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Prevalence and genetic diversity of *Enterocytozoon bieneusi* in nonhuman primates in Northern and Central China

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ABSTRACT

Background: *Enterocytozoon bieneusi* is a common enteric pathogen reported in human and many animals. But there are few reports of *E. bieneusi* in nonhuman primates (NHPs). The aim of this study was to examine the prevalence and molecular characterization of *E. bieneusi* in NHPs from Northern and Central China.

Results: A total of 299 specimens of NHPs were collected. The overall prevalence rate of *E. bieneusi* was 9.4% (28/299) in NHPs by ribosomal internal transcribed spacer (ITS) amplification, including 10.0% (16/160) in captive NHPs and 8.6% (12/139) in wild NHPs. In captive NHPs, the infection rate was 9.1% in male, 11.5% in female. Infection rate in juvenile ITS (6.7%) was higher than in the adult ITS (5.6%). In different regions, infection rate in Hubei (14.7%) was higher than in Henan (7.6%) and Beijing (7.9%). Five genotypes were found, including 4 known genotypes (D, HND-I, EbpC, SHW2) and a novel genotype named NHP1. Genotype D (8/28) and NHP1 (8/28) were the most prevalent, followed by EbpC (6/28), SHW2 (4/28), and HND-I (2/28). All the 5 genotypes belonged to zoonotic Group1.

Conclusion: These findings could deepen our understanding of *E. bieneusi* prevalence and genotype distribution in NHPs in China. Our study shows that NHPs may be the reservoir of zoonotic *E. bieneusi* and might present a potential serious threat.

1. Introduction

Enterocytozoon bieneusi is a common enteric parasitic pathogen, with a worldwide distribution and wide host range, including human, many domestic and wild mammals, birds, chickens, and others (Ding et al., 2018; Song et al., 2018; Tang et al., 2018; Zou et al., 2018). Even drinking source water (Guo et al., 2014) and fresh food (Jedrzejewski et al., 2007) have been detected. Clinical symptoms caused by *E. bieneusi* vary from asymptomatic to chronic diarrhea. As an opportunistic pathogen of human patients with acquired immune deficiency syndrome (AIDS), *E. bieneusi* can cause significant morbidity and even life-threatening diseases in immunodeficient patients (Conteas et al., 1998). Similar clinical symptoms could be found in NHPs and other natural hosts for *E. bieneusi* (Green et al., 2004; Mansfield et al., 1998; Słodkiewicz-Kowalska et al., 2007). However, no effective drugs and vaccine have been developed.

Up to now, more than 500 genotypes were identified through the internal transcribed spacer (ITS) sequence analysis of *E. bieneusi*. These genotypes have been classified into 11 genetically isolated clusters by phylogenetic analysis (Li et al., 2019). Group 1 is frequently reported in pets, nonhuman primates (NHPs), and livestock, have a wide range of host and extensive geographical distribution, posing a high potential risk of zoonotic (Li et al., 2016). Group 2 also implies public health concern related to the zoonotic potential for some genotypes (notably BEB4, BEB6, I, and J) within this group (Li et al., 2019). On the other hand, members in groups 3–11 were mainly found in specific hosts or wastewater, and thus have limited or unknown public health significance.

Currently, the prevalence of *E. bieneusi* in NHPs have been studied in a few countries, including China, Bangladesh, Kenya, Indonesia, Spain, Slovakia, and some European countries (Karim et al., 2020; Koster et al., 2021; Sak et al., 2011). However, most of them focused on captive NHPs,

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Table 1
Infection rate and genotypes of *E. bieneusi* in NHPs.

NHP Genus	No. of detected	No. of positive (%)	ITS genotypes (No.)
<i>Hylobates</i>	4	0	
<i>Hoolock</i>	4	0	
<i>Nomascus</i>	1	0	
<i>Rhinopithecus</i>	111	19 (17.1%)	HND-I (2), EbpC (6), SHW2 (4), D (1), NHP1 (6)
<i>Colobus</i>	8	1 (12.5%)	D (1)
<i>Lemur</i>	39	0	
<i>Trachypithecus</i>	12	0	
<i>Mandrillus</i>	10	0	
<i>Pan</i>	5	0	
<i>Macaque</i>	105	8 (7.6%)	D (6), NHP1 (2)

little information on the prevalence and genotypes of *E. bieneusi* in wild NHPs in China have been reported. Therefore, the aim of the present study was to examine the prevalence and genotype distribution of *E. bieneusi* in NHPs in different habitats in China.

2. Material and methods

2.1. Ethics statement

This study was reviewed and approved by the Animal Ethics Committee of the Institute of Zoology, Chinese Academy of Sciences, and was conducted strictly in accordance with the requirements of the Care and Use of Animals in Research, which are issued by the Institute of Zoology, Chinese Academy of Sciences.

2.2. Specimens collection

From September 2015 to February 2019, 299 fecal specimens were collected from NHPs, including 126 specimens from captive NHPs living in Beijing zoo, 34 specimens from captive NHPs and 34 from wild NHPs living in Shennongjia in Hubei, and 105 specimens from wild NHPs living in Wulongkou in Henan. Fresh specimens were immediately collected using individual polyethylene glove from the floor of the enclosures, marking number, age, gender, species. Age and gender information of captive NHPs was told by zookeepers and veterinarians, but as for wild NHPs, it was hard to get. At the time of collection, all the animals were clinically healthy. All the fecal specimens were transported to the laboratory in an ice box as soon as possible, and stored at $-80\text{ }^{\circ}\text{C}$ before DNA extraction.

2.3. DNA extraction

Total DNA was extracted from 200 mg fecal samples using Stool Mini Kit (Qiagen, Valencia, CA, USA) following the manufacturer's instruction. Extracted DNA was stored at $-20\text{ }^{\circ}\text{C}$ before PCR analysis.

Table 2
Genotype distribution of *E. bieneusi* in NHPs.

Region	Province	No. of detected	No. (%) of positive	Genotype (No.)	Habitats	Species
Northern China	Beijing	126	10 (7.9%)	HND-I (2)	Captive	<i>R. bieti</i>
				EbpC (6)	Captive	<i>R. bieti</i>
				D (2)	Captive	<i>Colobus</i> , <i>Rhinopithecus</i>
Central China	Henan	105	8 (7.6%)	NHP1 (2)	Wild	<i>Taihangshan macaques</i>
				D (6)	Wild	<i>Taihangshan macaques</i>
	Hubei	68	10 (14.7%)	SHW2 (4)	Captive	<i>R. roxellanae</i>
				NHP1 (2)	Captive	<i>R. roxellanae</i>
				NHP1 (4)	Wild	<i>R. Roxolani</i>

2.4. PCR amplification

For detection of *E. bieneusi*, a 392 bp fragment of the ITS region of the rRNA gene was amplified by nested PCR. The PCR mixtures (30 μL) included 3 μL of template DNA, 15 μL of 2 \times Rapid Taq Master Mix (Vazyme, China) and 0.5 μM (each) primers. Nested PCR was performed using the primers EBITS-1F (5'-GGTCATAGGGATGAAGAG-3') and EBITS-1R (5'-TTCGAGTT-CTTTCGCGCTC-3') for the first round PCR, and the primers EBITS-2F (5'-GCT-CTGAATATCTATGGCT-3') and EBITS-2R (5'-ATCGCCGACGGATCCAAGTG-3') for the secondary PCR. The reaction conditions were set as following: 94 $^{\circ}\text{C}$ for 5 min for denaturation, and 35 cycles of denaturation at 94 $^{\circ}\text{C}$ for 45 s followed by 55 $^{\circ}\text{C}$ for 45 s, and 72 $^{\circ}\text{C}$ for 1 min and final extension at 72 $^{\circ}\text{C}$ for 10 min. The conditions for the secondary PCR were identical to the primary PCR. The secondary PCR products were visualized on a 2.0% agarose gel with GoldViewTM (Solarbio, China) stained, using a UV transilluminator.

2.5. Sequencing and phylogenetic analysis

Positive products were sent the Sino Geno Max Company (Beijing, China) for sequencing. Mixed *E. bieneusi*, were detected by the presence of double peaks at the ITS region of the obtained chromatograms. Sequences were assembled using Seqman 7.1.0 software. The nucleotide sequences and reference sequences from NCBI database were aligned by ClustalX to identify the genotypes. The ITS sequences of *E. bieneusi* in this study and representative sequences available in GenBank database were used for phylogenetic analyses. Phylogenetic tree was constructed by MEGA 7.0 using neighbor-joining (NJ) method in the Kimura 2-parameter model with 1000 bootstrap replicates.

2.6. Statistical analysis

SPSS 20.0 was used for statistical analysis to compare differences in prevalence between age, gender, species and different places. The results were considered statistically significant when $P < 0.05$ under the chi-squared test.

2.7. Nucleotide sequence accession numbers

Unique sequences in this study were submitted to NCBI database under accession numbers MK965088-MK965095.

3. Results

3.1. Prevalence of *E. bieneusi*

The overall positive rate was 9.4%, and the infection rate of *Rhinopithecus*, *Macaque* and *Colobus* was 17.1%, 7.62% and 12.5%, respectively (Table 1). The infection rate in *Rhinopithecus* was significantly higher than in *Macaque* ($P < 0.05$). Age information of some specimens were known. The infection rate was 5.6% (4/71) in adult NHPs and 6.7% (2/30) in juvenile NHPs. Infection rates in juvenile NHPs were statistically higher than adult NHPs. Among the 3 provinces, the highest infection rate was discovered in Shennongjia (10/68, 14.7%), followed

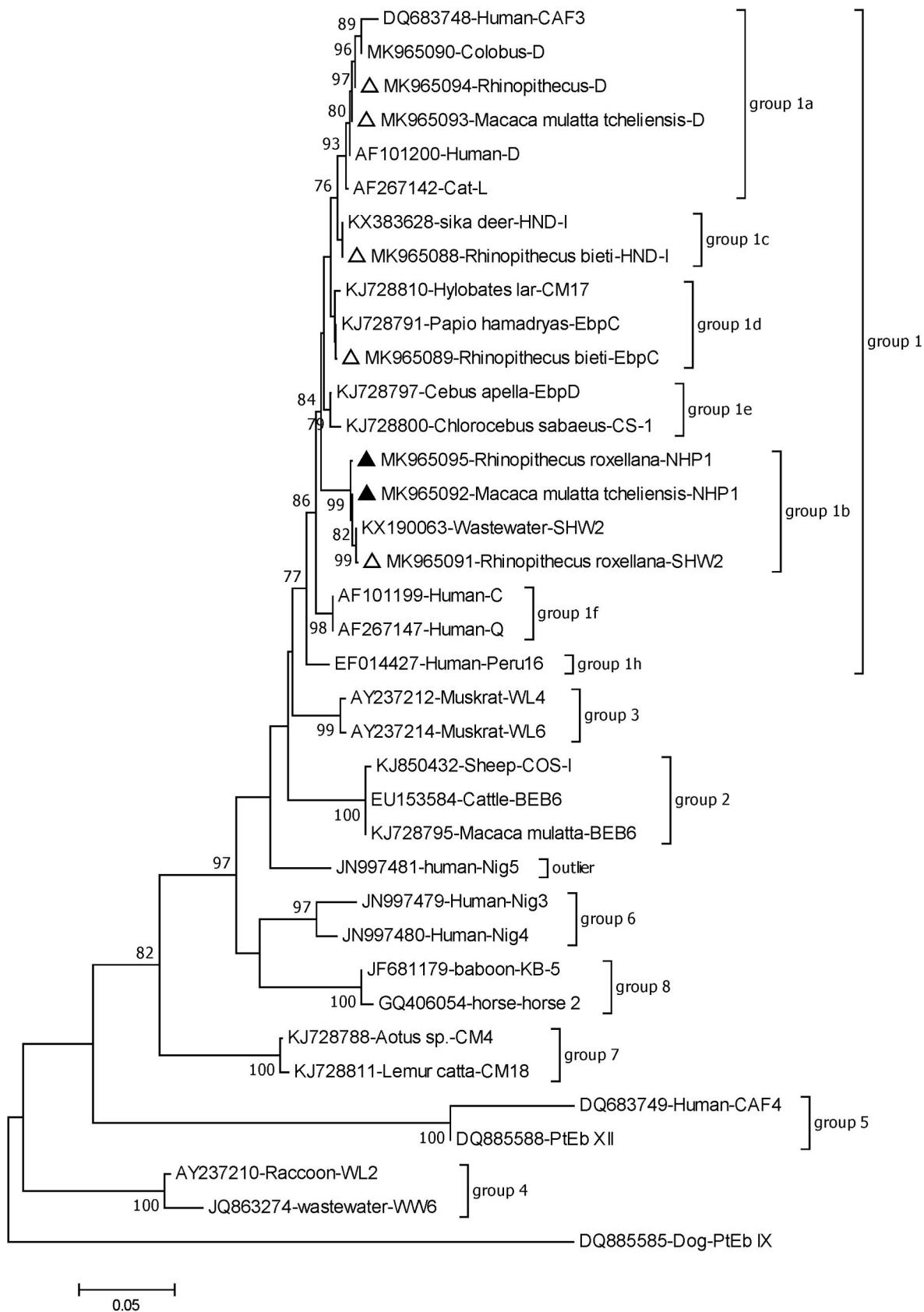


Fig. 1. Phylogenetic tree of *E. bieneusi* genotypes identified in this study and known genotypes based on the Neighbor-Joining analysis of the internal transcribed spacer of the rRNA gene. The numbers on the branches represent percent bootstrapping values from 500 replicates, with values of >70.0% shown in the tree. The genotypes identified in this study were marked, with \triangle for known genotypes and \blacktriangle for the novel genotypes.

by Beijing zoo (10/126, 7.9%) and Wulongkou (8/105, 7.6%). No significant differences were observed in the three provinces. The infection rate was 10.0% (16/160) in captive NHPs and 8.63% (12/139) in wild NHPs. The differences of infection rate in captive and wild NHPs were not statistically significant ($P > 0.05$). Five of 55 specimens (9.1%) from male, seven of 61 specimens (11.5%) from female were positive. The differences of infection rates in gender were not significant ($P > 0.05$).

According to ITS sequence analysis, 5 genotypes were found in this study, including 4 known genotypes (D, HND-I, EbpC, SHW2) and a novel genotype, named NHP1. No occurrence of mixed infections was identified. In Northern of China, three genotypes (D, HND-I and EbpC) were identified. Genotype SHW2, D and NHP1 were found in Central China (Table 2). The most common known genotype was genotype D ($n = 8$) which was detected in captive *Colobus*, *Rhinopithecus* and wild Taihangshan macaques (*Macaca mulatta tcheliensis*). Genotype EbpC ($n = 6$) and HND-I ($n = 2$) were found in captive *Rhinopithecus bieti*. SHW2 ($n = 4$) was detected in captive *Rhinopithecus roxellanae*. The novel genotype ($n = 8$) was discovered in captive and wild *R. roxellanae* and Taihangshan macaques.

3.2. Phylogenetic analysis

Phylogenetic tree was constructed based on ITS sequence. All ITS genotypes found in this study were classified into zoonotic Group 1. Further, Genotype D, HND-I and EbpC belong to subgroup 1a, 1c and 1d respectively. SHW2 and NHP1 belong to subgroup 1b. The novel genotype was included in the Group 1b (Fig. 1). The novel genotype had 98.2% identities compared to genotype Type IV(MF693832) with 7 single nucleotide polymorphisms at position 43 (an A to T change), 140 (C to T), 173 (C to T), 190 (G to A), 225 (T to C), 259 (G to A), 302 (G to A).

4. Discussion

Enterocytozoon bienersi is a common parasitic pathogen and has been found in many hosts. The overall infection rates of 11.4%–30.6% were recorded in different NHPs in China (Karim et al., 2014a, 2014b, 2015; Du et al., 2015; Li et al., 2017; Zhao et al., 2020; Zhong et al., 2017). Similar result was found in Central African Republic (13.9%) and Rwanda (11.0%) (Sak et al., 2013, 2014; Mynarova et al., 2016). Similar with previous study, the infection rates of different NHPs were 9.4% in this study. However, different from researches in Asian, infection rates of NHPs in Europe varied from 2.0% to 2.7% (Koster et al., 2021; Sak et al., 2011). The difference in prevalence may result from different ecological environments, feeding management, and NHPs species. Captive NHPs (10.0%) showed a higher rate of *E. bienersi* infection compared with wild NHPs (8.6%). In some research, the prevalence of *E. bienersi* in captive NHPs was statistically higher than in free-range (Karim et al., 2014a; Zhong et al., 2017). The different infection rates of captive and wild NHPs are probably influenced by source, number and region of specimens. In Karim's study, the prevalence of *E. bienersi* was significantly higher in captive NHPs (13.7%) than in free-range animals (5.0%) (Karim et al., 2015). In captive animals, the infection rate at research laboratories (26.5%) was higher than those at monkey farms (13.0%) and zoos (7.4%) (Robiul et al., 2014). These results suggest that feeding densities may cause the higher prevalence of *E. bienersi*, for the constant availability of susceptible animals and crowding in captivity. As for the region, a significantly higher infection rate was detected in Southwest China (Zhong et al., 2017) but not in Central and Northern China. Similar with previous study (Karim et al., 2014a), *Rhinopithecus*, *Colobus* and *Macaque*, which all belong to *Cercopithecidae* family, were positive for *E. bienersi*. However, there was no positive specimens in *Hylobatidae*, *Lemuridae* and *Hominidae* family. Infection rates may be connected with the diversity of species. The small number of specimens in these families might be the reason for this result. Consistently with previous reports, juvenile NHPs have a significantly

higher infection rate than adult NHPs (Li et al., 2011; Ye et al., 2014; Yang et al., 2017).

In this study, four known genotypes and a novel genotype were found. Genotype D and novel genotype (NHP1) were most common, followed by EbpC, SHW2 and NHP1. Genotype D and EbpC were prevalent genotype worldwide and detected in many species including NHPs (Ye et al., 2012, 2014; Karim et al., 2014a). Zoonotic genotype D was widely distributed and showed predominance in various types of NHPs in many studies (Yu et al., 2017). The genotype SHW2 was previously detected in wastewater (Huang et al., 2017a), and was first found in captive *R. roxellanae* in this study. In captive *R. bieti*, genotype HND-I was found, which was first detected in sika deer (Huang et al., 2017b). The novel genotype NHP1 was detected in *R. roxellanae* and Taihangshan macaques in Central China. Phylogenetic analysis implied that five genotypes found in this study were classified into zoonotic Group 1. These findings imply that NHPs could act as potential reservoir hosts for zoonotic *E. bienersi*.

5. Conclusion

Our data provides new information about the prevalence and genotype distribution of *E. bienersi* in Northern and Central China. Four known genotypes (D, HND-I, EbpC, SHW2) and 1 novel genotype (NHP1) have been identified. All of them are zoonotic, which suggests *E. bienersi* in these genotypes might be spilled over from NHPs into humans, or vice versa. Widespread public awareness should be aroused, especially people work with NHPs, such as animal attendants, scientists, and veterinarians, to diminish the risk of cross-species transmission of *E. bienersi*.

Declaration of competing interest

All authors declare no conflicts of interest.

References

- Conteas, C.N., Berlin, O.G., Speck, C.E., Pandhumas, S.S., Lariviere, M.J., Fu, C., 1998. Modification of the clinical course of intestinal microsporidiosis in acquired immunodeficiency syndrome patients by immune status and anti-human immunodeficiency virus therapy. *Am. J. Trop. Med. Hyg.* 58, 555–558.
- Ding, S., Huang, W., Qin, Q., Tang, J., Liu, H., 2018. Genotype identification and phylogenetic analysis of *Enterocytozoon bienersi* isolates from stool samples of diarrhetic children. *J. Parasitol.* 104, 297–301.
- Du, S.Z., Zhao, G.H., Shao, J.F., Fang, Y.Q., Tian, G.R., Zhang, L.X., Wang, R.J., Wang, H. Y., Qi, M., Yu, S.K., 2015. *Cryptosporidium* spp., *Giardia intestinalis*, and *Enterocytozoon bienersi* in captive non-human primates in Qinling Mountains. *Kor. J. Parasitol.* 53, 395–402.
- Green, L.C., Didier, P.J., Bowers, L.C., Didier, E.S., 2004. Natural and experimental infection of immunocompromised rhesus macaques (*Macaca mulatta*) with the microsporidian *Enterocytozoon bienersi* genotype D. *Microb. Infect.* 6, 996–1002.
- Guo, Y., Alderisio, K.A., Yang, W., Cama, V., Feng, Y., Xiao, L., 2014. Host specificity and source of *Enterocytozoon bienersi* genotypes in a drinking source watershed. *Appl. Environ. Microbiol.* 80, 218–225.
- Huang, C., Hu, Y., Wang, L., Wang, Y., Li, N., Guo, Y., Feng, Y., Xiao, L., 2017a. Environmental Transport of Emerging Human-Pathogenic Cryptosporidium Species and Subtypes through Combined Sewer Overflow and Wastewater. *Appl. Environ. Microbiol.* 83, e682–617.
- Huang, J., Zhang, Z., Yang, Y., Wang, R., Zhao, J., Jian, F., Ning, C., Zhang, L., 2017b. New genotypes of *Enterocytozoon bienersi* isolated from sika deer and red deer in China. *Front. Microbiol.* 8, 879.
- Jedrzejewski, S., Graczyk, T.K., Slodkiewicz-Kowalska, A., Tamang, L., Majewska, A.C., 2007. Quantitative assessment of contamination of fresh food produce of various retail types by human-virulent microsporidian spores. *Appl. Environ. Microbiol.* 73, 4071–4073.
- 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., 2015. Predominance and new genotypes of *Enterocytozoon bienersi* in captive nonhuman primates in zoos in China: high genetic diversity and zoonotic significance. *PLoS One* 10, e0117991.
- Karim, M.R., Rume, F.I., Rahman, A., Zhang, Z., Li, J., Zhang, L., 2020. Evidence for zoonotic potential of *Enterocytozoon bienersi* in its first molecular characterization in captive mammals at Bangladesh National zoo. *J. Eukaryot. Microbiol.* 67, 427–435.
- Karim, M.R., Wang, R., Dong, H., Zhang, L., Li, J., Zhang, S., Rume, F.I., Qi, M., Jian, F., Sun, M., Yang, G., Zou, F., Ning, C., Xiao, L., 2014a. Genetic polymorphism and

- zoonotic potential of *Enterocytozoon bienewisi* from nonhuman primates in China. *Appl. Environ. Microbiol.* 80, 1893–1898.
- Karim, M.R., Wang, R., He, X., Zhang, L., Li, J., Rume, F.I., Dong, H., Qi, M., Jian, F., Zhang, S., Sun, M., Yang, G., Zou, F., Ning, C., Xiao, L., 2014b. Multilocus sequence typing of *Enterocytozoon bienewisi* in nonhuman primates in China. *Vet. Parasitol.* 200, 13–23.
- Koster, P.C., Dashti, A., Bailo, B., Muadica, A.S., Maloney, J.G., Santin, M., Chicharro, C., Miguelanez, S., Nieto, F.J., Cano-Terriza, D., Garcia-Bocanegra, I., Guerra, R., Ponce-Gordo, F., Calero-Bernal, R., Gonzalez-Barrio, D., Carmena, D., 2021. Occurrence and genetic diversity of Protist parasites in captive non-human primates, zookeepers, and free-living sympatric rats in the Cordoba zoo conservation centre. *South. Spain. Anim. (Basel)* 11.
- Li, J., Dong, H., Wang, R., Yu, F., Wu, Y., Chang, Y., Wang, C., Qi, M., Zhang, L., 2017. An investigation of parasitic infections and review of molecular characterization of the intestinal protozoa in nonhuman primates in China from 2009 to 2015. *Int. J. Parasitol. Parasites Wildl.* 6, 8–15.
- Li, W., Deng, L., Yu, X., Zhong, Z., Wang, Q., Liu, X., Niu, L., Xie, N., Deng, J., Lei, S., Wang, L., Gong, C., Zhou, Z., Hu, Y., Fu, H., Xu, H., Geng, Y., Peng, G., 2016. Multilocus genotypes and broad host-range of *Enterocytozoon bienewisi* in captive wildlife at zoological gardens in China. *Parasites Vectors* 9, 395.
- Li, W., Feng, Y., Santin, M., 2019. Host specificity of *Enterocytozoon bienewisi* and public health implications. *Trends Parasitol.* 35, 436–451.
- Li, W., Kiulia, N.M., Mwenda, J.M., Nyachio, A., Taylor, M.B., Zhang, X., Xiao, L., 2011. *Cyclospora papionis*, *Cryptosporidium hominis*, and human-pathogenic *Enterocytozoon bienewisi* in captive baboons in Kenya. *J. Clin. Microbiol.* 49, 4326–4329.
- Mansfield, K.G., Carville, A., Hebert, D., Chalifoux, L., Shvetz, D., Lin, K.C., Tzipori, S., Lackner, A.A., 1998. Localization of persistent *Enterocytozoon bienewisi* infection in normal rhesus macaques (*Macaca mulatta*) to the hepatobiliary tree. *J. Clin. Microbiol.* 36, 2336–2338.
- Mynarova, A., Foitova, I., Kvac, M., Kvetonova, D., Rost, M., Morrogh-Bernard, H., Nurcahyo, W., Nguyen, C., Supriyadi, S., Sak, B., 2016. Prevalence of *cryptosporidium* spp., *Enterocytozoon bienewisi*, *Encephalitozoon* spp. and *Giardia intestinalis* in wild, semi-wild and captive orangutans (*Pongo abelii* and *Pongo pygmaeus*) on Sumatra and Borneo, Indonesia. *PLoS One* 11, e0152771.
- Robiul, K.M., Rongjun, W., Haiju, D., Longxian, Z., Jian, L., Sumei, Z., Islam, R.F., Meng, Q., Fuchun, J., Mingfei, S., Guangyou, Y., Fengcai, Z., Changshen, N., Lihua, X., 2014. Genetic polymorphism and zoonotic potential of *Enterocytozoon bienewisi* from nonhuman primates in China. *Appl. Environ. Microbiol.* 80.
- Sak, B., Kvac, M., Petzelkova, K., Kvetonova, D., Pomajbikova, K., Mulama, M., Kiyang, J., Modry, D., 2011. Diversity of microsporidia (Fungi: microsporidia) among captive great apes in European zoos and African sanctuaries: evidence for zoonotic transmission? *Folia Parasitol.* 58, 81–86.
- Sak, B., Petzelkova, K.J., Kvetonova, D., Mynarova, A., Pomajbikova, K., Modry, D., Cranfield, M.R., Mudakikwa, A., Kvac, M., 2014. Diversity of microsporidia, *cryptosporidium* and *Giardia* in mountain gorillas (*Gorilla beringei beringei*) in Volcanoes National park, Rwanda. *PLoS One* 9, e109751.
- Sak, B., Petzelkova, K.J., Kvetonova, D., Mynarova, A., Shutt, K.A., Pomajbikova, K., Kalousova, B., Modry, D., Benavides, J., Todd, A., Kvac, M., 2013. Long-term monitoring of microsporidia, *cryptosporidium* and *Giardia* infections in western lowland gorillas (*Gorilla gorilla gorilla*) at different stages of habituation in dzanga Sangha Protected areas, Central African Republic. *PLoS One* 8, e71840.
- Slodkiewicz-Kowalska, A., Graczyk, T.K., Tamang, L., Girouard, A.S., Majewska, A.C., 2007. Asymptomatic *Enterocytozoon bienewisi* microsporidiosis in captive mammals. *Parasitol. Res.* 100, 505–509.
- Song, Y., Li, W., Liu, H., Zhong, Z., Luo, Y., Wei, Y., Fu, W., Ren, Z., Zhou, Z., Deng, L., Cheng, J., Peng, G., 2018. First report of *Giardia duodenalis* and *Enterocytozoon bienewisi* in forest musk deer (*Moschus berezovskii*) in China. *Parasites Vectors* 11, 204.
- Tang, C., Cai, M., Wang, L., Guo, Y., Li, N., Feng, Y., Xiao, L., 2018. Genetic diversity within dominant *Enterocytozoon bienewisi* genotypes in pre-weaned calves. *Parasites Vectors* 11, 170.
- Yang, H., Lin, Y., Li, Y., Song, M., Lu, Y., Li, W., 2017. Molecular characterization of *Enterocytozoon bienewisi* isolates in laboratory macaques in north China: zoonotic concerns. *Parasitol. Res.* 116, 2877–2882.
- Ye, J., Xiao, L., Li, J., Huang, W., Amer, S.E., Guo, Y., Roellig, D., Feng, Y., 2014. Occurrence of human-pathogenic *Enterocytozoon bienewisi*, *Giardia duodenalis* and *Cryptosporidium* genotypes in laboratory macaques in Guangxi, China. *Parasitol. Int.* 63, 132–137.
- 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.
- Yu, F., Wu, Y., Li, T., Cao, J., Wang, J., Hu, S., Zhu, H., Zhang, S., Wang, R., Ning, C., Zhang, L., 2017. High prevalence of *Enterocytozoon bienewisi* zoonotic genotype D in captive golden snub-nosed monkey (*Rhinopithecus roxellanae*) in zoos in China. *BMC Vet. Res.* 13, 158.
- Zhao, W., Zhou, H., Jin, H., Sun, L., Li, P., Liu, M., Qiu, M., Xu, L., Li, F., Ma, T., Wang, S., Yin, F., Li, L., Cui, X., Chan, J.F., Lu, G., 2020. Genotyping of *Enterocytozoon bienewisi* among captive long-tailed macaques (*Macaca fascicularis*) in Hainan Province: high genetic diversity and zoonotic potential. *Acta Trop.* 201, 105211.
- Zhong, Z., Li, W., Deng, L., Song, Y., Wu, K., Tian, Y., Huang, X., Hu, Y., Fu, H., Geng, Y., Ren, Z., Peng, G., 2017. Multilocus genotyping of *Enterocytozoon bienewisi* derived from nonhuman primates in southwest China. *PLoS One* 12, e0176926.
- Zou, Y., Hou, J.L., Li, F.C., Zou, F.C., Lin, R.Q., Ma, J.G., Zhang, X.X., Zhu, X.Q., 2018. Prevalence and genotypes of *Enterocytozoon bienewisi* in pigs in southern China. *Infect. Genet. Evol.* 66, 52–56.