Archives of Razi Institute, Vol. 77, No. 6 (2022) 2345-2352

DOI: 10.22092/ARI.2022.358621.2271

Original Article

First Molecular Genotyping of *Cryptosporidium felis* in Cattle, Iraq

Al-Abedi, G. J. K[1 *], Al-Eodawee, E. M. M[1], Khalili, S[2], Gharban, H. A. J[1]

1. College of Veterinary Medicine, University of Wasit, Wasit, Iraq
2. Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran

Received 7 May 2022; Accepted 8 June 2022
Corresponding Author: ghjabar@uowasit.edu.iq

Abstract

To the best of our knowledge, this is the first Iraqi study to detect *C. felis* in cattle by the polymerase chain reaction (PCR) assay and to confirm the local isolates in the National Center for Biotechnology Information (NCBI). Overall, 130 diarrheic calves of different ages and sexes were selected randomly from rural and sub-urban areas in Wasit province (Iraq) from February to April (2021) and subjected to direct collection of fresh fecal samples for DNA extraction and PCR examination. Targeting the heat shock protein 70 (HSP70) gene of *C. felis* showed that 17.69% of the animals were positive. Findings from clinical examination revealed no significant differences in values of temperature, pulse, or respiratory rates between the positive and negative calves. The association between the positive results and demographic risk factors showed no significant differences in the prevalence rate of infection and risk of exposure to *C. felis* between the rural and sub-urban areas; however, higher significant values were reported in calves aged 6 months than in calves aged 12 months, as well as in females than in males. Five of the local *C. felis* isolates were documented under the accession numbers MZ964600.1, MZ964601.1, MZ964602.1, MZ964603.1, and MZ964604.1. Finally, the data presented here provide epidemiological and molecular evidence that the range of *C. felis* in cattle is wider than expected globally, with a high probability of infection transmission from cattle to humans.

Keywords: Calves, PCR, Phylogenetic Analysis, Cryptosporidiosis, Diarrhea

1. Introduction

Cryptosporidium genus is one of the most important intracellular protozoan parasites, which belongs to the *Eucoccidiorida* Order of *Apicomplexa* Phylum. In the 1907s, the parasite was detected for the first time in mice (1), while in cattle and humans, it was first isolated in 1970 (2) and 1976 (3), respectively. Approximately 30 species of *Cryptosporidium* are recently recognized to infect mammals, fish, reptiles and birds. These species are classified according to host specificity, life cycle, morphology and the size of the oocysts (4). Additionally, the species of this parasite shown to have several unique characteristics that differentiate them from other protozoa, such as innate resistance to disinfectants, ability to initiate self-infection, and their ability to be unusually located in the host cell, cell membrane and sequestrations between cytoplasm (5).

In the last 20 years, although there have been dramatic increases in studies about cryptosporidiosis, critical questions concerning the epidemiology, life cycle, transmission, cell invasion, and host-parasite interaction remain unclear. This parasite appears to be transmitted due to contact with infected animals (6). Canine and felines are the usual hosts of *Cryptosporidium* and are thought to act as a reservoir (7). In 1979, *C. felis* was discovered firstly in a cat that acts as a major host (8), but humans and cattle can be

infected as minor hosts (9). In cattle, newborns and calves showed a higher susceptibility to cryptosporidiosis shortly post birth and were still infected for weeks or months, as identified in dairy and beef cattle (10).

Early epidemiological reports were applied to detect the morphology and immunology of the parasite without sub-typing of *Cryptosporidium* spp.; but in the last decade, molecular diagnostic techniques have been used extensively as they are highly sensitive and specific in the identification of host species and genotyping (7). Polymerase chain reaction (PCR) assay was the almost molecular method employed for detecting *Cryptosporidium* oocyst and determining the species and genotype in many clinical and environmental samples (11). Sequencing of DNA considers a gold standard way for identification of mutation rapidly and for discovering polymorphism in a single nucleotide (12). To date, sequencing is used to enable *Cryptosporidium* classification, compare genetic materials and phenotypes differences, and evaluate genetic variations within *Cryptosporidium* spp. (13).

In Iraq, many studies were carried out to detect *Cryptosporidium* spp. (14, 15), however, there were neither online data nor reports about *C. felis* in any animal or human. Hence, this study was designed to demonstrate *C. felis* infections in cattle using the molecular PCR assay and to document some local isolates in the NCBI for the first time in Iraq. The relationship of positive results to clinical and demographic risk factors of study animals was also aimed.

2. Materials and Methods

2.1. Samples and Data Collection

Overall, 130 female and male diarrheic calves of ≤1 year old were selected from different sub-urban and rural regions in Wasit province (Iraq) from February to April (2021). Each study animal was subjected to directly sampling fresh fecal (about 50 gm) into a labelled plastic container that was transferred cooled to the laboratory. Also, all study animals were examined clinically to estimate their body temperature, pulse and respiratory rates.

2.2. Molecular Assay

About 1 gm of each fecal sample was subjected to extraction of the DNAs following the manufacturer's instructions for the Presto™ Stool DNA Extraction Kit (Geneaid, Korea). The purity and concentration of extracted DNAs were checked using the Nanodrop system (Thermo-Scientific, UK).

Targeting the HSP70 gene of *C. felis*, one set of specific primers [(F: 5′-AACCTGGTTGATCC TGCCAGTAGTC-3′) and (R: 5′-CCCATTTCCTTCGAAACAGGA-3′)] was designed (16) and provided by the Scientific Researcher Co. Ltd. (Iraq). Then, the Mastermix tubes were prepared using a ready-to-use Go Taq ® Green Master Mix Kit (Promega, USA) at a final volume of 25 µl, to be subjected later to the conditions of Thermal Cycler (Bio-Rad, USA) as follows: 1 cycle initial denaturation (95°C / 2 minutes), 33 cycles for denaturation (95°C / 30 seconds), annealing (60°C / 30 seconds) and extension (72°C / 50 seconds), and 1 cycle final extension (72°C / 5 minutes). The PCR products were analyzed using the stained 1.5% agarose gel with Ethidium bromide at 100 volts and 80 Am for 1 hour. The positive samples were amplified at 480bp.

Five positive PCR products were selected randomly for sequencing at the Macrogen Company (Korea) using the Sanger method, and the received data were submitted later for documentation in the NCBI. The local isolates were aligned with the NCBI-BLAST isolates for phylogenetic tree analysis and homologic identity.

2.3. Statistical Analysis

All data were analyzed using the GraphPad Prism (GraphPad Software Inc., USA) by One-way ANOVA test and *t*-test. Values of clinical examination were represented as mean±standard deviation (M±SD). Statistically, significant differences were detected at $P<0.05$ (17).

3. Results

23 (17.69%) diarrheic calves were positive for *C. felis* targeting the HSP70 gene (Figure 1). Concerning

clinical examination, positive calves to *C. felis* revealed insignificant variation (*P*>0.05) in values of temperature (38.72±1.23) ºC, pulse rate (89.25±2.52), beats/minute (bpm), and respiratory rate (39.04±1.88) breaths/minute (bpm), when compared to values of negative calves; (38.48±1.56) ºC, (85.61±2.34) bpm, and (37.93±1.79) bpm, respectively (Figure 2). Regarding demographic risk factors, the findings showed a significant variation (*P*<0.05) between the values of the study groups (Table 1). For region factor, the prevalence of *C. felis* and the risk of infection increased insignificantly (*P*≤0.078) in rural (18.18% and 1.09, respectively) areas when compared to sub-urban (16.67% and 0.92, respectively) areas. Significantly, a higher rate of infection and risk was shown in calves aged <6 months (24.56% and 2, respectively) than in calves aged ≥6-12 months (12.33% and 0.5, respectively) (*P*≤0.03). Among groups of sex factors, females revealed a significant elevation (*P*≤0.015) in prevalence rate (20.79%) and exposure risk (3.02) to *C. felis* infection more than males (6.9% and 0.33, respectively).

In this study, 5 local *C. felis* isolates were documented and named in the NCBI as *Cryptosporidium felis* Cattle No. 1, 2, 3, 4 and 5 isolates with the respective following accession numbers; MZ964600.1, MZ964601.1, MZ964602.1, MZ964603.1 and MZ964604.1. Multiple alignment analyses constructed using the NCBI-BLAST alignment tool showed a significant similarity (*) in HSP70 (Figure 3). Phylogenetic tree analysis based on HSP70 revealed that the genetic variant between the local *C. felis* isolates and NCBI-BLAST isolate was 0.0035-0.0005. An analysis of homology sequence identity between the local *C. felis* isolates and the NCBI-BLAST *C. felis* isolate detected a significant high identity between the local *C. felis* isolates and the Sweden *C. felis* (KP642069.1) isolate ranging 99.45% to 99.72% (Table 2, Figure 4).

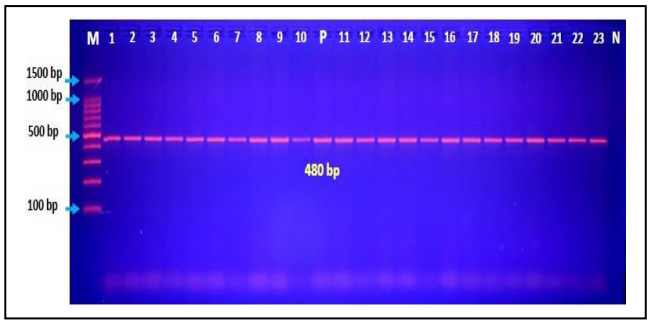

Figure 1. Agarose gel electrophoresis of PCR products targeting the HSP70 gene
Lane M: Ladder marker at 1500-100 bp; Lanes 1-23: Positive samples for *C. felis*; Lane P: Positive control; Lane N: Negative control

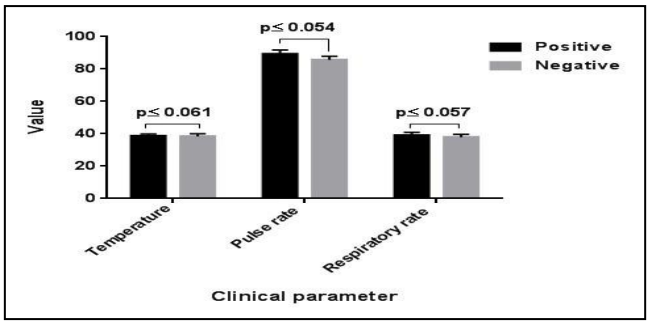

Figure 2. Results of clinical examination for positive and negative calves to *C. felis*

Table 1. Results of demographic risk factors for positive calves to *C. felis*

Factor	Total No.	Positive	Odd ratio	Risk	*P*-value
Region					
Rural	88	16 (18.18%)	1.3	1.09	0.078
Sub-urban	42	7 (16.67%)	0.77	0.92	
Age (Month)					
< 6	57	14 (24.56%) *	2.33	2	0.03
≥ 6-12	73	9 (12.33%)	0.43	0.5	
Sex					
Female	101	21 (20.79%) *	3.55	3.02	0.015
Male	29	2 (6.9%)	0.28	0.33	

Significance * (*P*<0.05)

Figure 3. Partial sequence of local *C. felis* isolates based on HSP70 gene

Table 2. Homology sequence identity between the local and NCBI-BLAST *C. felis* isolates

Local isolates of *C. felis*		NCBI-BLAST *C. felis* isolate		
Name	**Accession number**	**Country**	**Accession number**	**Identity (%)**
C. felis cattle No. 1	MZ964600.1	Sweden	KP642069.1	99.45
C. felis cattle No. 2	MZ964601.1	Sweden	KP642069.1	99.72
C. felis cattle No. 3	MZ964602.1	Sweden	KP642069.1	99.45
C. felis cattle No. 4	MZ964603.1	Sweden	KP642069.1	99.45
C. felis cattle No. 5	MZ964604.1	Sweden	KP642069.1	99.72

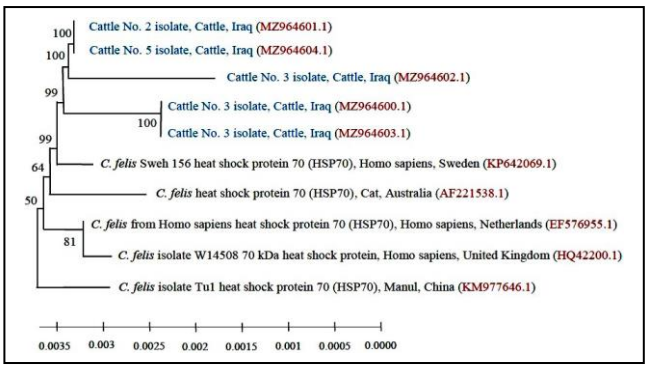

Figure 4. Phylogenetic tree analysis of the local and NCBI-BLAST *C. felis* isolates based on the HSP70 gene

4. Discussion

In the field, cryptosporidiosis can result in variable degrees of illness and poor health for both human and domestic animals due to direct and indirect contact with infected animals (7). Salyer, Silver (18) mentioned that infected companion animals, especially dogs and cats, risk transferring 60% of most infectious diseases. In this study, 17.69% of calves were positive for *C. felis* targeting the HSP70. However, several reports demonstrated infections of *Cryptosporidium* spp. (15), *C. hominis* (19), *C. parvum* (20), *C. andersoni* (21), *C. bovis* (22) and *C. ryanae* (15), little known are available about *C. felis* infection in cattle (23). Additionally, many studies have indicated significant variation in the prevalence of *Cryptosporidium* spp. between countries as *C. bovis* and *C. andersoni* are the almost detected species in African countries (24), while *C. parvum* is more prevalent in Australia (25) as well as the countries of Asia (26), North America (27) and Europe (28). However, the worldwide occurrence of cryptosporidiosis in cattle ranged 1.59-44.93% in African (29), 2.84-91.14% in American (30), 0.02-72.52% in Asian (31), and 2.56-55.6% in European (32) countries in addition to 0-71.85% in Australia (33) and 15.42-25.92% in New Zealand (34).

Based on our results, the HSP70 gene appeared as a highly sensitive tool for prevalence detection and genotyping of *C. felis*, which might be attributed to great levels of heterogeneity distribution over an entire sequence. Other studies have suggested other reasons for choosing of *Cryptosporidium* HSP70 gene, such as mRNA production that induces through simple heating to provide the desired inherent biological amplification; secondly, the HSP70 gene can be used as an indicator for oocyst viability, the half-life of HSP70 increases at least 10-fold upon heat shock, and easy to be identified from other related parasites which permitting for multiplexed detection (35).

We showed that values of the vital clinical parameters (temperature, pulse and respiratory rates) differed insignificantly, although all study animals were diarrheic. These findings indicate that *C. felis* infection in cattle does not result in a systemic reaction. Different studies have demonstrated that infections of *Cryptosporidium* spp. in cattle are commonly asymptomatic, particularly in adult animals (32, 36). Rieux, Paraud (37) summarized that the prevalence rate of infection and the level of oocysts excretion vary greatly with distinct patterns according to the year. Other previously (38) and recently (39) reports found that *C. parvum* is calves' most commonly detected enteropathogenic cryptosporidial agent. de Graaf, Vanopdenbosch (40) mentioned that diarrhea in calves is a multifactorial disease governed by a wide range of factors related to the animal, condition of the environment and husbandry, and a variety of viruses, bacteria and protozoan parasites.

Statistical analysis for demographic risk factors in this study revealed a significant variation in values of age and sex groups but not in region groups. Calves of <6 months appeared at a higher rate and risk of *C. felis* infection than calves ages ≥ 6 months. These results were consistent with those observed by Bawm, Kyi (41) and Hussin, Khalaf (42). However, the high infection rate of cryptosporidiosis in calves aged <6 months might be attributed to poor immunity and high susceptibility to infection. Rieux, Paraud (37) showed that the age-related prevalence of cryptosporidiosis in calves is variable with systemically almost observation at the 2nd and 3rd weeks of life. In the USA, Lemeteil, Roussel (43) indicated that *Cryptosporidium* spp. is responsible for about 85-97% of *Cryptosporidium* infections in pre-weaned calves; but 1-4% of *Cryptosporidium* infections in post-weaned calves. Other studies demonstrated that the prevalence of specific *Cryptosporidium* species/subtypes changes among the different age groups of cattle (44). Some factors related to age or previous *Cryptosporidium* exposure might have delayed the onset of shedding in that study (45). Mamedova and Karanis (19) recorded that in the farm communities from the vertical belts, lowlands, foothills, and mountains, the younger cattle

suffered higher consequences of *Cryptosporidium* infection than adult cattle. For sex factors, female calves showed a higher rate and risk of cryptosporidiosis than males. This result was similar to that reported by Mathew, David (46) and in contrast to those detected by Changizi, Salimi-Bejestani (47), who found no relationship between positive cases and sex of cattle, and to Ibrahim, Abdel-Ghany (48), who showed that *Cryptosporidium* prevalence was higher in males than females. Although the exact reason for the current observation is not apparent, we attributed this disparity between females and males to the usual practice of having a higher female-to-male ratio in a herd and the retention of female animals for breeding and milk production. The absence of significant differences in the prevalence of *C. felis* between calves of rural and suburban regions might presumably be due to direct contact between livestock, cattle, and cats.

In this study, genetic characterization of *C. felis* DNA revealed a significant association between the local *C. felis* isolates and NCBI-BLAST Sweden *C. felis* isolates (KP642069.1) at a 99.45 to 99.72% range of identity. In this Sweden study, the authors confirmed a case of human cryptosporidiosis is caused by the transmission of *C. felis* from a cat to a human (49). To a lesser extent, phylogenetic tree analysis also demonstrated a significant association between the local *C. felis* isolates and Australian *C. felis* isolates (AF221538.1) obtained from cats (50). The plausibility for passing *C. felis* from cats to calves was suggested in this study.

The nucleotide sequence of the HSP70 gene was helpful as a diagnostic tool for genotyping *C. felis*. However, additional studies are required to estimate the prevalence of infection and to detect the role of cats in the transmission of *C. felis* to animals and humans. The efficacy of the HSP70 gene and/or other genes in detecting *Cryptosporidium* spp. in fecal and environmental samples must be evaluated.

Authors' Contribution

G. J. K. A. and E. M. M. A. were responsible for collecting the samples of feces and for molecular testing, while H. A. J. G. was responsible for clinical evaluation of study animals, statistical analysis of results, and genetic sequence analysis. All authors participated equally in writing the final manuscript. No funds were received to complete this study.

Ethics

This study was approved by and performed under the license of the Scientific Committee of the College of Veterinary Medicine, Wasit University, Wasit province, Iraq.

Conflict of Interest

The authors declare that they have no conflict of interest.

References

1. Tyzzer E. A sporozoan found in the peptic glands of the common mouse. Proc Soc Exp Biol. Med. 1907;5(1):12-3.
2. Panciera R, Thomassen R, Garner F. Cryptosporidial infection in a calf. Vet Pathol. 1971;8(5-6):479-84.
3. Nime FA, Burek JD, Page DL, Holscher MA, Yardley JH. Acute enterocolitis in a human being infected with the protozoan Cryptosporidium. Gastroenterology. 1976;70(4):592-8.
4. Hassan EM, Örmeci B, DeRosa MC, Dixon BR, Sattar SA, Iqbal A. A review of Cryptosporidium spp. and their detection in water. Water Sci Technol. 2021;83(1):1-25.
5. Ghazy AA, Abdel-Shafy S, Shaapan RM. Cryptosporidiosis in animals and man: 1. Taxonomic classification, life cycle, epidemiology and zoonotic importance. Asian J Epidemiol. 2015;8(3):48.
6. Wang L, Zhang H, Zhao X, Zhang L, Zhang G, Guo M, et al. Zoonotic Cryptosporidium species and Enterocytozoon bieneusi genotypes in HIV-positive patients on antiretroviral therapy. J Clin Microbiol. 2013;51(2):557-63.
7. Li J, Ryan U, Guo Y, Feng Y, Xiao L. Advances in molecular epidemiology of cryptosporidiosis in dogs and cats. Int J Parasitol. 2021;51(10):787-95.
8. Iseki M. Cryptosporidium felis sp. n.(Protozoa Eimeriorina) from the domestic cat. Jpn J Parasitol. 1979;28:285.

9. Smith H, Caccio S, Cook N, Nichols R, Tait A. Cryptosporidium and Giardia as foodborne zoonoses. Vet Parasitol. 2007;149(1-2):29-40.
10. Ramirez NE, Ward LA, Sreevatsan S. A review of the biology and epidemiology of cryptosporidiosis in humans and animals. Microbes Infect. 2004;6(8):773-85.
11. Ahmed SA, Karanis P. Comparison of current methods used to detect Cryptosporidium oocysts in stools. Int J Hyg Environ Health. 2018;221(5):743-63.
12. Dwivedi S, Purohit P, Misra R, Pareek P, Goel A, Khattri S, et al. Diseases and molecular diagnostics: a step closer to precision medicine. Indian J Clin Biochem. 2017;32(4):374-98.
13. Jex AR, Gasser RB. Cryptosporidium: current state of genomics and systems biological research. Cryptosporidium: parasite and disease: Springer; 2014. p. 327-44.
14. Al-Robaiee I, Al-Farwachi M. Direct ELISA aided coprological diagnosis of Cryptosporidium parvum infection in diarrheic neonatal calves in Mosul city, Iraq. J Adv Vet Anim Res. 2014;1(1):8-10.
15. Alseady H, Kawan M. Prevalence and molecular identification of Cryptosporidium spp in cattle in Baghdad province, Iraq. Iraqi J Vet Sci. 2019;33(2):389-94.
16. Xiao L, Bern C, Limor J, Sulaiman I, Roberts J, Checkley W, et al. Identification of 5 types of Cryptosporidium parasites in children in Lima, Peru. J Infect Dis. 2001;183(3):492-7.
17. Berkman SJ, Roscoe EM, Bourret JC. Comparing self-directed methods for training staff to create graphs using Graphpad Prism. J Appl Behav Anal. 2019;52(1):188-204.
18. Salyer SJ, Silver R, Simone K, Behravesh CB. Prioritizing zoonoses for global health capacity building—themes from One Health zoonotic disease workshops in 7 countries, 2014–2016. Emerg Infect Dis. 2017;23(Suppl 1):S55.
19. Mamedova S, Karanis P. Cryptosporidium spp. infections in livestock and wild animals in Azerbaijan territory. J Water Health. 2021;19(4):545-62.
20. Tanriverdi S, Markovics A, Arslan MO, Itik A, Shkap V, Widmer G. Emergence of distinct genotypes of Cryptosporidium parvum in structured host populations. Appl Environ Microbiol. 2006;72(4):2507-13.
21. Ralston B, Thompson R, Pethick D, McAllister T, Olson M. Cryptosporidium andersoni in Western Australian feedlot cattle. Aust Vet J. 2010;88(11):458-60.
22. Åberg M, Emanuelson U, Troell K, Björkman C. A single-cohort study of Cryptosporidium bovis and Cryptosporidium ryanae in dairy cattle from birth to calving. Veterinary Parasitology: Regional Studies and Reports. 2020;20:100400.
23. Cardona GA, de Lucio A, Bailo B, Cano L, de Fuentes I, Carmena D. Unexpected finding of feline-specific Giardia duodenalis assemblage F and Cryptosporidium felis in asymptomatic adult cattle in Northern Spain. Veterinary parasitology. 2015;209(3-4):258-63.
24. Wegayehu T, Karim R, Anberber M, Adamu H, Erko B, Zhang L, et al. Prevalence and genetic characterization of Cryptosporidium species in dairy calves in Central Ethiopia. PLoS One. 2016;11(5):e0154647.
25. Waldron LS, Dimeski B, Beggs PJ, Ferrari BC, Power ML. Molecular epidemiology, spatiotemporal analysis, and ecology of sporadic human cryptosporidiosis in Australia. Applied and environmental microbiology. 2011;77(21):7757-65.
26. Pirestani M, Sadraei J, Zavvar M, Vaeznia H. Molecular characterization of Cryptosporidium isolates from human and bovine using 18s rRNA gene in Shahriar county of Tehran, Iran. Parasitology research. 2008;103(2):467-72.
27. Budu-Amoako E, Greenwood S, Dixon B, Barkema H, McClure J. G iardia and C ryptosporidium on Dairy Farms and the Role these Farms May Play in Contaminating Water Sources in P rince E dward I sland, C anada. Journal of veterinary internal medicine. 2012;26(3):668-73.
28. Kváč M, Hromadová N, Květoňová D, Rost M, Sak B. Molecular characterization of Cryptosporidium spp. in pre-weaned dairy calves in the Czech Republic: absence of C. ryanae and management-associated distribution of C. andersoni, C. bovis and C. parvum subtypes. Veterinary parasitology. 2011;177(3-4):378-82.
29. Parsons MB, Travis D, Lonsdorf EV, Lipende I, Roellig DMA, Kamenya S, et al. Epidemiology and molecular characterization of Cryptosporidium spp. in humans, wild primates, and domesticated animals in the Greater Gombe Ecosystem, Tanzania. PLoS neglected tropical diseases. 2015;9(2):e0003529.
30. Fayer R, Santín M, Trout JM, Greiner E. Prevalence of species and genotypes of Cryptosporidium found in 1–2-year-old dairy cattle in the eastern United States. Veterinary parasitology. 2006;135(2):105-12.

31. Karanis P, Eiji T, Palomino L, Boonrod K, Plutzer J, Ongerth J, et al. First description of Cryptosporidium bovis in Japan and diagnosis and genotyping of Cryptosporidium spp. in diarrheic pre-weaned calves in Hokkaido. Veterinary parasitology. 2010;169(3-4):387-90.
32. Razakandrainibe R, Diawara EHI, Costa D, Le Goff L, Lemeteil D, Ballet JJ, et al. Common occurrence of Cryptosporidium hominis in asymptomatic and symptomatic calves in France. PLoS neglected tropical diseases. 2018;12(3):e0006355.
33. Ng J, Yang R, McCarthy S, Gordon C, Hijjawi N, Ryan U. Molecular characterization of Cryptosporidium and Giardia in pre-weaned calves in Western Australia and New South Wales. Vet Parasitol. 2011;176(2-3):145-50.
34. Al Mawly J, Grinberg A, Velathanthiri N, French N. Cross sectional study of prevalence, genetic diversity and zoonotic potential of Cryptosporidium parvum cycling in New Zealand dairy farms. Parasit Vectors. 2015;8(1):1-7.
35. Javier DJ, Castellanos-Gonzalez A, Weigum SE, White Jr AC, Richards-Kortum R. Oligonucleotide-gold nanoparticle networks for detection of Cryptosporidium parvum heat shock protein 70 mRNA. J Clin Microbiol. 2009;47(12):4060-6.
36. Díaz P, Navarro E, Remesar S, García-Dios D, Martínez-Calabuig N, Prieto A, et al. The Age-Related Cryptosporidium Species Distribution in Asymptomatic Cattle from North-Western Spain. Animals. 2021;11(2):256.
37. Rieux A, Paraud C, Pors I, Chartier C. Molecular characterization of Cryptosporidium isolates from beef calves under one month of age over three successive years in one herd in western France. Vet Parasitol. 2014;202(3-4):171-9.
38. Moore D, Zeman D. Cryptosporidiosis in neonatal calves: 277 cases (1986-1987). J Am Vet Med Assoc. 1991;198(11):1969-71.
39. Holzhausen I, Lendner M, Göhring F, Steinhöfel I, Daugschies A. Distribution of Cryptosporidium parvum gp60 subtypes in calf herds of Saxony, Germany. Parasitol Res. 2019;118(5):1549-58.
40. de Graaf DC, Vanopdenbosch E, Ortega-Mora LM, Abbassi H, Peeters JE. A review of the importance of cryptosporidiosis in farm animals. Parasitol Int. 1999;29(8):1269-87.
41. Bawm S, Kyi S, Lay KK, Htun LL, Myaing TT. Prevalence and associated risk factors of Cryptosporidium and Giardia species in cattle within Mandalay Region, Myanmar. J Adv Parasitol. 2014;1:49-53.
42. Hussin A, Khalaf J, Ali H. Factors influencing the prevalence of Cryptosporidium spp. in cattle and their breeders. J Anim Health Prod. 2016;4(2):50-4.
43. Lemeteil D, Roussel F, Favennec L, Ballet JJ, Brasseur P. Assessment of candidate anticryptosporidial agents in an immunosuppressed rat model. J Infect Dis. 1993;167(3):766-8.
44. Gong C, Cao X-F, Deng L, Li W, Huang X-M, Lan J-C, et al. Epidemiology of Cryptosporidium infection in cattle in China: a review. Parasite. 2017;24.
45. Silverlås C, Näslund K, Björkman C, Mattsson JG. Molecular characterisation of Cryptosporidium isolates from Swedish dairy cattle in relation to age, diarrhoea and region. Vet Parasitol. 2010;169(3-4):289-95.
46. Mathew A, David O, Olubunmi F, Mosunmola O, Tiamiyu A, Gbenga A. Infection rate of Cryptosporidium parvum among diarrhoea children in Ibadan, Oyo State, Nigeria. Sch J App Med Sci. 2014;2:3127-31.
47. Changizi E, Salimi-Bejestani MR, Vayeghan A. The Cryptosporidium ryanae infection commence in Iranian cattle. J Vet Res. 2012;67(2):127-33.
48. Ibrahim M, Abdel-Ghany A, Abdel-Latef G, Abdel-Aziz S, Aboelhadid S. Epidemiology and public health significance of Cryptosporidium isolated from cattle, buffaloes, and humans in Egypt. Parasitol Res. 2016;115(6):2439-48.
49. Beser J, Toresson L, Eitrem R, Troell K, Winiecka-Krusnell J, Lebbad M. Possible zoonotic transmission of Cryptosporidium felis in a household. Infect Ecol Epidemiol. 2015;5(1):28463.
50. Sulaiman IM, Morgan UM, Thompson RA, Lal AA, Xiao L. Phylogenetic relationships of Cryptosporidium parasites based on the 70-kilodalton heat shock protein (HSP70) gene. Appl Environ Microbiol. 2000;66(6):2385-91.