Research Article

Molecular Identification of *Cryptosporidium* Spp. in Cows and Handlers in the Baghdad Province

Heba Ali Ghanim[1]*, Hydear Barakat Abbas[2], S Salam Ali[3], Sara Ayad Ahmed[4], Zaid Khalid Ahmed[5], Zeid Alsadoon[6]

[1]Department of Microbiology, College of Medicine, Ibn Sina University of Medical and Pharmaceutical Sciences, Iraq; [2]Department of Parasitology; College of Veterinary Medicine, University of Baghdad, Iraq; [3]Department of Pathological Analysis, College of Medical Technology, Al-Farahidi University, Iraq; [4]Department of Microbiology, College of Medicine, University of Anbar, Iraq; [5]College of Pharmacy, Al-Turath University, Iraq; [6]Microbiology Department, College of Veterinary Medicine, Wasit University, Iraq.

Abstract | This study aims to use molecular identification and phylogenetic analysis to identify the zoonotic *Cryptosporidium* spp. in human and cow. The total number 200 (100 human stool samples and 100 cow fecal samples were chosen at random) during the period from the September to November 2024 from different regions in private veterinary clinic and sent to the laboratory for nPCR diagnosis. The infection rate in cows was 29% out of 100 samples, while it was recorded in handler 10% out of 100 samples. Sequencing play an important role in the current study to verify the specificity and conduct a phylogenetic analysis of the samples. In this study, 10 nPCR products exhibited 100% similarity to the 18S ribosomal rRNA gene sequences of *Cryptosporidium* spp. from human and cow fecal samples available in GenBank. All sequences obtained were submitted to NCBI. The sequence identity between the 10 nPCR products from local isolates of *Cryptosporidium* spp. in cows and NCBI-BLAST *Cryptosporidium* spp. sequences showed 100% similarity for the cow species viz., *C. parvum*, *C. bovis*, *C. andersoni*, and *C. ryanae*. The sample also contained the species *C. hominis* and *C. parvum* for which the similarity of the 10 nPCR samples was 100%. This study explores zoonotic *Cryptosporidium* spp. in both human and cow fecal samples, highlighting the importance of molecular identification and phylogenetic analysis in understanding the transmission dynamics of these species; further research is recommended to explore the potential public health implications of these findings.

Keywords | Phylogenetic analysis, nPCR, *Cryptosporidium* spp, Handlers, Cows, Zoonotic protozoa

Received | January 22, 2025; **Accepted** | April 07, 2025; **Published** | May 26, 2025
***Correspondence** | Heba Ali Ghanim, Department of Microbiology, College of Medicine, Ibn Sina University of Medical and Pharmaceutical Sciences, Iraq; **Email:** heba.Ghanem.g@ibnsina.edu.iq
Citation | Ghanim HA, Abbas HB, Ali SS, Ahmed SA, Ahmed ZK, Alsadoon Z (2025). Molecular identification of *Cryptosporidium* spp. in cows and handlers in the Baghdad province. J. Anim. Health Prod. 13(2): 402-409.
DOI | https://dx.doi.org/10.17582/journal.jahp/2025/13.2.402.409
ISSN (Online) | 2308-2801

INTRODUCTION

Cryptosporidium species are obligate intracellular (extracytoplasmic), single-celled, apicomplexan parasites that infect both people and animals' respiratory and/or gastrointestinal systems. It is an important parasite, causes mild to severe profuse watery diarrhea in a wide variety of animals and humans (Checkley *et al*., 2015; Efstratiou *et al*., 2017). Cryptosporidiosis is a common zoonotic (Amphixenosis) disease, caused by the enteric pathogenic para-

site *Cryptosporidium* spp., widespread in humans and wide variety of animals, and recognized as one of the primary causes of diarrhea in newborn calves, which causes weight loss, growth retardation, and in extreme cases, illness and death, resulting in significant financial losses (Xiao, 2010; Striepen, 2013; Ryan *et al.*, 2014). Direct or indirect contact with an infected host can result in the transfer of *Cryptosporidium* oocysts, typically through the fecal oral pathway. Faecal-oral transmission is known to occur through person-to-person contact, zoonosis, and the ingestion of tainted food or drink (Ahmed and Karanis, 2020; Garcia, 2023). The epidemiology of cryptosporidiosis in cattle and human is not completely understood especially in the developing countries. *C. parvum* is most common species of zoonotic importance that identified in cattle, while several outbreaks of *C. parvum* in people have been associated with infected calves in United Kingdom, United States, Ireland, Germany, Belgium, Malaysia, Spain, Czech Republic, Japan, and Iran (Gait *et al.*, 2008; Silverla *et al.*, 2010). Contact with infected cattle has been found to be a major risk factor for the zoonotic transmission of *C. parvum* in youngsters and immunocompromised patients in Africa, particularly in Egypt and Ethiopia (Adamu *et al.*, 2014). Numerous factors, including age, cleanliness, colostrum feeding, management techniques, feed and water sources, diarrhea, and climate, have an impact on the occurrence of cryptosporidiosis (Ogendo *et al.*, 2017). Globally, prevalence data have confirmed the presence of cryptosporidiosis in cattle, with neonatal calves most frequently exhibiting the zoonotic *C. parvum* and adult cattle primarily exhibiting *C. andersoni* (Silverla *et al.*, 2010; Ryan *et al.*, 2014). According to the findings, calves younger than one month had a higher infection rate (25%) but calves two months and three months old had lower infection rates (10% and 12%, respectively). The aim of this study was to use a nested PCR molecular approach to detect *Cryptosporidium* infection in cows and handlers and to investigate the genotyping of *Cryptosporidium* species through sequencing.

MATERIALS AND METHODS

Ethical Approval

The Committee of Animal Ethics, Research of Scientific Deanship, College of Medicine, Ibn Sina University of Medical and Pharmaceutical Sciences, approved the experimental procedures of this study.

Samples Collection

Between September and November 2024, fecal samples (5–10 grams) were collected from 100 cows of various ages and sexes (male and female) from various parts of the province of Baghdad. Fecal samples were taken straight from the rectum or right after defecation and placed in a sterile plastic container. They were then securely sealed and labeled with the following information: age, sex, date of sampling, and sequential numbers. Ibn Sina University of Medical and Pharmaceutical Sciences' Department of Microbiology, College of Medicine, received the samples in a cold box.

Primers

The study used nested PCR primers from the NCBI-Genbank database to detect small subunit ribosomal RNA genes based on *Cryptosporidium* spp. Macrogen Company in Korea supplied these primers (Table 1).

Table 1: Nested PCR primers that used for detection of *Cryptosporidium* spp.

Primers	Sequence 5'-3'		Amplicon	Company/ Country
18SrRNA gene *Cryptosporidium* spp. PCR	F	AGACGGTAGGGTATTGGCCT	616bp	Macrogen/ Korea
	R	TCCTTGGCAAATGCTTTCGC		
Cryptosporidium spp. nCR18SrRNA gene	nF	AACGGGAATTAGGGTTCGA	567bp	
	nR	TGCTTTCGCATTAGTTTGTCTT		

Molecular Detection of *Cryptosporidium* by Nested PCR (PCR)

One hundred humans stool samples and one hundred cows' fecal samples were randomly selected for nPCR diagnosis. The nPCR technique was performed for detection *Cryptosporidium* spp. based 18S ribosomal rRNA gene from human and cow fecal samples. This method was carried out according to method described by Yu *et al.* (2009), Ruecker *et al.* (2013).

Genomic DNA Extraction

Following the guidelines provided by the company, genomic DNA was extracted from fecal samples using Geneaid Taiwan's Presto™ Stool DNA Extraction Kit.

Table 2: Components for PCR reaction.

PCR Master mix	Volume
DNA template 5-50ng	5µL
18SrRNA primary Forward primer (10pmol)	1 µL
18SrRNA primary Reverse primer (10pmol)	1 µL
Nuclease free water	13 µL
Total volume	20µL

PCR Reaction Preparation

AccuPower® PCR Premix was used to prepare the primary PCR master mix, which was completed in accordance with the company's instructions (Table 2). The components of the PCR master mix were added to standard AccuPow-

er® PCR Premix, which contained Taq DNA polymerase, dNTPs, Tris-HCl pH: 9.0, KCl, MgCl2, Stabilizer, and tracking dye. All of the PCR tubes were then placed in an Exispin vortex centrifuge and spun for three minutes at 3000 rpm. After that, it was put in a PCR thermocycler (BioRad USA, T100 Thermal cycler). The same conditions were used for the secondary PCR master mix.

PCR Thermocycler Conditions

The conditions for the PCR were set using a conventional thermocycler, as outlined in Table 3.

Table 3: Steps of PCR thermocycler system.

PCR step	Temp. °C	Time	Cycles
Initial Denaturation	95	5min	1
Denaturation	95	30sec.	35 cycle
Annealing	58	30sec	
Extension	72	30sec	
Final extension	72	5min	1
Hold	4	Forever	-

PCR Product Analysis

A 1.5% agarose gel was made by dissolving 1X TBE in a microwave at 100°C for one minute, then allowing it to cool to 50°C. The agarose gel solution was then treated with 3µ of ethidium bromide dye. After positioning the comb correctly, the agarose gel solution was put into the tray. The comb was then allowed to solidify for 15 minutes at room temperature before being carefully removed from the tray. Ten microliters of PCR product were added to each comb well, and five microliters of (100bp Ladder) were added to one well. The gel tray was filled with 1X TBE buffer and fixed in the electrophoresis chamber. After that, electric current was run for an hour at 100 volts and 80 AM. UV transilluminators were used to visualize convetional PCR products.

DNA Sequencing Method

Using an AB DNA sequencing system, ten PCR-positive samples from each human and cow were sequenced to identify *Cryptosporidium* species. These PCR small subunit ribosomal RNA gene-positive products were shipped by DHL in an ice bag to Macrogen Company in Korea. The c PCR technique was used to perform DNA sequencing for species typing of positive BLAST *Cryptosporidium* spp. isolates; the identified species isolates were then submitted to NCBI-GenBank, and phylogenetic tree analysis was conducted between the local *Cryptosporidium* species isolates and the NCBI-Blast submission *Cryptosporidium* species; Molecular Evolutionary Genetics Analysis Version X (MEGA X) was then used to perform the DNA sequencing analysis. The partial small subunit ribosomal RNA gene sequences were aligned multiple times using ClustalW alignment analysis, and the evolutionary distances were calculated using the Maximum Composite Likelihood Method by phylogenetic tree UPGMA method (Tamura *et al.*, 2013).

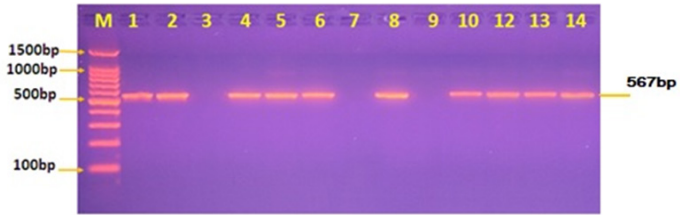

Figure 1: Agarose gel electrophoresis image that showed the nPCR product analysis of 18S rRNA gene of *Cryptosporidium* spp. in cow fecal samples. Where M: marker (1500-100bp) and Lane (1-14) some positive *Cryptosporidium* spp. was showed at (567bp) nPCR product.

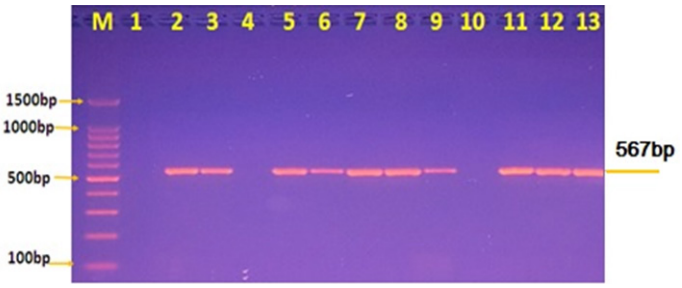

Figure 2: Agarose gel electrophoresis image that showed the nPCR product analysis of 18S rRNA gene of *Cryptosporidium* spp. in human stool samples. Where M: marker (1500-100bp) and Lane (1-13) some positive *Cryptosporidium* spp. was showed at (567bp) nPCR product.

RESULTS AND DISCUSSION

The nPCR technique was used to amplify the *Cryptosporidium* spp. infection. According to the marker ladder, the product of the 18S rRNA gene of *Cryptosporidium* spp. in cow fecal samples should have been 567 bp nPCR in length. Out of 100 samples, 29% of the cows had an infection (Figure 1). This result could not be compared to the findings of Benhouda *et al.* (2017), who found that the infection rate was 40% in young calves in Algeria and 35.5% in calves that had diarrhea in Sudan (Taha *et al.*, 2017). According to Zhang et al. (2018), the incidence was greater than the 14.4% recorded from dairy farms in China's Qinghai-Tibetan Plateau Area, 17.0% from cattle tested in Poland (Rzezutka and Kaupke, 2013), and 18.6% from cattle in Ethiopia (Manyazewal *et al.*, 2018). However, the infection rate was lower than the 42.85% recorded in Iraqi calves in Kut city (Mohammed, 2016), the 47.68% in Northeastern Chinese pre-weaned dairy calves (Zhang *et al.*, 2013), and the 52.2% in Algerian neonatal calves (Ouakli *et al.*, 2018). According to nPCR, 10% of

handlers in this study had cryptosporidiosis overall (Figure 2). Direct contact with cattle, a non-sterile water supply, and a lack of focus on parasite investigations, particularly those involving waterborne parasites like *Cryptosporidium* spp. were the causes of this incidence. The results of Altaee *et al*. (2014), who found that the infection rate among animal handlers was 47.72% in various parts of Baghdad city and 39.76% among youngsters in the Al-Ressafa neighborhood of Baghdad, were incomparable to this conclusion (Saeed and Khair, 2014). However, the prevalence was lower than the 50% among children in Wasit province (Rahi and Raheem, 2013), the 72% infection prevalence among handlers in Wasit province reported by Makawi and Al-Zubaidi (2017), and the 59% infection rate among children under 5 years in areas with high dairy cattle densities in New Zealand (Lal *et al*., 2016).

Table 4: NCBI -BLAST Homology sequence identity between local *Cryptosporidium* spp. cow isolates with NCBI-BLAST *Cryptosporidium* spp.

Local isolate	Genbank accession number	NCBI-BLAST Homology Sequence identity		
		NCBI BLAST *Cryptosporidium sp*	Accession number	Identity 100%
IQ.Cow No.1	PQ460369	*C. parvum*	MF074687.1	100%
IQ.Cow No.2	PQ460370	*C. parvum*	MF074687.1	100%
IQ.Cow No.3	PQ460371	*C. bovis*	KP334135.1	100%
IQ.Cow No.4	PQ460372	*C. parvum*	MF074687.1	100%
IQ.Cow No.5	PQ460373	*C. andersoni*	KJ531688.1	100%
IQ.Cow No.6	PQ460374	*C. andersoni*	KJ531688.1	100%
IQ.Cow No.7	PQ460375	*C. parvum*	MF074687.1	100%
IQ.Cow No.8	PQ460376	*C. ryanae*	MK501765.1	100%
IQ.Cow No.9	PQ460377	*C. andersoni*	KJ531688.1	100%
IQ.Cow No.10	PQ460378	*C. parvum*	MF074687.1	100%

However, compared to the 14% recorded in Al-Diwania city for youngsters, the infection rate was lower (Abas, 2011). 10.9% of children with diarrhea were reported in another study (Sadek, 2014); 8.35% of children who visited hospitals in the Mid-Euphrates Area were reported in another study (Abdul-Sada, 2015); 16.28% of displaced people in Kirkuk city were reported in another study (Salman et al., 2015); and 12.85% of children in Najaf province had persistent diarrhea (Tairsh *et al*., 2017). Hygiene systems, patient age and sex, sampling location (rural versus urban), environmental factors, sampling strategy and sample size, diagnostic methods used in various study locations, and direct contact with cattle could all be responsible for the discrepancies. Our findings are in line with those of other research. Only the density of dairy cattle was found to be significantly, positively linked with handlers' risk of contracting cryptosporidiosis in earlier research that included sheep, poultry, pigs, deer, and dairy cattle (Lal, 2014). Given the high prevalences of *C. parvum* and *C. andersoni* during the rainy season, water may be the primary means of transmission for the genotypes of *C. hominis, C. parvum,* and *C. andersoni* (Zahedi *et al*., 2018).

Table 5: NCBI -BLAST Homology sequence identity between local *Cryptosporidium* spp. isolates from handlers with NCBI-BLAST *Cryptosporidium* spp.

Local isolate	Genbank accession number	NCBI-BLAST Homology Sequence identity		
		NCBI BLAST *Cryptosporidium sp*	Accession number	Identity 100%
IQ.Human No.1	PQ460379	*C. parvum*	MF074687.1	100%
IQ.Human No.2	PQ460380	*C. parvum*	MF074687.1	100%
IQ.Human No.3	PQ460381	*C. hominis*	MH885553.1	100%
IQ.Human No.4	PQ460382	*C. parvum*	MF074687.1	100%
IQ.Human No.5	PQ460383	*C. hominis*	MH885553.1	100%
IQ.Human No.6	PQ460384	*C. hominis*	MH885553.1	100%
IQ.Human No.7	PQ460385	*C. parvum*	MF074687.1	100%
IQ.Human No.8	PQ460386	*C. parvum*	MK501765.1	100%
IQ.Human No.9	PQ460387	*C. hominis*	MH885553.1	100%
IQ.HumanNo.10	PQ460388	*C. parvum*	MF074687.1	100%

Sequencing and Phylogenetic Tree Analysis

Partial sequences of the 18S rRNA gene of isolates of *Cryptosporidium* spp. are identified using nPCR techniques. In order to do a phylogenetic analysis of the samples and confirm the specificity, sequencing is crucial to the current investigation. Ten nPCR products in this study showed 100% similarity to the 18S rRNA gene sequences of *Cryptosporidium* spp. data in GenBank and in fecal cow samples. All of the sequences were submitted to NCBI, and BLAST was used to look for similar published sequences using their reference numbers in GenBank. Local isolates samples sent to Bioneer company/Korea for analysis and compare with the NCBI-Genbank data Based on available information. Ten nPCR results from local isolates of *Cryptosporidium* spp. cows and NCBI-BLAST *Cryptosporidium* spp. showed 100% sequence identity for the following cow species: *C. parvum, C. bovis, C. andersoni,* and *C. ryanae* (Table 4). The sample also included the species *C. parvum and C. hominis*, for which the 10 nPCR results showed 100% similarity (Table 5). Phylogenetic tree UPGMA (MEGA X version) was used to calculate the evolutionary distances using the Maximum Composite Likelihood approach (Figure 3 and 4). The NCBI-Blast *C. parvum* isolate (MF074687.1) was shown to be closely related to the local *Cryptosporidium* spp. cow isolates No. 1,

2, 4, 7, and 10. At total genetic change (0.01-0.04%), the local *Cryptosporidium* spp. cow isolates of No. 5, 6, and 9 were linked to the NCBI-Blast *Cryptosporidium andersoni* isolate (KJ531688.1), the local *Cryptosporidium* spp. cow isolates of No. 3 were linked to the NCBI-Blast *Cryptosporidium* bovis isolate (KP334135.1), and the local *Cryptosporidium* spp. cow isolates of No. 8 were shown to be closed related to NCBI-Blast *Cryptosporidium* ryanae isolate (MK501765.1) (Figure 5).

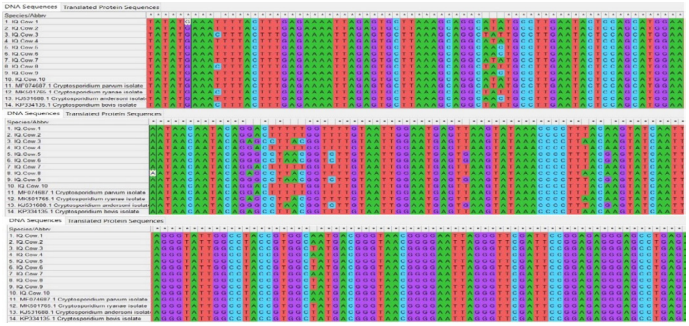

Figure 3: Multiple sequence alignment analysis of the partial 18 Small subunit rRNA gene sequence of local *Cryptosporidium* cow isolates and NCBI-Genbank *Cryptosporidium* spp. from different isolates based on ClustalW alignment analysis by using (MEGA X, multiple alignment analysis tool). The multiple alignment analysis similarity (*) and differences in 18 Small subunit rRNA gene nucleotide sequences.

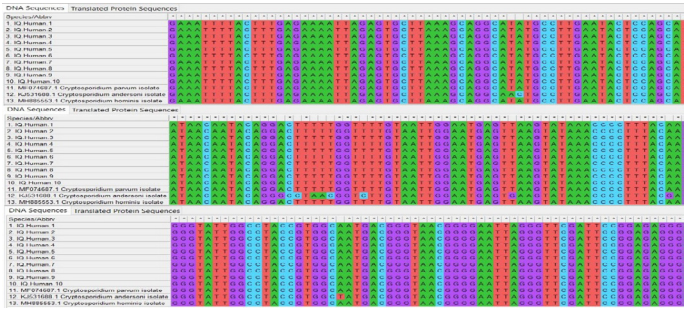

Figure 4: Multiple sequence alignment analysis of the partial 18 Small subunit rRNA gene sequence of local *Cryptosporidium* human isolates and NCBI-Genbank *Cryptosporidium* spp. from different isolates based on ClustalW alignment analysis by using (MEGA X, multiple alignment analysis tool). The multiple alignment analysis similarity (*) and differences in 18 Small subunit rRNA gene nucleotide sequences.

Human isolates were used to analyze the genetic identity of *Cryptosporidium* species. The Maximum Composite Likelihood technique via phylogenetic tree UPGMA (MEGA X version) was used to calculate the evolutionary distances. *Cryptosporidium* species in the area. The NCBI-Blast *C. parvum* isolate (MF074687.1) and the local *Cryptosporidium* spp. were shown to be closely related to the human isolates No. 1, 2, 4, 7, 8, and 10. At total genetic alteration (0.01-0.06%), human isolates No. 3, 5, 6, and 9 were shown to be closely related to NCBI-Blast *Cryptosporidium hominis* isolate (MH885553.1) (Figure 6).

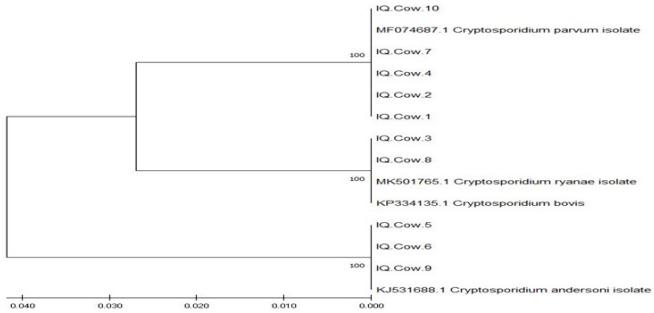

Figure 5: Phylogenetic tree analysis based on the partial sequence 18 small subunit rRNA gene in local *Cryptosporidium* cow isolates that used for *Cryptosporidium* species genetic identification analysis.

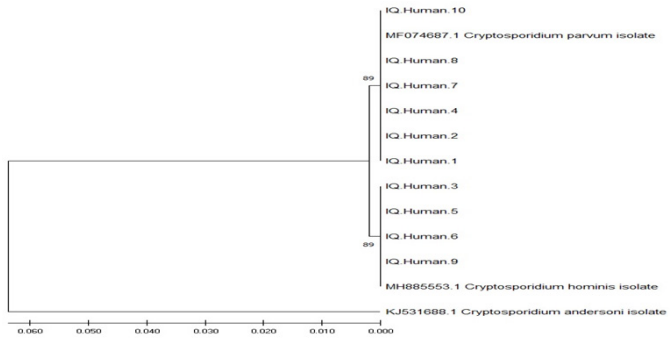

Figure 6: Phylogenetic tree analysis based on the partial sequence 18 small subunit rRNA gene in local *Cryptosporidium* isolates from handlers that used for *Cryptosporidium* species genetic identification analysis.

Recently, the most used method for typing *Cryptosporidium* isolates from various host and geographic sources is DNA sequencing (Xiao *et al*., 2000). The small subunit rRNA-based DNA sequencing used in this investigation was the same as that used in other earlier investigations (Xiao *et al*., 2004; Caccio *et al*., 2007; Fayer, 2010). The molecular study's findings indicated that four major species of *Cryptosporidium* were present in cattle: *C. parvum, C. andersoni,* and *C. bovis*. These findings were consistent with those of Khan *et al*. (2010) in Indian cattle, Zhang *et al*. (2013) in calves in northeast China, Rzezutka and Kaupke (2013) in Poland, and Taha *et al*. (2017) in calves in Sudan. Three *Cryptosporidium* species *C. bovis, C. ryanae* and *C. parvum*—were found in young calves in Algeria by Benhouda *et al*. (2017). The use of PCR techniques to amplify particular DNA sequences has shown great promise in the development of highly sensitive and specific diagnostic assays. Because of the gene's multi-copy nature and nested method, the polymerase chain reaction highlighted the sensitivity. Additional benefits of PCR include its capacity for batching, interpretability, and additional discrimination

at the species and genotype/subgenotype levels (Geurden *et al*., 2006). It is also essential to confirm the species detected using genetic methods because *Cryptosporidium* species cannot be identified just by morphology (Robinson *et al*., 2006). In order to identify species and diagnose *Cryptosporidium* infections, the current study also makes use of molecular detection techniques. Nested PCR and sequence analysis targeting the Small Subunit rRNA gene were used to identify the species of *Cryptosporidium* spp. in Baghdad province, Iraq. The amplified region was selected because it encompasses the major region of interspecies/genotype variability in the gene, enabling the identification of almost all *Cryptosporidium* spp. and genotypes through sequence analysis of the PCR product. The abundance of multi-copy hyper-variable sections indicates that this gene is highly conserved (Yoder and Beach, 2010). In the Iraqi province of Baghdad, three species of *C. hominis, C. parvum,* and *C. andersoni* were found in handlers. Our findings concur with those of Leoni *et al.* (2006), who identified eight species of *Cryptosporidium* in 2414 diarrheal individuals in England, including *C. hominis, C. parvum, C. meleagridis, C. andersoni, Cryptosporidium felis, C. canis, C. suis,* and *C. cervine* type. Only two species were found in Iraq by Abdul-Sada (2015) and Jawad (2015), including *C. parvum* and *C. hominis.*

CONCLUSIONS AND RECOMMENDATIONS

The results of this study indicate that *Cryptosporidium* spp. are among the most prevalent parasites that pose a significant threat to both human and animal health, as well as public safety. The widespread presence of the parasite in both humans and animals is concerning. From results of phylogenetic analysis ten local isolates from handlers and calves were deposited in the gene bank.

ACKNOWLEDGEMENTS

The authors would like to thank the Department of Microbiology, College of Medicine, Ibn Sina University of Medical and Pharmaceutical Sciences, as well as the field technicians who helped with the study.

NOVELTY STATEMENT

Molecular detection of *Cryptosporidium* in cows and handlers was carried out and ten new species of *Cryptosporidium* were registered in the gene bank.

AUTHOR'S CONTRIBUTIONS

All of the trials were designed by Heba Ali Ghanim and Hydear Barakat Abbas. Zaid Khalid Alani and Shahad Abdullah Shwan conducted all of the tests, gathered the data, and composed the manuscript draft. Sara Ayad Ahmed and Zeid Alsadoon helped with the data analysis that was done to prepare the work for submission to the journal. The final draft of the work was reviewed and approved by all authors for publication in the Journal of Animal and Health Production.

Ethical Consideration

Not applicable.

Conflict of Interest

The authors state that there is not conflict of notice.

REFERENCES

Abas Jasm G (2011). Prevalence Of Cryptosporidiosis in Calves and Child in Al Diwania City: Alaa mohammed abdul Razak. Ghaidaa abas Jasm. Iraqi J. Vet. Med., 35(1):190-194. https://doi.org/10.30539/iraqijvm.v35i1.623

Abdul-Sada KM (2015). Molecular and epidemiological study of *Cryptosporidium* spp. Mid-Euphrates Area. Kufa J. Nursing Sci., 5(1):179-89. https://doi.org/10.36321/kjns.vi20151.3163

Adamu H, Petros B, Zhang G, Kassa H, Amer S, Ye J, Feng Y, Xiao L (2014). Distribution and clinical manifestations of *Cryptosporidium* species and subtypes in HIV/AIDS patients in Ethiopia. PLoS Negl. Trop. Dis., 8(4):e2831. https://doi.org/10.1371/journal.pntd.0002831

Ahmed SA, Karanis P (2020). *Cryptosporidium* and cryptosporidiosis: the perspective from the Gulf countries. International journal of environmental research and public health, 17(18), 6824.

Altaee IA, Al-Ani JK, Al-Rubaie HM (2014). Prevalence of Giardia spp. and *Cryptosporidium* spp. in horses and animal handlers in some areas in Baghdad city. Al-Anbar J. Vet. Sci., 7(2).

Ali BA, Marif HF, Ali KN, Raoof HS, Sulaiman RR, Baba Sheikh MO (2024). Molecular Characterization and Phylogenic Analysis of *Cryptosporidium* Species Isolated from Cattle in Sulaymaniyah, Iraq. Assiut Vet. Med. J., 70(182): 125-135 https://doi.org/10.21608/avmj.2024.270775.1228.

Benhouda D, Hakem A, Sannella AR, Benhouda A, Cacciò SM (2017). First molecular investigation of *Cryptosporidium* spp. in young calves in Algeria. Parasite, 24. https://doi.org/10.1051/parasite/2017014

Cacciò SM, Rinaldi L, Cringoli G, Condoleo R, Pozio E (2007). Molecular identification of *Cryptosporidium parvum* and *Giardia duodenalis* in the Italian water buffalo (Bubalus bubalis). Vet. Parasitol., 150(1-2):146-9. https://doi.org/10.1016/j.vetpar.2007.09.013

Checkley W, White AC, Jaganath D, Arrowood MJ, Chalmers RM, Chen XM, Fayer R, Griffiths JK, Guerrant RL, Hedstrom L, Huston CD (2015). A review of the global burden, novel diagnostics, therapeutics, and vaccine targets for *Cryptosporidium*. Lancet Infect. Dis., 15(1):85-94. https://doi.org/10.1016/S1473-3099(14)70772-8

Efstratiou A, Ongerth JE, Karanis P (2017). Waterborne transmission of protozoan parasites: review of worldwide

outbreaks-an update 2011–2016. Water Res., 114:14-22. https://doi.org/10.1016/j.watres.2017.01.036

Fayer R, Santín M, Dargatz D (2010). Species of *Cryptosporidium* detected in weaned cattle on cow–calf operations in the United States. Vet. Parasitol., 170(3-4):187-92. https://doi.org/10.1016/j.vetpar.2010.02.040

Gait R, Soutar RH, Hanson M, Fraser C, Chalmers R (2008). Outbreak of cryptosporidiosis among veterinary students. Vet. Rec., 162(26):843-5. https://doi.org/10.1136/vr.162.26.843

Garcia LS (2023). Cryptosporidiosis. In Sexually Transmitted Diseases (pp. 189-204). CRC Press.

Geurden T, Goma FY, Siwila J, Phiri IG, Mwanza AM, Gabriël S, Claerebout E, Vercruysse J (2006). Prevalence and genotyping of *Cryptosporidium* in three cattle husbandry systems in Zambia. Vet. Parasitol., 138(3-4):217-22. https://doi.org/10.1016/j.vetpar.2006.02.009

Jawad TI (2015). Genotyping of *Cryptosporidium* isolates from clinical samples. Med. J. Babylon, 12(3):632-7.

Khan SM, Debnath C, Pramanik AK, Xiao L, Nozaki T, Ganguly S (2010). Molecular characterization and assessment of zoonotic transmission of *Cryptosporidium* from dairy cattle in West Bengal, India. Vet. Parasitol., 171(1-2):41-7. https://doi.org/10.1016/j.vetpar.2010.03.008

Lal A (2014). Evaluating the environmental and social determinants of enteric disease in New Zealand, Doctoral dissertation, University of Otago.

Lal A, Dobbins T, Bagheri N, Baker MG, French NP, Hales S (2016). Cryptosporidiosis risk in New Zealand children under 5 years old is greatest in areas with high dairy cattle densities. Eco. Health, 13:652-60. https://doi.org/10.1007/s10393-016-1187-8

Leoni F, Amar C, Nichols G, Pedraza-Diaz S, McLauchlin J (2006). Genetic analysis of *Cryptosporidium* from 2414 humans with diarrhoea in England between 1985 and 2000. J. Med. Microbiol., 55(6):703-7. https://doi.org/10.1099/jmm.0.46251-0

Makawi ZA, Al-Zubaidi MT (2017). Parasitic contamination of drinking water and its prevalence among handlers and sheep. Iraqi J. Vet. Med., 41(2): 7-14 https://doi.org/10.30539/iraqijvm.v41i2.41

Manyazewal A, Francesca S, Pal M, Gezahegn M, Tesfaye M, Lucy M, Teklu W, Getachew T (2018). Prevalence, risk factors and molecular characterization of *Cryptosporidium* infection in cattle in Addis Ababa and its environs, Ethiopia. Veterinary Parasitology: Reg. Stud. Rep., 13:79-84. https://doi.org/10.1016/j.vprsr.2018.03.005

Mohammed ST (2016). Detection the genotyping of *Cryptosporidium parvum* isolated from human and calves and studying in vivo effecte of *Sacharomyces boulardii* on parasite. Al-Mustansiriyah J. Sci., 27(3).

Ogendo A, Obonyo M, Wasswa P, Bitek A, Mbugua A, Thumbi SM (2017). *Cryptosporidium* infection in calves and the environment in Asembo, Western Kenya: 2015. Pan Afr. Med. J., 28(Suppl 1). https://doi.org/10.11604/pamj.supp.2017.28.1.9313

Ouakli N, Belkhiri A, de Lucio A, Köster PC, Djoudi M, Dadda A, Khelef D, Kaidi R, Carmena D (2018). *Cryptosporidium*-associated diarrhoea in neonatal calves in Algeria. Vet. Parasitol. Reg. Stud. Rep., 12:78-84. https://doi.org/10.1016/j.vprsr.2018.02.005

Rahi AA, Raheem HH (2013). Prevalence of *Cryptosporidium parvum* Among Children At Wasit Province. J. Wasit Sci. Med., 6(1):1-7. https://doi.org/10.31185/jwsm.202

Robinson G, Thomas AL, Daniel RG, Hadfield SJ, Elwin K, Chalmers RM (2006). Sample prevalence and molecular characterisation of *Cryptosporidium andersoni* within a dairy herd in the United Kingdom. Vet. Parasitol., 142(1-2):163-7. https://doi.org/10.1016/j.vetpar.2006.06.031

Rousseau A, La Carbona S, Dumètre A, Robertson LJ, Gargala G, Escotte-Binet S, Favennec L, Villena I, Gérard C, Aubert D (2018). Assessing viability and infectivity of foodborne and waterborne stages (cysts/oocysts) of *Giardia duodenalis*, *Cryptosporidium* spp., and *Toxoplasma gondii*: a review of methods. Parasite, 25. https://doi.org/10.1051/parasite/2018009

Ruecker NJ, Matsune JC, Lapen DR, Topp E, Edge TA, Neumann NF (2013). The detection of *Cryptosporidium* and the resolution of mixtures of species and genotypes from water. Infect. Genet. Evol., 15:3-9. https://doi.org/10.1016/j.meegid.2012.09.009

Ryan UN, Fayer R, Xiao L (2014). *Cryptosporidium* species in humans and animals: current understanding and research needs. Parasitology, 141(13):1667-85. https://doi.org/10.1017/S0031182014001085

Rzeżutka A, Kaupke A (2013). Occurrence and molecular identification of *Cryptosporidium* species isolated from cattle in Poland. Vet. Parasitol., 196(3-4):301-6. https://doi.org/10.1016/j.vetpar.2013.03.009

Sadek G (2014). Use of nested PCR-RFLP for genotyping of *Cryptosporidium* parasites isolated from calves and children suffering from diarrhea. Parasitologists United J., 7(2):129 https://doi.org/10.4103/1687-7942.149568

Saeed AK, Khairi NM (2014). Study The Prevalence of Giardiasis and Cryptosporidiosis Among Children at Al-Ressafa Side of Baghdad by Comparison Between the Efficiency of Some Diagnostic Methods. AL-Taqani. 27(2):82-93.

Salman YJ, Sadek WS, Rasheed ZK (2015). Prevalence of *Cryptosporidium parvum* among Iraqi displaced people in Kirkuk city using direct microscopy, flotation technique and ELISA-copro antigen test. Int. J. Curr. Microbiol. App. Sci., 4(11):559-572.

Silverlås C (2010). *Cryptosporidium* infection in dairy cattle. Prevalence, species distribution and associated management factors [PhD dissertation]. Swedish: Swedish University of Agricultural Sciences. 33-41.

Silverlås C, Näslund K, Björkman C, Mattsson JG (2010). Molecular characterisation of *Cryptosporidium* isolates from Swedish dairy cattle in relation to age, diarrhoea and region. Vet. Parasitol., 169(3-4):289-95. https://doi.org/10.1016/j.vetpar.2010.01.003

Striepen B (2013). Parasitic Infections: Time to tackle cryptosporidiosis. Nature, 503: 189-191. https://doi.org/10.1038/503189a

Taha S, Elmalik K, Bangoura B, Lendner M, Mossaad E, Daugschies A (2017). Molecular characterization of bovine *Cryptosporidium* isolated from diarrheic calves in the Sudan. Parasitol. Res., 116:2971-9. https://doi.org/10.1007/s00436-017-5606-8

Tairsh HR, Abdul AL, Al-A-asady RA, AL-zeyadi MJ, Ali H

(2017). Identification of *Cryptosporidium* spp. Infections in Children with Persistent Diarrhea by Modified Ziehl-Neelsen Stain Method and PCR Technique. Eur. J. Pharm. Med. Res., 4(2):208-15.

Tamura K, Stecher G, Peterson D, Filipski A, Kumar S (2013). MEGA6: molecular evolutionary genetics analysis version 6.0. Molecular Biol. Evol., 30(12):2725-9. https://doi.org/10.1093/molbev/mst197

Venu R (2010). *Molecular detection and typing of Cryptosporidium in dairy calves* (Doctoral dissertation, Tamil Nadu Veterinary and Animal Sciences University).

Xiao L (2010). Molecular epidemiology of cryptosporidiosis: an update. Exp. Parasitol., 124(1):80-9. https://doi.org/10.1016/j.exppara.2009.03.018

Xiao L, Alderisio K, Limor J, Royer M, Lal AA (2000). Identification of species and sources of *Cryptosporidium* oocysts in storm waters with a small-subunit rRNA-based diagnostic and genotyping tool. Appl. Environ. Microbiol., 66(12):5492-8. https://doi.org/10.1128/AEM.66.12.5492-5498.2000

Xiao L, Fayer R, Ryan U, Upton SJ (2004). *Cryptosporidium* taxonomy: recent advances and implications for public health. Clin. Microbiol. Rev., 17(1):72-97. https://doi.org/10.1128/CMR.17.1.72-97.2004

Yoder JS, Beach MJ (2010). *Cryptosporidium* surveillance and risk factors in the United States. Exp. Parasitol., 124(1):31-9. https://doi.org/10.1016/j.exppara.2009.09.020

Yu JR, Lee SU, Park WY (2009). Comparative sensitivity of PCR primer sets for detection of *Cryptosporidium parvum*. Korean J. Parasitol., 47(3):293. https://doi.org/10.3347/kjp.2009.47.3.293

Zahedi A, Monis P, Gofton AW, Oskam CL, Ball A, Bath A, Bartkow M, Robertson I, Ryan U (2018). *Cryptosporidium* species and subtypes in animals inhabiting drinking water catchments in three states across Australia. Water Res., 134:327-40. https://doi.org/10.1016/j.watres.2018.02.005

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. https://doi.org/10.1371/journal.pone.0054857

Zhang X, Jian Y, Li X, Ma L, Karanis G, Qigang C, Karanis P (2018). Molecular detection and prevalence of *Cryptosporidium* spp. infections in two types of domestic farm animals in the Qinghai-Tibetan Plateau Area (QTPA) in China. Parasitol. Res., 117:233-9. https://doi.org/10.1007/s00436-017-5697-2