Prevalence and Molecular Identification of *Entamoeba* spp. in Non-human Primates in a Zoological Garden in Nanjing, China

Xinchao Liu[1]*, Guangbin Bao[1], Menglong Yue[1], Yi Fang[1], Yueyue Gu[1], Wenchao Li[1], Youfang Gu[1], Wangkun Cheng[2] and Mingmin Lu[3]*

[1] Anhui Province Key Laboratory of Animal Nutritional Regulation and Health, College of Animal Science, Anhui Science and Technology University, Fengyang, China, [2] Nanjing Hongshan Forest Zoo, Nanjing, China, [3] MOE Joint International Research Laboratory of Animal Health and Food Safety, College of Veterinary Medicine, Nanjing Agricultural University, Nanjing, China

Objective: *Entamoeba* spp. are globally distributed zoonotic parasites that infect various hosts, among which non-human primates (NHPs) have been identified as one of the most common hosts of these parasites. Consequently, the infections of *Entamoeba* spp. in captive NHPs from Nanjing Hongshan Forest Zoo in China were investigated in order to assess their zoonotic potential.

Methods: A total of 120 fresh fecal samples, including 19 species of NHPs, were collected from four breeding bases of the zoo from May to June 2019. The infections of six species of *Entamoeba* spp. were detected by PCR using the 16S or 18S rDNA-specific primers, and the positive samples were sequenced and analyzed.

Results: *Entamoeba* spp. were detected as positive in 59 NHPs fecal samples (49.17%), including five *Entamoeba* species: *Entamoeba histolytica* (7.50%), *E. dispar* (22.50%), *E. coli* (22.50%), *E. chattoni* (10.00%) and *E. nuttalli* (1.67%). Infection with one *Entamoeba* species was more common (35%) than co-infections (13.33%) or infections with three *Entamoeba* species (0.83%). There was a significantly higher prevalence rate of *Entamoeba* spp. in the species *Pongo pygmaeus* and *Macaca mulatta* than in *Papio* sp., *Mandrillus sphinx,* and *Saimiri sciureus*.

Conclusion: *Entamoeba* spp. are highly prevalent in the NHPs raised in Nanjing Hongshan Forest Zoo. Therefore, attention should be paid to the development of containment strategies of *Entamoeba* spp. in this zoological garden.

Keywords: molecular identification, non-human primates, zoological garden, *Entamoeba* spp., infection

INTRODUCTION

Entamoeba histolytica, the causative agent of amebiasis, is a protozoan parasite that infects about 10% of the world' population and causes 100,000 deaths per year globally (1, 2). The genus *Entamoeba* includes several species, such as *E. dispar* and *E. moshkovskii*, which are morphologically similar to *E. histolytica* (3). Although *E. dispar* and *E. moshkovskii* have no apparent invasive potential, these two species exhibit some pathogenicity (4, 5). *E. coli, E. hatmanni,* and *E. nuttalli* are considered non-pathogenic; however, those species are frequently found in

the stools of several animals (6–8). As a reference method for parasitological diagnosis, the microscopic examination has been widely used to identify *Entamoeba* species, even though it lacks the ability to differentiate the infection caused by *E. histolytica* from non-pathogenic *Entamoeba* spp. (9). Therefore, molecular methods have been preferably used to identify and investigate the epidemiology of *Entamoeba* spp. in human and animal hosts (6, 7, 10, 11).

Among the hosts of *Entamoeba* spp., non-human primates (NHPs) have a close phylogenetic relationship with human, which increases the risk of *Entamoeba* spp. transmission between NHPs and humans. Previous studies showed that both captive and free-ranging NHPs are frequently infected with *Entamoeba* spp. (11, 12), and many *Entamoeba* spp. were identified in NHPs, especially *E. histolytica* (6, 13). Given that captive NHPs have more opportunities to contact with humans than free-ranging, they are a major concern group of host with a higher potential for zoonotic infections (6, 14).

Nanjing Hongshan Forest Zoo is one of the most distinctive zoological gardens in China with abundant NHPs animal resources and colorful theme activities, attracting millions of visitors every year. However, there is inadequate information on the epidemic of parasitic zoonoses within this zoological garden. The presence of zoonotic parasites poses a threat to the health of NHPs, which may in turn cause zoonotic transmission events between tourists and NHPs. Consequently, in this study, we aim to investigate the prevalence of *Entamoeba* spp. in NHPs in Nanjing Hongshan Forest Zoo to assess the potential for zoonotic transmission.

MATERIALS AND METHODS

Collection of Stool Samples

From May to June 2019, stool samples of 120 NHPs were collected from four breeding bases, including the headquarter (26/120), Gaochun base (26/120), Jing'an base (64/120), and rescue center (4/120) of Nanjing Hongshan Forest Zoo. There were 19 species included in 120 NHPs. About 15g fecal sample were collected immediately after defecation for each individual and placed in a plastic container marked with the name, species, gender, and breeding base. Fecal samples were then stored at 4°C for DNA extraction. The detailed information of the samples is shown in **Table 1**.

Detection of the Samples by Polymerase Chain Reaction

From each sample, 0.2 g of stool were used to isolate genomic DNA using the Stool DNA Kit (Tiangen Biotech, Beijing, China) according to the manufacturer's instructions. The genomic DNA was then detected by PCR assays based on the 16S or 18S rDNA sequence of six *Entamoeba* species according to previous studies (15–18). *E. chattoni* and *E. nuttalli* were detected by one round PCR, while *E. histolytica*, *E. dispar*, *E. moshkovskii,* and *E. coli* were detected by nested PCR using specific primers (**Table 2**). After the final PCR reaction, the products were examined by agarose gel electrophoresis, and the size of target fragments

TABLE 1 | Non-human primate (NHP) subjects sampled in the study.

Breeding bases	Hylobates hoolock	Hylobates leucogenys	Nomascus gabriellae	Rhinopithecus sp.	Macaca mulatta	Callithrix jacchus	Trachypithecus francoisi	Macaca assamensis	Erythrocebus patas	Macaca fascicularis	Colobus guereza	Eulemur fulvus	Papio sp.	Mandrillus sphinx	Pan troglodytes	Pongo pygmaeus	Sapajus apella	Lemur catta	Saimiri sciureus	No. of samples
Jing'an	5	0	11	11	0	0	5	4	6	1	8	5	2	4	0	0	2	0	0	64
Headquarter	0	2	7	5	0	4	0	0	0	0	0	0	0	0	5	3	0	0	0	26
Gaochun	0	0	10	3	0	0	0	0	0	0	0	0	0	0	0	0	6	5	2	26
Rescue center	0	0	0	0	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4
No. of samples	5	2	28	19	4	4	5	4	6	1	8	5	2	4	5	3	8	5	2	120

Genus/species	Primers (5'–3')	PCR product size (bp)	Reference	Gene
Entamoeba genus	E1-F: TAAGATGCAGAGCGAAA	*E. histolytica*: 897	(15)	16S-rDNA
	E1-R: GTACAAAGGGCAGGGACGTA	*E. dispar*: 898		
		E. moshkovskii: 896		
E. histolytica	EH-F: AAGCATTGTTTC TAGATCTGAG	439		
	EH-R: AAGAGGTCTAACCGAAATTAG			
E. dispar	ED-F: TCTAATTTCGATTAGAACTCT	174		
	ED-R: TCCCTACCTATTAGACATAGC			
E. moshkovskii	EM-F: GAAACCAAGAGTTTCACAAC	553		
	EM-R: CAATATAAGGCTTGGATGAT			
Entamoeba genus	Entam1: GTTGATCCTGCCAGTATTATATG	631	(16)	16S-rDNA
	Entam2: CTTTAAGTTTCAGCCTTGTGACC			
E. coli	EC-F: GAATGTCAAAGCTAATACTTGACG	159	(17)	16S-rDNA
	EC-R: GATTTCTACAATTCTCTTGGCATA			
E. chattoni	ECH-F: AGGATTTGTTTTATAACAAGTTC	215	(16)	16S-rDNA
	ECH-R: TAAATAACCTTTCTCCTTTTTCTATC			
E. nuttalli	EN-F: TTTTAACATTTTGAAGACTTTGATA	449	(18)	18S-rDNA
	EN-R: AAGGTAATATTGATATACTCAGATTA			

was validated by comparing them with DL2000 DNA Marker (TaKaRa, Dalian, China).

Sequence Analysis and Data Accessibility

Positive samples with the correct molecular size were purified and confirmed by two-directional sequencing using the specific PCR primers for each species by Sangon Biotech (Shanghai, China). The obtained nucleotide sequences were aligned with published international reference sequences available from the NCBI GenBank™ database using BLAST.

The assembled sequences obtained in this study were submitted to GenBank under accession number ON254797 for *E. histolytica* (9 samples), ON254798 (1 sample) and ON254799 (1 sample) for *E. nuttalli*, ON254800 for *E. chattoni* (12 samples), ON254801 (21 samples) and ON254802 (6 samples) for *E. coli*, and ON254803 for *E. dispar* (27 samples).

Statistical Analysis

The samples with the sequence that can be assigned to the reference sequences were defined as positive, and used to calculate the prevalence of *Entamoeba* spp. infections. The statistical analysis was performed to analyze the risk factors, including breeding bases and genders, for *Entamoeba* spp. infections in NHPs using SPSS 25.0. A chi-square test was used to analyze the non-metric variables in the form of frequency tables. $P < 0.05$ was considered significant.

RESULT

Entamoeba spp. Infection in NHPs

Among 120 stool samples from NHPs, 59 (49.17%) were detected as *Entamoeba* spp. positive. As shown in **Table 3**, the most common species detected were *E. coli* and *E. dispar* in 27 (22.50%) samples, respectively, followed by *E. chattoni* (10.00%,

12/120), *E. histolytica* (7.50%, 9/120) and *E. nuttalli* (1.67%, 2/120). None sample presented infection with *E. moshkovskii*. Statistical analysis showed that there was a significant difference in the infection rate of different *Entamoeba* spp. in the NHPs ($X^2 = 63.003$, $p = 0.000$).

Multiple Infections

Among the 59 positive stool samples, 17 presented multiple infections (14.17%), from which 16 corresponded to co-infections and 1 to triple infection. Co-infections *E. coli* + *E. dispar* represented 52.94% (9/17) of the co-infections, and the only case of triple infection was with *E. coli* + *E. dispar* + *E. chattoni* (**Table 3**).

Multiple infections were detected in 9 among the 19 species of NHPs. The highest rate of multiple infection was revealed in *M. mulatta* (75.0%, 3/4), followed by *P. pygmaeus* (66.7%, 2/3). The multiple infections of the other species are shown in **Table 3**.

Risk Factors for the Infection of *Entamoeba*. spp in NHPs

From the 19 species of NHPs, *M. mulatta*, *M. fascicularis*, and *P. pygmaeus* were all positive for *Entamoeba* spp. infections, while none of the fecal samples obtained from *Papio* sp., *M. sphinx*, and *S. sciureus* were detected as positive. The infection rates of other species of NHPs are shown in **Table 4**.

As shown in **Table 4**, all the samples from the rescue center presented *Entamoeba* infection, while the infection rates of the other three breeding bases were close to 50.0%. No statistical significance was observed among different bases ($X^2 = 4.987$, $p = 0.173$).

To assess the effect of NHPs gender on *Entamoeba* spp. infection, the infection rates of the gender categories (male, female, and unknown) were analyzed. It should be noted that there was no significant association of *Entamoeba* spp. infection

TABLE 3 | Mono- and mixed infection of *Entamoeba* spp. in NHPs in Nanjing Hongshan Forest Zoo.

Species of *Entamoeba*	Species of NHPs																			No. (%) of samples
	Hylobates hoolock	*Hylobates leucogenys*	*Nomascus gabriellae*	*Rhinopithecus sp.*	*Macaca mulatta*	*Callithrix jacchus*	*Trachypithecus francoisi*	*Macaca assamensis*	*Erythrocebus patas*	*Macaca fascicularis*	*Colobus guereza*	*Eulemur fulvus*	*Papio sp.*	*Mandrillus sphinx*	*Pan troglodytes*	*Pongo pygmaeus*	*Sapajus apella*	*Lemur catta*	*Saimiri sciureus*	
Species in monoinfections																				42(35.00)
E. histolytica	0	0	3	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4(3.33)
E. dispar	0	0	1	3	0	2	0	0	1	0	5	1	0	0	1	0	1	1	0	16(13.33)
E. moshkovskii	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
E. coli	2	0	3	0	0	0	3	0	0	0	0	0	0	0	1	1	0	2	0	12(10.00)
E. chattoni	1	1	1	1	1	0	0	1	2	1	0	0	0	0	0	0	0	0	0	9(7.50)
E. nuttalli	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1(0.83)
Species in mixed infections																				17(14.17)
E. dispar+ E. chattoni	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1(0.83)
E. coli+ E. dispar	0	0	4	0	0	0	1	1	0	0	0	0	0	0	0	2	1	0	0	9(7.50)
E. coli+ E. histolytica	0	0	1	1	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4(3.33)
E. chattoni+ E. histolytica	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1(0.83)
E. coli+ E. nuttalli	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1(0.83)
E. coli+ E. dispar+ E. chattoni	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1(0.83)

TABLE 4 | Factors associated with prevalence of _Entamoeba_ spp. in NHPS in this study.

Variable	No. of positive/tested	Prevalence (%)	X^2	_P_ value
Species			*	*
Hylobates hoolock	4/5	80.00		
Hylobates leucogenys	1/2	50.00		
Nomascus gabriellae	13/28	46.42		
Rhinopithecus sp.	6/19	31.58		
Macaca mulatta	4/4	100.0		
Callithrix jacchus	2/4	50.00		
Trachypithecus francois	4/5	80.00		
Macaca assamensis	2/4	50.00		
Erythrocebus patas	4/6	66.67		
Macaca fascicularis	1/1	100.0		
Colobus guereza	5/8	62.50		
Eulemur fulvus	1/5	20.00		
Papio sp.	0/2	0		
Mandrillus sphinx	0/4	0		
Pan troglodytes	4/5	80.00		
Pongo pygmaeus	3/3	100.0		
Sapajus apella	2/8	25.00		
Lemur catta	3/5	60.00		
Saimiri sciureus	0/2	0		
Breeding base			4.987	0.173
Jing'an	30/64	46.88		
Headquarter	14/26	53.85		
Gaochun	11/26	42.30		
Rescue center	4/4	100.0		
Gender			1.420	0.492
Male	18/39	46.15		
Female	25/54	46.30		
Unknown	16/27	59.26		

*Considering the small sample size of each species of NHPs, significance test was not analyzed among the species of NHPs._

with gender ($X^2 = 1.420$, $p = 0.492$) (**Table 4**). Nevertheless, no _E. histolytica_-positive samples were detected in the NHPs male category.

DISCUSSION

The _Entamoeba_ genus contains many species residing in the intestinal lumen, of which _E. histolytica_ is the main species associated with pathological sequelae (9). Nevertheless, other species are also frequently detected in a range of animals, such as goat, pig, yak, mice, and alpacas (7, 19–22). In addition, both captive and free-ranging NHPs have been identified as common hosts of _Entamoeba_ spp. (6, 11, 14).

The present study provides the first information on the epidemiological data of _Entamoeba_ spp. infections in NHPs from Nanjing Hongshan Forest Zoo (49.17%, 59/120). The prevalence rate of _Entamoeba_ spp. was lower than in the free-ranging NHPs

in savanna woodland (Tanzania, _Pan troglodytes_, 79%) (23) and Taihangshan (China, _Macaca mulatta tcheliensis_, 89.96%) (11). Compared to zoological gardens housing captive NHPs, the positivity was higher than those in Ibadan (Nigeria, _Erythrocebus patas_, _Cercocebus atys_, _Mandrillus sphinx_, _Cercopithecus sabaeus_, 13.90%) (14), Belgium (Belgium, prosimians, New World monkeys, Old World monkeys and apes, 44%) (24), and the experimental macaques in Yunnan (China, _M. mulatta_, _M. nemestrina and M. fascicularis_, 9.31%) (13). These results indicate that free-ranging NHPs may have a much higher _Entamoeba_ occurrence rate than captive ones. It is likely that captive NHPs are largely housed in zoological gardens and research facilities, and the captive management may interrupt the transmission of parasitic pathogens to hosts. Of note, the infection rate of _Entamoeba_ spp. in captive NHPs from Nanjing Hongshan Forest Zoo is much higher than those reported in several epidemiological studies (seven NHPs species) (13, 14) suggesting that this attraction should strengthen the housing and husbandry practices to improve the health of NHPs.

Except for _E. moshkovskii_, the other five _Entamoeba_ spp. species were found in this study, presenting different infection rates. Compared with the studies in Belgium and the Netherlands (four _Entamoeba_ species among 36 NHPs species), China (three _Entamoeba_ species among three NHPs species) and Italy (three _Entamoeba_ species among nine NHPs species), more species of _Entamoeba_ spp. were detected in Nanjing Hongshan Forest Zoo (6, 13, 25). The infection rate of _E. histolytica_ was 7.50%, which is consistent with the data reported by the zoological gardens in Belgium and Netherlands (36 NHPs species, 8.1%) and the experimental macaques (_M. mulatta_, _M. nemestrina and M. fascicularis_, 6.38%) in Yunnan Province in China (6, 13). In contrast, no _E. histolytica_ infection was detected in savanna woodland (Tanzania, _Pan troglodytes_), Taihangshan (China, _Macaca mulatta tcheliensis_), and an Italian zoological garden (Italy, nine NHP species) (11, 23, 25). Our results revealed higher diversity of _Entamoeba_ species in NHPs in Nanjing Hongshan Forest Zoo China, and these NHPs may be potential sources for human infection with _E. histolytica_.

Entamoeba dispar and _E. coli_ were the more prevalent species revealed in this study, consistent with other 13 zoos/parks in China (26). While in the NHPs from zoological gardens in the UK and the experimental NHPs from Japan and China, only _E. dispar_/_E. coli_ was detected as the main species for infections (27–29). In addition, _E. hartmanni_ was the most prevalent _Entamoeba_ species shed in the NHPs in Belgium and Netherlands, and in savanna woodland chimpanzees (6, 23), and _E. chattoni_ was the predominant species shed in macaques from wild Taihangshan and southwest China (11, 30). These results indicated that different regions had their specific prevalent species of _Entamoeba_, which might be related to the parameters including lifestyles, and the species of NHPs. The observation needs to be further studied. There is, to date, no report indicating the _E. moshkovskii_ infections in NHPs (11, 25, 31). Likewise, no _E. moshkovskii_-positive sample was detected in the NHPs in this study. However, the cases of human infection with _E. moshkovskii_ have been reported in Malaysia, Japan and Colombia (32–34), and _E. moshkovskii_ has also been detected

in animal hosts, such as cattles (UK), elephants (Namibia), and chelonian (Mexico) (35, 36). Further investigations are needed to demonstrate whether NHPs can be infected by *E. moshkovskii*.

The multiple infection rate of *Entamoeba* spp. in the NHPs in this study was 14.17%. It was lower than that in the other 13 zoos/parks in China (45.4%) and in the free-ranging Taihangshan Macaques (84.93%) (11, 25). The differences in the prevalence of multiple infections may be related to the method used for *Entamoeba* detection. Microscopic examination was used in those studies, which may not allow correct discriminations of *Entamoeba* species. Compared with the studies using molecular techniques, the multiple infection rate in this study was consistent with the studies in Tanzania (16.67%) (22), higher than that in Italian (0) (24), and lower than that in Belgium and the Netherlands (51.90%) (6, 22, 24). The difference in *Entamoeba* multiple infections in NHPs may be associated with the climate, the species of NHPs or breeding and management conditions, which need to be further studied. *Entamoeba* spp. was prevalent in all four breeding bases of the zoo, especially in the rescue center. It could attribute to the poor conditions in which the NHPs were raised before being rescued. Importantly, both males and females have a high infection rate, whereas no significant difference was observed ($X^2 = 1.42$, $p = 0.492$), which was consistent with the finding in the experimental macaques in Yunnan (13). It indicates that the infection of *Entamoeba* spp. was not correlated to the gender of NHPs. However, all the *E. histolytica*-positive samples were detected from females, which warrants further investigations.

In the 19 species of NHPs, no *M. sphinx* and *Papio* sp. was confirmed with *Entamoeba* spp. infections. In a previous study, both the semi-free-ranging (100%) and captive (81.20%) *M. sphinx* presented a high infection rate of *Entamoeba* spp. (26, 37), and *Papio* sp. was verified to be a natural host of *Entamoeba* spp. (38). It is likely that our sampling size was too small to identify the positive infection of *Entamoeba* spp. in these two NHPs species. In Martinez et al.'s research, *S. sciureus* was not susceptible to *E. histolytica* (39). In our study, *S. sciureus* was found to be *Entamoeba* spp. negative, which could be explained by the small sample size. The infection rate of *Entamoeba* spp. ranged from 25% (*Sapajus apella*) to 100% (*M. mulatta, Macaca fascicularis*, and *P. pygmaeus*), indicating that the infection of *Entamoeba* spp. was widespread in most species of NHPs in this zoo.

In conclusion, this study revealed that *Entamoeba* spp. are widely prevalent in the captive NHPs in Nanjing Hongshan Forest Zoo. Furthermore, five species (*E. coli, E. dispar, E. histolytica, E. chattoni*, and *E. nuttalli*) were identified, with *E. coli* and *E. dispar* as the predominant species. The epidemiology of *Entamoeba* spp. infection is complex, and these captive NHPs could potentially participate in zoonotic transmission, and the *Entamoeba* spp. infection of other animals from this zoological garden. The prevention and control of *Entamoeba* spp. infection in this zoological garden should be strengthened to reduce disease exposure risk of public health.

DATA AVAILABILITY STATEMENT

The datasets supporting the findings of this article are included within the article. Additional datasets generated for this study are available on reasonable request to the corresponding authors.

ETHICS STATEMENT

The animal study was reviewed and approved by the Animal Care and Welfare Committee of Anhui Science and Technology University, Fengyang, China. Written informed consent was obtained from the owners for the participation of their animals in this study.

AUTHOR CONTRIBUTIONS

XL designed this study, performed data analysis, and drafted the manuscript. GB, MY, YYG, and YF helped to carry out the study. WL and YFG helped to design this study and perform data analysis. WC participated in the collection of experimental materials. ML critically revised the manuscript. All authors read and approved the final version of the manuscript.

FUNDING

REFERENCES

1. Hung CC. Amoebiasis: current status in Australia. *Med J Aust.* (2007) 187:412–6. doi: 10.5694/j.1326-5377.2007.tb0 1293.x

2. World Health Organization. WHO/Pan American Health Organization/UNESCO Report of a Consultation of Experts on Amoebiasis. *Wkly Epidemiol Rec.* (1997) 72:97–9.

3. Lauren DM. Distinguishing between pathogenic and non-pathogenic species of *Entamoeba*. *Lab Med.* (2004) 35:613–5. doi: 10.1309/B81NPVAW8Y4BGY11

4. Jackson T. *Entamoeba histolytica* and *Entamoeba dispar* are distinct species, clinical, epidemiological and serological evidence. *Int J Parasitol.* (1998) 28:181. doi: 10.1016/S0020-7519(97)00177-X

5. Tengku SA, Norhayati M. Public health and clinical importance of amoebiasis in Malaysia: a review. *Trop Biomed.* (2011) 28:194–222. doi: 10.1371/journal.ppat.1002177

6. Levecke B, Dreesen L, Dorny P, Verweij JJ, Vercammen F, Casaert S, et al. Molecular identification of *Entamoeba* spp. in captive nonhuman primates. *J Clin Microbiol.* (2010) 48:2988–00. doi: 10.1128/JCM.00013-10

7. Al-Habsi K, Yang R, Ryan U, Jacobson C, Miller DW. Morphological and molecular characterization of an uninucleated cyst-producing *Entamoeba*

spp. in captured rangeland goats in western Australia. *Vet Parasitol.* (2017) 235:41–6. doi: 10.1016/j.vetpar.2017.01.013

8. Matsubayashi M, Suzuta F, Terayama Y, Shimojo K, Yui T, Haritani M, et al. Ultrastructural characteristics and molecular identification of *Entamoeba suis* isolated from pigs with hemorrhagic colitis: implications for pathogenicity. *Parasitol Res.* (2014) 113:3023–8. doi: 10.1007/s00436-014-3965-y

9. Fotedar R, Stark D, Beebe N, Marriott D, Harkness J. Laboratory diagnostic techniques for *Entamoeba* species. *Clin Microbiol Rev.* (2007) 20:511–3. doi: 10.1128/CMR.00004-07

10. Roshdy HM, El-Kader NA, Tammam M, Fuentes I, Mohamed M, EI-Sheikh NA, et al. Molecular diagnosis of *Entamoeba* spp. versus microscopy in the Great Cairo. *Acta Parasitol.* (2017) 62:188–91. doi: 10.1515/ap-2017-0022

11. Zhang Q, Liu K, Wang C, Luo J, Lu J, He H. Molecular characterization of *Entamoeba* spp. in wild Taihangshan Macaques (*Macaca mulatta tcheliensis*) in China. *Acta Parasitol.* (2019) 64:228–31. doi: 10.2478/s11686-019-00026-y

12. David EB, Patti M, Coradi ST, Oliveira-Sequeira TCG, Ribolla PEM, Guimaraes S. Molecular typing of *Giardia duodenalis* isolates from nonhuman primates housed in a Brazilian zoo. *Rev Inst Med Trop Saõ Paulo.* (2014) 56:49–54. doi: 10.1590/S0036-46652014000100007

13. Pu LH, Li Z, Wu J, Zhang YL, Zou FC. Prevalence, molecular epidemiology and zoonotic risk of *Entamoeba* spp. from experimental macaques in Yunnan Province, southwestern China. *Parasitol Res.* (2020) 119:2733–40. doi: 10.1007/s00436-020-06762-9

14. Adetunji VE. Prevalence of gastro-intestinal parasites in primates and their keepers from two zoological gardens in Ibadan, Nigeria. Sokoto. *J Vet Sci.* (2014) 12:25–30. doi: 10.4314/sokjvs.v12i2.5

15. Khairnar K, Parija SC, A. novel nested multiplex polymerase chain reaction (PCR) assay for differential detection of *Entamoeba histolytica*, *E. moshkovskii and E. dispar* DNA in stool samples. *BMC Microbiol.* (2007) 7:47. doi: 10.1186/1471-2180-7-47

16. Verweij JJ, Polderman AM, Clark CG. Genetic variation among human isolates of uninucleated cyst-producing *Entamoeba* species. *J Clin Microbiol.* (2001) 39:1644–6. doi: 10.1128/JCM.39.4.1644-1646.2001

17. Tachibana H, Yanagi T, Akatsuka A, Kobayashi S, Kanbara H, Tsutsumi V. Isolation and characterization of a potentially virulent species *Entamoeba nuttalli* from captive Japanese macaques. *Parasitology.* (2009) 136:1169–77. doi: 10.1017/S0031182009990576

18. Tachibana H, Yanagi T, Pandey K, Cheng XJ, Kobayashi S, Sherchand JB, et al. An *Entamoeba* spp. strain isolated from rhesus monkey is virulent but genetically different from *Entamoeba histolytica. Mol Biochem Parasit.* (2007) 153:107–14. doi: 10.1016/j.molbiopara.2007.02.006

19. Ren M, Yang F, Gou JM, Wang PX, Lin Q. First detection and molecular identification of *Entamoeba* in yaks from China. *Acta Parasitol.* (2020) 66. doi: 10.1007/s11686-020-00258-3

20. Hirashima Y, Manchanayake T, Yano T, Kitahara S, Koreeda T, Kamimura S, et al.. Development of molecular diagnostic protocols for detecting three types of *Entamoeba* from diarrheal and asymptomatic pigs and environmental moist soils. *Parasitol Res.* (2017) 116:2001–7. doi: 10.1007/s00436-017-5483-1

21. Chikako S, Mamun K, Mami T, Dinesh M, Seiki K, Ali IKM, et al. *Entamoeba moshkovskii* is associated with diarrhea in infants and causes diarrhea and colitis in mice. *J Infect Dis.* (2012) 206:744–51. doi: 10.1093/infdis/jis414

22. Gao WW, Ma YT, Ma YY, Li RL, Zhu XQ. First report of *Eimeria* and *Entamoeba* infection in alpacas (*Vicugna pacos*) in Shanxi Province, northern China. *Parasitol Res.* (2021) 120:2031–5. doi: 10.1007/s00436-021-07157

23. Pomajbíková KJ, Cepicka I, Kalousová B, Jirku M, Stewart F, Levecke B, et al. Molecular identification of *Entamoeba* species in savanna woodland chimpanzees (*Pan troglodytes schweinfurthii*). *Parasitology.* (2016) 143:741–8. doi: 10.1017/S0031182016000263

24. Levecke B, Dorny P, Geurden T, Vercammen F, Vercruysse J. Gastrointestinal protozoa in non-human primates of four zoological gardens in Belgium. *Vet Parasitol.* (2007) 148:236–46. doi: 10.1016/j.vetpar.2007.06.020

25. Berrilli F, Prisco C, Friedrich KG, Cerbo PD, Cave D, Liberato CD. *Giardia duodenalis* assemblages and *Entamoeba* species infecting non-human primates in an Italian zoological garden: zoonotic potential and management traits. *Parasit Vectors.* (2011) 4:119. doi: 10.1186/1756-3305-4-199

26. Dong H, Li J, Qi M, Wang R, Yu F, Jian F, et al. Prevalence, molecular epidemiology, and zoonotic potential of *Entamoeba* spp. in

nonhuman primates in China. *Infect Genet Evol.* (2017) 54:216–20. doi: 10.1016/j.meegid.2017.07.002

27. Regan CS, Yon L, Hossain Z, Elsheikha HM. Prevalence of *Entamoeba* species in captive primates in zoological gardens in the UK. *Peer J.* (2014) 2:e492. doi: 10.7717/peerj.492

28. Tachibana H, Cheng XJ, Kobayashi S, Matsubayashi N, Gotoh S, Matsubayashi K. High prevalence of infection with *Entamoeba dispar*, but not *E. histolytica,* in captive macaques. *Parasitol Res.* (2001) 87:14–7. doi: 10.1007/s004360000289

29. Feng M, Yang B, Yang L, Fu Y, Tachibana H. High prevalence of *Entamoeba* infections in captive long-tailed macaques in China. *Parasitol Res.* (2011) 109:1093–7. doi: 10.1007/s00436-011-2351-2

30. Feng M, Cai J, Min X, Fu Y, Xu Q, Tachibana H, Cheng X. Prevalence and genetic diversity of *Entamoeba* species infecting macaques in southwest China. *Parasitol Res.* (2013) 112:1529–36. doi: 10.1007/s00436-013-3299-1

31. Elsheikha HM, Regan CS, Clark CG. Novel *Entamoeba* findings in nonhuman primates. *Trends Parasitol.* (2017) 1724:12. doi: 10.1016/j.pt.2017.12.008

32. Anuar TS, Al-Mekhlafi HM, Ghani M, Azreen SN, Salleh FM, Ghazali N, et al. First molecular identification of *Entamoeba moshkovskii* in Malaysia. *Parasitol.* (2012) 139:1521–5. doi: 10.1017/S0031182012001485

33. Zebardast N, Yeganeh F, Gharavi MJ, Abadi A, Tabaei SS, Haghighi A. Simultaneous detection and differentiation of *Entamoeba histolytica*, *E. dispar*, *E. moshkovskii*, *Giardia lamblia* and *Cryptosporidium* spp. in human fecal samples using multiplex PCR and qPCR-MCA. *Acta Trop.* (2016)162:233–8. doi: 10.1016/j.actatropica.2016.07.004

34. Consuelo LM, León C, Jairo F, Patricia R, Ligia M, Olivera MJ, et al. Molecular epidemiology of *Entamoeba*: first description of *Entamoeba moshkovskii* in a rural area from central Colombia. *PLoS ONE.* (2015) 10: e0140302. 0140302 doi: 10.1371/journal.pone.0140302

35. Jacob AS, Busby EJ, Levy A, Komm N, Clark CG. Expanding the *Entamoeba* universe: new hosts yield novel ribosomal lineages. *J Eukaryotic Microbiol.* (2016) 63:69–78. doi: 10.1111/jeu.12249

36. Garcia G, Ramos F, Perez RG, Yanez J, Estrada MS, Mendoza LH et al. Molecular epidemiology and genetic diversity of *Entamoeba* species in a chelonian collection. *J Med Microbiol.* (2014) 63:271–83. doi: 10.1099/jmm.0.061820-0

37. Setchell JM, Bedjabaga IB, Goossens B, Reed P, Wickings EJ, Knapp LA. Parasite prevalence, abundance, and diversity in a semi-free-ranging colony of *Mandrillus sphinx. Int J Primatol.* (2007) 28:1345–62. doi: 10.1007/s10764-007-9225-6

38. Myers BJ, Malherbe KH. Intestinal commensals and parasites of the South African baboon (*Papio cynocephalus*). *Trans Am Microsc Soc.* (1971) 90:80–3. doi: 10.2307/3224900

39. Martinez Reyes R, Gonzalez Pacheco M, Gomez Reyes I, Torre MDL, Sepulveda B. Induction of anti-amoebic immunity in non-human primates with lysosomal antigen from *Entamoeba histolytica*. VI. Intrahepatic inoculation of virulent *E. histolytica* in monkeys. *Arch Invest Med.* (1980) 11:267–73.