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Molecular characterization of *Cryptosporidium* spp. in goats (*Capra hircus*) from Zhejiang Province, China: evidence of zoonotic species and subtypes

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Abstract

Background *Cryptosporidium* spp. are protozoan pathogens that infect the gastrointestinal tracts of humans and animals, posing significant zoonotic risks. Goats are recognized as important hosts; however, their role in the epidemiology and transmission of *Cryptosporidium* remains inadequately characterized in certain regions of China.

Methods To investigate the occurrence and species composition of *Cryptosporidium* in goats (*Capra hircus*), fecal samples ($n=386$) were collected from multiple farms in Zhejiang Province, China. Genomic DNA was extracted and screened for *Cryptosporidium* using PCR amplification of the small subunit ribosomal RNA (SSU rRNA) gene, followed by Sanger sequencing for species identification. Positive samples of *C. parvum*, *C. xiaoi*, and *C. ubiquitum* were further subtyped via nested PCR targeting the 60-kilodalton glycoprotein *(gp60)* gene.

Results Overall, 31 (8.0%) of the 386 samples were positive for *Cryptosporidium* spp. Four species were identified: *C. xiaoi* ($n=14$), *C. ubiquitum* ($n=12$), *C. parvum* ($n=3$), and *C. occultus* ($n=2$). Subtyping revealed *C. xiaoi* XXIIIa, *C. ubiquitum* XIIa, and *C. parvum* IIdA19G1. Both *C. parvum* and *C. ubiquitum* are zoonotic species that have been previously reported in humans and rodents, indicating potential cross-species transmission within the region.

Conclusions This study reveals the presence of multiple *Cryptosporidium* species, including zoonotic *C. parvum* and *C. ubiquitum*, in goats from Zhejiang Province. These findings indicate that goats may serve as potential reservoirs for environmental contamination and zoonotic transmission. It is recommended to implement proper management practices and hygienic disposal of goat manure to mitigate the risk of cryptosporidiosis outbreaks.

Keywords Cryptosporidium, Goat, Genotyping, Zoontoic, Zhejiang

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Background

Cryptosporidium is a globally distributed gastrointestinal protozoan pathogen that poses a substantial threat to human health, particularly in developing countries. It is a major contributor to the burden of diarrheal disease among children under two years of age, often leading to malnutrition and diarrhea-associated mortality [1]. In addition to its impact on humans, *Cryptosporidium* infections are commonly detected in animals, with more than 260 species identified as hosts or carriers of the pathogen [2]. *Cryptosporidium* follows a fecal-oral transmission cycle, during which infectious oocysts are excreted into the environment, enabling subsequent host infection [3]. Outbreaks often result from contamination of food or water with oocysts [4], leading to *Cryptosporidium* being classified as a priority pathogen for monitoring in drinking water supplies in many countries [5]. Direct contact with infected animals has also been implicated in outbreaks among high-risks occupational groups such as veterinary students and farmers [6, 7]. As a result, the World Health Organization classifies *Cryptosporidium* as a major zoonotic pathogen. Given the absence of effective vaccines or specific therapeutic agents for cryptosporidiosis, prevention and control primarily depend on interrupting transmission pathways [3]. Therefore, identifying infection sources through advanced molecular techniques is essential for developing targeted strategies to mitigate the spread of this significant pathogen.

Over the past decade, numerous molecular tools have been developed to detect and differentiate *Cryptosporidium* spp. at the species/genotype and subtype levels [2]. These techniques have been increasingly applied in epidemiological studies, significantly advancing our understanding of the transmission dynamics of *Cryptosporidium* spp. To date, over 50 species within the genus *Cryptosporidium* have been identified. Among them, *C. hominis* and *C. parvum* are the two most prevalent species infecting humans, exhibiting distinct epidemiological patterns linked to geographical regions and host-pathogen interactions [8]. Specifically, *C. hominis* is predominantly transmitted through person to person contact in urban environments, such as contact with contaminated recreational water (e.g., swimming pools). In contrast, *C. parvum* is more commonly associated with zoonotic transmission in rural areas, such as contact with infected animals [9]. Nevertheless, accumulating evidence suggests that the diversity of *Cryptosporidium* species infecting humans is increasing, and there are more cases of infections caused by species such as *C. andersoni* and *C. ubiquitum* in recent years [10]. Without strengthened multisectoral surveillance and molecular source tracing, emerging *Cryptosporidium* threats may remain undetected. Therefore, urgent molecular surveillance of animal-derived *Cryptosporidium* is essential within a "One Health" framework to identify zoonotic spillover early [11]. Continuous monitoring of animals in close contact with humans—particularly in regions where livestock–human interactions are common—can facilitate the timely detection of high-risk hosts and potential transmission pathways.

Goats are a livestock species that frequently come into close contact with humans and are globally valued for the production of milk, meat, and fiber. As a result, they can act as potential reservoirs for zoonotic pathogens, including *Cryptosporidium* [12]. Although adult goats infected with *Cryptosporidium* may be asymptomatic, Cryptosporidiosis, a common infection in neonatal goats, causes watery yellow diarrhea, weight loss, and poor growth. It can even lead to the death of infected animals and significant economic losses [13]. Molecular investigations have revealed a wide diversity of *Cryptosporidium* species identified in goats, such as *C. parvum*, *C. andersoni*, *C. muris*, *C. baileyi*, *C. hominis*, *C. xiaoi*, *C. ubiquitum*, and *Cryptosporidium* rat genotype II (Table 1). Notably, all of these species have also been detected in human infections [8, 14]. This genetic concordance underscores the zoonotic potential of *Cryptosporidium* isolates originating from goats, particularly *C. parvum* and *C. ubiquitum*, which have been repeatedly implicated in human cryptosporidiosis outbreaks [8].

China is a major contributor to the global animal husbandry industry, with extensive goat-farming operations distributed throughout the country. By the end of 2023, the national goat population had reached 129.34 million. In rural areas, goats are often raised in close proximity to human settlements, creating favorable conditions for the zoonotic transmission of *Cryptosporidium* spp. Previous studies have documented the widespread occurrence of cryptosporidiosis in goats across several provinces in northern, western, and central China [51]. However, no comprehensive investigation of *Cryptosporidium* infections in goats has yet been carried out in Zhejiang Province. Therefore, the present study aimed to determine the prevalence and species composition of *Cryptosporidium* spp. in goats from this region, thereby providing new insights into their molecular epidemiology and potential public health significance.

Materials and methods

Collection of fecal samples

From September 2021 to May 2023, a total of 386 fresh fecal samples were collected from goats housed in five communities within Zhejiang Province, China (Fig. 1; Table 2). Farms were selected based on owner consent and sampling accessibility. All farms contained a single species of animals. Fecal samples were aseptically collected using sterile, disposable latex gloves immediately

Table 1 The rate of detection by PCR and the species distribution of *Cryptosporidium* spp. In goats across the Globe

Locations	No. positive/No. sampled (%)	Species (no.)	References
[a]Algeria	13.6 (76/559)	*C. xiaoi* (66)	[15]
	8.7 (8/92)	*C. xiaoi* (6); *C. ubiquitum* (2)	[16]
[a]Belgium	9.5 (14/148)	*C. parvum* (11)	[17]
China	0.8 (3/398)	*C. xiaoi* (3)	[18]
	3.14 (19/541)	*C. andersoni* (5); *C. xiaoi* (8); *C. ubiquitum* (6)	[19]
	1.91 (9/352)	*C. xiaoi* (5); *C. andersoni* (1); *C. ubiquitm* (3)	[20]
	18.0 (55/305)	*C. muris* (40); *C. xiaoi* (15)	[21]
	8.7 (68/781)	*C. parvum* (68)	[22]
	4.7 (16/342)	*C. xiaoi* (11); *C. suis* (5)	[23]
	16.5 (104/629)	*C. parvum* (8); *C. xiaoi* (94); *C. ubiquitum* (2)	[24]
	11.4 (69/604)	*C. parvum* (4); *C. xiaoi* (45); *C. ubiquitum* (10)	[25]
	3.48 (44/1256)	*C. ubiquitum* (24); *C. andersoni* (16); *C. xiaoi* (4)	[26]
	37.6 (76/202)	*C. parvum* (41); *C. xiaoi* (35)	[27]
	4.8 (2/42)	*Cryptosporidium* sp. (1); *C. bovis*-like genotype (1)	[28]
Cyprus	50 (5/10)	*C. parvum* (5)	[29]
[a]Ghana	33.3 (95/285)	*C. xiaoi* (20); *C. baileyi* (1); *C. parvum* (1)	[30]
[a]Greece	27.7 (41/148)	*C. parvum* (16); *C. xiaoi* (1)	[31]
	7.1 (18/255)	*C. xiaoi* (7); *C. ubiquitum* (5); *C. parvum* (2)	[32]
Guinea	4.4 (10/228)	*C. hominis* (6); *C. parvum* (2); *C. xiaoi* (1); *Cryptosporidium* rat genotype II (1)	[33]
India	0.5 (1/207)	*C. ubiquitum* (1)	[34]
Iran	2 (2/100)	*C. xiaoi* (2)	[35]
Jordan	3.9 (2/51)	*C. xiaoi* (2)	[36]
Kenya	4.5 (4/88)	*C. baileyi* (1); *C. xiaoi* (1); *C. ubiquitum* (1)	[37]
Korea	53.8 (35/65)	*C. parvum* (28); *C. xiaoi* (7)	[38]
	42.9 (3/7)	*C. hominis*	[39]
[a]Kuwait	7.2 (16/222)	*C. parvum* (7); *C. ubiquitum* (2); *C. xiaoi* (1)	[40]
Nigeria	21.4 (3/14)	*C. muris* (1); *C. parvum* (2)	[41]
Poland	37.1 (39/105)	*C. parvum* (1); *C. xiaoi* (38)	[42]
Portugal	12.7 (8/63)	*C. xiaoi* (6); *C. parvum* (1); *C. ubiquitum* (1)	[43]
[a]South Africa	47.7 (42/88)	*C. parvum* (5); *C. andersoni* (1)	[44]
Spain	23.3 (31/240)	*C. xiaoi* (16); *C. parvum* (13); *C. ubiquitum* (2)	[45]
	6.0 (14/234)	*C. parvum* (3); *C. ubiquitum* (5); *C. xiaoi* (5)	[46]
	89.2 (66/118)	*C. parvum* (61); *C. xiaoi* (5)	[47]
Tanzania	8.9 (5/56)	*C. xiaoi* (5)	[48]
Turkey	13.4 (2/15)	*C. parvum* (2)	[49]
Zambia	1.2 (3/245)	*C. xiaoi* (3)	[50]

[a]In certain countries, the quantities of species do not align with the quantities of positive cases, as not all isolates were successfully genotyped

following defecation and deposited into individually labeled sterile tubes. Any portions of the samples contacting the ground were discarded to minimize contamination risk. Samples were transported to the laboratory in chilled containers with ice and subsequently stored at 4 °C until processing. All sampled animals were clinically healthy at the time of collection, however, no information regarding their age and gender was recorded.

DNA extraction

Distilled water was used to homogenize fecal samples, which were then filtered through a 45-µm pore-size sieve with a diameter of 10 cm. Filtrates were concentrated by centrifugation at 1,500 *g* for 10 min. Genomic DNA was extracted directly from 200 mg of processed fecal material using the QIAamp Fast DNA Stool Mini Kit (Qiagen, Hilden, Germany), in accordance with the manufacturer's protocol. This included a 95 °C incubation step to enhance oocyst lysis and maximize DNA yield [52]. Purified DNA extracts were stored at -20 °C for subsequent PCR analysis.

PCR amplification

Identification of *Cryptosporidium* species was accomplished through nested PCR for the amplification and subsequent sequence analysis of an approximately 830-base pair (bp) fragment of the small subunit ribosomal RNA (SSU rRNA) gene [53]. Samples positive for *C. parvum*, *C. xiaoi*, and *C. ubiquitum* underwent additional nested PCR targeting the 60 kDa glycoprotein (*gp60*) gene [54–56]. All PCR primers and conditions employed in this study have been previously reported and summarized in Table 3. Each sample was analyzed by PCR at least twice, using 2×TransTaq®-T PCR SuperMix (+ dye). *Cryptosporidium bovis* DNA served as the positive control for the SSU rRNA PCR, while DNA of *C. parvum* and *C. ubiquitum* isolated in rodents was used for the *gp60* PCR. A negative control with reagent water was included in each PCR analysis. PCR products were visualized on a UV transilluminator after electrophoresis on 1.5% agarose gels stained with GelStrain. All the employed reagents were purchased from TransGen Biotech Co., Beijing, China.

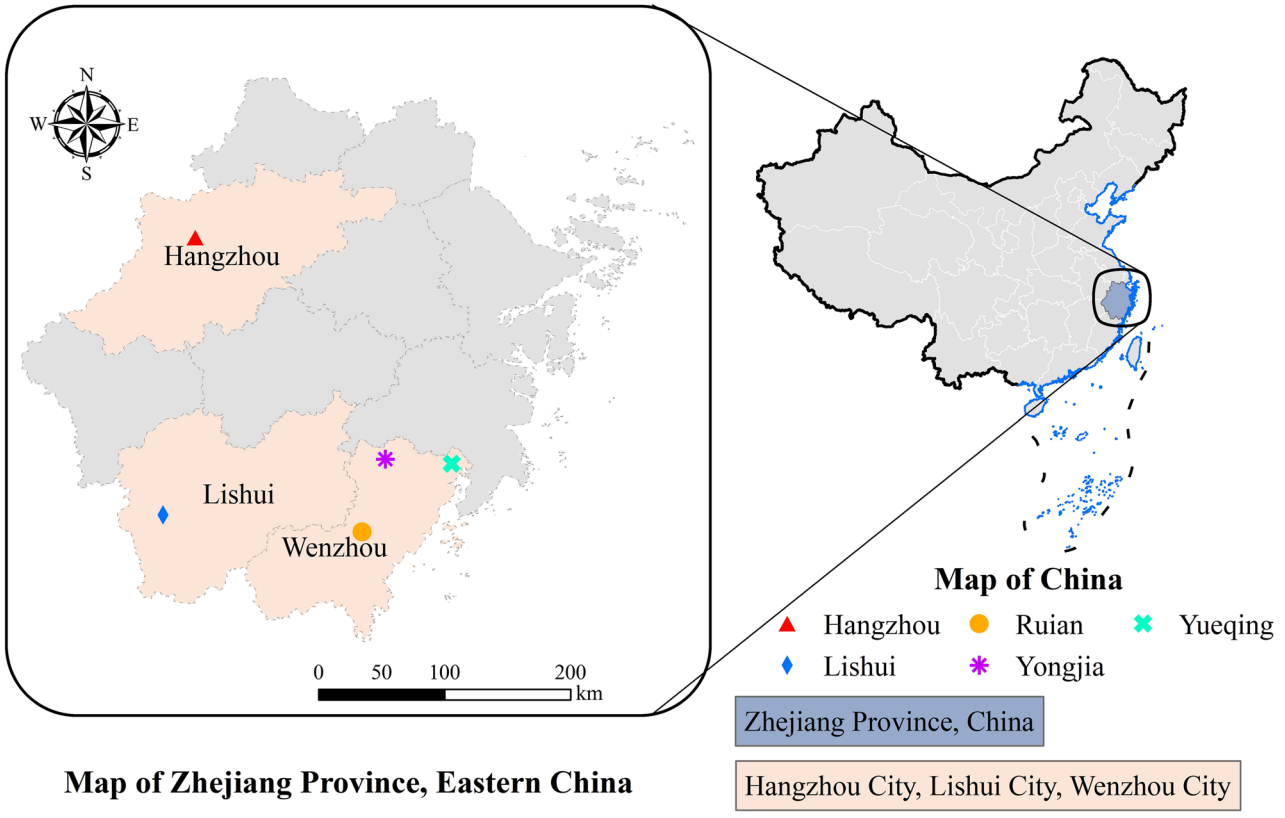

Fig. 1 This is a map for collecting goat feces samples in this study. The blue - highlighted area was Zhejiang Province, and the orange - highlighted areas were Hangzhou, Lishui, and Wenzhou Cities. The legend shows a red triangle for the Hangzhou farm, an orange dot for the Ruian farm, a cyan cross for the Yueqing farm, a blue diamond for the Lishui farm, and a purple star for the Yongjia farm. The authors initially conceptualized and designed it using ArcGIS 10.4 software. The original vector diagram imported into ArcGIS came from the National Geomatics Center of China (http://www.ngcc.cn). However, the final map was modified and assembled to meet specific attribution and permission guidelines, which was achieved by using both Microsoft PowerPoint 2003 and Adobe Photoshop CS6

Table 2 The rate of detection and species distribution of *Cryptosporidium* spp. In goats at different locations In Zhejiang Province, China

Location	No. Positive/ No. sample (%)	Species (*n*)	*P*-value
Hangzhou	4/92 (4.3)	*C.xiaoi* (2), *C. occultus* (2)	$P=0.103$
Lishui	8/88 (9.1)	*C.xiaoi* (4), *C. ubiquitum* (4)	
Ruian	4/69 (5.8)	*C. parvum* (3), *C. ubiquitum* (1)	
Yongjia	10/63 (15.9)	*C.xiaoi* (4), *C. ubiquitum* (6)	
Yueqing	5/74 (6.8)	*C.xiaoi* (4), *C. ubiquitum* (1)	
Total	31/386 (8.0)	*C.xiaoi* (14), *C. ubiquitum* (12), *C. parvum* (3), *C. occultus* (2)	

Nucleotide sequencing and analyzing

The positive PCR amplicons were dispatched to Saiheng Biotechnology Company Limited (Shanghai, China), for the purpose of sequencing. The accuracy of the sequencing outcomes was verified through bidirectional sequencing. The sequences acquired from the sense and antisense strands were aligned by means of CLUSTAL W and then edited with MEGA version 11.0.13 (http://www.megasoftware.net/). The species and *gp60* subtypes of *Cryptosporidium* were determined by comparing the nucleotide sequences with those stored in the National Center for Biotechnology Information (NCBI) database, employing the BLAST tool (http://blast.ncbi.nlm.nih.gov/Blast.cgi).

Statistical analyses

The data underwent statistical analysis employing SPSS version 22.0 (SPSS, Chicago, IL, USA). A chi-square test was implemented to examine and contrast the prevalence of *Cryptosporidium* spp. across various goat farm groups. Significance was determined for differences at a *P*-value threshold of ≤ 0.05.

Nucleotide sequence accession numbers

The nucleotide sequences acquired in this research were submitted to the GenBank database, with accession numbers PX427217 to PX427228 for *SSU rRNA* gene and PX471837 to PX471841 for *gp 60* gene.

Table 3 Details of the primers employed for the characterization of *Cryptosporidium* spp. In the current study

Loci	Species	Primer ID	Primer Sequences (5′-3′)	Fragment Length (bp)	Temperature Annealing (°C)	References
SSU rRNA	*Cryptosporidium* spp.	*SSU*U1	TTCTAGAGCTAATACATGCG	830	55	[51]
		S*SU*D1	CCCATTTCCTTCGAAACAGGA			
		*SSU*U2	GGAAGGGTTGTATTTATTAGATAAAG		55	
		*SSU*D2	AAGGAGTAAGGAACAACCTCCA			
gp60	*C. parvum*	*gp60*-F1	ATAGTCTCCGCTGTATTC	850	50	[52]
		gp60-R1	GGAAGGAACGATGTATCT			
		gp60-F2	TCCGCTGTATTCTCAGCC		50	
		gp60-R2	GCAGAGGAACCAGCATC			
	C. ubiquitum	Ubi-*gp60*-F1	TTTACCCACACATCTGTAGCGTCG	850	58	[53]
		Ubi- *gp60*-R1	ACGGACGGAATGATGTATCTGA			
		Ubi- *gp60*-F2	ATAGGTGATAATTAGTCAGTCTTTAAT		55	
		Ubi- *gp60*-R2	TCCAAAAGCGGCTGAGTCAGCATC			
	C. xiaoi	Xiaoi-*gp60*-F1	CCTCTCGGCACTTATTGCCCT	~ 1437	55	[54]
		Xiaoi-*gp60*-R1	ATACCTGAGATCAAATGCTGATGAA			
		Xiaoi-*gp60*-F2	CCTCTTAGGGGTTCATTGTCTA		55	
		Xiaoi-*gp60*-R2	TACCTTCAAAGATGACATCAC			

Results

Prevalence of Cryptosporidium species across participating farms

Based on PCR amplification and sequencing of the partial SSU rRNA gene, 31 out of 386 (8.0%) fecal samples tested positive for *Cryptosporidium* spp. The highest positive rate was detected in Yongjia (15.9%, 10/63), followed by Lishui (9.1%, 8/88), Yueqing (6.8%, 5/74), Ruian (5.8%, 4/69), and Hangzhou (4.3%, 4/92). Although the prevalence of *Cryptosporidium* varied among the five surveyed goat farms, the difference was not statistically significant ($\chi^2 = 7.698$, $P = 0.103$) (Table 2).

Identified species of *Cryptosporidium* from participating farms

A total of 31 *Cryptosporidium*-positive samples were further analyzed by nucleotide sequencing of an ~830 bp fragment of the *SSU rRNA* gene. Four distinct *Cryptosporidium* species were identified: *C. xiaoi*, *C. ubiquitum*, *C. parvum*, and *C. occultus*. The most prevalent species was *C. xiaoi*, accounting for 45.2% (14/31) of the isolates, followed by *C. ubiquitum* at 38.7% (12/31). *C. parvum* and *C. occultus* were detected in three and two isolates, respectively. No co-infections with different *Cryptosporidium* species were detected in the same isolate.

Notable differences in species distribution were observed among the five goat farms. Specifically, *C. xiaoi* was detected in four farms, excluding Rui'an, while *C. ubiquitum* was found in four farms (Lishui, Yongjia, Rui'an, and Yueqing). *C. parvum* and *C. occultus* were detected exclusively in Rui'an and Hangzhou, respectively (Table 2).

Genetic diversity of *Cryptosporidium* spp

Among the 31 recognized sequences, 12 distinct sequences were obtained. These included five sequences classified as *C. xiaoi* (PX427217 to PX427221), five as *C. ubiquitum* (PX427222 to PX427226), and one each as *C. occultus* (PX427227) and *C. parvum* (PX427228) (Table 4).

Among the five *C. xiaoi* sequences identified, three corresponded to previously reported genotypes. Sequence PX427217, detected in nine samples, was identical to KM199754 (and five other GenBank entries) derived from goats in Hubei, China. Two additional known sequences, PX427218 and PX427219, were obtained from two and one samples, respectively. PX427218 was identical to KM199750 (and 12 other GenBank sequences) originating from a goat in Guangdong, while PX427219 matched KM199748 (and three other sequences) from a goat in Shanghai. The remaining two sequences, PX427220 and PX427221, each recovered from a single sample, were novel and showed 99.88% and 99.02% similarity, respectively, to the reference sequence KM199754.

Among the five representative *C. ubiquitum* sequences, 16 polymorphic loci were identified, including two base insertions, two deletions, and 12 substitutions (Table 5). Two of these sequences, PX427222 and PX427223, corresponded to previously reported genotypes and were derived from eight and one samples, respectively. PX427222 was identical to KT027448, a sequence obtained from *Sciurus carolinensis* in the United States. PX427223 showed 100% identity with OL376589, which was reported from Tibetan sheep in China. The remaining three sequences (PX427224 to PX427226) were novel,

Table 4 Similarity analysis of the SSU rRNA and *gp60* gene sequences of *Cryptosporidium* isolates acquired in this study

Species	Location (*n*)	Accession number(s)	Similarity	Ref accession numbers-host-country
C. xiaoi	Yongjia (2); Lishui (4); Yueqing (3)	PX427217	100%	KM199754-goat-China
	Hangzhou (2)	PX427218	100%	KM199750-goat-China
	Yongjia (1)	PX427219	100%	KM199748-goat-China
	Yongjia (1)	PX427220	99.88%	KM199754-goat-China
	Yongjia (1)	PX427221	99.02%	KM199754-goat-China
C. ubiquitum	Lishui (4); Yongjia (2); Ruian (1); Yueqing (1)	PX427222	100%	KT027448-Sciurus carolinensis-the USA
	Yongjia (1)	PX427223	100%	OL376589-Tibetan sheep-China
	Yongjia (1)	PX427224	99.25%	EU827399-sheep-China
	Yongjia (1)	PX427225	99.88%	KT027448-Sciurus carolinensis-the USA
	Yongjia (1)	PX427226	99.15%	KT027448-Sciurus carolinensis-the USA
C. occultus	Hangzhou (2)	PX427227	99.64%	OL912797-Buffaloes-China
C. parvum	Ruian (3)	PX427228	100%	HQ651731-rats-Iran
IIdA19G1	Ruian (3)	PX471837	100%	MF074731-cattle-China
XIIa	Lishui (4); Yongjia (5); Ruian (1); Yueqing (1)	PX471838	100%	MH049733-sheep-China
	Yongjia (1)	PX471839	99.77%	MH049733-sheep-China
XXIIIa	Yongjia (2); Lishui (4); Yueqing (3); Hangzhou (2)	PX471840	100%	MW815186-goat-China
	Yongjia (3)	PX471841	99.71%	MW815186-goat-China

Table 5 Nucleotide polymorphisms at 16 polymorphic loci within the five distinct *SSU rRNA* sequences of *C. ubiquitum* acquired in this investigation

Accession no.	Nucleotide at position															
	70	263	299	411	486	487	516	545	673	722	753	756	773	779	802	804
PX427222	-	-	A	G	T	A	G	G	G	A	G	G	G	A	A	T
PX427223	T	-	A	G	T	A	G	G	G	A	G	G	G	A	A	T
PX427224	-	A	G	G	-	-	G	G	G	T	C	A	T	T	-	G
PX427225	-	-	G	G	-	-	G	G	G	A	G	G	G	A	A	T
PX427226	-	-	A	A	T	A	A	C	T	T	C	G	C	A	A	T

exhibiting 99.15%–99.88% similarity to their closest known references (Table 4).

Two identical *C. occultus* sequences (PX427227) obtained from goats were identified in this study. These sequences have not been previously reported and exhibit 99.64% similarity to OL912797, a sequence derived from buffaloes in China. In addition, three identical *C. parvum* sequences (PX427228) were detected, showing complete identity with the previously reported HQ651731 sequence from free-ranging rats in Iran (Table 4).

Gp60 subtyping of *Cryptosporidium* spp

Subtyping analysis of three *Cryptosporidium* species (*C. parvum*, *C. xiaoi*, and *C. ubiquitum*) was conducted based on sequencing of the *gp60* gene. All samples positive for these species were successfully amplified and genotyped at the *gp60* locus. A single subtype was identified for each species: IIdA19G1 for *C. parvum*, XIIa for *C. ubiquitum*, and XXIIIa for *C. xiaoi*.

The four *C. parvum* sequences of subtype IIdA19G1 obtained in this study were identical and exhibited 100% homology with the previously reported sequence MF074731 (derived from a cow in Shanghai), as well as with 69 other identical sequences available in GenBank. Among the 12 *C. ubiquitum* XIIa sequences, two representative sequences were identified, originating from 11, and 1 sample, respectively. The first sequence showed 100% homology with sequence MH049733 from sheep in China. The remaining sequence, although previously unreported, demonstrated the highest similarity to MH049733, with 99.77% identity, involving two base substitutions. The 14 *C. xiaoi* XXIIIa sequences were grouped into two distinct variants. The first variant, representing 11 samples, was identical to MW815186 from goats in China, while the second was a novel sequence representing three samples, showing 99.71% similarity to MW815186, differing by three base substitutions (Table 4).

Discussion

The detection rate of *Cryptosporidium* in goats in this study was 8.0%, exceeding the national average reported in China [52]. This detection rate was higher than those observed in Jiangsu (0.8%) [18], Inner Mongolia (3.14%) [19], Sichuan (4.7%) [21], Chongqing (3.5%) [26], and Qinghai (4.8%) [28], but lower than those reported in Yunnan (18.0%) [21], Henan and Shaanxi (16.5%) [24], Shaanxi (37.6%) [27], and Anhui (8.7%) [22]. Globally, *Cryptosporidium* infection rates in goats exhibit substantial variation. High prevalence rates have been documented in Korea (53.8% and 42.9%) [38, 39], Spain (23.3% and 89.2%) [45, 47], Ghana (33.3%) [30], Poland (37.1%) [42], Greece (27.7%) [31], and South Africa (47.7%) [44]. Conversely, relatively low rates were reported in India (0.5%) [34], Iran (2.0%) [35], Jordan (3.9%) [36], Papua New Guinea (4.4%) [33], and Kenya (4.5%) [37]. Given that most countries have conducted only limited investigations, it remains challenging to accurately characterize the regional prevalence of *Cryptosporidium* in goats. Therefore, strengthened and systematic molecular surveillance across diverse geographic regions is warranted to better assess the actual infection burden and transmission potential of this pathgeon in goat populations.

Four *Cryptosporidium* species were identified in the examined goat population, with *C. xiaoi* being the most prevalent. This result aligns with numerous previous studies that consistently recognize *C. xiaoi* as the dominant *Cryptosporidium* species infecting goats worldwide (Table 1). *C. xiaoi* was initially described as a *C. bovis*-like genotype and formally designated as a distinct species in 2009 [57]. It primarily infects herbivorous artiodactyls, including cattle, sheep, Alpine musk deer, Tibetan antelope, deer, and yaks [56, 58–61]. Beyond these typical hosts, *C. xiaoi* has also been detected in a variety of other animals, such as wild rodents [62], muskoxen [63], whiting [64], kangaroos [65], and even in a *C. xiaoi*-like genotype found in domestic free-range chickens in China [66]. In addition, *C. xiaoi* is generally regarded as a rare zoonotic species, with sporadic infections documented in individuals with HIV/AIDS patients [67], indicating a potential susceptibility among immunocompromised populations. Moreover, *C. xiaoi* has been detected in environmental samples, including water sources and ready-to-eat produce such as salads and berries [68, 69]. These findings indicate that *C. xiaoi* has the potential to infect humans indirectly through food- or waterborne transmission, underscoring its underestimated public health significance.

Cryptosporidium ubiquitum was identified as the second most common species in the examined goat population, accounting for 38.7% (12/31) of the detected sequences. Previous studies have also identified *C. ubiquitum* as one of the most prevalent *Cryptosporidium* species in goats, potentially ranking second only to *C. parvum* and *C. xiaoi* (Table 1). The high detection rate observed in this study highlights the potential role of goats as important reservoirs in the zoonotic transmission of *Cryptosporidium* to humans. Human infections with *C. ubiquitum* have been reported worldwide, with documented cases in more than 13 countries, including the United States, Ethiopia, Canada, Colombia, Cambodia, Sweden, the United Kingdom, New Zealand, Peru, Slovenia, Spain, Turkey, and Venezuela [55, 70–72]. Although the precise transmission routes remain uncertain, substantial evidence suggests that infection commonly occurs through the ingestion of contaminated water or food. *Cryptosporidium ubiquitum* has been frequently detected in various environmental matrices

such as drinking water, source water, stormwater runoff, wastewater, irrigation reservoirs, lake water, recreational water, and even ready-to-eat salads and berries [69, 73–75]. Given its broad host range, including primates, carnivores, ruminants, and various other animal taxa, the transmission dynamics of *C. ubiquitum* are complex and remain incompletely understood [76]. Nevertheless, the high infection rate of *C. ubiquitum* in goats observed in this study reinforces their significant role in maintaining and transmitting this zoonotic pathogen.

Cryptosporidium parvum and *C. occultus* were detected in three and two of the examined goats, respectively. *C. parvum* is widely recognized as a major etiological agent of human cryptosporidiosis, with its zoonotic potential well established through numerous epidemiological and molecular studies [77, 78]. This species has been implicated in both human outbreaks and large-scale epidemics among livestock populations [79], and direct evidence supports its transmission between humans and animals [6]. Goats are considered important reservoirs for *C. parvum*, and previous studies have shown that this species accounts for over one-third of all reported cryptosporidiosis cases in goats (Table 1). Therefore, the risk of zoonotic transmission between goats and humans warrants serious attention.

Cryptosporidium occultus, initially regarded as a rodent-specific species, has been increasingly detected in other hosts such as cattle, reflecting its expanding host range as research progresses [80]. Although less commonly reported than *C. parvum*, *C. occultus* has occasionally been identified in immunocompromised individuals, suggesting its potential public health relevance [81]. Notably, this study represents the first detection of *C. occultus* in goats, suggesting that goats have the potential to be hosts—or at least carriers—of this species. This finding not only broadens the recognized host spectrum of *C. occultus* but also highlights the need for strengthened surveillance of small ruminants, particularly in areas with frequent human–animal contact and inadequate hygiene practices. The emergence of *C. occultus* in goats underscores the need for further research into its transmission pathways and environmental persistence through fecal contamination.

The *gp60* gene has become one of the most widely used molecular markers for differentiating *Cryptosporidium* species, providing critical insights into intra-species diversity and zoonotic transmission dynamics [82]. In this study, the *gp60* gene was successfully sequenced and analyzed from *C. xiaoi*, *C. parvum*, and *C. ubiquitum*. The results revealed highly conserved subtypes among the examined goats, with only a single subtype identified for each species: IIdA19G1 for *C. parvum*, XXIIIa for *C. xiaoi*, and XIIa for *C. ubiquitum*. The IIdA19G1 subtype has been widely reported in livestock and has been implicated in outbreaks among neonatal calves [83]. Remarkably, this same subtype was previously detected as the sole subtype in diarrheic children from the same region [84]. This overlap strongly suggests that goats may serve as a significant reservoir in the *Cryptosporidium* transmission chain, particularly in rural or peri-urban areas where frequent close contact between children and livestock facilitates zoonotic transmission.

To date, twelve *C. xiaoi* subtypes (XXIIIa–XXIIIl) have been characterized [56]. Among them, the XXIIIa subtype is the most frequently detected in goats and displays a wide geographical distribution worldwide. Interestingly, this subtype has not been reported in sheep from the same regions, suggesting potential host specificity—although infections have been documented in Alpine musk deer [60, 85]. Due to the limited availability of subtype data from other hosts, evaluating the cross-species transmissibility and zoonotic potential of *C. xiaoi* remains challenging. Further research is needed to elucidate its host adaptability and epidemiological dynamics. In contrast, the *C. ubiquitum* XIIa subtype is well recognized for its zoonotic nature, characterized by a broad host range and confirmed human infections [55]. A similar pattern is observed for the zoonotic subtype *C. parvum* IIdA19G1, which was also identified in goats in this study, underscoring their potential role in zoonotic transmission. Collectively, the detection of *C. ubiquitum* XIIa and *C. parvum* IIdA19G1 subtypes in goats highlights their significance in public health and underscores the need for integrated One Health strategies to monitor and control zoonotic cryptosporidiosis at the human–animal interface.

There are several limitations in this study. First, the selection criteria for farms were solely based on the owners' consent to participate and the ease of accessing the animals for sampling. This may limit the generalizability of the findings to the conditions prevailing in Zhejiang Province, China. Second, it can be difficult to determine whether certain low-frequency species represent true infection/colonization or are simply carried by the animals. This is because molecular methods were used without morphological evidence. Furthermore, the infection rate may be underestimated. Since we only collected one fecal sample per animal, it may not have captured intermittent oocyst discharge. Third, the present study lacks a comparative study of different ages, genders, and clinical symptoms such as diarrhoea. Moreover, the risk factors of goats' infection with *Cryptosporidium* are not analyzed and judged in detail. Last, the study did not include a population survey near these animals or environmental sampling around the farms. This means it did not provide direct evidence of *Cryptosporidium* contamination and its transmission to humans from a One Health perspective. Despite these limitations, this study has successfully

identified the presence of four *Cryptosporidium* species in goats in the surveyed areas and provided initial observations regarding the species and subtype characteristics of their genes.

Conclusions

This study revealed a notable prevalence of *Cryptosporidium* infection (8.0%) among goats in Zhejiang Province, China, thereby contributing to the limited epidemiological data on its regional distribution. Four distinct species were identified: *C. parvum* (subtype IIdA19G1), *C. xiaoi* (subtype XXIIIa), *C. occultus*, and *C. ubiquitum* (subtype XIIa). The detection of the zoonotic species *C. parvum* and *C. ubiquitum*, together with their corresponding subtypes IIdA19G1 and XIIa, highlights a potential risk of zoonotic transmission. These findings enhance the current understanding of *Cryptosporidium* epidemiology in caprine hosts and provide critical baseline data for assessing infection risks and implementing targeted prevention and control measures. Ongoing surveillance is essential to mitigate potential threats to both animal and public health.

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Authors' contributions
Conceptualization, YJ and WZ, Methodology, XH, YL, AJ and BY, Software, XH, LS, HD and WZ, Validation, YJ and WZ, Formal analysis, XH, LS, FS, HD and WZ, Investigation, LS, AJ and BY, Resources, HY, HH and YJ, Data curation, YJ and WZ, Visualization, YJ and WZ, Supervision, WZ and YJ, Project administration, YJ and WZ, Funding acquisition, YJ and WZ, Writing—original draft, XH, LS, YL, AJ and WZ, Writing—review & editing, XH, LS, FS, HY, BY, HH, YJ and WZ. All authors have read and agreed to the published version of the manuscript.

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Data availability
The representative nucleotide sequences of *Cryptosporidium* obtained in the present study were deposited in the GenBank database under the following accession nos.: PX427217 to PX427228 for *SSU rRNA* gene and PX471837 to PX471841 for *gp 60* gene.

Declarations

Ethical approval and consent to participate
The study protocol was reviewed and approved by the Research and Animal Ethics Committees of Wenzhou Medical University after a thorough review (SCILLSC-2021-01). All fecal samples were collected with the explicit informed consent of animal owners or managers, ensuring strict adherence to non-harm protocols for the animals.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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References

1. 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.
2. Ryan UM, Feng Y, Fayer R, Xiao L. Taxonomy and molecular epidemiology of *Cryptosporidium* and *Giardia*—a 50 year perspective (1971–2021). Int J Parasitol. 2021;51(13–14):1099–119.
3. 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.
4. Bourli P, Eslahi AV, Tzoraki O, Karanis P. Waterborne transmission of protozoan parasites: a review of worldwide outbreaks—an update 2017–2022. J Water Health. 2023;21(10):1421–47.
5. GB/T 5750.12–2023. Standard examination methods for drinking water—Part 12: Microbiological indices. Beijing: National Administration for Market Regulation; 2023.
6. Jones G, Matizanadzo J, Nelson A, Chalmers RM, Thomas DR, Williams S, et al. A large *Cryptosporidium parvum* outbreak associated with a lamb-feeding event at a commercial farm in South Wales, March–April 2024: a retrospective cohort study. Epidemiol Infect. 2025;153:e82.
7. Hovd LBN, Jiménez-Meléndez A, Varegg MS, Woolsey ID, Olstad I, Mathisen SJ, et al. From the field: a cryptosporidiosis outbreak among veterinary students associated with activities during the lambing period in Norway during 2024. Epidemiol Infect. 2024;152:e178.
8. Ryan U, Zahedi A, Feng Y, Xiao L. An update on zoonotic *Cryptosporidium* species and genotypes in humans. Anim (Basel). 2021;11(11):3307.
9. Mendonça RLM, Miranda MS, Guimarães AC, Queiroga AP, Câmara HFM, Anjos PHLTS, et al. Clinical, laboratory and epidemiological aspects of *Cryptosporidium parvum* and *Cryptosporidium hominis* infection: a integrative review. Braz J Biol. 2025;85:e283306.
10. 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.
11. Helmy YA, Hafez HM, Cryptosporidiosis. From prevention to Treatment, a narrative review. Microorganisms. 2022;10(12):2456.
12. Utaaker KS, Chaudhary S, Kifleyohannes T, Robertson LJ. Global goat! Is the expanding goat population an important reservoir of *Cryptosporidium*? Front Vet Sci. 2021;8:648500.
13. Mammeri M, Obregón DA, Chevillot A, Polack B, Julien C, Pollet T, et al. *Cryptosporidium parvum* infection depletes butyrate producer bacteria in goat kid Microbiome. Front Microbiol. 2020;11:548737.
14. Hatam-Nahavandi K, Ahmadpour E, Carmena D, Spotin A, Bangoura B, Xiao L. *Cryptosporidium* infections in terrestrial ungulates with focus on livestock: a systematic review and meta-analysis. Parasit Vectors. 2019;12(1):453.
15. Bedjaoui S, Baroudi D, Adjou KT, Davoust B, Laidoudi Y. Molecular and epidemiological investigation of *Cryptosporidium* infection in goat population from Bouira Province. Algeria Pathogens. 2025;14(6):597.
16. Baroudi D, Hakem A, Adamu H, Amer S, Khelef D, Adjou K, et al. Zoonotic *Cryptosporidium* species and subtypes in lambs and goat kids in Algeria. Parasit Vectors. 2018;11(1):582.
17. Geurden T, Thomas P, Casaert S, Vercruysse J, Claerebout E. Prevalence and molecular characterisation of *Cryptosporidium* and *Giardia* in lambs and goat kids in Belgium. Vet Parasitol. 2008;155(1–2):142–5.
18. Cheng C, Fan Z, Cheng D, Tao J. Prevalence of *Cryptosporidium* spp. In sheep and goats In Jiangsu, China. Vet Sci. 2024;11(4):144.
19. Lang J, Han H, Dong H, Qin Z, Fu Y, Qin H, et al. Molecular characterization and prevalence of *Cryptosporidium* spp. In sheep and goats In Western inner Mongolia, China. Parasitol Res. 2023;122(2):537–45.
20. Wang P, Zheng L, Liu L, Yu F, Jian Y, Wang R, et al. Genotyping of Cryptosporidium spp., Giardia duodenalis and Enterocytozoon bieneusi from sheep and goats in China. BMC Vet Res. 2022;18(1):361.
21. Zhang ZW, Chen D, Zou Y, Hou JL, Sun LX, Li Z, et al. First report of *Cryptosporidium* spp. Infection and risk factors in black-boned goats and black-boned sheep in China. Parasitol Res. 2020;119(9):2813–9.

22. Li WC, Wang K, Tang L, Chen M, Li H, Kan ZZ, et al. Molecular characterization of *Cryptosporidium* species in sheep and goats in Anhui Province and neighboring provinces. Chin J Schisto Control. 2019;31(5):474–8. (in Chinese).
23. Zhong Z, Tu R, Ou H, Yan G, Dan J, Xiao Q, et al. Occurrence and genetic characterization of *Giardia duodenalis* and *Cryptosporidium* spp. From adult goats in Sichuan Province, China. PLoS ONE. 2018;13(6):e0199325.
24. Peng XQ, Tian GR, Ren GJ, Yu ZQ, Lok JB, Zhang LX, et al. Infection rate of *Giardia duodenalis*, *Cryptosporidium* spp. And *Enterocytozoon bieneusi* in cashmere, dairy And meat goats in China. Infect Genet Evol. 2016;41:26–31.
25. Mi R, Wang X, Huang Y, Zhou P, Liu Y, Chen Y, et al. Prevalence and molecular characterization of *Cryptosporidium* in goats across four provincial level areas in China. PLoS ONE. 2014;9(10):e111164.
26. Wang R, Li G, Cui B, Huang J, Cui Z, Zhang S, et al. Prevalence, molecular characterization and zoonotic potential of *Cryptosporidium* spp. In goats In Henan and Chongqing, China. Exp Parasitol. 2014;142:11–6.
27. Yang X, Wang J, Huang S, Song J, Fan Y, Zhao G. Molecular characterization of Cryptosporidium spp., giardia duodenalis, enterocytozoon bieneusi and Escherichia coli in dairy goat kids with diarrhea in partial regions of Shaanxi Province, China. Anim (Basel). 2023;13(18):2922.
28. Karanis P, Plutzer J, Halim NA, Igori K, Nagasawa H, Ongerth J, et al. Molecular characterization of *Cryptosporidium* from animal sources in Qinghai Province of China. Parasitol Res. 2007;101(6):1575–80.
29. Schou C, Hasapis K, Karanis P. Molecular identification of *Cryptosporidium* species from domestic ruminants and wild reptiles in Cyprus. Parasitol Res. 2022;121(7):2193–8.
30. Squire SA, Yang R, Robertson I, Ayi I, Ryan U. Molecular characterization of *Cryptosporidium* and *Giardia* in farmers and their ruminant livestock from the coastal Savannah zone of Ghana. Infect Genet Evol. 2017;55:236–43.
31. Papanikolopoulou V, Baroudi D, Guo Y, Wang Y, Papadopoulos E, Lafi SQ, et al. Genotypes and subtypes of *Cryptosporidium* spp. In diarrheic lambs and goat kids In Northern Greece. Parasitol Int. 2018;67(4):472–5.
32. Tzanidakis N, Sotiraki S, Claerebout E, Ehsan A, Voutzourakis N, Kostopoulou D, et al. Occurrence and molecular characterization of *Giardia duodenalis* and *Cryptosporidium* spp. In sheep and goats reared under dairy husbandry systems In Greece. Parasite. 2014;21:45.
33. Koinari M, Lymbery AJ, Ryan UM. *Cryptosporidium* species in sheep and goats from Papua new Guinea. Exp Parasitol. 2014;141:134–7.
34. Utaaker KS, Myhr N, Bajwa RS, Joshi H, Kumar A, Robertson LJ. Goats In the city: prevalence of *Giardia duodenalis* and *Cryptosporidium* spp. In extensively reared goats In Northern India. Acta Vet Scand. 2017;59(1):86.
35. Firoozi Z, Sazmand A, Zahedi A, Astani A, Fattahi-Bafghi A, Kiani-Salmi N, et al. Prevalence and genotyping identification of *Cryptosporidium* in adult ruminants in central Iran. Parasit Vectors. 2019;12(1):510.
36. Hijjawi N, Mukbel R, Yang R, Ryan U. Genetic characterization of *Cryptosporidium* in animal and human isolates from Jordan. Vet Parasitol. 2016;228:116–20.
37. Essendi WM, Muleke C, Miheso M, Otachi E. Genetic diversity of *Cryptosporidium* species in Njoro sub County, Nakuru, Kenya. J Parasit Dis. 2022;46(1):262–71.
38. Kim AY, Alkathiri B, Lee S, Min KD, Kim S, Lee SM, et al. Outbreak of severe diarrhea due to zoonotic *Cryptosporidium parvum* and *C. xiaoi* in goat kids in Chungcheongbuk-do, Korea. Parasitol Res. 2023;122(9):2045–54.
39. Park JH, Guk SM, Han ET, Shin EH, Kim JL, Chai JY. Genotype analysis of *Cryptosporidium* spp. Prevalent in a rural village in Hwasun-gun, Republic of Korea. Korean J Parasitol. 2006;44(1):27–33.
40. Majeed QAH, El-Azazy OME, Abdou NMI, Al-Aal ZA, El-Kabbany AI, Tahrani LMA, et al. Epidemiological observations on cryptosporidiosis and molecular characterization of *Cryptosporidium* spp. In sheep and goats In Kuwait. Parasitol Res. 2018;117(5):1631–6.
41. Ayinmode AB, Oliveira BCM, Obebe OO, Dada-Adgebola HO, Ayede AI, Widmer G. Genotypic characterization of *Cryptosporidium* species in humans and Peri-Domestic animals in Ekiti and Oyo States, Nigeria. J Parasitol. 2018;104(6):639–44.
42. Kaupke A, Michalski MM, Rzeżutka A. Diversity of *Cryptosporidium* species occurring in sheep and goat breeds reared in Poland. Parasitol Res. 2017;116(3):871–9.
43. Gomes-Gonçalves S, Palmeira JD, Ferreira H, Santos-Silva S, Mesquita JR. Occurrence and phylogenetic analysis of zoonotic enteropathogenic protist parasites in asymptomatic domestic ruminants from Portugal. Pathogens. 2023;12(11):1341.
44. Samie A, Hlungwani AH, Mbati PA. Prevalence and risk factors of *Cryptosporidium* species among domestic animals in rural communities in Northern South Africa. Trop Biomed. 2017;34(3):636–47.
45. Del Río MC, Martín S, Quílez J, Molina JM, Ferrer O, Molina JA, et al. Molecular epidemiology of cryptosporidiosis on lamb and goat kid farms in Gran Canaria, Canary Islands (Spain). Microorganisms. 2025;13(3):644.
46. Díaz P, Navarro E, Prieto A, Pérez-Creo A, Viña M, Díaz-Cao JM, et al. *Cryptosporidium* species in post-weaned and adult sheep and goats from N.W. Spain: public and animal health significance. Vet Parasitol. 2018;254:1–5.
47. Díaz P, Quílez J, Prieto A, Navarro E, Pérez-Creo A, Fernández G, et al. *Cryptosporidium* species and subtype analysis in diarrhoeic pre-weaned lambs and goat kids from north-western Spain. Parasitol Res. 2015;114(11):4099–105.
48. Parsons MB, Travis D, Lonsdorf EV, Lipende I, Roellig DM, Collins A, et al. Epidemiology and molecular characterization of *Cryptosporidium* spp. In humans, wild primates, and domesticated animals In the greater Gombe Ecosystem, Tanzania. PLoS Negl Trop Dis. 2015;9(2):e0003529.
49. Kabir MHB, Ceylan O, Ceylan C, Shehata AA, Bando H, Essa MI, et al. Molecular detection of genotypes and subtypes of *Cryptosporidium* infection in diarrheic calves, lambs, and goat kids from Turkey. Parasitol Int. 2020;79:102163.
50. Mwaba F, Robertson LJ, Tembo R, Zulu M, Ngalamika O, Phiri AM, et al. Occurrence and factors associated with *Cryptosporidium* infection in livestock in three districts of Zambia. Vet Parasitol Reg Stud Rep. 2024;52:101057.
51. Yang XY, Gong QL, Zhao B, Cai YN, Zhao Q. Prevalence of *Cryptosporidium* infection in sheep and goat flocks in China during 2010–2019: A systematic review and Meta-Analysis. Vector Borne Zoonotic Dis. 2021;21(9):692–706.
52. Zhao W, Zhang W, Yang F, Cao J, Liu H, Yang D, et al. High prevalence of *Enterocytozoon bieneusi* in asymptomatic pigs and assessment of zoonotic risk at the genotype level. Appl Environ Microbiol. 2014;80(12):3699–707.
53. 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.
54. Alves M, Xiao L, Sulaiman I, Lal AA, Matos O, Antunes F. Subgenotype analysis of *Cryptosporidium* isolates from humans, cattle, and zoo ruminants in Portugal. J Clin Microbiol. 2003;41(6):2744–7.
55. 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.
56. Fan Y, Huang X, Guo S, Yang F, Yang X, Guo Y, et al. Subtyping *Cryptosporidium xiaoi*, a common pathogen in sheep and goats. Pathogens. 2021;10(7):800.
57. Fayer R, Santín M. *Cryptosporidium Xiaoi* n. sp. (Apicomplexa: Cryptosporidiidae) in sheep (*Ovis aries*). Vet Parasitol. 2009;164(2–4):192–200.
58. Zhao W, Xu J, Xiao M, Cao J, Jiang Y, Huang H, et al. Prevalence and characterization of *Cryptosporidium* species and genotypes in four farmed deer species in the Northeast of China. Front Vet Sci. 2020;7:430.
59. Qin SY, Sun HT, Lyu C, Zhu JH, Wang ZJ, Ma T, et al. Prevalence and characterization of *Cryptosporidium* species in Tibetan antelope (*Pantholops hodgsonii*). Front Cell Infect Microbiol. 2021;11:713873.
60. Li P, Zhang B, Zhao Y, Chen R, Yu F, Qi M, et al. Molecular detection of *Cryptosporidium* in alpine musk deer (*Moschus chrysogaster*) in Gansu Province, Northwest China. Parasitol Res. 2024;123(6):231.
61. 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.
62. Li X, Atwill ER. Diverse genotypes and species of *Cryptosporidium* in wild rodent species from the West Coast of the USA and implications for Raw produce safety and microbial water quality. Microorganisms. 2021;9(4):867.
63. Davidson RK, Amundsen H, Lie NO, Luyckx K, Robertson LJ, Verocai GG, et al. Sentinels in a Climatic outpost: endoparasites in the introduced muskox (*Ovibos moschatus wardi*) population of Dovrefjell, Norway. Int J Parasitol Parasites Wildl. 2014;3(2):154–60.
64. Reid A, Lymbery A, Ng J, Tweedle S, Ryan U. Identification of novel and zoonotic *Cryptosporidium* species in marine fish. Vet Parasitol. 2010;168(3–4):190–5.
65. Yang R, Fenwick S, Potter A, Ng J, Ryan U. Identification of novel *Cryptosporidium* genotypes in kangaroos from Western Australia. Vet Parasitol. 2011;179(1–3):22–7.
66. Gong Z, Kan ZZ, Huang JM, Fang Z, Liu XC, Gu YF, et al. Molecular prevalence and characterization of *Cryptosporidium* in domestic free-range poultry in Anhui Province, China. Parasitol Res. 2021;120(10):3519–27.
67. Adamu H, Petros B, Zhang G, Kassa H, Amer S, Ye J, et al. Distribution and clinical manifestations of *Cryptosporidium* species and subtypes in HIV/AIDS patients in Ethiopia. PLoS Negl Trop Dis. 2014;8(4):e2831.

68. Hatam-Nahavandi K, Mohebali M, Mahvi AH, Keshavarz H, Najafian HR, Mirjalali H, et al. Microscopic and molecular detection of *Cryptosporidium andersoni* and *Cryptosporidium Xiaoi* in wastewater samples of Tehran Province, Iran. Iran J Parasitol. 2016;11(4):499–506.
69. Barlaam A, Sannella AR, Ferrari N, Temesgen TT, Rinaldi L, Normanno G, et al. Ready-to-eat salads and berry fruits purchased in Italy contaminated by *Cryptosporidium* spp., *Giardia duodenalis*, and *Entamoeba histolytica*. Int J Food Microbiol. 2022;370:109634.
70. Bujila I, Troell K, Ögren J, Hansen A, Killander G, Agudelo L, et al. *Cryptosporidium* species and subtypes identified in human domestic cases through the National Microbiological surveillance programme in Sweden from 2018 to 2022. BMC Infect Dis. 2024;24(1):146.
71. Guy RA, Yanta CA, Muchaal PK, Rankin MA, Thivierge K, Lau R, et al. Molecular characterization of *Cryptosporidium* isolates from humans in Ontario, Canada. Parasit Vectors. 2021;14(1):69.
72. Blanco MA, de Lucio A, Fuentes I, Carmena D. *Cryptosporidium ubiquitum* in venezuela: first report in a paediatric patient with acute diarrhoea. Enferm Infecc Microbiol Clin. 2016;34(2):142–3.
73. Xiao S, Zhang Y, Zhao X, Sun L, Hu S. Presence and molecular characterization of *Cryptosporidium* and *Giardia* in recreational lake water in Tianjin, china: a preliminary study. Sci Rep. 2018;8(1):2353.
74. 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.
75. Liu A, Ji H, Wang E, Liu J, Xiao L, Shen Y, et al. Molecular identification and distribution of *Cryptosporidium* and *Giardia duodenalis* in Raw urban wastewater in Harbin, China. Parasitol Res. 2011;109(3):913–8.
76. 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.
77. Guo Y, Ryan U, Feng Y, Xiao L. Emergence of zoonotic *Cryptosporidium parvum* in China. Trends Parasitol. 2022;38(4):335–43.
78. Oladele DB, Swain M, Robinson G, Clare A, Chalmers RM. A review of recent *Cryptosporidium hominis* and *Cryptosporidium parvum gp60* subtypes. Curr Res Parasitol Vector Borne Dis. 2025;8:100292.
79. Garcia-R JC, Hayman DTS. A review and analysis of cryptosporidiosis outbreaks in new Zealand. Parasitology. 2023;150(7):606–11.
80. Kváč M, Vlnatá G, Ježková J, Horčičková M, Konečný R, Hlásková L, et al. *Cryptosporidium occultus* sp. n. (Apicomplexa: Cryptosporidiidae) in rats. Eur J Protistol. 2018;63:96–104.
81. Xu N, Liu H, Jiang Y, Yin J, Yuan Z, Shen Y, et al. First report of *Cryptosporidium viatorum* and *Cryptosporidium occultus* in humans in China, and of the unique novel *C. viatorum* subtype XVaA3h. BMC Infect Dis. 2020;20(1):16.
82. Robinson G, Chalmers RM, Elwin K, Guy RA, Bessonov K, Troell K, et al. Deciphering a cryptic minefield: A guide to *Cryptosporidium gp60* subtyping. Curr Res Parasitol Vector Borne Dis. 2025;7:100257.
83. Li N, Wang R, Cai M, Jiang W, Feng Y, Xiao L. Outbreak of cryptosporidiosis due to *Cryptosporidium parvum* subtype IIdA19G1 in neonatal calves on a dairy farm in China. Int J Parasitol. 2019;49(7):569–77.
84. Zhao W, Ren G, Jiang W, Wang L, Wang J, Yuan Z, et al. Genetic characterizations of *Cryptosporidium* spp. From children with or without diarrhea in Wenzhou, china: high probability of zoonotic transmission. BMC Microbiol. 2024;24(1):113.
85. Chen Y, Qin H, Huang J, Li J, Zhang L. The global prevalence of *Cryptosporidium* in sheep: a systematic review and meta-analysis. Parasitology. 2022;149(12):1652–65.

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