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Short communication

# Identification of *Enterocytozoon bieneusi* and *Cryptosporidium* spp. in farmed wild boars (*Sus scrofa*) in Beijing, China

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ABSTRACT

*Enterocytozoon bieneusi* and *Cryptosporidium* spp. are opportunistic pathogen that can infected humans and other animals. However, the data on the prevalence and genotypes of the parasites in captive wild boars is not available in Beijing, China. In this study, a total of 257 fecal specimens of wild boars were collected. The overall prevalence of *E. bieneusi* and *Cryptosporidium* spp. was 42.0% (108/257) and 5.8%, respectively. Higher infection rate of *E. bieneusi* was discovered in the wild boar ≤2 months old (58.3%). The differences between the feeding pattern and gender were not significant. Furthermore, eight genotypes of *E. bieneusi* were determined by analyzing the internal transcribed spacer (ITS) of the rRNA gene, including seven known genotypes and one novel genotype. Phylogenetic analysis revealed that all the eight genotypes belonged to the zoonotic potential Group 1. For *Cryptosporidium* spp., no significant differences were found between groups of gender, age and feeding pattern. Only *C. scrofarum* was identified in the investigated samples. The findings suggest that wild boar could be reservoirs of *E. bieneusi* and *C. scrofarum* which could be potentially transmitted to humans and other animals.

## 1. Introduction

Microsporidiosis and Cryptosporidiosis caused by *Enterocytozoon bieneusi* and *Cryptosporidium* spp., are emerging infectious diseases in humans and animals (Feng et al., 2018; Li et al., 2019). Infection occurs when the food or water contaminated with the spores and oocyst are ingested. The symptoms of infected host may present from mild to severe, depending on their health level (Li et al., 2012). It is noteworthy that the parasites can cause life-threatening diarrhea in immunocompromised individuals (Checkley et al., 2015; Wang et al., 2013b).

Because of the difficulty of morphological differentiation, molecular methods have become the ideal method for characterizing the genotypes of *E. bieneusi* (Santin and Fayer, 2011). To date, approximately 500 genotypes of *E. bieneusi* have been confirmed in humans, livestock, companion animals, wildlife and water source worldwide through analysis of the ribosomal internal transcribed spacer (ITS) sequences (Li et al., 2019). The known genotypes are divided into 11 groups, group1–11 (Li et al., 2019). Of the 11 groups, the group 1 is considered zoonotic and the remaining group 2–11 mostly contain host-specific genotypes (Li et al., 2019; Li et al., 2015; Yang et al., 2016).

Currently, at least 38 species of *Cryptosporidium* have been identified humans, animals and environment (Efstratiou et al., 2017; Khan et al., 2018). Though most *Cryptosporidium* species/genotypes have host specificity, some *Cryptosporidium* species/genotypes can be found in other hosts once in a while (Khan et al., 2018). For example, *C. canis* are almost detected in dogs, however, the parasite was also isolated from children (Xiao et al., 2007), which confer it with zoonotic significance.

*E. bieneusi* and *Cryptosporidium* spp. have been reported in various hosts worldwide. However, the information concerning *E. bieneusi* and *Cryptosporidium* spp. circulating among wild boar populations in northern China is generally scarce. To our knowledge, only captive wild boars from Sichuan, a province in southern China, have been reported to be infected with *E. bieneusi*. and *C. scrofarum* (Li et al., 2017). The objective of the present study was to determine the prevalence and genetic diversity of *E. bieneusi* and *Cryptosporidium* spp. in farmed wild boars from Beijing, China.

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**Table 1**
Primers used for identification of *E. bieneusi* and *Cryptosporidium* spp. in the present study.

| Parasite | Primer | Sequence (5′-3′c) | Reference |
|---|---|---|---|
| *E. bieneusi* | EBITS3 | GGTCATAGGGATGAAGAG | Li et al. (2017) |
| | EBITS4 | TTCGAGTTCTTTCGCGCTC | |
| | EBITS1 | GCTCTGAATATCTATGGCT | |
| | EBITS2.4 | ATCGCCGACGGATCCAAGTG | |
| *Cryptosporidium* spp. | XF2f | GGAAGGGTTGTATTTATTAGATAAAG | Xiao et al. (2001) |
| | XF2r | AAGGAGTAAGGAACAACCTCCA | |
| | pSSUf | AAAGCTCGTAGTTGGATTTCTGTT | |
| | pSSUr | ACCTCTGACTGTTAAATACRAATGC | |

**Table 2**
The prevalence of *E. bieneusi* in wild boars in Beijing, China.

| Factors | Category | No. tested | No. positive | Prevalence (%) (95%CI) | *P*-value |
|---|---|---|---|---|---|
| Feeding pattern | Semi-captivity | 92 | 41 | 44.6% (34.2–54.9) | 0.538 |
| | Captivity | 165 | 67 | 40.6% (33.0–48.2) | |
| Age | ≤ 2 months | 108 | 63 | 58.3% (48.9–67.8) | 0.000 |
| | > 2 months | 149 | 45 | 30.2% (22.7–37.7) | |
| Gender | Female | 118 | 56 | 47.5% (38.3–56.6) | 0.104 |
| | Male | 139 | 52 | 37.4% (29.3–45.6) | |
| Total | | 257 | 108 | 42.0% (35.9–48.1) | |

**Table 3**
Distribution of *E. bieneusi* in wild boars in the present study.

| Factors | Category | Genotypes(n) |
|---|---|---|
| Feeding pattern | Semi-captivity | CAM5(2); wildboar12(4); CM8(1); CTS3(4); EbpC(12); Henan-IV(3); pigEBITS5(12) |
| | Captivity | CAM5(3); wildboar12(1); CM8(7); CTS3(10); EbpC(23); Henan-IV(8); pigEBITS5(6); CHS12(9) |
| Age | ≤ 2 months | CAM5(3); wildboar12(2); CHS12(7); CM8(6); CTS3(9); EbpC(17); Henan-IV(7); pigEBITS5(10) |
| | > 2 months | CAM5(2); wildboar12(3); CHS12(2); CM8(2); CTS3(5); EbpC(18); Henan-IV(4); pigEBITS5(8); |
| Gender | Female | CAM5(1); wildboar12(4); CHS12(5); CM8(3); CTS3(7); EbpC(22); Henan-IV(4); pigEBITS5(8); |
| | Male | CAM5(4); wildboar12(1); CHS12(4); CM8(5); CTS3(7); EbpC(13); Henan-IV(7); pigEBITS5(10) |
| Total | | CAM5(5); wildboar12(5); CHS12(9); CM8(8); CTS3(14); EbpC(35); Henan-IV(11); pigEBITS5(18) |

## 2. Materials and methods

### 2.1. Ethics statement

This study was conducted in accordance with the Guidelines for the Care and Use of Animals in Research, which are issued by the Institute of Zoology, Chinese Academy of Sciences. This work was reviewed and approved by the Animal Ethics Committee of the Institute of Zoology, Chinese Academy of Sciences. For sample collection, we got permission from animal owners.

### 2.2. Sample collection

The wild boars included in this study belonged to three farms (A, B and C) located in different regions of Beijing, China. There are two feeding patterns in the three farms. Wild boars from farm A and C were kept in pigsty, while wild boars from farm B were raised in an outdoor enclosure which is about 0.5 ha. With the help of the breeders, the age and gender of the wild boar being sampled were recorded.

A total of 257 fresh fecal samples were obtained in randomly selected wild boar farms from September 2015 to November 2018 in Beijing area, China. Briefly, the top layer of the feces was collected immediately after being defecated on the ground with a sterile separate container, numbered, recorded the age and gender of the individual by asking the breeder. The samples were put into box filled with ice packs, and transported to laboratory within two hours. All the wild boars were apparently healthy, and no diarrhea was observed during the collection.

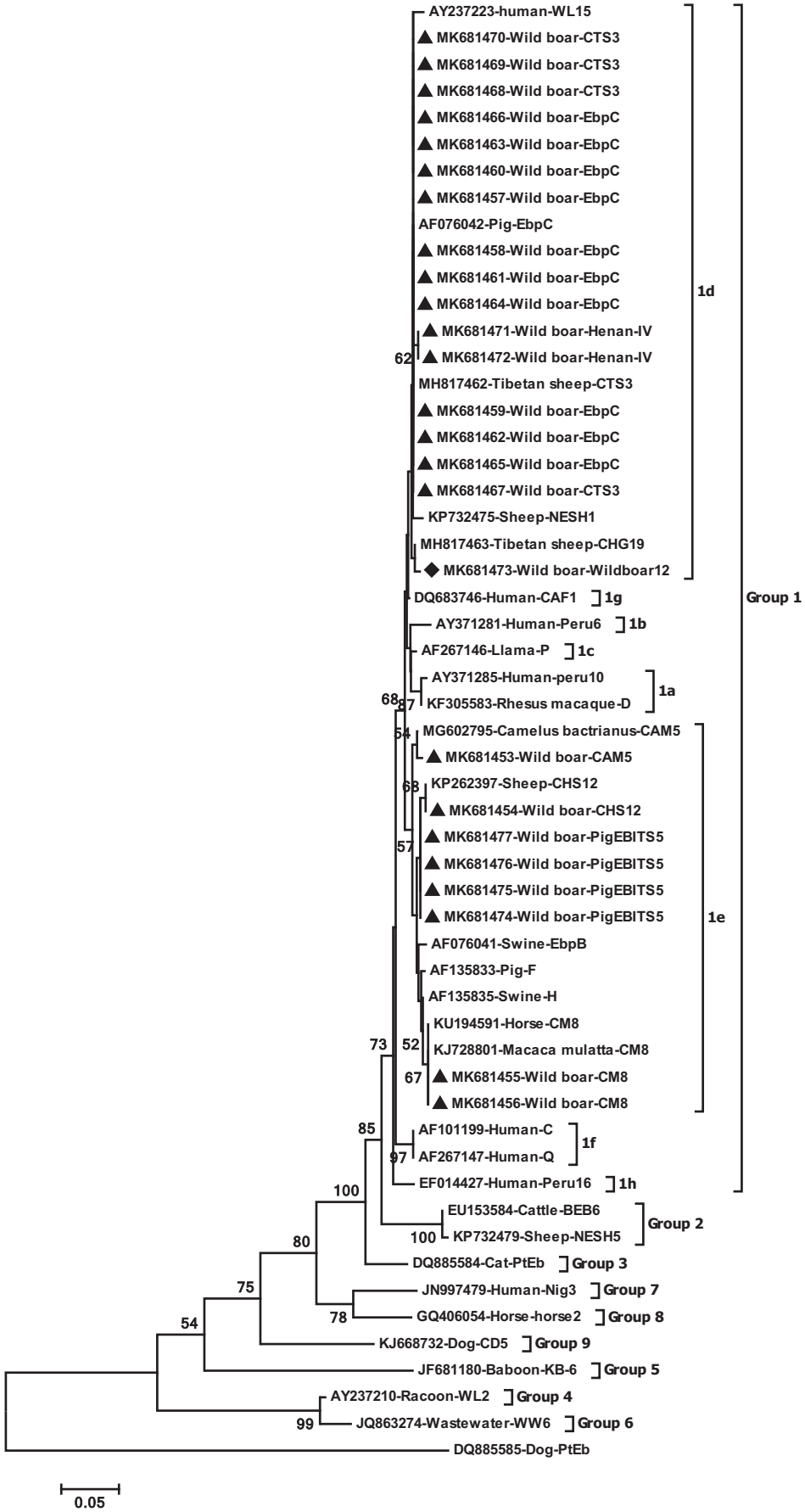
### 2.3. DNA extraction and detection of E. bieneusi and Cryptosporidium spp.

Fecal genomic DNA was extracted from 200 mg of each sample with the E.Z.N.A.® Stool DNA Kit (Omega Biotek Inc., Norcross, USA) according to the manufacturer's recommendations. And the extracted DNA was stored at −20 °C until further PCR assay. The presence of *E. bieneusi* and *Cryptosporidium* spp. were determined by amplifying the internal transcribed spacer (ITS) and the small subunit (SSU) rRNA genes according to studies by (Li et al., 2017) and (Xiao et al., 2001) respectively. All the primers used in this study were listed in Table 1. Positive control (PCR template from *E. bieneusi* genotype D and *C. baileyi*) and negative control (reagent-grade water) were included in each run to ensure the accuracy of the results. The secondary PCR products were detected by 2% agarose gel electrophoresis with GoldView™ (Solarbio, China) stained.

### 2.4. Genotyping and phylogenetic analysis

The second PCR-positive samples were sequenced from both directions by the Sino Geno Max Company (Beijing, China). Three sequencing repeats were conducted for each sample. Chromatograms of the forward sequences and reverse sequences were confirmed and the sequences were manually assembled with Lasergene SeqMan software (DNASTAR, Madison, Wisconsin, USA). The sequences obtained in the present study were compared with the reference sequences downloaded from the GenBank database using the ClustalX 1.83 software package to determine the genotypes/species of *E. bieneusi* and *Cryptosporidium* spp.

Phylogenetic tree was constructed under MEGA 7.0 (Kumar et al., 2016) with the Neighbor-joining algorithm in a Kimura2-parameter model, and the branch reliability was analyzed using a bootstrap of




*(caption on next page)*

**Fig. 1.** Phylogenetic analysis of ITS nucleotide sequences of *Enterocytozoon bieneusi* obtained in this study and reference genotypes. The phylogenetic tree was constructed with Neighbor-Joining method under the Kimura 2-parameter model. Bootstrap values > 50% from 1000 replicates are shown on the nodes. The *E. bieneusi* genotype PtEb (DQ885585) was used as outgroup. The genotypes detected in the current study are labeled with solid triangle.

1,000 replicates (Zhang et al., 2018).

Nucleotide sequences generated in this study were submitted to GenBank under accession numbers MK681453-MK681477 (*E. bieneusi*) and MN453368-MN453371 (*Cryptosporidium* spp.).

### 2.5. Statistical analysis

The $\chi 2$ test was used to compare the infection rates between feeding pattern, gender and age. Differences were considered to be statistically significant at $P < .05$.

## 3. Results and discussion

*E. bieneusi* and *Cryptosporidium* spp. can infect a broad range of host, including domestic animals, wildlife and humans worldwide (Ghoyounchi et al., 2017; Lesnianska and Perec-Matysiak, 2017; Qiu et al., 2019). In the present study, the epidemiology of the two parasites in farmed wild boar from Beijing area was first investigated.

Overall, the infection rate was 42.0% (108/149) (Table 2), which was similar to Sichuan Province, China (Li et al., 2017). However, the prevalence was much higher than that in central European countries (Nemejc et al., 2014), which suggests that *E. bieneusi* is more prevalent in wild boar in China. The size of living area of the wild boar between China and Central European countries may be the causation of the difference, because wild boars from central European countries inhabited natural surroundings, while wild boars from China were kept in captivity in a certain area.

Thus, the wild boar in captivity has a greater chance to contact the contaminated environment. On the other hand, wild boars in the natural surroundings may have evolved stronger immune systems, making them stronger resistance to *E. bieneusi*. A higher prevalence was observed in young piglets (< 2 month) (Table 2), which might be caused by their naive immune status and stress during weaning process. No significant difference in the infection rate was found between gender groups and feeding patterns (Table 2).

In the present study, 108 out of 111 *E. bieneusi*-positive samples were successfully sequenced. A total of 8 distinct genotypes were identified by analyzing the ITS sequences, including 7 known genotypes (CAM5, CHS12, CM8, CTS3, EbpC, Henan-IV and pigEBITS5) and one novel genotype, termed wildboar12 (Table 3). The predominant known genotype was EbpC ($n = 35$), followed by PigEBITS5 ($n = 18$), CTS3 ($n = 14$), Hennan-IV ($n = 11$), CHS12 ($n = 9$), CM8 ($n = 8$) and CAM5 ($n = 5$). Moreover, the genotype EbpC was also the most frequent in wild boars in the studies conducted by Nemejc et al. (2014) and Li et al. (2017), indicating that wild boar might be susceptible to this genotype regardless of from captivity or wild. Other host, such as HIV-positive/negative patients, non-human primates, Bactrian camels, cattle, sika deer, horse, minks, foxes and pig infected with EbpC have also been reported (Hu et al., 2017; Karim et al., 2015; Li et al., 2014; Liu et al., 2017; Qi et al., 2018; Qi et al., 2016; Shi et al., 2016; Wang et al., 2013a; Zhang et al., 2016; Zhang et al., 2018; Zhao et al., 2015). The genotype PigEBITS5 were mainly detected in humans (Leelayoova et al., 2006), Non-human primates (Karim et al., 2015), pigs (Wang et al., 2018), dogs (Karim et al., 2014) and mice (Sak et al., 2011). Advertently, the genotype Henan-IV has been found in Children patients (Yang et al., 2014) and diarrheic Chicken (Li et al., 2014), suggesting its stronger pathogenicity. Besides Henan-IV, other genotypes, such as CAM5, CHS12, CM8 and CTS3 were first confirmed in wild boars in the present study, and their danger to the hosts should be further evaluated.

Phylogenetic tree was constructed with the genotypes found in this study and other reference sequences. The four known genotypes (EbpC, CTS3, Hennan-IV and CHG19) as well as the novel genotype obtained in the present study were grouped into group 1d. Likewise, the genotype CAM5, CHS12, CM8 and pigITS5 fell into the genotype 1e (Fig. 1). Both group 1d and 1e are the subgroup of group 1, the major zoonotic potential group (Widmer and Akiyoshi, 2010). Which suggests that wild boars are potential source of human microsporidiosis. Breeders of captive wild boar, who are in close contact with wild boar during feeding and nursing wild boars as well as cleaning the pigsty, are more likely exposed to infection. And whether the *E. bieneusi* carried by wild boar can be transmitted to humans should be further confirmed.

For *Cryptosporidium* spp., the overall prevalence was 5.8% (15/257), which was similar to the studies performed by Nemejc et al. (2013) and Nemejc et al. (2012). Higher infection rate was observed in Spain (Garcia-Presedo et al., 2013), Czech Republic (Castro-Hermida et al., 2011) and Austria (Nemejc et al., 2012), while lower prevalence was detected in Poland (Paziewska et al., 2007) and southern China (Li et al., 2017). Many factors may contribute to the differences, such as management level, detection methods and climate. Moreover, only *C. scrofarum* was identified in the present study, suggesting a lower diversity of *Cryptosporidium* species in wild boars in Beijing area. On the one hand, it is possible that more than one species was identified but only the dominant species was confirmed by molecular methods (Garcia-Presedo et al., 2013). On the other hand, the wild boars in this study may have little contact with other infected animals, which resulted in low diversity of *Cryptosporidium* infection. *C. scrofarum* has been detected in humans (Ryan et al., 2017) and domestic animals (Zahedi et al., 2018) and surface water (Xiao et al., 2012), suggesting the transmission potential of *C. scrofarum* from wild boar to humans or other animals.

## 4. Conclusions

In this study, the prevalence and the involved genotype/species distribution of *E. bieneusi* and *Cryptosporidium* spp. in farmed wild boar in northern China was first investigated. More significantly, all of the genotypes/species identified in the present study were potentially zoonotic. Thus, wild boar can be an important source of human infection with *E. bieneusi* and *C. scrofarum*. In order to know more about the epidemiology of *E. bieneusi* and *C. scrofarum* in wild boar population, a more comprehensive survey in captive and natural wild boar populations is needed.

## Declaration of Competing Interest

The authors have no conflict of interest.

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## References

Castro-Hermida, J.A., Garcia-Presedo, I., Gonzalez-Warleta, M., Mezo, M., 2011. Prevalence of *Cryptosporidium* and *Giardia* in roe deer (*Capreolus capreolus*) and wild boars (*Sus scrofa*) in Galicia (NW, Spain). Vet. Parasitol. 179, 216–219.

Checkley, W., White Jr., A.C., Jaganath, D., Arrowood, M.J., Chalmers, R.M., Chen, X.M., Fayer, R., Griffiths, J.K., Guerrant, R.L., Hedstrom, L., Huston, C.D., Kotloff, K.L., Kang, G., Mead, J.R., Miller, M., Petri Jr., W.A., Priest, J.W., Roos, D.S., Striepen, B., Thompson, R.C., Ward, H.D., Van Voorhis, W.A., Xiao, L., Zhu, G., Houpt, E.R., 2015. A review of the global burden, novel diagnostics, therapeutics, and vaccine targets for

*Cryptosporidium*. Lancet Infect. Dis. 15, 85–94.
Efstratiou, A., Ongerth, J.E., Karanis, P., 2017. Waterborne transmission of protozoan parasites: review of worldwide outbreaks-An update 2011-2016. Water Res. 114, 14–22.
Feng, Y., Ryan, U.M., Xiao, L., 2018. Genetic diversity and population structure of *Cryptosporidium*. Trends Parasitol. 34, 997–1011.
Garcia-Presedo, I., Pedraza-Diaz, S., Gonzalez-Warleta, M., Mezo, M., Gomez-Bautista, M., Ortega-Mora, L.M., Castro-Hermida, J.A., 2013. Presence of *Cryptosporidium scrofarum*, *C. suis* and *C. parvum* subtypes IIaA16G2R1 and IIaA13G1R1 in Eurasian wild boars (*Sus scrofa*). Vet. Parasitol. 196, 497–502.
Ghoyounchi, R., Ahmadpour, E., Spotin, A., Mahami-Oskouei, M., Rezamand, A., Aminisani, N., Ghojazadeh, M., Berahmat, R., Mikaeili-Galeh, T., 2017. Microsporidiosis in Iran: a systematic review and meta-analysis. Asian Pac J Trop Med 10, 322–330.
Hu, S., Liu, Z., Yan, F., Zhang, Z., Zhang, G., Zhang, L., Jian, F., Zhang, S., Ning, C., Wang, R., 2017. Zoonotic and host-adapted genotypes of *Cryptosporidium* spp., *Giardia duodenalis* and *Enterocytozoon bieneusi* in dairy cattle in Hebei and Tianjin, China. Vet. Parasitol. 248, 68–73.
Karim, M.R., Dong, H., Yu, F., Jian, F., Zhang, L., Wang, R., Zhang, S., Rume, F.I., Ning, C., Xiao, L., 2014. Genetic diversity in *Enterocytozoon bieneusi* isolates from dogs and cats in China: host specificity and public health implications. J. Clin. Microbiol. 52, 3297–3302.
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. 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.
Khan, A., Shaik, J.S., Grigg, M.E., 2018. Genomics and molecular epidemiology of *Cryptosporidium* species. Acta Trop. 184, 1–14.
Kumar, S., Stecher, G., Tamura, K., 2016. MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. Mol. Biol. Evol. 33, 1870–1874.
Leelayoova, S., Subrungruang, I., Suputtamongkol, Y., Worapong, J., Petmitr, P.C., Mungthin, M., 2006. Identification of genotypes of *Enterocytozoon bieneusi* from stool samples from human immunodeficiency virus-infected patients in Thailand. J. Clin. Microbiol. 44, 3001–3004.
Lesnianska, K., Perec-Matysiak, A., 2017. Wildlife as an environmental reservoir of *Enterocytozoon bieneusi* (microsporidia)-analyses of data based on molecular methods. Ann. Parasitol. 63, 265–281.
Li, W., Cama, V., Feng, Y., Gilman, R.H., Bern, C., Zhang, X., Xiao, L., 2012. Population genetic analysis of *Enterocytozoon bieneusi* in humans. Int. J. Parasitol. 42, 287–293.
Li, W., Tao, W., Jiang, Y., Diao, R., Yang, J., Xiao, L., 2014. Genotypic distribution and phylogenetic characterization of *Enterocytozoon bieneusi* in diarrheic chickens and pigs in multiple cities, China: potential zoonotic transmission. PLoS One 9, e108279.
Li, W., Li, Y., Song, M., Lu, Y., Yang, J., Tao, W., Jiang, Y., Wan, Q., Zhang, S., Xiao, L., 2015. Prevalence and genetic characteristics of *Cryptosporidium*, *Enterocytozoon bieneusi* and *Giardia duodenalis* in cats and dogs in Heilongjiang province, China. Vet. Parasitol. 208, 125–134.
Li, W., Deng, L., Wang, K., Huang, X., Song, Y., Su, H., Hu, Y., Fu, H., Zhong, Z., Peng, G., 2017. Presence of zoonotic *Cryptosporidium scrofarum*, *Giardia duodenalis* assemblage A and *Enterocytozoon bieneusi* genotypes in captive Eurasian wild boars (*Sus scrofa*) in China: potential for zoonotic transmission. Parasit. Vectors 10, 10.
Li, W., Feng, Y., Santin, M., 2019. Host specificity of *Enterocytozoon bieneusi* and public health implications. Trends Parasitol. 35, 436–451.
Liu, H., Jiang, Z., Yuan, Z., Yin, J., Wang, Z., Yu, B., Zhou, D., Shen, Y., Cao, J., 2017. Infection by and genotype characteristics of *Enterocytozoon bieneusi* in HIV/AIDS patients from Guangxi Zhuang autonomous region, China. BMC Infect. Dis. 17, 684.
Nemejc, K., Sak, B., Kvetonova, D., Hanzal, V., Jenikova, M., Kvac, M., 2012. The first report on *Cryptosporidium suis* and *Cryptosporidium* pig genotype II in Eurasian wild boars (*Sus scrofa*) (Czech Republic). Vet. Parasitol. 184, 122–125.
Nemejc, K., Sak, B., Kvetonova, D., Hanzal, V., Janiszewski, P., Forejtek, P., Rajsky, D., Ravaszova, P., McEvoy, J., Kvac, M., 2013. *Cryptosporidium suis* and *Cryptosporidium scrofarum* in Eurasian wild boars (*Sus scrofa*) in Central Europe. Vet. Parasitol. 197, 504–508.
Nemejc, K., Sak, B., Kvetonova, D., Hanzal, V., Janiszewski, P., Forejtek, P., Rajsky, D., Kotkova, M., Ravaszova, P., McEvoy, J., Kvac, M., 2014. Prevalence and diversity of *Encephalitozoon* spp. and *Enterocytozoon bieneusi* in wild boars (*Sus scrofa*) in Central Europe. Parasitol. Res. 113, 761–767.
Paziewska, A., Bednarska, M., Nieweglowski, H., Karbowiak, G., Bajer, A., 2007. Distribution of *Cryptosporidium* and *Giardia* spp. in selected species of protected and game mammals from North-Eastern Poland. Ann. Agric. Environ. Med. 14, 265–270.
Qi, M., Wang, R., Wang, H., Jian, F., Li, J., Zhao, J., Dong, H., Zhu, H., Ning, C., Zhang, L., 2016. *Enterocytozoon bieneusi* genotypes in grazing horses in China and their zoonotic transmission potential. J. Eukaryot. Microbiol. 63, 591–597.
Qi, M., Li, J., Zhao, A., Cui, Z., Wei, Z., Jing, B., Zhang, L., 2018. Host specificity of *Enterocytozoon bieneusi* genotypes in Bactrian camels (*Camelus bactrianus*) in China. Parasit. Vectors 11, 219.
Qiu, L., Xia, W., Li, W., Ping, J., Ding, S., Liu, H., 2019. The prevalence of microsporidia in China: a systematic review and meta-analysis. Sci. Rep. 9, 3174.
Ryan, U., Paparini, A., Oskam, C., 2017. New technologies for detection of enteric parasites. Trends Parasitol. 33, 532–546.
Sak, B., Kvac, M., Kvetonova, D., Albrecht, T., Pialek, J., 2011. The first report on natural *Enterocytozoon bieneusi* and *Encephalitozoon* spp. infections in wild East-European house mice (*Mus musculus musculus*) and West-European house mice (*M. m. domesticus*) in a hybrid zone across the Czech Republic-Germany border. Vet. Parasitol. 178, 246–250.
Santin, M., Fayer, R., 2011. Microsporidiosis: *Enterocytozoon bieneusi* in domesticated and wild animals. Res. Vet. Sci. 90, 363–371.
Shi, K., Li, M., Wang, X., Li, J., Karim, M.R., Wang, R., Zhang, L., Jian, F., Ning, C., 2016. Molecular survey of *Enterocytozoon bieneusi* in sheep and goats in China. Parasit. Vectors 9, 23.
Wang, L., Xiao, L., Duan, L., Ye, J., Guo, Y., Guo, M., Liu, L., Feng, Y., 2013a. Concurrent infections of *Giardia duodenalis*, *Enterocytozoon bieneusi*, and *Clostridium difficile* in children during a cryptosporidiosis outbreak in a pediatric hospital in China. PLoS Negl. Trop. Dis. 7, e2437.
Wang, L., Zhang, H.W., Zhao, X.D., Zhang, L.X., Zhang, G.Q., Guo, M.J., Liu, L.L., Feng, Y.Y., Xiao, L.H., 2013b. Zoonotic *Cryptosporidium* species and *Enterocytozoon bieneusi* genotypes in HIV-positive patients on antiretroviral therapy. J. Clin. Microbiol. 51, 557–563.
Wang, S.S., Li, J.Q., Li, Y.H., Wang, X.W., Fan, X.C., Liu, X., Li, Z.J., Song, J.K., Zhang, L.X., Zhao, G.H., 2018. Novel genotypes and multilocus genotypes of *Enterocytozoon bieneusi* in pigs in northwestern China: a public health concern. Infect. Genet. Evol. 63, 89–94.
Widmer, G., Akiyoshi, D.E., 2010. Host-specific segregation of ribosomal nucleotide sequence diversity in the microsporidian *Enterocytozoon bieneusi*. Infect. Genet. Evol. 10, 122–128.
Xiao, L., Bern, C., Limor, J., Sulaiman, I., Roberts, J., Checkley, W., Cabrera, L., Gilman, R.H., Lal, A.A., 2001. Identification of 5 types of *Cryptosporidium* parasites in children in Lima, Peru. J. Infect. Dis. 183, 492–497.
Xiao, L., Cama, V.A., Cabrera, L., Ortega, Y., Pearson, J., Gilman, R.H., 2007. Possible transmission of *Cryptosporidium canis* among children and a dog in a household. J. Clin. Microbiol. 45, 2014–2016.
Xiao, S., An, W., Chen, Z., Zhang, D., Yu, J., Yang, M., 2012. Occurrences and genotypes of *Cryptosporidium* oocysts in river network of southern-eastern China. Parasitol. Res. 110, 1701–1709.
Yang, J., Song, M., Wan, Q., Li, Y., Lu, Y., Jiang, Y., Tao, W., Li, W., 2014. *Enterocytozoon bieneusi* genotypes in children in Northeast China and assessment of risk of zoonotic transmission. J. Clin. Microbiol. 52, 4363–4367.
Yang, Z., Zhao, W., Shen, Y., Zhang, W., Shi, Y., Ren, G., Yang, D., Ling, H., Yang, F., Liu, A., Cao, J., 2016. Subtyping of *Cryptosporidium cuniculus* and genotyping of *Enterocytozoon bieneusi* in rabbits in two farms in Heilongjiang Province, China. Parasite 23, 52.
Zahedi, A., Monis, P., Gofton, A.W., Oskam, C.L., Ball, A., Bath, A., Bartkow, M., Robertson, I., Ryan, U., 2018. *Cryptosporidium* species and subtypes in animals inhabiting drinking water catchments in three states across Australia. Water Res. 134, 327–340.
Zhang, X.X., Cong, W., Liu, G.H., Ni, X.T., Ma, J.G., Zheng, W.B., Zhao, Q., Zhu, X.Q., 2016. Prevalence and genotypes of *Enterocytozoon bieneusi* in sika deer in Jilin province, Northeastern China. Acta Parasitol. 61, 382–388.
Zhang, X.X., Jiang, R.L., Ma, J.G., Xu, C., Zhao, Q., Hou, G., Liu, G.H., 2018. *Enterocytozoon bieneusi* in minks (*Neovison vison*) in Northern China: a public health concern. Front. Microbiol. 9, 1221.
Zhao, W., Zhang, W., Yang, Z., Liu, A., Zhang, L., Yang, F., Wang, R., Ling, H., 2015. Genotyping of *Enterocytozoon bieneusi* in farmed blue foxes (*Alopex lagopus*) and raccoon dogs (*Nyctereutes procyonoides*) in China. PLoS One 10, e0142611.