

Feral Cats: Parasitic Reservoirs in Our Zoos?

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Abstract

Up until the recent past, zoos served limited function, primarily existing for entertainment value. Today's zoos, however, are serving many roles, chief among them: species conservation of captive animals. The biggest zoo in Brazil, São Paulo Zoological Park Foundation, has among its 2000 animals and many species of wild cats. The presence of domestic cats living freely in zoos is common and can be a source of spreading disease. The aim of this study was to verify the variety and prevalence of parasites found in the feces of felids (feral and wild) living in the São Paulo Zoo. The results of this parasitological analysis have been obtained from the laboratory of clinical analysis and correspond to the 4-year period beginning January/2009 and ending December/2012. Eight species of parasites were identified in the feces of captive wild cats and three in the feces of feral cats. For those captives, *Toxocara cati* (7.95%) had the highest prevalence, followed by *Toxascaris leonina* (7.58%), *Isospora* sp. (2.03%), *Hymenolepis nana* (0.92%), *Eimeria* sp., *Giardia* sp. and *Blastocystis* sp. (0.37% each) and *Ascaris* sp. (0.18%). Among the feral cats, we found *Toxocara cati* (59.26%), *Giardia* sp. (22.22%) and *Isospora* sp. (11.11%). For the captive group, we also distinguished natives from exotic species, finding native species to be more frequently parasitized than the exotic ones. Key to our findings, though, was the fact that a few parasite species were found among all groups of felids, specifically (*Toxocara cati*, *Giardia* sp. and *Isospora* sp). Further research is needed, however, to confirm that transmission of these parasites is occurring between and among these groups.

Keywords

Enteroparasites, Felids, *Felis catus*, *Toxocara cati*, Wild Animals

1. Introduction

Frequent discussions involving zoos are due to the origin of these institutions,

which is directly linked to different human attitudes towards animals [1]. The basic purpose of zoos was to expose exotic species to society as a source of entertainment, but nowadays they have as main goals: species conservation, scientific research and entertainment [2]. In Brazil, the history of zoos begins around 1882 in the city of Belém where the first zoo was created, (Pará, Brazil). Created in 1957 and inaugurated in 1958, the São Paulo Zoological Institute is an important Brazilian zoo; in 1959, it became the São Paulo Zoological Park Foundation (FPZSP), with legal authority, autonomy and defined objectives [3]. In addition to providing entertainment, encouraging research and developing environmental awareness, FPZSP also develops and acts in *in-situ* and *ex-situ* species conservation projects [4].

Among the animals in the FPZSP, there exists a large diversity of species, and one of the largest groups are felids (Mammalia) [4] [5] [6]. Felids are members of Felidae and have, in general, nocturnal and solitary habits, being intolerant to other adults of the same sex [7] [8]. When kept in captivity, these species often exhibit maladaptive, or locomotive stereotyped behavior (repetitive and without apparent function), owing to small enclosures and limited environmental interaction [6] [9].

Being top-chain predators, they also may be affected by anthropic changes in the environment, making them vulnerable. In this way, all felids present some degree of extinction threat according to the International Union for Conservation of Nature (IUCN) [8] [10].

Zoos help in the prevention of wild species rapid extinction, as they are considered great *ex-situ* breeding centers and have species conservation as one of their main goals. Indeed, studies with captive felids are easier to perform than with wild animals and can play an important role in the conservation of these animals [9] [11].

Common to zoos, felids can be divided into two groups: exotic and native felid species, dependent on the location of the institution and animal species. Exotic felids kept in FPZSP are native to Africa and Asia; native species are found throughout Brazil [12].

Another species of felid that is common in the São Paulo Zoo is the feral domestic cat (*Felis catus*). These animals organize themselves in groups, finding the zoo environment resourceful as a means for scouring food and providing physical structure for shelter; these qualities make the zoo environment ideal for the development of large colonies and all of these facts contribute to increasing the possibility of pathogen transmission due to the proximity of wild captive animals and feral domestic cats [13].

The biggest concern related to the presence of feral domestic cats in zoos is related to transmission of felid retroviruses, toxoplasmosis and mycoplasmosis, but enteroparasites are also important, due primarily to their zoonotic potential [13]-[18]. In Brazil, many studies have reported infections due to gastrointestinal parasites in feral cats, resulting in the disease that has the potential to be

transmitted to a nearby captive population [14] [15] [19]-[25].

Several species that parasitize cats may have high zoonotic potential. Although the majority of parasitic zoonoses are not fatal in humans, they can cause unpleasant clinical symptoms and must be combated [15]. However, it must be taken into account that some serious infections are caused by parasites commonly found in cats, as is the case of toxocariasis. This disease is caused by the nematodes *Toxocara canis* (which has the domestic dog as its definitive host) and *Toxocara cati*, whose definitive host is the domestic or wild cat. Although they can survive for years in animals without causing clinical changes, very intense infections can lead to diarrhea, dehydration, growth retardation (for puppies) and even death [26]. Other parasites such as *Ancylostoma* sp., *Giardia* sp. and *Cryptosporidium* sp. they can also cause diseases and deserve more attention, since they have been previously described in Brazil and in other parts of the world because they parasitize domestic cats [14] [15] [19]-[25] and wild felids [27] [28] [29] [30] [31]. In Brazil, much has been studied in regards to the gastrointestinal parasites that most affect stray domestic cats and the possible diseases that can be transmitted to the population, [19] [20] [22] [23] [24] as well as data on enteroparasites present in stray and domestic cats have also been compared [14] [15] [21] [24] [25].

With regard to felids and captive animals, research has already been carried out in several parts of the world, such as Tanzania [30], India [17] [18] [31] [32], Malaysia [28], Iran [33] [34], Italy [35], Spain [36] and Zambia [37], which demonstrates the importance of this subject. However, little is known about the prevalence of enteroparasites in Brazil [27] [29] [38], indicating the need for more research in this area in the country.

To contribute to the knowledge of gastrointestinal parasites affecting wild felids in captivity in the São Paulo Zoo, the present study intends to bring the coproparasitological profile of felid species kept in the Foundation, considering feral cats (*F. catus*) as a possible reservoir.

2. Material and Methods

2.1. Samples

The present study analyzed results of coproparasitological exams from 13 species of Felidae kept in captivity and from feral domestic cats that are circulating in São Paulo Zoo; this was performed over a period of 4 years beginning January/2009 and ending December/2012, and included all samples received by the Clinical Analysis Laboratory. As a general protocol followed by this institution, fresh fecal samples were obtained from the animal enclosure, using clean, uncontaminated material and all samples were processed through the following techniques: direct method, fecal flotation with sodium chlorite solution and fecal sedimentation. The smears were analyzed under optical microscopy and the parasite identification was performed according to morphological characteristics using the literature available [39] [40]. Captive species were divided into native

and exotic, depending on the current distribution and native location of studied species, according to IUCN Red List of Threatened Species online database.

2.2. Data Analysis

This retrospective study analyzed 587 results of processed samples from 13 captive species and 27 results from feral cats. To make the survey more accurate and safer, only the data that presented parasite identification in the minimum level of genus was used. This resulted in a total of 541 samples of captive animals and 27 of feral domestic cats.

After initial screening, three different types of analysis were performed: comparison between results of native and exotic felids; prevalence of infections in feral domestic cats and analyses of all felid captive species in the FPZSP. The analyses were performed quantitatively, based on the percentage of samples that showed positive results for a given parasite.

These analyses allowed us to identify which parasites were most frequently found in each group (native felids, exotic felids, captive felids in general and wandering domestic cats), compare the results among these groups and identify the parasite that is most commonly found in each species.

3. Results

3.1. Captive Wild Felids

Among 541 results analysed, 105 (19.41%) were positive for at least one parasite. Mixed infections were found in two samples from *Leopardus colocolo* and in both of them protozoa *Isospora* sp. and *Eimeria* sp. were associated. Samples indicating the presence of helminths corresponded to 16.63% (90/541), while 3.14% (17/541) were positive for protozoans. The results show that helminth infections are more frequent than protozoan infections in both native, 14.78% (51/345), and exotic species, 19.9% (39/196).

Considering felids in general, *Toxocara cati* was the helminth with the highest prevalence with 43 (7.95%) positive samples, followed by *Toxascaris leonina* with 41 (7.58%) positive samples, *Hymenolepis nana* with five (0.92%), and *Ascaris* sp. with only one (0.18%). Among protozoans, *Isospora* sp. was the most common, with 11 (2.03%) positive samples; *Eimeria* sp., *Giardia* sp. and *Blastocystis* sp. were the second most frequent with two (0.37%) positive samples each (**Table 1**).

From the 541 results analyzed, 345 (63.77%) were referring to the native species, while 196 (36.22%) were related to the exotic species.

Considering only native species samples, 80.87% (279/345) were negative and 19.13% (66/345) were positive for any parasite. The most frequent parasite found in this group was *T. cati*, found in 43 (12.46%) samples, followed by *Isopora* sp. (3.19%), *T. leonina* (1.74%), *Eimeria* sp. (0.58%), *Giardia* sp. (0.58%), *Blastocystis* sp. (0.58%) and *H. nana* (0.58%) (**Table 2**). Considering only positive results, *Toxocara cati* was found in 65.15% of the samples, followed by *Isopora* sp.

Table 1. Prevalence of the different parasites identified in samples of felids of the FPZSP.

	Parasites	Number of positive cases	Prevalence (%)
Protozoa	<i>Isospora</i> sp.	11	2.03
	<i>Eimeria</i> sp.	2	0.37
	<i>Giardia</i> sp.	2	0.37
	<i>Blastocystis</i> sp.	2	0.37
Helminths	<i>Toxocara cati</i>	43	7.95
	<i>Toxascaris leonina</i>	41	7.58
	<i>Hymenolepis nana</i>	5	0.92
	<i>Ascaris</i> sp.	1	0.18

Table 2. Occurrence of enteroparasites in native and exotic species present at FPZSP.

	Analysed samples	<i>Toxocara cati</i> , n (%)	<i>Isospora</i> sp., n (%)	<i>Toxascaris leonina</i> , n (%)	<i>Hymenolepis nana</i> , n (%)	<i>Eimeria</i> sp., n (%)	<i>Giardia</i> sp., n (%)	<i>Blastocystis</i> sp., n (%)	<i>Ascaris</i> sp., n (%)
Native felids									
Pampas cat (<i>Leopardus colocolo</i>)	28	2 (7.14)	4 (14.28)	-	-	2 (7.14)	1 (3.57)	-	-
Geoffroy's cat (<i>Leopardus geoffroyi</i>)	95	13 (13.68)	-	-	-	-	1 (1.05)	2 (2.1)	-
Ocelot (<i>Leopardus pardalis</i>)	1	-	-	-	-	-	-	-	-
Northern tiger cat (<i>Leopardus tigrinus</i>)	122	17 (13.93)	5 (4.10)	2 (1.64)	-	-	-	-	-
Margay (<i>Leopardus wiedii</i>)	37	-	-	-	2 (5.40)	-	-	-	-
Jaguar (<i>Panthera onca</i>)	18	-	-	-	-	-	-	-	-
Puma (<i>Puma concolor</i>)	15	9 (60.0)	1 (6.66)	-	-	-	-	-	-
Jaguarundi (<i>Puma yagouaroundi</i>)	29	2 (6.9)	1 (3.45)	4 (13.79)	-	-	-	-	-
Total (native felids)	345	43 (12.46)	11 (3.19)	6 (1.74)	2 (0.58)	2 (0.58)	2 (0.58)	2 (0.58)	-
Exotic felids									
Serval (<i>Leptailurus serval</i>)	54	-	-	-	3 (5.55)	-	-	-	-
Lion (<i>Panthera leo</i>)	53	-	-	30 (56.6)	-	-	-	-	-
Leopard (<i>Panthera pardus mela</i>)	2	-	-	-	-	-	-	-	-
Amur tiger (<i>Panthera tigris altaica</i>)	65	-	-	-	-	-	-	-	-
Bengal tiger (<i>Panthera tigris tigris</i>)	22	-	-	5 (22.73)	-	-	-	-	1 (4.54)
Total (exotic felids)	196	-	-	35 (17.86)	3 (1.53)	-	-	-	1 (0.51)

n = number of positive samples.

(16.67%), *T. leonina* (9.09%), *Eimeria* sp. (3.03%), *Giardia* sp. (3.03%), *Blastocystis* sp. (3.03%) and *H. nana* (3.03%).

All native felids were positive for at least one parasite, except for ocelot (*Leopardus pardalis*) and jaguar (*Panthera onca*), which had negative results. The only parasite found in margay (*Leopardus wiedii*) was the helminth belonging to the class Cestoda, *H. nana*. The protozoans *Eimeria* sp. and *Blastocystis* sp. were

identified only in pampas cat (*Leopardus colocolo*) and geoffroy's cat (*Leopardus geoffroyi*) samples, respectively (Table 2).

Samples related to exotic species, from 196 results analyzed, 157 (80.1%) were negative for the presence of parasites and 39 (19.9%) were positive. In this group, *Toxocara leonina* had the highest prevalence, found in 35 (17.86%) samples. *Hymenolpis nana* was the second most prevalent with 1.53% (3/196), followed by *Ascaris* sp. found only in one sample (0.51%) (Table 2). Considering positive samples, *Toxocara leonina* was the most common parasite (89.74%), followed by *H. nana* with 7.69% and *Ascaris* sp. with 2.56%. All samples were negative for protozoan and *T. cati* infections. Although the prevalence was high for *T. leonina*, this parasite was found in only two species, *Panthera leo* and *Panthera tigris tigris*. *Hymenolepis nana* and *Ascaris* sp. were identified in only in *Leopardus serval* and *P. tigris*, respectively. All samples of leopard (*Panthera pardus mela*) and amur tiger (*P. tigris altaica*) were negative (Table 2).

Among all analyzed samples, *T. cati* had the highest prevalence (7.95%), where it was identified in 43 samples. The second most common was *T. leonina* found in 35 samples (7.58%), followed by *Isospora* sp. with prevalence of 2.03% (11/541), *H. nana* found in five samples (0.92%), *Eimeria* sp., *Giardia* sp. and *Blastocystis* sp. found in two samples and with a prevalence of 0.37% each and *Ascaris* sp. identified in only one sample (0.18%) (Table 1). Considering only positive samples, *T. cati* had a prevalence of 40.95%. The second most common was *T. leonina* (39.05%), followed by *Isospora* sp. (10.48%), *H. nana* (4.76%), *Eimeria* sp., *Giardia* sp. and *Blastocystis* sp. (1.90% each) and *Ascaris* sp. (0.95%).

Toxocara cati was the most common parasite in this study, but it was present only in native animal samples, while *Ascaris* sp. appeared only in exotic animals. Some species did not have parasite infections, these were: ocelot (*L. pardalis*), jaguar (*P. onca*), leopard (*P. pardus mela*) and amur tiger (*P. tigris altaica*) (Table 2).

3.2. Feral Cat (*Felis catus*)

Twenty-seven coproparasitological exam results were analyzed, of which seven (25.93%) showed negative results and twenty (74.07%) were shown to be positive for at least one parasite species. Mixed infections were identified in six samples (22.22%) and the most frequent association was between *T. cati* and *Giardia* sp. in four samples (14.81%) (Table 3).

Samples from this group showed that infections by helminths were more common than for protozoans. Helminths were present in 16 samples (59.26%) and protozoans in nine (33.33%) (Table 3). Three different parasite species were identified: *T. cati*, in 16 samples (59.26%), followed by *Giardia* sp. in six samples (22.22%) and *Isospora* sp. in three samples (11.11%) (Table 3). Considering only positive samples, *T. cati* appeared in 80% of samples, followed by *Giardia* sp. in 30% and *Isospora* sp. identified in 15%.

Table 3. Occurrence of enteroparasites (helminths and protozoa) and associations in feral cats found at FPZSP.

Parasite	Positive samples	Prevalence (%)
<i>Toxocara cati</i> (helminth)	16	59.26
<i>Giardia</i> sp. (protozoa)	6	22.22
<i>Isospora</i> sp. (protozoa)	3	11.11
Associations		
<i>Toxocara cati</i> + <i>Giardia</i> sp.	4	14.81
<i>Toxocara cati</i> + <i>Isospora</i> sp.	2	7.41

4. Discussion

4.1. Captive Wild Felids

The present study found a prevalence of parasitic infection in captive animals of 19.41%. This prevalence was lower than has previously been reported in Brazil, with prevalence ranging from 20.2% to 64.3%, and other studies conducted around the world, which reported infection rates between 36.7% and 89.3% [17] [18] [28] [29] [35] [38]. Although some studies included other Carnivora species, if only felids are considered, the prevalence is still higher than the one observed in the present study [17] [18] [28] [29] [35] [38]. This is probably due to the adoption of careful sanitary measures, including frequent cleaning of enclosures, removal of food debris and use of footbaths, all accepted preventive veterinary medicine techniques [35] [41].

The prevalence of helminth infections (16.63%) in the present study was higher than that of protozoan (3.14%), a fact also observed in zoos in Malaysia, Bangladesh and Italy [17] [28] [35].

Considering the coproparasitological profiles, *T. cati* was reported as one of the most common parasites in felids, not only in Brazil, but also around the world [38]. Our study corroborates that, with this being the parasite with the highest prevalence. This demonstrates the cosmopolitan characteristic of this parasite, the resistance of its eggs and its infective capacity [28] [35] [41] [42]. These infections were already observed in several reports [17] [27] [32] [35] [38]. The striking presence in lions (*P. leo*) confirms the findings in India and Italy [32] [35]. The results in the northern tiger (*L. tigrinus*) confirm the finding in Paraná (Brazil) [38]. The existence of *T. leonina* in bengal tiger (*P. tigris tigris*) differs from previous findings, where *T. cati* predominated [17] [28] [35]. The frequent presence of *T. leonina* in captive animals and absence in wild cats is frequently reported in the literature, a fact that could be explained by the difference of food offered to captive lions and the proximity of these animals to a great variety of other host species [30]. It is also known that the life cycle of this parasite greatly influences the persistence of infections since its eggs become infective on the soil in one week, facilitating the contamination of captive animals [42].

Isospora sp. was found in four species in a relatively low prevalence (2.03%),

when compared to another study realized in Paraná, Brazil, that was of 14.28% [38]. Despite the low prevalence, the presence of this parasite in captive animals may represent a risk, since stress or other infection may lead to development of main symptoms of isosporosis [43].

The low prevalence of *H. nana* (0.92%), *Eimeria* sp. (0.37%), *Blastocystis* sp. (0.37%) and *Ascaris* sp. (0.18%) found in this study corroborates with other studies [36]. *Eimeria* sp. was not reported as infecting felids, however some studies did not specify the coccidian genera found in their studies [17] [30]. *Blastocystis* sp. was found only in geoffroyi's cat (*L. geoffroyi*), the literature reports the presence of this parasite only in primates [28]. *Ascaris* sp. was present only in bengal tiger (*Panthera tigris tigris*); this parasite has been reported to exhibit high prevalence in herbivores [18].

The prevalence of *Giardia* sp. (0.37%) was low when the present study is compared to others [29] [30]. Presence of *Giardia* sp. was already reported in other zoos, mainly in Brazilian native species [29] [44].

Among the 13 captive species analyzed in this study, only four had negative results for all samples: ocelot (*L. pardalis*), jaguar (*P. onca*), leopard (*P. pardus mela*) and amur tiger (*P. tigris altaica*). The small number of samples of ocelot and leopard could be one of the reasons for these findings. The results observed for jaguar are different from those found in other studies that report infections by helminths and protozoans [29] [38]. Amur tigers were already found to have parasite infections in eastern Russia [45].

Spirometra sp. is a common helminth in zoological animals, constantly found in felids and is considered the most frequent parasite in some studies [17] [28] [30] [31] [37] [38]. The absence of this cestode in samples from FPZSP could be attributed to the absence of intermediate hosts necessary for completing the parasite life cycle [17] [41].

The difference between coproparasitological profiles of native and exotic species is considerable regarding the number of parasite species and prevalence. Among native felids, seven parasite species were identified: *T. cati* (12.46%), *Isospora* sp. (3.19%) and *Toxascaris leonina* (1.74%). Among the exotic felids, only three species were observed: *T. leonina* (17.86%), *H. nana* (1.53%) and *Ascaris* sp. (0.51%). A similar occurrence was observed in Pomerode Zoo (Santa Catarina, Brazil), where all exotic felids were free of gastrointestinal parasite infections, while native species had infections by *Giardia* sp. and *Trichuris* sp. [29].

Although the parasite species infecting the two groups were different, infection rates were similar. Among the native samples, 19.13% were positive to parasites, compared to 19.9% of exotic species samples. In addition, helminths were the most frequent infections in both groups, representing 14.78% of native samples and 19.9% of exotic samples.

4.2. Feral Cat (*Felis catus*)

In the present study, an infection rate of 74.07% was observed in *Felis catus*

samples, much higher than the prevalence reported for domiciled cats, where prevalence ranged from 31.5% to 56.8% [14] [15] [21] [24] [25]. However, it was lower when compared to the prevalence obtained by studies with wandering cats, that was of 87.9% and 100% [19] [24].

Multiple parasitism was observed in 22.22% of the samples, and association between *T. cati* and *Giardia* sp. was the most frequent. Previous studies in Rio de Janeiro (Brazil) reported associations between *Ancylostoma* sp. and *Toxocara* sp. in 32.8% of samples [24].

Considering the coproparasitological profile of these animals, three species were identified, a lower diversity than reported by other authors when six to nine species were identified [14] [15] [19] [21] [25].

In the present study, helminth *T. cati* was the most common, with a prevalence of 59.26%; similar results were obtained in other cities in São Paulo state [19] [23]. However, there was a big difference from other studies with wandering and domiciled cats, in which *T. cati* appears with a prevalence ranging from 0.86% to 19.1% [14] [15] [20] [21] [24] [25]. One explanation for this low prevalence in domiciled animals is due to good nutrition and periodic antiparasitic treatment. The low values reported in other studies with wandering animals may reflect the lack of fixed habitat of these cats, since the eggs of *T. cati* need four weeks to reach the infecting stage, a time surpassing to the presence of the animals [42].

Despite other studies presenting greater infection by protozoa [21], in this study, *Giardia* sp. had a prevalence of 22.22% and is the second most prevalent parasite. Although there was a similar result in Botucatu (São Paulo, Brazil), a lower prevalence is commonly reported in the literature [15] [19] [21] [24] [25]. This parasite affects not only companion animals, but also other mammals, including humans. This parasite is considered to have zoonotic potential, causing severe enteritis, so understanding its distribution among animals that share the same environment with humans is essential for our health [38] [42].

While the present study reports a low prevalence of *Isospora* sp. (11.11%), other studies found it to have higher prevalence between 45.3% - 50.72%, and being the most common parasite [23] [24] [25].

It is important to highlight the absence of *Ancylostoma* sp. in the studied population, a parasite commonly mentioned in studies with domestic cats [19] [24]. It is suggested that the absence of this parasite is due either to non-infection or the fact that cats are still not releasing eggs of hookworms in their feces.

Although the number of identified parasite species in the captive felids samples was higher than those of feral felids, the same parasites were found in both groups but in different prevalence. *Toxocara cati* had the highest prevalence in both feral and captive groups. *Giardia* sp. was the second most common parasite among feral cats (22.22%), however its prevalence was significantly lower (0.37%) in the captive group. *Isospora* sp. was the third most common in both captive and feral animals, but more frequent among feral cats than captive

(11.11% and 2.03%, respectively). While these shared species are significant, their presence alone is not sufficient evidence to claim that feral cats are their reservoirs. As has been noted, past studies have shown that these parasites are frequently found in felids in general and all species have already been reported for both groups. While the existence of a proximally relevant transmission route between feral and captive felids is legitimate, it does not appear to be the predominant method by which captive felids are acquiring infection. This must, of course, be afforded additional studies and ideally, alternative sampling and analyzing methodologies. Indeed, the authors recognize that the absence of an active sampling methodology was a limitation of this study. Nonetheless, it is hoped the work has provided a foundation for what is needed, and adds to our understanding of the parasitic profile of these animals. Going forward, we also recognize the importance of maintaining control over the feral colonies living in the FPZSP area given their close contact with humans and the potential for disease spread within the community.

5. Conclusions

We were able to identify eight different parasite species infecting captive felids in FPZSP and three infecting feral cats. *Toxocara cati* was the most frequent parasite in both groups.

There was a considerable difference between the coproparasitological profile of exotic and native captive felids. We found there was both a greater diversity of parasites found in native felids than in exotic, along with major differences in the overall prevalence of parasite species among these two groups.

While our study found feral cats and captive felids to share many of the same parasite species, it is not possible to claim that those in the feral group act as a reservoir; we base this on the fact that the species identified are common felid parasites, found in all environments, along with the inability of our study to confirm direct link(s) of transmission between the two groups.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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