103/7066). With exception of amphibians, all other classes had species with positive results for *Giardia* sp. Mammals had the

Table 1. Prevalence of *Giardia* infection in positive sampled species

Order	Family	Host	N (N+)	P (%)
Casuariformes	Dromaidae	*Dromaius novaehollandiae*	27 (1)	3.7
Galliformes	Phasianidae	*Pavo muticus*	36 (1)	2.8
Piciformes	Ramphastidae	*Baillonius bailloni*	22 (1)	4.5
		Pteroglossus aracari	35 (5)	14.3
		P. inscriptus	3 (1)	33.3
		Ramphastos tucanus	55 (2)	3.6
		R. vitellinus	44 (4)	9.1
		Selenidera maculirostris	66 (10)	15.2
Artiodactyla	Cervidae	*Dama dama*	53 (1)	1.9
	Giraffidae	*Giraffa camelopardalis*	53 (4)	7.5
Carnivora	Canidae	*Cerdocyon thous*	126 (4)	3.2
	Felidae	*Felis catus*	30 (6)	20
		Leopardus colocolo	46 (1)	2.2
		L. geoffroyi	62 (1)	1.6
		Panthera tigris altaica	107 (1)	0.9
	Herpestidae	*Suricata suricatta*	37 (8)	21.6
Pilosa	Myrmecophagidae	*Myrmecophaga tridactyla*	255 (1)	0.4
Primates	Callithrichidae	*Callithrix penicillata*	32 (1)	3.1
		Leontopithecus chrysopygus	237 (6)	2.5
		L. rosalia	37 (5)	13.5
	Cebidae	*Alouatta caraya*	37 (1)	2.7
		A. clamitans	122 (15)	12.2
		Ateles sp.	68 (4)	5.9
		Brachyteles arachnoides	29 (1)	3.4
		Cebus kaapori	22 (1)	4.5
		Lagothrix lagothricha	59 (13)	22
	Hylobatidae	*Hylobates lar*	22 (3)	13.6
Squamata	Dipsadidae	*Oxyrhopus guibei*	38 (1)	2.6

N: Number of analysed samples; N+: Number of positive samples; P: prevalence of infections

higher prevalence of infections with 2% (77/3872), followed by birds with 1.1% (25/2186) and reptiles with only one positive sample (1/894). Considering all positive samples, prevalence encountered when all three methods were used was of 94.2% (97/103), while using direct method and flotation solution was of 5.8% (6/103). There was no positive sample processed using only direct method.

In mammals, 19 species were positive for *Giardia*, these species belong to four orders (Artiodactyla, Carnivora, Pilosa and Primates) and nine families (Cervidae, Giraffidae, Canidae, Felidae, Herpestidae, Myrmecophagidae, Callithrichidae, Cebidae, Hylobatidae). It is possible to highlight positive results in Primates, especially Cebidae, with the higher number of positive samples and species presenting *Giardia* sp. infections. Avian presented positive results for eight species, and infected animals belong to three orders (Casuariformes, Galliformes and Piciformes) and three families (Dromaidae, Phasianidae and Ramphastidae). It is worth to note that Ramphastidae had the higher prevalence of infections and the majority of positive samples among birds. Reptile, with the lower prevalence, had only one positive species of Squamata order, Dipsadidae family. All positive results and prevalence are shown in Table 1, for negative species see Supplementary material (S1 and S2).

Discussion

The present study analysed samples from a wide variety of species, from different taxa, and with a high number of samples. This study will be useful to wildlife professionals, as veterinarians and biologists, in the management of captive species and in conservational decisions. Coproparasitological exams are non-invasive and easy to perform, even in small laboratories, but for obtaining reliable results it is necessary to have experienced and well-trained professionals performing them.

The overall *Giardia* sp. prevalence in our study was of 1.5% (103/7066), lower than prevalence registered in other zoos around the world: 37,8% in a Colombian zoo [35]; 29% in Zagreb zoo in Croatia [19], 24% in Ogród Zoologiczny in Poland [30], 2.5% in Zhengzhou Zoo in China [36], but similar to one study in Osaka Municipal Tennoji Zoological Gardens in Japan, which prevalence was of 1.1% [37] and in Poznań zoo in Poland, with 1.8% [38]. One explanation for the difference of prevalence in different zoological gardens may be due to the methods used. Some of them used molecular biology techniques and concentration methods, which are more sensitive than when only direct smear is used. However, even when PCR was used to diagnose infections, such as in the study performed by Li et al. [36] the prevalence was low (2.5%), when compared to other studies that used the same techniques. The differences are more liked to be related to the group of hosts studied, usually Primates and Carnivora present higher infection rates than other animals [19,32,39]; and due to hygiene conditions in each facility. In Poznań zoo two different studies were realized in an interval of almost 30 years (compare [38] with [39]) and was possible to notice that the prevalence of infections decreased along the years, and according to the author this was related to the higher hygiene levels in the enclosures [38]. Prophylactic measures are well known to prevent parasitic infections, and hygiene of enclosures was mentioned in the past as an important factor to diminish infections rates in captive [40,41].

In birds group, there were three families with positive results: Dromaidae (*Dromaius novahollandiae*), Phasianidae (*Pavo muticus*) and Ramphastidae (*Baillonius bailloni*, *Pteroglossus aracari*, *Pteroglossus inscriptus*, *Ramphastos tucanus*, *Ramphastos vitellinus* and *Selenidera maculirostris*). The presence of *Giardia* sp. in ratites (ostriches, emus and rheas) is not well documented in the literature, infections were reported in *Struthio* sp. [42] and *D. novaehollandiae* in a captive in Brazil [43]. Concerning *Pavo muticus*, prevalence of *Giardia* sp. in the present study was 2.8%. In a zoo in India, it was reported a prevalence of 4.2% in *Pavo cristatus* [44]; while in Michigan zoo, in United States, all studied animals were seronegative for *Giardia* sp. [45]. Samples from *P. cristatus* were also analysed, but all of them were negative. This also may be the first report of *Giardia* in *P. muticus*. Ramphastidae species are well known for harbouring asymptomatic *Giardia* sp. infections, making then reservoir of this protozoan for other species [46]. The presence of *Giardia* sp. was already reported in the studied population in *Pteroglossus aracari* and *Selenidera maculirostris* [47]. The present study, shows that the infections can affect not these two toucanet species, but four more Ramphastidae species. This group of birds can have a high prevalence of *Giardia* sp. infection when in captive [48].

Mammals samples showed a high diversity of families infected by *Giardia* sp. Herpestidae showed a high prevalence (21.6%) of infection, followed by Hylobatidae (13.6%) and Cebidae (10.3%). In total, 19 mammalian species were found to harbour *Giardia* infections.

Two species of Cervidae, *Giraffa camelopardalis* and *Dama dama*, were positive during the period studied. Several attempts have been made to detect the presence of *Giardia* sp. in *G. camelopardalis* [18,37,39], in all these studies the host did not show any infection. So far, the only description in the literature reporting *Giardia* infections in *G. camelopardalis* was in a zoo in Chile [49]. This is the second time that this parasite is mentioned for this host in the literature. The presence of *Giardia* in *D. dama* was reported using molecular techniques and for wild populations in Italy [50] and in Poland [51]. In Italy, it was identified as belonging to assemblage A, which may represent a risk to humans due to its zoonotic characteristic [50]. Other assemblages were detected in *D. dama* samples [13], reinforcing the importance of performing molecular analysis to identify correctly the species and assemblage involved in the infections, since it is not possible to access this information using conventional techniques, and this knowledge is important when management decisions are needed.

Concerning Canidae species, we only found one

host species, *Cerdocyon thous.* Holsback et al. [52] sampled this species, but all samples were negative for this parasite. The presence of *Giardia* in this group of animals was reported in wild population of *Lycaon pictus*, affecting 26% of studied population and in an Australian zoo, with a prevalence of 62% [8]. Literature present report of *Giardia* in *Canis lupus* and *Chrysocyon brachyurus* [19], but during the studied period we did not found such infections.

Concerning Felids, this study found infection in *Leopardus colocolo*, *Leopardus geoffroyi*, *Panthera tigris altaica* and in domestic cats (*Felis catus*). *Giardia* infections in captive felids were already reported in *Panthera tigris* in a zoo in Peru [31] in a higher prevalence (33.3%) than the present study (0.9%), and in a zoo in China [36]. The presence of *Giardia* in a wild *Leopardus wiedii* that was brought to a zoo from the wild was described [21]. This parasite was already found in other felids that are common in Brazilian zoos, such as *Panthera onca*, *Leopardus pardalis*, *Leopardus tigrinus*, *Leopardus wiedii* and *Leopardus colocolo* [28,31]. Other species such as *Leptailurus serval*, *Panthera leo* and *Uncia uncia* were also reported to harbour such infections [19], but in the present study, all samples from these animals were negative. The presence of *Giardia* sp. in domestic cats have been widely reported [11,12,14]. The circulation of domestic/feral cats in zoos are common [49,53,54] and this can represent a risk to the health of captive animals, since they can harbour different pathogenic agents [49,53,55].

In this study, the higher prevalence of infection was encountered in *Suricata suricatta* (Herpestidae) with 21.6% (8/37). There are few studies with this species, and none of them show the presence of this parasite in meerkats [19,56]. The only work that describes the presence of *Giardia* in *S. suricatta* was done in a zoo in Chile, South America [57].

Concerning *Myrmecophaga tridactyla*, the presence of *Giardia* sp. was already reported in the studied population [58]. Previously, the prevalence was higher (1%) than the one found now (0.4%). The former study analysed samples from *Tamandua tetradactyla* and *M. tridactyla* but does not specify the prevalence for each species. In the present study, all *T. tetradactyla* were negative. Infections by *G. duodenalis,* zoonotic assemblage B, was also reported in a *T. tetradactyla* in Poznań zoo, in Poland [38].

In Callithichidae prevalence of infections was of 3.9% and they were present in *Callithrix penicillata*, *Leontopithecus chrysopygus* and *Leontopithecus rosalia*. Carmo and Salgado [41] studied two population of *Callithrix* sp., in Brazil, and found different results in each population, wild animals harboured high rates of infections while animals kept under captive conditions were all negative for *Giardia* sp. The prevalence among different populations of *Callithrix* may vary greatly, in some studies all analysed samples were negative [25] while in others they were present in all samples [22]. Other studies failed to detect the presence of *Giardia* spp. in *Leontopithecus* [25], while in the present study the prevalence of this parasite in *L. chryopygus* and *L. rolasia* were 2.5 and 13.5%, respectively. These animals are considered as endangered species by IUCN [59,60], and parasites can compromise their health in captive and even in conservation and reintroduction programs.

The present study found that 10.4% of Cebidae were positive for this protozoan, in a wide variety of hosts. *Giardia* infections were already reported in other Brazilian zoo in Cebidae, in this previously study the prevalence was higher (18%) [25] that the one reported here. The presence of *Giardia* sp. in *Alouatta* spp. was already reported in Brazilian zoos [24,25], being 25% for *Alouatta clamitans* and 12.5% for *Alouatta guariba* [24], in the present, study the prevalence varied from 2.7 to 12.2%, being lower than the one previously reported. In *Cebus* genus, the most commonly species reported to harbour parasite infection is *Cebus apella* [17,22,30], despite of that, all animals from this species, sampled during this study, were negative. *Giardia* was found in *Cebus kaapori*. Among Cebidae, the species with the highest prevalence was *Lagothrix lagothricha*, with 22% of tested samples. The presence of *Giardia* was already reported in this host but in a lower prevalence (12.5%), in a Colombia zoo [29]. *Brachyteles arachnoides* is one rare species, endemic in Atlantic Rain Forest, classified as endangered by IUCN [61]. In our study we identified a prevalence of 3.4% (1/29) of samples positive for *Giardia* sp. Infections by this protozoan was already reported in wild *Brachyteles hypoxanthus* [62]. This may be the first report of *Giardia* in *B. arachnoides*. Other Brazilian zoos reported the presence of *Giardia* in *Ateles* sp. [25].

Concerning old world primates, the only species of Hylobatidae kept at the FPZSP during this studied period was *Hylobates lar*, and a prevalence of infection were of 13.6%. *Giardia* sp. infections were

already reported in this species in Croatia zoo [19], and in 12% of samples in four studied Belgium zoos [17]. *Pan troglodytes* (Hominidae) kept in captive was already reported to have infections by *Giardia* sp. [17,19], sometimes even in high prevalence such as 39% of samples. That was not the case not only in our study but also in other reports [17,20]. *Pan troglodytes* and *Pongo pygmaeus* were negative during the entire studied period, as demonstrated by Berrilli et al. [20] in a zoo in Italy and Maesano et al. [30] in a zoo in Poland. Presence of *Giardia* sp. in captive *Pan troglodytes* was reported by many studies all over the world [17,19,39,63,64] as in other primate species.

In FPZSP reptile population studied, only one sample was identified with *Giardia* sp., from an *Oxyrhopus guibei* (Dipsididae). There is one report of *Giardia* in snakes in Brazil, but the host was a *Bothrops jararaca* [26], all animals tested of this species were negative. Few studies with captive and wild lizards were done, but only few of them reports the presence of *Giardia* infections [27,65,66], and in other studies all tested animals were negative [67]. Reptiles are not considered as potential hosts for *Giardia* sp. [68].

Biological characteristics of this parasite are what makes it difficult to fight against in zoos, the cysts are resistant and intermittently released in the feces of infected hosts in the environment [7]. This intermittently release of cysts also makes it hard to diagnose using simple methods. It is important to analyse more than one sample when possible, increasing the chance of diagnosing the parasitic infection. Professional training, use of sensitive techniques (molecular diagnose and immunoassay) and application of more than one method are also recommended.

Captive environment can be stressing to animals, compromising their immune system and usually animals live in high densities and limited space, favouring the presence of parasitic diseases, especially those with direct life cycle, as is the case of *Giardia* sp. Performing preventive and diagnose exams, to better know which parasites can be found in captive animals it is essential to guarantee zoological-management strategies and to give insight into host-parasite interaction. Results produced with microscopical analyses of feces must be analysed carefully, and not considered lonely in determining if *Giardia* sp. has zoonotic potential, since it is impossible to differentiate between the different assemblages morphologically, being necessary to use molecular tools for this purpose [4]. It is essential to determine the species and assemblages of *Giardia duodenalis* involved in infection of wild animal and identify potential reservoirs for human infection [4].

The presence of zoonotic assemblage of *Giardia duodenalis* has already been reported in wild animals [5,6,19,25]. This must be considered when dealing with these infections, mainly because this parasite can be transmitted by and for humans (zoo staff and visitors) and captive animals. In zoos, wild animals are in constant contact with humans and other species of animals. The literature reports that in a zoo were zookeepers harboured *Giardia* infections, and in more detailed analysis, harbouring infections with the same sub-assemblage as the captive animals in their care, suggesting that zoonotic transmission may have occurred in such places and reinforcing the importance of performing more detailed analysis [8], not only via animal-human, but also human-animal.

Another common practice in zoos is exchanging animals between institutions. This practice may facilitate the introduction of a parasite species or new assemblage in the captive population, since many institutions have animals infected by *Giardia* sp. This reinforces the necessity to perform exams and establish quarantine protocols to avoid the transmission of giardiosis between institutions [64]. The presence of feral cats is common in Brazilian zoos, and this may be an additional source of infection that must be considered when dealing with infection in zoos [53].

This research has thrown up some questions for further investigations, such as identifying which genotypes are involved in the *Giardia* infections detected in FPZSP. This will bring more information about species that are infected by this parasite and help professionals involved with animal management to establish sanitary protocols to prevent the transmission of zoonotic pathogens.

In conclusion, this investigation has discussed the results of positive samples for the presence of *Giardia* sp. infection in wild captive animals during ten years in the biggest zoological park in South America using conventional parasitological techniques. This study has found that the prevalence of the parasite is relatively low, when compared to many other studies worldwide, but they are present in important group of animals, representing a risk for the health of captive animals. Findings of this study indicate that this is the first report of *Giardia*

sp. in *Pavo muticus* and *Brachyteles arachnoides*. Infections were prevalent in Cebidae and Ramphastidae species. The insights gained from this study may be of assistance in further studies addressing the identification of *Giardia* assemblages involved in infection of studied population.

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Supplement 1. Animal species with negative results for *Giardia* infections

Order	Family	Host	N
Accipitriformes	Accipitridae	*Buteo melanoleucus*	4
		Buteogallus urubitinga	3
		Harpia harpyja	6
		Harpyhaliaetus coronatus	4
		Heterospizia meridionalis	1
		Leptodon cayanensis	2
		Leucopternis lacernulata	1
		Spizaetus ornatus	14
		Spizaetus tyrannus	6
		Trigoneceps occipitalis	3
Anseriformes	Anatidae	*Aix sponsa*	2
		Alopochen aegyptiacus	9
		Amazonetta brasiliensis	1
		Anser cygnoides	1
		Branta canadensis	1
		Cereopsis novaehollandiae	21
		Chenonetta jubata	2
		Cygnus atratus	107
		Cygnus melanocoryphus	49
		Coscoroba coscoroba	40
		Dendrocygna bicolor	3
		Dendrocygna viduata	10
		Neochen jubata	3
		Netta erythrophthalma	2
		Plectropterus gambensis	8
		Tadorna ferruginea	12
		Tadorna radjah	5
		Tadorna tadornoides	12
		Tadorna variegata	7
	Anhimidae	*Chauna torquata*	22
Bucerothiformes	Bucerotidae	*Ceratogymna brevis*	1
		Buceros rhinoceros	5
	Bucorvidae	*Bucorvus abyssinicus*	11
Casuariformes	Casuaridae	*Causarius casuarius*	11

Catharthiformes	Cathartidae	*Sarcoramphos papa*	6
		Vultur gryphus	3
Ciconiformes	Ciconiidae	*Jabiru mycteria*	1
Columbiformes	Columbidae	*Columbina talpacoti*	4
Cuculiformes	Cuculidae	*Piaya cayana*	1
Falconiformes	Falconidae	*Falco femoralis*	4
		Falco sparverius	3
		Polyborus plancus	7
Galliformes	Cracidae	*Crax blumenbachii*	10
		Crax rubra rubra	6
		Mitu tomentosa	12
		Mitu tuberosa	10
		Nothocrax urumutum	12
		Penelope obscura	6
		Pipile jacutinga	5
	Odonthophoridae	*Odontophorus capoeira*	12
	Phasianidae	*Pavo cristatus*	60
Gruiformes	Cariamidae	*Cariama cristata*	43
		Chunga burmeisteri	32
	Gruidae	*Balearica regulorum*	40
	Ralidae	*Gallinula chloropus*	3
		Aramides cajanea	1
Musophagiformes	Musophagidae	*Musophaga violacea*	36
		Tauraco leucotis	14
Nyctibiiformes	Nyctibiidae	*Nyctibius griseus*	2
Passeriformes	Corvidae	*Cyanocorax cristatellus*	4
	Cotingidae	*Pyroderus scutatus*	6
		Procnias averano	3
		Procnias sp.	1
		Rupicola rupicola	6
	Pipridae	*Antilophia galeata*	5
	Icteridae	*Cacicus cela*	3
		Gnorimopsar chopi	1
		Molothrus bonariensis	3
		Psarocolius decumanus	7
	Sturnidae	*Acridotheres cristatellus*	3
	Thraupidae	*Coereba flaveola*	2
		Coryphospingus sp.	4
		Dacnis cayana	2
		Paroaria coronata	4
		Paroaria dominicana	2
		Saltator atricollis	2
		Sicalis flaveola	7
		Sicalis luteola	3
		Sporophila caerulescens	1
		Sporophila collaris	2
		Thraupis ornata	1
		Traupis palmarum	7
	Tyrannidae	*Pitangus sulphuratus*	10
	Turdidae	*Turdus rufiventris*	5
Pelecaniformes	Ardeidae	*Tigrisoma fasciatum fasciatum*	1
	Pelecanidae	*Pelecanus onocrotalus*	1
	Threskiornithidae	*Ajaia ajaja*	5
Phoenicopteriformes	Phoenicopteridae	*Phoenicopterus chilensis*	25
		Phoenicopterus minor	1
Piciformes	Picidae	*Dryocopus lineatus*	5
		Melanerpes candidus	1

Order	Family	Host	N
	Ramphastidae	*Pteroglossus castanotis*	4
		Ramphastos dicolorus	102
		Ramphastos toco	58
Psittaciformes	Cacatuidae	*Cacatua alba*	1
		Cacatua citrinocristata	1
		Cacatua galerita	8
		Cacatua goffiniana	2
		Cacatua mollucensis	7
		Nymphicus hollandicus	19
	Psittacidae	*Amazona aestiva*	45
		Amazona amazonica	21
		Amazona brasiliensis	17
		Amazona farinosa	6
		Amazona festiva	23
		Amazona ochrocephala	10
		Amazona rhodocorytha	12
		Amazona sp.	10
		Amazona tucumana	2
		Amazona vinacea	16
		Amazona xanthops sp.	2
		Anodorhynchus hyacinthinus	67
		Anodorhynchus leari	93
		Ara ararauna	14
		Ara chloroptera	9
		Ara macao	50
		Ara militaris	12
		Ara rubrogenys	11
		Ara severa	3
		Aratinga acuticaudata	6
		Aratinga aurea	2
		Aratinga auricapillus	1
		Aratinga jandaya	5
		Aratinga leucophthalmus	19
		Aratinga solstitialis	8
		Brotogeris tirica	7
		Cyanopsitta spixii	20
		Deroptyus accipitrinus	21
		Diopsittaca nobilis	3
		Ecletus roratus	1
		Eos bornea	2
		Graydidascalus brachyurus	3
		Guaruba guarouba	39
		Myopsitta monachus	1
		Nandayus nenday	7
		Pianopsitta pileata	4
		Pionites leucogaster	12
		Pionites melanocephala	3
		Pionopsitta pileata	1
		Pionus fuscus	10
		Pionus maximiliani	2
		Pionus menstruus	13
		Pyrrhura frontalis	4
		Pyrrhura lepida	10
		Pyrrhura perlata	1
		Pyrrhura rhodogaser	2

		Pyrrhura sp.	4
		Poichephalus senegalus	8
		Primolius auricolis	2
		Primolius maracana	2
		Propyrrhura maracana	1
Rheiformes	Rheidae	*Rhea americana*	30
Sphenisciformes	Spheniscidae	*Spheniscus magellanicus*	2
Strigiformes	Strigidae	*Asio clamator*	27
		Asio stygius	4
		Bubo virginianus	8
		Ketupa zeylonensis	1
		Otus choliba	35
		Pulsatrix koeniswaldiana	3
		Pulsatrix perspicillata	6
		Pulsatrix sp.	1
	Tytonidae	*Tyto alba*	2
Struthioniformes	Struthionidae	*Struthio camelus*	27
Tinamiformes	Tinamidae	*Crypturellus obsoletus*	8
		Tinamus solitarius	6
Artiodactyla	Bovidae	*Addax nasomaculatus*	17
		Aepyceros melampus	1
		Ammotragus lervia	29
		Bison bonasus	11
		Hippotragus niger	10
		Kobus ellipsiprymnus	18
		Oryx gazella	8
		Tragelaphus strepsiceros	6
	Camelidae	*Camelus bactrianus*	30
		Camelus dromedarius	86
		Lama glama	27
		Lama pacos	13
	Cervidae	*Cervus elaphus*	15
		Cervus unicolor	4
		Mazama americana	1
		Mazama gouazoupira	11
	Hippopotamidae	*Hippopotamus amphibius*	13
	Suidae	*Phacochoerus africanus*	9
	Tayassuidae	*Tayassu pecari*	6
		Tayassu tajacu	11
Carnivora	Canidae	*Canis lupus*	30
		Chrysocyon brachyurus	52
		Lycaon pictus	5
		Speothos venaticus	33
	Felidae	*Caracal caracal*	14
		Leopardus pardalis	17
		Leopardus tigrinus	220
		Leopardus wiedii	60
		Leptailurus serval	72
		Panthera leo	66
		Panthera onca	45
		Panthera pardus melas	4
		Panthera tigris tigris	33
		Prionailurus viverrinus	1
		Puma concolor	19
		Puma yagouaroundi	104
		Uncia uncia	3
	Mustelidae	*Eira barbara*	11
		Galictis vittata	7
		Lontra longicaudis	63
	Otariidae	*Otaria byronia*	45

Order	Family	Host	N
	Procyonidae	*Nasua nasua*	62
		Potos flavus	21
	Ursidae	*Tremarctos ornatus*	23
		Ursus americanus	12
		Ursus arctos	8
Cingulata	Dasypodidae	*Dasypus novemcinctus*	19
Didelphimorphia	Didelphidae	*Didelphis aurita*	15
Lagomorpha	Leporidae	*Oryctolagus* sp.	4
Perissodactyla	Equidae	*Equus burchelli antiquorum*	6
		Equus grevyi	11
	Rhinocerotidae	*Ceratotherium simum*	8
	Tapiridae	*Tapirus terrestris*	26
Pilosa	Bradypodidae	*Bradypus variegatus*	10
	Megalonychidae	*Choloepus didactylus*	16
		Choloepus hoffmanni	15
	Myrmecophagidae	*Tamandua tetradactyla*	164
Primates	Callithrichidae	*Callithrix argentata*	15
		Callithrix jacchus	34
		Callithrix kuhlii	6
		Callithrix sp.	14
		Leontopithecus chrysomelas	194
		Saguinus fuscicollis weddelli	2
		Saguinus midas niger	29
		Saguinus oedipus	7
	Cebidae	*Aotus trivirgatus*	41
		Ateles chameck	25
		Ateles marginatus	11
		Ateles paniscus	28
		Cebus apella	26
		Cebus flavius	43
		Cebus olivaceus	21
		Cebus xanthosternos	20
		Saimiri sciureus	17
	Cercopithecidae	*Papio cynocephalus*	40
		Papio hamadryas	3
	Hominidae	*Pan troglodytes*	40
		Pongo pygmaeus	57
	Pitheciidae	*Pithecia albicans*	8
		Pithecia pithecia	19
Proboscidae	Elephantidae	*Elephas maximus*	11
		Loxodonta africana	6
Rodentia	Caviidae	*Cavia porcellus*	13
	Dasyproctidae	*Dasyprocta azarae*	8
		Dasyprocta fuliginosa	1
	Erethizontidae	*Sphiggurus villosus*	29
	Muridae	*Mus musculus*	1
		Rattus norvegicus	14
Crocodylia	Alligatoridae	*Caiman latirostris*	1
		Paleosuchus sp.	1
Gymnophiona	Siphonopidae	*Siphonops annulatus*	2
Squamata	Agamidae	*Physignathus lesueurii*	9
		Pogona viticeps	8
		Uromastyx maliensis	3
	Amphisbaenidae	*Amphisbaena alba*	4
	Anguidae	*Dipoglossus lessonae*	1

	Boidae	*Boa constrictor*	40
		Corallus caninus	8
		Corallus hortulanus	23
		Epicrates cenchria	29
		Epicrates crassus	23
		Epicrates sp.	4
		Eunectes murinus	14
	Colubridae	*Drymarchon corais*	10
		Elaphe guttata	50
		Elaphe obsoleta	2
		Hidrodynastes gigas	1
		Lampropeltis californiae	28
		Lampropeltis getulus mexicanus	1
		Lampropeltis sp.	5
		Lampropeltis triangulum	10
		Liophis miliaris	10
		Pseudoboa nigra	11
		Spilotes pullatus	17
	Dactyloidae	*Anolis carolinensis*	4
	Dipsadidae	*Hydrodinastes gigas*	1
		Philodryas olfersii	19
		Philodryas patagoniensis	1
		Tropidrodryas striaticeps	10
		Xenodon merremii	5
	Gekkonidae	*Eublepharis macularius*	25
		Gekko ulikovskii	3
		Gekko vittatus	2
		Hemitheconyx caudicinctus	6
	Hoplocercidae	*Hoplocercus spinosus*	1
	Iguanidae	*Iguana iguana*	67
	Lacertidae	*Lacerta lepida*	1
	Pythonidae	*Morelia spilota*	3
		Python curtus	6
		Python molurus	9
		Python regius	60
		Python reticulatus	1
	Scincidae	*Corucia zebrata*	9
		Tiliqua scincoides	13
	Teiidae	*Ameiva ameiva*	1
		Tupinambis merianae	11
		Tupinambis rufescens	3
		Tupinambis sp.	10
	Varanidae	*Varanus doreanus*	1
		Varanus exanthematicus	4
	Viperidae	*Bothrops alternatus*	8
		Bothrops insularis	22
		Bothrops jararaca	15
		Bothrops jararacussu	11
		Bothrops moojeni	13
		Crotalus durissus	7
Testudines	Chelidae	*Mesoclemmys tuberculata*	3
		Phrynops geoffroanus	4
		Phrynops hilarii	2
	Chelydridae	*Chelydra serpentina*	1
	Emydidae	*Pseudemys concinna*	1
		Trachemys dorbigni	2
		Trachemys scripta elegans	11

Order	Family	*Host*	N
		Trachylepis atlantica	9
	Geoemydidae	*Cuora amboinensis*	1
	Kinosternidae	*Kinosternon scorpioides*	2
	Podocnemidiae	*Podocnemis unifilis*	2
	Testudinidae	*Chelonoidis carbonaria*	64
		Chelonoidis chilensis	3
		Chelonoidis denticulata	22
		Chelonoides sp.	41
		Dipsochelys dussumieri	8
		Testudo graeca	4
Anura	Bufonidae	*Bufo marinus*	2
		Rhinella ornata	2
		Rhinella schneideri	12
	Ceratophyidae	*Ceratophrys ornata*	1
	Dendrobatidae	*Adelphobates galactonotus*	25
	Hylidae	*Dendropsophus minutus*	1
		Hypsiboas albomarginatus	2
		Hypsiboas bischofii	2
		Hypsiboas faber	2
		Hypsiboas sp.	1
		Scinax fuscovarius	3
		Scinax perpucillus	8
		Trachycephalus mesophaeus	1
		Trachycephalus venulosus	3
	Leptodactylidae	*Cycloramphus eleutherodactylus*	1
		Eupemprix nattereri	1
		Hylodes asper	1
		Leptodactylus labyrinthicus	4
		Leptodactylus podicipinus	4
		Physalaenuis cuvieri	1
	Microhylidae	*Chiasmocleis albopunctata*	2
	Odontophrynidae	*Proceratophrys boiei*	14
	Pyxicephalidae	*Pyxicephalus adspersus*	1
	Ranidae	*Lithobates catesbeianus*	7
Unknown	unknown	frogs	13

N: Number of analysed samples

Supplement 2. Samples from multi-species enclosures with negative results for *Giardia* infections

Order	Family	Host	N
Anseriformes	Anatidae	*Alopochen aegyptiacus – Cygnus atratus – Plectropterus gambensis*	1
		Amazonetta brasiliensis – Chenonetta jubata – Tadorna radjah	2
		Amazonetta brasiliensis – Dendrocygna viduata – Netta erythrophthalma – Tadorna ferruginea	4
Cuculiformes	–	*Piaya cayana – Pyroderus scutatus – Pitangus sulphuratus – Dryocopus lineatus*	1
Passeriformes	–	*Agelaius ruficapillus – Antilophia galeata – Ramphocelus carbo*	1
		Agelaius ruficapillus – Carduelis magellanicus – Molothrus bonariensis – Paroaria coronata – Paroaria dominicana – Sicalis luteola	1
		Agelaius ruficapillus – Paroaria coronata	1
		Agelaius ruficapillus – Ramphocelus carbo	2
		Cacicus cela – Pseudoleistes guirahurro	3
		Carduelis magellanicus – Gnorimopsar chopi – Paroaria coronata – Sicalis flaveola – Sicalis luteola – Sporophila collaris – Tangara sayaca – Thraupis palmarum	1
		Carduelis magellanicus – Sicalis luteola – Sporophila collaris	1
		Gnorimopsar chopi – Saltator similis – Sicalis flaveola – Thraupis palmarum	1
		Tangara sayaca – Thraupis palmarum	1
Pelecaniformes	–	*Ajaia ajaja – Eudocimus ruber – Plegadis chihi – Theristicus caudatus*	2
Psittaciformes	Psittacidae	*Amazona amazonica – Amazona rhodocorytha*	1
		Aratinga aurea – Pianopsitta pileata	1
		Aratinga jandaya – Aratinga solstitialis	1
		Aratinga leucophthalmus – Propyrrhura maracana	1
		Aratinga solstitialis – Triclaria malachitacea	1
		Cyanopsitta spixii – Primolius maracana	5
		Myopsitta monachus – Pyrrhura rhodogaser	1
		Pyrrhura lepida – Pyrrhura rhodogaser	3
Primates	Callithrichidae	*Callithrix jacchus – Callithrix penicillata*	1
	Cebidae	*Alouatta clamitans – Cebus xanthosternos*	4
Squamata / Testudines		*Chelonoidis carbonaria – Tupinambis rufescens*	1
Testudines	Testudinidae	*Astrochelys radiata – Chelonoidis denticulata*	1
		Astrochelys radiata – Chelonoidis denticulata – Psammobates pardalis	5
		Chelonoidis carbonaria – Chelonoidis denticulata	2

N: Number of analysed samples