International Journal for Parasitology: Parasites and Wildlife 6 (2017) 8–15

Contents lists available at ScienceDirect

International Journal for Parasitology: Parasites and Wildlife

journal homepage: www.elsevier.com/locate/ijppaw

Invited article

An investigation of parasitic infections and review of molecular characterization of the intestinal protozoa in nonhuman primates in China from 2009 to 2015

Junqiang Li [a, b], Haiju Dong [a], Rongjun Wang [a, b], Fuchang Yu [a, b], Yayun Wu [a, b], Yankai Chang [a, b], Chenrong Wang [a, b], Meng Qi [a, b], Longxian Zhang [a, b, *]

[a] College of Animal Science and Veterinary Medicine, Henan Agricultural University, Zhengzhou 450002, PR China
[b] International Joint Research Laboratory for Zoonotic Diseases of Henan, Zhengzhou 450002, PR China

ARTICLE INFO

Article history:
Received 1 October 2016
Received in revised form
6 November 2016
Accepted 1 December 2016

Keywords:
Nonhuman primates
Intestinal parasites
Prevalence
Molecular characterization review

ABSTRACT

Parasites are a well-known threat to nonhuman primate (NHP) populations, and potentially cause zoonotic diseases in humans. In this study, the basic data was provided of the parasites in NHPs and the molecular characterization of the *Enterocytozoon bieneusi*, *Giardia duodenalis*, *Cryptosporidium* spp., and *Entamoeba* spp. were reviewed, which were found in these samples. A total of 3349 fecal samples were collected from 34 species reared at 17 districts in zoos, farms, free-range, or research laboratories, and examined microscopically. Eleven genera of intestinal parasites were detected: five genera of protozoans (*Isospora* spp., *Entamoeba* spp., *Giardia* sp., *Cryptosporidium* spp., and *Cyclospora* spp.) and six genera of helminths (*Trichuris* spp., *Strongyloides* spp., *Ascaris* spp., *Physaloptera* spp., *Ancylostoma* spp., and *Enterobius* spp.). The overall sample prevalence of parasitic infection was 54.1% (1811/3349). *Entamoeba* spp. was the most prevalent (36.4%, 1218/3349). The infection rate was the highest in free-range animals (73.0%, 670/918) ($P < 0.01$) and Guangxi Zhuang autonomous region (64.8%, 566/873). Mixed infections were mostly detected for *Entamoeba* spp., *Trichuris* spp., and *Strongyloides* spp.. Molecular characterization was reviewed of *Enterocytozoon bieneusi*, *Giardia duodenalis*, *Cryptosporidium* spp., and *Entamoeba* spp., as these are zoonotic species or genotypes. This parasitological data for NHPs in China, provides important information for veterinarians and public health authorities for the elimination of such parasites and monitor the potential transmission of zoonotic infections from NHPs.

1. Introduction

Nonhuman primates (NHPs), with their high level of genetic homology to humans, make them invaluable experimental models for biomedical research (Messaoudi et al., 2011; Zhang et al., 2014). However, they are also an increasingly important source of emerging zoonotic diseases in humans, including human immunodeficiency virus (HIV), Ebola virus, malaria, etc (Poinar, 2009; Miller et al., 2013).

Several intestinal parasites occur in NHPs, causing asymptomatic or only mild disorders (Karim et al., 2014a; Kouassi et al., 2015; Li et al., 2015a). Potentially zoonotic protozoans (including *Enterocytozoon bieneusi*, *Giardia duodenalis*, *Cryptosporidium* spp., and *Entamoeba* spp.) could be maintained and transmitted with the attendant risk of human outbreaks originating in such animal reservoirs (Legesse and Erko, 2004; Ye et al., 2012). The health of NHPs is therefore important not only in terms of management objectives, but also concerning public health.

Compared with developed countries in America and Europe, China has relatively rich primate resources and is currently a leading producer and major supplier of NHPs to the international market (Zhang et al., 2014). NHPs are commonly maintained in zoos, natural reserves, and zoological gardens by different feeding habitats in China (Karim et al., 2014a). Therefore, it is important to understand the epidemiology of such intestinal parasites and their potential transmission from NHPs to humans.

The molecular characterization of NHP parasites is increasingly being studied (Berrilli et al., 2011; Iñiguez et al., 2012; Betson et al.,

* Corresponding author. College of Animal Science and Veterinary Medicine, Henan Agricultural University, 95 Wenhua Road, Zhengzhou 450002, PR China.
E-mail addresses: zhanglx8999@henau.edu.cn, zhanglx8999@gmail.com (L. Zhang).

http://dx.doi.org/10.1016/j.ijppaw.2016.12.003

2014; Li et al., 2015a, 2015b), but there is a lack of comprehensive studies on the intestinal parasites in NHPs. Here, the prevalence of parasites in NHPs in China has been reported and the molecular characterization of the *Enterocytozoon bieneusi*, *Giardia duodenalis*, *Cryptosporidium* spp., and *Entamoeba* spp. found in these samples also had been reviewed.

2. Materials and methods

2.1. Ethics statement

This study was conducted in accordance with the Chinese Laboratory Animal Administration Act (1988). The research protocol was reviewed and approved by the Research Ethics Committee of Henan Agricultural University. Appropriate permission was obtained from the director of animals and properties before the samples were collected. Veterinarians were notified of the parasitic infections identified in NHPs as soon as possible to expedite their management.

2.2. Study area

A total of 3349 fresh fecal specimens were collected from 17 districts in two cities (Beijing and Shanghai), one autonomous region (Guangxi Zhuang autonomous region), and eight provinces (Hebei, Henan, Hubei, Hunan, Guangdong, Sichuan, Yunnan, and Shanxi) in China during the period between July 2009 to April 2015 (Fig. 1). This study included 34 NHP species (Table 1S). NHPs were grouped according to their feeding habits. 912 fecal specimens were subsequently collected from animals in zoos, 1402 from farms, 918 from free-range, and 117 from those in research laboratories (Table 1).

2.3. Sampling

Fresh fecal samples from captive NHPs, which were kept in separate pens during the day, were collected in the early morning. The specimens from free-living animals were immediately collected from the ground after defecation.

Each specimen (about 10 g) was collected into a plastic container and labelled with the number, district, species, and clinical symptoms of the animal. Specimens were transported to the laboratory as soon as possible and stored in 2.5% (w/v) potassium dichromate solution at 4 °C prior to microscopy. No animal exhibited any obvious clinical symptoms during the collection period.

2.4. Microscopy

The fecal specimens were sieved through a sieve (7.62 cm diameter) with a pore size of 245 μm, transferred into a 50 ml centrifuge tube containing water, and precipitated by centrifugation at 5000 rpm for 10 min. A portion of each specimen was microscopically examined to detect protozoan and helminthic parasites with both Sheather's sugar flotation technique and Lugol's iodine staining (Huang et al., 2014). Wet smears were examined with a bright-field microscope at 100 × and 400 × magnification to determine the shape, size, and colour of the eggs/cysts.

2.5. Review on molecular characterization of the intestinal protozoan

For *Giardia duodenalis*, a total of 1882 fecal specimens from NHPs were examined and characterized by ssrRNA (Appelbee et al., 2003), triosephosphate isomerase (*tpi*) (Sulaiman et al., 2003a), glutamate dehydrogenase (*gdh*) (Cacciò et al., 2008) and beta-giardin (*bg*) gene (Cacciò et al., 2002). 2660 specimens were

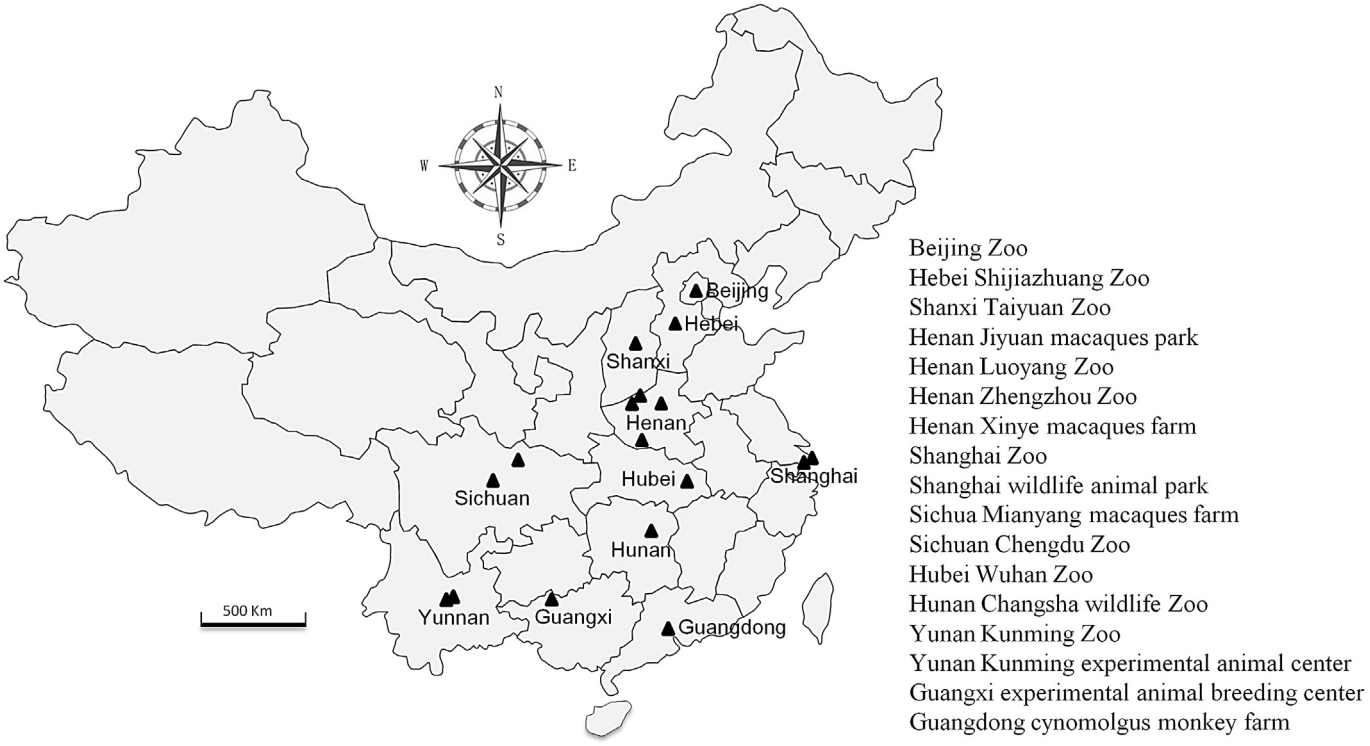

Fig. 1. Locations of the study area in China. Filled triangles indicate sampling sites.

Table 1
Prevalence of intestinal parasites in NHPs according to geography and feeding habitats by microscopy.

Locations	Zoos	Farms	Free-range	Research laboratories	Protozoans					Helminths					
					Isospora	*Giardia*	*Cryptosporidium*	*Cyclospora*	*Entamoeba*	*Trichuris*	*Strongyloides*	*Ascaris*	*Physaloptera*	*Ancylostoma*	*Enterobius*
Beijing	33/72				5	10	1	0	11	14	2	0	0	0	0
Shanghai	49/128				4	3	0	0	22	22	2	0	0	0	5
Hebei	53/102				22	1	0	2	17	27	0	0	0	0	0
Henan	161/303	221/357	178/254		14	25	7	1	332	238	88	32	25	9	6
Hubei	41/66				2	0	0	0	21	27	1	0	0	0	0
Hunan	35/75				1	0	4	0	22	10	5	0	0	0	0
Guangxi		184/360	382/513		6	0	5	1	471	189	2	0	0	0	0
Guangdong		107/328			1	1	1	0	102	3	1	0	0	0	0
Shanxi	24/65				0	0	0	0	18	20	1	0	0	0	0
Sichuan	10/73	133/357	110/151		3	3	0	1	148	100	92	0	0	7	1
Yunnan	16/28			74/117	6	0	0	2	54	36	12	0	0	0	0
Total	422/912	645/1402	670/918	74/117	64	43	18	7	1218	686	206	32	25	16	12
Infection ratio	46.3%	46.0%	73.0%	63.2%	1.9%	1.3%	0.5%	0.2%	36.4%	20.5%	6.2%	1.0%	0.8%	0.5%	0.4%

identified for *Cryptosporidium* spp. by PCR amplification of the 18S rRNA (Xiao et al., 2001), 70 kDa heat shock protein (*hsp70*) (Xiao and Ryan, 2008) and genotyped by 60 kDa glycoprotein (*gp60*) gene (Alves et al., 2003). For *Enterocytozoon bieneusi*, there were a total of 1882 fecal specimens from NHPs that were screened and genotyped by SSU rRNA ITS gene (Sulaiman et al., 2003b); For *Entamoeba* spp., 531 specimens from 1059 *Entamoeba* spp. positive samples by microscopy, were randomly selected for PCR amplification based on SSU rRNA, using the specific primers of *E. histolytica* (Clark and Diamond, 1991), *E. dispar* (Clark and Diamond, 1991), *E. moshkovskii* (Ali et al., 2003), *E. nuttulli* (Verweij et al., 2001), *E. coli* (Tachibana et al., 2009) and *E. chattoni* (Tachibana et al., 2009) in order to identify the molecular characterization.

2.6. Statistical analysis

The statistical analysis was performed with SPSS software 19.0. The infection rates were compared with a χ^2 test, and differences were considered significant at $P < 0.01$.

3. Results

3.1. Occurrence of intestinal parasites

Eleven genera of intestinal parasites (five protozoan and six helminths genera) were found in the NHPs (Fig. 2). The overall sample prevalence of parasitic infection was 54.1% (1811/3349). *Entamoeba* spp. were the most frequently detected species, with an incidence of 36.4% (1218/3349), followed by *Trichuris* spp. (20.5%, 686/3349), *Strongyloides* spp. (6.2%, 206/3349), *Isospora* spp. (1.9%, 64/3349), *Giardia* sp. (1.3%, 43/3349), *Ascaris* spp. (1.0%, 32/3349), *Physaloptera* spp. (0.8%, 25/3349), *Cryptosporidium* spp. (0.5%, 18/3349), *Ancylostoma* spp. (0.5%, 16/3349), *Enterobius* spp. (0.4%, 12/3349), and *Cyclospora* spp. (0.2%, 7/3349) (Table 1).

3.2. Infection rate according to feeding habitats

The ratio of intestinal parasitic infections ranged from 46.0% to 73.0% among the four feeding habitats (zoos, farms, free-range, and research laboratories) (Table 1). The highest infection rate was found in those animals that were the free-range (73.0%, 670/918), followed by those in research laboratories (63.2%, 74/117), with lower infection rates at zoos (46.3%, 422/912) and farms (46.0%, 645/1402) ($p < 0.01$).

3.3. Geographic distribution of intestinal parasites

The sample prevalence of infection ranged from 32.6% to 64.8% among the 11 sampling locations. The Guangxi Zhuang autonomous region had the highest rate (64.8%, 566/873), and the lowest was found in Guangdong Province (32.6%, 107/328) (Table 2).

3.4. Mixed infections

The majority (74.3%, 1345/1811) of infected NHPs carried one parasitic species, 22.2% (402/1811) carried two parasitic species, and only 3.5% (64/1811) carried three or more parasite species (Table 2). The parasites most often involved in mixed infections were *Entamoeba* spp., *Trichuris* spp., and *Strongyloides* spp. (Table 1S).

3.5. Distribution patterns of infections among species

Six families, 20 genera, 34 species of NHPs, and 3349 individual specimens were detected, and the infections rates ranged from 0%

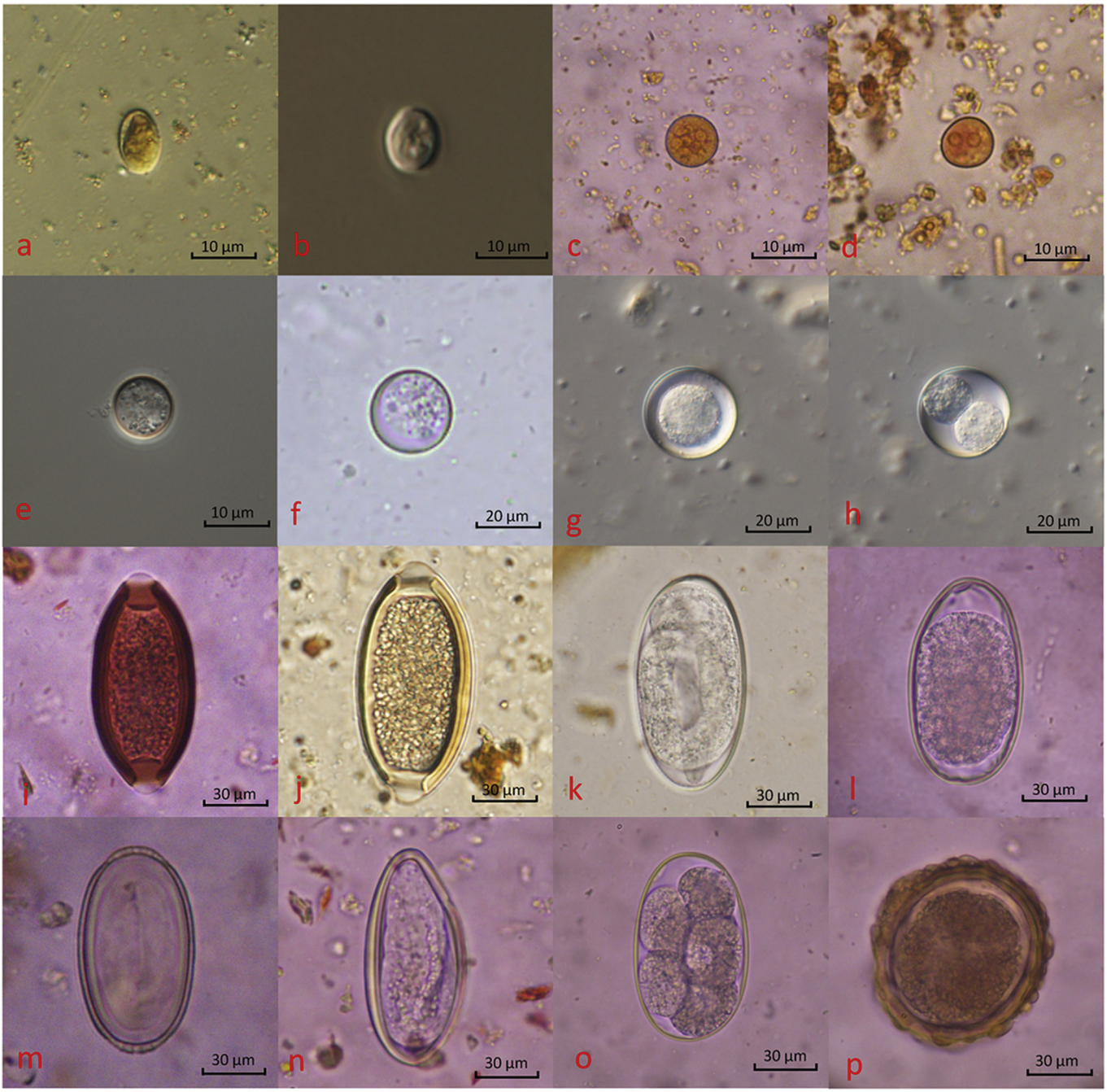

Fig. 2. Parasites identified in stool samples from NHPs. (a): *Giardia* sp.; (b): *Cryptosporidium* spp.; (c–d): *Entamoeba* spp.; (e): *Cyclospora* spp.; (f–h): *Isospora* spp.; (i–j): *Trichuris* spp.; (k–l): *Strongyloides* spp.; (m): *Physaloptera* spp.; (n): *Enterobius* spp.; (o): *Ancylostoma* spp.; (p): *Ascaris* spp.

to 100% in different NHP species (Table 1S). Macaques monkey had the highest rate of parasitic infection with 80.1% (1908/2381). Interestingly, *Ascaris* spp. were only found in this species.

3.6. Molecular characterization of the intestinal protozoan

6.5% (122/1882) of specimens tested for *Giardia duodenalis* were positive by PCR analysis. The assemblages A (n = 4) and B (n = 118) were found, both which have zoonotic potential (Table 3). Assemblage A included subtypes A1, A2 and one novel subtype. Thirty-two assemblage B isolates with data at all three loci yielded 15 multi-locus genotypes (MLGs) (including 2 known and 13 new) (Karim et al., 2014a, 2015a). The occurrence of *Giardia duodenalis* assemblages in different species of nonhuman primate species are shown in Table 2S.

For *Cryptosporidium* spp., 0.7% (19/2660) were positive by PCR amplification (Karim et al., 2014a). 73.7% (14/19) of the positive specimens were found to be *Cryptosporidium hominis*, whilst 26.3% (5/19) were *C. muris*. The subtypes of the *C. hominis* were identified as IbA12G3 (7/14) and IiA17 (1/14) by *gp60* gene sequence analysis (Table 4). The occurrence of *Cryptosporidium* spp. and subtypes in nonhuman primate species based on PCR analysis are shown in Table 3S.

For *Enterocytozoon bieneusi*, there were 16.3% (306/1882)

Table 2
Geographic distribution and mixed infections of intestinal parasites in NHPs by microscopy.

Locations	No. of specimens tested	No. (%) of positive specimens	Single	Double	3 or above
Beijing	72	33 (45.8)	24	8	1
Shanghai	128	49 (38.3)	39	10	0
Hebei	102	53 (52.0)	37	16	0
Henan	914	560 (61.3)	379	140	41
Hubei	66	41 (62.1)	31	10	0
Hunan	75	35 (46.7)	29	6	0
Guangxi	873	566 (64.8)	456	108	2
Guangdong	328	107 (32.6)	105	2	0
Shanxi	65	24 (36.9)	19	5	0
Sichuan	581	253 (43.5)	168	68	17
Yunnan	145	90 (62.1)	58	29	3
Total	3349	1811 (54.1)	1345	402	64

Table 3
Occurrence of *Giardia duodenalis* assemblages by PCR analysis in NHPs by Karim et al. (2014a and 2015a).

Locations	Habitats	No. of specimens tested	Microscopy (%)	No. (%) of positive specimens	Assemblages (*n*)
Hebei	Zoos	89	1 (1.1)	10 (11.2)	B (10)
Hubei	Zoos	66	0	5 (7.6)	B (5)
Shanxi	Zoos	66	0	9 (13.6)	B (9)
Hunan	Zoos	75	0	33 (44.0)	B (31)/A (2)
Beijing	Zoos	72	10 (13.9)	16 (22.2)	B (15)/A (1)
Shanghai	Zoos	128	3 (2.3)	19 (8.2)	B (18)/A (1)
Guangdong	Farms	57	1 (1.8)	1 (1.8)	B (1)
Guangxi	Farms	363	0	9 (2.5)	B (9)
Henan	Farms/Zoos/Free range	518	12 (2.3)	20 (3.9)	B (20)
Yunnan	Zoos/Research lab	144	0	0	–
Sichuan	Farms/Zoos/Free range	304	0	0	–
Total		1882	27 (1.4)	122 (6.5)	B (118)/A (4)

n: Number of specimens.

Table 4
Occurrence of *Cryptosporidium* spp. and subtypes distribution by PCR analysis in NHPs by Karim et al. (2014a).

Locations	Habitats	No. of specimens tested	Microscopy (%)	PCR (%)	18S rRNA (*n*)	*Gp 60* (*n*)
Henan	Zoos/Farms	786	5 (0.6)	5 (0.6)	*C. Hominis* (5)	IbA12G3 (3)
Guangdong	Farms	57	1 (1.8)	1 (1.8)	*C. Hominis* (1)	IiA17 (1)
Guangxi	Farms	1079	5 (0.5)	11 (1.0)	*C. hominis* (7)/*C. muris* (4)	IbA12G3 (4)
Shanghai	Zoos/Farms	290	0	2 (0.7)	*C. hominis* (1)/*C. muris* (1)	PN
Sichuan	Free-range	304	0	0	PN	–
Yunnan	Zoos/Research lab	144	0	0	PN	–
Total		2660	11 (0.4%)	19 (0.7)	*C. hominis* (14)/*C. muris* (5)	IbA12G3 (7)/IiA17 (1)

PN: PCR-negative; *n*: Number of specimens.

positive specimens detected by PCR analysis. Altogether, 34 ITS genotypes were observed, including 16 known genotypes (Type IV, D, O, Henan V, Henan-IV, Peru8, PigEBITS5, PigEBITS7, EbpA, EbpC, EbpD, Peru11, BEB4, BEB6, I, and CS-1) and 18 new genotypes (CM1 to CM18) (Table 5). The new genotypes CM1 to CM3, CM6, CM 8, CM 10 to CM 17 belong to the previously described group 1, which have zoonotic potential. Genotypes CM5, CM7, and CM9 clustered with group 2, whereas genotypes CM4 and CM18 formed new cluster (Karim et al., 2014b, 2015b). The occurrence of *Enterocytozoon bieneusi* and genotypes in different species of nonhuman primate species are shown in Table 4S.

For *Entamoeba* spp., the overall amplification efficiency was 87.19% (463) among the 531 positive specimens but only *Entamoeba dispar* (72.69%, 386/531) and *Entamoeba coli* (54.05%, 287/531) were amplified successfully. The mixed infections with *E. dispar* and *E. coli* were 27.1% (144/531) (Unpublished data).

4. Discussion

This study demonstrates a high sample prevalence (54.1%, 1811/3349) and diversity (five protozoan genera and six helminths genera) of intestinal parasites in NHPs in China. The prevalence varied with feeding habitats, NHP species, and geographic region. Similar infection ratio was found in pet macaques (59.1%, 52/88) in Indonesia (Jones-Engel et al., 2004), a zoo in Malaysia (54.5%, 54/99) (Lim et al., 2008), and pet monkeys in Cameroon (51.1%, 24/47) (Pourrut et al., 2011).

A diversity of intestinal parasites is frequently reported to infect NHPs (Jones-Engel et al., 2004; Legesse and Erko, 2004; Gillespie et al., 2005; Lim et al., 2008; Pourrut et al., 2011). Greater parasite species diversity was observed in Taï National Park, Côte d'Ivoire (with nine protozoans and 14 helminths in 3142 specimens) (Kouassi et al., 2015). Several studies had reported *Entamoeba* spp. as the most prevalent intestinal parasites in NHPs (Pourrut et al.,

Table 5
Occurrence of *Enterocytozoon bieneusi* and ITS genotypes distribution by PCR analysis in NHPs by Karim et al. (2014b and 2015b).

Locations	Habitats	No. of specimens tested	No. (%) of positive specimens	ITS genotypes (*n*)
Hebei	Zoos	89	24 (27.0)	CM1 (15), Type IV (3), Henan-IV (2), D (1), EbpC (1), EbpA (1), CM8 (1)
Hubei	Zoos	66	10 (15.2)	D (5), EbpC (3), BEB6 (2)
Shanxi	Zoos	66	12 (18.2)	D (6), CM4 (4), Henan-IV (1), CM9 (1)
Hunan	Zoos	75	28 (37.3)	D (15), EbpC (4), O (3), CM12 (2), Type IV (1), BEB6 (1), CM13 (1), CM14 (1)
Beijing	Zoos	72	21 (29.2)	O (8), EbpA (4), EbpC (2), Type IV (1), EbpD (1), Peru8 (1), PigEBITS5 (1), CS-1 (1), CM10 (1), CM11 (1)
Shanghai	Zoos	128	53 (41.4)	CM4 (16), D (13), CM16 (13), O (2), CM17 (2), BEB4 (2), Henan-IV (1), CM15 (1), CM18 (1), EbpA (1), EbpC (1),
Guangdong	Farms	57	40 (70.2)	Type IV (15), CM1 (14), Peru8 (3), CM2 (3), D (2), Peru11 (2), CM3 (1)
Guangxi	Farms	363	31 (8.5)	D (14), CM1 (12), Peru8 (2), Type IV (1), CM2 (1), Peru11 (1)
Henan	Farms/Zoos/ Free range	518	39 (7.5)	Henan V (10), D (8), CM4 (7), EbpC (5), PigEBITS7 (4), Type IV (1), I (1), CM5 (1), CM6 (1), CM7 (1)
Yunnan	Zoos/ Research lab	144	31 (21.5)	Type IV (13), CM1 (12), Peru8 (4), D (2)
Sichuan	Farms/Zoos/ Free range	304	17 (5.6)	CM1 (5), BEB6 (5), D (4), Type IV (1), PigEBITS7 (1), CM4 (1)
Total		1882	306 (16.3)	D (70), CM1 (58), Type IV (36), CM4 (28), EbpC (16), O (13), CM16 (13), Henan V (10), Peru8 (10), BEB6 (8), EbpA (6), PigEBITS7 (5), CM2 (4), Henan-IV (4), Peru11 (3), BEB4 (2), CM12 (2), CM17 (2), PigEBITS5 (1), EbpD (1), CS-1 (1), CM3 (1), CM5 (1), CM6 (1), CM7 (1), CM8 (1), CM9 (1), CM10 (1), CM11 (1), CM13 (1), CM14 (1), CM15 (1), CM18 (1), I (1)

n: Number of specimens.

2011), whereas others reported that *Strongyloides* spp. were the most prevalent (Gillespie et al., 2005).

All five genera of protozoans detected by microscopy, as well as *Enterocytozoon bieneusi*, are zoonotic (Mansfield and Gajadhar, 2004; Ye et al., 2012; Karim et al., 2014b; Plutzer and Karanis, 2016). *Giardia duodenalis* is a particularly zoonotic parasitic protozoan that infects a wide range of mammals, including NHPs (Feng and Xiao, 2011). Animals are infected when they ingest food or water contaminated with *Giardia* cysts (Graczyk et al., 2003). The assemblage B were the NHPs host-adaptated, in 96.7% (118/122) of the positive isolates, which were zoonotic assemblage (Karim et al., 2015a).

The zoonotic *Cryptosporidium* spp. are usually associated with intestinal pathology, resulting in diarrhea in both humans and animals (Ryan and Hijjawi, 2015). They are transmitted via the fecal-oral route by either direct contact or the ingestion of contaminated food or water. The protozoan can disperse rapidly because they have a monoxenous life cycle, a low infective dose, and a short prepatent period (Graczyk et al., 2003; Smith et al., 2006). However, the prevalence rate found in this study is much lower than that found in Sri Lanka (27.2%, 27/125) (Ekanayake et al., 2006) and Ethiopia (29.3%, 17/59) (Legesse and Erko, 2004).

E. bieneusi is a common parasitic pathogen in NHPs with high prevalence (16.3%) which difficult to detect by light microscopy. A lower infection rate (12.3%) of *E. bieneusi* was reported in Kenya (Li et al., 2011), while higher infection rates (28.2% and 18.5%) were found in free-range macaque monkeys in Guizhou and cynomolgus monkeys in Guangxi, China, respectively (Ye et al., 2012, 2014). Altogether, 34 *E. bieneusi* ITS genotypes were found involving 26 genotypes (263/306, 86.0%) belonged to group 1 with zoonotic potential (Karim et al., 2014b, 2015b). Thus, the genotypes in NHPs had zoonotic potential, and NHPs could act as reservoirs of human microsporidiosis.

The *Entamoeba* spp. had the highest infection rate (36.4%) by microscopy, and was observed in the majority of the NHP species (25/34) examined (Table 1S). They are also known to be a highly prevalent intestinal parasite in Ethiopia, Uganda, Senegal, Tanzania, Italian, Cameroon, etc (Legesse and Erko, 2004; Gillespie et al., 2005; Petrášová et al., 2010; Berrilli et al., 2011; Howells et al., 2011; Pourrut et al., 2011). *Entamoeba* spp. are human pathogens that are transmitted by various forms of contact due to their direct life cycle (Pedersen et al., 2005; Berrilli et al., 2011; Morf and Singh, 2012). Although, only *E. dispar* and *E. coli* were found in this study (Unpublished data) which were non-pathogenic species with low risk of zoonotic transmission from NHPs to human, the zoonotic transmit also should be pay attention.

Cyclospora spp. are obligate intracellular parasites that inhabit the bile duct or intestinal mucosal epithelial cells of various vertebrates (Legua and Seas, 2013). Until now, four *Cyclospora* species had been found in NHPs (Eberhard et al., 1999; Ortega and Sanchez, 2010; Li et al., 2015b) and one in humans (Zhou et al., 2011). The highest prevalence of *Cyclospora* spp. were found in Ethiopia (22.0%, 13/59) (Legesse and Erko, 2004). And, *Cyclospora*-like organisms were also detected in monkeys (Zhao et al., 2013).

The helminths, including *Trichuris* spp., *Strongyloides* spp., *Ascaris* spp., *Physaloptera* spp., *Ancylostoma* spp., and *Enterobius* spp., are parasitic with a high potential for transmission to humans because of their simple life cycles. They have been reported in several populations of primates (Ocaido et al., 2003; Legesse and Erko, 2004; Gillespie et al., 2005; Bezjian et al., 2008; Petrášová et al., 2010; Kouassi et al., 2015). The macaque monkeys displayed a very high sample prevalence of *Trichuris* spp. (Table 1S), in contrast to the colobus monkeys in Côte d'Ivoire (Kouassi et al., 2015). The *Strongyloides* are also mainly detected in macaques (Table 1S). Unfortunately, it is difficult to identify the helminths' species only based on morphology of oocysts or eggs. A comprehensive study of their genetic diversity is necessary to confidently distinguish the species and genotypes of these intestinal parasites.

In conclusion, this is an investigation of the parasites in NHPs in China, which detailed parasites infection status and reviewed of molecular characterization of four intestinal protozoans. Our preliminary results demonstrate their high prevalence and diversity parasitic infection amongst NHPs. This baseline parasitological data provides important information for the elimination of such parasites and monitor the potential transmission of zoonotic infections from NHPs.

Conflict of interest

The authors declare no conflicts of interest.

Acknowledgments

This study was supported, in part, by the National Natural Science Foundation of China (U1404327), the Key Program of the National Natural Science Foundation of China (31330079), and the Key National Science and Technology Specific Projects (2012ZX10004220-001).

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ijppaw.2016.12.003.

References

Ali, I.K., Hossain, M.B., Roy, S., Ayeh-Kumi, P.F., Petri Jr., W.A., Haque, R., Clark, C.G., 2003. *Entamoeba moshkovskii* infections in children. Bangladesh. Emerg. Infect. Dis. 9, 580–584.

Alves, M., Xiao, L., Sulaiman, I., Lal, A.A., Matos, O., Antunes, F., 2003. Subgenotype analysis of *Cryptosporidium* isolates from humans, cattle, and zoo ruminants in Portugal. J. Clin. Microbiol. 41, 2744–2747.

Appelbee, A.J., Frederick, L.M., Heitman, T.L., Olson, M.E., 2003. Prevalence and genotyping of *Giardia duodenalis* from beef calves in Alberta, Canada. Vet. Parasitol. 112, 289–294.

Berrilli, F., Prisco, C., Friedrich, K.G., Di Cerbo, P., Di Cave, D., De Liberato, C., 2011. *Giardia duodenalis* assemblages and *Entamoeba* species infecting non-human primates in an Italian zoological garden: zoonotic potential and management traits. Parasit. Vectors 4, 199.

Betson, M., Nejsum, P., Bendall, R.P., Deb, R.M., Stothard, J.R., 2014. Molecular epidemiology of ascariasis: a global perspective on the transmission dynamics of Ascaris in people and pigs. J. Infect. Dis. 210, 932–941.

Bezjian, M., Gillespie, T.R., Chapman, C.A., Greiner, E.C., 2008. Coprologic evidence of gastrointestinal helminths of forest baboons, *Papio anubis*, in Kibale National Park, Uganda. J. Wildl. Dis. 44, 878–887.

Cacciò, S.M., Beck, R., Lalle, M., Marinculic, A., Pozio, E., 2008. Multilocus genotyping of *Giardia duodenalis* reveals striking differences between assemblages A and B. Int. J. Parasitol. 38, 1523–1531.

Cacciò, S.M., De Giacomo, M., Pozio, E., 2002. Sequence analysis of the ß-giardin gene and development of a polymerase chain reaction-restriction fragment length polymorphism assay to genotype *Giardia duodenalis* cysts from human fecal samples. Int. J. Parasitol. 32, 1023–1030.

Clark, C.G., Diamond, L.S., 1991. The Laredo strain and other '*Entamoeba histolytica*-like' amoebae are *Entamoeba moshkovskii*. Mol. Biochem. Parasitol. 46, 11–18.

Eberhard, M.L., Da Silva, A.J., Lilley, B.G., Pieniazek, N.J., 1999. Morphologic and molecular characterization of new *Cyclospora* species from Ethiopian monkeys: *C. cercopitheci* sp.n., *C. colobis* sp.n., and *C. papionis* sp.n. Emerg. Infect. Dis. 5, 651–658.

Ekanayake, D.K., Arulkanthan, A., Horadagoda, N.U., Sanjeevani, G.K., Kieft, R., Gunatilake, S., Dittus, W.P., 2006. Prevalence of *Cryptosporidium* and other enteric parasites among wild non-human primates in Polonnaruwa, Sri Lanka. Am. J. Trop. Med. Hyg. 74, 322–329.

Feng, Y., Xiao, L., 2011. Zoonotic potential and molecular epidemiology of *Giardia* species and giardiasis. Clin. Microbiol. Rev. 24, 110–140.

Gillespie, T.R., Greiner, E.C., Chapman, C.A., 2005. Gastrointestinal parasites of the colobus monkeys of Uganda. J. Parasitol. 91, 569–573.

Graczyk, T.K., Grimes, B.H., Knight, R., Da Silva, A.J., Pieniazek, N.J., Veal, D.A., 2003. Detection of *Cryptosporidium parvum* and *Giardia lamblia* carried by synanthropic flies by combined fluorescent in situ hybridization and a monoclonal antibody. Am. J. Trop. Med. Hyg. 68, 228–232.

Howells, M.E., Pruetz, J., Gillespie, T.R., 2011. Patterns of gastro-intestinal parasites and commensals as an index of population and ecosystem health: the case of sympatric western chimpanzees (*Pan troglodytes verus*) and Guinea baboons (*Papio hamadryas papio*) at Fongoli, Senegal. Am. J. Primatol. 73, 173–179.

Huang, J., Yue, D., Qi, M., Wang, R., Zhao, J., Li, J., Shi, K., Wang, M., Zhang, L., 2014. Prevalence and molecular characterization of *Cryptosporidium* spp. and *Giardia duodenalis* in dairy cattle in Ningxia, northwestern China. BMC Vet. Res. 10, 292.

Iñiguez, A.M., Leles, D., Jaeger, L.H., Carvalho-Costa, F.A., Araújo, A., Amazonas Research Group, 2012. Genetic characterisation and molecular epidemiology of *Ascaris* spp. from humans and pigs in Brazil. Trans. R. Soc. Trop. Med. Hyg. 106, 604–612.

Jones-Engel, L., Engel, G.A., Schillact, M.A., Froehlich, J., Paputungan, U., Kyes, R.C., 2004. Prevalence of enteric parasites in pet macaques in Sulawesi, Indonesia. Am. J. Primatol. 62, 71–82.

Karim, M.R., Zhang, S., Jian, F., Li, J., Zhou, C., Zhang, L., Sun, M., Yang, G., Zou, F., Dong, H., Li, J., Rume, F.I., Qi, M., Wang, R., Ning, C., Xiao, L., 2014a. Multilocus typing of *Cryptosporidium* spp. and *Giardia duodenalis* from non-human primates in China. Int. J. Parasitol. 44, 1039–1047.

Karim, M.R., Wang, R., Dong, Zhang, L., Li, J., Zhang, S., Rume, F.I., Qi, M., Jian, F., Sun, M., Yang, G., Zou, F., Ning, C., Xiao, L., 2014b. Genetic polymorphism and zoonotic potential of *Enterocytozoon bieneusi* from nonhuman primates in China. Appl. Environ. Microbiol. 80, 1893–1898.

Karim, M.R., Wang, R., Yu, F., Li, T., Dong, H., Li, D., Zhang, L., Li, J., Jian, F., Zhang, S., Rume, F.I., Ning, C., Xiao, L., 2015a. Multi-locus analysis of *Giardia duodenalis* from nonhuman primates kept in zoos in China: geographical segregation and host-adaptation of assemblage B isolates. Infect. Genet. Evol. 30, 82–88.

Karim, M.R., Dong, H., Li, T., Yu, F., Li, D., Zhang, L., Li, J., Wang, R., Li, S., Li, X., Rume, F.I., Ning, C., 2015b. Predomination and new genotypes of *Enterocytozoon bieneusi* in captive nonhuman primates in zoos in China: high genetic diversity and zoonotic significance. PLoS One 10, e0117991.

Kouassi, R.Y., McGraw, S.W., Yao, P.K., Abou-Bacar, A., Brunet, J., Pesson, B., Bonfoh, B., N'goran, E.K., Candolfi, E., 2015. Diversity and prevalence of gastrointestinal parasites in seven non-human primates of the Taï National Park, Côte d'Ivoire. Parasite 22, 1.

Legesse, M., Erko, B., 2004. Zoonotic intestinal parasites in *Papio anubis* (baboon) and *Cercopithecus aethiops* (vervet) from four localities in Ethiopia. Acta Trop. 90, 231–236.

Legua, P., Seas, C., 2013. *Cystoisospora* and *Cyclospora*. Curr. Opin. Infect. Dis. 26, 479–483.

Li, J., Qi, M., Chang, Y., Wang, R., Li, T., Dong, H., Zhang, L., 2015a. Molecular characterization of *Cryptosporidium* spp., *Giardia duodenalis*, and *Enterocytozoon bieneusi* in captive wildlife at Zhengzhou Zoo, China. J. Eukaryot. Microbiol. 62, 833–839.

Li, N., Ye, J., Arrowood, M.J., Ma, J., Wang, L., Xu, H., Feng, Y., Xiao, L., 2015b. Identification and morphologic and molecular characterization of *Cyclospora macacae* n. sp. from rhesus monkeys in China. Parasitol. Res. 114, 1811–1816.

Li, W., Kiulia, N.M., Mwenda, J.M., Nyachieo, A., Taylor, M.B., Zhang, X., Xiao, L., 2011. *Cyclospora papionis*, *Cryptosporidium hominis*, and human-pathogenic *Enterocytozoon bieneusi* in captive baboons in Kenya. J. Clin. Microbiol. 49, 4326–4329.

Lim, Y.A., Ngui, R., Shukri, J., Rohela, M., Mat Naim, H.R., 2008. Intestinal parasites in various animals at a zoo in Malaysia. Vet. Parasitol. 157, 154–159.

Mansfield, L.S., Gajadhar, A.A., 2004. *Cyclospora cayetanensis*, a food- and waterborne coccidian parasite. Vet. Parasitol. 126, 73–90.

Messaoudi, I., Estep, R., Robinson, B., Wong, S.W., 2011. Nonhuman primate models of human immunology. Antioxid. Redox Signal 14, 261–273.

Miller, R.S., Farnsworth, M.L., Malmberg, J.L., 2013. Diseases at the livestock-wildlife interface: status, challenges, and opportunities in the United States. Prev. Vet. Med. 110, 119–132.

Morf, L., Singh, U., 2012. *Entamoeba histolytica*: a snapshot of current research and methods for genetic analysis. Curr. Opin. Microbiol. 15, 469–475.

Ocaido, M., Dranzoa, C., Cheli, P., 2003. Gastrointestinal parasites of baboons (*Papio anubis*) interacting with humans in west bugwe forest reserve, Uganda. Afr. J. Ecol. 41, 356–359.

Ortega, Y.R., Sanchez, R., 2010. Update on *Cyclospora cayetanensis*, a food-borne and waterborne parasite. Clin. Microbiol. Rev. 23, 218–234.

Pedersen, A.B., Altizer, S., Poss, M., Cunningham, A.A., Nunn, C.L., 2005. Patterns of host specificity and transmission among parasites of wild primates. Int. J. Parasitol. 35, 647–657.

Petrášová, J., Modrý, D., Huffman, M.A., Mapua, M.I., Bobáková, L., Mazoch, V., Singh, J., Kaur, T., Petrželková, K.J., 2010. Gastrointestinal parasites of indigenous and introduced primate species of Rubondo Island National Park, Tanzania. Int. J. Primatol. 31, 920–936.

Plutzer, J., Karanis, P., 2016. Neglected waterborne parasitic protozoa and their detection in water. Water Res. 101, 318–332.

Poinar, G., 2009. In: Huffman, Michael A., Chapman, Colin A. (Eds.), Review of "Primate Parasite Ecology: the Dynamics and Study of Host-parasite Relationships", vol. 2. Parasit Vectors, p. 49.

Pourrut, X., Diffo, J.L., Somo, R.M., BilongBilong, C.F., Delaporte, E., LeBreton, M., Gonzalez, J.P., 2011. Prevalence of gastrointestinal parasites in primate bushmeat and pets in Cameroon. Vet. Parasitol. 175, 187–191.

Ryan, U., Hijjawi, N., 2015. New developments in *Cryptosporidium* research. Int. J. Parasitol. 45, 367–373.

Smith, H.V., Cacciò, S.M., Tait, A., McLauchlin, J., Thompson, R.C., 2006. Tools for investigating the environmental transmission of *Cryptosporidium* and *Giardia* infections in humans. Trends Parasitol. 22, 160–167.

Sulaiman, I.M., Fayer, R., Bern, C., Gilman, R.H., Trout, J.M., Schantz, P.M., Das, P., Lal, A.A., Xiao, L., 2003a. Triosephosphate isomerase gene characterization and potential zoonotic transmission of *Giardia duodenalis*. Emerg. Infect. Dis. 9, 1444–1452.

Sulaiman, I.M., Fayer, R., Lal, A.A., Trout, J.M., Schaefer III, F.W., Xiao, L., 2003b. Molecular characterization of microsporidia indicates that wild mammals harbor host-adapted *Enterocytozoon* spp. as well as humanpathogenic *Enterocytozoon bieneusi*. Appl. Environ. Microbiol. 69, 4495–4501.

Tachibana, H., Yanagi, T., Akatsuka, A., Kobayashi, S., Kanbara, H., Tsutsumi, V., 2009. Isolation and characterization of a potentially virulent species *Entamoeba nuttalli* from captive Japanese macaques. Parasitology 136, 1169–1177.

Verweij, J.J., Polderman, A.M., Clark, C.G., 2001. Genetic variation among human isolates of uninucleated cyst-producing *Entamoeba* species. J. Clin. Microbiol. 39, 1644–1646.

Xiao, L., Ryan, U., 2008. Molecular epidemiology. In: Fayer, R., Xiao, L. (Eds.), *Cryptosporidium* and Cryptosporidiosis. CRC Press and IWA Publishing, Boca Raton, pp. 119–171.

Xiao, L., Singh, A., Limor, J., Graczyk, T.K., Gradus, S., Lal, A., 2001. Molecular characterization of *Cryptosporidium* oocysts in samples of raw surface water and wastewater. Appl. Environ. Microbiol. 67, 1097–1101.

Ye, J., Xiao, L., Ma, J., Guo, M., Liu, L., Feng, Y., 2012. Anthroponotic enteric parasites in monkeys in public park, China. Emerg. Infect. Dis. 18, 1640–1643.

Ye, J., Xiao, L., Li, J., Huang, W., Amer, S.E., Guo, Y., Roellig, D., Feng, Y., 2014. Occurrence of human-pathogenic *Enterocytozoon bieneusi*, *Giardia duodenalis* and *Cryptosporidium* genotypes in laboratory macaques in Guangxi, China. Parasitol. Int. 63, 132–137.

Zhang, X., Pang, W., Hu, X., Li, J., Yao, Y., Zhang, Y., 2014. Experimental primates and non-human primate (NHP) models of human diseases in China: current status and progress. Zoological Res. 35, 447–464.

Zhao, G., Cong, M., Bian, Q., Cheng, W., Wang, R., Qi, M., Zhang, L., Lin, Q., Zhu, X., 2013. Molecular characterization of *Cyclospora*-like organisms from golden snub-nosed monkeys in qinling mountain in Shaanxi province, Northwestern China. PLoS One 8, e58216.

Zhou, Y., Lv, B., Wang, Q., Wang, R., Jian, F., Zhang, L., Ning, C., Fu, K., Wang, Y., Qi, M., Yao, H., Zhao, J., Zhang, X., Sun, Y., Shi, K., Arrowood, M.J., Xiao, L., 2011. Prevalence and molecular characterization of *Cyclospora cayetanensis*, Henan, China. Emerg. Infect. Dis. 17, 1887–1890.