Host-Adaptation of Rare *Enterocytozoon bieneusi* Genotype CHN4 in Coypu (*Myocastor Coypus*) in China

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Abstract

Background: *Enterocytozoon bieneusi* is a zoonotic gastrointestinal pathogen and can infect both humans and animals. Coypus (*Myocastor coypus*) is a semi-aquatic rodent, in which few *E. bieneusi* infections have been reported.

Methods: A total of 308 fresh fecal samples were collected from seven coypu farms in China to determine the infection status and the zoonotic potential of *E. bieneusi* from coypus using nested-PCR amplification of the internal transcribed spacer (ITS) region.

Results: *E. bieneusi* was detected with an infection rate of 41.2% (n = 127). Four genotypes were identified, including CHN4 (n = 111), EbpC (n = 8) and EbpA (n = 7) and a novel genotype named CNCP1 (n = 1).

Conclusions: The rare genotype CHN4 was the most dominant genotype in this study, and the transmission dynamics of *E. bieneusi* in coypus were different from other rodents. This is the first report of *E. bieneusi* infections in coypus in China. Our study reveals that *E. bieneusi* in coypus may be potentially transmissible from coypus to humans.

Background

Enterocytozoon bieneusi is an obligate intracellular pathogen, which has been detected in a broad range of hosts, including humans, livestock, companion animals, birds and wildlife, even in some water bodies [1, 2]. Hosts can be infected by ingesting infective spores through foodborne and waterborne routes or direct contact with infected humans or animals [3]. To date, over 500 genotypes of *E. bieneusi* were identified in the world by molecular genotyping based on internal transcribed spacer (ITS) region of the rRNA gene [1, 4]. These genotypes can be placed into 11 distinct groups (groups 1 to 11) in the phylogenetic analysis [5]. The majority of the zoonotic genotypes are clustered in group 1 [5]. Meanwhile, an increasing number of reports have been showing that some genotypes (I, J, BEB4 and BEB6) in group 2 which were firstly detected in livestock or wild animals can also infect humans, indicating a low host specificity and zoonotic inherence of this group [1, 6, 7]. Other groups mostly contain host-adapted genotypes [6].

About 40–50% of the mammalian species are rodents, which are distributed throughout the world except the Antarctic and a handful of islands [8]. Because of their abundant population and broad active range, rodents infected with *E. bieneusi* pose an unneglectable threat to public health. In a previous study, the zoonotic transmission of *E. bieneusi* occurred between a child and guinea pigs in Peru [9]. More than 35 *E. bieneusi* genotypes have been detected in more than 20 rodent species, including zoonotic ones (BEB6, C, D, EbpA, EbpC, H, Peru8, Peru11, Peru16, PigITS5, S6 and Type IV) [1, 10]. Coypu (*Myocastor coypus*) is a large rodent adapted to amphibious environments, and nowadays they have been widely raised in farms as important fur-bearing animals. However, there are limited information regarding the prevalence

and genetic characteristics of *E. bieneusi* in coypu worldwide. Therefore, this study aimed to determine the genotypes and infection rate and assess the zoonotic potential of *E. bieneusi* from coypus in China.

Methods

Sample collection

A total of 308 fresh fecal samples were collected from asymptomatic coypus from seven farms in Anyang and Kaifeng in Henan province, Yongzhou in Hunan province, Laibin in Guangxi Zhuang Autonomous Region, Baoding in Hebei province, Chengdu in Sichuan province and Ganzhou in Jiangxi province in China (Table 1; Fig. 1). Each farm was sampled on one occasion from August 2018 to March 2019. In each farm, about 2-4 coypus were kept in one accommodation, which was surrounded by 80 cm-high walls to fence from each other. The ground of the accommodations was hardened with cement. An accommodation is typically composed of a piece of vacant land as the playground and a pool in which the coypus can swim. The samples were collected when the handlers finished the ground using a high-pressure water gun. All the fecal samples were collected immediately after they excreted using sterile polyethylene gloves and marked with animal information. To avoid duplicate sampling of animals, only one fecal sample was collected from one location of the ground in each accommodation, and all deposits from each accommodation pooled as a single sample. All the samples were transferred to the laboratory in a cooler with ice packs within 36 hours and stored at 4°C.

DNA extraction and PCR amplification

Genomic DNA (gDNA) was directly extracted from 200 mg of each sample using *E.Z.N.A.* Stool DNA Kit (Omega Biotek Inc., Norcross, GA, USA) according to the manufacturer's protocol with minor modification.

All samples were screened for the presence of *E. bieneusi* using a nested PCR that targets ITS region (~389bp fragment) using a previously described assay by Sulaiman et al [11]. Reagent-grade water and known positive DNA derived from Golden snub-nosed monkey (genotype D, Accession no.: KU604932) were used as negative and positive controls, respectively. The secondary PCR products were separated electrophoretically on 1% agarose (Life Technologies Corporation, CA, USA) gel stained with DNAGreen (Tiandz, Beijing, China) and visualized under UV light.

Sequencing and data analyses

Positive secondary PCR products were sequenced bidirectionally by Sangon Biotech Co. Ltd., Shanghai, China. The obtained sequences were assembled and edited using DNASTAR Lasergene EditSeq version 7.1.0 (http://www.dnastar.com/) and aligned with the reference sequences downloaded from GenBank by Clustal X version 2.1 (http://www.clustal.org/).

All statistical analyses were performed with IBM SPSS Statistics (www.ibm.com/products/spssstatistics). Fisher's exact test was used to compare the prevalence of *E.*

bieneusi among different age groups, and the odds ratios (ORs) with the 95% confidence interval (CI) were also calculated. A two-sided *P* value of 0.05 or less was set as significant.

To reveal the evolutionary relationships and zoonotic potential of *E. bieneusi* isolates, a phylogenetic tree was constructed by the Neighbor-Joining (NJ) method using the Kimura-2-parameter algorithm in MEGA version 7.0.26 (http://www.megasoftware.net). The robustness of the nodes was tested by a bootstrap analysis of 1,000 iterations.

Results

Occurrence of E. bieneusi in coypu

E. bieneusi was detected in 127 of 308 coypus with an infection rate of 41.2%. *E. bieneusi* was found in every farm, and the highest infection rate of *E. bieneusi* in coypu was detected in Anyang (72.3%, 73/101), followed by Baoding (62.9%, 22/35), Kaifeng (17/52, 30.8%), Ganzhou (7/35, 20.0%), Chengdu (6/40, 15.0%), Laibin (2/22, 9.1%) and Yongzhou (1/23, 4.3%) (Table 1). The differences in infection rates of *E. bieneusi* in coypus among different farms were statistically significant ($P < 0.0001$).

The highest infection rate (76.9%, 50/65) was detected in < 3-month-old group, followed by coypus aged 3-6 months (51.1%, 24/47) and aged > 6 months (28.5%, 53/186) (Table 2) ($P < 0.0001$). The correlations between age and the infection rates were evaluated by calculating the ORs and their 95% CIs, which are shown in Table 2. There was a significant negative correlation between the infection rate and age in this study, as an OR of 0.31 (95% CI: 0.14-0.70, $P = 0.005$) was associated with the 3-6-month-old group, and 0.12 (95% CI: 0.06–0.23, $P < 0.0001$) was associated with the > 6-month-old group.

E. bieneusi ITS genotypes

Four distinct *E. bieneusi* genotypes, including three previously reported genotypes [CHN4 (n = 111), EbpC (n = 8), EbpA (n = 7)], and one novel genotype (named CNCP1, n = 1) were observed. Genotype CHN4 was the most common genotype and detected in six farms except the farm in Yongzhou. Genotype EbpC was distributed in Yongzhou, Laibin and Kaifeng, while genotype EbpA and novel genotype CNCP1 were only detected in the specimens from Kaifeng.

CHN4 was the only genotype detected in the < 3-month-old group (n =50). In the 3-6-month-old group, CHN4 (n = 16) was also the predominant genotype, which was detected in 16 samples, followed by EbpA (n = 4), EbpC (n = 3) and CNCP1 (n = 1). In the age group > 6 months, three genotypes (CHN4, EbpC and EbpA) were detected in 45, 5 and 3 samples, respectively.

Phylogenetic analysis of E. bieneusi

The evolutionary relationships and zoonotic potential of *E. bieneusi* genotypes were analyzed by the NJ phylogenetic tree. Genotype CNCP1 had one single nucleotide polymorphism (SNP) at nucleotide position

274 (G to A) compared to genotype EbpA (Accession no.: MK968834). All the genotypes identified in this study were clustered in group 1 (Fig. 2).

Discussion

The infection rate of *E. bieneusi* in rodent species varies from 2.5–100% worldwide [12, 13]. To the best of our knowledge, this is the first report of *E. bieneusi* infections in coypus in China. In the present study, the infection rate of *E. bieneusi* was 41.2% in coypu, which is higher than the infection rate of *E. bieneusi* reported in brown rats (7.9%) [10], bamboo rats (5.1%) [14], experimental rats (4.8%) [15], commensal rodents (4.0%) [13] and pet chinchillas (3.6%) in China [16]. In addition, lower infection rates were also reported in wild house mice (10.7%) from a hybrid zone across the Czech Republic-Germany border [17], and beavers (15.3%) and muskrats (8.4%) from USA [18]. However, higher infection rates of *E. bieneusi* were reported in chipmunks (71.4%) and woodchucks (100%) from USA [12]. Similar infection rates of *E. bieneusi* have been reported in small rodents (38.9%) from southwestern Poland [19], and a laboratory prairie dog colony (37.9%) from USA [20]. The infection rates of *E. bieneusi* in rodents could be influenced by many factors, such as animal immune status, age distribution, sample size, detection method, feeding environment, management system and population density [15]. Although the high infection rate was detected in coypus in our study, we cannot come to an inference that coypus are more susceptible to *E. bieneusi* than many other rodent species due to the lack of more investigations.

In the present study, the dominant genotype of *E. bieneusi* was CHN4, which was detected in six cities except Yongzhou, indicating that genotype CHN4 is commonly found in coypus in China. Interestingly, genotype CHN4 has not been reported in rodents previously. However, it was firstly identified in three human and two cattle samples [21] and four pre-weaned calf samples (1.9%) [22] from China. Genotype D was identified in squirrels from China [23] and USA [12], chipmunks [24], bamboo rats [14] and brown rats [10, 25] from China, house mice from Czech Republic-Germany border [17] and striped field mice from Poland [19], and genotype WL4 was observed in squirrels, chipmunks and muskrats from USA [12, 18] (Table 3). EbpA, EbpC, PigEBITS7, S7, Peru16 and CHG4 have also been reported as the most common genotypes [9, 13, 15, 18, 25, 26]. The rare genotype CHN4 was the dominant genotype, indicating that the transmission dynamic of *E. bieneusi* in coypus is different from other rodents. This may be explained by the unique life habits of coypus as aquatic rodents compared to other rodents involved in previous studies.

Genotypes EbpA and EbpC have been detected in several rodent species (squirrel, house mouse, experimental rat, muskrat, bamboo rat and beaver) worldwide [14, 15, 17, 18, 23] (Table 3). EbpA and EbpC are two of the most common genotypes detected in both immunocompetent and immunocompromised people worldwide [1]. Meanwhile, genotypes EbpA and EbpC have a vast host range, such as non-human primates (NHPs), livestock (cattle, buffalo, sheep and goat), pets (dog and horse), wild animals (deer, fox, raccoon, bear, panda and otter) and birds (pigeon, crane and parrot) [1]. These two genotypes also have been observed in lake water [27], river water [28] and wastewater treatment plants [29, 30]. According to these data, the interspecies transmission of genotypes EbpA and

EbpC pose a zoonotic risk to human or other animals, and coypus may serve as a reservoir of EbpA and EbpC in the *E. bieneusi* transmission.

In the phylogenetic analysis, an NJ tree was constructed and the novel genotype CNCP1 clustered with CHN4, EbpC and EbpA in group 1. The majority of the zoonotic genotypes belongs to the group 1, and genotypes CHN4, EbpC and EbpA have been reported in humans [21, 31, 32], indicating that genotype CNCP1 maybe have zoonotic potential and the *E. bieneusi* isolates in coypus detected in this study can be transmissible from coypus to humans, especially the animal handlers, or vice versa.

Conclusion

E. bieneusi infection was highly observed in coypus from China, with the prevalence of rare genotype CHN4. The presence of zoonotic genotypes EbpC and EbpA revealed the role of coypus as a reservoir of *E. bieneusi* and posed a threat to the public health. To further characterize the role of coypus in the transmission of microsporidiosis, more intensive research of *E. bieneusi* should be devised and employed.

List Of Abbreviations

ITS: internal transcribed spacer; gDNA: Genomic DNA; CI: confidence interval; OR: odds ratio; NJ: Neighbor-Joining; NHP: non-human primate; SNP: single-nucleotide polymorphism;

Declarations

Ethics approval and consent to participate

The present study was carried out in accordance with the Chinese Laboratory Animal Administration Act of adopted in 1988. The research protocol was reviewed and approved by the Institutional Review Board of Henan Agricultural University (Approval No. IRB-HENAU-20190424-01). Specimens were collected after acquiring the permission of animal owners and no animals were injured during this procedure.

Consent for publication

Not applicable.

Availability of data and material

The nucleotide sequences from this study were deposited in GenBank (Accession no.: MT549052, and MT557703-MT557705).

Competing interests

The authors declare that they have no competing interests.

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Authors' Contributions

Collected samples: YC, HW and QL. Analysis and interpretation: FY, YC, HW and QL. Methodology: FY, YC, HW, QL and AZ. Conceptualization: HW, AZ, MQ and LZ. Wrote the paper: FY, MQ and LZ. Supervision of project: AZ and LZ. Grant funding: MQ. All authors read and approved the final manuscript.

References

1. Li W, Feng Y, Santin M. Host specificity of *Enterocytozoon bieneusi* and public health implications. Trends Parasitol. 2019;35:436-451. https://doi.org/10.1016/j.pt.2019.04.004.
2. Santín M, Fayer R. Microsporidiosis: *Enterocytozoon bieneusi* in domesticated and wild animals. Res Vet Sci. 2011;90:363-371. https://doi.org/10.1016/j.rvsc.2010.07.014.
3. Thellier M, Breton J. *Enterocytozoon bieneusi* in human and animals, focus on laboratory identification and molecular epidemiology. Parasite. 2008;15:349-358. https://doi.org/10.1051/parasite/2008153349.
4. Li W, Xiao L. Multilocus sequence typing and population genetic analysis of *Enterocytozoon bieneusi*: host specificity and its impacts on public health. Front Genet. 2019;10:307. https://doi.org/10.3389/fgene.2019.00307.
5. Li N, Ayinmode AB, Zhang H, Feng Y, Xiao L. Host-adapted *Cryptosporidium* and *Enterocytozoon bieneusi* genotypes in straw-colored fruit bats in Nigeria. Int J Parasitol Parasites Wildl. 2019;8:19-24. https://doi.org/10.1016/j.ijppaw.2018.12.001.
6. Karim MR, Rume FI, Rahman A, Zhang Z, Li J, Zhang L. Evidence for zoonotic potential of *Enterocytozoon bieneusi* in its first molecular characterization in captive mammals at Bangladesh national zoo. J Eukaryot Microbiol. 2020. https://doi.org/10.1111/jeu.12792.
7. Wang S, Wang R, Fan X, Liu T, Zhang L, Zhao G. Prevalence and genotypes of *Enterocytozoon bieneusi* in China. Acta Trop. 2018;183:142-152. https://doi.org/10.1016/j.actatropica.2018.04.017.
8. Drewes S, Straková P, Drexler JF, Jacob J, Ulrich RG. Assessing the diversity of rodent-borne viruses: exploring of high-throughput sequencing and classical amplification/sequencing approaches. Adv Virus Res. 2017;99:61-108. https://doi.org/10.1016/bs.aivir.2017.08.002.
9. 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;45:2708-2710. https://doi.org/10.1128/JCM.00725-07.

10. Zhao W, Wang J, Ren G, Yang Z, Yang F, Zhang W et al. Molecular characterizations of *Cryptosporidium* spp. and *Enterocytozoon bieneusi* in brown rats (*Rattus norvegicus*) from Heilongjiang Province, China. Parasit Vectors. 2018;11:313. https://doi.org/10.1186/s13071-018-2892-7.
11. Sulaiman IM, Fayer R, Yang C, Santín M, Matos O, Xiao L. Molecular characterization of *Enterocytozoon bieneusi* in cattle indicates that only some isolates have zoonotic potential. Parasitol Res. 2004;92:328-334. https://doi.org/10.1007/s00436-003-1049-5.
12. Guo Y, Alderisio 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;80:218-225. https://doi.org/10.1128/AEM.02997-13.
13. Yu F, Qi M, Zhao Z, Lv C, Wang Y, Wang R et al. The potential role of synanthropic rodents and flies in the transmission of *Enterocytozoon bieneusi* on a dairy cattle farm in China. J Eukaryot Microbiol. 2019;66:435-441. https://doi.org/10.1111/jeu.12687.
14. Wang H, Liu Q, Jiang X, Zhang Y, Zhao A, Cui Z et al. Dominance of zoonotic genotype D of *Enterocytozoon bieneusi* in bamboo rats (*Rhizomys sinensis*). Infect Genet Evol. 2019;73:113-118. https://doi.org/10.1016/j.meegid.2019.04.025.
15. Li J, Jiang Y, Wang W, Chao L, Jia Y, Yuan Y et al. Molecular identification and genotyping of *Enterocytozoon bieneusi* in experimental rats in China. Exp Parasitol. 2020;210:107850. https://doi.org/10.1016/j.exppara.2020.107850.
16. Qi M, Luo N, Wang H, Yu F, Wang R, Huang J et al. Zoonotic *Cryptosporidium* spp. and *Enterocytozoon bieneusi* in pet chinchillas (*Chinchilla lanigera*) in China. Parasitol Int. 2015;64:339-341. https://doi.org/10.1016/j.parint.2015.05.007.
17. Sak B, Kváč M, Květoňová D, Albrecht T, Piálek J. 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. 2011;178:246-250. https://doi.org/10.1016/j.vetpar.2010.12.044.
18. Sulaiman IM, Fayer R, Lal AA, Trout JM, Schaefer FW 3rd, Xiao L. Molecular characterization of microsporidia indicates that wild mammals Harbor host-adapted *Enterocytozoon* spp. as well as human-pathogenic *Enterocytozoon bieneusi*. Appl Environ Microbiol. 2003;69:4495-4501. https://doi.org/10.1128/aem.69.8.4495-4501.2003.
19. Perec-Matysiak A, Buńkowska-Gawlik K, Kváč M, Sak B, Hildebrand J, Leśniańska K. Diversity of *Enterocytozoon bieneusi* genotypes among small rodents in southwestern Poland. Vet Parasitol. 2015;214:242-246. https://doi.org/10.1016/j.vetpar.2015.10.018.
20. Roellig DM, Salzer JS, Carroll DS, Ritter JM, Drew C, Gallardo-Romero N et al. Identification of *Giardia duodenalis* and *Enterocytozoon bieneusi* in an epizoological investigation of a laboratory colony of prairie dogs, Cynomys ludovicianus. Vet Parasitol. 2015;210:91-97. https://doi.org/10.1016/j.vetpar.2015.03.022.

21. Zhang X, Wang Z, Su Y, Liang X, Sun X, Peng S et al. Identification and genotyping of *Enterocytozoon bieneusi* in China. J Clin Microbiol. 2011;49:2006-2008. https://doi.org/10.1128/JCM.00372-11.
22. Tang C, Cai M, Wang L, Guo Y, Li N, Feng Y et al. Genetic diversity within dominant *Enterocytozoon bieneusi* genotypes in pre-weaned calves. Parasit Vectors. 2018;11:170. https://doi.org/10.1186/s13071-018-2768-x.
23. Deng L, Li W, Yu X, Gong C, Liu X, Zhong Z et al. First Report of the Human-Pathogenic *Enterocytozoon bieneusi* from Red-Bellied Tree Squirrels (*Callosciurus erythraeus*) in Sichuan, China. PloS one. 2016;11:e0163605. https://doi.org/10.1371/journal.pone.0163605.
24. Deng L, Li W, Zhong Z, Chai Y, Yang L, Zheng H et al. Molecular characterization and new genotypes of *Enterocytozoon bieneusi* in pet chipmunks (*Eutamias asiaticus*) in Sichuan province, China. BMC Microbiol. 2018;18:37. https://doi.org/10.1186/s12866-018-1175-y.
25. Wang J, Lv C, Zhao D, Zhu R, Li C, Qian W. First detection and genotyping of *Enterocytozoon bieneusi* in pet fancy rats (*Rattus norvegicus*) and guinea pigs (*Cavia porcellus*) in China. Parasite. 2020;27:21. https://doi.org/10.1051/parasite/2020019.
26. Gui B, Zou Y, Chen Y, Li F, Jin Y, Liu M et al. Novel genotypes and multilocus genotypes of *Enterocytozoon bieneusi* in two wild rat species in China: potential for zoonotic transmission. Parasitol Res. 2020;119:283-290. https://doi.org/10.1007/s00436-019-06491-8.
27. Ye J, Xiao L, Ma J, Guo M, Liu L, Feng Y. Anthroponotic enteric parasites in monkeys in public park, China. Emerg Infect Dis. 2012;18:1640-1643. https://doi.org/10.3201/eid1810.120653.
28. Hu Y, Feng Y, Huang C, Xiao L. Occurrence, source, and human infection potential of *Cryptosporidium* and *Enterocytozoon bieneusi* in drinking source water in Shanghai, China, during a pig carcass disposal incident. Environ Sci Technol. 2014;48:14219-14227. https://doi.org/10.1021/es504464t.
29. 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. https://doi.org/10.1371/journal.pntd.0001809.
30. Ye J, Ji Y, Xu J, Ma K, Yang X. Zoonotic *Enterocytozoon bieneusi* in raw wastewater in Zhengzhou, China. Folia Parasitol (Praha). 2017;64. https://doi.org/10.14411/fp.2017.002.
31. Sak B, Brady D, Pelikánová M, Květoňová D, Rost M, Kostka M et al. Unapparent microsporidial infection among immunocompetent humans in the Czech Republic. J Clin Microbiol. 2011;49:1064-1070. https://doi.org/10.1128/JCM.01147-10.
32. Wang L, Zhang H, Zhao X, Zhang L, Zhang G, Guo M et al. Zoonotic *Cryptosporidium* species and *Enterocytozoon bieneusi* genotypes in HIV-positive patients on antiretroviral therapy. J Clin Microbiol. 2013;51:557-563. https://doi.org/10.1128/JCM.02758-12.

Tables

Table 1. Distribution of *E. bieneusi* genotypes in coypu from different farms in China

Location	No. of sample	No. of positive	Infection rate (95% CI) (%)	Genotype (n)
Anyang	101	73	72.3 (63.0-81.5)	CHN4 (73)
Yongzhou	23	1	4.3 (0-14.8)	EbpC (1)
Laibin	22	2	9.1 (0-23.4)	CHN4 (1), EbpC (1)
Baoding	35	22	62.9 (45.3-80.3)	CHN4 (22)
Chengdu	40	6	15.0 (2.7-27.3)	CHN4 (6)
Ganzhou	35	7	20.0 (5.3-34.7)	CHN4 (7)
Kaifeng	52	16	30.8 (17.3-44.3)	CHN4 (2), EbpA (7), EbpC (6), CNCP1 (1)
Total	308	127	41.2 (35.6-46.9)	CHN4 (111), EbpA (7), EbpC (8), CNCP1 (1)

Table 2. Occurrence of *E. bieneusi* in coypu by age

Age (month)	No. of sample	Infection rate (95% CI) (%)	*P* value	OR (95% CI)
< 3	65	76.9 (65.9-87.9)	< 0.0001	1.00
3-6	47	51.1 (35.7-66.4)	0.005	0.31 (0.14-0.70)
> 6	196	27.0 (20.1-33.5)	< 0.0001	0.11 (0.06-0.21)

Table 3
Prevalence and genotype distribution of *Enterocytozoon bieneusi* in rodents worldwide [1]

Host	Location	Infection rate (%) (positive no./total no.)	Genotype (n)	Reference
Squirrel	USA	29.7 (11/37)	WL4 (5), Type IV (3), PtEb V (1), WL21 (1), WW6 (2)	[12]
	China	16.7 (24/144)	D (18), EbpC (3), SC02 (1), CE01 (1), horse2 (1)	[23]
Chipmunk	USA	71.4 (5/7)	WL4 (3), Type IV (1), WL23 (1)	[12]
	China	17.6 (49/279)	D (6), Nig7 (4), CHG9 (2), CHY1 (5), SCC-1 (17), SCC-2 (9), SCC-3 (5), SCC-4 (1)	[24]
Prairie dog	USA	48.3 (14/29)	Row[a] (14)	[20]
Woodchuck	USA	100 (5/5)	Type IV (1)[b], WL20 (1), WL4 (2), WL22 (1), WW6 (1)	[12]
House mouse	Czech/German border	10.7 (31/289)	D (10), PigEBITS5 (7), CZ3 (4), Peru8 (4), C (2), EbpA (2), H (1), S6 (1)	[17]
	Poland	28.6 (6/21)	WR3 (1)	[19]
	China	3.2 (1/31)	D (1)	[13]
Deer mouse	USA	23.6 (13/55)	WL4 (10), WL23 (2), WL25 (1)	[12]
Striped field mouse	Poland	42.9 (79/184)	D (6), gorilla 1 (1), WR5 (1), WR8 (2), WR7 (1)	[19]
Yellow-necked mouse	Poland	30.0 (18/60)	D (2), WR1 (1), WR4 (1), WR6 (6), WR9 (1)	[19]
Brown rat	China	7.9 (19/242)	D (17), Peru6 (2)	[10]
	China	2.5 (7/277)	CHG14(3), BEB6(2), D(1), CHG2(1)	[13]
	China	17.2 (17/152)	D (12), Peru11(3), S7 (1), SCC-2 (1)	[25]
Experimental rat[c]	China	4.8 (14/291)	EbpA (7), EbpC (3), CHY1 (2), N (1), SHR1 (1)	[15]

[a] Invalid genotype.

[b] One sample was co-infected with Type IV and WL20.

[c] Including Wistar rat, Sprague Dawley rat and Spontaneously Hypertensive rat.

Host	Location	Infection rate (%) (positive no./total no.)	Genotype (n)	Reference
White-toothed rat/giant rat	China	33.3 (76/228)	PigEBITS7 (22), D (14), K (8), Peru8 (2), CQR1 (10), CQR2 (15), CQR3 (1), GDR1(2), GDR2 (1)	[26]
Muskrat	USA	8.4 (20/239)	WL4 (8), WL15 (4), EbpC (3), D (2), WL10 (1), WL14 (1), WL6 (1)	[18]
Vole	USA	26.7 (4/15)	Peru11 (2), WL21(2), type IV (1), WL20 (1)	[12]
Bank vole	Poland	39.1 (18/46)	D (2), WR2 (1), WR6 (2), WR10 (2)	[19]
Bamboo rat	China	5.1 (22/435)	D (17), J (1), BR1 (1), BR2 (1), EbpA (1), PigEBITS7 (1)	[14]
Beaver	USA	15.3 (13/85)	EbpC (5), D (4), WL7, WL9, WL12, and WL15 (1 each)	[18]
Chinchilla	China	3.6 (5/140)	D (2), BEB6 (3)	[16]
Guinea pig	Peru	14.9 (10/67)	Peru16 (10)	[9]
	China	20.2 (35/173)	S7 (30), PGP (5)	[25]

[a] Invalid genotype.

[b] One sample was co-infected with Type IV and WL20.

[c] Including Wistar rat, Sprague Dawley rat and Spontaneously Hypertensive rat.

Figures

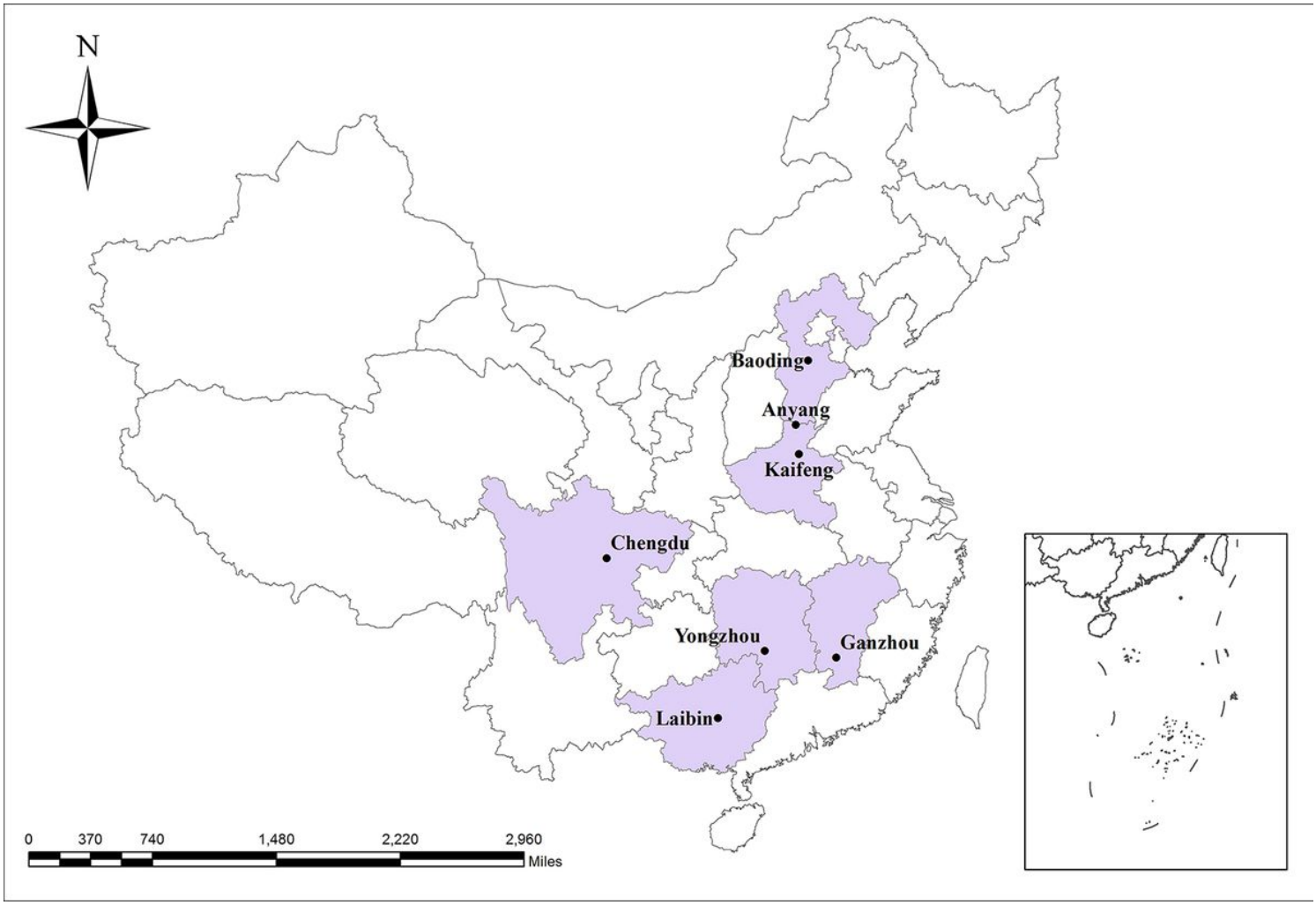

Figure 1

Geographic map of the sampling locations. The figure was originally designed by the authors under the software ArcGIS 10.2. The original vector diagram imported in ArcGIS was adapted from Natural Earth (http://www.naturalearthdata.com). Note: The designations employed and the presentation of the material on this map do not imply the expression of any opinion whatsoever on the part of Research Square concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. This map has been provided by the authors.

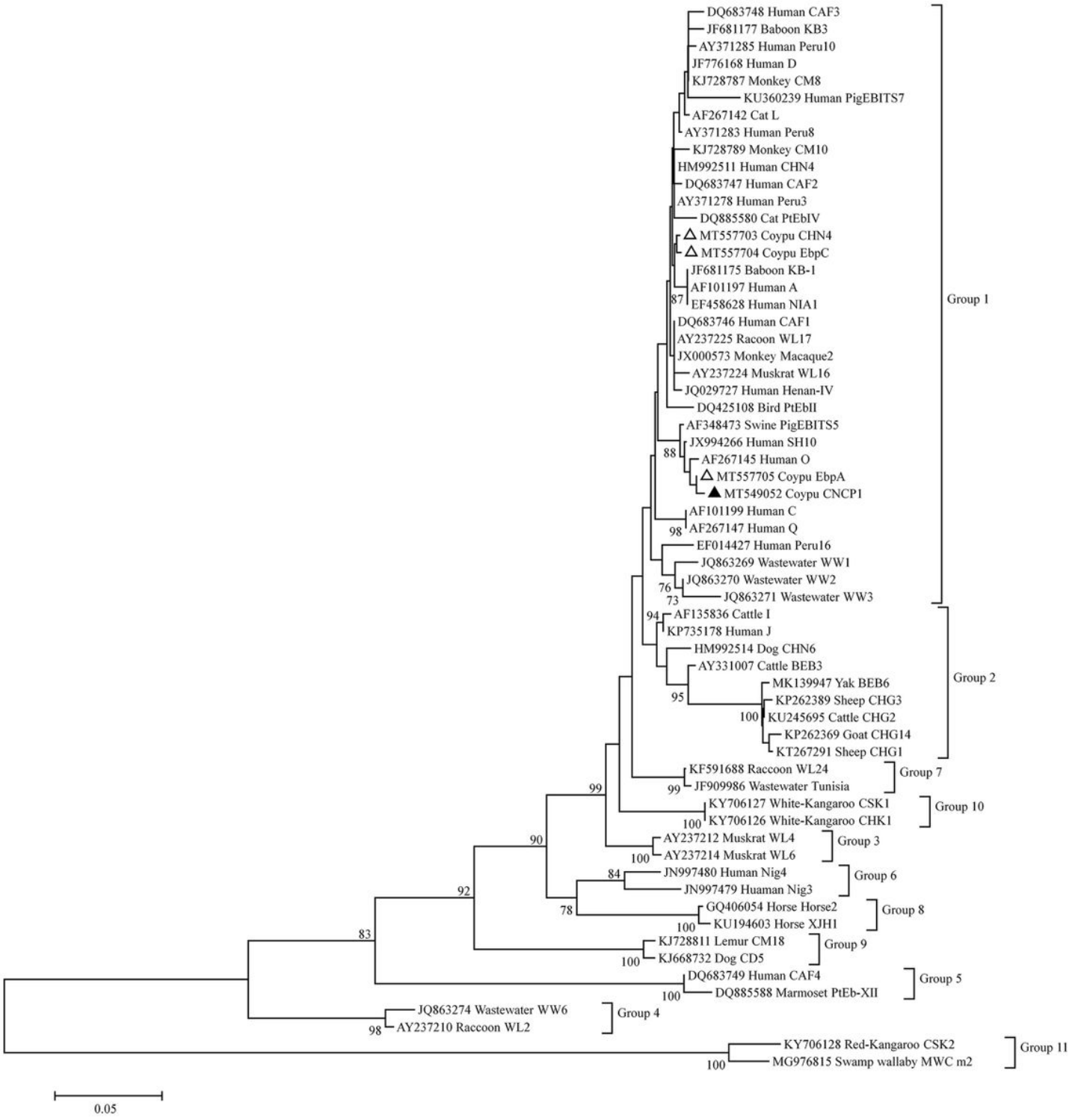

Figure 2

Neighbor-joining tree of Enterocytozoon bieneusi ITS genotypes. Phylogenetic relationships of Enterocytozoon bieneusi genotypes of this study and other genotypes previously deposited in GenBank. Bootstrap values >50% from 1,000 are shown on nodes. Sample names include GenBank accession number followed by host and then genotype designation. Known and novel genotypes identified in this study are indicated by empty and filled triangles, respectively.