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Infections and genetic diversity of *Cryptosporidium* spp. and *Giardia* spp. in small wild mammals on the Eastern Tibetan plateau: public health implications

Qingqiu Zuo[1†], Zhuoma Bianba[2†], Chuizhao Xue[1], Hua Liu[1], Xiaoxue Peng[1], Hao Zhou[1], Xiaocheng Zhang[1], Quan Chen[1], Mengqing Li[1], Yang Yang[3], Yan Zhou[1], Jianping Cao[1,3], Jianhai Yin[1], Xu Wang[1*] and Yujuan Shen[1*]

Abstract

Background *Cryptosporidium* and *Giardia* are protozoan parasites that cause significant diarrheal diseases. Small mammals are potential reservoirs for their zoonotic transmission. The Tibetan Plateau alpine ecosystem harbors diverse and abundant small wild mammals, but their roles in the transmission of *Cryptosporidium* and *Giardia* remain inadequately studied.

Methods Field sampling was conducted in Shiqu County, Sichuan Province, on the eastern Tibetan Plateau, in 2023. Small wild mammals were captured and morphologically identified. Fecal DNA was screened for *Cryptosporidium* and *Giardia* with nested-PCR targeting the small subunit ribosomal RNA (SSU rRNA) gene and the beta-giardin (*bg*) gene, respectively. Positive DNA samples were sequenced and analyzed phylogenetically. Differences in prevalence were assessed with the chi-square test.

Results In total, 336 small wild mammals were collected, dominated by *Neodon fuscus* (175/336, 52.1%) and *Ochotona curzoniae* (106/336, 31.5%). *Cryptosporidium* infection was detected in 22.3% (75/336) of the small mammals, with marginally higher prevalence in *N. fuscus* (44/175, 25.1%) than in *O. curzoniae* (17/106, 16.0%; $\chi^2 = 3.220$, $p = 0.073$). Six species/genotypes were identified: zoonotic *C. suis* ($n = 25$) and *C. canis* ($n = 6$), and non-zoonotic muskrat genotype II ($n = 26$), yak genotype ($n = 10$), muskrat genotype I ($n = 4$), and *Cryptosporidium* sp. MT524977 ($n = 4$). The overall prevalence of *Giardia* was 8.3% (28/336), with similar prevalence in *N. fuscus* (17/175, 9.7%) and *O. curzoniae* (10/106, 9.4%, $\chi^2 = 0.006$, $p = 0.938$). Non-zoonotic *G. microti* ($n = 3$) and four unclassified *Giardia* spp.: *Giardia* sp. OR770651 ($n = 13$), *Giardia* sp. PQ604631 ($n = 7$), *Giardia* sp. MG676959 ($n = 3$), and *Giardia* sp. OP963933 ($n = 2$) were

†Qingqiu Zuo and Zhuoma Bianba have contributed equally to this work.

*Correspondence:
Xu Wang
Wangxu@nipd.chinacdc.cn
Yujuan Shen
Shenyj@nipd.chinacdc.cn

Full list of author information is available at the end of the article

identified. *Neodon fuscus* harbored greater *Giardia* diversity (all five *Giardia* spp.) than *O. curzoniae* (only *Giardia* sp. OR770651).

Conclusions This study demonstrates a high *Cryptosporidium* prevalence in small wild mammals on the eastern Tibetan Plateau, suggesting potential zoonotic risks to humans and livestock. *Giardia* exhibited host-specific infection patterns, indicating possible niche adaptation that warrants further investigation. These findings expand the host range and geographic distribution of these parasites and help elucidate their transmission dynamics in alpine ecosystems.

Keywords *Cryptosporidium*, *Giardia*, Rodentia, Lagomorpha, Zoonotic potential, Shiqu

Background

Cryptosporidium spp. and *Giardia* spp. are protozoan parasites of significant global public health concern. They are responsible for widespread gastrointestinal diseases in humans and animals. Infections caused by these pathogens are typically characterized by asymptomatic or self-limiting diarrhea in healthy individuals. However, they can progress to severe, life-threatening diarrheal illness in vulnerable populations, particularly children and immunocompromised individuals such as those with HIV/AIDS [1]. *Cryptosporidium* species are the fourth leading etiological agent of diarrhea in children under 5 years old, accounting for 68,500 (38,900–110,000) deaths globally in 2021 [2]. *Giardia* species are responsible for approximately 280 million cases of diarrhea in humans annually [3]. These pathogens are primarily transmitted via the fecal–oral route in contaminated water or food, especially in regions with inadequate sanitation infrastructure and low-resource settings [4].

Recent evidence indicates that small mammals, particularly Rodentia, are important reservoirs for zoonotic *Cryptosporidium* spp. and *Giardia* spp. To date, more than 170 species/genotypes of *Cryptosporidium* spp. have been identified. Of these, more than 26 species and 59 genotypes have been reported in rodents [5]. Among them, 17 species/genotypes commonly infect humans, including *C. viatorum*, *C. mortiferum*, *C. muris*, and *C. andersoni* [5]. Nine *Giardia* species have been recognized to date [6], four of which have been identified in rodents: *G. duodenalis*, *G. muris*, *G. microti*, and *G. cricetidarum* [7]. *Giardia duodenalis*, which includes eight assemblages (A to H), is the only zoonotic species complex. Assemblages A and B are the main causative agents of giardiasis in humans, whereas assemblage E is occasionally found in humans [5]. Lagomorpha, another highly abundant group of small mammals, although understudied, can also transmit zoonotic protozoan parasites, including *C. parvum*, *C. cuniculus*, and *G. duodenalis* (assemblages A and B), which can contaminate drinking water [8]. Nowadays, the high prevalence of *Cryptosporidium* spp. (up to 50% in Hainan) and *Giardia* spp. (up to 52.6% in Ningxia) in small wild mammals in China has attracted significant attention (Table 1). The distribution and prevalence of these pathogens show significant variations influenced by complex environmental factors, including geography, climate, and host species [13]. Understanding the infection status of specific hosts across different ecological environments is crucial for clarifying the transmission dynamics of these pathogens.

The Tibetan Plateau is a typical alpine meadow ecosystem characterized by its high altitude (average elevation > 4,000 m) and frigid climate (annual mean temperature < 0 °C) [33]. This ecosystem harbors an abundance of endemic small mammals, including the plateau pika (*Ochotona curzoniae*), Tibetan hamster (*Cricetulus kamensis*), and mountain vole (*Neodon* spp.) [34–36]. These mammals play pivotal roles in the ecosystem due to their large population sizes, extensive distribution ranges, and strong migratory capabilities [37]. In the eastern Tibetan Plateau, where dense rodent populations intersect with human and livestock activities, these species also serve as key reservoirs for the transmission of various zoonotic diseases, including plague [38], leptospirosis, babesiosis [39], echinococcosis [40], and others [41]. However, to date, limited studies have investigated the infection of small mammals on the Tibetan Plateau with *Cryptosporidium* spp. and *Giardia* spp. Since Zhang et al. [21] first reported the presence of *Cryptosporidium* spp. in *N. fuscus* (8.9%, 8/90) and *O. curzoniae* (6.25%, 4/64) on the Tibetan Plateau, only three additional studies have investigated these parasites in rodents in this area [13, 22, 23]. Except for the plateau zokor (*Myospalax baileyi*) [22] and Himalayan marmot (*Marmota himalayana*) [23], the infection of endemic small mammals with *Cryptosporidium* spp. or *Giardia* spp. has yet to be investigated. Furthermore, the potential influence of the host species and other host traits (e.g., sex and age) on the infection dynamics of these two pathogens in this alpine meadow ecosystem has not been examined. Because humans on the Tibetan Plateau primarily engage in nomadic pastoralism, frequently interact with wildlife, and often rely on untreated surface water [42], *Cryptosporidium* oocysts and *Giardia* cysts from wildlife (such as rodents and pikas) may pose an elevated threat to the transmission of zoonotic diseases to humans.

Table 1 Prevalence of *Cryptosporidium* spp. And *Giardia* spp. In wild Rodentia And Lagomorpha in China

Pathogens	Province	Published year	Host taxa (No.)	Detection methods	Target gene	No. tested	No. positive	Prevalence (%)	Species/genotypes/subtypes (No.)	Reference
Cryptosporidium	Chongqing	2019	*Leopoldamys edwardsi* (111)	Nested-PCR	SSU rRNA, *gp60*	111	4	3.6	*C. viatorum* (4, XVaA6 (2)) [a]	[9]
	Fujian	2009	*Rattus tanezumi* (33)	Nested-PCR	SSU rRNA, *gp60*	33	6	1.8	mouse genotype I (1), rat genotype II (1), rat genotype III (1), mouse genotype I plus rat genotype II (1), mouse genotype I plus rat genotype III (1)	[10]
		2015	*Rattus tanezumi* (33), *Rattus norvegicus* (168), *Mus musculus* (31)	Nested PCR	SSU rRNA, *gp60*	232	19	8.2	*C. parvum* (12, IIdA15G1(9)), *C. muris* (7)	[11]
	Gansu	2022	*Marmota himalayana* (399), *Spermophilus alashanicus* (99)	Nested-PCR	SSU rRNA, *gp60*	498	11	2.2	*C. rubeyi* (2), ground squirrel genotype II (7), chipmunk genotype V (1), horse genotype VIbA10 (1)	[12]
	Guangdong	2019	*Berylmys bowersi* (117)	Nested-PCR	SSU rRNA, *gp60*	117	21	18.0	*C. viatorum* (21, XVaA3g (3), XVaA3h (7), XVcA2G1 (1))	[9]
		2024	Wild rodents (125)	Nested-PCR	SSU rRNA, *gp60*	125	30	24.0	rat genotype IV (10), *C. muris* (5), rat genotype III (4), *Cryptosporidium* sp. MT561515 (3), *C. tyzzeri* (2), w19 (3), *C. parvum* (1, IIdA20G1 (1)), *C. baileyi* (1), *Cryptosporidium* sp. KY483983 (1)	[13]
	Guangxi	2021	*Mus musculus* (74)	Nested-PCR	SSU rRNA	74	7	9.5	*C. viatorum* (1), rat genotype II/III (5), *Cryptosporidium* sp. novel2 (1)	[14]
		2025	Wild rodents (103)	Nested-PCR	SSU rRNA	103	5	4.9	*C. viatorum* (1), rat genotype II (1), rat genotype IV (3)	[15]
	Hainan	2019	*Rattus tanezumi* (46), *Rattus norvegicus* (56), *Leopoldamys edwardsi* (38), *Niviventer fulvescens* (10)	Nested-PCR	SSU rRNA, *gp60*	150	75	50.0	rat genotype IV (47), rat genotype III (13), *C. viatorum* (11, XVcA2G1a (4), XVcA2G1b (1) and XVdA3 (1)), *C. occultus* (2), *C. muris* (1), *C. erinacei* (1)	[16]
	Heilongjiang	2018	*Rattus norvegicus* (242)	Nested-PCR	SSU rRNA	242	22	9.1	rat genotype I (14), rat genotype IV (6), *C. suis*-like genotype (1)	[17]
		2021	*Spermophilus dauricus* (39), *Rattus norvegicus* (2)	Nested-PCR	SSU rRNA	41	5	12.2	*C. felis* (1), *Cryptosporidium* sp. novel 1 (4)	[14]
		2022	*Rattus norvegicus* (242)	Nested-PCR	SSU rRNA	231	8	3.5	*C. felis* (2), *Cryptosporidium* sp. novel 2 (3)	[18]
	Henan	2009	*Hystrix hodgsoni* (8), *Myocastor coypus* (1), *Marmota sibirica* (1), *Rattus norvegicus* (64), *Mus musculus* (12)	Nested-PCR	SSU rRNA, *gp60*	86	4	0.5	mouse genotype I (3), mouse genotype I plus rat genotype III (1)	[10]
	Hunan	2025	Wild rodents (319)	Nested-PCR	SSU rRNA	319	2	0.6	vole genotype VII (2)	[15]

Table 1 (continued)

Pathogens	Province	Published year	Host taxa (No.)	Detection methods	Target gene	No. tested	No. positive	Prevalence (%)	Species/genotypes/subtypes (No.)	Reference
	Inner Mongolia	2021	*Lasiopodomys brandtii* (22)	Nested-PCR	SSU rRNA	22	0	0.0	-	[14]
		2024	*Lasiopodomys brandtii* (678)	Nested-PCR	SSU rRNA	678	127	18.7	*C. suis*, *Cryptosporidium* environmental sequence, muskrat genotype II, Brandt's voles genotype I	[19]
		2024	*Apodemus agrarius* (27), *Cricetulus barabensis* (60), *Mus musculus* (78), *Rattus norvegicus* (92)	Nested-PCR	SSU rRNA	257	20	7.8	*C. ubiquitum* (7), rat genotype IV (5), rat genotype III (5), *C. muris* (1), *C. occultus* (1), *C. ratti* (1)	[20]
	Jilin	2022	*Rattus norvegicus* (242)	Nested-PCR	SSU rRNA	119	8	6.7	rat genotype Ⅱ/Ⅲ (6), *Cryptosporidium* sp. novel1 (2)	[18]
	Liaoning	2024	*A. agrarius* (62), *Cricetulus barabensis* (36), *Mus musculus* (28), *Rattus norvegicus* (103)	Nested-PCR	SSU rRNA	229	16	7.0	rat genotype III (5), *C. occultus* (4), rat genotype IV (4), *C. muris* (1), *C. ubiquitum* (1), *C. viatorum* (1)	[20]
	Qinghai	2018	*Microtus fuscus* (90), *Ochotona curzoniae* (64)	Nested-PCR	SSU rRNA	154	12	7.8	*C. parvum* (5), *C. canis* (1), *C. ubiquitum* (1), Qinghai vole genotype (3), pika genotype (2)	[21]
		2022	*Myospalax baileyi* (98)	Nested-PCR	SSU rRNA	98	1	1.0	*Cryptosporidium* sp. (1)	[22]
		2024	*Marmota himalayana* (243)	Nested-PCR	SSU rRNA	243	12	4.9	marmot genotype I (3), marmot genotype II (9)	[23]
	Shandong	2021	*Rattus norvegicus* (227)	Nested-PCR	SSU rRNA	227	0	0.0	-	[14]
	Shanxi	2021	*Rattus norvegicus* (53)	Nested-PCR	SSU rRNA	53	2	3.8	*Cryptosporidium* sp. novel2 (2)	[14]
	Sichuan	2009	*Rattus nitidus* (2), *Apodemus agrarius* (26)	Nested-PCR	SSU rRNA, *gp60*	28	0	0.0	-	[10]
		2019	*Callosciurus erythraeus* (28)	Nested-PCR	SSU rRNA	287	4	1.8	rat genotype II (2), *C. parvum* (1), *C. wrairi* (1)	[24]
		2024	Wild rodents (185)	Nested-PCR	SSU rRNA, *gp60*	185	38	20.5	*C. viatorum* (14, XVeA4 (4), XVeA6 (2), XVfA4 (2), XVfA5 (2), XVeA5 (1)), *C. occultus* (6), Vole genotype II (4), C. *microti* (2), *C. muris* (2), *C. ubiquitum* (2, XIIh (1), XIIj (1)), bear genotype (3), vole genotype 1 (2), W25 (1), *Cryptosporidium* sp. KY644567 (1), *C. sciurinum* (1)	[13]
	Xinjiang	2023	*Ochotona pallasi* (83)	Nested-PCR	SSU rRNA	83	12	14.5	*C. canis* (2), *C. ubiquitum* (2), *C. ryanae* (2), yak genotype (4), *Cryptosporidium* sp. Mongolian pika (2)	[25]
		2024	*Rattus norvegicus* (435)	Nested-PCR	SSU rRNA, *gp60*	435	24	5.5	*C. occultus* (10), *C. parvum* (2, IIdA19G1(2)), *C. ditrichi* (1), rat genotype IV (9)	[26]
		2024	Wild rodents (132)	Nested-PCR	SSU rRNA, *gp60*	132	20	15.2	chipmunk genotype III (7), sw1 (7), *C. alticolis* (3), vole genotype V (3)	[13]
		2024	*Marmota baibacina* (378)	Nested-PCR	SSU rRNA, *gp60*	378	15	4.0	chipmunk genotype V (9), chipmunk genotype III (1), *C. rubeyi* (1), *C. ubiquitum* (3, XIIa (3)), *C. bovis* (1)	[27]

Table 1 (continued)

Pathogens	Province	Published year	Host taxa (No.)	Detection methods	Target gene	No. tested	No. positive	Prevalence (%)	Species/genotypes/subtypes (No.)	Reference
	Yunnan	1991	*Rattus tanezumi* (70), *Rattus norvegicus* (85), *Mus musculus* (6), *Apodemus chevrier* (20), *Eothenomys miletus* (6)	Microscopic examination	-	187	44	23.5	-	[28]
		2018	*Rattus norvegicus* (207), *Rattus tanezumi* (17)	Nested-PCR	SSU rRNA	287	13	4.5	*C. muris* (13)	[29]
		2025	Wild rodents (88)	Nested-PCR	SSU rRNA	88	2	2.3	*C. muris* (1), *C. ratti* (1)	[15]
	Zhejiang	2021	*Rattus norvegicus* (119)	Nested-PCR	SSU rRNA	119	4	3.4	*Cryptosporidium* sp. novel 2 (4)	[14]
		2024	*Apodemus agrarius* (36), *Niviventer niviventer* (75), *Rattus losea* (18), *Rattus norvegicus* (155), *Rattus tanezumi* (86)	Nested-PCR	SSU rRNA, *gp60*	370	26	7.0	*C. parvum* (4, IIdA15G1 (2), IIpA6 (1)), *C. viatorum* (1, XVdA3, (1)), *C. muris* (1), rat genotype IV (16), *C. mortiferum*-like (4).	[30]
Giardia	Fujian	2015	*Rattus tanezumi* (33), *Rattus norvegicus* (168), *Mus musculus* (31)	Nested PCR	*bg*, *tpi*, *gdh*	232	14	6.0	G. *duodenalis* (G (14))	[11]
	Guangdong	2024	Wild rodents (61)	Nested-PCR	*bg*, *tpi*, *gdh*	61	3	4.9	G. *duodenalis* (3, G (2), A (1))	[13]
	Guangxi	2025	Wild rodents (103)	Nested-PCR	*bg*	103	2	1.9	G. *duodenalis* (2, G (1) F (1))	[31]
	Gansu	2022	*Marmota himalayana* (399), *Spermophilus alashanicus* (99)	Nested-PCR	*bg*, *tpi*, *gdh*	498	8	1.6	G. *duodenalis* (8, A (1), B (6), E (1))	[12]
	Hunan	2025	Wild rodents (153)	Nested-PCR	*bg*	153	45	29.4	G. *microti* (45)	[31]
		2000	*Microtus fortis* (142)	Microscopic examination	-	142	73	51.4	*Giardia* spp.	[32]
	Ningxia	2000	*Microtus fortis* (142)	Microscopic examination	-	38	20	52.6	*Giardia* spp.	[32]
	Qinghai	2022	*Myospalax baileyi* (98)	Nested-PCR	*bg*	98	0	0	-	[22]
		2024	*Marmota himalayana* (243)	Nested-PCR	18 s rRNA	243	2	0.8	G. *duodenalis* (2, A (2))	[23]
	Sichuan	2024	Wild rodents (185)	Nested-PCR	*bg*, *tpi*, *gdh*	185	15	8.1	*G. muris* (15)	[13]
	Xinjiang	2024	Wild rodents (132)	Nested-PCR	*bg*, *tpi*, *gdh*	132	57	43.2	G. *microti* (57)	[13]
	Yunnan	2025	Wild rodents (88)	Nested-PCR	*bg*	88	1	1.1	G. *duodenalis* (1, F (1))	[31]

[a] All data in this column follow the format: species/genotype (total, subtype$_1$ (count), subtype$_2$ (count), …). The first number (total) represents the total instances of the detection of the species or genotype, and subsequent values indicate the counts of individual subtypes

b. The study of *Lasiopodomys brandtii* in Inner Mongolia reported *Cryptosporidium* infections but did not provide species/genotype counts

Therefore, in the present study, we (1) investigated the prevalence of *Cryptosporidium* spp. and *Giardia* spp. in small wild mammals on the eastern Tibetan plateau; (2) assessed the genetic diversity of these pathogens and evaluated their zoonotic risks; and (3) investigated the influence of host traits on their prevalence.

Materials and methods

Study area

Field studies were conducted in Yongbo Valley (32°19′–34°20′N, 97°20′–99°15′E), a typical alpine meadow ecosystem located in Sexu town, Shiqu County, Ganzi Tibetan Autonomous Prefecture, Sichuan Province, China. This area has an average elevation of 4,250 m above sea level (asl), and is characterized by gently rolling hills and broad valleys, with rivers flowing through the valley floor. The vegetation is primarily dominated by *Kobresia* spp. It provides a suitable habitat for endemic small mammals, including *O. curzonia* and several Circetidae rodents, primarily *N. fuscus*.

Sample collection

This study and research protocols were reviewed and approved by the Laboratory Animal Welfare & Ethics Committee of the National Institute of Parasitic Diseases, Chinese Center for Disease Control and Prevention, China (IPD-2022-010). All procedures involving the trapping, handling, and euthanasia of wild rodents were conducted following the Regulation on the Administration of Laboratory Animals (2013 Revision). All small mammals used in this study were sourced from free-living populations in the wild. No animals were privately owned by any institution, individual, or farm.

In 2023, small mammals were collected during the warm season (from July to August), when these small Mammals are most active. Four 50 m × 50 m quadrats were randomly set on the grassland at Yongbo Gou. Within each quadrat, 400 snap traps (size: 12 cm × 6.5 cm) were placed at the entrances of active dens for 24 h. The majority of the captured small mammals were dead due to the mechanical force of the snap traps. Only a small number of individuals were alive, and they were euthanized by rising carbon dioxide (CO_2) concentration in a sealed chamber, with death confirmed by exsanguination. The heads of the small Mammals were removed and stored separately in 50-ml centrifuge tubes containing 95% ethanol for further species identification. Fecal samples were collected in separate 50 ml centrifuge tubes and stored at − 80℃ for subsequent DNA extraction.

Identification of small mammal species

In Shiqu County, small mammals primarily include *O. curzoniae* and rodents of the family Cricetidae. The morphological identification of rodent species was based on pelage color patterns and body measurements, especially the skull–mandible morphology (particularly the molars), following the methods described by Luo et al. [43] and Smith and Xie [35].

DNA extraction

Genomic DNA was extracted from ~200 mg of each rodent fecal sample using the QIAamp DNA Stool Mini Kit (cat. #51604; Qiagen, Hilden, Germany), according to the manufacturer's instructions. The DNA was stored at −20 ℃ until PCR analysis.

PCR amplification

Based on preliminary experiments evaluating the amplification efficiency of genetic loci for *Cryptosporidium* spp. and *Giardia* spp. in DNA extracted from fecal samples of small wild mammals, the small subunit ribosomal RNA gene (SSU rRNA gene, for *Cryptosporidium* spp.) and the beta-giardin gene (*bg* gene, for *Giardia* spp.) were selected as the most efficient and consistent targets for detection. In formal experiments, all DNA samples were tested with nested-PCR, amplifying an 880-bp fragment of the SSU rRNA gene for *Cryptosporidium* spp [44]. and a 470-bp fragment of the *bg* gene for *Giardia* spp [13]., using previously reported primers [13, 44]. All PCR amplifications were performed with TaKaRa Taq DNA Polymerase (TaKaRa Bio Inc., Tokyo, Japan). To ensure the validity of the reactions, RNase-free water and DNA from *C. parvum* and *G. duodenalis* assemblage D, isolated from a stray dog, were used as negative and positive controls, respectively. At least two replicates were performed of each PCR assay. All inner PCR amplicons were visualized with 1% agarose gel electrophoresis.

Sequencing and analysis

All secondary positive PCR amplicons were sent to Sangon Biotech (Shanghai) Co., Ltd. (Shanghai, China) for bidirectional sequencing. All nucleotide sequences were then analyzed with MEGA version 11.0.13 [45] and compared with the National Center for Biotechnology Information (NCBI) GenBank database (http://www.ncbi.nlm.nih.gov/) to identify the species and/or genotypes.

Phylogenetic analysis

To determine the phylogenetic relationships of the *Cryptosporidium* spp. and *Giardia* spp. and to assess their potential zoonotic risk, neighbor-joining (NJ) trees were constructed with the Kimura-2-parameter model based on the SSU rRNA gene for *Cryptosporidium* spp. and the *bg* gene for *Giardia* spp. The robustness of the nodes was tested with a bootstrap analysis using 1000 iterations.

Statistical analysis

The prevalence of *Cryptosporidium* spp. and *Giardia* spp. was calculated as the proportion of positive samples among the samples collected. The 95% confidence interval (CI) was calculated to quantify the precision of the prevalence estimates by the Clopper-Pearson method. Variations in the positive rates of pathogens across host species and sexes were analyzed using the Chi-square (χ^2) test in R version 4.3.2 [46]. *P* values ≤ 0.05 were considered statistically significant.

Results

Small mammal species composition

In total, 336 small mammals were captured from two orders (Rodentia, $n = 230$; Lagomorpha, $n = 106$), and four species were identified. *Neodon fuscus* was the most abundant species ($n = 175$), followed by *O. curzoniae* ($n = 106$), *N. leucurus* ($n = 23$), and *N. irene* ($n = 9$), as well as 23 rodents that could not be classified to the species level due to ambiguous morphological characteristics (Table 2).

Infections with Cryptosporidium spp

Molecular screening targeting the SSU rRNA gene revealed a *Cryptosporidium* spp. prevalence of 22.3% (75/336) in the small mammals sampled (Table 3). The highest prevalence was observed in *N. leucurus* (34.8%, 8/23), followed by *N. fuscus* (25.1%, 44/175) and *O. curzoniae* (16.0%, 17/106). Due to sample size limitations (*N. leucurus*: $n = 23$; *N. irene*: $n = 9$), the comparative statistical analysis was restricted to *N. fuscus* and *O. curzoniae*, and revealed a marginally higher prevalence in *N. fuscus* than in *O. curzoniae* ($\chi^2 = 3.220$, $p = 0.073$). No significant difference in *Cryptosporidium* prevalence was observed between males and females ($\chi^2 = 0.364$, $p = 0.546$).

Genetic identification of Cryptosporidium species/genotypes

Analysis of the SSU rRNA gene sequences from 75 *Cryptosporidium*-positive samples identified five known species/genotypes and one unclassified *Cryptosporidium* sp. (Table 3). These included muskrat genotype II (i.e., genotype w16, 34.7%, 26/75), *C. suis* (33.3%, 25/75), yak genotype (13.3%, 10/75), *C. canis* (8.0%, 6/75), muskrat genotype I (i.e., genotype w7, 5.3%, 4/75), and an unclassified *Cryptosporidium* sp. (5.3%, 4/75). At the SSU rRNA locus, 26 sequences of muskrat genotype II showed 100% identity to AY737567 (isolated from a stormwater sample in the United States), and 10 yak genotype sequences were identical to KF971356 (from a yak in China). Twenty-five *C. suis* sequences exhibited 96.9% identity to OQ456430 (from a pig in China), with 23 nucleotide differences, while six *C. canis* sequences demonstrated 98.8% identity to OR557506 (from a Mongolian pika), differing by Nine nucleotides. Four muskrat genotype I sequences shared 99.2% identity with JQ178277 (from a water sample in Canada), with six nucleotide differences, and four *Cryptosporidium* sp. sequences shared 98.8% identity with an unclassified *Cryptosporidium* sp. sequence (MT524977) isolated from a western spotted skunk (*Spilogale gracilis*), differing by nine nucleotides. For clarity of description, each unclassified sequence was labeled "*Genus* sp." with the accession number of its closest NCBI match (e.g., *Cryptosporidium* sp. MT524977; see Table 3). A phylogenetic analysis confirmed that sequences of the same species/genotype formed distinct clusters (Fig. 1).

Distribution of Cryptosporidium species/genotypes

The distribution of *Cryptosporidium* spp. varied across the small mammal taxa. *Neodon fuscus* showed the highest diversity, with six *Cryptosporidium* species/genotypes (including *Cryptosporidium* sp. MT524977), whereas *O. curzoniae* contained five (Table 3). Among all the sampled mammals, muskrat genotype II (34.7%, 26/75) and *C. suis* (33.3%, 25/75) were the most frequently detected species/genotype. In the two most abundant host species, *N. fuscus* predominantly carried muskrat genotype II (43.2%, 19/44), whereas *O. curzoniae* was primarily infected with yak genotype (29.4%, 5/17).

Infections with Giardia spp

Molecular screening based on the *bg* gene revealed an overall prevalence of *Giardia* spp. of 8.3% (28/336). The highest prevalence was observed in *N. irene* (11.1%, 1/9), followed by *N. fuscus* (9.7%, 17/175) and *O. curzoniae* (9.4%, 10/106). No significant difference in the prevalence of *Giardia* spp. was detected between *N. fuscus* and *O. curzoniae* ($\chi^2 = 0.006$, $p = 0.938$). Similarly, no significant difference in prevalence was observed between male and female hosts ($\chi^2 = 0.009$, $p = 0.924$).

Table 2 Information on small wild mammals captured in Shiqu County

	N. fuscus (Rodentia)	*N.* irene (Rodentia)	*N.* leucurus (Rodentia)	Unclassified (Rodentia)	O. curzoniae (Lagomorpha)	Total
Male	82	7	17	13	59	178
Female	92	2	6	10	39	149
Unknown	1	0	0	0	8	9
Total	175	9	23	23	106	336

Table 3 *Cryptosporidium* spp. And *Giardia* spp. In small wild mammals in Shiqu county

Category	No. tested	Cryptosporidium spp.		Giardia spp.	
		No. positive (%, 95%CI)	Species/genotypes (No.)	No. positive (%, 95%CI)	Species/genotypes (No.)
Taxa					
Lagomorpha *O. curzoniae*	106	17 (16.0, 9.6–24.3)	yak genotype (5), *C. canis* (4), *C. suis* (4), muskrat genotype II (2), *Cryptosporidium* sp. MT524977 (2)	10 (9.4, 4.8–16.6)	*Giardia* sp. OR770651 (10)
Rodentia	230	58 (25.2, 19.8–31.3)	muskrat genotype II (w16) (24), *C. suis* (21), yak genotype (5), muskrat genotype I (4), *C. canis* (2), *Cryptosporidium* sp. MT524977 (2)	18 (7.8, 4.7–12.1)	*Giardia* sp. PQ604631 (7), *G. microti* (3), *Giardia* sp. OR770651 (3), *Giardia* sp. MG676959(3), *Giardia* sp. OP963933 (2)
N. fuscus	175	44 (25.1, 19.0–32.2)	muskrat genotype II (19), *C. suis* (14), muskrat genotype I (4), yak genotype (4), *C. canis* (2), *Cryptosporidium* sp. MT524977 (1)	17 (9.7, 5.8–15.1)	*Giardia* sp. PQ604631 (6), *G. microti* (3), *Giardia* sp. OR770651 (3), *Giardia* sp. MG676959(3), *Giardia* sp. OP963933 (2)
N. irene	9	1 (11.1, 0.6–44.3)	*C. suis* (1)	1 (11.1, 0.6–44.3)	*Giardia* sp. PQ604631 (1)
N. leucurus	23	8 (34.8, 17.2–57.5)	*C. suis* (4), muskrat genotype II (4)	0 (0)	/
Unclassified	23	5 (21.7, 8.3–43.0)	*C. suis* (2), muskrat genotype II (1), yak genotype (1), *Cryptosporidium* sp. MT524977 (1)	0 (0)	/
Sex					
Male	178	42 (23.6, 17.7–30.4)	muskrat genotype II (19), *C. suis* (14), yak genotype (6), muskrat genotype I (2), *C. canis* (1)	15 (8.4, 4.813.6)	*Giardia* sp. OR770651 (6), *Giardia* sp. PQ604631 (3), *G. microti* (2), *Giardia* sp. MG676959(2), *Giardia* sp. OP963933 (2)
Female	149	31 (20.8, 14.7–28.2)	*C. suis* (11), muskrat genotype II (6), *C. canis* (5), *Cryptosporidium* sp. MT524977 (4), yak genotype (3), muskrat genotype I (2)	13 (8.7, 4.7–14.5)	*Giardia* sp. OR770651 (7), *Giardia* sp. PQ604631 (4), *Giardia* sp. MG676959(1), *Giardia* sp. OP963933 (1)
Unknown	9	2 (22.2, 3.9–55.1)	muskrat genotype II (1), yak genotype (1)	1 (11.1, 0.6–44.3)	*G. microti* (1)
Total	336	75 (22.3, 18.1–27.0)	muskrat genotype II (26), *C. suis* (25), yak genotype (10), *C. canis* (6), muskrat genotype I (4), *Cryptosporidium* sp. MT524977 (4)	28 (8.3, 5.6–11.8)	*Giardia* sp. OR770651 (13), *Giardia* sp. PQ604631 (7), *G. microti* (3), *Giardia* sp. MG676959(3), *Giardia* sp. OP963933 (2)

Unclassified sequences are labeled "*Genus* sp." with the accession number of its closest NCBI match

Genetic identification of Giardia species

Based on the analysis of *bg* gene sequences, *G. microti* (10.7%, 3/28) and four unclassified *Giardia* spp. (as listed in NCBI) were identified. Three *G. microti* sequences showed 98.3% identity to PQ318270 (from wild rodents in the USA), differing by eight nucleotides. For the unclassified *Giardia* spp. sequences, 13 sequences shared 99.0% identity with OR770651 (isolated from a canine in Riduoxiang township, adjacent to our study area), with five nucleotide differences. Seven *Giardia* sp. sequences exhibited 99.6% identity to PQ604631 (from *Alexandromys fortis* in China), differing by two nucleotides. Three sequences displayed 99.2% identity to MG676959 (from *Myodes glareolus* in Germany), with four nucleotide differences, and two sequences shared 99.6% identity with OP963933 (from *Arvicola sapidus* in Portugal), differing by five bases. A phylogenetic analysis confirmed that all the sequences clustered with their corresponding reference sequences, and that the unclassified *Giardia* spp. formed a distinct clade adjacent to *G. microti*, suggesting a close genetic relationship (Fig. 2).

Giardia species distribution

The distribution of *Giardia* spp. varied across different small mammal taxa. *Neodon fuscus* showed the highest diversity, carrying five *Giardia* species (including unclassified *Giardia* spp.), whereas *O. curzoniae* was only infected with *Giardia* sp. OR770651. *Giardia* sp. OR770651 dominated the entire sample of small mammals (46.4%, 13/28), whereas *Giardia* sp. PQ604631 was the predominant species in *N. fuscus* (35.3%, 6/17) and Rodentia (38.9%, 7/18).

Nucleotide sequence accession numbers

All gene sequences of *Cryptosporidium* spp. and *Giardia* spp. determined in this study were submitted to the NCBI GenBank database under accession numbers PV523158-PV523163, and PV711367-PV711371.

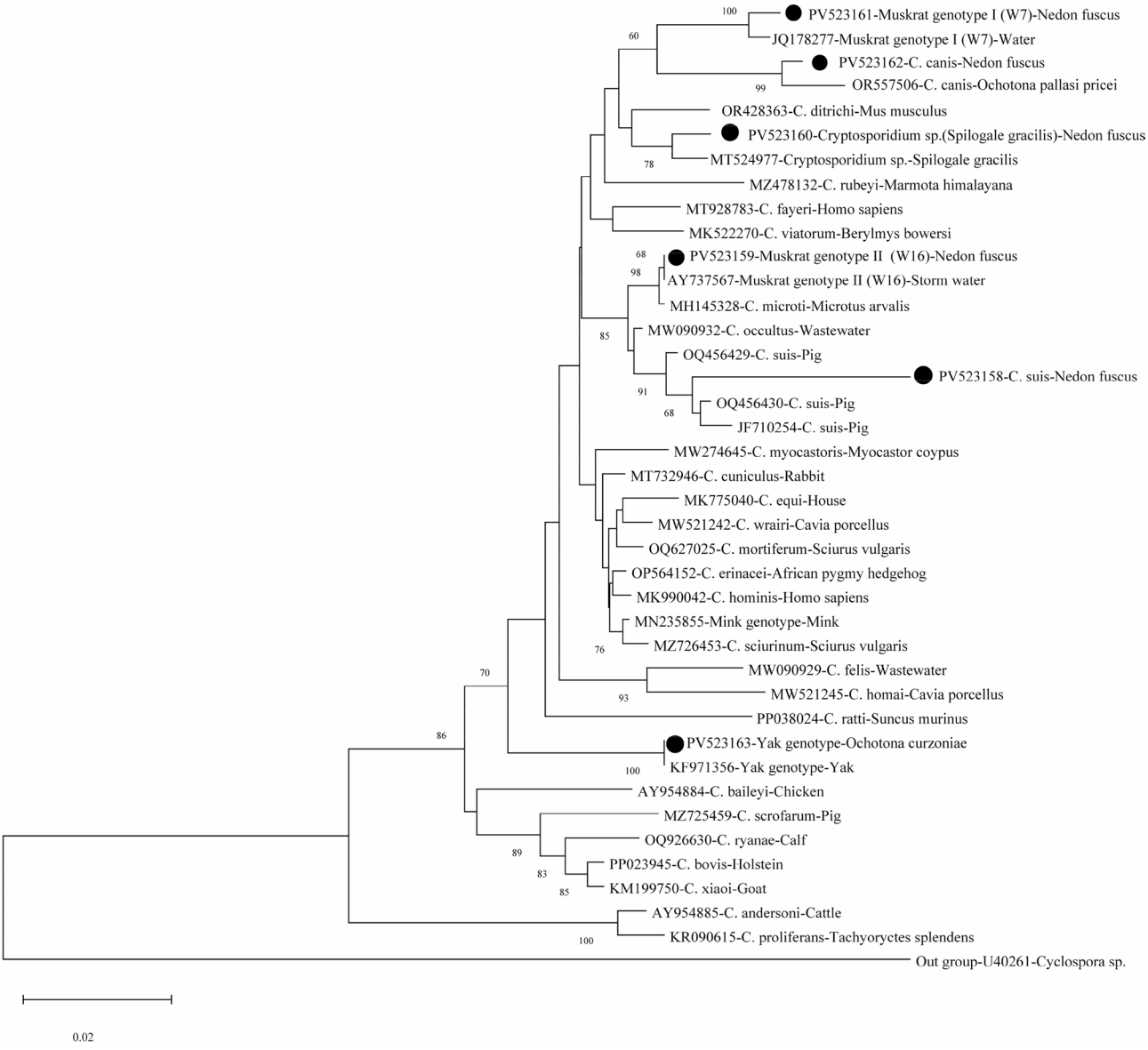

Fig. 1 Phylogenetic relationships of *Cryptosporidium* spp. based on a neighbor-joining analysis of the SSU rRNA gene, Bootstrap values > 60% from 1,000 replications are shown. Blank circles indicate the sequences identified in the present study. Sequences are identified by accession number, *Cryptosporidium* species/genotype, and host name

Discussion

Cryptosporidium spp. in small wild mammals

Small mammals, particularly in the order Rodentia, serve as critical reservoirs for zoonotic pathogens such as *Cryptosporidium* spp. This study revealed a *Cryptosporidium* prevalence of 22.3% (75/336) in the small Mammals of Shiqu County on the eastern Tibetan Plateau, with 25.2% (58/230) in rodents and 16.0% (17/106) in lagomorphs. The prevalence in rodent (25.2%) surpasses both the global average of 20.5% (3848/18,804) and China's national average of 9.78% (345/3,526) [47, 48], ranking as the second-highest reported in China, exceeded only by that in wild rodents in Hainan (50.0%, 75/150, Table 1). For lagomorphs, research remains limited, with most studies focusing on commercial and dwarf rabbits [8]. Zhang et al. [21] reported a prevalence of 6.25% (4/64) of *Cryptosporidium* spp. in *O. curzoniae* in Qinghai Province, a neighboring region to our study area, which is lower than that of 16.0% (17/106) observed in the present study. The prevalence of *Cryptosporidium* spp. detected in this study is also higher than that reported from adjacent areas in previous studies, such as in *M. baileyi* (1.0%, 1/98) [22], and *M. himalayana* (4.9%, 12/243) [23]. It is well-known that the prevalence of *Cryptosporidium* pathogens shows spatiotemporal variation, influenced by geographic distribution, climatic conditions, and host

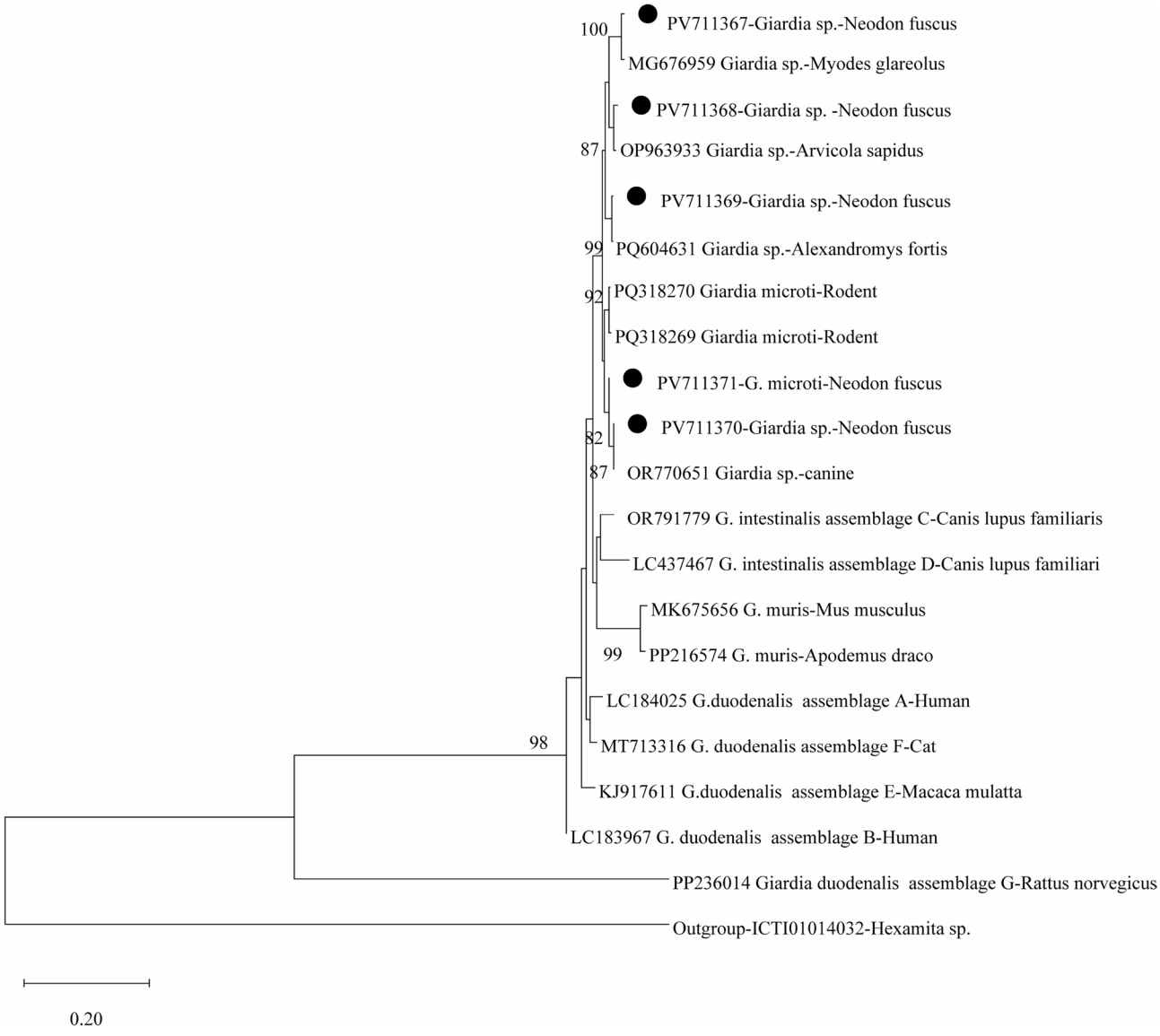

Fig. 2 Phylogenetic relationships of *Giardia* spp. based on a neighbor-joining analysis of the *bg* gene. Bootstrap values > 60% from 1,000 replications are shown. The blank circles indicate the sequences identified in the present study. Sequences are identified by accession number, *Giardia* species, and host name

population structure [13, 49, 50]. The high prevalence of *Cryptosporidium* spp. in this alpine meadow ecosystem may be driven by the relatively low temperatures, which promote prolonged oocyst survival in the environment [51]. The dense populations of burrowing mammals (both rodents and pikas) in the area also probably facilitate the transmission of *Cryptosporidium* spp.

In this study, we identified six *Cryptosporidium* species/genotypes in small wild mammals: *C. suis*, *C. canis*, yak genotype, muskrat genotype II (w16), muskrat genotype I (w7), and unclassified *Cryptosporidium* sp. MT524977. Among these, *C. suis* and *C. canis* are zoonotic species that are frequently reported in domestic animals and humans. *Cryptosporidium suis* was the second most frequently detected species (33.3%, 25/75) in our study area. In addition to the detection of *C. suis* in *N. fuscus*, *N. irene*, *N. leucurus*, and *O. curzoniae* in the present study, this parasite has also been reported in other rodent species, including the yellow-throated mouse (*A. flavicollis*) in eastern Slovakia [52] and Brandt's vole (*Lasiopodomys brandtii*) in Inner Mongolia, China [19]. Whereas *C. suis* is primarily reported in swine (*Sus scrofa*) [53, 54], its detection in rodents is controversial in so far as it is unclear whether it represents true infection or mechanical carriage is contentious, particularly in livestock-rodent sympatric environments. This ambiguity is reinforced by the identification of *C. suis* in sympatric livestock (*Bos grunniens*, *Ovis aries*, and *Equus caballus*)

in our parallel study [55]. Although the zoonotic potential of rodent-associated *C. suis* requires further clarification, its high detection rate (33.3%, 25/75), coupled with high densities of small mammals (e.g., pikas reach up to 300 individuals/ha in summer) and their seasonal migration patterns [35], strongly supports their role in facilitating *Cryptosporidium* spp. transmission within the region.

The detection of *C. canis* in both rodents and pikas in the present study is consistent with previous reports of their presence in small wild mammals, such as the Qinghai vole (*N. fuscus*) [21] and Mongolian pika (*Ochotona pallasi*) [25]. However, *C. canis* is more commonly detected in canids such as the domestic dog (*Canis lupus*) [56], coyote (*C. latrans*) [57], arctic fox (*Vulpes lagopus*) [58], red fox (*V. vulpes*) [59], mink (*Mustela vison*) [60], and raccoon dog (*Nyctereutes procyonoides*) [59]. In our study area, domestic dogs serve as herding companions, maintaining close contact with humans, while frequently preying on rodents and pikas [35]. This suggests a potential pathway for the transmission of *C. canis* between small wild mammals and domestic dogs. The zoonotic transmission of *C. canis* from domestic dogs to humans has been confirmed by Jiang et al. [61], so the potential spillover of this parasite from small wild mammals to humans via dogs warrants urgent assessment.

We also identified two *Cryptosporidium* genotypes that have been previously characterized as specific to rodent hosts. The most abundant genotype identified was muskrat genotype II (w16), accounting for 34.7% (26/75) of positive samples. Muskrat genotype I (w7) was also found in four *N. fuscus* samples. As rodent-adapted species, muskrat genotypes I and II primarily infect muskrats (*Ondatra zibethicus*) and voles (including *Microtus* spp. and *Apodemus* spp.) across North America, Europe, and Asia [52, 62–64]. Moreover, these two genotypes have frequently been detected in environmental samples from the United States [65, 66], the United Kingdom [67, 68], and Canada [62, 69], indicating that these parasites contribute significantly to water contamination, which drives their transmission dynamics. The findings of the present study contribute to the geographic and host distribution records of these genotypes.

The remaining two isolates identified (yak genotype and *Cryptosporidium* sp. MT524977) have been less frequently reported. The yak genotype was initially identified in yaks (*B. grunniens*) in Qinghai, China [70], and subsequently detected in Mongolian pikas in Xinjiang, China [25]. *Cryptosporidium* sp. MT524977 was first reported in a western spotted skunk (*S. gracilis*) from the Pacific Northwest of the USA [71], and shares 98.7% genetic similarity with a *Cryptosporidium* environmental sequence (i.e., genotype w12) and 98.3% similarity with an isolate from a meadow vole (GenBank KY644661). To the best of our knowledge, neither of these isolates has been reported in other animal species or human populations within the study area. The zoonotic potential of these isolates remains undetermined and warrants further research to assess the possible risks they pose to livestock and human populations.

In summary, the findings of this study, particularly the detection and prevalence of known zoonotic *Cryptosporidium* species, highlight a potential public health risk on the eastern Tibetan Plateau. Crucially, two zoonotic species, *C. suis* and *C. canis*, were identified, accounting for a substantial proportion (41.3%, 31/75) of all positive samples. These species are well-documented to cause infections in humans and livestock, indicating a potential for cross-species transmission [72]. This potential risk is amplified by the unique socio-ecological context of the study area. The eastern Tibetan Plateau is a human-livestock-wildlife interface ecosystem, where nomadic pastoralists share overlapping habitats and untreated water sources with domestic animals (e.g., dogs, yaks, and sheep) and a variety of wild animals. This sympatric relationship may facilitate the environmental transmission of oocysts. Our hypothesis of a potential transmission cycle of *Cryptosporidium* is further supported by parallel findings: we recently detected the same zoonotic species, *C. suis*, in free-ranging livestock in this area [55] and identified *Cryptosporidium* oocysts in hand-rinsing water used by local pastoralists (genotyping in progress). Research in Madagascar and the United States has also demonstrated that rodents may be a key source of human *Cryptosporidium* infections, with identical subtypes found in rodents, humans, and water sources [73, 74]. Although no human cryptosporidiosis cases have been clinically documented in this region, likely due to limited surveillance, our findings underscore the critical need for a "One Health" approach to investigate and monitor *Cryptosporidium* transmission dynamics among wildlife, livestock, and human populations on the Tibetan Plateau.

Giardia spp. in small wild mammals

Compared with research into *Cryptosporidium*, research into *Giardia* in rodents remains Limited, despite its high global pooled molecular prevalence of 21.2% (1471/6931) [7]. In the present study, we revealed that the prevalence of *Giardia* spp. in the small Mammal community in Shiqu County was 8.3%, substantially lower than the global average (21.2%), but close to the national prevalence in China of 10.4% (241/2313, derived from Li et al. [7]). *Giardia* infection in wild rodents varies geographically across China, with its prevalence ranging from 0% (0/98) in Qinghai (*Myospalax baileyi*) to 52.6% (20/38) in Ningxia (*Microtus fortis*). High prevalence was also observed in Hunan (51.4%, 73/142 in 2000; 29.4%, 45/153 in 2025) and Xinjiang (43.2%, 57/132). Our study revealed a lower prevalence of *Giardia* spp. in both *N.*

fuscus (9.4%, 10/106) and *O. curzoniae* (9.7%, 17/175) in Shiqu County in Sichuan. Similarly, low prevalence has been observed in neighboring regions, including Sichuan (8.1%, 15/185), Gansu (1.6%, 8/498), Yunnan (1.1%, 1/88), and Qinghai (0.8%, 2/243). Comparably low prevalence has also been found in southern China provinces, including Guangdong (4.9%, 3/61and Guangxi (1.9%, 2/103) (Table 1). However, our current understanding of these spatial patterns is limited because most studies have been performed with small sample sizes ($n < 200$ per region) and uneven geographic coverage. Therefore, this observed spatial heterogeneity requires validation with systematic surveys incorporating geographic covariates, including altitude, land use, and other factors.

In this study, *G. microti* was identified based on an analysis of *bg* gene sequences. To the best of our knowledge, *G. microti* was initially considered a rodent-specific species [75, 76], documented in rodents of various genera, including *Rattus*, *Mus*, *Apodemus* [77], *Eothenomys* [78], *Ondatra* [79], *Peromyscus*, *Microtus*, and *Myodes* [7]. However, subsequent studies revealed a broader host range for this protozoan, extending to Canidae (e.g., dogs, *Canis familiaris*), Felidae (e.g., leopards, *Panthera pardus japonensis*), Cervidae (e.g., red deer, *Cervus elaphus*), and even fish (e.g., barramundi, *Lates calcarifer*) [80]. Although no human or non-human primate infections have been reported to date, the observed cross-species transmission raises concerns for the potential spillover of *G. microti*. The mechanisms facilitating host adaptation, particularly the ecological overlap between rodent reservoirs and potential novel hosts, require further investigation to assess the spillover risks, particularly in regions with an intense domestic animal–wildlife interface.

In addition to *G. microti*, four unique *Giardia* sp. sequences were identified in local small mammals, which share > 99.0% identity with four unclassified *Giardia* sp. sequences in the NCBI database. Three of these—*Giardia* sp. PQ604631 (originally isolated from *Alexandromys fortis*), *Giardia* sp. MG676959 (*M. glareolus*), and *Giardia* sp. OP963933 (*Arvicola sapidus*)—were previously reported in rodents, whereas *Giardia* sp. OR770651 was isolated from canines. Although none of these sequences have yet been formally classified as known *Giardia* species, in a phylogenetic analysis of the *bg* gene, these sequences (from both our study and the NCBI database) clustered closely with *G. microti*. These findings suggest that the isolates identified in the present study represent local variants of *G. microti*. However, this conclusion must be interpreted with caution. Definitive taxonomic classification of *Giardia* ideally relies on a multilocus sequence typing (MLST) approach, which typically includes more conserved markers like the glutamate dehydrogenase (*gdh*) and small subunit ribosomal RNA (SSU rRNA) genes [81]. Unfortunately, our attempts to amplify these additional loci from the positive samples were unsuccessful, likely due to low oocyst concentrations or DNA degradation, thus limiting the conclusiveness of our phylogenetic analysis. This challenge is compounded by the broader context of the field; as noted by Ryan and Zahedi (2019), the diversity of *Giardia* in wildlife remains poorly characterized, with largely opportunistic studies [82]. Furthermore, the scarcity of reference data—with only 26 *G. microti* sequences currently available in GenBank—severely hampers the precise taxonomic placement of our isolates. Given these limitations, we have conservatively designated these isolates as "*Giardia* sp. accession number", pending more comprehensive genomic characterization. Future research employing more sensitive methods or whole-genome sequencing will be essential to overcome these amplification challenges and definitively resolve the taxonomic status and evolutionary relationships of these potentially novel *Giardia* isolates/variants.

Notably, *Giardia* sp. OR770651 was previously reported in herding dogs in Rido township (29.8°N, 92.3°E; 4300 m asl), Xizang Autonomous Region, which shares similar plateau ecological conditions (alpine meadow ecosystem) and pastoral practices (dog-dependent pastoralism) with our study area. The detection of *Giardia* sp. OR770651 in *O. curzoniae* (9.4%, 10/106) and rodents (1.3%, 3/230) suggests the potential cross-species transmission of *Giardia* from small mammals (sylvatic) to canines (synanthropic). Further investigations of the prevalence and genetic characteristics of *Giardia* spp. in wildlife, domestic animals, and humans will clarify its transmission dynamics in this pastoral system.

Our study also reveals the host adaptation patterns of *Giardia* spp. Although only *Giardia* sp. OR770651 was detected in *O. curzoniae*, the rodent *N. fuscus* harbored all five *Giardia* spp. (*C. microti* and four unclassified *Giardia* spp.). Beyond host susceptibility, this difference may be attributable to the broader trophic niche width of Rodentia compared with that of Lagomorpha [83], which probably increases the exposure of rodents to diverse *Giardia* cysts through various foraging sources. These infection patterns suggest distinct ecological roles of these taxa in *Giardia* transmission, with lagomorphs (i.e., *O. curzoniae*) potentially acting as obligate hosts for some specific *Giardia* spp. (e.g., *Giardia* sp. OR770651), whereas rodents (i.e., *N. fuscus*) may act as reservoir hosts, maintaining multiple *Giardia* spp. The data implicate rodents as likely key drivers in preserving the genetic diversity of *Giardia* within this ecosystem.

However, rodents are also known hosts of several other *Giardia* species, as well as *G. microti* identified in this study, including the zoonotic *G. duodenalis* complex and rodent-specific species such as *G. muris* (primarily circulating in Muridae) and *G. cricetidarum* (reported only

in hamsters) [5, 6, 78]. Our findings, together with those of Helmy et al. [84], suggest that rodent-adapted *Giardia* species are more prevalent than zoonotic *G. duodenalis* in wild rodents, which is consistent with the findings in wild rodents in Xinjiang [13] and Hunan [32]. However, other studies have documented the *G. duodenalis* assemblage as the predominant species in wild rodents, such as beavers (*Castor canadensis*, assemblage B) in the US [85], wild urban rodents (assemblages G and B) in Iran [86], *R. rattus* (assemblage B) in Spain [87], and wild rodents (assemblages A, B, E, F, G) in some provinces of China (Table 1). These discrepancies indicate potential geographic and ecological influences on the transmission dynamics of *Giardia* and highlight the need to investigate further the factors driving the distribution of *Giardia* species.

Conclusions

This study demonstrates the high prevalence of *Cryptosporidium* spp. (22.3%) and diversity of *Giardia* spp. (8.3%, including G. *microti* and four unclassified *Giardia* spp.) in small wild mammals on the Tibetan Plateau. The detection of zoonotic species (*C. suis* and *C. canis*) suggests significant public health risks. These findings underscore the fact that wildlife reservoirs are critical components of parasite transmission cycles. Targeted surveillance of these wild mammal populations and their zoonotic pathogens should be prioritized to prevent the emergence of potential disease in human and domestic animal populations.

Abbreviations

PCR	Polymerase chain reaction
SSU rRNA	Small subunit ribosomal RNA
bg	Beta-giardin
χ^2 tests	Chi-square tests

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12866-025-04363-z.

Supplementary Material 1.

Acknowledgements

Not applicable.

Authors' contributions

YS, XW and QZ designed this study. QZ, HL, XP, YZ, ML, and YY performed the experiments. QZ, ZB, CX, XP, HZ, XZ, QC, XW, ML, and YY collected the fecal samples. QZ, ZB, XW, HL, YJ and XP analyzed the data. XW, BZ, JC, and YS contributed reagents/materials. QZ, BZ, XW wrote the original draft of the manuscript. QZ, XW, JC and YS revised the manuscript. XW and YS made the final revision. All authors read the manuscript and approved the submitted version.

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Data availability

The representative nucleotide sequences obtained in the present study were deposited in GenBank database under the following accession numbers: PV523158-PV523163 for Cryptosporidium spp., and PV711367-PV711371 for Giardia spp.

Declarations

Ethics approval and consent to participate

This study and research protocols were reviewed and approved by the Laboratory Animal Welfare & Ethics Committee of the National Institute of Parasitic Diseases, Chinese Center for Disease Control and Prevention, China (IPD-2022-010). All procedures involving the trapping, handling, and euthanasia of wild rodents were conducted by the Regulation on the Administration of Laboratory Animals (2013 Revision). All animals were free-living and sourced from wild populations. As no animals were privately owned, there was no need for informed consent from any institution or individual to use the animals in this study.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

Author details

[1]National Key Laboratory of Intelligent Tracking and Forecasting for Infectious Diseases, National Institute of Parasitic Diseases, Chinese Center for Disease Control and Prevention (Chinese Center for Tropical Diseases Research), NHC Key Laboratory of Parasite and Vector Biology, WHO Collaborating Centre for Tropical Diseases, National Center for International Research on Tropical Diseases, Shanghai 200025, China
[2]National Health Commission Key Laboratory of Echinococcosis Prevention and Control, Xizang Center for Disease Control and Prevention, Xizang Autonomous Region, Lhasa City 850000, China
[3]The School of Global Health, Chinese Center for Tropical Diseases Research, Shanghai Jiao Tong University School of Medicine, Shanghai 200025, China

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References

1. Ryan U, Hijjawi N. New developments in *Cryptosporidium* research. Int J Parasitol. 2015;45(6):367–73.
2. GBD2021DDC. Global, regional, and national age-sex-specific burden of diarrhoeal diseases, their risk factors, and aetiologies, 1990–2021, for 204 countries and territories: a systematic analysis for the global burden of disease study 2021. Lancet Infect Dis. 2025;25(5):519–36.
3. Ryan U, Hijjawi N, Feng Y, Xiao L. *Giardia*: an under-reported foodborne parasite. Int J Parasitol. 2019;49(1):1–11.
4. Checkley W, White AC Jr., Jaganath D, Arrowood MJ, Chalmers RM, Chen XM, et al. A review of the global burden, novel diagnostics, therapeutics, and vaccine targets for *Cryptosporidium*. Lancet Infect Dis. 2015;15(1):85–94.
5. Egan S, Barbosa AD, Feng Y, Xiao L, Ryan U. Critters and contamination: zoonotic protozoans in urban rodents and water quality. Water Res. 2024;251:121165.
6. Cai W, Ryan U, Xiao L, Feng Y. Zoonotic giardiasis: an update. Parasitol Res. 2021;120(12):4199–218.
7. Li J, Qin H, Li X, Zhang L. Role of rodents in the zoonotic transmission of giardiasis. One Health. 2023;16:100500.
8. Egan S, Barbosa AD, Feng Y, Xiao L, Ryan U. Rabbits as reservoirs: an updated perspective of the zoonotic risk from Cryptosporidium and giardia. Vet Parasitol. 2024;327:110151.

9. Chen YW, Zheng WB, Zhang NZ, Gui BZ, Lv QY, Yan JQ, et al. Identification of Cryptosporidium viatorum XVa subtype family in two wild rat species in China. Parasit Vectors. 2019;12(1):502.
10. 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(24):7692–9. https://doi.org/10.1128/AEM.01386-09.
11. 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(6):800–6.
12. Xu J, Liu H, Jiang Y, Jing H, Cao J, Yin J, et al. Genotyping and subtyping of *Cryptosporidium* spp. and *Giardia duodenalis* isolates from two wild rodent species in Gansu province, China. Sci Rep. 2022;12(1):12178.
13. Feng K, Yang S, Xu Y, Wen L, Chen J, Zhang W, et al. Molecular characterization of *Cryptosporidium* spp., *Giardia* spp. and *Enterocytozoon bieneusi* in eleven wild rodent species in China: common distribution, extensive genetic diversity and high zoonotic potential. One Health. 2024;18:100750.
14. Ni HB, Sun YZ, Qin SY, Wang YC, Zhao Q, Sun ZY, et al. Molecular detection of *Cryptosporidium* spp. and *Enterocytozoon bieneusi* infection in wild rodents from six provinces in China. Front Cell Infect Microbiol. 2021;11:783508.
15. Gao ZQ, Wang HT, Li JH, Song YX, Hou QY, Qin SY, et al. Prevalence and genotype analysis of *Cryptosporidium* spp. in nine species of wild rodents in China. Parasite. 2025;32:19.
16. Zhao W, Zhou H, Huang Y, Xu L, Rao L, Wang S, et al. *Cryptosporidium* spp. in wild rats (*Rattus* spp.) from the Hainan province, china: molecular detection, species/genotype identification and implications for public health. Int J Parasitol Parasites Wildl. 2019;9:317–21.
17. 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(1):313.
18. Wang N. Investigation and genotyping of four zoonotic parasitic diseases in Brown rat. MSc Thesis, Jilin Agricultural University, Changchun (in Chinese). 2022.
19. Feng S, Chang H, Wang Y, Huang C, Han S, He H. Molecular characterization of *Cryptosporidium* spp. in brandt's vole in China. Front Vet Sci. 2020;7:300.
20. Liu L, Xu Q, Jiang A, Zeng F, Zhao W, Tan F. Molecular characterization of *Cryptosporidium* in wild rodents from the inner Mongolian autonomous region and Liaoning province, china: assessing host specificity and the potential for zoonotic transmission. Front Vet Sci. 2024;11:1406564.
21. Zhang X, Jian Y, Li X, Ma L, Karanis G, Karanis P. The first report of *Cryptosporidium* spp. in *Microtus fuscus* (Qinghai vole) and *Ochotona curzoniae* (wild plateau pika) in the Qinghai-Tibetan plateau area, China. Parasitol Res. 2018;117(5):1401–7. https://doi.org/10.1007/s00436-018-5827-5.
22. Hu B, Wang J, Zhang S, Wang B, Xing Y, Han S, et al. Novel genotypes of *Cryptosporidium* and *Enterocytozoon bieneusi* detected in plateau Zokors (*Myospalax baileyi*) from the Tibetan plateau. Int J Parasitol Parasites Wildl. 2022;19:263–8.
23. Ma L, Jian Y, Wang G, Cai Q, Wang G, Li X et al. The Prevalence of Cryptosporidium spp. and Giardia duodenalis in Marmota himalayana (Rodentia: Sciuridae) in the Qinghai Tibetan Plateau area, China. Iran J Parasitol. 2024;19(2):162–70.
24. Chai Y, Deng L, Liu H, Yao J, Zhong Z, Xiang L, et al. First detection of *Cryptosporidium* spp. in red-bellied tree squirrels (*Callosciurus erythraeus*) in China. Parasite. 2019;26:28.
25. Wang S, Wang H, Han X, Zhao SS, Tan WB, Wang SW, et al. Mongolian Pika (*Ochotona pallasi*) a important *Cryptosporidium* species vector at China-mongolia border of China. Res Square. 2023. https://doi.org/10.21203/rs.3.rs-3553611/v1.
26. Li J, Yuan Z, Xu J, Xin X, Liu J, Zhang X, et al. Molecular detection and genetic variability of *Cryptosporidium* spp. in wild Asian house shrews (*Suncus murinus*) from Southern Zhejiang province, China. Heliyon. 2024;10(12):e33334.
27. Tuo HX. Molecular detection characterization of Cryptoridium and Enterocytozoon bieneusi in wild gray marmot (Marmota baibacina) in Xinjiang, China. MSc Thesis, Tarim University, Aral (in Chinese). 2024.
28. Lian ZQ, Zuo YX. Investigation of *Cryptosporidium* spp. Infection in wild rodents and wildlife in yunnan, China. Chin J Zoonoses. 1991;7(6):33–4.
29. Wang QQ. Molecular epidemiology of *Enterocytozoon* bieneusi and *Cryptosporidium* in the Bai ethnic people and wild rodents from Dali area. MSc Thesis, Dali University, Dali; 2018. (in Chinese).
30. Jiang Y, Jiang A, Ren G, Wang L, Xin X, Yua Z, et al. Cross-species transmission of *Cryptosporidium* in wild rodents from the Southern region of Zhejiang Province of China and its possible impact on public health. Parasite. 2024;31:34.
31. Qin Y, Gao ZQ, Wang HT, Hou QY, Qin SY, Zhao Q et al. Prevalence of giardia in nine species of wild rodents in guangxi, hunan, and Yunan provinces, China. Foodborne Pathog Dis. 2024. https://doi.org/10.1089/fpd.2024.0185.
32. Gao C, Shen ZM, Liu X, Lu GP, Shao WJ, Wang SC, et al. Detection of microorganisms and parasites in field vole (*Microtus fortis*) from different regions in China. Shanghai Lab Anim Sci. 2000;20(1):22–5. (in Chinese).
33. Shiqu County Chronicles Compilation Committee; 2.3 Climate. In Shiqu County Chronicles; Sichuan People's Publishing House: Chengdu, China. 2000. p. 665–73.
34. Zhang YZ, Zheng CL. The geographical distribution of mammals and the evolution of mammalian fauna in Qinghai-Xizang plateau. Acta Geogr Sin. 1985;40(3):225–31.
35. Smith AT, Xie Y. A guide to the mammals of China. Changsha: Hunan Education; 2009. pp. 234–308.
36. Wei W, He J, Zheng Q, He Y, Oosthuizen MK. A comparison of density estimation methods in Plateau pika populations in an alpine meadow ecosystem. J Mamm. 2020;101:1091–109.
37. Harris RB, Zhou J, Ji Y, Zhang K, Yang C, Yu DW. Evidence that the Tibetan fox is an obligate predator of the plateau pika: conservation implications. J Mammal. 2014;95(6):1207–21. https://doi.org/10.1644/14-MAMM-A-021.
38. Wang LM, Song XY, Zhu XP, Xie F, Li GQ, Luozhi DB, et al. Monitoring the microtus fuscus plague epidemic in Sichuan Province during 2000–2008. Chin J Epidemiol. 2009;30(11):1175–8.
39. Chen HB, Liu YP, Cui J, Rao HX, Li DM, Yu J. Prevalence of seven zoonotic pathogens in small mammals in the Qinghai plateau region. Chin J Zoonoses. 2024;40(9):880–6. (in chinese). https://doi.org/10.3969/j.issn.1002-2694.2024.00.131.
40. Wang X, Liu JY, Zuo QQ, Mu ZQ, Weng XD, Sun XH, et al. *Echinococcus multilocularis* and *Echinococcus shiquicus* in a small mammal community on the Eastern Tibetan plateau: host species composition, molecular prevalence, and epidemiological implications. Parasit Vectors. 2018;11:302.
41. He X, Wang X, Fan G, Li F, Wu W, Wang Z, et al. Metagenomic analysis of viromes in tissues of wild Qinghai vole from the Eastern Tibetan plateau. Sci Rep. 2022;12(1):17239. https://doi.org/10.1038/s41598-022-22134-y.
42. Men J, Ran DZ, Wu YF, Kong XQ. Investigation and statistical analysis of drinking water quality in pastoral areas of the Tibetan plateau. Tibet Sci Technol. 2020;10:42–6. (in Chinese).
43. Luo ZX, Chen W, Gao W. Fauna sinica Mammalia. Beijin: Science; 2000. pp. 35–50. (in Chinese).
44. 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(4):1578–83.
45. Tamura K, Stecher G, Kumar S. MEGA11: molecular evolutionary genetics analysis version 11. Mol Biol Evol. 2021;38(7):3022–7.
46. R Core Team. R: A Language and Environment for Statistical Computing. 2023. www.R-project.org
47. Zhang K, Fu Y, Li J, Zhang L. Public health and ecological significance of rodents in *Cryptosporidium* infections. One Health. 2022;14:100364.
48. Zhou SS, Jiang YY, Cao JP. *Cryptosporidium* infection in rodents in China. Chin J Parasitol Parasit Dis. 2024;42(4):512–20.
49. Wang X, Jiang Y, Wu W, He X, Wang Z, Guan Y, et al. *Cryptosporidiosis* threat under climate change in China: prediction and validation of habitat suitability and outbreak risk for human-derived *Cryptosporidium* based on ecological niche models. Infect Dis Poverty. 2023;12(1):35.
50. Feng Y, Ryan UM, Xiao L. Genetic diversity and population structure of *Cryptosporidium*. Trends Parasitol. 2018;34(11):997–1011.
51. Michael BJ, Dwight DB, Elizabeth AF, William CG. *Cryptosporidium parvum* oocyst inactivation in three soil types at various temperatures and water potentials. Soil Biol Biochem. 2002;34(8):1101–9.
52. Danišová O, Valenčáková A, Stanko M, Luptáková L, Hatalová E, Čanády A. Rodents as a reservoir of infection caused by multiple zoonotic species/genotypes of *C. parvum*, *C. hominis*, *C. suis*, *C. scrofarum*, and the first evidence of *C. muskrat* genotypes I and II of rodents in Europe. Acta Trop. 2017;172:29–35.
53. Yui T, Nakajima T, Yamamoto N, Kon M, Abe N, Matsubayashi M, et al. Age-related detection and molecular characterization of *Cryptosporidium suis* and *Cryptosporidium scrofarum* in pre- and post-weaned piglets and adult pigs in Japan. Parasitol Res. 2014;113(1):359–65.
54. Lin Q, Wang XY, Chen JW, Ding L, Zhao GH. *Cryptosporidium suis* infection in post-weaned and adult pigs in Shaanxi province, Northwestern China. Korean J Parasitol. 2015;53(1):113–7.

55. Peng X, Wang X, Jian J, Zuo Q, Liu H, Wang Y, et al. Investigation of *Cryptosporidium* spp. and *Enterocytozoon bieneusi* in free-ranged livestock on the southeastern Qinghai-Xizang plateau, China. BMC Infect Dis. 2025;25(1):356.
56. Fayer R, Trout JM, Xiao L, Morgan UM, Lai AA, Dubey JP. *Cryptosporidium canis* n. sp. from domestic dogs. J Parasitol. 2001;87:1415–22.
57. Trout JM, Santín M, Fayer R. *Giardia* and *Cryptosporidium* species and genotypes in Coyotes (*Canis latrans*). J Zoo Wildl Med. 2006;37:141–4.
58. Zhang XX, Cong W, Ma JG, Lou ZL, Zheng WB, Zhao Q, et al. First report of Cryptosporidium canis in farmed Arctic foxes (*Vulpes lagopus*) in China. Parasit Vector. 2016;3:126.
59. Perec-Matysiak A, Hildebrand J, Popiołek M, Buńkowska-Gawlik K. The occurrence of *Cryptosporidium* spp. in wild-living carnivores in Poland-a question concerning its host specificity. Pathogens. 2023;12:198.
60. Wang W, Wei Y, Cao S, Wu W, Zhao W, Guo Y, et al. Divergent *Cryptosporidium* species and host-adapted *Cryptosporidium canis* subtypes in farmed minks, raccoon dogs and foxes in shandong, China. Front Cell Infect Microbiol. 2022;22:980917.
61. Jiang W, Roellig DM, Guo Y, Li N, Feng Y, Xiao L. Development of a subtyping tool for zoonotic pathogen *Cryptosporidium canis*. J Clin Microbiol. 2021;59(3):e02474-20.
62. Ruecker NJ, Matsune JC, Wilkes G, Lapen DR, Topp E, Edge TA, et al. Molecular and phylogenetic approaches for assessing sources of *Cryptosporidium* contamination in water. Water Res. 2012;46(16):5135–50.
63. Zhou L, Fayer R, Trout JM, Ryan UM, Schaefer FW 3rd, Xiao L. Genotypes of *Cryptosporidium* species infecting fur-bearing mammals differ from those of species infecting humans. Appl Environ Microbiol. 2004;70(12):7574–7.
64. Feng Y, Alderisio KA, Yang W, Blancero LA, Kuhne WG, Nadareski CA, et al. *Cryptosporidium* genotypes in wildlife from a new York watershed. Appl Environ Microbiol. 2007;73(20):6475–83.
65. Jiang J, Alderisio KA, Xiao L. Distribution of *Cryptosporidium* genotypes in storm event water samples from three watersheds in New York. Appl Environ Microbiol. 2005;71(8):4446–54.
66. Yang W, Chen P, Villegas EN, Landy RB, Kanetsky C, Cama V, et al. *Cryptosporidium* source tracking in the Potomac River watershed. Appl Environ Microbiol. 2008;74(21):6495–504.
67. Nichols RA, Connelly L, Sullivan CB, Smith HV. Identification of *Cryptosporidium* species and genotypes in Scottish raw and drinking waters during a one-year monitoring period. Appl Environ Microbiol. 2010;76(17):5977–86.
68. Chalmers RM, Robinson G, Elwin K, Hadfield SJ, Thomas E, Watkins J, et al. Detection of *Cryptosporidium* species and sources of contamination with *Cryptosporidium hominis* during a waterborne outbreak in North West Wales. J Water Health. 2010;8(2):311–25.
69. Wilkes G, Ruecker NJ, Neumann NF, Gannon VPJ, Jokinen C, Sunohara M, et al. Spatiotemporal analysis of *Cryptosporidium* species/genotypes and relationships with other zoonotic pathogens in surface water from mixed-use watersheds. Appl Environ Microbiol. 2013;79(2):434–48.
70. Ma J, Cai J, Ma J, Feng Y, Xiao L. Occurrence and molecular characterization of *Cryptosporidium* spp. in yaks (*Bos grunniens*) in China. Vet Parasitol. 2014;202(3–4):113–8.
71. Li X, Nguyen T, Xiao C, Levy A, Akagi Y, Silkie S, et al. Prevalence and genotypes of *Cryptosporidium* in wildlife populations co-located in a protected watershed in the Pacific Northwest, 2013 to 2016. Microorganisms. 2020;8(6):914.
72. Liu A, Gong B, Liu X, Shen Y, Wu Y, Zhang W, et al. A retrospective epidemiological analysis of human *Cryptosporidium* infection in China during the past three decades (1987–2018). PLoS Negl Trop Dis. 2020;14(3):e0008146.
73. Li N, Xiao L, Alderisio K, Elwin K, Cebelinski E, Chalmers R, et al. Subtyping *Cryptosporidium ubiquitum*, a zoonotic pathogen emerging in humans. Emerg Infect Dis. 2014;20(2):217–24.
74. Bodager JR, Parsons MB, Wright PC, Rasambainarivo F, Roellig D, Xiao L, et al. Complex epidemiology and zoonotic potential for *Cryptosporidium suis* in rural Madagascar. Vet Parasitol. 2015;207(1–2):140–3.
75. Feely DE. Morphology of the cyst of *Giardia microti* by light and electron microscopy. J Protozool. 1988;35(1):52–4.
76. Thompson RC, Monis P. *Giardia*-from genome to proteome. Adv Parasitol. 2012;78:57–95.
77. Backhans 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(9):1885–91.
78. Lyu Z, Shao J, Xue M, Xue M, Ye QQ, Chen B, et al. A new species of *Giardia* künstler, 1882 (Sarcomastigophora: Hexamitidae) in hamsters. Parasit Vectors. 2018;11(1):202.
79. van Keulen H, Feely DE, Macechko PT, Jarroll EL, Erlandsen SL. The sequence of giardia small subunit rRNA shows that voles and muskrats are parasitized by a unique species *Giardia microti*. J Parasitol. 1998;84(2):294–300.
80. Yang R, Reid A, Lymbery A, Ryan U. Identification of zoonotic *Giardia* genotypes in fish. Int J Parasitol. 2010;40(7):779–85.
81. Wielinga C, Williams A, Monis P, Thompson RCA. Proposed taxonomic revision of *Giardia duodenalis*. Infect Genet Evol. 2023;111:105430.
82. Ryan U, Zahedi A. Molecular epidemiology of giardiasis from a veterinary perspective. Adv Parasitol. 2019;106:209–54.
83. Zhang CJ, Wang XY, Yao BH, Wang C, Kang YK, Zhang Q, et al. Diet composition and trophic niche characteristics of three rodents in Gannan meadow. Acta Agrestia Sinica. 2021;29(7):1484–90.
84. Helmy YA, Spierling NG, Schmidt S, Rosenfeld UM, Reil D, Imholt C, et al. Occurrence and distribution of *Giardia* species in wild rodents in Germany. Parasit Vectors. 2018;11(1):213.
85. Fayer R, Santín M, Trout JM, DeStefano S, Koenen K, Kaur T. Prevalence of microsporidia, *Cryptosporidium* spp., and *Giardia* spp. in beavers (*Castor canadensis*) in Massachusetts. J Zoo Wildl Med. 2006;37(4):492–7.
86. Asghari A, Motazedian MH, Asgari O, Shamsi L, Sarkari B, Shahabi S, et al. Occurrence, genetic characterization, and zoonotic importance of giardia duodenalis in various species of rodents (*Mus musculus*, *Rattus norvegicus*, and *Rattus rattus*). Comp Immunol Microbiol Infect Dis. 2022;85:101812.
87. Fernández-Álvarez Á, Martín-Alonso A, Abreu-Acosta N, Carlos F, Hugot J-P, Valladares B, et al. Identification of a novel assemblage G subgenotype and a zoonotic assemblage B in rodent isolates of *Giardia duodenalis* in the Canary islands, Spain. Parasitology. 2014;141(2):206–15.

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