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Gastrointestinal protozoa in non-human primates of four zoological gardens in Belgium

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Abstract

Gastrointestinal parasites are important infectious causes of diarrhoea in captive non-human primates (NHP). However, prevalence data of gastrointestinal parasites in zoological gardens are scarce. Therefore, a cross-sectional survey was conducted to estimate the occurrence of gastrointestinal parasites in NHP of four zoological gardens in Belgium. Between August 2004 and April 2006, 910 faecal samples were collected from 222 animals housed in 39 groups. The 31 species involved were representatives of prosimians, New World (NW) monkeys, Old World (OW) monkeys and apes. Because individual sampling was impossible, a statistical simulation was performed to estimate a sufficient sample size. All samples were microscopically examined after an acetic acid–ether concentration. Differences in host species susceptibility were examined by non-parametric tests. *Entamoeba* spp. (44%) and *Giardia* spp. (41%) were the most prevalent species. Other parasites detected were *Endolimax nana* (36%), *Chilomastix mesnili* (21%), *Balantidium coli* (13%), *Trichuris* spp. (10%), *Iodamoeba bütschlii* (5%) and *Strongyloides* spp. (5%). Parasites for which a significant difference in susceptibility at the level of host taxonomy was noted were *Entamoeba* spp. ($p < 0.001$) and *C. mesnili* ($p < 0.05$). Samples containing *Entamoeba* spp. were the most prevalent in OW monkeys ($p < 0.0083$). Samples collected from OW monkeys contained the highest number of parasite species ($p < 0.0083$).

Keywords: Gastrointestinal protozoa; Non-human primates; Zoological gardens; Prevalence; Host species susceptibility

1. Introduction

Infections with gastrointestinal parasites are widespread among non-human primates (NHP). However, as a consequence of regular deworming and hygienic measures helminth infections are uncommon in captive NHP (Gómez et al., 1996; Verweij et al., 2003a). In contrast, protozoa such as *Entamoeba histolytica* (Pang et al., 1993; Verweij et al., 2003a), *Giardia* spp. (Peisert et al., 1983; Hamlen and Lawrence, 1994; Kalishman et al., 1996), C*ryptosporidium* spp. (Gómez et al., 1992; Kalishman et al., 1996; da Silva et al., 2003) and *Balantidium coli* (Nakauchi, 1999) are frequently reported in captive NHP, and are considered as important causes of gastro-enteritis in NHP. Infection by these gastrointestinal parasites may cause watery diarrhoea, hemorrhagic dysentery, extra-intestinal pathologies, such as liver abscesses, and even death. *E. histolytica*, the causative organism of invasive intestinal and extra-intestinal amebiasis, is of major

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concern. Clinical outbreaks and deaths caused by this parasite are frequently reported (Loomis et al., 1983; Beaver et al., 1988; Marquez-Monter et al., 1991; Verweij et al., 2003a). Giardiasis and cryptosporidiosis are of less clinical importance, but are considered as a cause of diarrhoea or failure to thrive in young animals (Miller et al., 1990; Kalishman et al., 1996). *B. coli* is probably harmless in most of the NHP species. However, according to Lee et al. (1990) and Hänichen et al. (1995) care should be taken when great apes are involved.

Because of the importance of *E. histolytica* (Stauffer and Ravdin, 2003), *Giardia* spp. (Thompson, 2000) and *Cryptosporidium* spp. (O'Donoghue, 1995) in humans, the role of NHP as potential reservoirs for zoonotic transmission should not be underestimated. Transmission between animals and humans in association with clinical outbreaks in animal caretakers has been reported in various studies (Miller et al., 1990; Hamlen and Lawrence, 1994).

Although the above-mentioned studies have shown the clinical importance of protozoa for both NHP and animal caretakers, studies to quantify the prevalence and importance of these parasites in zoological gardens are scarce. Most studies describe clinical outbreaks and were based on a limited number of animal and/or parasite species (Teare and Loomis, 1982; Loomis et al., 1983; Lee et al., 1990). Moreover, the strategies used to collect faecal samples may thwart the prevalence results.

In this study, the prevalence of gastrointestinal protozoa in NHP of four Belgian zoological gardens was estimated based on a new sampling strategy. In order to examine all animals within a group a statistical simulation was performed to estimate a sufficient sample size. The 31 species involved were representatives of NHP of four Belgian zoological gardens and included members of *Lemuridae*, *Galagonidae*, *Cebidae*, *Atelidae*, *Cercopithecidae, Hylobatidae* and *Hominidae*. A second objective was to examine differences in host species susceptibility to gastrointestinal protozoan infections.

2. Materials and methods

2.1. Study sites

This study was conducted at four Belgian zoological gardens. These included the Antwerp Zoo, the Animal Park Planckendael, the Olmen Zoo and the Park Paradisio. Because of confidentiality, a letter was randomly assigned to the four study sites. Study site A was founded in 1843 and is a typical urban zoo of about 10 ha where most of the NHP are accommodated in indoor enclosures. At study site B (12 ha), study site C (55 ha) and study site D (40 ha) the animals are kept on a large verdant outdoor enclosure. At all sites animals are kept indoor when the temperature drops below 10 °C.

2.2. Animals and husbandry

From August 2004 to April 2006, 222 animals belonging to 31 NHP species and seven families were studied (Table 1). The families involved were two of prosimians (*Lemuridae*, *Galagonidae*), two of New World (NW) monkeys (*Cebidae* and *Atelidae*), one of the Old World (OW) monkeys (*Cercopithecidae*) and both families of the apes (*Hylobatidae* and *Hominidae*) (Groves, 2001). The animals are housed in 39 groups of 2–20 individuals (median of four animals). Apart from six groups where two NHP species are mixed, all groups consisted of one species. Of all sites, study site A lodges the largest and most diverse population of NHP. This site includes half of the animals and 75% of the species included in this study representing all seven families. A total of 37 animals (11 species) were examined at study site B, 38 animals (5 species) at study site C and 17 animals (3 species) at study site D.

Overall, the median (25th quantile (25Q); 75th quantile (75Q)) of the stocking density was 13.5 m^2 per animal (7.5; 51.1). The lowest values were found at study site A (median of 8.3 m^2 per animal).

2.3. Sampling strategy

Due to the group housing of semi-wild NHP individual sampling was impossible. However, a statistical simulation in R (version 2.4.0, The R Foundation for Statistical Computing) was used to estimate the sample size needed to examine all animals within a group with a probability of at least 95% (Appendix A). Because little is known about the defecation behaviour of the different NHP species, an equal probability of sampling was assumed in this simulation ($\pi_1 = \pi_2 = \cdots = \pi_j = 1/n\_\text{NHP}$). To correct for this unknown variability, a sampling method with replacement was performed (group $\sim$ *Mult*(n_sim, π)). The estimated sample sizes in function of the group size obtained by this simulation are presented in Table 2.

For each particular group all samples were picked up from the ground during consecutive days until the estimated sample size was approximated.

Table 1
Animal species and husbandry conditions of NHP kept at four Belgian zoological gardens

Groups		Number of animals	Stocking density (animal/m^2)
Common name	Scientific name		
Study site A			
Ring-tailed lemur	*Lemur catta*	9	12.0
Red ruffed lemur	*Varecia rubra*	2	7.5
Red ruffed lemur	*Varecia rubra*	4	3.5
Brown greater galago	*Otolemur crassicaudatus*	2	60.0
Goeldi's marmoset	*Callimico goeldii*	2	3.7[a]
Golden-headed lion tamarin	*Leontopithecus chrysomelas*	2	
Common marmoset	*Callithrix jacchus*	1	3.3[a]
Golden-headed lion tamarin	*Leontopithecus chrysomelas*	2	
Common marmoset	*Callithrix jacchus*	2	4.0[a]
Golden-headed lion tamarin	*Leontopithecus chrysomelas*	2	
Pygmy marmoset	*Callithrix pygmae*	10	1.2
Emperor tamarin	*Saguinus imperator*	2	7.5
Black-headed spider monkey	*Ateles fusciceps*	5	13
Celebes crested macaque	*Macaca nigra*	2	19.9
Mandrill	*Mandrillus sphinx*	14	17.9
Hamadryas baboon	*Papio hamadryas*	20	8.1
Mantled guereza	*Colobus guereza*	7	4.3
Hamlyn's monkey	*Cercopithecus hamlyni*	8	8.5
Northern plains gray langur	*Semnopithecus entellus*	5	7.9
Javan lutung	*Trachypithecus auratus*	4	3.7
Javan lutung	*Trachypithecus auratus*	6	11.4
Siamang	*Hylobates syndactylus*	4	12.0
Western lowland gorilla	*Gorilla gorilla*	1	78.7[a]
Mountain gorilla	*Gorilla beringei*	2	
Common chimpanzee	*Pan troglodytes*	10	15.7
Bornean Orangutan	*Pongo pygmaeus*	2	83.0
Study site B			
Black-and-white ruffed lemur	*Varecia variegata*	6	31.0[a]
Ring-tailed lemur	*Lemur catta*	3	19.5[a]
Brown lemur	*Eulemur fulvus*	1	
Tufted capuchin	*Cebus apella*	5	44.4
Common squirrel monkey	*Saimiri sciureus*	6	2.5
Common marmoset	*Callithrix jacchus*	2	1.0
Black crested mangabey	*Lophocebus aterrimus*	5	9.3
Black crested gibbon	*Hylobates concolor*	1	14.0[a]
Red-cheeked gibbon	*Hylobates gabriellae*	1	
White-handed gibbon	*Hylobates lar*	4	44.5
Common chimpanzee	*Pan troglodytes*	3	161.7
Study site C			
Ring-tailed lemur	*Lemur catta*	8	253.0
Red ruffed lemur	*Varecia rubra*	4	257.4
Black-and-white ruffed lemur	*Varecia variegata*	4	127.2
Common squirrel monkey	*Saimiri sciureus*	20	51.1
Siamang	*Hylobates syndactylus*	2	NA
Study site D			
White-handed gibbon	*Hylobates lar*	4	132.2
Northern white-cheeked gibbon	*Hylobates leucogenys*	4	32.3
Bonobo	*Pan paniscus*	9	347.9

NA: not available.
[a] Both NHP species are housed in the same enclosures.

Table 2
Estimated sample sizes and probabilities in relation to different group sizes

Group size	Samples size	Probability (%) (95% confidence interval)
2	6	96.8 (96.5; 97.2)
3	11	96.6 (96.2; 96.9)
4	16	96.4 (96.0; 96.8)
5	21	95.6 (95.1; 96.0)
6	27	95.6 (95.2; 96.0)
7	33	95.8 (95.4; 96.2)
8	38	95.3 (95.0; 95.8)
9	45	95.4 (95.0; 95.8)
10	50	95.2 (95.0; 95.6)
11	58	95.5 (95.1; 95.9)
14	76	95.4 (95.0; 95.8)
20	116	95.4 (95.0; 95.8)

2.4. Coprological examination

An acetic acid–ether concentration method on faeces was used to demonstrate the presence of gastrointestinal parasites in NHP (Thienpont et al., 1986). Half a gram of faeces was suspended in 5 ml acetic acid (5%) and strained through a brush wire sieve to remove debris. The fat in the resulting filtrate was removed by emulsifying the sample with 5 ml of ether followed by centrifugation at 1500 rpm for 2 min. The resulting supernatant (ether, debris and acetic acid) was discarded and two drops of diluted iodine (1:100) were added to the remaining sediment. The stained sediment was thoroughly mixed after which it was transferred onto a glass microscope slide and covered with a cover glass. Each sample was examined at a 400× magnification for the presence of eggs, larvae, trophozoites and/or cysts.

2.5. Statistical analysis

'Prevalence' is defined as the percentage of groups of NHP infected with a particular parasite species and 'diversity' as the number of parasite species within the same group. 'Proportion' describes the proportion of samples containing a particular parasite species within the same group and 'multiple infections' the median number of parasite species found in these samples. *Entamoeba* spp. was treated as one species. The Kruskal–Wallis test was performed to test for differences in proportion and mixed infections between host species (SAS 9.1.3, SAS Institute Inc., Cary, NC, USA). The host species' classes involved were prosimians, NW monkeys, OW monkeys and apes. The level of significance was set at $P < 0.05$. In addition, a Bonferroni pair wise comparison procedure was performed. For this, the Wilcoxon test was employed and the level of significance was set at 0.0083 (SAS 9.1.3, SAS Institute Inc., Cary, NC, USA).

3. Results

In total, 910 individual samples were collected. The number of times a group was sampled varied between 1

Table 3
Prevalence and diversity of parasites in NHP at four Belgian zoological gardens

	Number of groups	*Entamoeba* spp. (%)	*E. nana* (%)	*I. bütschlii* (%)	*Giardia* spp. (%)	*C.mesnili* (%)	*B. coli* (%)	*Trichuris* spp. (%)	*Strongyloides* spp. (%)	Diversity (25Q–75Q)
Prosimians	9	33	11	0	44	11	0	0	0	0–2
Study site A	4	50	0	0	25	25	0	0	0	
Study site B	2	50	50	0	100	0	0	0	0	
Study site C	3	0	0	0	33	0	0	0	0	
NW monkeys	10	40	40	0	40	0	0	0	0	0–2
Study site A	6	17	17	0	33	0	0	0	0	
Study site B	3	67	67	0	33	0	0	0	0	
Study site C	1	100	100	0	100	0	0	0	0	
OW monkeys	9	100	44	22	22	55	33	33	0	1–5
Study site A	8	100	50	12	25	50	37	37	0	
Study site B	1	100	0	100	0	100	0	0	0	
Apes	11	64	45	0	54	18	18	1	18	1–3
Study site A	4	100	25	0	75	25	25	0	25	
Study site B	3	67	0	0	33	33	0	33	0	
Study site C	1	100	100	0	100	0	0	0	0	
Study site D	3	0	100	0	33	0	33	0	33	
	39	59	36	5	41	21	13	10	5	1–3

and 11 occasions and the median number of faecal samples produced by the groups measured 1 per animal, ranging from 0.15 to 8. The median (25Q; 75Q) probability of sampling all animals within a group was 96% (93–97).

3.1. Prevalence and diversity of parasites

A total of eight parasite species were identified, two protozoan and two nematode species. Prevalence and diversity of gastrointestinal parasites are reported in Table 3. Infections with trematodes and cestodes were not detected. In 33 out of the 39 groups, infection with at least one parasite species was detected. The most prevalent gastrointestinal parasites were: *Entamoeba* spp. (59%), and *Giardia* spp. (41%). Other parasites detected were *Endolimax nana* (36%), *Chilomastix mesnili* (21%), *B. coli* (13%), *Trichuris* spp. (10%), *Iodamoeba bütschlii* (5%) and *Strongyloides* spp. (5%). All parasite species were detected in no less than two study sites. *Giardia* spp. and *E. nana* were the only parasite species observed at all the four study sites. Groups of OW monkeys harboured the most parasite species. In these groups the median number of parasite species detected was three. The smallest diversity was observed in NW monkeys and prosimians (median of one parasite species).

3.2. Proportion and multiple infections

Tables 4–7 provide an overview of the results. Overall, there was a large variation in proportion of parasite infections. For the most prevalent parasite species, the proportion ranged from 0 to 100% for *Entamoeba* spp., and from 0 to 94% for *Giardia* spp. For *Entamoeba* spp., the highest proportion was found in OW monkeys. The median (25Q; 75Q) of proportion in these host species measured 87.5% (80.0; 96.0). In Apes, *Entamoeba* spp. occurred in 12.5% (0; 62.5) of the samples. Prosimians and NW monkeys were the least infected with this parasite species. *Entamoeba* spp. was not detected in 75% of these host species. Samples containing cysts of *Giardia* spp. were mainly found in apes. The median proportion in these NHP species was 4% (0; 38.8). The least proportion of this infection was observed in OW monkeys, in which no cysts were found in 75% of the groups. The less common parasites were mainly found in OW monkeys. Overall, the median of the multiple infections measured 1, ranging from 0 to 3. OW monkeys harboured the most species of endoparasites. In these groups the median (25Q; 75Q) number of infections detected was 1 (1; 2), 1 in apes (0; 1) and 0 in both NW monkeys (0; 1) and prosimians (0; 0).

Table 4
Proportion and multiple infections in prosimians

	Group size	Sample size	Probability (%)	*Entamoeba* spp. (%)	*E. nana* (%)	*C. mesnili* (%)	*Giardia* spp. (%)	*I. bütschlii* (%)	*B. coli* (%)	*Trichuris* spp.	*Strongyloides* spp. (%)	Mixed infections
Study site A												
Ring-tailed lemur	9	34	83	29	0	0	94	0	0	0	0	1
Red ruffed lemur	2	4	87	0	0	25	0	0	0	0	0	0
Red ruffed lemur	4	16	96	1	0	0	0	0	0	0	0	0
Brown greater galago	2	5	93	0	0	0	0	0	0	0	0	0
Study site B												
B & W ruffed lemur[a]	6	25	93	24	0	0	16	0	0	0	0	0
Ring-tailed lemur	3	15[b]	94	0	7	0	27	0	0	0	0	0
Brown lemur	1											
Study site C												
Ring-tailed lemur	8	40	96	0	0	0	15	0	0	0	0	0
Red ruffed lemur	4	16	96	0	0	0	0	0	0	0	0	0
B & W ruffed lemur[a]	4	16	96	0	0	0	0	0	0	0	0	0

[a] Black and white ruffed lemur.
[b] Both NHP species are housed in the same enclosure.

Table 5
Proportion and multiple infections in NW monkeys

	Group size	Sample size	Probability (%)	*Entamoeba* spp. (%)	*E. nana* (%)	*C. mesnili* (%)	*Giardia* spp. (%)	*I. bütschlii* (%)	*B. coli* (%)	*Trichuris* spp. (%)	*Strongyloides* spp. (%)	Multiple infections
Study site A												
Goeldi's monkey	2	16[a]	96	0	0	0	0	0	0	0	0	0
Golden-headed lion tamarin	2											
Common marmoset	1	4[a]	44	0	0	0	0	0	0	0	0	0
Golden-headed lion tamarin	2											
Common marmoset	2	12[a]	86	8	0	0	0	0	0	0	0	0
Golden-headed lion tamarin	2											
Pygmy marmoset	10	5	0	0	80	0	20	0	0	0	0	1
Emperor tamarin	2	4	87	0	0	0	0	0	0	0	0	0
Black-headed spider monkey	5	20	94	0	0	0	45	0	0	0	0	0
Study site B												
Tufted capuchin	5	21	96	5	0	0	5	0	0	0	0	0
Common squirrel monkey	6	14	69	7	7	0	0	0	0	0	0	0
Common marmoset	2	6	97	0	67	0	0	0	0	0	0	1
Study site C												
Common squirrel monkey	20	127	97	1	56	0	9	0	0	0	0	1

[a] Both NHP species are housed in the same enclosure.

Table 6
Proportion and multiple infections in OW monkeys

	Group size	Sample size	Probability (%)	*Entamoeba* spp. (%)	*E. nana* (%)	*C. mesnili* (%)	*Giardia* spp. (%)	*I. bütschlii* (%)	*B. coli* (%)	*Trichuris* spp. (%)	*Strongyloides* spp. (%)	Multiple infections
Study site A												
Celebes crested macaque	2	10	99	30	60	0	0	0	70	20	0	2
Mandrill	14	75	93	99	56	49	7	0	52	0	0	3
Hamadryas baboon	20	102	89	96	42	23	2	0	2	16	0	2
Mantled guereza	7	30	93	80	0	0	0	0	0	0	0	1
Hamlyn's monkey	8	35	92	83	54	11	0	37	0	3	0	2
Northern plains gray langur	5	16	86	94	0	0	0	0	0	0	0	1
Javan lutung	4	14	94	64	0	0	0	0	0	0	0	1
Javan lutung	6	24	93	88	0	8	0	0	0	0	0	1
Study site B												
Black crested mangabey	5	23	97	100	0	13	0	30	0	0	0	1

No significant effects were found of study sites on proportion of infections ($\chi^2(3) = 0.80$–6.0, $p = 0.84$–0.11) and multiple infections ($\chi^2(3) = 4.2$, $p > 0.2$), neither for stocking density ($R = -0.17$ to 0.001, $p = 0.32$–0.99; $\chi^2(3) = 0.77$, $p > 0.8$).

3.3. Differences in host species susceptibility

Both proportion and multiple infections ($\chi^2(3) = 19.9$, $p = 0.0002$) differed between host species. Parasites for which a significant difference in susceptibility at the level of taxonomy was noted were *Entamoeba* spp. ($\chi^2(3) = 21.4$, $p < 0.0001$) and *C. mesnili* ($\chi^2(3) = 9.3$, $p < 0.05$). However, a marginal p-value was also found for *I. bütschlii* ($\chi^2(3) = 6.8$, $p = 0.08$), *Trichuris* spp. ($\chi^2(3) = 6.8, p = 0.08$) and *B. coli* ($\chi^2(3) = 6.4, p = 0.09$). Samples containing cysts of *Entamoeba* spp. were more prevalent in OW monkeys than in prosimians ($|Z| = 3.6$, $p < 0.0005$), in NW monkeys ($|Z| = 3.7$, $p < 0.0005$) and in apes ($|Z| = 2.7$, $p < 0.0083$). OW monkeys had a marginal significantly higher number of infected samples with *C. mesnili* than NW monkeys ($|Z| = 2.6$, $p = 0.0099$). Samples collected from OW monkeys contained the highest number of parasite species (prosimians: $|Z| = 3.5$, $p < 0.0005$; NW monkeys: $|Z| = 3.2$, $p < 0.005$; apes: $|Z| = 2.8$, $p < 0.0083$). No significant differences were observed between the other host species.

4. Discussion

In the present study, the majority of the examined groups were infected with at least one parasite species. All the parasite species identified in our study have previously been described in both captive and free ranging NHP (Gómez et al., 1996; Ashford and Wrangham, 2000; Legesse and Erko, 2004). Protozoa were most prevalent, which confirms the findings of other studies performed in captive NHP (Gómez et al., 1996; Verweij et al., 2003a). The occurrence of these parasites can be explained by the simplicity of their lifecycle, because they need no intermediate hosts and are immediately infective when excreted. Moreover, the low infective dose and the short prepatent period, obviously ease transmission (Tanyuksel and Petri, 2003; Thompson and Monis, 2004). The most prevalent parasite species in this study were *Entamoeba* spp. and *Giardia* spp. More than 40% of the examined groups were infected with one of these endoparasites. However, a large variation in proportion and multiple infections was observed between the examined groups. This variation may be caused by differences in host species

Table 7
Proportion and multiple infections in apes

	Group size	Sample size	Probability (%)	*Entamoeba* spp. (%)	*E. nana* (%)	*C. Mesnili* (%)	*Giardia* spp. (%)	*I. bütschlii* (%)	*B. coli* (%)	*Trichuris* spp. (%)	*Strongyloides* spp. (%)	Multiple infections
Study site A												
Siamang	4	15[b]	94	27	0	0	47	0	0	0	7	1
Western lowland gorilla[a]	1	3[b]	100	67	0	0	67	0	0	0	0	1
Mountain gorilla[a]	2											
Common chimpanzee	10	49	94	8	8	2	39	0	29	0	0	1
Bornean orang-utan[a]	2	8	100	62	0	0	0	0	0	0	0	1
Study site B												
White-handed gibbon	4	16	96	12	0	0	12	0	0	0	0	0
Black crested gibbon[a]	1	3[b]	100	0	0	0	0	0	0	33	0	0
Red-cheeked gibbon[a]												
Common chimpanzee	3	11	97	100	0	45	0	0	0	0	0	1
Study site C												
Siamang	2	10	99	60	10	0	20	0	0	0	0	1
Study site D												
White-handed gibbon	4	18	97	0	6	0	0	0	0	0	0	0
Northern w-c gibbon[c]	4	26	99	0	12	0	4	0	11	0	42	1
Bonobo[a]	9	22	100	0	41	0	0	0	0	0	0	0

[a] Individual samples.
[b] Both species are housed in the same enclosure.
[c] Northern white-cheeked gibbon.

susceptibility, since OW monkeys were at higher risk of infections, including those with *Entamoeba* spp. and harboured the highest number of parasite species. Differences in host species susceptibility have been hypothesized and might be explained by differences in behaviour, because the majority of the OW monkeys are ground dwellers (Beaver et al., 1988; Munene et al., 1998).

Using the sample strategy described in this study, more insights were gained in the epidemiology of gastrointestinal parasites in NHP of zoological gardens. First of all, it is likely to result in more accurate estimates of the group's prevalence, because all animals were examined with a high probability and none of the samples were pooled. Moreover, it allowed a more powerful risk factor analysis, since proportion within groups could be estimated.

Of all parasites identified in this study, *Giardia* spp., *B. coli*, *Trichuris* spp. and *Strongyloides* spp. might cause gastrointestinal enteritis in NHP (Lee et al., 1990; Hamlen and Lawrence, 1994; Hänichen et al., 1995; Kalishman et al., 1996). However, due to the study design the clinical importance of these infections could not be studied more in depth, in particular for *E. histolytica*. *E. histolytica* has been previously described in various species involved in this study (Loomis et al., 1983; Beaver et al., 1988; Verweij et al., 2003a; Mätz-Rensing et al., 2004), but differentiation between other *Entamoeba* spp. (*E. coli*, *E. hartmanii*, *E. polecki*-like) is difficult (Verweij et al., 2001, Kebede et al., 2003) and even impossible when *E. dispar* or *E. moshkovskii* are involved (Diamond and Clark, 1993, Tanyuksel and Petri, 2003). For this purpose molecular diagnostic techniques are more appropriate (Verweij et al., 2003b). Among other pathogenic parasites that cannot be ruled out in the present study was *Cryprosporidium* spp. (Miller et al., 1990; Muriuki et al., 1997; Gómez et al., 2000), because no appropriate detection technique was used.

Although all parasites found are recognised zoonotic pathogens, the high prevalence of *Giardia* warrants special attention. Zoonotic assemblages (*G. duodenalis* assemblage A and B) have been described in NHP (Thompson et al., 2000; Graczyk et al., 2002; Nizeyi et al., 2002, Vitazkova and Wade, 2006). However, differentiation of the assemblages of *G. duodenalis* based morphological features is impossible, and therefore additional prevalence data based on molecular techniques are needed to confirm this reservoir function of NHP involved in this study. In conclusion, the results of this study demonstrated that gastrointestinal protozoa and multiple infections are highly prevalent in NHP of Belgian zoos. There was a large variation in proportion and multiple infections between groups of NHP, which might be explained by differences in host species susceptibility. The used sample strategy is likely to result in more accurate epidemiological data. This study also emphasizes the need for molecular diagnostic tools in NHP to evaluate the clinical importance and zoonotic risk of these infections.

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Appendix A

n_NHP <-number of animals within a group;

min_n_samples <- minimum number of samples;

max_n_samples <- maximum number of samples;

n_sim <- number of iterations needed to obtain estimates. This was set on 10000;

miss <- rep(0,max_n_samples)

miss[i] = the number of times at least one animal within a group was not sampled when i faecal samples were collected. This vector contains 0's on the onset of the simulation.

for (i in min_n_samples:max_n_samples)

{

For each sample size i varying between min_n_samples and max_n_samples a simulation will be performed.

for (simlus in 1:n_sim)

{

Each simulation consists of 10000 iterations

group <- rep(0,n_NHP);

group[j] equals the number of faecal samples collected of animal j within a group. This vector contains only 0's on the onset of the sampling.

for (lus in 1:i)

{

j <-sample(1:n_NHP,1);

group(j) = group(j)+1;

group ~ *Mult*(n_sim, π), where $\pi = (\pi_1\ \pi_2 \ldots \pi_j)$ and $\pi_1 = \pi_2 = \ldots = \pi_j = 1/n\_NHP$

}

ifelse(any(group==0,na.rm = TRUE) == TRUE, miss[i] <- miss[i]+1, miss[i] <- miss[i]+0)

}

The sample size i will increase with one unit

}

prob = miss/n_sim;

prob[i] equals the probability of missing at least one animal in a group of n_NHP animals when i faecal samples were collected.

prob[i] ~ *binom*(n_sim, prob[i])

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