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ORIGINAL PAPER

Study of the gastrointestinal parasitic fauna of captive non-human primates (*Macaca fascicularis*)

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Abstract The aim of this study was to examine helminths and protozoans in cynomolgus macaques (*Macaca fascicularis*) imported from registered breeding facilities in China and their relation to health risks for non-human primate handlers in biomedical research centers and in breeding facilities. Fresh fecal samples were collected from a total of 443 *M. fascicularis* and analyzed by copromicroscopical analysis, immunoenzymatic, or molecular assays. As to helminths, whose eggs were shed in 2.03% of the samples, *Trichuris* and *Oesophagostomum* were the only two taxa found, with low prevalence and low eggs per gram (EPG) values. Protozoans were more frequently detected (87.40%), with *Entamoeba coli* (85.19%) and *Endolimax nana* (79.26%) as the most prevalent species shed. Other parasites found by fecal smear examination were uninucleated-cyst-producing Entamoebas (78.52%), *Iodamoeba bütschlii* (42.96%), and *Chilomastix mesnili* (24.44%), while cysts of *Balantidium coli* (22.2%) were only observed by sedimentation. No coproantigens of *Giardia duodenalis*, *Cryptosporidium* spp., and *Entamoeba histolytica* complex were detected. *Blastocystis* sp. infection was noticed in 87.63% of macaques by PCR. These cynomolgus monkeys were infected with many subtypes (ST1, ST2, ST3, ST5, and ST7), where the predominant *Blastocystis* sp. subtypes were ST2 (77.5%), followed by ST1 (63.5%). Data collected confirmed the presence of potentially zoonotic parasites and a high parasite diversity, suggesting the need for appropriate and sensitive techniques to adequately control them and related health risks for handlers of non-human primates in biomedical research centers and in breeding facilities.

Keywords *Trichuris* sp. · Protozoans · Parasites · *Oesophagostomum* sp. · *Macaca fascicularis* · *Blastocystis* sp.

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Introduction

Gastrointestinal parasitism in colonies of non-human primates (NHPs) used for research is a common occurrence (Sano et al. 1980; Takano et al. 2005; da Silva Barbosa et al. 2015). *Strongyloides* spp., *Oesophagostomum* spp., and *Trichuris trichiura* were previously considered among the most common pathogens causing poor development, anemia, and diarrhea in macaques and in other NHPs (Honjo et al. 1963; Wong and Conrad 1978; Abbott and Majeed 1984). Regular deworming and high-standard hygienic measures currently adopted in breeding facilities and research centers should lower helminthic infections. Nevertheless, *Trichuris* could persist in NHPs for biomedical research due to their egg high resistance and to the long life span of their adults, which requires a specific treatment strategy to eradicate them (Reichard et al. 2007). Recently, captive NHPs were also demonstrated as frequently affected by several species of intestinal protozoans that resulted pathogenic for their hosts (Lee et al. 1990; Muriuki et al. 1997; Vogel et al. 1996; Zanzani et al. 2014). Moreover, most parasites recorded in NHPs represent a potential zoonotic risk for researchers and caretakers in breeding centers (Loomis 1983;

Roperto et al. 1985; Muriuki et al. 1998; Pedersen et al. 2005; Yoshikawa et al. 2009; Meloni et al. 2012). This investigation aimed at evaluating the following in cynomolgus macaques, a non-human primate species: (i) the prevalence of gastrointestinal parasites (helminths and protozoans), (ii) the degree of infection (helminths), and (iii) the prevalence of *Blastocystis* genotypes in order to improve both health management and welfare of this species of monkey and to prevent the transmission of zoonotic parasites to researchers and staff.

Material and methods

The study was approved by the Animal Care and Use Committee of the University of Milan.

Sampling

Fresh fecal samples from 443 cynomolgus macaques (*Macaca fascicularis*) imported from registered breeding facilities in China (F2 purpose-bred) were obtained on four occasions throughout 2013. The feces were stored at +4 °C and, under refrigerated condition, were sent for examination to a laboratory within 48 hours.

Fecal examination

Fecal samples (n=443) were microscopically analyzed; feces were previously added with formol-ether and centrifuged and their sediment analyzed by FLOTAC® basic technique using a flotation solution with sucrose and sodium nitrate (specific gravity: 1.20) for the detection of nematode eggs, and their eggs per gram (EPG) number was calculated. Cysts of *Balantidium coli* and trematode eggs were detected by sedimentation. Following a few cases of severe diarrhoeic diseases, the analysis focused also on protozoan infections. Fecal smears stained with Lugol's solution were performed in 135 samples out of 443 for protozoan detection; the nomenclature for protozoans partially used for Entamoebas was the one proposed by Stensvold et al. (2011). Eventually, two aliquots were stored at −20 °C until immunoenzymatic and molecular analysis was carried out.

Immunoenzymatic assay

One hundred and thirty-five fecal samples were screened by commercial available kits (RIDASCREEN® *Cryptosporidium*, RIDASCREEN® *Giardia* and RIDASCREEN® *Entamoeba*, R-Biopharm, Darmstadt, Germany) for the antigens of *Cryptosporiudm parvum*, *Giardia duodenalis,* and *Entamoeba histolytica* complex.

PCR assay

A group of 97 samples were processed by a commercial kit (QIAamp DNA Stool Mini Kit, QIAGEN®, Valencia, CA, USA) for DNA extraction. A PCR protocol was applied to amplify a fragment of the nucleotide SSU rDNA of *Blastocystis*. For external PCR, the forward primer Blast 505–532 (5′ GGA GGT AGT GAC AAT AAA TC 3′) and the reverse primer Blast 998–1017 (5′ TGC TTT CGC ACT TGT TCA TC 3) were used. A ca. 500 (479)-bp fragment, containing a variable region that allows subtyping of *Blastocystis* specimens was amplified (Santín et al. 2011). Amplification products were run on 1% ethidium bromide agarose gels and visualized under ultraviolet light. Bands were excised from agarose gels and purified using a QIAquick Gel Extraction Kit (QIAGEN®, Valencia, CA, USA). Amplification products were sequenced using the seven pairs of STS primers (SB83, SB155, SB227, SB332, SB340, SB336, and SB337) to genotype *Blastocystis* sp. as employed by Yoshikawa et al. (2004) and Stensvold (2013).

Results

Parasitological analysis

Prevalence of gastrointestinal parasites is reported in Table 1. Copromicroscopic analysis demonstrated helminth eggs shedding in 2.03% of 443 fecal samples from cynomolgus monkeys; both found taxa, *Trichuris* and *Oesophagostomum*, showed low prevalence and low EPG values (Table 1). Protozoans (87.40%) were more frequently detected than helminths. *Entamoeba coli* (85.19%) and *Endolimax nana* (79.26%) were the most prevalent species shed. Other parasites found by examination of fecal smears were uninucleated-cyst-producing Entamoebas (78.52%), *Iodamoeba bütschlii* (42.96%), and *Chilomastix mesnili* (24.44%), while cysts of *B. coli* (22.2%) were only observed by sedimentation. No coproantigens of *G. duodenalis*; *Cryptosporidium* spp., and *E. histolytica* complex were detected.

Genotyping of *Blastocystis* sp.

Blastocystis sp. infection was noticed in 87.63% of the macaques (85/97) under investigation. The most predominant *Blastocystis* sp. subtype was ST2 (77.5%), followed by ST1 (63.5%), ST7 (41.2%), ST3 (38.8%), and ST5 (1.2%). ST4 and ST6 were not isolated. A mixed infection with two or more subtypes (76.5%) occurred more frequently than an infection sustained by a single subtype (23.5%). Combinations of ST1 and ST2 or ST1, ST2, and ST3 were the most common forms of mixed infection (16.47 and 11.76%, respectively). A

Table 1 Prevalence (%) and 95 % confidence interval (CI) of intestinal parasites in macaque fecal samples

	Number	Positives	% (95 % CI)	EPG (min–max)	Test
Trichuris sp.	443	7	1.58 (0.78–3.22)	0.35 (0–50)	Flotation by FLOTAC® basic technique
Oesophagostomum sp.	443	2	0.45 (0.14–1.62)	0.18 (0–70)	Flotation by FLOTAC® basic technique
Balantidium coli	443	98	22.12 (18.50–26.22)	nd	Sedimentation
Entamoeba coli	135	115	85.19 (78.23–90.2)	nd	Lugol-stained fecal smear
Uninucleated-cyst-producing Entamoebas (*Entamoeba polecki*)	135	106	78.52 (70.85–84.61)	nd	Lugol-stained fecal smear
Endolimax nana	135	107	79.26 (71.66–85.24)	nd	Lugol-stained fecal smear
Iodamoeba bütschlii	135	58	42.96 (34.92–51.39)	nd	Lugol-stained fecal smear
Chylomastix mesnili	135	33	24.44 (17.96–32.33)	nd	Lugol-stained fecal smear

nd not determined

mixed infection with ST1, ST2, ST3, and ST7 showed a percentage of 11.76% (Table 2).

Discussion

Eight distinct taxa of enteric parasites were detected by microscopic examination, showing a faunal diversity consistent with previously published data (Bezubik and Furmaga 1960; Matsubayashi et al. 1992; Tachibana et al. 2001; Lee et al. 2010; MacIntosh et al. 2010). Captive non-human primates are frequently infected with parasites having a direct life cycle and show a lower number of parasitic species in comparison with wild NHPs; besides, the majority of their enteric parasites are protozoans, as recently demonstrated (Lane et al. 2011; Ye et al. 2014; da Silva Barbosa et al. 2015).

In our study, helminth infections were represented only by nematodes belonging to *Trichuris* spp. and *Oesophagostomum* spp. *Trichuris* spp. inhabits the ceca and colons of a variety of simians. *T. trichiura*, a species infecting humans (Dinh 2002; Melfi and Poyser 2007), is thought to be present in *M. fascicularis*, *M. mulatta* (Taylor et al. 1994), and in other Old and New World primate species. Further, *Trichuris* is highly pathogenic for monkeys, as previously observed in *M. fascicularis* and in other monkeys (Janagi 1981; Loomis and Wright 1986; Emikpe et al. 2002).

Table 2 Occurrence of different subtypes (ST) of *Blastocystis* sp. in 85 macaque fecal samples

Infection	Subtypes (ST)	Number	Percent
Single	ST1	4	4.71
	ST2	14	16.47
	ST7	2	2.35
Double	ST2+ST1	14	16.47
	ST2+ST3	5	5.88
	ST2+ST7	5	5.88
	ST3+ST1	3	3.53
	ST5+ST2	1	1.17
	ST7+ST1	7	8.23
	ST7+ST3	1	1.17
Triple	ST1+ST2+ST3	10	11.76
	ST1+ST2+ST7	5	5.88
	ST2+St3+ST7	3	3.53
	ST1+ST3+ST7	1	1.17
Quadruple	ST1+ST2+ST3+ST7	10	11.76

Oesophagostomum is a parasitic nodular worm whose larvae can encyst in the wall of the large intestines of macaques (Honjo et al. 1963). Among captive monkeys, infections with *Oesophagostomum* show lower frequency, likely due to the epidemiology of its infective larvae (Abbott and Majeed 1984) than *Trichuris*, its larvae being protected by egg shells. Though both *Trichuris* and *Oesophagostomum* infections showed low prevalence and EPG values, the relevance of these parasites in monkeys managed for biomedical purposes should be carefully considered. In fact, even light infections could somehow interfere with biomedical research despite the fact that, as recently observed, *Trichuris* infections can improve colitis in rhesus monkeys by restoring their mucosal barrier functions, reducing the overall bacterial attachment, and altering the community of attached bacteria (Broadhurst et al. 2012). It should be considered that available drugs cannot be equally effective in eradicating *Trichuris* infections in monkeys; thus monitoring of these parasites is crucial to their control and should be warranted in animals not individually caged (Reichard et al. 2007). As recently revealed by our unpublished data, infections with *Trichuris* were heavier in cynomolgus monkeys housed in groups where EPG varied from 6 to 184 and whose prevalence values (9/20, 45%) were higher than those in the present survey.

Regarding protozoans, Entamoebas cysts are more frequently shed by monkeys than other parasitic species. Non-pathogenic *Entamoeba* species, such as *E. coli* and uninucleated-cyst-producing Entamoebas, were the most prevalent in our samples as observed also in previous surveys on captive and wild-trapped non-human primates (Rivera et al. 2010; Tachibana et al. 2001; Regan et al. 2014).

Particularly, in cynomolgus monkeys from Japan, higher prevalence of non-pathogenic *Entamoeba* species rather than of *E. histolytica* was detected both by microscopy and by PCR (Takano et al. 2005; Feng et al. 2011). However, *E. histolytica* NHP variant was detected in captive non-human primates, including the macaques, with moderate prevalence (36%) (Levecke et al. 2010). In the present study, cynomolgus monkeys harbored *E. nana*, *I. bütschlii*, and *E. polecki*; these are generally considered harmless for NHPs even though some pathological consequences were reported (Loomis 1983; Vogel et al. 1996). Also in humans, these parasites species are regarded as nonpathogenic intestinal protozoans, and when detected in symptomatic people, other etiologies should be considered (http://www.cdc.gov/parasites/nonpathprotozoa/).

Additionally, our survey revealed the presence of *B. coli*, a cosmopolitan ciliate colonizing the intestine of many animals, humans included, with pigs serving as reservoir hosts (Schuster and Ramirez-Avila 2008). In humans, *B. coli* infections are considered zoonotic and are generally associated with close proximity to swine. Nevertheless, NHPs are often infected with this ciliate showing large variations in the prevalence values (Nakauchi 1999; Schuster and Ramirez-Avila 2008; da Silva Barbosa et al. 2015). Recently, a broad genetic diversity of isolates of *B. coli* from several species of NHPs was found; however, the high risk for humans from these ciliates inhabiting the intestines of NHPs seems to be confirmed (Pomajbíkova et al. 2013).

In the current study, tested NHPs were *G. duodenalis*, *Cryptosporidium* spp., and *E. histolytica* free. Recently, Ye et al. (2014) detected *G. duodenalis* and *Cryptosporidium* spp. in laboratory macaques at very low prevalence by molecular tools. Particularly, *G. duodenalis* infection was detected in 5 of 205 animals mainly young and housed in groups; in contrast, macaques from the present survey were adult and located in single cages where the risk of infection could be low. *E. histolytica* prevalence of infection varied in different studies: 26 % of *M. fascicularis* harbored in a Philippinian facility were infected (Rivera et al. 2010), while it was not detected in *Macaca mulatta* and *M. fascicularis* reared in China (Feng et al. 2011). The latter finding agrees with results of the current study that were obtained from *M. fascicularis* imported from China.

Finally, molecular analysis performed in our study revealed that cynomolgus monkeys were infected with *Blastocystis*, a well-known microeukaryote infecting the large intestine of possibly more than one billion people from both developed and developing countries (Boorom et al. 2008). Human-to-human transmission of *B. hominis* frequently occurs, but most *Blastocystis* isolates from animals and humans are shown to be genetically similar or identical indicating a possible animal-to-human transmission, a hypothesis also supported by the occurrence of *Blastocystis* infections with high prevalence in people in close contact with animals (Yoshikawa et al. 2009). Non-human primates are often infected with *Blastocystis*, and they host subtypes (ST) identified also in human feces. In a recent survey on the distribution of *Blastocystis* subtypes in 260 NHPs, the major groups of NHPs were found infected with eight subtypes; six of these (ST1, ST2, ST3, ST4, ST5, and ST8) were also found in humans (Alfellani et al. 2013a, b). According to previous studies, ST1 and ST2 were the most frequent subtypes of *Blastocystis* commonly identified in fecal samples of cynomolgus monkeys (Abe et al. 2002). However, cynomolgus monkeys were found infected with a number of ST (ST1, ST2, ST3, ST5, and ST7) higher than in other investigations where only ST1, ST2, and ST3 had been found in macaques and ST5 in *Gorilla gorilla* and *Pan troglodytes* (Alfellani et al. 2013a, b; Stensvold 2013). In NHPs, ST7 had not yet been identified. It is a subtype rarely occurring in humans; being considered of avian origin, both NHPs and humans could be infected through bird feces exposure. Humans are natural hosts of nine subtypes (ST1 through ST9), of which ST1 to ST4 are by far the most common; all subtypes found in macaques are likely to infect people. While humans and NHPs can host the same ST with the same alleles, a few ST alleles appeared to be NHPs specific (Stensvold et al. 2012; Alfellani et al. 2013a, b). *Blastocystis* sp. infection can cause acute and chronic intestinal diseases both in humans and in monkeys (Cekin et al. 2012; Zanzani et al. 2014); nevertheless, several single and mixed *Blastocystis* STs infections were detected in healthy humans and the possibility that *Blastocystis* could be a member of the normal gut microbiota should be evaluated also in NHPs (Scanlan et al. 2014; Scanlan et al. 2015). Acquisition of the infection seems to be more likely due to the conditions of animals in breeding farms rather than in research centers, where they are kept in single cages. The occurrence of asymptomatic *Blastocystis* sp. infections that in presence of stressors (i.e., transport, separation of animals from a group into single cages, dietary changes) can turn symptomatic should also be considered (Liu et al. 2008).

Conclusion

This study reports the prevalence of helminth and protozoan infections of the gastrointestinal tract in macaques employed in biomedical research. Data collected showed a high parasite diversity and the presence of potentially zoonotic parasites that can pose health risks to people involved in the care and use of non-human primates in biomedical research centers or to staff managing them in breeding facilities. The rich parasite fauna detected suggests the importance of a periodic coprological examination in order to minimize related health risks. Further, the panel of such fecal examination should include protozoans; in fact, they resulted to be more frequent parasites than helminths. In particular, protozoans can be

zoonotic or cause acute diarrhea when NHPs are delivered to research centers. Finally, to enhance the control of helmintic infections, the evaluation of EPG by a highly sensitive technique, e.g., the FLOTAC technique, should be recommended, mainly in captive monkeys bred in groups. In fact, parasitic infections can be more frequent as the number of animals in social groups increases (Phillippi and Clarke 1992) and even latest regulations underline that research centers had better consider small NHP groups. As a consequence, minor health risks for humans would derive.

Notably, this is the first study to report a high prevalence of *Blastocystis* sp. infection in monkeys involved in biomedical research and to confirm the presence of zoonotic subtypes in NHPs.

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Conflict of interests The authors declare that they have no competing interests.

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