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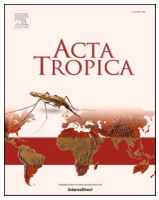

Molecular detection of *Cryptosporidium* spp. in cattle in southern Zhejiang Province, China: Prevalence and genetic characteristics

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ABSTRACT

Cryptosporidium sp. is a genus of parasitic protozoa that infects the gastrointestinal tracts of humans and animals, potentially leading to zoonotic transmission. A molecular investigation was carried out on *Cryptosporidium* spp. in cattle reared in southern Zhejiang Province, China, to elucidate its epidemiology. A total of 265 fresh cattle fecal specimens were collected and subjected to detection of *Cryptosporidium* spp. via polymerase chain reaction (PCR) and Sanger sequencing of its small subunit ribosomal RNA (*SSU rRNA)* gene. Specimens testing positive for *C. parvum* were further analyzed using nested PCR targeting its 60 kDa glycoprotein (*gp60*) gene. Among the 265 samples, 19 (7.2%) yielded positive test results. Four species of *Cryptosporidium* were identified: *C. bovis* (n = 8), *C. ryanae* (n = 7), *C. occultus* (n = 3), and *C. parvum* (n = 1) with subtype IIdA19G1. As the first report of *Cryptosporidium* in cattle from southern Zhejiang Province, the detection of zoonotic *C. parvum* at low prevalence suggests a limited but non-negligible public health risk rather than an urgent threat.

1. Introduction

Globally, *Cryptosporidium* spp. are prevalent unicellular eukaryotes in the intestines of humans and animals (Widmer et al., 2020). Their presence can cause moderate to severe diarrhoea in humans, particularly in children younger than 5 years in low- to middle-income countries, leading to malnutrition and diarrhoea-related deaths (GBD 2021 Diarrhoeal Diseases Collaborators, 2025; Ghonaim et al., 2024). The most likely mode of transmission occurs through the fecal-oral route, specifically via contaminated food and water, which results in outbreaks of foodborne and waterborne diseases in high-income countries (Ryan et al., 2018; Ali et al., 2024). Simultaneously, zoonotic infection makes a substantial contribution to its epidemiology (Guo et al., 2022). Contact with farm animals, such as cattle, is identified as a recognized risk factor, as demonstrated by outbreaks among veterinary students, farmers, and other relevant populations (Alsmark et al., 2018; Hovd et al., 2024). As there are no specific medications or vaccinations, understanding infection pathways and transmission sources is crucial for control.

Previously, fecal specimens were examined under a light microscope for the diagnosis of *Cryptosporidium* spp. In recent years, polymerase chain reaction (PCR) followed by sequencing has often been utilized to identify and characterize this parasite at the molecular level (Mendonça et al., 2025). *Cryptosporidium* spp. are genotyped through the examination of polymorphic regions of its small subunit ribosomal RNA (*SSU rRNA)* gene (Ryan et al., 2021a). Globally, over 50 species of *Cryptosporidium* have been identified in humans or animals, with at least 21 of these species detected in humans. All these species found in humans are also present in animals, indicating their zoonotic potential (Ryan et al., 2021b). Due to host-range differences, these *Cryptosporidium* species have different transmission routes in humans (Guo et al., 2021). Prioritizing the genetic characterization of *Cryptosporidium* spp. isolates from animal hosts with frequent human interaction in epidemiological surveys can improve our understanding of human cryptosporidiosis epidemiology and our comprehension of animal populations in human transmission contexts (Checkley et al., 2015).

Cattle as farm animals that live in close proximity to humans in rural areas are significant hosts for various species of *Cryptosporidium* (Buchanan et al., 2025). Several studies have investigated the presence

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of different *Cryptosporidium* species in cattle and more than 15 species have been identified: *C. andersoni, C. bovis, C. parvum, C. canis, C. felis, C. hominis, C. meleagridis, C. muris, C. occultus, C. ryanae, C. scrofarum, C. serpentis, C. suis, C. ubiquitum*, and *C. viatorum*. With the exception of *C. serpentis* and *C. ryanae*, all these species are considered zoonotic to varying degrees (Ryan et al., 2021b; Buchanan et al., 2025). This indicates that cattle play a crucial role in the transmission of cryptosporidiosis to humans, underscoring the need for ongoing surveillance of *Cryptosporidium* infections in cattle.

China is a major contributor to the animal husbandry sector, boasting extensive cattle raising industries nationwide. These animals, which are often found in rural areas near humans, provide numerous opportunities for the spread of *Cryptosporidium* spp. to other animals and humans (Wang et al., 2017; Guo et al., 2022). Results showed cryptosporidiosis was widespread in cattle in 29 provinces which mainly were in northern, western, and central China (Gong et al., 2017; Wang et al., 2017; Cai et al., 2019; Yan et al., 2025). In Zhejiang Province, a renowned coastal region of China characterized by its warm and humid climate and significant transient population, no studies on *Cryptosporidium* spp. in cattle have been conducted. In the southern part of Zhejiang, human infections with *Cryptosporidium* have been reported in Wenzhou and its surrounding areas. Local wild rodents have also been identified as carriers or infected with the parasite, yet the etiology remains unclear (Zhao et al., 2024; Jiang et al., 2024; Li et al., 2024). Therefore, this study aimed to screen for the presence and species distribution of *Cryptosporidium* in cattle in Zhejiang, China. These data will provide fundamental information for a deeper understanding of the infection sources and transmission routes of *Cryptosporidium*.

2. Materials and methods

2.1. Ethical approval

The study protocol was approved by Wenzhou Medical University's Research and Animal Ethics Committees after a comprehensive review (SCILLSC-2021-01). All fecal samples were obtained with the consent of cattle owners or managers, ensuring no animals were harmed.

2.2. Sample collection and size determination

The sample size was determined using the formula proposed by Thrusfield (Thrusfeld, 1997), with a 95% confidence interval and a 5% precision value considered. Based on the previously reported prevalence of *Cryptosporidium* in cattle in China (Cai et al., 2019), an expected prevalence of 17.0% was assumed. Using this formula, we calculated a minimum sample size of 217 cattle. From September 2021 to May 2023, 265 fresh fecal samples were collected from adult beef cattle raised under intensive farming systems with a herd size of 300-500 cattle in four regions of Zhejiang Province, China: Jingning County (56 samples), Ruian City (71 samples), Yongjia County (71 samples), and Yueqing City (67 samples) (Fig. 1 and Table 1). Farms were selected based on owner consent and ease of sample collection, with each farm housing only one animal per pen. The number of collected samples accounted for 20–30% of the total number of animals in each farm. Fecal samples were collected using sterile, disposable latex gloves and placed into individually labeled sterile tubes immediately after defecation. Any parts of the samples that had touched the ground were discarded to minimize the risk of cross-contamination. The test tubes were transferred to the laboratory within a cold container filled with ice and stored at 4°C until processing. All the sampled cattle were ≥2 years old, and all the experimental animals exhibited normal health statuses during the

Table 1
The infection rate and species distribution of *Cryptosporidium* in cattle at different locations in Zhejiang Province, China.

Location	No. Positive/ No. sample	Prevalence (%, 95% CI)	Species (n)	*P*-value
Jingning	3/56	5.4 (1.1 – 14.9)	*C. bovis* (3)	0.582
Ruian	6/71	8.5 (3.2 – 17.5)	*C. ryanae* (3), *C. bovis* (3)	
Yongjia	7/71	9.9 (4.0 – 19.3)	*C. occultus* (3), *C. ryanae* (3), *C. bovis* (1)	
Yueqing	3/67	4.5 (0.9 – 12.6)	*C. parvum* (1), *C. ryanae* (1), *C. bovis* (1)	
Total	19/265	7.2 (4.3 – 11.0)	*C. bovis* (8), *C. ryanae* (7), *C. occultus* (3), *C. parvum* (1)	

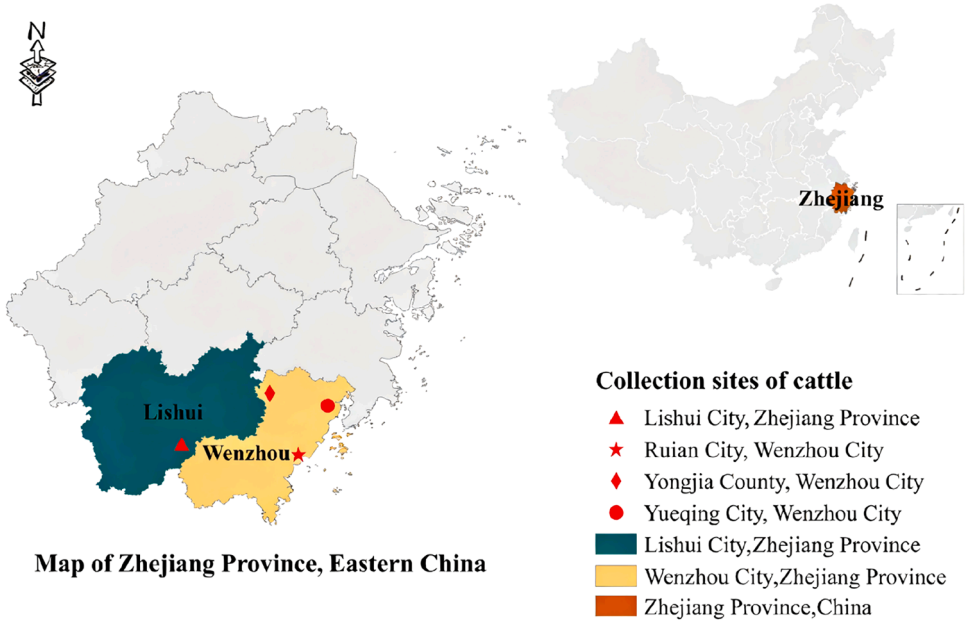

Fig. 1. A cartographic representation of the sampling locations within Zhejiang Province, China. The researchers initially conceptualized and devised the map using ArcGIS 10.4 software. The original vector diagram imported into ArcGIS came from the National Geomatics Center of China (http://www.ngcc.cn). However, to meet specific attribution and permission criteria, the final map version was revised and assembled by combining Microsoft PowerPoint 2003 and Adobe Photoshop CS6.

sampling process. No information related to the gender of the cattle was collected.

2.3. DNA extraction

Distilled water was used to dilute the feces, which were then immediately filtered through an 8.0-cm-diameter sieve with a 45 μm pore size. Subsequently, the filtrates were concentrated through centrifugation at 1,500 g for 10 minutes. Genomic DNA was directly extracted from 200 mg of each processed fecal sample using the QIAamp DNA Stool Mini Kit (QIAgen, Hilden, Germany), in accordance with the manufacturer's guidelines. An additional step was added to the protocol: the lysis temperature was set at 95 °C to optimize DNA yield (Zhao et al., 2014). The isolated DNA was preserved at -20 °C until subsequent PCR analysis.

2.4. PCR amplification

Cryptosporidium was identified using nested PCR to amplify an approximately 830-bp fragment of its *SSU rRNA* gene (Xiao et al., 1999). Species identification was achieved through sequence analysis of the PCR products. Samples positive for *C. parvum* underwent additional nested PCR targeting its *60 kDa glycoprotein* (*gp60*) gene (Alves et al., 2003). Each specimen was amplified by PCR at least twice, using TaKaRa Taq DNA polymerase (TaKaRa Bio Inc., Tokyo, Japan). *C. bovis* DNA served as the positive control for the *SSU rRNA* PCR, whereas DNA from *C. parvum* isolated in rodents (Jiang et al., 2024) was utilized for the *gp60* PCR. A negative control with reagent water was included in each PCR analysis. Following electrophoresis on 1.5% agarose gels stained with GelStain (Trans Gen Biotech, Beijing, China), PCR products were visualized using a UV transilluminator.

2.5. Nucleotide sequencing and analyzing

The positive PCR amplicons were dispatched to Sangon Biotech Co. Ltd. located in Shanghai, China, for the purpose of sequencing. The precision of the sequencing results was ascertained via a process of bi-directional sequencing. The species and *gp60* subtypes of *Cryptosporidium* were determined by comparing the nucleotide sequences with those in the National Center for Biotechnology Information (NCBI) database via the BLAST tool (http://blast.ncbi.nlm.nih.gov/Blast.cgi).

2.6. Statistical analyses

Statistical analysis of the data was conducted using SPSS version 22.0 (SPSS, Chicago, IL, USA). A chi - square test was utilized to analyze and compare the prevalence of *Cryptosporidium* spp. among different farm groups of cattle. Statistical significance was established for differences with P - values ≤ 0.05.

2.7. Nucleotide sequence accession numbers

The representative nucleotide sequences acquired in this study were submitted to the GenBank database, with accession numbers ranging from PX419104 to PX419109.

3. Results

3.1. Positivity rates of Cryptosporidium

Based on PCR and sequencing of the partial *SSU rRNA* gene, 19 of 265 (7.2%, 95% CI: 4.3-11.0%) fecal samples were positive for *Cryptosporidium*. The highest infection rate was found in Yongjia (9.9%, 7/71, 95% CI: 4.0-19.3%), followed by Ruian (8.5%, 6/71, 95% CI: 3.2-17.5%), Jingning (5.4%, 3/56, 95% CI: 1.1-14.9%), and Yueqing (4.5%, 3/67, 95% CI: 0.9-12.6%). There were no statistically significant differences in infection rates among the four cattle farms examined ($\chi^2 = 1.953$, df = 3, $P = 0.58$) (Table 1). All sampled animals were adult cattle, and no comparison by age, sex, or breed was applicable.

3.2. Species of Cryptosporidium isolates

A total of 19 *Cryptosporidium* positive-samples were analyzed using nucleotide sequence analysis of the partial sequence of the *SSU rRNA* gene (~830bp). A total of four distinct *Cryptosporidium* species were identified: *C. bovis, C. ryanae, C. occultus*, and *C. parvum* (Table 1). *C. bovis* emerged as the predominant species, detected in 42.1% of samples (8/19). *C. ryanae* followed in prevalence, accounting for 36.8% (7/19). *C. occultus* and *C. parvum* demonstrated relatively lower frequencies, with *C. occultus* at 15.8% (3/19) and *C. parvum* at 5.3% (1/19). Notably, distinct patterns of species prevalence were observed across different cattle ranches. For example, Yueqing demonstrated *C. parvum, C. ryanae*, and *C. bovis*, whereas Yongjia presented *C. occultus, C. ryanae*, and *C. bovis*. In contrast, Rui'an farm hosted only two species: *C. ryanae* and *C. bovis*. Meanwhile, Jingning exhibited exclusively *C. bovis* (Table 1).

3.3. Genetic diversity of Cryptosporidium spp. in SSU rRNA Gene

Among 19 recognized sequences, six representative sequences were observed. There were three sequences labeled as *C. bovis,* one sequence labeled as *C. occultus, C. parvum* and *C. ryanae*, respectively (Table 2). Six samples of *C. bovis* possess a known sequence (PX419104) that was previously documented, originating from Tibetan sheep (OL376597) in Qinghai, China. The sequence of another *C. bovis* (PX419105) sample is entirely consistent with that of Chinese cattle (MW767057). The remaining *C. bovis* (PX419106) sequence is newly identified and differs from sequence OL376597 by only a single base. The samples of *C. occultus* (PX419107) obtained from cattle possess a sequence that exhibits remarkable similarity to OL912797, which is derived from buffaloes in Yunnan Province, China. Remarkably, all seven samples of *C. ryanae* (PX419108) demonstrated identical sequences and showed 100% similarity to the OQ001439 sequence of Korean cattle. Additionally, the sequence of *C. parvum* (PX419109) was completely aligned with the previously reported HQ651731 sequence of free - ranging rats in Iran.

3.4. Subtyping of Cryptosporidium spp. based on the gp60 Gene

The subtype of *C. parvum* was analyzed by sequencing the *gp60* gene. Subtype IIdA19G1 was identified. The sequence of IIdA19G1 obtained here is consistent with the previously reported sequence MF074731

Table 2
Similarity analysis of 18S sequences of *Cryptosporidium* obtained in this study.

Species	No. of samples containing variant	Accession number(s)	Similarity	Host (Ref accession numbers) country
C. bovis	6	PX419104	100%	Tibetan sheep (OL376597) in China
	1	PX419105	100%	Chinese cattle (MW767057) in China
	1	PX419106	99.88%	Tibetan sheep (OL376597) in China
C. occultus	3	PX419107	100%	Buffaloes (OL912797) China
C. ryanae	7	PX419108	100%	Cattle (OQ001439) in Korea
C. parvum	1	PX419109	100%	Rat (HQ651731) in Iran

(derived from a cow in Shanghai) as well as with the other 69 sequences stored in GenBank.

4. Discussion

The overall prevalence of *Cryptosporidium* infection in cattle in this study was 7.2%, which is significantly lower than the global average infection rate of 26.5% reported in recent meta-analyses (Buchanan et al., 2025). Compared with other provinces in China, the 7.2% prevalence in southern Zhejiang is also lower than that reported in most regions, including 22.4% in Yunnan, 19.4% in Ningxia, 48.7% in Xinjiang, 29.90% in Inner Mongolia, and 23.5% in Guizhou (Zhao et al., 2023;Qin et al., 2025; Li et al., 2025; Wang et al., 2024; Zhang et al., 2022). Only a few regions, such as Guangdong (4.4%) and Anhui (2.4%), have documented slightly lower prevalence than observed in the present study (Liu et al., 2022; Liang et al., 2019).

Indeed, determining the true infection rate of *Cryptosporidium* in specific host species remains challenging due to multiple confounding factors, including immune status of animals, sampling strategies, seasonal variations, and diagnostic methods. The lower prevalence noted in this study may reflect regional disparities or improved farm management practices in the surveyed areas. All animals included in this study were adult cattle raised under intensive farming systems with good hygiene and management conditions. Adult cattle generally have relatively strong immune systems and higher natural resistance to pathogens; in addition, all sampled animals were clinically healthy at the time of collection, which may further reduce the probability of detecting active infection. Notably, each animal was sampled only once, which may have affected the detection rate to some extent and potentially led to an underestimation of the true infection prevalence. Furthermore, the high specificity of the *SSU rRNA* sequencing method used in this study may also contribute to the relatively low observed prevalence compared with studies using conventional microscopy with lower specificity (Buchanan et al., 2025).

In the present study, we also performed a preliminary comparative analysis of infection rates among the four investigated regions in Zhejiang Province. Although no significant geographical differences were detected ($\chi^2 = 1.953$, $P = 0.58$), the observed variation in prevalence (4.5%–9.9%) highlights the complexity of local epidemiological patterns. These regional comparisons, together with published data, emphasize the need for more comprehensive investigations: the greater the number of studies performed, the closer the results will reflect the actual epidemiological situation. Future studies should include animal populations from more diverse regions and different rearing systems, and efforts should be made to standardize sampling protocols and detection methods. Only through such integrated approaches can we accurately clarify the epidemiological characteristics of *Cryptosporidium* infection and provide a scientific basis for formulating targeted prevention and control strategies.

The present study identified four *Cryptosporidium* species (*C. bovis, C. ryanae, C. occultus*, and *C. parvum)*, with *C. bovis* being the predominant one. This species distribution is consistent with that commonly reported in adult cattle globally (Buchanan et al., 2025; Bulumulla et al., 2025). Generally, pre-weaned calves are mostly infected by *C. parvum*, the primary zoonotic species causing clinical cryptosporidiosis in young animals (Hu et al., 2022). In contrast, adult cattle usually carry *C. bovis, C. ryanae*, and *C. occultus*, which are host-adapted with lower pathogenicity and zoonotic risk (Díaz et al., 2021). The low prevalence of *C. parvum* in this study supports the age-related distribution pattern, as all sampled animals were adult cattle with mature immune systems and low susceptibility to *C. parvum* (Hu et al., 2022). These results show that host age is a key factor in shaping *Cryptosporidium* species composition, with different species dominating in calves and adult cattle.

This finding aligns with previous data, showing *C. bovis* is the most prevalent *Cryptosporidium* species in cattle globally (Bulumulla et al., 2025; Buchanan et al., 2025). Furthermore, *C. bovis* is also commonly found in herbivorous artiodactyls, including goats, sheep, donkeys, and camels (Mahdavi et al., 2024; Hatam-Nahavandi et al., 2019; de Aquino et al., 2020). Although *C. bovis* is generally considered non-zoonotic, it has been detected in humans in several instances, including an Indian dairy farmer, two individuals from Australia, a child from Egypt, and a cattle ranch worker from Mongolia (Khan et al., 2010; Ng et al., 2012; Helmy et al., 2013; Mo et al., 2024). These findings suggest that while *C. bovis* may have limited zoonotic potential, human exposure through close contact with infected livestock cannot be ignored.

This study identified seven cattle infected with *C. ryanae*, a species previously recognized as the *Cryptosporidium* deer-like genotype and officially named in 2018 (Fayer et al., 2018). Previous research indicates that *C. ryanae* primarily infects members of the Bovidae family, including cattle, yaks, and water buffaloes (Buchanan et al., 2025). Although *C. ryanae* has not yet been detected in humans, it can also infect non-bovid animals, such as marsh deer, horses, sika deer, red deer, roe deer, wild boar, and cats (Yun et al., 2023; Bartley et al., 2025; Mirhashemi et al., 2016). The presence of *C. ryanae* in such a broad range of hosts underscores the complexity of its transmission dynamics and highlights the potential for cross-species spillover under favorable ecological conditions and evolving host-pathogen interactions.

Cryptosporidium occultus ranked third in this study, being detected in three samples. Previously referred to as *C. suis*-like in some earlier reports, it was officially named in 2018 (Kváč et al., 2018). This species has been reported in various rodent species, including bamboo rats, and has successfully infected laboratory rats and mice, making rodents the considered primary hosts (Zhao et al., 2019; Wei et al., 2019; Kváč et al., 2018). Additionally, *C. occultus* can infect a variety of other hosts, including cattle, water buffalo, yaks, alpacas, Bactrian camels, red deer, and the Iberian lynx (Buchanan et al., 2025; Matas-Méndez et al., 2024; Mi et al., 2024; Sahin et al., 2025; Cao et al., 2020; Zhang et al., 2020). Furthermore, *C. occultus* has been identified in humans in Canada, England, and China (Xu et al., 2020; Ong et al., 2002). This indicates *C. occultus* can infect humans and has a wide host range, suggesting its host range is expanding from rodents to other animals, including humans, which should raise more concern.

This research identified *C. parvum* in a single cattle. This species has been widely detected in cattle globally, with a prevalence of 21.9% (Buchanan et al., 2025; Bulumulla et al., 2025). In China, the *C. parvum* infection rate in calves varies among provinces and cities: for example, the rate was 2.5% in dairy calves from Beijing and 45.5% in those from Xinjiang (Zhang et al., 2022). Although the prevalence of *C. parvum* in the cattle investigated in this study was low, it cannot be ignored because this species is dominant in local children (Zhao et al., 2024).

Overall, the *Cryptosporidium* species in the studied cattle were mainly bovine-specific (*C. bovis* and *C. ryanae)*, while the zoonotic *C. parvum* was rare. This implies that cross-transmission of *Cryptosporidium* among cattle mainly occurs within the cattle population, perhaps related to the rearing methods. The cattle are intensively farmed, and the crowded environment may facilitate cross-infection. The surveyed cattle had no clinical symptoms, and most previous studies found no link between *C. bovis* or *C. ryanae* infection and diarrhea, suggesting the cattle may have developed tolerance to these infections (Wang et al., 2017). Thus, the true pathogenicity of these two species requires further investigation.

The subtype families of *C. parvum* infection in cattle are primarily IIa and IId (Buchanan et al., 2025). Moreover, the intraspecific subtype diversity is continuously increasing, as evidenced by the heterogeneity in the ACATCA repeat sequence found in *C. parvum* subtypes isolated in cattle from Egypt (Hosein et al., 2024). There may be regional differences in subtype composition; for instance, *C. parvum* IIaA15G2R1 is widely distributed in cattle in European and American countries (Buchanan et al., 2025). In contrast, *C. parvum* IIdA15G1 and IIdA19G1 are the dominant subtypes in cattle in China (Wang et al., 2017). In this study, the subtype of *C. parvum* was identified as IIdA19G1, which has been detected in cattle from Ningxia, Heilongjiang, Inner Mongolia,

Guangdong, and Gansu (Wang et al., 2017). It was also associated with an outbreak in neonatal calves in Jiangsu, China (Li et al., 2019). Globally, infections caused by subtype IIdA19G1 have only been reported in cattle in Sudan and Sweden (Buchanan et al., 2025). This suggests a potential for regional spread of the *Cryptosporidium* subtype IIdA19G1. The recent discovery of subtype IIdA19G1 in children and HIV-positive patients in China further bolsters this hypothesis (Yu et al., 2019; Zhao et al., 2024). These findings indicate that infected cattle represent a potential concern not only for animal health but also for public health due to the zoonotic potential of *Cryptosporidium*. Although *C. parvum* was detected in only one animal in this study, this zoonotic species and its subtype IIdA19G1 have been previously documented in local human cases. Nevertheless, given the extremely low prevalence observed, strong conclusions regarding ongoing zoonotic transmission cannot be drawn. This result should be regarded merely as an alert to potential spillover risk that warrants long-term monitoring, rather than evidence of a substantial or widespread public health threat.

5. Conclusions

This study reveals the occurrence of *Cryptosporidium* spp. infection in cattle from Zhejiang Province, China, and supplements the limited epidemiological data on *Cryptosporidium* distribution in the investigated region. Four *Cryptosporidium* species (*C. parvum, C. bovis, C. occultus*, and *C. ryanae*) were identified, with *C. bovis* as the dominant species. The detection of zoonotic *C. parvum* and its subtype IIdA19G1 in cattle in this region, combined with its previous occurrence in local human cases, suggests a potential zoonotic risk, even though only one isolate was detected. The low prevalence of zoonotic *C. parvum* IIdA19G1 indicates limited but noteworthy zoonotic potential, supporting the need for continuous surveillance to monitor trends for both animal and public health, rather than requiring immediate public health intervention.

Data availability statement

The data presented in the study are deposited in the Genbank repository, accession numbers: PX419104 to PX419109

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CRediT authorship contribution statement

Wei Zhao: Writing – review & editing, Writing – original draft, Project administration, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Yongli Jian:** Formal analysis, Investigation, Funding acquisition, Writing – original draft. **Huilan Wang:** Formal analysis, Investigation, Writing – original draft, Writing – review & editing. **Lisha Xie:** Investigation, Writing – review & editing. **Yijia Huang:** Investigation, Writing – review & editing. **Xinyu Hu:** Supervision, Writing – review & editing. **Haowen Dong:** Supervision, Writing – review & editing. **Baolong Yan:** Validation, Writing – review & editing. **Yanyan Jiang:** Conceptualization, Formal analysis, Funding acquisition, Project administration, Resources, Writing – review & editing. **Huicong Huang:** Conceptualization, Resources, Supervision, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

No data was used for the research described in the article.

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