DOI: 10.1002/vms3.1024

ORIGINAL ARTICLE

WILEY

Molecular evaluation of *Cryptosporidium* spp. among breeding calves of Lorestan province Western Iran

Razieh Talebi[1] | **Amirreza Javadi Mamaghani**[1,2] | **Farnaz Kheirandish**[1,3] | **Azadeh Karimi**[1] | **Farzad Ebrahimzadeh**[4] | **Mohamad Kazempour**[5] | **Nozhat Zebardast**[6] | **Shirzad Fallahi**[1,2]

[1]Department of Medical Parasitology and Mycology, School of Medicine, Lorestan University of Medical Sciences, Khorramabad, Iran

[2]Hepatitis Research Center, School of Medicine, Lorestan University of Medical Science, Khorramabad, Iran

[3]Razi Herbal Medicines Research Center, School of Medicine, Lorestan University of Medical Sciences, Khorramabad, Iran

[4]Department of Biostatistics and Epidemiology, School of Health and Nutrition, Lorestan University of Medical Sciences, Khorramabad, Iran

[5]Department of Chemistry, Tabriz Branch, Islamic Azad University, Tabriz, Iran

[6]Cellular and Molecular Research Center, School of Medicine, Guilan University of Medical Sciences, Rasht, Iran

Correspondence
Shirzad Fallahi, Department of Medical Parasitology and Mycology, School of Medicine, Lorestan University of Medical Sciences, Khorramabad, Iran.
Email: Falahi.sh@lums.ac.ir

Nozhat Zebardast, Cellular and Molecular Research Center, School of Medicine, Guilan University of Medical Sciences, Rasht, Iran.
Email: nszebardast@gmail.com

Abstract

Background: *Cryptosporidium* spp. are opportunistic intestinal protozoans with global distribution and are of great importance as zoonotic protozoans are common to humans and domestic animals, including cattle and calves. Identification and detection of parasite species using precise methods including molecular methods can be an effective step in treating and controlling parasites.

Objectives: This study aimed to investigate the prevalence of *Cryptosporidium* among breeding calves of Khorramabad city, Lorestan province, Western Iran, using PCR.

Methods: The faecal samples were taken from 181 healthy and diarrhoeal calves and after the Ziehl Neelsen Acid-fast staining and microscopic evaluation, the genomic DNA was extracted for molecular evaluations. To detect *Cryptosporidium* species, specific primers targeting the SAM-1 gene of *Cryptosporidium* and a commercial master mix were used for PCR.

Results: Out of 181 faecal samples of breeding calves in Khorramabad city, 9 samples (5%) were positive for *Cryptosporidium* spp. using the PCR method. Statistical analysis of the data showed that there was no significant statistical relationship between *Cryptosporidium* infection of the calves and variables of age, breed, type of water consumption, clinical signs of diarrhoea, and sampling location, while parasite infection had a significant relationship with calf gender so that all *Cryptosporidium* positive samples were from male calves ($p \leq 0.05$).

Conclusions: Considering the presence of *Cryptosporidium* infection, the region's traditional grazing system, and the close relationship between livestock and humans, there is a possibility of human infection in the region. So preventive measures such as periodic animal testing with sensitive and accurate diagnostic techniques including PCR, pharmacological treatment of livestock, water hygiene and the use of industrial

Shirzad Fallahi and Nozhat Zebardast contributed equally to this work.

grazing instead of traditional grazing to improve the hygiene of food consumed by livestock are recommended.

KEYWORDS
breeding calves, *Cryptosporidium*, Iran, PCR, SAM-1 gene

1 | INTRODUCTION

Cryptosporidium, an obligate intracellular coccidian protozoan parasite of the phylum of *Apicomplexa*, is an essential intestinal pathogen with a wide distribution in young animals and humans (Sunnotel et al., 2006). *Cryptosporidium* is easily spread in the environment due to its simple transmission through contaminated water, air, and dust (Haghi et al., 2020). Cryptosporidiosis is a major economic problem in many countries, including Iran (Azami, 2007; Haghi et al., 2020; Hassanpour, 2008; Maleki & Nayebzadeh, 2008; Parsa, 2007). This infection has emerged as a major cause of diarrhoea worldwide and is a significant threat to young children and immunocompromised patients (Desai et al., 2012; Utami et al., 2020). There are numerous annual reports of this infection in children, immunocompromised and young (Desai et al., 2012). It is estimated that *Cryptosporidium* spp. are responsible for approximately 7.6 million cases and between 48,000 and 202,000 annual deaths among young children in low-resource environments (Haghi et al., 2020; Love & Choy, 2021; O'Leary et al., 2021). *Cryptosporidium* has the potential to cause water-borne epidemics and widespread outbreaks in developing and developed countries (Gururajan et al., 2021). The human-to-human and livestock routes of transmission are well defined, with food and water contaminated with faeces being a common source of infection (O'Leary et al., 2021).

The genus *Cryptosporidium* has different species that are found in a large number of domestic animals and humans. This protozoan has been associated with diarrhoea in calves, lambs, piglets, foals, puppies, kittens, and turkeys (Sadrebazzaz et al., 2021). Four species are capable of causing infection in cows: *C. parvum*, *C. bovis*, *C. andersoni* and *C. ryanae* (Wegayehu et al., 2016). *C. parvum* is one of the most common species in cattle and calves that can cause gastroenteritis and diarrhoea in humans. *C. andersoni* can be substituted in the abomasum of cattle and buffaloes, causing weight loss and reduced milk production in animals, creating a chronic degenerative state which is economically significant (Naghibi & Vahedi, 2002). The oocyst resistance of this parasite to environmental, physical and chemical factors makes it important for public health (Brownstein et al., 1977). Parasite-infected calves can contaminate their environment by excreting large amounts of oocysts in their faeces; because this protozoan is mainly spread through water (90% of the spread is through water and the rest through food) infection of calves can cause water pollution as a result of the transmission of the infection to human, and other animals (Chalmers & Katzer, 2013; Tyzzer, 1910; Tyzzer, 1912). The lack of effective treatment and resistance of parasite oocysts to common disinfectants has made this disease one of the major problems of industrial farms. More than half of newborn calves from broiler and dairy cows are at risk of being infected with *Cryptosporidium*, which in the first week of infection develops severe diarrhoea, dehydration, stunted growth, and sometimes death (Ungar, 2018). Due to the lack of sufficient information about different *Cryptosporidium* spp. in livestock as a reservoir and an important source of human infections, in the Lorestan province, Khorramabad city, Western Iran, which is one of the most important livestock hubs in the region, the present study aimed to molecularly investigate the *Cryptosporidium* species in breeding calves of this area.

2 | MATERIALS AND METHODS

2.1 | The study population and sampling

The target population of this cross-sectional study was farmed calves in Khorramabad township of Lorestan province, Western Iran, 2020. Considering that the nature of the study is mainly descriptive and based on the study of Maleki et al. study (151), the number of the initial sample was estimated at 151 samples, which considering the effect of the design was determined by 20% more, i.e., 181 samples. A classified cluster sampling method was used in the sampling process. The classes were north, centre, east, and South of Khorramabad Township, within each class, several livestock formed clusters. From each geographical class, 2 to 3 livestock were systematically selected then, on each farm, systematic random sampling was performed based on the livestock identification code.

2.2 | Microscopic evaluation

Stool samples collected from calves were transferred to the Parasitology Laboratory, Lorestan University, Khorramabad, West of Iran. A part of each sample was kept for microscopic examination of the wet mount stained by the Ziehl Neelsen Acid-fast staining method, and the other part was used for DNA extraction in a special container containing 2.5% potassium dichromate in a ratio of 1:1 at 4°C.

2.3 | Molecular assays

DNA extraction was performed from each stool sample after three washes with Phosphate-buffered saline (PBS) using a DNA

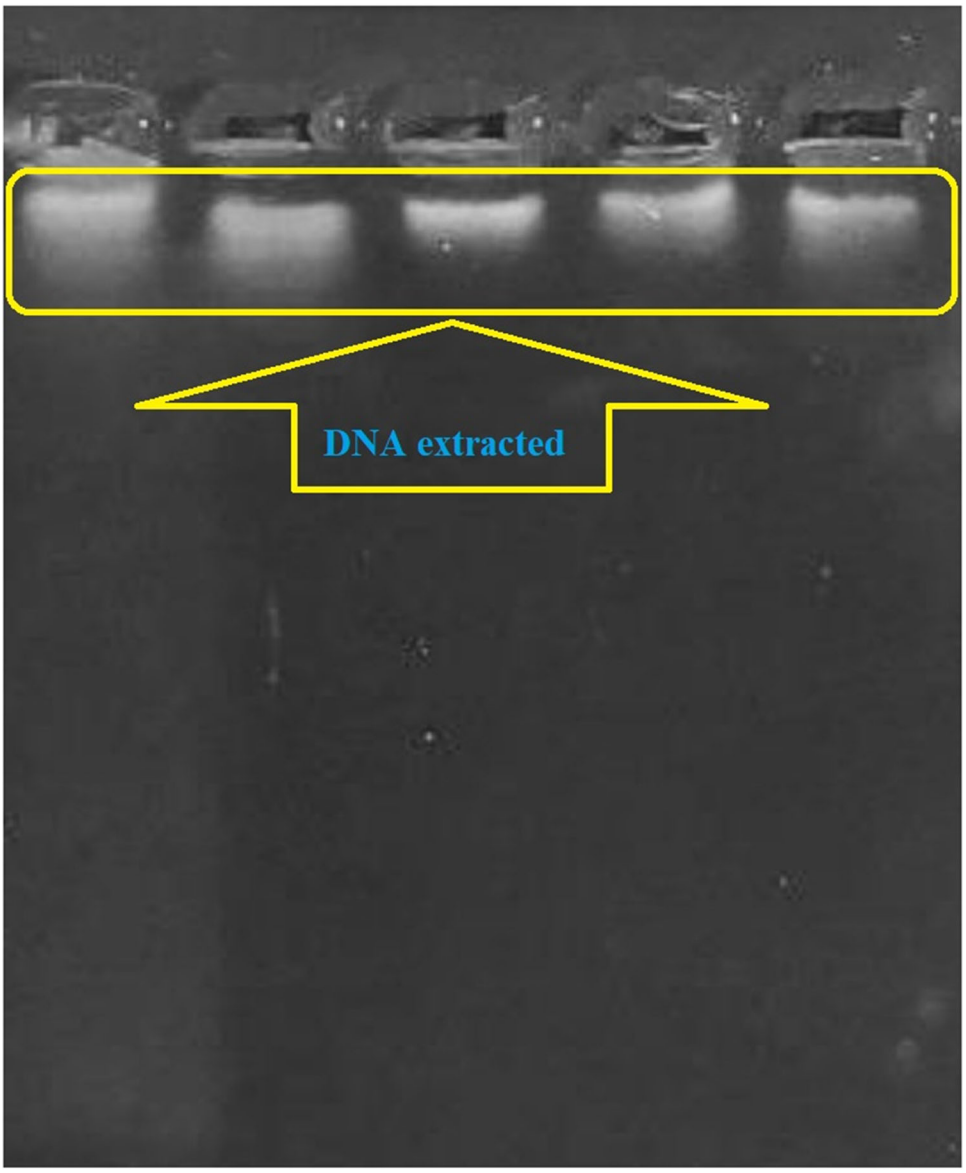

FIGURE 1 Evaluation of DNA quality on 2% agarose gel

TABLE 1 The sequences of primers in PCR from the S-adenosyl-l-methionine synthetase (SAM-1) gene of *Cryptosporidium spp*.

Primer type	Target gene	Sequence of primers
Forward	S-adenosyl-l-methionine synthetase (SAM-1)	5ť-ATTTGATRGACAAAGAAACTAG-3ť
Revers		5ť-CGATTGACTTTGCAACAAG-3ť

isolation stool mini kit (Yekta Tajhiz Azma Co., Iran) according to the manufacturer's protocol. DNA quality was measured by agarose gel electrophoresis (Figure 1) and its concentration was measured by Nanodrop spectrophotometry (NanoDrop 2000, Thermo). The extracted DNA was stored at −20°C until use. Single-cycle PCR amplification was performed on genomic DNA extracted to amplify a 200 bp fragment of *Cryptosporidium* S-adenosyl-l-methionine synthetase (SAM-1) gene using *Cryptosporidium* spp.-specific primers (Table 1) (Bakheit et al., 2008). PCR was performed using Amplicon (Taq DNA Polymerase Master Mix Red, Denmark) as the ready solution. The reaction mixture contained 9 μl of distilled water, 12.5 μl of the amplicon, 0.5 μl of forward and reverse primers (10 pm/l), 1 μl of magnesium chloride (mM), and 2 μl of the extracted DNA template (100 ng) to reach a final volume of 25 μl. Amplification of the DNA fragment began with an initial denaturation at 95°C for 5 min, followed by 35 cycles each including 95°C for 45 s, 54.2°C for 45 s, 72°C for 45 s, and a final step

extension at 72°C for 5 min. Amplified products were observed after electrophoresis on 2% agarose gel with safe stain staining (DNA Safe stain Sinaclon Co. IRAN).

2.4 | Statistical analysis

The data analysis was performed using SPSS software version 18. Results were reported using appropriate ratios and graphs. Frequency distribution tables were used to describe the data. A chi-square comparison test was used for correlation and Fisher's exact test and Mann–Whitey test were used if necessary. A *p*-value lower than 0.05 was considered statistically significant.

3 | RESULTS

3.1 | Demographic information of the studied animals

In the present study, 181 faecal samples were collected from breeding calves in Khorramabad, Western Iran, 2020. Most of the collected samples were from calves in the Imanabad region (23.20%, 42), male (66.9%, 121), hybrid breed (70.7%, 128), without clinical signs of diarrhoea (64.1%, 116) and consumed tap water (50.8%, 92).

3.2 | Microscopic evaluation

The microscopic examination of the wet mounts stained by the Ziehl Neelsen Acid-fast staining method revealed that out of 181 faecal samples collected from breeding calves, 3 samples (1.65%) were positive for *Cryptosporidium* spp. (data not shown).

3.3 | PCR

The forward primer combination with the reverse primer amplified the 200 bp PCR product. The results showed that out of 181 samples, 9 DNA samples (5%) were positive for *Cryptosporidium* species. PCR products were electrophoresed on 2% agarose gel are shown in Figure 2.

3.4 | Statistical analysis

Statistical analysis of the results using Mann–Whitey test showed that the age distribution of the studied calves was normal. Also, statistical analysis of data using the chi-square test with Monte Carlo simulation showed that there was no significant relationship between infection with *Cryptosporidium* species and the variable of sampling location, breed, type of water consumption, and clinical signs of diarrhoea in farmed calves ($p = 0.219$, $p = 0.134$, $p = 0.087$, respectively) (Table 2).

While statistical analysis of the data using the same test showed that there is a statistically significant relationship between infection with *Cryptosporidium* and the gender variable in the studied calves ($p = 0.031$). So that all 9 positive samples in terms of *Cryptosporidium* species infection were male calves (Table 2, Figure 3). Mann–Whitney test did not show a statistically significant relationship between infection with *Cryptosporidium* species and the age variable of farmed calves ($p = 0.439$) (Table 2).

4 | DISCUSSION

Up to now, Cryptosporidial infections have been reported in most countries of the world with urban (non-zoonotic) and rural (zoonotic) patterns. As previously mentioned, most vertebrates are infected with this parasite, which is especially common among domestic animals. Different studies conducted in Iran and around the world confirm the theory that domestic animals, especially livestock, are an important reservoir of infection for humans (Kiani et al., 2017). The rate of infection of different animals with this protozoan varies depending on the type of animal, clinical situation, and geographical distribution in the world. *Cryptosporidium* is the most common intestinal pathogen in young animals and the cause of ruminant diarrhoea syndrome, especially chronic diarrhoea of calves, which causes heavy economic losses to the livestock industry every year (Maleki & Nayebzadeh, 2007). According to review studies conducted in the world, *Cryptosporidium* has a high prevalence in farm animals, which is most important in cows with a prevalence of 29.1% compared to sheep at 24.4%, goats at 8.2%, pigs at 22.6%, horses at 7.4%, and buffalo 26% (Hatam-Nahavandi et al., 2019). Molecular diagnostic assays are more accurate than morphological diagnostic methods. The error rate in morphological methods is high, such as the structural similarity of different coccidia, which in most cases can cause the lack of differentiation between two different species. So, in some cases, cryptosporidium cannot be distinguished from yeasts, *Blastocystis* spp., and artefacts. On the other hand, morphological diagnostic methods are time-consuming, such as microscopic settings such as condenser adjustment, lens magnification, proper exposure and lens cleanliness. In the present study, the main goal was diagnosis using molecular methods. In this study, we first detected the parasite by Nelson's staining method and observation under the microscope, but for definitive diagnosis and final confirmation, we used the PCR method using specific primers.

In the present study, out of a total of 181 samples, 9 samples (5%) were positive for *Cryptosporidium* spp. In some cases, the present result is consistent with other studies conducted in Lorestan province, and it is not consistent in some cases. Numerous epidemiological factors such as age, gender, climatic and geographical factors, number of livestock per herd, and livestock care conditions play an important role in the prevalence of *Cryptosporidium* infection (Fayer et al., 2000). Despite the relatively high prevalence of *Cryptosporidium* species obtained in the present study (5%), considering the high potential of livestock contamination and the climatic conditions of the region, it seems that a higher

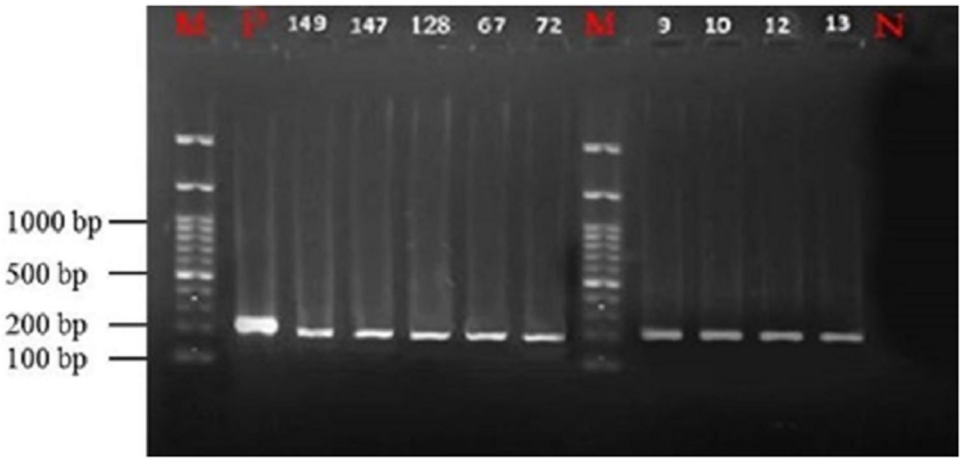

FIGURE 2 Electrophoresis of PCR products of faecal samples of calves studied on 2% agarose gel column M: 100 bp marker; column: P positive control; column N: negative control; columns 149-147-128-67-9-10-12-13: Positive samples of *Cryptosporidium* in the studied calves

TABLE 2 Frequency distribution of Cryptosporidium species infection in the studied calves using PCR technique based on demographic variables

			PCR					
			Positive		Negative			
No	Variable	Explanation	No.	%	No.	%	Total	*p* Value
1	Sampling location	Mehr Ali Khan	3	25.0%	9	75.0%	12 (100)	0.087
		Posht Meleh Rimaleh	1	14.3%	6	85.7%	7 (100)	
		Robatnamaki	0	0.0%	6	100.0%	6 (100)	
		Sarabchangaei	0	0.0%	15	100.0%	15 (100)	
		Kakasharaf	2	5.7%	33	94.3%	35 (100)	
		Sepiddasht	0	0.0%	8	100.0%	8 (100)	
		Bishehchenar	0	0.0%	8	100.0%	8 (100)	
		Bisheh	0	0.0%	9	100.0%	9 (100)	
		Cheshmeh Parian	0	0.0%	6	100.0%	6 (100)	
		Imanabad	2	4.8%	40	95.2%	42 (100)	
		Biran Shahr	1	25.0%	3	75.0%	4 (100)	
		Zagha-Kaka Reza	0	0.0%	14	100.0%	14 (100)	
		Taleghan	0	0.0%	15	100.0%	15 (100)	
2	Sex	Female	0	0.0%	60	100.0%	60 (100)	0.031
		Male	9	7.4%	112	92.6%	121 (100)	
3	Breed	Holstein	0	0.0%	6	100.0%	6 (100)	0.134
		Hybrid	9	7.0%	119	93.0%	128 (100)	
		Native	0	0.0%	47	100.0%	47 (100)	
4	Clinical symptoms	Diarrhoeal	2	3.1%	63	96.9%	65 (100)	0.493
		Healthy	7	6.0%	109	94.0%	116 (100)	
5	Type of consumed water	Tap water	7	7.6%	85	0.493	92 (100)	0.219
		Spring water	0	0.0%	31	100.0%	31 (100)	
		Unknown	2	3.4%	56	96.6%	58 (100)	
6	Age	Average (5 months)	4	5	5	2		0.439

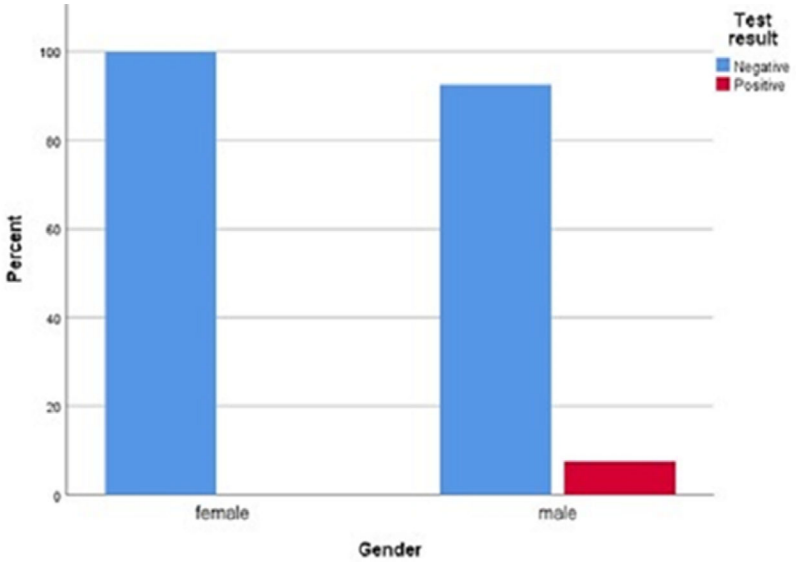

FIGURE 3 Distribution of frequency of *Cryptosporidium* species infection in the studied calves using PCR technique by gender

percentage of livestock infection with *Cryptosporidium* species may be obtained by testing more samples. This finding indicates the infection of Khorramabad township livestock with *Cryptosporidium* species and the potential to transmit the infection to humans and other animals. Due to the zoonotic nature of the infection, the traditional livestock system of the region, and the close relationship between livestock and humans, the chances of human infection in the region are very high. In the present study, there was no statistically significant relationship between cryptosporidiosis in the studied calves and variables of sampling location, breed and type of water consumed. This means that livestock in Khorramabad city in all areas of the city have the potential to be infected with *Cryptosporidium* species and the sampling area did not affect livestock infection. Studies conducted in Northwestern China, Ethiopia and France at 19 calf farms, ruminant farms and 26 animal farms, respectively, reported no statistically significant association between the prevalence of *Cryptosporidium* and animal habitat (Ayana & Alemu, 2015; Mammeri et al., 2019; Qi et al., 2015). Cryptosporidiosis is logically related to the type of cattle breeding, calf care, type of nutrition, drinking water, hygiene and livestock management. Also, in some studies conducted in Iran and other countries, a statistically significant relationship has been reported between the prevalence of *Cryptosporidium* and storage (Chen & Huang, 2012; Dalimi et al., 2015; Hamzavi, 2014; Zhang et al., 2013). The cause for the inconsistency is due to various reasons such as the geographical location of each region, the way of agriculture, livestock breeding and most importantly the movement of water from streams and contaminated beds following seasonal rains, which are effective factors in increasing the prevalence of *Cryptosporidium* and other intestinal parasites in the region (Gholami et al., 2012). As mentioned above, in the present study, different breeds of livestock were susceptible to one *Cryptosporidium* infection. Similar to the results of the current study, studies conducted in Iran and other parts of the world have not reported a statistically significant relationship between livestock breeds and cryptosporidiosis (Dalimi et al., 2015; Lombardelli et al., 2019; Mammeri et al., 2019; Noorani Kolije et al., 2020; Sannella et al., 2019). In contrast, some studies have reported a statistically significant relationship between the prevalence of *Cryptosporidium* and livestock breeds (pure, mixed, and native breeds) (Baghban & Moradimofrad, 2009; Qi et al., 2015). Given that innate immunity plays an important role in cryptosporidiosis infection, and most studies have been performed on mice, therefore, more studies are needed regarding cryptosporidiosis and breeding in other animals. Because most of the animals studied in the present study used tap water, the rate of cryptosporidiosis in these animals was higher due to the large number of samples. However, due to the possibility of water contamination, the rate of infection with *Cryptosporidium* species is expected to be higher in livestock that uses well and aqueduct water. Similar to the present study, several studies conducted in other parts of Iran did not report any statistically significant relationship between the prevalence of *Cryptosporidium* and water consumption (Asadi et al., 2014; Changizi et al., 2012). On the other hand, some studies in Iran and other parts of the world have reported a statistically significant relationship between the infection with *Cryptosporidium* and untreated pipe-borne drinking or recreational water (Dabirzadeh et al., 2017; Khan et al., 2019). Parasite protozoa are transmitted by water and food (Zahedi et al., 2016). Water pollution by domestic and wild animals and unsanitary disposal of human wastewater is the main way of water pollution (Zahedi et al., 2016). The small size of *Cryptosporidium* oocysts, its ability to pass through filtered water filters, as well as its resistance to commonly used disinfectants, its high environmental life, and its surface water and river water are reasons for the easy transfer of *Cryptosporidium* through contaminated water. Several studies in Iran and the world have reported water contamination with *Cryptosporidium* (Feng et al., 2007; Gallas-Lindemann et al., 2016; Koloren et al., 2013; Mahmoudi, 2020; Mahmoudi et al., 2013; Mohammadi Ghaleh Bin et al., 2007; Parva & Baharvand, 2018). The use of wastewater in agricultural irrigation and the transmission of contamination to livestock pastures and human pastures is a significant point that must be considered. Contrary to expectations, there was no statistically significant relationship between *Cryptosporidium* infection in calves and the clinical symptom variable of diarrhoea in the present

study. However, in most studies, the prevalence of Cryptosporidium infection in domains with clinical signs of diarrhoea has been reported higher (Asadpour et al., 2013; Avendaño et al., 2018; Baroudi et al., 2018; Khezri & Khezri, 2013; Lombardelli et al., 2019; Mammeri et al., 2019; Mirzai et al., 2014). Watery diarrhoea is one of the most common and visible clinical signs of *Cryptosporidium* infection in animals. *Cryptosporidium* species are zoonotic endoparasites that cause watery diarrhoea in humans, domestic, and wild animals by infecting small intestinal epithelial cells (Thomson et al., 2017). Acute diarrhoea is of particular economic importance to farm ruminants, which can be asymptomatic or associated with severe, fatal and high mortality. *Cryptosporidium* infection is often asymptomatic in domestic animals but in animals such as dogs, cats, horses and reptiles it can cause severe infections at the same time (Deming et al., 2008). In this study, the prevalence of infection in male livestock was significantly higher than in female livestock. This finding could indicate the possible sensitivity of male calves to infection. Statistical analysis of the results showed that the age distribution of the tested calves was normal. *Cryptosporidium* is 13 times more common in young animals, especially calves under 4 months of age, compared to older ages. Aging in livestock makes them more resistant to disease (Thomson et al., 2017). The protozoan has been identified in calves in the first 24 to 72 h of birth (postpartum infection and environmental contamination) (Thomson, 2016), and 7- to 5-day-old calves (Tzipori et al., 1983). *Cryptosporidium* species are infected according to the age of the animal, *C. parvum* is found in dairy calves, *C. bovis* and *C. ryanae* in calves older than 2 months, and *C. andersoni* in adult cows without clinical signs. An age-related distribution of *Cryptosporidium* species has been reported in cattle, with *C. parvum* being predominant in suckling calves, *C. bovis* and *C. ryanae* being predominant in postweaning calves and *C. andersoni* being predominant in adults. However, variants of this pattern have recently been reported (Díaz et al., 2021). Some studies have not reported any statistically significant relationship between age and cryptosporidiosis (Ayana & Alemu, 2015; Keyvanloo Shahrestanakey et al., 2017; Qi et al., 2015). Several studies in Iran have reported the highest rates of *Cryptosporidium* infection in calves less than 6 months (Mirzai et al., 2014), under 30 days (Asadpour et al., 2013), 6–8 weeks (Normohamadzadeh et al., 2010) and cows under 1 year (Heidari and Gharakhani, 2012). In other parts of the world, cryptosporidiosis has also been reported in calves: from 50 to 41 days (Mammeri et al., 2019), less than 45 days, 14–8 days (Avendaño et al., 2018), lambs less than 11 days (Mammeri et al., 2019; Mammeri et al., 2019) and less than 2 weeks (Baroudi et al., 2018). Age is one of the most important factors in the occurrence of cryptosporidiosis in animals. Of course, other factors such as livestock management, nutrition and animal husbandry conditions also have a direct impact (Thomson, 2016).

5 | CONCLUSION

Based on the findings of the present study, it can be concluded that farmed calves in Khorramabad township have the potential for infection with *Cryptosporidium* species and can play an important role in spreading the infection to the environment and ultimately transmitting the infection to humans. Preventive measures such as periodic animal testing with sensitive and accurate diagnostic techniques including PCR, pharmacological treatment of livestock, water hygiene and the use of industrial grazing instead of traditional grazing to improve the hygiene of food consumed by livestock are recommended.

ACKNOWLEDGEMENTS

We hereby acknowledge all colleagues who participated in the collection of animal feces samples.

AUTHOR CONTRIBUTIONS

Razieh Talebi: conceptualisation; methodology; writing – review & editing. Amirreza Javadi Mamaghani: conceptualisation; investigation; writing – review & editing. Farnaz Kheirandish: conceptualisation; data curation; investigation; validation; writing – review & editing. Azadeh Karimi: data curation; investigation. Farzad Ebrahimzadeh: formal analysis; methodology. Mohamad Kazempour: investigation. Nozhat Zebardast: conceptualisation; data curation; investigation; writing – original draft. Shirzad Fallahi: conceptualisation; investigation; project administration; resources; supervision; validation, writing – review & editing.

FUNDING

This study was extracted from a master's thesis and was supported financially by Vice Chancelor for Research and Technology, Lorestan University of Medical Sciences, Khorramabad, Iran.

CONFLICT OF INTEREST

On behalf of all authors, the corresponding author states that there is no conflict of interest or competing interests.

ETHICS STATEMENT

Ethical approval was received from the Ethics Committee of Lorestan University of Medical Sciences (Ethical clearance for research was approved by the Medical Ethics Committee of Lorestan University of Medical Sciences) (IR.GUMS.REC.1396.40). All procedures were performed in accordance with relevant guidelines. In this study, written informed consent was obtained from farm animal owners to enter the farm and use clinical animal samples.

DATA AVAILABILITY STATEMENT

The data sets generated for this study are available on request to the corresponding author.

ORCID

Nozhat Zebardast https://orcid.org/0000-0003-3791-9312
Shirzad Fallahi https://orcid.org/0000-0003-1826-7910

PEER REVIEW

The peer review history for this article is available at https://publons.com/publon/10.1002/vms3.1024.

REFERENCES

Asadi, M., Sedighi, I., Fallah, M., Saidijam, M., & Maghsood, A. (2014). A survey study of Cryptosporidium infection in children under 10 years old referred to the health care centers of Hamadan district in 2013. *Avicenna Journal of Clinical Medicine*, *21*(3), 211–217.

Asadpour, M., Razmi, G., Mohhammadi, G., & Naghibi, A. (2013). Prevalence and molecular identification of Cryptosporidium spp. in pre-weaned dairy calves in Mashhad area, Khorasan Razavi province, Iran. *Iranian Journal of Parasitology*, *8*(4), 601–607.

Avendaño, C., Ramo, A., Vergara-Castiblanco, C., SÃąNchez-Acedo, C., & Quã-Lez, J. -N. (2018). Genetic uniqueness of *Cryptosporidium parvum* from dairy calves in Colombia. *Parasitology research*, *117*(5), 1317–1323.

Ayana, D., & Alemu, B. (2015). Cryptosporidiosis in calves, lambs and goat kids in Bishoftu, Oromia Regional State, Ethiopia. *African Journal of Basic & Applied Sciences*, *7*(5), 233–239.

Azami, M. (2007). Prevalence of Cryptosporidium infection in cattle in Isfahan, Iran. *Journal of Eukaryotic Microbiology*, *54*(1), 100–102.

Baghban, F., & Moradimofrad, M. (2009). The survey of the abundance of Cryptosporidium spp. oocysts in diarrhoeic calves in Yasuj city. *Clinical Research of Large Animals*, *3*, 17–22.

Bakheit, M. A., Torra, D., Palomino, L. A., Thekisoe, O. M. M., Mbati, P. A., Ongerth, J., & Karanis, P. (2008). Sensitive and specific detection of *Cryptosporidium* species in PCR-negative samples by loop-mediated isothermal DNA amplification and confirmation of generated LAMP products by sequencing. *Veterinary Parasitology*, *158*(1–2), 11–22.

Baroudi, D., Hakem, A., Adamu, H., Amer, S., Khelef, D., Adjou, K., Dahmani, H., Chen, X., Roellig, D., Feng, Y., & Xiao, L. (2018). Zoonotic *Cryptosporidium* species and subtypes in lambs and goat kids in Algeria. *Parasites & Vectors*, *11*(1), 1–8.

Brownstein, D., Strandberg, J. D., Montali, R. J., Bush, M., & Fortner, J. (1977). *Cryptosporidium* in snakes with hypertrophic gastritis. *Veterinary Pathology*, *14*(6), 606–617.

Chalmers, R. M., & Katzer, F. (2013). Looking for *Cryptosporidium*: The application of advances in detection and diagnosis. *Trends in Parasitology*, *29*(5), 237–251.

Changizi, E., Salimi-Bejestani, M. R., & Vayeghan, A. J. (2012). The *Cryptosporidium ryanae* infection commence in Iranian cattle. *Journal of Veterinary Research*, *67*(2), 127–133.

Chen, F., & Huang, K. (2012). Prevalence and molecular characterization of *Cryptosporidium* spp. in dairy cattle from farms in China. *Journal of Veterinary Science*, *13*(1), 15–22.

Dabirzadeh, M., Rostami, D., & Bagheri, S. (2017). Prevalence of *Cryptosporidium* species in children referred to central and hospital laboratories of Zabol city, south east of Iran. *International Journal of Pediatrics*, *5*(12), 6359–6364.

Dalimi, A., Tahvildar, F., & Kazemi, B. (2015). Molecular identification of Cryptosporidium andersoni in Shahriar calves. *Veterinary Research and Biological Products (Research and Development)*, *28*, 24–30.

Deming, C., Greiner, E., & Uhl, E. W. (2008). Prevalence of *Cryptosporidium* infection and characteristics of oocyst shedding in a breeding colony of leopard geckos (*Eublepharis macularius*). *Journal of Zoo and Wildlife Medicine*, *39*(4), 600–607.

Desai, N. T., Sarkar, R., & Kang, G. (2012). Cryptosporidiosis: An underrecognized public health problem. *Tropical Parasitology*, *2*(2), 91.

Díaz, P., Navarro, E., Remesar, S., García-Dios, D., Martínez-Calabuig, N., Prieto, A., López-Lorenzo, G., López, C. M., Panadero, R., Fernández, G., Díez-Baños, P., & Morrondo, P. (2021). The age-related *Cryptosporidium* species distribution in asymptomatic cattle from North-Western Spain. *Animals*, *11*(2), 256.

Fayer, R., Morgan, U., & Upton, S. J. (2000). Epidemiology of *Cryptosporidium*: Transmission, detection and identification. *International Journal for Parasitology*, *30*(12-13), 1305–1322.

Feng, Y., Alderisio, K. A., Yang, W., Blancero, L. A., Kuhne, W. G., Nadareski, C. A., Reid, M., & Xiao, L. (2007). *Cryptosporidium* genotypes in wildlife from a New York watershed. *Applied and Environmental Microbiology*, *73*(20), 6475–6483.

Gallas-Lindemann, C., Sotiriadou, I., Plutzer, J., Noack, M. J., Mahmoudi, M. R., & Karanis, P. (2016). Giardia and *Cryptosporidium* spp. dissemination during wastewater treatment and comparative detection via immunofluorescence assay (IFA), nested polymerase chain reaction (nested PCR) and loop mediated isothermal amplification (LAMP). *Acta Tropica*, *158*, 43–51.

Gholami, S., Ali Aliasghar, H., Ali Reza, K., Mahdi, F., Ahmad, D., Mahdi, S., Shahban, G., & Ahsan, A. (2012). The frequency of cryptosporidiosis among gastroenteritic patients referred to Mazandaran University of Medical Science Hospitals, during 2010–2011. *Journal of Mazandaran University of Medical Sciences*, *21*, 263–272.

Gururajan, A., Rajkumari, N., Devi, U., & Borah, P. (2021). *Cryptosporidium* and waterborne outbreaks – A mini review. *Tropical Parasitology*, *11*(1), 11–15.

Haghi, M. M., Khorshidvand, Z., Khazaei, S., Foroughi-Parvar, F., Sarmadian, H., Barati, N., Etemadifar, F., & Ghasemikhah, R. (2020). *Cryptosporidium* animal species in Iran: A systematic review and meta-analysis. *Tropical Medicine and Health*, *48*(1), 1–15.

Hamzavi, Y. (2014). Cryptosporidiosis in children with and without diarrhea in Kermanshah from 2011–12. *Journal of Clinical Research in Paramedical Sciences*, *3*(1), e82087.

Hassanpour, A. (2008). Prevalence of cryptosporidiosis in calves and humans to be in contact them in Tabriz area in Iran. *International Journal of Infectious Diseases*, *12*, e126.

Hatam-Nahavandi, K., Ahmadpour, E., Carmena, D., Spotin, A., Bangoura, B., & Xiao, L. (2019). *Cryptosporidium* infections in terrestrial ungulates with focus on livestock: A systematic review and meta-analysis. *Parasites & Vectors*, *12*(1), 1–23.

Heidari, H., & Gharakhani, J. (2012). Study of *Cryptosporidium* infection in the livestock (cattle, sheep, dogs, fowls) and humans, in Hamadan city and its suburbs during 2006–2011. *Avicenna Journal of Clinical Medicine*, *19*(3), 67–74.

Keyvanloo Shahrestanakey, R., Taghavi Razavizadeh, A., & Ramzi, G. (2017). A survey on *Cryptosporidium* spp. infection in lambs with and without clinical signs of diarrhea in Jovein area. *Veterinary Clinical Pathology The Quarterly Scientific Journal*, *11*(43), 233–241.

Khan, A., Shams, S., Khan, S., Khan, M. I., Khan, S., & Ali, A. (2019). Evaluation of prevalence and risk factors associated with *Cryptosporidium* infection in rural population of district Buner, Pakistan. *PLoS One*, *14*(1), e0209188.

Khezri, M., & Khezri, O. (2013). The prevalence of *Cryptosporidium* spp. in lambs and goat kids in Kurdistan, Iran. *Veterinary World*, *6*(12), 974–977.

Kiani, H., Haghighi, A., Seyyedtabaei, S. J., Azargashb, E., Zebardast, N., Taghipour, N., Rostami, A., & Xiao, L. (2017). Prevalence, clinical manifestations and genotyping of *Cryptosporidium* spp. in patients with gastrointestinal illnesses in Western Iran. *Iranian Journal of Parasitology*, *12*(2), 169–176.

Koloren, Z., Kaya, D., & Avsar, C. (2013). Detection of *Cryptosporidium* species in the sea and tap water samples of Black Sea, Turkey. *The Journal of parasitology*, *99*(3), 554–557.

Lombardelli, J. A., Tomazic, M. L., Schnittger, L., & Tiranti, K. I. (2019). Prevalence of *Cryptosporidium parvum* in dairy calves and GP60 subtyping of diarrheic calves in central Argentina. *Parasitology Research*, *118*(7), 2079–2086.

Love, M. S., & Choy, R. K. (2021). Emerging treatment options for cryptosporidiosis. *Current Opinion in Infectious Diseases*, *34*(5), 455.

Mahmoudi, M. R. (2020). Molecular detection and characterization of *Cryptosporidium* spp. in the sewage-contaminated rivers entering Bandar-e Anzali Lagoon in Guilan Province, Iran. *Journal of Advances in Environmental Health Research*, *8*(2), 102–106.

Mahmoudi, M. - R., Kazemi, B., Mohammadiha, A., Mirzaei, A., & Karanis, P. (2013). Detection of *Cryptosporidium* and *Giardia* (oo) cysts by IFA, PCR

and LAMP in surface water from Rasht, Iran. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, *107*(8), 511–517.

Maleki, S., & Nayebzadeh, H. (2007). A survey on prevalence rate of cryptosporidiosis among diarrheic and healty cattle and calves in Khoram-Abad, Iran. *Journal of Veterinary Research*, *62*(4), 423–426.

Maleki, S., & Nayebzadeh, H. (2008). A survey on the prevalence rate of cryptosporidiosis among diarrheic and healthy cattle and calves in Khoram-Abad, Iran. Veterinary Research Journal, *62*, 43–426.

Mammeri, M., Cartou, L., Chevillot, A., Thomas, M., Julien, C., Vallée, I., Polack, B., Follet, J., & Adjou, K. T. (2019). First identification of *Cryptosporidium parvum* zoonotic subtype IIaA15G2R1 in diarrheal lambs in France. *Veterinary Parasitology: Regional Studies and Reports*, *18*, 100355.

Mammeri, M., Chevillot, A., Chenafi, I., Thomas, M., Julien, C., Vallée, I., Polack, B., Follet, J., & Adjou, K. T. (2019). Molecular characterization of *Cryptosporidium* isolates from diarrheal dairy calves in France. *Veterinary Parasitology: Regional Studies and Reports*, *18*, 100323.

Mirzai, Y., Yakhchali, M., & Mardani, K. (2014). *Cryptosporidium parvum* and *Cryptosporidium andersoni* infection in naturally infected cattle of northwest Iran. In *Veterinary research forum: An international quarterly journal*. Faculty of Veterinary Medicine, Urmia University, Urmia, Iran.

Mohammadi Ghaleh Bin, B., Fallah, E., Asgharzadeh, M., & Kazemi, A. (2007). Detection of *Cryptosporidium* species in water resources of Ardabil province by PCR, RFLP. *Journal of Ardabil University of Medical Sciences*, *7*(24), 177–183.

Naghibi, A., & Vahedi, H. (2002). Prevalence of cryptosporidial infection in horse and man in Mashhad, Iran. *Archives of Razi Institute*, *54*(1), 101–106.

Noorani Kolije, O., Yakhchali, M., & Malekifard, F. (2020). Study on *Cryptosporidium* Infection in cattle and water buffaloes of farms in Mahabad Suburb, Iran. *Journal of Veterinary Research*, *75*(2), 147–155.

Normohamadzadeh, F., Davoodi, Y., Jamali, R., & Nowrouzian, I. (2010). Epidemiological study on cryptosporidiosis in newborn calves in eastern Azarbaijan Province. *Journal of Veterinary Research*, *65*(3), .

O'Leary, J. K., Sleator, R. D., & Lucey, B. (2021). *Cryptosporidium* spp. diagnosis and research in the 21st century. *Food and Waterborne Parasitology*, *24*, e00131.

Parsa, A. (2007). Prevalence of Cryptosporidium muris–like (andersoni) infection in cattle and buffalo slaughtered in Tabriz area Abattoir. *Clinical Research of Large Animals*, *1*, 49–55.

Parva, E. B., & Baharvand, P. (2018). Parasitic contamination of drinking water wells in Rumeshgan, Lorestan province. *International Journal of Pharmaceutical Research*, *10*(3), .

Qi, M., Fang, Y. Q., Wang, X. T., Zhang, L. X., Wang, R. J., Du, S. Z., Guo, Y. X., Jia, Y. Q., Yao, L., Liu, Q. D., & Zhao, G. H. (2015). Molecular characterization of *Cryptosporidium* spp. in pre-weaned calves in Shaanxi Province, northwestern China. *Journal of Medical Microbiology*, *64*(1), 111–116.

Sadrebazzaz, A., Hoseinioun, H., Yousesfi, M., & Noaman, V. (2021). Evaluation of *Cryptosporidium* spp. infection in cattle in industrial farms in North Khorasan Province, Iran. *Veterinary Researches & Biological Products*, *34*(3), 62–69.

Sannella, A. R., Suputtamongkol, Y., Wongsawat, E., & Cacciò, S. M. (2019). A retrospective molecular study of *Cryptosporidium* species and genotypes in HIV-infected patients from Thailand. *Parasites & Vectors*, *12*(1), 1–6.

Sunnotel, O., Lowery, C. J., Moore, J. E., Dooley, J. S. G., Xiao, L., Millar, B. C., Rooney, P. J., & Snelling, W. J. (2006). Cryptosporidium. *Letters in Applied Microbiology*, *43*(1), 7–16.

Thomson, S. (2016). *Cryptosporidiosis in farm livestock*. University of Glasgow.

Thomson, S., Hamilton, C. A., Hope, J. C., Katzer, F., Mabbott, N. A., Morrison, L. J., & Innes, E. A. (2017). Bovine cryptosporidiosis: Impact, host-parasite interaction and control strategies. *Veterinary Research*, *48*(1), 1–16.

Tyzzer, E. (1912). *Cryptosporidium parvum*, a coccidiuin found in the small intestine of the common mouse. *Arch. Protistenked*, *26*, 394–412.

Tyzzer, E. E. (1910). An extracellular coccidium, *Cryptosporidium muris* (gen. et sp. nov.), of the gastric glands of the common mouse. *The Journal of Medical Research*, *23*(3), 487.

Tzipori, S., Smith, M., Halpin, C., Angus, K., Sherwood, D., & Campbell, I. (1983). Experimental cryptosporidiosis in calves: Clinical manifestations and pathological findings. *The Veterinary Record*, *112*(6), 116–120.

Ungar, B. L. (2018). Cryptosporidiosis in humans (Homo sapiens). In J. P. Dubey (Ed.), *Cryptosporidiosis of man and animals* (pp. 59–82). CRC Press.

Utami, W. S., Murhandarwati, E. H., Artama, W. T., & Kusnanto, H. (2020). *Cryptosporidium* infection increases the risk for chronic diarrhea among people living with HIV in Southeast Asia: A systematic review and meta-analysis. *Asia Pacific Journal of Public Health*, *32*(1), 8–18.

Wegayehu, T., Karim, R., Anberber, M., Adamu, H., Erko, B., Zhang, L., & Tilahun, G. (2016). Prevalence and genetic characterization of *Cryptosporidium* species in dairy calves in Central Ethiopia. *PLoS One*, *11*(5), e0154647.

Zahedi, A., Paparini, A., Jian, F., Robertson, I., & Ryan, U. (2016). Public health significance of zoonotic *Cryptosporidium* species in wildlife: Critical insights into better drinking water management. *International Journal for Parasitology: Parasites and Wildlife*, *5*(1), 88–109.

Zhang, W., Wang, R., Yang, F., Zhang, L., Cao, J., Zhang, X., Ling, H., Liu, A., & Shen, Y. (2013). Distribution and genetic characterizations of *Cryptosporidium* spp. in pre-weaned dairy calves in Northeastern China's Heilongjiang Province. *PLoS One*, *8*(1), e54857.

How to cite this article: Talebi, R., Javadi Mamaghani, A., Kheirandish, F., Karimi, A., Ebrahimzadeh, F., Kazempour, M., Zebardast, N., & Fallahi, S. (2022). Molecular evaluation of *Cryptosporidium* spp. among breeding calves of Lorestan province Western Iran. *Veterinary Medicine and Science*, 1–9. https://doi.org/10.1002/vms3.1024