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Research article
Ερευνητικό άρθρο

Evaluation of Risk Factors and Diagnostic Methods for *Cryptosporidium parvum* in Neonatal Dairy Calves in Iran

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ABSTRACT: *Cryptosporidium* is a protozoan parasite that affects a wide range of hosts, including humans and animals. This study aimed to evaluate risk factor assessment and compare diagnostic methods for detecting *Cryptosporidium parvum* in neonatal Holstein calves across various farms in Iran. A total of 301 fecal samples were collected from 23 dairy farms located in six provinces. Samples were examined using immunochromatography (ICG), the modified Ziehl-Neelsen (MZN) staining method, and species-specific PCR assays. The ICG technique was performed immediately after sample collection, while the remaining samples underwent laboratory processing. The prevalence of *Cryptosporidium parvum* was 50.5% and 45.85% based on the MZN and ICG techniques, respectively. PCR analysis revealed a 70% positivity rate among 50 randomly selected samples. Infection rates were higher in diarrheic calves compared to non-diarrheic ones, with rates of 39.52% versus 8.55% by MZN and 41.9% versus 7.6% by ICG. Key factors influencing infection included annual rainfall, hutch hygiene, fecal consistency, and calf-cow contact. While the ICG method showed acceptable diagnostic performance in diarrheic calves, its accuracy was lower for detecting infections in non-diarrheic calves. The high prevalence of *Cryptosporidium parvum* among neonatal calves in Iran highlights the need for improved diagnostic and preventive measures. The ICG method can be a practical diagnostic tool but is less effective in identifying subclinical infections in non-diarrheic calves.

Keyword: Cryptosporidiosis; Calf; Diarrhea; Immunochromatography; PCR

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INTRODUCTION

Cryptosporidium is a protozoan parasite from the phylum Apicomplexa, capable of infecting a wide range of hosts, including mammals, birds, and fish (Santin, 2020; Kumar Dhal et al., 2022). Among its many species, *Cryptosporidium parvum* is predominant in pre-weaned calves and poses significant zoonotic and economic concerns (Cai et al., 2017). This parasite's ability to cause diarrhea and growth retardation in neonatal calves underscores its impact on livestock production.

Despite its clinical relevance, the role of *C. parvum* in healthy versus diarrheic calves remains contentious. While commonly associated with diarrhea, the parasite has been detected in asymptomatic animals, suggesting its pathogenicity may depend on host factors and co-infections (De Graaf et al., 1999; Icen et al., 2013). Moreover, outbreaks linked to *Cryptosporidium* have emphasized its importance in public health, particularly due to waterborne and foodborne transmission routes (Ryan et al., 2018).

In calves, cryptosporidiosis presents as diarrhea, dehydration, and anorexia, with clinical severity influenced by immune status and environmental factors (Castro-Hermida et al., 2002a). Infected calves shed oocysts into the environment, contaminating water and soil, which exacerbates transmission risks. Identifying and managing risk factors such as hygiene, calf-cow interactions, and climatic conditions are crucial for controlling the spread of this disease.

Diagnostic approaches for cryptosporidiosis vary in accuracy and feasibility. Microscopy using the modified Ziehl-Neelsen (MZN) method remains the gold standard but requires time and expertise. Rapid diagnostic tests like immunochromatography (ICG) offer field applicability but may lack sensitivity for subclinical cases. Molecular techniques, particularly PCR assays, provide high specificity and are invaluable for epidemiological studies (Mahmoudi et al., 2020; Bairami et al., 2018).

Despite prior studies on cryptosporidiosis in Iran, comprehensive investigations into its prevalence, associated risk factors, and diagnostic efficacy remain scarce. This study addresses these gaps by evaluating *C. parvum* infection in neonatal Holstein calves using three diagnostic methods and assessing the impact of environmental and management factors on infection rates. Therefore, the objective of this study was to comprehensively assess the prevalence of Cryptosporidium parvum in neonatal Holstein calves across different geographical regions in Iran, identify and analyze potential intrinsic and extrinsic risk factors associated with infection, and evaluate the diagnostic performance of three commonly used techniques—Modified Ziehl-Neelsen (MZN) staining, immunochromatography (ICG), and species-specific PCR. By integrating epidemiological, environmental, and diagnostic perspectives, this study aimed to provide a holistic understanding of C. parvum infection dynamics to inform more effective surveillance and control strategies.

MATERIALS AND METHODS

Ethical Statement, Study Area, and Sample Size

All procedures conducted in this study adhered to the ethical guidelines outlined by ARRIVE 2.0 (Percie du Sert et al., 2020) and were approved by the Shahid Chamran University Ethics Committee (Protocol No. 94/3/24/60865). Sample collection was carried out by licensed veterinarians following national regulations on animal welfare.

The study was performed across 23 industrial dairy farms in six provinces of Iran, chosen to represent diverse climatic and geographical conditions. These farms were selected based on their accessibility and the lack of prior research on *Cryptosporidium* prevalence in these areas. Sampling occurred between October 2015 and February 2016, covering both dry and wet seasons to account for potential environmental influences on infection rates.

To determine the required sample size, the formula for estimating proportions in a finite population was employed. Based on an expected prevalence of 23% (Mirzai et al., 2014), an absolute error of 5%, and a confidence level of 95%, a minimum of 272 calves were needed. Ultimately, fecal samples were collected from 301 calves to ensure robust statistical power.

Sampling and Definition of Variables

Calves aged 1 to 30 days were included in the study, with fecal samples collected directly from the rectum using sterile equipment. Each sample was divided into two portions: one for immediate analysis using the ICG method and the other for laboratory-based tests, including MZN staining and PCR.

Clinical examinations were conducted to classify calves as diarrheic or non-diarrheic based on fecal consistency scores. Diarrhea was defined as a fecal score of 3 or above, following established guidelines

(Larson et al., 1977). Additional data on intrinsic factors (e.g., calf age and sex) and extrinsic factors (e.g., herd size, hutch hygiene, contact with mature cows, and environmental conditions) were recorded.

Environmental variables, such as mean annual rainfall, ambient temperature, and relative humidity, were sourced from local meteorological data. Management practices, including disinfection protocols and feeding regimes, were documented through farm surveys.

Immunochromatographic Technique

A small amount of fecal sample was collected directly from the rectum using the stopper plug and then added to the diluent buffer in the provided sample tube. In cases where the feces were solid prior to analysis, any excess material was removed with a spatula. The sample dilution buffer was thoroughly mixed to ensure a uniform stool suspension. The tube was then tapped on a flat surface to eliminate air bubbles before being placed into a strip tube and securely sealed. The device was set upright, and the results were read after 10 minutes. Diagnostic interpretation of the ICG RAINBOW calf scours kit (BIO K 288, BIO-X Diagnostics, Rochefort, Belgium) was carried out according to the manufacturer's guidelines. A visible control band confirmed a valid test result. In the test line, the yellow, blue, red, and green strips correspond to Rotavirus, Coronavirus, Escherichia coli attachment factor K99, and *Cryptosporidium parvum*, respectively (Hamedian-Asl et al., 2022).

Modified Ziehl–Neelsen (MZN) Method

Fecal samples were collected directly from the rectum of calves into clean plastic cups and subsequently analyzed using the concentration flotation technique with sugar, as described by Georgi and Georgi (1990). Smears were prepared, allowed to dry, fixed in methanol, and stained according to the Modified Ziehl–Neelsen (MZN) method (Henriksen and Pohlenz, 1981). The detection and identification of *Cryptosporidium* were performed under a light microscope (Figure 1). Morphologically, *C. parvum* oocysts (5.0 × 4.5 μm) were distinguished from *C. andersoni* oocysts (7.6 × 5.6 μm) based on their size (Soulsby, 1982).

DNA Extraction

Slide preparations were first washed with PBS, then subjected to centrifugation at 10,000 ×g for 5 minutes (repeated three times) to remove any residual stains. The pellet was resuspended in 250 μL of TE-SDS buffer (Tris-HCl 10 mM, EDTA 1 mM, SDS 1%). The oocyst suspension underwent seven freeze-thaw cycles using liquid nitrogen and a water bath at 80–95 °C. Next, 50 μL of proteinase K (Cianneg, Tehran) was added, and the mixture was incubated overnight at 60–65 °C in a water bath. DNA was extracted using the Genomic DNA isolation kit (Denazist Asia, Tehran, Iran) according to the manufacturer's protocol. The concentration of purified DNA was determined at 260 nm and 280 nm using a Nanodrop Lite spectrophotometer (Thermo Scientific, Waltham, USA). The extracted DNA was stored at -20 °C until further analysis. For PCR amplification, the following primer sequences were used (5'-3'): TAAACGGTAGGGTATTGGCCT and CAGACTTGCCCTCCAATTGATA, which amplify a 240 bp fragment of the rRNA (18S) gene (Bairami et al., 2018). The final PCR product was visualized on a 1.2% agarose gel under UV light.

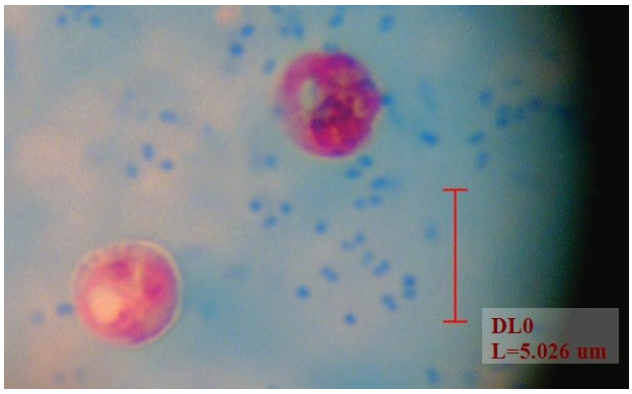

Figure 1. *Cryptosporidium parvum* in feces sample of diarrohic calf (MZN stain).

PCR Detection Setup

The PCR reaction was prepared in a total volume of 25 μL, containing 12.5 μL of master mix (Amplicon, Denmark), 8.5 μL of DNA-free Milli-Q water, 20 pmol/μL of each primer, and 1 μL of DNA. The PCR cycling conditions were as follows: initial denaturation at 94 °C for 5 minutes, followed by 30 amplification cycles consisting of 94 °C for 30 seconds, 55 °C for 1.5 minutes, and 72 °C for 1.5 minutes, with a final extension at 72 °C for 5 minutes. A 100 bp DNA ladder (Cinaclon, Tehran) was used as the size marker.

Statistical Analysis

The apparent prevalence for each diagnostic method was calculated by dividing the number of positive animals by the total number of animals included in the study. The differences in the proportion of fecal

samples testing positive or negative for *C. parvum* were assessed using the chi-square and Fisher's exact tests, with a 95% confidence level. Both intrinsic factors (age and sex) and extrinsic factors (farm size, location, sanitation practices, calf-cow interaction, colostrum management, milk pasteurization, environmental conditions such as temperature, rainfall, and relative humidity) were considered as potential risk factors. Variables with a p-value ≤ 0.2 in univariate analysis were further analyzed using logistic regression (Hosmer and Lemeshow, 2000) with the MedCalc software (version 19.2; MedCalc Software Ltd., Ostend, Belgium) to explore the relationships between independent and dependent variables. The fit of the final model was confirmed using the Hosmer and Lemeshow test, and potential collinearity between independent variables was evaluated through correlation analysis. Significance for both univariate and multivariate tests was set at $p < 0.05$.

For method comparison, the minimum sample size recommended was 40 subjects from the target population (Jensen and Kjelgaard-Hansen, 2006). To compare the performance of the ICG (rapid test) with that of the MZN (standard method), results were categorized as positive or negative for *Cryptosporidium* spp. The diagnostic accuracy of the ICG was evaluated against MZN using receiver operating characteristic (ROC) curves, McNemar's test, inter-rater agreement (Kappa), and concordance correlation coefficient (CCC) tests (Thrusfield, 2005). Concordance correlation was interpreted using McBride's (2005) guidelines as poor (<0.90), moderate (0.90–0.95), substantial (0.95–0.99), and perfect (>0.99). Additionally, the precision and bias of each method were compared to PCR results. The agreement between the three methods (MZN, ICG, and PCR) in diagnosing *Cryptosporidium parvum* was evaluated using the κ statistic (Thrusfield, 2005). Cohen's Kappa (κ) was calculated to assess the degree of agreement between each diagnostic method and the gold standard. Agreement levels were interpreted as follows: $\kappa < 0.2$ for poor agreement, $0.2 < \kappa \leq 0.4$ for fair agreement, $0.4 < \kappa \leq 0.6$ for moderate agreement, $0.6 < \kappa \leq 0.8$ for substantial agreement, and $\kappa > 0.8$ for almost perfect agreement (Bablok and Passing, 1985).

RESULTS

Disease Prevalence and Association with Risk Factors

Table 1 provides an overview of geographical data, weather indices, and the number of calves sampled across various provinces and farms in Iran. The mean

Table 1. Geographical information, weather indexes [mean±SD with 95% confidence interval (CI) in parenthesis], number of tested farms and animals of different studied districts for *Cryptosporidium parvum* prevalence in Iran (Based on Synoptic Automatic Weather Station)

District	Geographical information	Mean annual rainfall (mm)	Number of sunny hours (h)	ambient air temperature (°C)	Relative humidity (%)	average elevation (m)	No. of tested farms	No. of tested animals	
								Diarrheic	Healthy
Isfahan	32°38′41″N 51°40′03″E	7.5±12.7 (0.5-15.6)	286±55 (250-321)	17.9±8.6 (12.5-23.5)	32.6±12.5 (24.6-40.5)	1590	6	46 (44.23%)	58 (55.77%)
Tehran	35°41′21″N 51°23′20″E	24.4±29.6 (5.6-43.3)	244±68 (201-288)	17.8±10.7 (11-24.7)	42.7±14.9 (33.2-52.3)	1350	4	21 (51.22%)	20 (48.78%)
Karaj	35°50′08″N 51°00′37″E	17.8±25.2 (1.8-33.8)	258±73 (211-304)	15.3±9.6 (9.2-21.5)	46.2±11.9 (38.6-53.7)	1312	3	15 (51.72%)	14 (48.27%)
Hamedan	34°48′N 48°31′E	19.7±26.3 (3.03-36.5)	250±75 (202-298)	13±9.4 (7.02-19)	47.7±20.9 (34.4-60.9)	1850	2	10 (55.55%)	8 (44.45%)
Khorramabad	33°29′16″N 48°21′21″E	47.1±58.1 (10.1-83.9)	242±76 (193-291)	17.7±9.4 (11.8-23.7)	42.4±19.1 (30.3-54.5)	1147	3	16 (51.61%)	15 (48.38%)
Babol	36°33′05″N 52°40′44″E	69.8±63.7 (29.4-110)	176±67 (133-219)	16.62 (12.7-22.5)	81.4±2.84 (79.6-83.2)	-2	5	37 (47.43%)	41 (52.56%)

age of the animals was 14.76 ± 5.2 days (mean ± standard deviation), with ages ranging from 1 to 30 days. In this study, 40.86% of the calves were under 2 weeks of age, and approximately 52% of the animals tested were female. The largest and smallest *Cryptosporidium* oocysts observed in the study measured 6.4 × 4.5 µm and 3.5 × 3.3 µm, respectively.

A total of 301 fecal samples were analyzed, with the overall prevalence of cryptosporidiosis in neonatal Holstein calves found to be 50.5% (152/301) based on the MZN method and 45.85% (138/301) based on the ICG method. In calves with diarrhea, the prevalence according to the MZN method was 39.52% (95% CI: 37.23-41.25), while in non-diarrheic calves, it was 8.55% (95% CI: 6.62-9.84). For the ICG method, the prevalence in diarrheic calves was 41.9% (95% CI: 38.13-44.29) and 7.64% (95% CI: 7.05-8.62) in non-diarrheic calves. Among the 23 farms tested, 91.3% showed infection, with no contamination detected in two farms located in Tehran and Khorramabad. Additionally, the prevalence of other key enteropathogens in both mono- and co-infection forms is depicted in Figure 2.

The highest prevalence was recorded in calves aged 15 to 30 days (30.23%) and in female calves (26.91%) compared to calves aged 1 to 14 days (19.27%) and male calves (22.59%). However, no significant correlation (P > 0.05) was found between these intrinsic factors and cryptosporidiosis prevalence. As shown in Table 2, extrinsic factors such as location (P = 0.0001), annual rainfall (P = 0.0001), relative humidity (P = 0.01), herd size (P = 0.007), fecal consistency (P = 0.0001), and cow contact (P = 0.002) were significantly associated with infection rates. Additionally, hutch hygiene (P = 0.0001) emerged as a protective factor against *Cryptosporidium* infection.

In the multivariate analysis, a backward deletion method was applied to exclude non-significant factors from the model. The results indicated that with over 62% confidence, the disease prevalence could be explained using 9 independent variables. The Omnibus test results confirmed the model's good fit, showing statistical significance at an error level of less than 0.05. The extrinsic variables accounted for 45.5% to 60.6% of the variation in *Cryptosporidium* infection rates. In the final analysis, mean annual rainfall (P = 0.05), hutch hygiene (P = 0.03), fecal consistency (P = 0.0001), and contact with mature cows (P = 0.04) remained significantly associated with the infection (Table 3).

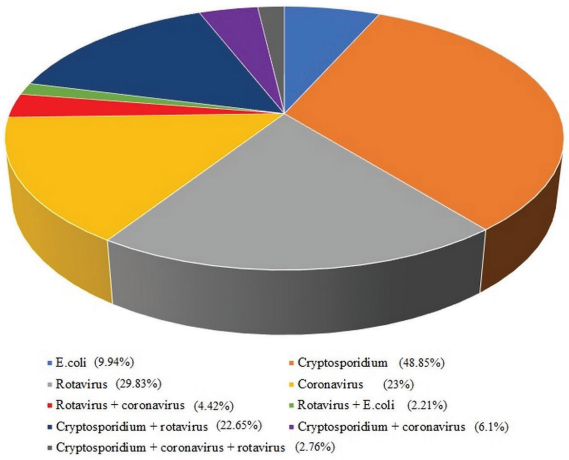

Figure 2. Prevalence of four enteropathogens by using ICG technique in feces samples of diarrheic and non-diarrheic calves. Mono- and co-infection of pathogens with each other's presented.

PCR Analysis and Genome Sequencing

Out of 50 samples, 35 tested positive in the PCR assay (Figure 3), and these were subsequently analyzed through sequence analysis. The amplified genomic region was sent for sequencing (Macrogen, South Korea). The nucleotide sequence of the amplified rRNA region exhibited a genomic identity of 99.87% with Cryptosporidium parvum (GenBank accession No. MK880570).

Method Comparison Analysis

As molecular techniques were not applied to fecal samples in our study, the MZN method was considered the reference standard for evaluating the specificity and sensitivity of the immunochromatography (ICG) method. The sensitivity, specificity, predictive values, and likelihood ratios of the ICG-based diagnostic assay were calculated for diarrheic (145 cases) and non-diarrheic (156 cases) calves, with respect to the standard diagnostic method (MZN), as shown in Table 4.

The results indicated that the concordance correlation and accuracy of the ICG method for detecting *C. parvum* were lower in non-diarrheic calves (0.62 and 0.64, respectively) compared to diarrheic calves (0.72 and 0.73, respectively). Additionally, the sensitivity of the ICG method was higher in non-diarrheic calves (85.26%) than in diarrheic calves (84.6%). False-positive results were more common in diarrheic calves (12.6%) compared to

Table 2. *Cryptosporidium parvum* infection rate [percentage with 95% confidence interval in parenthesis] of newborn Holstein dairy calves based on intrinsic and extrinsic risk factors in Iran

Risk factor	No.	MZN detection	ICG detection	χ^2	P-value
Sex					
Male	144	22.59 (19.4-25.6)	21.3 (18.75-23.15)	0.57	0.44
Female	157	26.91 (24.1-28.97)	24.6 (22.3-26.2)		
Age (days)					
1-14	123	19.27 (17.77-21.09)	14 (12.55-15.8)	11.4	0.5
15-30	178	30.23 (28.45-33.6)	31.9 (29.3-33.23)		
Location					
Isfahan	104	21.6 (20.09-23.40)	22.4 (20.81-24.49)		
Tehran	41	6.3 (5.97-6.88)	3.3 (2.29-4.03)		
Karaj	29	4.3 (3.36-4.79)	3.3 (2.36-3.87)	20.42	0.0001
Hamedan	18	4.3 (3.33-4.68)	4.3 (3.59-5.2)		
Khorramabad	31	4.3 (3.37-4.74)	3.7 (3.11-4.89)		
Babol	78	8.3 (7.55-9.12)	8.6 (6.9-9.56)		
Temperature (°C)					
>16	44	8.6 (8.05-9.11)	7.6 (6.65-8.45)	1.89	0.17
≥16	257	40.9 (37.65-43.25)	38.2 (36.23-40.12)		
Mean annual rainfall **(mm)**					
>20	151	30.6 (28.9-31.5)	30.2 (28.41-31.23)	15.77	0.0001
≥20	150	18.9 (17.22-19.4)	15.6 (14.3-16.68)		
Relative humidity **(%)**					
>45	179	32.6 (30.45-36.5)	29.6 (28.45-32.12)	2.66	0.01
≥45	122	16.9 (15.2-18.7)	16.3 (14.78-17.9)		
No. of sunny hours					
>250	154	23.48 (21.87-24.6)	24.59 (23.12-26.32)	6.88	0.34
≥250	147	26.59 (25.25-28.2)	26.01 (25.03-27.32)		
Average elevation (m)					
>1300	109	12.6 (10.62-13.84)	12.3 (10.07-13.51)	14.6	0.0001
≥1300	192	36.88 (33.34-38.12)	33.6 (30.56-34.8)		
Herd size					
>4000	153	21.3 (19.66-22.8)	19.9 (18.5-21.25)	7.3	0.007
≥4000	148	28.2 (27.5-30.3)	25.9 (24.03-27)		
Hutch hygiene					
Yes	165	16.6 (14.85-17.6)	16.9 (15.68-17.87)	18.66	0.0001
No	136	20.9 (18-22.54)	28.9 (27-29.8)		
Calf-cow contact					
Yes	109	36.9 (34.45-38.9)	33.6 (32.2-34.77)	9.72	0.002
No	192	12.6 (11.5-13.6)	12.3 (11.59-13.22)		
Fecal consistency	145				
Diarrheic	156	39.52 (37.23-41.25)	41.86 (38.13-44.29)	156	0.0001
Non-diarrheic		8.55 (6.62-9.84)	7.64 (7.05-8.62)		

Table 3. Final model of multivariate analysis of *Cryptosporidium parvum* infection risk factors. Factors had been eliminated step by step (Akaike Information Criterion) until best model was built.

Risk factor	Odd ratio (95% CI)	SE	Regression equation (B)	P-value
Location				
Isfahan	2.89 (1.96-5.85)	0.37	-0.39	0.13
Tehran	1.18 (0.78-2.59)			0.2
Karaj	-			reference
Hamedan	1.21 (0.65-2.28)			0.24
Khorramabad	1.16 (0.59-2.26)			0.52
Babol	2.02 (1.63-5.58)			0.18
Temperature (°C)				
>16	-	1.37	-0.04	reference
≥16	1.68 (0.9-3.52)			0.52
Mean annual rainfall (mm)				
>20	2.1 (1.87-4.93)	1.11	0.74	0.05
≥20	-			reference
***Relative humidity* (%)**				
>45	1.26 (0.58-3.49)	0.73	0.06	0.93
≥45	-			reference
Average elevation (m)				
>1300	3.22 (1.27-10.89)	0.7	-0.54	0.45
≥1300	-			reference
Herd size				
>4000		1.01	-0.25	reference
≥4000	1.57 (0.8-3.32)			0.8
Hutch hygiene				
Yes	-	1.16	-0.15	reference
No	1.44 (1.09-2.59)			0.03
Calf-cow contact				
Yes	2.32 (1.45-4.9)	0.89	0.25	0.04
No	-			reference
Fecal consistency				
Diarrheic	39.4 (2.56-107.23)	0.35	3.67	0.0001
Non-diarrheic	-			reference

non-diarrheic calves (9.72%). The area under the curve (AUC) for the ICG method was 0.86 in diarrheic calves and 0.87 in non-diarrheic calves, both of which were acceptable and did not show significant differences when compared to the MZN method ($P > 0.05$).

As detailed in Table 5, the Bias Correction factor (0.999 vs 0.997) and Pearson's ρ (0.84 vs 0.79) for the MZN method were higher than those for the ICG method when compared to the gold standard technique (PCR). Furthermore, the linear kappa statistic and concordance correlation for the MZN method in comparison with PCR were perfect (0.94) and substantial (0.97), while those for the ICG method were perfect (0.86) and poor (0.77), respectively.

DISCUSSION

Gastrointestinal infections, particularly Cryptosporidium spp., are significant health concerns in

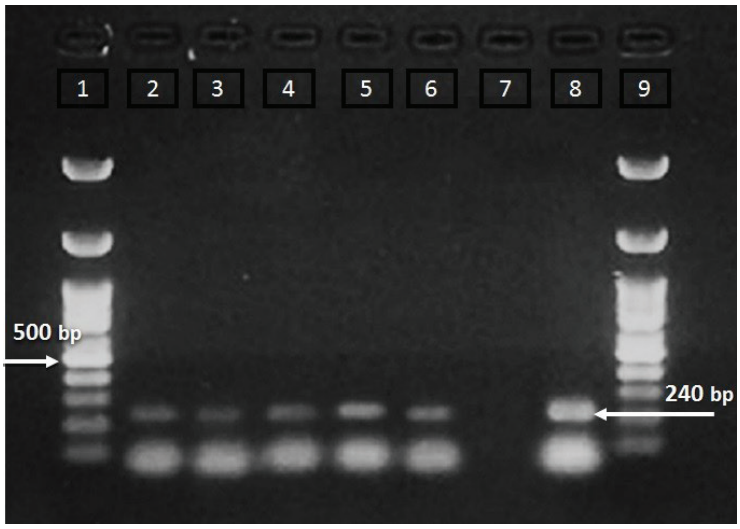

Figure 3. Gel electrophoresis of *Cryptosporidium* SSU rRNA. products resulting from digestion of the PCR assay. Lines 1 and 8: 100 bp molecular marker, Line 2 through 5: *C. parvum,* Line 7: Positive control, Line 8: Negative control (no template).

Table 4. Test performance characteristics and area under curve (AUC) of immunochromatography (ICG) method in comparison to modified Ziehl-Neelsen (MZN) for the diagnosis of Cryptosporidiosis among diarrheic, and non-diarrheic Holstein dairy calves in Iran

Test / Parameters	ICG technique	
	Diarrheic calves	Non-diarrheic calves
Sensitivity (95% CI)	84.6 (77.7-90.0)	85.26 (78.7-90.4)
Specificity (95% CI)	87.5 (81.2-92.3)	86.9 (80.3-92.0)
NPV (95% CI)	85.3 (79.8-89.4)	84.6 (78.9-88.9)
PPV (95% CI)	86.9 (81.2-91.0)	87.5 (82.1-91.5)
+LR (95% CI)	6.77 (4.4-10.4)	6.51 (4.3-9.9)
-LR (95% CI)	0.18 (0.1-0.3)	0.17 (0.1-0.2)
AUC* (95% CI)	0.86 (0.82-0.89)	0.87 (0.81-0.90)
Difference (95% CI)	0.14 (-0.1-0.19)	0.14 (-0.22-0.19)
P-value	0.26	0.09

* DeLong et al., 1988

young livestock, especially in tropical and subtropical regions (Burd and Hinrichs, 2016). Diarrhea in calves can be caused by nutritional factors or infectious agents, including viruses, bacteria, fungi, and protozoa. Among these, Cryptosporidium is a protozoan parasite known to cause substantial economic losses due to diarrhea, poor growth, and even death in calves (Bamaiyi and Redhuan, 2017). The prevalence of Cryptosporidium infections varies by region, with reports in Iran ranging from 3.6% to 50% (Azami, 2007; Mahami-Oskouei et al., 2014; Noorani Kolije et al., 2020), and globally from 1.61% to 96.6% (Akinkuotu et al., 2014; Huang et al., 2014; Thompson et al., 2017). In our study, we found an overall prevalence of 50.5% based on the MZN method, which aligns with similar studies in Iran (Asadpour et al., 2013; Noorani Kolije et al., 2020). Comparable high prevalence rates have also been observed in neonatal calves in the USA, Vietnam, and Italy (Santín et al., 2004; Nguyen et al., 2007; Diaz et al., 2018). On infected farms, prevalence rates in 1- to 3-week-old calves often exceed 50%, with some studies indicating that over 90% of farms harbor this parasite (Anderson and Rings, 2008).

When assessing risk factors for Cryptosporidium infection, we identified several key factors that contribute to the spread of this parasite. First, our study found that the infection rate was higher in calves older than 2 weeks (30.23%) compared to younger calves (19.27%), although this difference was not statistically significant. This observation is consistent with previous studies, which suggest that older calves are more susceptible to Cryptosporid-

Table 5. Concordance of results between MZN and ICG against diagnostic 'gold' standard (PCR) method and ICG against standard diagnostic technique (MZN) by using linear kappa factor, reliability of kappa statistics, bias correction rate and Pearson correlation for the diagnosis of *Cryptosporidium parvum* in newborn Holstein dairy calves in Iran.

Test \ Parameter	Prevalence (%)	Reciprocal tests results				Linear Kappa (95% CI)	Reliability of average of Kappa (95% CI)	Concordance Correlation (95% CI)	Bias Correction factor (%) *	Pearson ρ‡ (95% CI)
		+/+	+/-	-/+	-/-					
MZN vs PCR	35/50 (70%)	35/50	4/50	8/50	3/50	0.94 (0.89-0.97)	0.93 (0.88-0.96)	0.97 (0.95-0.98)	0.999	0.84 (0.79-0.91)
ICG vs PCR	32/50 (64%)	32/50	5/50	6/50	7/50	0.86 (0.78-0.88)	0.89 (0.85-0.92)	0.77 (0.67-0.84)	0.997	0.79 (0.71-87)
ICG vs MZN	30/50 (60%)	30/50	2/50	4/50	14/50	0.73 (0.53-0.93)	0.8 (0.75-0.86)	0.71 (0.62-0.78)	0.996	0.7 (0.67-0.77)

* C_b Accuracy
‡ Precision

ium infections (Doungmala et al., 2019). The peak shedding of oocysts typically occurs around day 12 of life, which could explain the higher infection rates in older calves (Anderson and Rings, 2008). This suggests that optimal sampling for detecting oocysts might occur during this window.

Regarding sex distribution, we found that more female calves were infected with Cryptosporidium, which aligns with other studies reporting a higher infection rate in females compared to males (Ranjbar and Fattahi, 2017). This finding warrants further investigation, as some studies have not observed any significant sex-related differences in Cryptosporidium prevalence (Shobhamani, 2005).

Environmental factors also play a crucial role in the transmission of Cryptosporidium. We found that the infection rate was higher in regions with low mean annual rainfall (<20 mm) and low relative humidity (<45%), which is consistent with other studies reporting increased oocyst shedding in warm and humid climates (Paul et al., 2008; Joute et al., 2016). However, some studies have shown higher prevalence rates in colder, winter months (Mohammad et al., 1999; Ranjbar and Fattahi, 2017), likely due to the confinement of animals in smaller spaces, leading to increased contamination (Hatam-Nahavandi et al., 2019). This contradiction may be due to the fact that warmer, humid conditions may accelerate oocyst transmission, while colder conditions may cause oocysts to become more resilient due to freeze/thaw cycles (Jenkins et al., 1999; Kato et al., 2002).

Our study also revealed a positive correlation between altitude and Cryptosporidium prevalence, which supports previous findings indicating that fecal shedding and infection rates are associated with altitude (Montecino-Latorre et al., 2015). This may be due to factors such as temperature, relative humidity, and UV radiation, which vary with altitude and influence oocyst survival and transmission. Cryptosporidium oocysts are known to be resilient to environmental conditions, but the geographical location influences their ability to survive and spread, often due to climate and weather patterns.

Other risk factors identified in this study include herd management practices such as hygiene and animal density. We found that farms with unhygienic conditions, where calves had close contact with older cattle, and larger herd sizes had a higher risk of Cryptosporidium infection. This aligns with previous studies suggesting that high herd density increases the likelihood of infection due to greater

contamination of the environment (Quigley et al., 1994; Mohammed et al., 1999). Additionally, the use of disinfectants like bleach or lime was found to reduce infection risk (Castro-Hermida et al., 2002a), highlighting the importance of proper hygiene and sanitation in controlling Cryptosporidium infections.

Comparison of Diagnostic Methods

There are no internationally standardized methods for diagnosing Cryptosporidium infections, and various diagnostic techniques, both low- and high-tech, are used in the field. One of the most common methods for detecting Cryptosporidium is microscopic examination of stool samples, where oocysts are colorless, spherical, or ovoid, smooth, thick-walled, and contain four sporozoites (Mead and Arrowood, 2020). In our study, only *C. parvum* oocysts were considered, as the immunochromatographic (ICG) method employed in this study detects only this species. The average size of *C. parvum* oocysts was found to be 4.6±0.3 × 5.2±0.3 µm, which is consistent with previous reports (Noorani Kolije et al., 2020).

We compared two diagnostic methods in this study: the MZN method and the ICG method. The MZN method demonstrated higher sensitivity, particularly for detecting infections in diarrheic calves, likely due to its ability to detect subclinical infections and lower oocyst shedding in non-diarrheic animals. Conversely, the ICG method showed lower sensitivity in non-diarrheic calves, which could be attributed to the lower excretion of oocysts in subclinical cases. However, the ICG method proved to be a useful on-farm tool, providing acceptable diagnostic power in clinical settings, particularly for identifying *C. parvum* infections in diarrheic calves. Sensitivity and specificity estimates for both methods in other studies suggest that the carbolfuchsin smear method, for example, has a sensitivity of 78% and specificity of 79%, while immunological methods like ICG can achieve higher specificity (Geurden et al., 2006).

The findings from our comparison indicate that while the MZN method may be more effective for detecting subclinical infections and providing overall diagnostic accuracy, the ICG method remains a valuable tool for rapid on-farm diagnosis, especially in acute clinical cases. For the detection of *C. parvum* and other Cryptosporidium species, species-specific PCR assays followed by sequencing provide the highest diagnostic sensitivity and specificity, offering reliable tools for characterizing infections in clinical specimens.

CONCLUSION

Cryptosporidium parvum is a common pathogen in neonatal calves on Iranian dairy farms, and its prevalence is influenced by several environmental and management factors. Key risk factors for infection include poor hygiene, close contact between mature and neonatal cattle, and high herd density. Our study also emphasized the need for effective herd management practices, including improving sanitation and separating calves from mature animals to reduce infection risk. While the MZN method demonstrated higher sensitivity for detecting infections, the ICG method remains a useful, accessible tool for on-farm diagnosis, particularly for acute cases. Species-specific PCR assays offer the most reliable diagnostic approach for identifying Cryptosporidium species in clinical settings.

Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work the author(s) used ChatGPT in order to paraphrase and grammar check some sections of the text. After using this tool/service, the author(s) reviewed and edited the content as needed and take(s) full responsibility for the content of the publication.

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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Ethical statement

All procedures in this study were performed following the ARRIVE guidelines 2.0 and approved by the Ethics Committee on Animal Use of Shahid Chamran University of Ahvaz, under protocol number 94/3/24/60865. Sample collection and clinical examinations were conducted by licensed veterinarians, ensuring adherence to ethical standards of animal welfare and husbandry regulations. No animals were harmed, and all efforts were made to minimize distress and discomfort during sample collection.

REFERENCES

Akinkuotu, OA., Fagbemi, BO., Otesile, EB., Dipeolu, MA., Ayinmode, AB. (2014). *Cryptosporidium* infection in cattle in Ogun state, Nigeria. *Sokoto J. Vet. Sci*, 12(2): 52-56.

Anderson, D., Rings, M. (2008). Current Veterinary Therapy: Food Animal Practice. (8th Edn.), Saunders Publishing, USA.

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. *Iran J. Parasito*, 8(4): 601-607

Azami, M. (2007). Prevalence of *Cryptosporidium* infection in cattle in Isfahan, Iran. *J. Euk. Microbiol*, 54(1): 100-102.

Bablok, W., Passing, H. (1985). Application of statistical procedures in analytical instrument testing. *J. Auto. Chem*, 2: 74-79.

Bairami, S., Rezaei, S., Rezaeian, M. (2018). Synchronous Identification of *Entamoeba histolytica*, *Giardia intestinalis*, and *Cryptosporidium* spp. in Stool Samples Using a Multiplex PCR Assay. *Iran J. Parasitol*, 13: 24-30.

Bamaiyi, PH., Redhuan, NE. (2017). Prevalence and risk factors for cryptosporidiosis: a global, emerging, neglected zoonosis. *Asian Biomed*, 10(4): 309-325

Bartley, P.M., Standar, J.H., Katzer, F. (2024). Genetic characterisation of *Cryptosporidium parvum* in dairy cattle and calves during the early stages of a calving season. *Current Res Parasito Vector-Borne Dis*, 5:100160

Burd, EM., Hinrichs, BH. (2016). Gastrointestinal Infections. In: Leonard D. (eds) Molecular Pathology in Clinical Practice. Springer, Cham.

Cai, M., Guo, Y., Pan, B., Li, N., Wang, XL., Tang, CX. (2017). Longitudinal monitoring of *Cryptosporidium* species in pre-weaned dairy calves on five farms in Shanghai, China. *Vet. Parasitol*, 241: 14–19

Castro-Hermida, JA., Gonzalez-Losada, YA., Ares-Mazas, E. (2002a). Prevalence of and risk factors involved in the spread of neonatal bovine cryptosporidiosis in Galicia (NW Spain). *Vet. Parasitol*, 106: 1-10

Castro-Hermida, JA., Gonzalez-Losada, YA., Mezo-Menedez, M., Ares-Mazas, E. (2002b). A study of cryptosporidiosis in a cohort of neonatal calves. *Vet. Parasitol*, 106: 11-17

Chako, CZ., Tyler, JW., Schultz, LG., Chiguma, L., Beerntsen, BT. (2010). Cryptosporidiosis in people: it's not just about the cows. *J. Vet. Int. Med*, 24(1): 37-43

Constable, PD., Hinchcliff, KW., Done, SH., Gruenberg, W. (2017). Parasitic diseases. In: Veterinary medicine: A textbook of the disease of cattle, horses, sheep, pigs and goats. (11th Edn.), Missouri, Saunders Elsevier, Philadelphia, PA, USA.

De Graaf, DC., Vanopdenbosch, E., Ortega-Mora, LM., Abbassi, H., Peeters, JE. (1999). A review of the importance of cryptosporidiosis in farm animals. *Int. J. Parasitol*, 29(8): 1269-1287.

DeLong, ER., DeLong, DM., Clarke-Pearson, DL. (1988). Comparing the areas under two or more correlated receiver operating characteristic curves: a nonparametric approach. *Biometrics*, 44: 837-845

Diaz, P., Varcasia, A., Pipia, PA., Tamponi, C., Sanna, G., Prieto, A., Ruiu, A., Spissu, P., Diez-Banos, P., Morrondo, P., Scala, A. (2018). Molecular characterization and risk factor analysis of *Cryptosporidium* spp. In calves from Italy. *Parasitol. Res*, 117: 3081-3090

Doungmala, P., Phuektes, P., Taweenan, W., Sangmaneedet, S., Japa, O. (2019). Prevalence and species identification of *cryptosporidium* spp. In the newborn dairy calves from Muang district, Khon Kaen province, *Thailand. Vet. Worl*, 12(9): 1454-1459.

Fasihi Harandi, M., Fotouhi, AR. (2008). *Cryptosporidium* infection of sheep and goats in Kerman: epidemiology and risk factor analysis. *J Vet Res*, 63(1):47–51.

Firoozi, Z., Sazmand, A., Zahedi, A., Astani, A., Fattahi-Bafghi, A., Kiani-Salmi, N., Ebrahimi, B., Dehghani-Tafti, A., Ryan, U., Akrami-Mohajeri, F. (2019). Prevalence and genotyping identification of *Cryptosporidium* in adult ruminants in central Iran. *Parasites Vec*, 12, 510.

Georgi, JE., Georgi, ME. (1990). Parasitology for veterinarians. W.B. Saunders, Philadelphia, Pennsylvania, USA.

Geurden, T., Goma, FY., Siwila, J., Phiri, IGK., Mwanza, AM., Gabriel, S., Claerebout, E., Vercruysse, J. (2006). Prevalence and genotyping of *Cryptosporidium* in three cattle husbandry system in Zambia. *Vet. Parasitol*, 138: 217-222

Geurden, T., Berkvens, D., Geldhof, P., Vercruysse, J., Claerebout, E. (2006). A Bayesian approach for the evaluation of six diagnostic assays. *Vet. Res*, 37: 671-682.

Hamedian-Asl, M., Zakian, A., Azimpour, S., Davoodi, F., Kahroba, Houman (2022). Evaluation of diagnostic methods for the detection of Bovine Coronavirus and Rotavirus in faeces of diarrhoeic calves. *J. Hellenic Vet. Med. Soc*, 73(2):3951-3960

Harp, JA., Woodmansee, DB., Moon, HW. (1990). Resistance of calves to *cryptosporidium parvum*: effects of age and previous exposure. *Infec. Immu*, 58(7): 2237-2240.

Hatam-Nahavandi, K., Ahmadpour, E., Carmena, D., Spotin, A., Bangoura, B., Xia, L. (2019). *Cryptosporidium* infections in terrestrial ungulates with focus on livestock: a systematic review and meta-analysis. *Para. Vect*, 12: 453.

Henricksen, SA., Polenz, JF. (1981). Staining of *Cryptosporidia* by modified Ziehl-Neelsen technique. *Acta Vet. Scand*, 22: 594-596.

Hosmer, DW., Lemeshow, S. (2000). Applied Logistic Regression. (2nd Edn.), John Wiley and Sons, Inc. USA.

Icen, H., Berrin Arserim, N., Isik, N., Ozkan, C., Kaya, A. (2013). Prevalence of four enteropathogens with immunochromatographic rapid test in the feces of diarrheic calves in east and southeast of Turkey. *Pakistan Vet. J*, 33: 496-499.

Jenkins, M., Walker, M., Bowman, D., Anthony, L., Ghiorse, W. (1999). Use of a sentinel system for field measurements of *Cryptosporidium parvum* oocyst inactivation in soil and animal waste. *Appl. Env. Mic*, 65: 1998-2005.

Jensen, AL., Kjelgaard-Hansen, M. (2006). Method comparison in the clinical laboratory. *Vet Clin. Path*, 35: 276-286.

Joute, JR., Gill, J.P.S., Singh, BB. (2016). Prevalence and molecular epidemiology of *cryptosporidium parvum* in dairy calves in Punjab (India). *J. Parasite Dis*, 40(3): 745-749.

Kasyap, G., Sindhoora, K., Singh, S., Banerji, PS., Gupta, D., Kumar, P., Singh, R., Singh, V. (2019). Occurrence and diagnosis of cryptosporidiosis in cattle calves with clinical diarrhoea. *Biol. Rhy. Res*, DOI: 10.1080/09291016.2019.1603687

Kato, S., Jenkins, MB., Fogarty, EA., Bowman, DD. (2002). Effects of freeze-thaw events on the viability of *Cryptosporidium parvum* oocysts in soil. *J. Parasitol*, 88: 718-722.

Kumar Dhal, A., Panda, C., Yun, S.I., Mahapatra, R.K. (2022). An update on *Cryptosporidium* biology and therapeutic avenues. *J. Parasit. Dis*, 46(3): 923-939.

Larson, LL., Owen, FG., Albright, JL., Appleman, RD., Muller, LD. (1977). Guidelines toward more uniformity in measuring and reporting calf experimental data. *J. Dairy Sci*, 60: 989-991.

Li, X., Atwill, ER., Dunbar, LA., Tate, KW. (2010). Effect of daily temperature fluctuation during the cool season on the infectivity of *Cryptosporidium parvum*. *Appl. Environ. Mic*, 76: 989-993.

Li, N., Wang, R., Cai, M., Feng, Y., Xiao, L. (2019). Outbreak of cryptosporidiosis due to *cryptosporidium parvum* subtype IIdA19G1 in neonatal calves on a dairy farm in China. *Int. J. Parasit*, 49: 569-577.

Mahami Oskouei, M., Fallah, E., Ahmadi, M., Safaiyan, A., Bakhtiyari, S., Naserifar, R. (2014). Molecular and parasitological study of *Cryptosporidium* isolates from cattle in Ilam, West of Iran. *Iranian J. Parasit*, 9(3): 435-40.

Mahmoudi, M., Hazrati Tappeh, K., Abasi, E., Aminpour, A. (2020). Utility of different diagnostic tools for detection of *cryptosporidium* in children with diarrhea. *Arch. Ped. Inf. Dis*, 8(1): e92033.

Majeed, QAH., El-Azazy, OME., Abdou, NMI., Al-Aal, ZA., El-Kabbany, AI., Tahrani, LMA., AlAzemi, MS., Wang, Y., Feng, Y., Xiao, L. (2018). Epidemiological observations on cryptosporidiosis and molecular characterization of *Cryptosporidium* spp. in sheep and goats in Kuwait. *Parasitol. Res*, 117: 1631-1636.

McBride, G.B. (2005). A proposal for strength-of-agreement criteria for Lin's concordance correlation coefficient. NIWA Client Report: HAM2005-062. Report to Ministry of Health.

Mead, JR., Arrowood, MJ. (2020). Methods in molecular biology: *Cryptosporidium*. Springer protocols, Humana press, London. UK.

Mirzai, Y., Yakhchali, M., Mardani, K. (2014). *Cryptosporidium par-*

vum and *Cryptosporidium andersoni* infection in naturally infected cattle of northwest Iran. *Vet. Res. Forum.* 5(1): 55-60.

Mohammad, HO., Wade, SE., Schaaf, S. (1999). Risk factors associated with *Cryptosporidium parvum* infection in dairy cattle in southeastern New York State. *Vet. Parasitol*, 83(1): 15-22.

Montecino-Latorre, D., Li, X., Xiao, C., Atwill, ER. (2015). Elevation and vegetation determine *Cryptosporidium* oocysts shedding by yellow-bellied marmots (Marmota flaviventris) in the Sierra Nevada Mountains. *Int. J. Parasitol.: Parasit. Wild*, 4: 171-177.

Nguyen, ST., Nguyen, DT., Le, DQ., Le Hua, LN., Van Nguyen, T., Honma, H., Nakai, Y. (2007). Prevalence and first genetic identification of *Cryptosporidium* spp. in cattle in central Viet Nam. *Vet. Parasitol*, 150: 357-361.

Noorani Kolije, O., Yakhchali, M., Malekifard, F. (2020). Study on *Cryptosporidium* infection in cattle and water Buffaloes of farms in Mahabad Suburb, Iran. *J. Vet. Res*, 75(2): 147-155

Ortega-Mora, L.M., Wright, S.E. (1994). Age-related resistance in ovine Cryptosporidiosis: patterns of infection and humoral immune response. *J. Inf. Imm*, 62(11): 5003-5009.

Papanikolopoulou, V., Baroudi, D., Guo, Y., Wang, Y., Papadopoulos, E., Lafi, SQ., Abd El-Tawab, MM., Diakou, A., Giadinis, ND., Feng, Y., Xiao, L. (2018). Genotypes and subtypes of *Cryptosporidium* spp. in diarrheic lambs and goat kids in northern Greece. *Parasitol. Int*, 67: 472-475.

Paul, S., Chandra, D., Ray, DD., Tewari, A.K., Rao, JR., Banerjee, PS., Baidya, S., Raina, OK. (2008). Prevalence and molecular characterization of bovine *Cryptosporidium* isolates in India. *Vet. Parasitol*, 153: 143-146.

Percie du Sert, N., Hurst, V., Ahluwalia, A., Alam, S., Avey, M. T., Baker, M., Browne, W. J., Clark, A., Cuthill, I. C., Dirnagl, U., Emerson, M., Garner, P., Holgate, S. T., Howells, D. W., Karp, N. A., Lazic, S. E., Lidster, K., MacCallum, C. J., Macleod, M., . . . Würbel, H. (2020). The ARRIVE guidelines 2.0: Updated guidelines for reporting animal research. *J Cereb Blood Flow Metab*, 40(9): 1769–1777.

Quigley, JD., Martin, KR., Bemis, DA., Potgiete, LND., Reinemeyer, CR., Rohrbach, BW., Dowlen, HH., Lamar, KC. (1994). Effects of housing and colostrum feeding on the prevalence of selected infectious organisms in feces of Jersey calves. *J. Dairy Sci*, 77: 3124-3131.

Ranjbar, R., Fattahi, R. (2017). Prevalence of *Cryptosporidium* spp. In calves under one year old in Ilam County (Iran), from March 2014 to February 2015. *Elec. Phys*, 9(6): 4631-4635.

Ranjbar-Bahadori, S., Sangsefidi, H., Shemshadi, B., Kashefinejad, M. (2011) Cryptosporidiosis and its potential risk factors in children and calves in Babol, north of Iran. *Trop. Biomed*, 28(1):125–31.

Ryan, U., Hijjawi, N., Xiao, L. (2018). Foodborne Cryptosporidiosis. *Int. J. Parasitol*, 48: 1-12.

Santin, M. (2020). *Cryptosporidium* and *Giardia* in ruminants. *North America: Vet. Clin. Food Anim*, 36: 223-238.

Shafieyan, H., Alborzi, A., Hamidinejat, H., Tabandeh, MR., Haji Hajikolaei, MR. (2014). Prevalence of Cryptosporidium spp. In ruminants of Lorestan Province, Iran. *J. parasitic Dis*, 40(4): 1165-1169.

Shivley, CB., Lombard, JE., Urie, NJ., Kopral, CA., Santin, M., Earlywine, TJ., Olson, JD., Garry, FB. (2018). Preweaned heifer management on US dairy operations: part VI. Factors associated with average daily gain in preweaned dairy heifer calves. *J. Dairy Sci*, 101: 9245-9258.

Shobhamani, B. (2005). Epidemiological studies on diarrhoea in calves with particular reference to diagnosis and treatment of Cryptosporidiosis. *J. Vet. Parasitol*, 19: 77.

Soulsby, EJL. (1982). Helminths, Arthropods and Protozoa of Domesticated Animals. (7th Edn.), London, UK: Bailliere Tindall.

Swai, ES. (2010). Investigation into the Prevalence of *Cryptosporidium* Infection in Calves among Small-Holder Dairy and Traditional Herds in Tanzania. *Vet. Med. Int*, 676451.

Thomson, S., Hamilton, CA., Hope, JC., Katzer, F., Mabbott, NA., Morrison, LJ., Innes, E.A. (2017). Bovine Cryptosporidiosis: impact, host-parasite interaction and control strategies. *Vet. Res*, 48.

Thompson, RCA., Ash, A. (2019). Molecular epidemiology of *Giardia* and *Cryptosporidium* infections - What's new? Infection, *Genetics and Evolution*, 75: 103951.

Thrusfield, MV. (2005). Diagnostic testing. In Veterinary Epidemiology. (3rd Edn.), Oxford, Blackwell Publishing, UK.

Thrusfield, MV. (2007). Veterinary Epidemiology. (4th Edn.), Blackwell Sci. Ltd. Royal School of Veterinary Studies, University of Edinburgh, UK.

Ye, J., Xiao, L., Wang, Y., Amer, S., Roellig, DM., Guo, Y., Feng, Y. (2013). Periparturient transmission of *Cryptosporidium xiaoi* from ewes to lambs. *Vet. Parasitol*, 197: 627-633.

Zhou, L., Singh, A., Jiang, J., Xiao, L. (2003). Molecular Surveillance of Cryptosporidium spp. in Raw Wastewater in Milwaukee: Implications for Understanding Outbreak Occurrence and Transmission Dynamics. *J. Clin. Microbiol*., 41: 5254-525