Molecular characterizations of Cryptosporidium spp. and Enterocytozoon bieneusi in brown rats (Rattus norvegicus) from Heilongjiang Province, China

Wei Zhao1,2, Jianguang Wang1, Guangxu Ren1, Ziyin Yang1, Fengkun Yang1, Weizhe Zhang1, Yingchu Xu2, Aiqin Liu1* and Hong Ling1,2,3*

Abstract

Background: Cryptosporidium spp. and Enterocytozoon bieneusi are prevalent zoonotic pathogens responsible for the high burden of diarrheal diseases worldwide. Rodents are globally overpopulated and are known as reservoirs or carriers of a variety of zoonotic pathogens including Cryptosporidium spp. and E. bieneusi. However, few data are available on genetic characterizations of both pathogens in rodents in China. The aim of the present work was to determine the prevalence and genetic characterizations of Cryptosporidium spp. and E. bieneusi in brown rats (Rattus norvegicus) from Heilongjiang, China.

Methods: A total of 242 wild brown rats were captured in Heilongjiang Province of China. A fresh fecal specimen was collected directly from the intestinal and rectal content of each brown rat. All the fecal specimens were examined for the presence of Cryptosporidium spp. and E. bieneusi by PCR and sequencing of the partial small subunit (SSU) rRNA gene and the internal transcribed spacer (ITS) region of the rRNA gene of the two pathogens, respectively.

Results: The infection rate was 9.1% (22/242) for Cryptosporidium spp. and 7.9% (19/242) for E. bieneusi. Sequence analysis confirmed the presence of C. ubiquitum (1/22, 4.5%) and three genotypes of Cryptosporidium, including Cryptosporidium rat genotype I (14/22, 63.6%), Cryptosporidium rat genotype IV (6/22, 27.3%) and Cryptosporidium suis-like genotype (1/22, 4.5%). Meanwhile, two E. bieneusi genotypes were identified, including D (17/19, 89.5%) and Peru6 (2/19, 10.5%).

Conclusions: To the best of our knowledge, Enterocytozoon bieneusi genotype Peru6 was identified in rodents for the first time globally and Cryptosporidium rat genotype I and Cryptosporidium rat genotype IV were found in rats in China for the first time. The finding of zoonotic C. ubiquitum and C. suis-like genotype, as well as E. bieneusi genotypes, suggests that brown rats pose a threat to human health. It is necessary to control brown rat population in the investigated areas and improve local people’s awareness of the transmission risk of the two pathogens from brown rats to humans.

Keywords: Cryptosporidium, Enterocytozoon bieneusi, Zoonotic, Brown rats, Genotyping
Background

*Cryptosporidium* spp. and *Enterocytozoon bieneusi* are two common opportunistic pathogens in humans and have been reported to be associated with diarrhea [1, 2]. However, clinical symptoms of the infection are variable depending on the health status of the infected hosts, displaying asymptomatic infection or self-limiting diarrhea in healthy people, and chronic or life-threatening diarrhea in immunocompromised individuals [2, 3]. In addition to humans, numerous animal species are also the hosts of the two pathogens, suggesting a zoonotic nature of the two parasitic diseases [4, 5]. Meanwhile, they have also been identified in some water bodies and food products, indicating the possibility of water-borne and food-borne transmission [3, 6, 7]. Because of the nature of the two parasitic diseases [4, 5]. Meanwhile, *Cryptosporidium* spp. and *E. bieneusi*, both have been ranked on category B list, in which the pathogens are defined as the second highest priority organisms/biological agents by the National Institutes of Health (NIH) of the USA [8]. Meanwhile, *Cryptosporidium* spp. is ranked fifth among the 24 most important food-borne parasites in a global ranking by a joint Food and Agriculture Organization (FAO)/World Health Organization (WHO) expert committee [9]. *Enterocytozoon bieneusi* is listed on the Environmental Protection Agency (EPA) microbial contaminant candidate list of concern for waterborne transmission [10].

*Cryptosporidium* is a complex genus. To date, at least 31 species and more than 40 genotypes of *Cryptosporidium* have been identified by sequencing the small subunit (SSU) rRNA gene [2, 11]. Of them, 21 *Cryptosporidium* species/genotypes have been isolated in humans and *C. parvum* is generally considered to be zoonotically transmitted [2]. Some zoonotic outbreaks of cryptosporidiosis caused by *C. parvum* have been reported and confirmed at a subtype level, such as calf-derived IlaA15G2R1 in the UK [12] and sheep-derived IlaA17G1R1, IlaA15G2R1 in the UK and IlaA20G2R1 in Italy [12, 13]. Thus, molecular epidemiological investigations of animal cryptosporidiosis have been paid more attention. However, most of them focus on farm animals (pigs, sheep and cattle) or pets (dogs and cats) [5, 14]. Rodents, as the most widespread groups of mammals, have been reported to carry at least 11 species and more than 20 genotypes of *Cryptosporidium* as vectors or reservoirs, including *C. parvum, C. muris, C. ubiquitum, C. meleagrisid, C. scrofa, C. vairi, C. tyszleri, C. rubeyi, C. andersoni, C. hominis, C. suis* and rat genotypes (I-IV), mouse genotypes (II, III) and the Naruko genotype, ferret genotype, chipmunk genotypes (I, II), skunk genotype, hamster genotype, deer mouse genotypes (I-IV), vole genotype, bear genotype, muskrat genotypes (I, II) and ground squirrel genotypes (I-III) [15–38]. Among them, *Cryptosporidium* species except for *C. rubeyi* and two genotypes (chipmunk genotype I and skunk genotype) have also been found in humans [11].

For *E. bieneusi*, at least 240 genotypes have been identified by analyzing the internal transcribed spacer (ITS) region (~243 bp) of the rRNA gene and they have been classified into nine groups (groups 1-9) by phylogenetic analysis [39, 40]. Group 1 is composed of the common zoonotic genotypes and groups 2-9 mostly contain host-adapted genotypes [11]. To date, more than 70 genotypes have been found in humans, 33 of which are also found in animals, supporting presumption of zoonotic potential [1, 5]. In fact, zoonotic transmission of *E. bieneusi* has been reported in Peru, which occurred between a child and guinea pigs [41]. *Enterocytozoon bieneusi* has been detected in many rodent species and 35 genotypes have been identified, including 12 zoonotic genotypes (BEB6, C, D, EbpA, EbpC, H, Peru8, Peru11, Peru16, PigITS5, S6 and TypeIV) [22, 41–48].

Currently, due to limited effect of nitazoxanide on cryptosporidiosis and fumagillin on microsporidiosis caused by *E. bieneusi* [11], transmission control and prevention of infection of the two pathogens targeting the epidemiologic cycles are the key effective strategies. Understanding *Cryptosporidium* spp. and *E. bieneusi* epidemiology in wide range of hosts, exploring the molecular phylogeny and assessing zoonotic potential of animal-derived isolates are the key steps to prevent and reduce occurrence of the two parasitic diseases. Brown rats (*Rattus norvegicus*) are one of the most common rodent species, and usually live almost everywhere humans are. The dynamic activity of rodents facilitates the transmission and spread of various diseases including cryptosporidiosis and microsporidiosis caused by *E. bieneusi* [49].

In Heilongjiang Province of China, *Cryptosporidium* spp. and *E. bieneusi* are prevalent in a variety of species of animals [50, 51]. Moreover, they have been found in human immunodeficiency virus (HIV)-infected and acquired immunodeficiency syndrome (AIDS)-patients (unpublished data), cancer patients and children [52, 53]. The source of the human infection is still unclear. This study aimed to determine the prevalence of *Cryptosporidium* spp. and *E. bieneusi* in brown rats from various regions of Heilongjiang, China, to characterize the isolates and assess their zoonotic potential.

Methods

Study sites and rodent collections

During a three-year period from April 2014 to June 2017, 242 brown rats were captured from five distinct regions of Heilongjiang Province, China, including 55 from a granary in Xingren Town, 30 from a cattle farm in Xingren Town, 73 from a pig farm in Mingshui County, 27 from a pig farm in Qinggang County, 37 from a sheep farm in Baqing County and 20 from a
subdistrict in Harbin City (Table 1). All rats were captured in cage traps baited with sunflower seeds and peanut/sesame butter. In each location, 20 cage traps were installed at sunset and gathered before sunrise, with traps 5 m apart in transects. All rats were transported to the laboratory within 48 h after being captured and were killed by CO₂ inhalation.

Fecal sample collection and DNA extraction
A fresh fecal specimen (approximately 500 mg) was collected directly from the intestinal and rectal content of each brown rat. All specimens were washed with distilled water by centrifugation for 10 min at 1,500×g at room temperature. Genomic DNA was extracted directly from approximately 200 mg of each processed specimen using QIAamp DNA Mini Stool Kit (Qiagen, Hilden, Germany) according to the manufacturer’s procedures. The lysis temperature was increased to 95 °C in order to obtain high DNA yield. DNA was eluted in 200 μl of AE elution buffer (provided with the kit) and stored at -20 °C prior to PCR analysis.

Genotyping of Cryptosporidium spp. and E. bieneusi
Cryptosporidium spp. in the fecal specimens was identified by nested PCR amplification of a SSU rRNA gene fragment of ~830 bp designed by Xiao et al. [54]. Each PCR consisted of 35 cycles of denaturation at 94 °C for 45 s, annealing at 60 °C for 45 s, and extension at 72 °C for 60 s; an initial denaturation step consisting of incubation at 94 °C for 5 min and a final extension step consisting of incubation at 72 °C for 10 min were also included [54]. Enterocytozoon bieneusi was identified and genotyped by nested PCR amplification of an approximately 390 bp nucleotide fragment of the rRNA gene, containing 76 bp of the 3’ end of the SSU rRNA gene, 243 bp of the ITS region, and 70 bp of the 5’ end of the large subunit (LSU) rRNA gene designed by Buckholt et al. [55]. The two sets of cycling parameters were as follows: 35 cycles of 94 °C for 30 s, 57 °C for 30 s, and 72 °C for 40 s and 30 cycles of 94 °C for 30 s, 55 °C for 30 s, and 72 °C for 40 s, with both of them having an initial denaturation step at 94 °C for 5 min and a final extension step at 72 °C for 10 min [55]. TaKaRa Taq DNA polymerase (TaKaRa Bio Inc., Tokyo, Japan) was used for all the PCR amplifications. All PCR amplification tests were carried out with positive controls (chicken-derived C. bailey DNA for Cryptosporidium spp. and deer-derived genotype BEB6 DNA for E. bieneusi) and negative controls which contained no DNA.

DNA sequencing and analysis
All nested PCR products were sequenced using the same PCR primers used for the secondary PCRs on an ABI PRISM™ 3730 DNA Analyzer (Applied Biosystems, Carlsbad, CA, USA), using a BigDye Terminator v3.1 Cycle Sequencing kit (Applied Biosystems). The accuracy of the sequencing data was confirmed by sequencing of the PCR products in both directions. Further PCR products were sequenced for some DNA preparations, from which we obtained the sequences with single nucleotide substitutions, deletions or insertions compared to those published in GenBank. The species and genotypes of Cryptosporidium spp. and E. bieneusi isolates were identified by aligning and analyzing the nucleotide sequences with each other and with reference sequences from GenBank using the Basic Local Alignment Search Tool (BLAST) and Clustal X 1.83 [56]. All the E. bieneusi genotypes were named based on 243 bp of the ITS region according to the established nomenclature system [57].

Table 1 Prevalence and distribution of Cryptosporidium species/genotypes and E. bieneusi genotypes in brown rats in Heilongjiang Province of China

| Source     | Location | No. examined | Cryptosporidium Positive (%) | Species/Genotype (n) | E. bieneusi Positive (%) | Genotype (n) |
|------------|----------|--------------|-----------------------------|----------------------|--------------------------|--------------|
| Farms      |          |              |                             |                      |                          |              |
| Cattle farm| Xingren  | 30           | 2 (6.7)                     | Rat genotype I (2)   | 1 (3.3)                  | D (1)        |
| Pig farm   | Mingshui | 73           | 12 (16.4)                   | Rat genotype I (9);  | 3 (4.1)                  | D (3)        |
|            |          |              |                             | Rat genotype IV (3)  |                          |              |
| Pig farm   | Qinggan  | 27           | 3 (11.1)                    | Rat genotype I (3)   | 3 (11.1)                 | D (3)        |
| Sheep farm | Baoqing  | 37           | 1 (2.7)                     | C. ubiquitum (1)     | 10 (27.0)                | D (8); Peru6 (2) |
| Granary    | Xingren  | 55           | 4 (7.3)                     | Rat genotype IV (3); | 1 (1.8)                  | D (1)        |
|            |          |              |                             | Suis-like genotype (1)|                          |              |
| Subdistrict| Harbin   | 20           | 0                           | –                    | 1 (5.0)                  | D (1)        |
| Total      |          | 242          | 22 (9.1)                    | Rat genotype I (14); | 19 (7.4)                 | D (17); Peru6 (2) |
Results
Prevalence of Cryptosporidium spp. and E. bieneusi
Totals of 22 (9.1%) and 19 (7.9%) out of 242 brown rats were found to be infected with Cryptosporidium spp. and E. bieneusi, respectively. Cryptosporidium spp. was found in five areas, with infection rates ranging between 6.7–16.4% except the subdistrict of Harbin City. Enterocytozoon bieneusi was found in all the six areas investigated, with infection rates ranging between 1.8–27.0%. No mixed infections of the two pathogens were found in rodents in our study (Table 1).

Cryptosporidium species/genotypes
Sequence analysis of SSU rRNA gene products of 22 Cryptosporidium isolates identified four Cryptosporidium species and genotypes, including Cryptosporidium rat genotype I (14/22, 63.6%), Cryptosporidium rat genotype IV (6/22, 27.3%), Cryptosporidium suis-like genotype (1/22, 4.5%) and C. ubiquitum (1/22, 4.5%). Cryptosporidium rat genotype I showed dominance in brown rats in the investigated regions (Table 1).

At the SSU rRNA locus, 14 Cryptosporidium rat genotype I isolates had 100% homology between each other and were identical to that (FJ205699) from waste water in the UK (GQ183517). The sixth sequence had 100% homology between each other investigated regions (Table 1).

Cryptosporidium suis-like genotype (1/22, 4.5%) and C. ubiquitum (1/22, 4.5%). Cryptosporidium rat genotype I showed dominance in brown rats in the investigated regions (Table 1).

The difference in prevalence may be related to rodent species, detection methods, sample size, animal age and study locations [58]. In the present study, four Cryptosporidium species/ge- notypes were identified including Cryptosporidium rat genotype I and IV, Cryptosporidium suis-like genotype and C. ubiquitum. Previous molecular epidemiological data revealed the presence of at least 11 species and 20 genotypes of Cryptosporidium spp. in rodents worldwide [15–38]. In China, five Cryptosporidium species (C. parvum, C. muris, C. andersoni, C. ubiquitum and C. wrairi) and six Cryptosporidium genotypes (mouse genotype I, rat genotypes II and III, ferret genotype, chipmunk genotype III and hamster genotype) have been found in rodents [19, 20, 22]. The species and genotype identification of rodent-derived Cryptosporidium spp. will be helpful to understand the roles that rodents play in the transmission of cryptosporidiosis.

Several studies have revealed that rats appear to be a major animal host for Cryptosporidium rat genotype I and Cryptosporidium rat genotype IV. Cryptosporidium rat genotype I (previously rat genotype) has been found in brown rats from Philippines, Sweden and Nigeria, and Cryptosporidium rat genotype IV (previously W19) in brown rats from Japan and Sweden [26, 30, 31, 35]. Furthermore, the two genotypes have also been detected in environmental samples, including a stream in the USA [48], raw water in the UK and China [60, 61] and the South Nation River watershed in Canada [62]. However, Cryptosporidium rat genotype I and Cryptosporidium rat genotype IV were found in brown rats in China for the first time. To date, there are only eight studies of Cryptosporidium infection in rodents worldwide [22, 41–47]. In general, the prevalence of E. bieneusi in brown rats here was higher than those in chinchillas (3.6%) and mice (1.1%) [22, 45], but lower than those in hamster family (24.3%), prairie dogs (48.3%), squirrel family (16.7–42.9%), voles (39.1%) and mice (10.5–87.5%) [41–44, 46, 47].

Discussion
In the present study, 9.1% of the brown rats examined were found to be infected with Cryptosporidium spp. The infection rate of Cryptosporidium was lower than those reported in brown rats in Iran (17.1%), Japan (38.0%), the Philippines (18.6%) and Sweden (12.0%) [23, 26, 31, 35], but higher than those reported in China (5.6% and 7.1%), Iran (4.1%) and Nigeria (1.5%) [19, 20, 24, 30]. Cryptosporidium spp. has been detected in various rodent species. Variable prevalence rates have been observed, such as 8.0–31.4% in mice, 2.1–63.0% in rats and 0.8–73.0% in voles [58], with the lowest and the highest prevalence rates in muskrats (0.7%) and in guinea pigs (85.0%), respectively [19, 59]. In the present study, E. bieneusi was detected in brown rats for the first time, with a prevalence of 7.9%. To date, there are only eight studies of Cryptosporidium infection in rodents worldwide [22, 41–47]. In general, the prevalence of E. bieneusi in brown rats here was higher than those in chinchillas (3.6%) and mice (1.1%) [22, 45], but lower than those in hamster family (24.3%), prairie dogs (48.3%), squirrel family (16.7–42.9%), voles (39.1%) and mice (10.5–87.5%) [41–44, 46, 47].
the future, more systematic molecular epidemiological investigations of Cryptosporidium spp. in more hosts need to be carried out to understand the true host range of the two genotypes.

Cryptosporidium ubiquitum, previously known as the Cryptosporidium cervine genotype, infects the largest number of host species of animals [63]. It has been found in domesticated and wild ruminants, a colony of non-human primates (lemurs) and a variety of rodents [22, 29, 32, 35, 38, 63]. To date, human cases of cryptosporidiosis caused by C. ubiquitum have been documented in more than ten countries [64]. In addition, this species has also been found in some water bodies, including source water, storm water and raw waste-water [63]. Cryptosporidium suis-like genotype has been recorded in rats in Philippines [31] and other animal species including cattle in Denmark, India and China, and yaks in China [65–68]. This genotype was also found in humans in Canada [69]. Although C. ubiquitum and Cryptosporidium suis-like genotype only accounted for 9.1% of all the Cryptosporidium isolates in investigated brown rats, we still need to consider them as a threat to human health, especially Cryptosporidium suis-like genotype, for this genotype was found in a granary.

To date, 35 E. bieneusi genotypes have been identified in rodents worldwide, 12 of which (BEB6, C, D, EbpA, EbpC, H, Peru 8, Peru11, Peru 16, PigITS5, S6 and TypeIV) have been detected in humans [22, 41–48]. In the present study, two known zoonotic E. bieneusi genotypes, D and Peru 6, were identified in brown rats. Genotype D was identified in 88.9% of E. bieneusi isolates and was found to have a wide distribution; it was found in all areas investigated. Genotype D is reported to be responsible for most human infections and it has been found in humans from more than 40 countries or areas [1]. It is also isolated in at least 15 species of animals as well as in some water bodies [70, 71]. In the present study Peru6 was only identified in two E. bieneusi isolates from two rats captured in the sheep farm. This genotype has been recorded in humans in Peru and Portugal [72–74], and some mammalian animal species and bird species [5, 75]. It has also been detected in wastewater in China [71]. To our knowledge, genotype Peru6 was found in rodents for the first time globally, indicating that this genotype might have more reservoir hosts than expected. The finding of two genotypes previously reported in humans suggests the possibility of rodents in the transmission of E. bieneusi to humans [1, 5].

Conclusions

The present study demonstrated the occurrence of Cryptosporidium spp. and E. bieneusi in brown rats in Heilongjiang, China and genetically characterized the isolates. We identified E. bieneusi genotype Peru6 in rodents for the first time, and Cryptosporidium rat genotype I and Cryptosporidium rat genotype IV in rats in China for the first time. The finding of zoonotic C. ubiquitum and Cryptosporidium suis-like genotype as well as two E. bieneusi genotypes suggests that brown rats pose a threat to human health. Thus, it is strongly recommended to take measures to control brown rat populations in the areas investigated and improve local people’s awareness of the transmission risk of these two diseases from brown rats to humans.

Abbreviations

AIDS: Acquired immunodeficiency syndrome; BLAST: Basic Local Alignment Search Tool; EPA: Environmental Protection Agency; HIV: Human immunodeficiency virus; ITS: Internal transcribed spacer; NIH: National Institutes of Health; SSU: Small subunit

Funding

The study was supported partially by the Graduate Student Innovation Foundation of Harbin Medical University (YSJC2016-41HYD) and the Heilongjiang Province Education Bureau (No. 12531266). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Availability of data and materials

All data generated or analysed during this study are included in this published article. Sequences were submitted to the GenBank database under the accession numbers MG917670 and MG917671.

Authors’ contributions

Experiments were conceived and designed by HL and AL. Experiments were performed by WZ, J-G W, G-X R, Z-Y Y and Y-C X. The data were analyzed by WZ. FY and W-Z Z contributed reagents/materials/analysis tools. The manuscript was written by WZ, and revised by AL and HL. All authors read and approved the final manuscript.

Ethics approval and consent to participate

All the animals were handled and cared for according to the Chinese Laboratory Animal Administration Act of 1998. The research of protocol was reviewed and approved by the Research Ethics Committee and the Animal Ethical Committee of Harbin Medical University (HMIURB20130009).

Competing interests

The authors declare that they have no competing interests.

Publisher’s Note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Author details

1Department of Parasitology, Harbin Medical University China, Harbin 150081, Heilongjiang, China. 2Department of Microbiology, Wu Lien-Teh Institute, Harbin Medical University, Heilongjiang Provincial Key Laboratory of Infection and Immunity, Key Laboratory of Pathogen Biology, Harbin 150081, China. 3Department of Immunology, Harbin Medical University, Harbin 150081, China.

Received: 8 February 2018 Accepted: 8 May 2018

Published online: 24 May 2018

References

1. Matos O, Lobo ML, Xiao L. Epidemiology of Enterocytozoon bieneusi infection in humans. J Parasit Res. 2012;2012:981424.
2. Ryan U, Fayer R, Xiao L. Cryptosporidium species in humans and animals: Current understanding and research needs. Parasitology. 2014;141:1667–85.
3. Didier ES, Weiss LM. Microsporidiosis: current status. Curr Opin Infect Dis. 2006;19:485–92.
4. Ryan U, Zahedi A, Paparini A. Cryptosporidium in humans and animals - a One Health approach to prophylaxis. Parasite Immunol. 2016;38:535–47.

5. Santin M, Fayer R. Microsporidiosis. Enterocytozoon bineusi in domesticated and wild animals. Res Vet Sci. 2011;90:363–71.

6. Ryan U, Hiljawi N, Xiao L. Foodborne cryptosporidiosis. Int J Parasitol. 2018;48:1–12.

7. Speich B, Croll D, Fürst T, Utzinger J, Keiser J. Effect of sanitation and water treatment on intestinal protozoa infection: a systematic review and meta-analysis. Lancet Infect Dis. 2016;16:87–99.

8. NIAID Emerging Infectious Diseases/Pathogens. (https://www.niaid.nih.gov/research/emerging-infectious-diseases-pathogens). Accessed 7 May 2018.

9. Report of a Joint FAO/WHO Expert Meeting, 3–7 September 2012, FAO Headquarters, Rome, Italy. (http://www.fao.org/food/food-safety-quality/a2-index/foodborne-parasites/en/). Accessed 7 May 2018.

10. Didier ES, Weiss LM, Cali A, Marciano-Cabral F. Overview of the presentations on microsporidia and free-living amebae at the 10th International Workshops on Opportunistic Protists. Eukaryot Cell. 2009;8:441–5.

11. Yang Z, Zhao W, Shen Y, Zhang W, Shi Y, Ren G, et al. Subtyping of Cryptosporidium cuniculus and genotyping of Enterocytozoon bineusi in rabbits in two farms in Heilongjiang Province, China. Parasite. 2016;23:52.

12. Chalmers RM, Giles M. Zoonotic cryptosporidiosis in the UK - challenges for control. J Appl Microbiol. 2010;109:1487–97.

13. Cacció SM, Sannella AR, Mariano V, Valentini S, Berti F, Tosiini F, Pozio E. A rare Cryptosporidium parvum genotype associated with infection of lambs and zoonotic transmission in Italy. Vet Parasitol. 2015;219:128–31.

14. Feng Y, Xiao L. Molecular epidemiology of Cryptosporidium in China. Front Microbiol. 2017;8:1701.

15. Paparini A, Jackson B, Ward S, Young S, Ryan UM. Multiple Cryptosporidium genotypes detected in wild black rats (Rattus norvegicus) from northern Australia. Exp Parasitol. 2012;131:404–12.

16. Foo C, Farrell J, Bowell A, Robertson I, Ryan UM. Novel Cryptosporidium genotype in wild Australian mice (Mus domesticus). Appl Environ Microbiol. 2007;73:7693–6.

17. Warren KS, Swan RA, Morgan-Ryan UM, Friend JA, Elliot A. Cryptosporidium muris infection in bilbies (Macrotis lagotis). Aust Vet J. 2003;81:739–41.

18. Meilees MV, Soares RM, Bonello F, Gennari SM. Natural infection with zoonotic subtype of Cryptosporidium parvum in capybara (Hydrochoerus hydrochaeris) from Brazil. Vet Parasitol. 2007;147:166–70.

19. Lv C, Zhang L, Wang R, Jian F, Zhang S, Ning C, et al. Cryptosporidium spp. in wild, laboratory, and pet rodents in China: prevalence and molecular characterization. Appl Environ Microbiol. 2009;75:7692–9.

20. Zhao Z, Wang R, Zhao W, Qi M, Zhao J, Zhang L, et al. Genotyping and subtyping of Giardia and Cryptosporidium isolates from commensal rodents in China. Parasitology. 2015;142:800–7.

21. Li X, Zhang L, Wang R, Jian F, Zhang S, Ning C, et al. Cryptosporidium spp. in farmed blue foxes (Vulpes vulpes) in China. Vet Parasitol. 2016;219:258–61.

22. Kowalska-Gawlik K, Zaczyk R, Konior I, Szczepaniak J, Kowalska M, Kowal M. Cryptosporidium and Giardia spp. in farmed red-bellied tree squirrels (Callosciurus erythraeus) in Sichuan, China. PLoS One. 2016;11:e0163605.

23. Sak B, Kváčová O, Valenčáková A, Stanko M, Luptáková L, Hatalová E, Čanády A. Rodents as a reservoir of infection caused by multiple zoonotic species/genotypes of Cryptosporidium parvum, Cryptosporidium cuniculus, and the first evidence of Cryptosporidium muskar genotypes I and II of rodents in Europe. Acta Trop. 2017;172:29–35.

24. Backans A, Jacobson M, Hansson I, Lebbad M, Lambertz ST, Gammelgård E, et al. Occurrence of pathogens in wild rodents caught on Swedish pig and chicken farms. Epidemiol Infect. 2013;141:1885–91.

25. Stenger BLS, Clark ME, Kváč M, Khan E, Giddings CW, Prediger J, et al. North American tree squirrels and ground squirrels with overlapping ranges host different Cryptosporidium species and genotypes. Infect Genet Evol. 2015;36:287–93.

26. Stenger BL, Clark ME, Kváč M, Khan E, Giddings CW, Dyer NW, et al. Highly divergent 18S rRNA gene paralogs in a Cryptosporidium genotype from eastern chipmunks (Tamias striatus). Infect Genet Evol. 2015;32:113–23.

27. Feng Y, Alderioia K, Yang W, Blancero LA, Kuhne WG, Nadareski CA, et al. Cryptosporidium genotypes in wildlife from a New York watershed. Appl Environ Microbiol. 2007;73:6475–83.

28. Karim MR, Dong H, Li Y, Fu L, Li D, Zhang L, et al. Prevalence and new genotypes of Enterocytozoon bieneusi in captive nonhuman primates in zoos in China: high genetic diversity and zoonotic significance. PLoS One. 2015;10:e0117991.

29. Zhao W, Zhang W, Yang Z, Liu A, Zhang L, Yang F, et al. Genotyping of Enterocytozoon bieneusi in farmed blue foxes (Alopex lagopus) and raccoon dogs (Nyctereutes procyonoides) in China. PLoS One. 2015;10:e0142611.

30. Cama VA, Pearson J, Cabrera L, Pacheco L, Gilman R, Meyer S, et al. Transmission of Enterocytozoon bieneusi between a child and guinea pigs. J Clin Microbiol. 2007;45:2708–10.

31. Feng L, Li W, Yu X, Geng C, Liu J, Zhong Z, et al. First report of the human-pathogenic Enterocytozoon bieneusi from red-bellied tree squirrels (Cauculus corbetti) in Sichuan, China. PLoS One. 2016;11:e0163605.

32. Sak B, Kváč M, Kvetňová D, Albrecht T, Pilák E. The first report on natural Enterocytozoon bieneusi and Encephalitozoon spp. infections in wild East-European house mice (Mus musculus musculus) and West-European house mouse (M. m. domesticus) in a hybrid zone across the Czech Republic-Germany border. Vet Parasitol. 2011;178:246–50.

33. Perec-Matysiak A, Burkówaska-Gawluk K, Kváč M, Sak B, Hildebrand J, Leśniarska K. Diversity of Enterocytozoon bieneusi genotypes among small rodents in southwestern Poland. Vet Parasitol. 2015;214:242–6.

34. Karlová O, Valenčáková A, Stanko M, Luptáková L, Hasálová J. First report of Enterocytozoon bieneusi and Encephalitozoon intestinalis infections of wild mice in Slovakia. Ann Agric Environ Med. 2015;22:51–2.

35. Guo Y, Alderioia KA, Yang W, Cama V, Feng Y, Xiao L. Host specificity and source of Enterocytozoon bieneusi genotypes in a drinking source watershed. Appl Environ Microbiol. 2014;80:218–25.

36. Rodrig DM, Salzer JS, Carroll DS, Ritter JM, Drew C, Gallardo-Romo N, et al. Identification of Giardia duodenalis and Enterocytozoon bieneusi in an epizootiological investigation of a laboratory colony of prairie dogs, Cynomys ludovicianus. Vet Parasitol. 2015;215:109–17.

37. Jiang J, Alderioia KA, Xiao L. Distribution of Cryptosporidium genotypes in storm event water samples from three watersheds in New York. Appl Environ Microbiol. 2005;71:4456–44.

38. Meiring BG, Singleton GR, Klijsta A. Rodent-borne diseases and their risks for public health. Crit Rev Microbiol. 2009;35:221–70.
50. Li W, Li Y, Song M, Lu Y, Yang J, Tao W, et al. Prevalence and genetic characteristics of Cryptosporidium, Enterocytozoon bieneusi and Giardia duodenalis in cats and dogs in Heilongjiang province, China. Vet Parasitol. 2015;208:125–34.

51. Zhao W, Zhang W, Yang F, Cao J, Liu H, Yang D, et al. High prevalence of Enterocytozoon bieneusi in asymptomatic pigs and assessment of zoonotic risk at the genotype level. Appl Environ Microbiol. 2014;80:3699–707.

52. Zhang W, Ren G, Zhao W, Yang Z, Shen Y, Sun Y, et al. Genotyping of Enterocytozoon bieneusi and subtyping of Blastocystis in cancer patients: relationship to diarrhea and assessment of zoonotic transmission. Front Microbiol. 2017;8:1835.

53. Yang J, Song M, Wan Q, Li Y, Lu Y, Jiang Y, et al. Enterocytozoon bieneusi genotypes in children in Northeast China and assessment of risk of zoonotic transmission. J Clin Microbiol. 2014;52:3463–7.

54. Xiao L, Escalante L, Yang C, Sulaiman I, Escalante AA, Montali RJ, et al. Phylogenetic analysis of Cryptosporidium parasites based on the small-subunit rRNA gene locus. Appl Environ Microbiol. 1999;65:1578–83.

55. Buckholt MA, Lee JH, Tzipori S. Prevalence of Enterocytozoon bieneusi in swine an 18-month survey at a slaughterhouse in Massachusetts. Appl Environ Microbiol. 2002;68:2595–9.

56. Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG. The Clustal X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Res. 1997;24:4876–82.

57. Santín M, Fayer R. Cryptosporidium ubiquitum genotypes in wild placental mammals. Exp Parasitol. 2010;124:128–37.

58. Ziegler PE, Wade SE, Schaaf SL, Chang YF, Mohammed HO. Cryptosporidium spp. from small mammals in the New York City watershed. J Wildl Dis. 2007;43:586–96.

59. Chalmers RM, Robinson G, Elwin K, Hadfield SJ, Thomas E, Watkins J, et al. Characterization and assessment of zoonotic transmission of Cryptosporidium from dairy cattle in West Bengal, India. Vet Parasitol. 2010;172:23

60. Chalmers RM, Robinson G, Elwin K, Hadfield SJ, Thomas E, Watkins J, et al. Characterization and assessment of zoonotic transmission of Cryptosporidium from dairy cattle in West Bengal, India. Vet Parasitol. 2010;172:23

61. Feng Y, Li N, Duan L, Xiao L. Detection of Enterocytozoon bieneusi and subtyping of Blastocystis in cancer patients: relationship to diarrhea and assessment of zoonotic transmission. Front Microbiol. 2017;8:1835.

62. Ruecker NJ, Matsune JC, Lapen DR, Topp E, Edge TA, Neumann NF. The epidemiology of intestinal microsporidiosis in patients with HIV/AIDS in Lima, Peru. J Infect Dis. 2015;190:1658–64.

63. Lobo ML, Xiao L, Antunes F, Matos O. Microsporidia as emerging pathogens and the implication for public health: a 10-year study on HIV-positive and -negative patients. Int J Parasitol. 2012;42:197–205.

64. Sulaiman IM, Bern C, Gilman R, Carma V, Kawai V, Vargas D, et al. A molecular biologic study of Enterocytozoon bieneusi in HIV-infected patients in Lima, Peru. J Eukaryot Microbiol. 2003;50:91–6.

65. Blanco MA, de Lucio A, Fuentes I, Carmena D. Distribution of Enterocytozoon bieneusi genotypes in children in Northeast China and assessment of risk of zoonotic transmission. J Clin Microbiol. 2014;52:3463–7.

66. Tao W, Li Y, Yang H, Song M, Wu W, Li W. Widespread occurrence of Enterocytozoon bieneusi genotypes in children in Northeast China and assessment of risk of zoonotic transmission. J Clin Microbiol. 2014;52:3463–7.

67. Li P, Cai J, Cai M, Wu W, Li W. Prevalence and genetic characteristics of previously considered ruminant-adapted genotypes. Appl Environ Microbiol. 2015;81:3326–35.

68. Jiang Y, Tao W, Wan Q, Li Q, Yang Y, Lin Y, et al. Zoonotic and potentially host-adapted Enterocytozoon bieneusi genotypes in sheep and cattle in northeast China and an increasing concern about the zoonotic importance of previously considered ruminant-adapted genotypes. Appl Environ Microbiol. 2015;81:3326–35.

69. Ong CSL, Eister DL, Alkilani A, Fung Vicki WK, Tomblin J, Bowie WR, et al. Novel Cryptosporidium genotypes in sporadic cryptosporidiosis cases: first report of human infections with a cervine genotype. Emerg Infect Dis. 2002;8:263–8.

70. Jiang Y, Tao W, Wan Q, Li Q, Yang Y, Lin Y, et al. Zoonotic and potentially host-adapted Enterocytozoon bieneusi genotypes in sheep and cattle in northeast China and an increasing concern about the zoonotic importance of previously considered ruminant-adapted genotypes. Appl Environ Microbiol. 2015;81:3326–35.

71. Li N, Xiao L, Wang L, Zhao S, Zhao X, Duan L, et al. Molecular surveillance of Cryptosporidium spp., Giardia duodenalis, and Enterocytozoon bieneusi by genotyping and subtyping parasites in wastewater. PLoS Negl Trop Dis. 2012;6:e1809.

72. Bern C, Kawai V, Vargas D, Rabke-Verani J, Williamson J, Chavez-Valdez R, et al. The epidemiology of intestinal microsporidiosis in patients with HIV/AIDS in Lima, Peru. J Infect Dis. 2015;190:1658–64.

73. Lobo ML, Xiao L, Antunes F, Matos O. Microsporidia as emerging pathogens and the implication for public health: a 10-year study on HIV-positive and -negative patients. Int J Parasitol. 2012;42:197–205.

74. Sulaiman IM, Bern C, Gilman R, Carma V, Kawai V, Vargas D, et al. A molecular biologic study of Enterocytozoon bieneusi in HIV-infected patients in Lima, Peru. J Eukaryot Microbiol. 2003;50:91–6.

75. Zhao W, Zhang W, Yang D, Zhang L, Wang R, Liu A. Prevalence of Enterocytozoon bieneusi and genetic diversity of ITS genotypes in sheep and goats in China. Infect Genet Evol. 2015;32:265–70.