

Cross-species transmission of *Cryptosporidium* in wild rodents from the southern region of Zhejiang Province of China and its possible impact on public health

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Abstract – Wild rodents serve as reservoirs for *Cryptosporidium* and are overpopulated globally. However, genetic data regarding *Cryptosporidium* in these animals from China are limited. Here, we have determined the prevalence and genetic characteristics of *Cryptosporidium* among 370 wild rodents captured from three distinct locations in the southern region of Zhejiang Province, China. Fresh feces were collected from the rectum of each rodent, and DNA was extracted from them. The rodent species was identified by PCR amplifying the vertebrate *cytochrome b* gene. *Cryptosporidium* was detected by PCR amplification and amplicon sequencing the small subunit of ribosomal RNA gene. Positive samples of *C. viatorum* and *C. parvum* were further subtyped by analyzing the 60-kDa glycoprotein gene. A positive *Cryptosporidium* result was found in 7% (26/370) of samples, involving five rodent species: *Apodemus agrarius* (36), *Niviventer niviventer* (75), *Rattus losea* (18), *R. norvegicus* (155), and *R. tanezumi* (86). Their respective *Cryptosporidium* positive rates were 8.3%, 5.3%, 11.1%, 7.1%, and 7.0%. Sequence analysis confirmed the presence of three *Cryptosporidium* species: *C. parvum* (4), *C. viatorum* (1), and *C. muris* (1), and two genotypes: *Cryptosporidium* rat genotype IV (16) and *C. mortiferum*-like (4). Additionally, two subtypes of *C. parvum* (IIdA15G1 and IIpA19) and one subtype of *C. viatorum* (XVdA3) were detected. These results demonstrate that various wild rodent species in Zhejiang were concurrently infected with rodent-adapted and zoonotic species/genotypes of *Cryptosporidium*, indicating that these rodents can play a role in maintaining and dispersing this parasite into the environment and other hosts, including humans.

Key words: *Cryptosporidium*, Molecular detection, Wild rodents, Zoonotic, Public health, China.

Résumé – Transmission interspécifique de *Cryptosporidium* chez les rongeurs sauvages de la région sud de la province chinoise du Zhejiang et son impact possible sur la santé publique. Les rongeurs sauvages servent de réservoirs à *Cryptosporidium* et ont des grandes populations à l'échelle mondiale. Cependant, les données génétiques concernant *Cryptosporidium* chez ces animaux en Chine sont limitées. Ici, nous avons déterminé la prévalence et les caractéristiques génétiques de *Cryptosporidium* parmi 370 rongeurs sauvages capturés dans trois endroits distincts de la région sud de la province du Zhejiang, en Chine. Des excréments frais ont été collectés dans le rectum de chaque rongeur et l'ADN en a été extrait. L'espèce de rongeur a été identifiée par amplification par PCR du gène du cytochrome b des vertébrés. *Cryptosporidium* a été détecté par amplification PCR et séquençage d'amplicons de la petite sous-unité du gène de l'ARN ribosomal. Les échantillons positifs de *C. viatorum* et *C. parvum* ont ensuite été sous-typés en analysant le gène de la glycoprotéine de 60 kDa. Un

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résultat positif pour *Cryptosporidium* a été trouvé dans 7 % (26/370) des échantillons, impliquant cinq espèces de rongeurs : *Apodemus agrarius* (36), *Niviventer niviventer* (75), *Rattus losea* (18), *R. norvegicus* (155) et *R. tanezumii* (86). Leurs taux respectifs de positivité pour *Cryptosporidium* étaient de 8,3 %, 5,3 %, 11,1 %, 7,1 % et 7,0 %. L'analyse des séquences a confirmé la présence de trois espèces de *Cryptosporidium* : *C. parvum* (4), *C. viatorum* (1) et *C. muris* (1), et de deux génotypes : *Cryptosporidium* génotype IV de rat (16) et *C. mortiferum*-like (4). De plus, deux sous-types de *C. parvum* (IIaA15G1 et IIpA19) et un sous-type de *C. viatorum* (XVdA3) ont été détectés. Ces résultats démontrent que diverses espèces de rongeurs sauvages du Zhejiang sont simultanément infectées par des espèces/génotypes de *Cryptosporidium* zoonotiques et adaptés aux rongeurs, ce qui indique que ces rongeurs peuvent jouer un rôle dans le maintien et la dispersion de ce parasite dans l'environnement et d'autres hôtes, y compris les humains.

Introduction

Cryptosporidium, a protozoan parasite that colonizes the intestines, is a significant contributor to moderate to chronic diarrhea and related fatalities among children under two years of age and immunocompromized patients (HIV-positive) [4, 34]. Additionally, *Cryptosporidium* has been identified as able to infect over 260 species of animals [24]. Humans can acquire this parasite through various routes, encompassing direct contact with infected individuals or animals, and ingestion of contaminated water and food [35]. The public health importance of cryptosporidiosis became evident with the global recognition of *Cryptosporidium* as the predominant waterborne parasite [8]. To effectively minimize the frequency of *Cryptosporidium* outbreaks, it is imperative to identify potential sources of infection and likely modes of transmission [13]. Thus, monitoring *Cryptosporidium* in different hosts becomes critical, especially in animal hosts in close contact with humans.

A wide range of molecular epidemiological strategies have been used to characterize *Cryptosporidium* species at species/genotype and subtype levels [24]. Currently, this parasite has been identified with an estimated 120 genotypes and 50 valid species [23, 26]. Moreover, almost 21 distinct species/genotypes of this parasite have been found in humans, primarily as a result of zoonotic transmission, where the infection is transmitted from animals to humans [23]. Rodents, as a key reservoir of *Cryptosporidium*, have attracted widespread attention, particularly wild ones, considering their involvement alongside various animals (domestic, stray, and wild) and water sources in maintaining the stability and continuity of the *Cryptosporidium* transmission cycle. The presented data indicate that rodents harbor a minimum of 25 species and 48 genotypes of *Cryptosporidium*. Among these, wild rodents harbor 21 species and 32 genotypes, with *C. parvum* being the most common species [36]. Therefore, wild rodents potentially play a pivotal role in the transmission of zoonotic *Cryptosporidium* species. Despite this understanding, significant gaps exist concerning the incidence of *Cryptosporidium* infection in various nations and territories. For instance, in China, molecular studies on *Cryptosporidium* species in wild rodent species have been restricted to a small number of species [15, 22, 36].

Cryptosporidium species have been observed to exhibit a high prevalence in diverse animal species, including pigs, cattle, chickens, and horses, within the geographical region of Zhejiang Province, China [9, 28, 33, 40]. Moreover, they have also been found in patients with diarrhea, as well as in the source water of several cities of this province [2, 21, 37]. However, currently, there is only one study that confirms the presence of this parasite in *R. norvegicus* from Jiaying City in

Zhejiang [22]. The objective of the present study was to investigate the distribution, prevalence, and genetic characteristics of *Cryptosporidium* species among wild rodents residing in southern Zhejiang Province.

Materials and methods

Ethics

The present study was conducted as per the recommendations of the Chinese Laboratory Animal Administration Act (1988), which regulates the ethical handling and use of animals in scientific studies. All protocols were carefully examined and approved by the Research Ethics Committee of Wenzhou Medical University (SCILLSC-2021-01).

Sample collection

A total of 370 wild rodents were trapped in three distinct locations within rural areas immediately adjacent to human habitations in Zhejiang Province, between April 1 and October 14, 2023 (specifically, encompassing the second week of April, June, August, and October in the year 2023) (Fig. 1). Among these, 68 were caught in Yueqing (Hongqiao), 102 in Yongjia (Yantou), and 200 in Rui'an (Tangxia, Pandai, Shangwang). All wild rodents were trapped in cage traps baited with deep-fried dough sticks. In each designated location, around 50 cage traps were deployed at sunset and collected before sunrise. The traps were positioned in a linear setup, with 5 m between each trap, forming transects. All rodents were shifted to the controlled laboratory environment within 48 h following their capture and euthanized *via* CO₂ inhalation. Data related to the collection time and region was noted after these rodents were captured *via* trapping. Following that, a fresh feces sample (500 mg) was obtained immediately from the rectum of each rodent. The sample was then stored in ice boxes and shifted to the laboratory, where its DNA was extracted within a week.

DNA extraction

As per the manufacturer's recommendations, genomic DNA was isolated from each processed sample (200 mg) *via* a QIAamp DNA Mini Stool Kit (QIAGEN, Hilden, Germany). To achieve a significant yield of DNA, the lysate temperature was elevated to 95 °C. Before the PCR analysis, the DNA reconstituted in 200 µL of AE elution buffer (supplied with the kit) was kept at −20 °C.

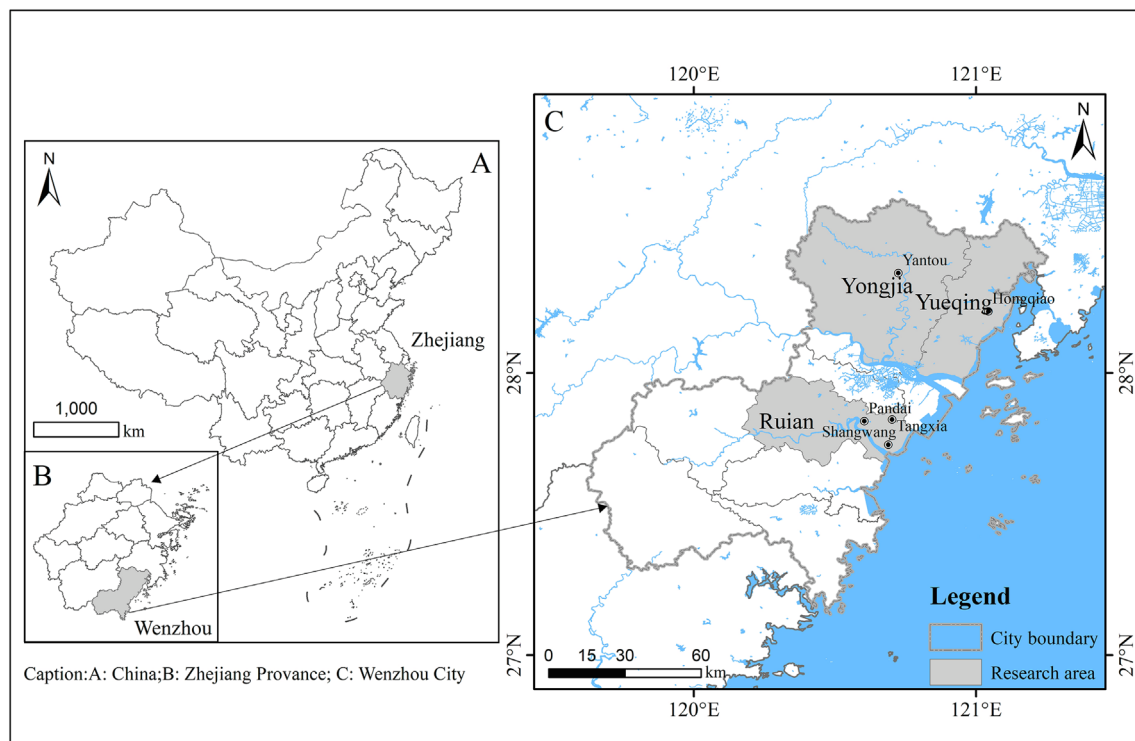


Figure 1. Map of rodent sampling locations in Wenzhou, Zhejiang Province, China. The figure was originally designed by the authors under ArcGIS 10.4 software. The original vector diagram imported in ArcGIS was adapted from the National Geomatics Center of China (<http://www.ngcc.cn>). The map has been modified and assembled according to permission and attribution guidelines.

Identification of rodent species

The rodent species were identified *via* PCR amplification of the vertebrate *cytochrome b* (*cytb*) gene with 421 bp amplified from fecal DNA. The primer design and PCR conditions were in line with the guidelines defined by Verma and Singh (2003) [27]. Each PCR reaction was comprised of 35 cycles, which included denaturation at 94 °C for 30 s, annealing at 51 °C for 30 s, and extension at 72 °C for 30 s. An initial denaturation step was also performed at 94 °C for 5 min, followed by the completion of a final extension at 72 °C for 5 min.

Cryptosporidium genotyping and subtyping

Nested PCR was performed on all isolated DNA with a specific target, using an 830 bp fragment of the partial small subunit of ribosomal RNA (*SSU rRNA*) gene of *Cryptosporidium* for amplification. Based on a previous description, primers were synthesized [32]. The 60-kDa glycoprotein (*gp60*) gene was amplified using nested PCR, enabling the further subtyping of positive isolates of *C. parvum* and *C. viatorum* using the same primers previously designed by Alves et al. (2003) and Stensvold et al. (2015), respectively [1, 25]. In every PCR amplification process, TaKaRa Taq DNA Polymerase (TaKaRa Bio Inc., Tokyo, Japan) was utilized. To ensure the validity of the reactions, positive controls, which contain *C. bailey* DNA derived from chickens, and negative controls, where no DNA template is included, were incorporated in every PCR reaction. Before sequencing, secondary PCR products were observed on

1.5% agarose gels, followed by staining with GelRed (Biotium, Fremont, CA, USA).

Sequencing and phylogenetic analysis

The commercial sequencing of amplified products of *SSU rRNA* and *gp60* genes of *Cryptosporidium* spp. was performed by Sangon Biotech (Shanghai) Co., Ltd. (Shanghai, China). Two-way sequencing was used to validate the accuracy of the sequence. To examine the species and subtype of *Cryptosporidium* species, the identified sequences were aligned with the reference sequences obtained from the National Center for Biotechnology (<https://www.ncbi.nlm.nih.gov/>) using ClustalX 2.0 (<http://www.clustal.org/>). In MEGA 11, a neighbor-joining (NJ) method with a Kimura 2-parameter model was used to conduct phylogenetic analyses, with the objective of assessing the phylogenetic relationships among the sequences obtained in this study and pertinent reference sequences available in GenBank. The clusters' stability was evaluated using 1000 replicates and Bootstrap analysis.

Statistical analyses

Data analysis was performed with SPSS version 22.0 (SPSS Inc., Chicago, IL, USA). The chi-square test was utilized to compare the prevalence of *Cryptosporidium* spp. between areas, gender, rodent species and season groups, respectively. A *p*-value < 0.05 was considered indicative of statistical significance.

Table 1. Prevalence and species/genotype of *Cryptosporidium* in the investigated rodent by species, season, gender, and location.

Category	Positive/examined (%)	<i>Cryptosporidium</i> spp./genotype (n)
Rodent species		
<i>Apodemus agrarius</i>	3/36 (8.3)	<i>C. parvum</i> (2), <i>C. muris</i> (1)
<i>Niviventer niviventer</i>	4/75 (5.3)	<i>Cryptosporidium</i> rat genotype IV (4)
<i>Rattus losea</i>	2/18 (11.1)	<i>Cryptosporidium</i> rat genotype IV (2)
<i>Rattus norvegicus</i>	11/155 (7.1)	<i>C. mortiferum</i> -like (4), <i>Cryptosporidium</i> rat genotype IV (4), <i>C. parvum</i> (2), <i>C. viatorum</i> (1)
<i>Rattus tanezumi</i>	6/86 (7.0)	<i>Cryptosporidium</i> rat genotype IV (6)
Season		
Spring	11/119 (9.2)	<i>Cryptosporidium</i> rat genotype IV (4), <i>C. mortiferum</i> -like (4), <i>C. parvum</i> (2), <i>C. viatorum</i> (1)
Summer	8/160 (5.0)	<i>Cryptosporidium</i> rat genotype IV (5), <i>C. parvum</i> (2), <i>C. muris</i> (1)
Autumn	7/91 (7.7)	<i>Cryptosporidium</i> rat genotype IV (7)
Gender		
Female	9/195 (4.6)	<i>Cryptosporidium</i> rat genotype IV (3), <i>C. mortiferum</i> -like (4), <i>C. parvum</i> (1), <i>C. muris</i> (1)
Male	17/175 (9.7)	<i>Cryptosporidium</i> rat genotype IV (13), <i>C. parvum</i> (3), <i>C. viatorum</i> (1)
Location		
Yueqing	10/68 (14.7)	<i>Cryptosporidium</i> rat genotype IV (7), <i>C. parvum</i> (2), <i>C. viatorum</i> (1)
Yongjia	3/102 (2.9)	<i>Cryptosporidium</i> rat genotype IV (2), <i>C. mortiferum</i> -like (1)
Rui'an	13/200 (6.5)	<i>Cryptosporidium</i> rat genotype IV (7), <i>C. mortiferum</i> -like (3), <i>C. parvum</i> (2), <i>C. muris</i> (1)
Total	26/370 (7.0)	<i>Cryptosporidium</i> rat genotype IV (16), <i>C. parvum</i> (4), <i>C. mortiferum</i> -like (4), <i>C. viatorum</i> (1), <i>C. muris</i> (1)

Nucleotide sequence accession numbers

The nucleotide sequences of *Cryptosporidium* obtained in this study were deposited in the GenBank database under accession numbers PP038021 to PP038023 and PP038026 to PP038028 for *SSU rRNA*, and PP104938 to PP104940 for *gp60*.

Results

Study population

This study used PCR and sequencing analysis of the *cytb* gene to identify five species of rodents, including *Rattus norvegicus* ($n = 155$), *R. tanezumi* ($n = 86$), *Niviventer niviventer* ($n = 75$), *Apodemus agrarius* ($n = 36$) and *R. losea* ($n = 18$) (Table 1 and Table S1). The majority of samples were obtained in the summer (43.2%, 160/370), then in spring (32.2%, 119/370) and autumn (24.6%, 91/370); none were collected in the winter. The sex of the rodents was reported as 52.7% (195/370) females and 47.3% (175/370) males (Table 1 and Table S1).

Prevalence of *Cryptosporidium* infection

Nested PCR was conducted on 370 fecal samples to evaluate the existence of *Cryptosporidium* species via the *SSU rRNA* gene. A total of 26 samples tested positive for this parasite with an average (7.0%) infection rate. *Cryptosporidium* existed in all three areas, with infection rates of 14.7% (Yueqing), 2.9% (Yongjia), and 6.5% (Rui'an) (Table 1 and Table S1). Statistical analysis revealed significant variations in the prevalence of *Cryptosporidium* among the three regions ($\chi^2 = 8.829$; $df = 2$; $p = 0.012$).

The infection rates of *Cryptosporidium* vary among rodent species, ranging from 5.3% (4/75) in *N. niviventer* to 11.1% (2/18) in *R. losea*, with 7.1% (11/155) in *R. norvegicus*, 7.0% (6/86) in *R. tanezumi*, and 8.3% (3/36) in *A. agrarius* (Table 1 and Table S1). The highest detection rate of *Cryptosporidium* in rodents collected in spring, reaching 9.2% (11/119), followed by 7.7% (7/91) in autumn, and 5.5% (8/160) in summer (Table 1 and Table S1). However, the difference between infection rates of *Cryptosporidium* in the groups of rodent species and seasons was not regarded as statistically significant ($p > 0.05$). In relation to gender, the incidence of *Cryptosporidium* was comparatively lower in female (4.6%) (9/195) than in male (9.7%; 17/175) rodents, but without statistical significance ($\chi^2 = 3.67$; $df = 1$; $p = 0.06$) (Table 1 and Table S1).

Cryptosporidium species/genotypes distribution

Five *Cryptosporidium* species or genotypes were detected, including *Cryptosporidium* rat genotype IV ($n = 16$), *C. parvum* ($n = 4$), *C. mortiferum*-like genotype ($n = 4$), *C. viatorum* ($n = 1$), and *C. muris* ($n = 1$) (Table 1 and Table S1). The prevalence of *Cryptosporidium* rat genotype IV was observed to be predominant (61.5%; 16/26) among the wild rodent population. This genotype was detected in four out of the five rodent species, except *A. agrarius* (Table 1 and Table S1). Of the four *C. parvum* isolates, two each were found in *R. norvegicus* and *A. agrarius*. *Cryptosporidium mortiferum*-like was only found in *R. norvegicus*, while *C. viatorum* and *C. muris* were identified in a single *R. norvegicus* and *A. agrarius*, respectively (Table 1 and Table S1).

The sampling sites exhibit differences in the distribution of *Cryptosporidium* species. Specifically, Yueqing yielded the *Cryptosporidium* rat genotype IV, *C. viatorum* and *C. parvum*. Yongjia yielded *C. mortiferum*-like and *Cryptosporidium* rat

genotype IV. While *Cryptosporidium* rat genotype IV, *C. parvum*, *C. muris* and *C. mortiferum*-like were discovered in Rui'an (Table 1 and Table S1).

Meanwhile, *Cryptosporidium* rat genotype IV was detected throughout all three seasons, whereas *C. muris* was exclusively detected in the summer. Conversely, *C. viatorum* and *C. mortiferum*-like were only detected in the spring (Table 1). In terms of gender, both male and female rodents were found to harbor *Cryptosporidium* rat genotype IV and *C. parvum*, whereas only female rodents were found to possess *C. muris* and *C. mortiferum*-like, and male rodents were found to harbor *C. viatorum* (Table 1 and Table S1).

Genetic identification of *Cryptosporidium* species/genotypes

At the *SSU rRNA* locus, among the 16 sequences of *Cryptosporidium* rat genotype IV, 10 and six sequences were identical and had 100% similarity with the sequence AY737582 of genotype W19 variant in storm water from the USA and MG917671 of genotype W19 variant in brown rats from China, respectively (Table S2). Four sequences of *C. parvum* were identical and had 100% similarity with the sequence OM146539 of *C. parvum* in humans from Sweden, as well as eight other sequences in *Macaca mulatta* or bamboo rats from China (Table S2). The four *C. mortiferum*-like isolates possessed identical sequences which have not been documented previously and exhibit a sequence similarity of 98.78% to the *C. mortiferum* sequence (OP935211) detected in humans from the USA (Table S2). The sequence of *C. viatorum* was similar and it has not been documented previously and exhibited homology of 99.61% to the sequence MK522270 of *C. viatorum* isolated in *Berylmys bowersi* from China (Table S2). The sequence of *C. muris* exhibited 100% identity to KF419208, which is found in *R. norvegicus* from China (Table S2). In the phylogenetic tree, the sequences belonging to the same species were shown to form distinct clusters, as depicted in Figure 2.

At the *gp60* gene locus, successful amplification was achieved for *C. viatorum* and three *C. parvum*-positive isolates. Two of the three *gp60* sequences obtained from *C. parvum* exhibited 100% resemblance to the sequence of *M. mulatta* in China, from which *C. parvum* subtype IIIdA15G1 (KJ917586) was identified (Table S2). The other one, *gp60* sequence of *C. parvum*, shared 100% similarity to subtype IIpA6 (MK956001) from bamboo rats in China (Table S2). The *C. viatorum* sample had the same *gp60* sequence which has not been documented in the previous literature and possesses a nucleotide similarity of 99.51% with the well-documented subtype XVdA3 (MK433560) of *C. viatorum* originating from *Leopoldamys edwardsi* in China (Table S2). Figure 3 presents the phylogenetic tree, showing the genetic correlations among the *gp60* subtypes of *C. parvum* and *C. viatorum*.

Discussion

In the present study, the average rate of *Cryptosporidium* infection among the identified rodents was 7.0%, which was found to be lower than the aggregated global rate for wild

rodents (20.5%), as determined by Zhang et al. [36]. In China, *Cryptosporidium* has been found in a variety of rodents, where the infection rates vary by their types, such as 4.0–73.9% in wild rodents, 2.1–29.5% in farmed rodents, 0.6–8.6% in lab rodents and 1.4–100% in pet rodents [22, 36]. The differences in rodent species, detection strategies, animal age, sample size, and study locations might be responsible for the disparity in prevalence.

The present study identified five *Cryptosporidium* species/genotypes including *Cryptosporidium* rat genotype IV, *C. mortiferum*-like, *C. parvum*, *C. viatorum* and *C. muris*. Multiple studies have demonstrated that rats serve as a predominant host species for *Cryptosporidium* rat genotype IV (formerly known as *Cryptosporidium* environmental sequence, *Cryptosporidium* genotypes W19, or W19 variant) [36, 39]. *Cryptosporidium* rat genotype IV has previously been found in Asian house rats, Edward's long-tailed rats, Muridae, and brown rats from China [6, 38], and it has also been identified in rats from Japan, Spain and Sweden [3, 14, 17]. However, despite the discovery of *Cryptosporidium* rat genotype IV in Asiatic black bears and cats from China and in one-humped camels from Egypt, limited data exist regarding the chances for infection of humans and other animals by *Cryptosporidium* rat genotype IV [7, 19, 29]. Consequently, the possibility of this genotype inducing disease in livestock or humans remains uncertain. Further systemic molecular epidemiological studies into *Cryptosporidium* species with a wider range of hosts are required in the future to identify the exact host distribution of *Cryptosporidium* rat genotype IV.

Zoonotic species include *C. muris*, *C. viatorum* and *C. parvum*, due to the extensive documentation of their infections in humans and a diverse array of mammalian hosts [23]. For example, *C. parvum*, which is prevalent in rodents worldwide, has been consistently identified in wildlife, having infected over 40 species of wild animals [23, 36]. In China 16.7% of human cases (44/263) of cryptosporidiosis were attributed to *C. parvum*, a prevalent pathogen in farmed animals, including cattle, sheep, and goats [12, 20]. Further, 18.7% of rodent-derived *Cryptosporidium* cases (189/1010) had been confirmed to be caused by *C. parvum* [36]. Initially, *C. viatorum* was detected in travelers from the Indian subcontinent who had arrived in the United Kingdom [7]. More than 13 countries, including China, have reported cases of *C. viatorum* in humans [25, 31]. Further analysis revealed the presence of *C. viatorum* in several rodent species, including *R. rattus* from France, *R. lutreolus* from Australia and *Leopoldamys edwardsi* and *Berylmys bowersi* from China [10, 16, 38]. Additionally, *C. muris* has been extensively documented in various mammalian hosts, such as rodents, felids, canids, equids, suids, non-human primates, etc. [23]. The transmission of *C. parvum*, *C. viatorum* and *C. muris* from wild rodent species to humans and other animals via cross-species contact could, therefore, not be ignored.

This study identified a novel genotype in *R. norvegicus* that shares genetic similarities to *C. mortiferum* (*Cryptosporidium* chipmunk genotype I), named *C. mortiferum*-like. The sequences of *C. mortiferum*-like discovered in this study have not been previously reported in the literature. However, it is well known that *C. mortiferum* can infect people, and several

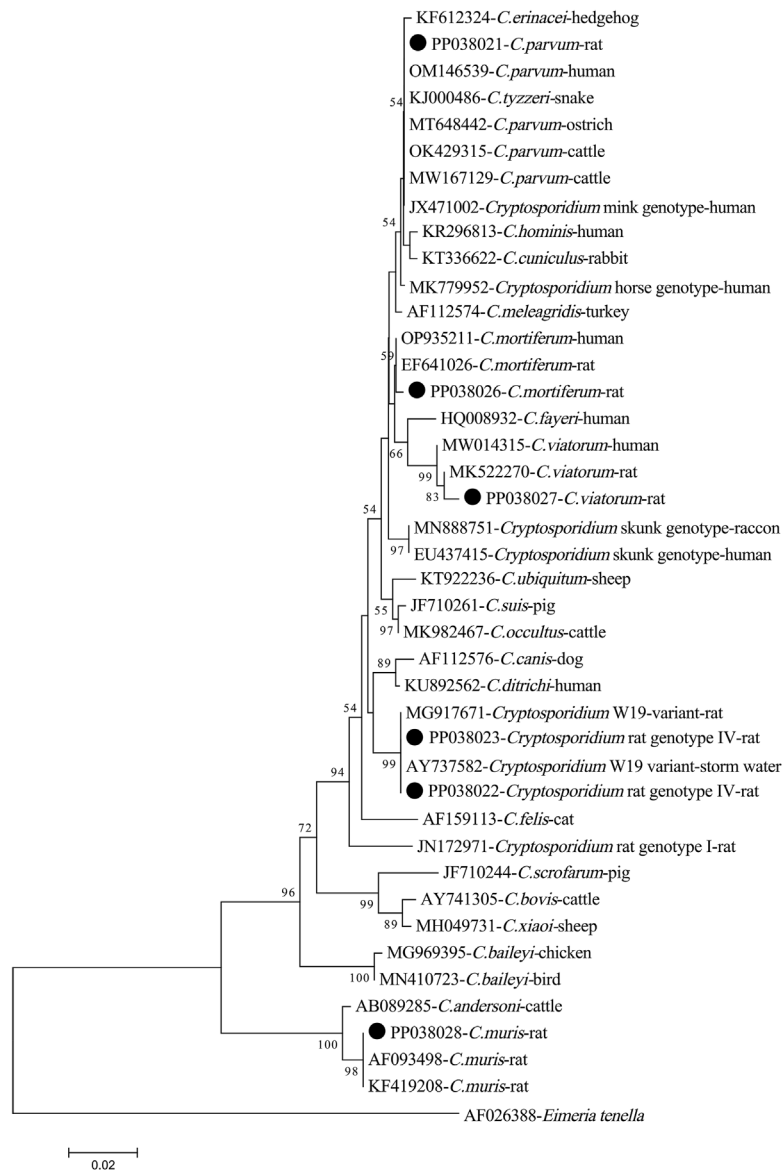


Figure 2. Phylogenetic tree of *Cryptosporidium* species based on SSU rRNA sequences. The tree was generated using a neighbor-joining analysis, with genetic distances calculated via the Kimura 2-parameter model. Bootstrap values (>50%) derived from 1000 replicates are displayed to the left of the nodes for reliability assessment. The sequences generated in the present study are indicated with the solid circles.

human cases have been reported [11, 23, 26]. Therefore, *C. mortiferum*-like is highly likely to also have the ability to infect people, and of course, clear evidence needs to be provided through more research in the future. The discovery of novel sequences of *Cryptosporidium* in *R. norvegicus* suggests the existence of some novel *Cryptosporidium* species/genotypes in wild rodents. This is primarily due to the order of rodents having the most diverse of all mammalian groups.

In the evaluation of *C. parvum* in both animals and humans, subtyping tools are frequently used. The transmission of *C. parvum* between animals and humans was enhanced via the application of subtype-specific molecular diagnostic tools [12, 24]. At least 15 subtype families for *C. parvum* were detected via *gp60* gene analysis, including Ila-IIi and IIk-IIp [12]. In China, at least 20 subtypes have been identified, with

IlaA15G2R1, IlaA15G2R2, IlaA13G2R2, IIdA15G1 and IIdA14G1 being found in humans [12, 20]. Wild rodents were examined in the present study that observed two subtypes (IIdA15G1 and IIpA6) of *C. parvum*. The subtype IIdA15G1 is one of the prevalent subtypes found in cattle, exhibiting a diverse geographic distribution across China [12]. Its elevated mortality rate among pre-weaned dairy calves in China has been attributed to multiple outbreaks of cryptosporidiosis [5, 12]. Furthermore, the subtype was subsequently detected in non-human primates and humans in China, which provides further evidence for the possibility of zoonotic transmission [12, 30, 37]. Thus, IIdA15G1-infected wild rodents pose a potential risk to both humans and other animals. However, IIpA6 has only been detected in bamboo rodents thus far, and its potential to infect humans and livestock is unknown [18].



Figure 3. Phylogenetic relationships of gp60 subtypes of *C. parvum* and *C. viatorum* identified in the study and other gp60 subtype sequences deposited in GenBank, as inferred by a neighbor-joining analysis of gp60 sequences based on the genetic distance by the Kimura 2-parameter model. The numbers displayed on the branches represent the percentage bootstrapping outcomes derived from 1000 replicates. The sequences generated in the present study are indicated with solid circles.

A comprehensive understanding of the host range of subtype IIpA6 of *Cryptosporidium* species would require systematic molecular epidemiological studies across a wider range of hosts.

Conclusions

The present study provided evidence of the presence of *Cryptosporidium* in five species of wild rodents in Zhejiang, China, with an average infection rate of 7.0%. The presented molecular findings suggest that *Cryptosporidium* rat genotype IV predominantly infect wild rodents. As a result, these rodents have a restricted capacity to serve as natural reservoirs for human infections. In contrast, the discovery of *C. muris*, *C. parvum*, *C. mortiferum*-like, *C. viatorum* and *C. viatorum* suggests a connection between rodents and humans. This finding demonstrates that animals infected with *Cryptosporidium* have substantial zoonotic potential and indicates that wild rodents could serve as a reservoir for human cryptosporidiosis caused by the *Cryptosporidium* species above.

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Conflict of interest

The authors do not have a commercial or other association that represents a conflict of interest.

Supplementary Tables

The supplementary Tables S1 and S2 for this article can be found at <https://www.parasite-journal.org/10.1051/parasite/2024033/olm>.

Table S1: Prevalence and species of *Cryptosporidium* in the investigated wild rodents by species, season, gender, and location.

Table S2: Similarity analysis of SSUrRNA and Gp60 sequences of *Cryptosporidium* obtained in this study.

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