Iraqi Journal of Veterinary Sciences

www.vetmedmosul.com

Molecular analysis of *Cryptosporidium* species in domestic goat in central Iraq

A.K. Al-Musawi[1], A.H. Awad[2] and M.J. Alkhaled[3]

[1]Department of Parasitology, College of Veterinary Medicine, Al-Qasim Green University, Babylon, [2]Department of Biology, College of Education for Pure Sciences, Basrah University, Basrah, [3]Department of Parasitology, College of Veterinary Medicine, University of Al-Qadisiyah, Al-Qadisiyah, Iraq

Article information

Article history:
Received February 21, 2022
Accepted May 8, 2022
Available online July 6, 2022

Keywords:
Cryptosporidium spp.
Genotype
Subtypes
Domestic goat
Iraq

Correspondence:
M.J. Alkhaled
mansoor.ali@qu.edu.iq

Abstract

Cryptosporidium spp. is a significant parasitic disease that results in diarrhea and gastroenteritis in humans and animals worldwide. The present study aimed to investigate the molecular diversity of *Cryptosporidium* species in domestic goats. A total of a hundred feces samples were collected from four locations in Babylon city in central Iraq. All the samples were investigated phenotypically using a modified Ziehl-Neelsen stain method and genotypically using conventional and nested PCR methods based on a partial sequence of heat shock protein 70 (Hsp70) and 60 kDa glycoprotein (gp60) genes, and finally, phylogenetic analysis method. The molecular results showed five species of *Cryptosporidium*, including *C. parvum, C. hominis, C. ryana, C. xiaoi,* and *C. bovis*. The phylogenetic results of partial sequence of gp60 gene for *C. parvum* and C. hominis isolate two subtypes were established IIdA21G1 and IIdA19G1 belong to *C. parvum*. For *C. hominis*, three subtypes were detected: IbA21G2, IbA13G3, and IbA19G2. This study showed that *Cryptosporidium parvum* (zoonotic) is more prevalent than other *Cryptosporidium* species in goats from this area. This suggests that zoonotic transmission is the primary mode of transmission of *Cryptosporidium* infection in Babylon province.

DOI: 10.33899/ijvs.2022.132974.2155,

Introduction

Cryptosporidiosis disease has been considered a global problem in humans and animals, caused by coccidian protozoan parasite *Cryptosporidium* species (1,2). The most prevalent species of the parasite present in ruminants are *C. parvum*, *C. xiaoi*, *C. bovis*, *C. andersoni*, *C. ryanae*, and *C. ubiquitum* (3). The common gene used for the identification and characterization of *Cryptosporidium* species is a heat shock protein 70 (HSP70) and glycoprotein (gp60) genes (4). The (HSP70) gene belongs to multigene families and is highly conserved across the eukaryotes and prokaryotes. Hsp70 protects cells and keeps them alive when exposed to various stress conditions (5-7). The gp60 gene is commonly used for subtyping *Cryptosporidium* species and is attributable to having tandem iterate of the trinucleotide coding- serine TCG, TCT, or TCA at the 50 (gp40) end of the locus (8).

Materials and methods

One hundred fecal samples were collected from diseased and clinically healthy domestic goats from June 2020 to January 2021. The samples were collected from domestic goat farms located in four districts in Babylon city (Hilla city, Al-Hashmiyah, Mahaweel, and Al-Musayyib). The samples were collected directly from the rectum using gloves and plastic containers.

Phenotypic identification of *Cryptosporidium* species

Fecal samples were investigated to detect *Cryptosporidium* oocysts using the Ziehl Neelsen stain smears method (9). We will take 50 positive samples for molecular examination.

DNA extraction

DNA was extracted from fecal samples using AddPrep Genomic DNA Extraction Kit (addbio, Daejeon, Korea) according to the manufacturer's instruction (9).

Nested PCR

The nested PCR method was used to detect *Cryptosporidium* species by targeting the (HSP70) gene, and the same reaction was used to diagnose the subtypes of *C. hominins* and *C. parvum* by targeting the (gp60) gene. Various primers were used for each process, as shown in (Table 1).

Table 1: Oligonucleotide primer sequences used for *Cryptosporidium* species PCR methods.

Gene	Target	PCR	Oligonucleotide primer 5'- 3'	size (bp)	°C	Reference
HSP-70	Universal	conventional	F: GGTGGTGGTACTTTTGATGTAT R: GCCTGAACCTTTGGAATACG	448	52	(10)
		nested	F: GCTGSTGATACTCACTTGGGTGG R: CTCTTGTCCATACCAGCATCC	325	52	(10)
GP60	*C. parvum*	conventional	F: ATAGTCTCCGCTGTATTC R: GGAAGGAACGATGTATCT	1400	50	(11)
		nested	F: TCCGCTGTATTCTCAGCC R: GCAGAGGAACCAGCATC	800	51	(11)
GP60	*C. hominis*	conventional	F: ATAGTCTCGCTGTATTC R: GCAGAGGAACCAGCATC	1400	50	(11)
		nested	F: TCCGCTGTATTCTCAGCC R: GAGATATATCTTGGTGCG	800	53	(11)

Statistical analysis

The Chi-square test was used to compare the results. Differences were considered statistically significant at $P<0.05$.

Results

Detection of genotyping of *Cryptosporidium* spp.

The results of nested PCR for detection of *Cryptosporidium* spp. and the bands appear on the agarose gel (Figure1). They are sent to the sequencing to know the *Cryptosporidium* spp. in goats. The sequencing results for *Cryptosporidium* spp. showed different ratios between the species, with *C. parvum* being the most common. The total percentage of *C. parvum* in goats was 46.15% of the examined with the accession number (MZ787781, MZ787782, MZ787783, MZ787784, MZ787785, and MZ787786). The total percentage of *C. hominis* and *C. Xiao* in goats was 23.07% of the samples sent, with the accession number (MZ787787, MZ787788, MZ787789, MZ787791, MZ787792, and MZ787793) respectively. The lowest common is *C. ryanae* and *C. bovis*, which were recorded with a rate of 07.14% with the accession number (MZ787790 and MZ787794), respectively.

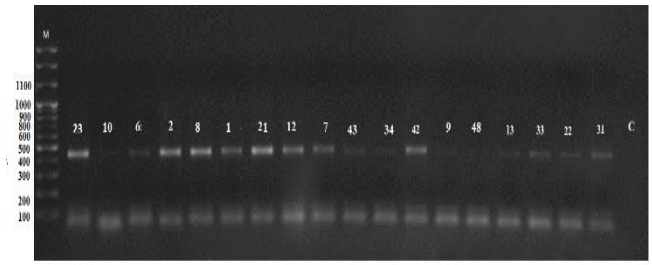

Figure 1: Gel electrophoresis 1% shows some positive PCR in fecal samples of goats (size= 448 bp) using safe gel stain dye and using universal primers (first round). M is a molecular marker (100- 1100 bp) from (ADDBIO, Korea); C is a control negative.

Detection of subtype of *C. parvum* and *C. hominis*

The results of nested PCR for *C. parvum* and *C. hominis* and the bands that appear on the agarose gel are sent to the sequencing to know the *C. hominis* and *C. parvum* subtype (Figure 2) in goats. *C. parvum* alignments with reference sequences classify all isolates into one family: IId. Extra sub-classification led to five different subtypes, the subtype IIdA21G1 being the most common, present in six cases in goats with accession numbers (MZ787819,

MZ787820, MZ787821, MZ787822. MZ787823, and MZ787824) and subtype IIdA19G1 identified in four cases in goats, it has accession number (MZ787815, MZ787816, MZ787817, and MZ787818). *C. hominis* alignments with reference sequences classify all isolates into one family: Ib. Extra sub-classification led to 3 subtypes was detected with IbA21G2 subtype is common, found in six cases in goats with accession numbers (MZ787825, MZ787826, MZ787827, MZ787828, MZ787829, and MZ787830). Subtype IbA13G3 was identified in four cases in goats with accession numbers (MZ787831, MZ787832, MZ787833, and MZ787834), and subtype IbA19G2 was identified in two cases in goats with the accession numbers (MZ787835 and MZ787836).

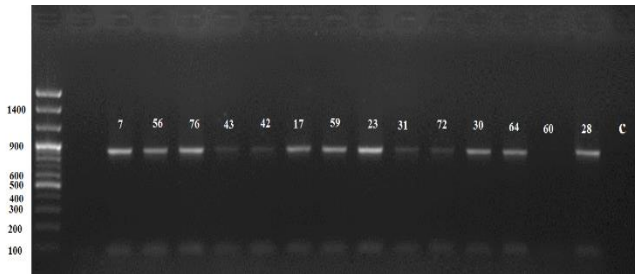

Figure 2: Gel electrophoresis 1% using species-specific primers, using safe gel stain dye shows some positive amplicons for the subtypes of *Cryptosporidium hominis* (size= 800 bp).

Phylogenetic characterization

14 *Cryptosporidium* sequences were evaluated to understand the relationship of *Cryptosporidium* species isolated in the present study. A Six *C. parvum*, three isolates for both *C. hominis* and C. xiaoi, and one isolate for both *C. ryanae* and *C. bovis* had been checked in the GenBank database in accession no. MZ787781 to MZ787794. There is closely 100% of similarity/ Sequence homology) related between *C. hominis* and *C. parvum.* The *C. parvum* isolate was the same as *the C. parvum* isolate from the Netherlands under accession no ABD60355.1, and the *C. hominis* isolates were the same as the *C. hominis* isolate from Brazil under accession no AMR08234.1 (Figure 3).

Ten isolates of the C. *parvum* subtype had been checked in GeneBank base data in accession no. MZ787815 - MZ787824, twelve isolates for *C. hominis* subtype, were checked in GeneBank base data in accession no. MZ787825 - MZ787836. *C. parvum* IIdA21G1 subtype isolate was the same as *the C. parvum* isolate from Italy under accession no. ALH22624.1 and the *C. parvum* IIdA19G1 subtype isolate were identical to the *C. hominis* isolate from China under accession no. QIC04069.1. C. *hominis* IbA13G3 subtype isolate was the same as *C. hominis* isolate from Australia under accession no. AEN71166.1 and UK under accession no. ADK92641.1, the *C. hominis* IbA19G2, and IbA32G2 subtype showed high relation (Figure 4).

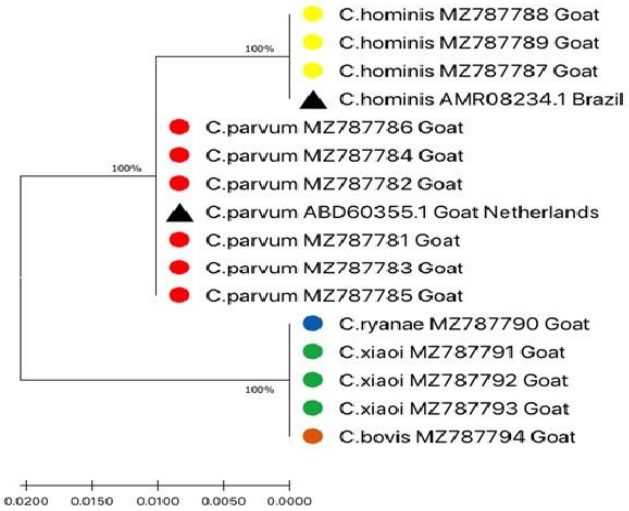

Figure 3: Phylogenetic tree analysis of *Cryptosporidium* spp. created on the sequence of the Hsp70 gene of goat isolates used for local *Cryptosporidium* spp. (referred to as circular) compared with global *Cryptosporidium* spp. (referred to as triangle).

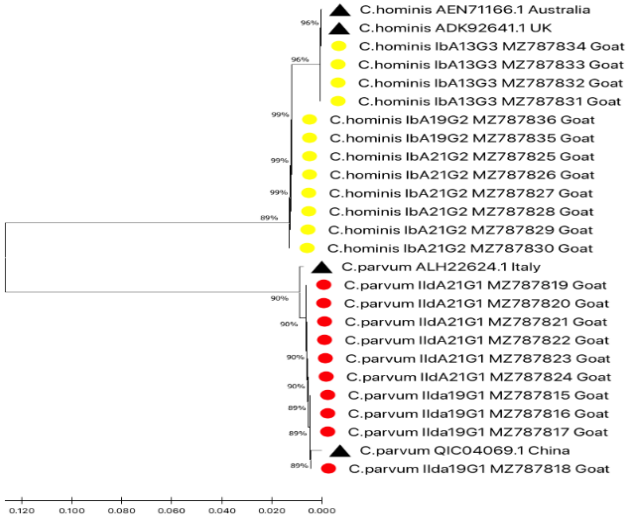

Figure 4: Phylogenetic tree analysis of *C. parvum* and *hominis* subtype based on the partial sequence of the gp60 gene of goat isolates that used for local of *C. parvum* and *hominis* subtype (referred to as circular) compared with global *C. parvum* and *hominis* subtype (referred to as triangle).

Discussion

The nested PCR and identifying the species through sequence analysis found that *C. parvum* has the highest occurrence, followed by *C. hominis*, and the lowest was *C. ryana* and *C. bovis*. The present study results are similar to those Alkhaled and Hamad (12) in goats in Al-Qadisiyah province. They found that *C. parvum* was the highest, followed by *C. hominis.* The high percentage of *C. parvum* compared to the other species of *Cryptosporidium* can be attributed to the fact that *C. parvum* is not specific to a host, and it is the most prevalent species in other animals and the second most prevalent after *C. hominis* in humans, which is consistent with what was mentioned by Alkhaled and Hamad (12). Ten GP60 sequences belonging to *C. parvum* indicate that a unique subtype family is IId in goats, and two subtypes were achieved by the GenBank database. They are IIdA21G1and IIdA19G1. IIdA21G1 subtypes were previously recorded in Iraq (13), and IIdA19G1 subtypes were recorded for the first time in Iraq. The first *C. parvum* subtype IIdA21G1 sequences were isolated from goats. The present results agreed with results recorded by Alves *et al.* (14) in Portugal in HIV patients -infected and sheep. In sheep in Al-Diwaniyah Province - Iraq (13). The second subtype of *C. parvum* is IIdA19G1 found in goats. These results agreed with Alves *et al.* (14) in HIV patients infected in Portugal. those Wang *et al.* (15) in patients with AIDS in China. (16) in China, meat goats. Taha *et al.* (17) in diarrheic calves in Sudan.

According to the current results, the subtype families IId are considered zoonotic subtypes. This agrees with what was mentioned by Taghipour *et al.* (18). Compared with other studies mentioned previously have been reported in humans, and one can say that goats may be a possible source of animal and human infection with *C. parvum* 12 GP60 sequences belonging to *C. hominis* indicate that one subtype family is, Ib belongs to the subtype family in goats. The GenBank database achieved three subtypes they are IbA21G2, IbA19G2 and IbA13G3. one subtype was previously recorded in Iraq (IbA21G2) (13), and two subtypes were recorded for the first time in Iraq (IbA13G3 and IbA13G3). The first *C. hominis* subtype IbA21G2 sequences in isolates from goats were agreed with results recorded by Feng *et al*. (19) in water in Shanghai, China. Al-Jabbar (13) in sheeps at Al-Diwaniyah province. The second *C. hominis* subtype IbA19G2 sequence is isolated from goats were agree with the results of Feng *et al*. (19) in water in Shanghai, China. In China (20), dairy cattle in Henan. The third *C. hominis* subtype IbA13G3 sequence is isolated from goats were agreed with Cama *et al.* (21) in persons with HIV in Peru. In Nigeria, Molloy *et al.* (22) in human. Razakandrainibe *et al.* (23) and in calves in five geographic regions of France. The Ib subtype family is widespread and that cause infection in human and animal, and the subtype in the current study, when compared with other studies mentioned above, maybe *C. hominis* in goats, perhaps a source of human infection with *C. hominis*, and this agree with Razakandrainibe *et al.* (23), who mentioned that animals might be a source of *C. hominis.*

Conclusion

These results indicate a common occurrence of five species of *Cryptosporidium* in goats (*C. parvum*, *C. hominis*, *C. ryana*, *C. xiaoi*, and *C. bovis*) and two subtypes of *C. parvum* IIdA21G1 and IIdA19G1 and three subtypes of C. *hominis* IbA21G2, IbA13G3 and IbA19G2.

Acknowledgments

The author would like to thank the College of veterinary, Al-Qasim Green University, for providing facilities.

Conflict of interest

The authors declare that no conflict of interest exists.

References

1. Mahdi NK, Ali N H. Intestinal parasitic (Including Cryptosporidium) infections in day-care centers. Bahrain Med Bull. 2002;24(4):1-7. [available at]
2. Kadim AH, Al-Emarah YA, Ali NH. The effect of breast-feeding on Cryptosporidium infection rate in children below two years age. Int J Res Med Appl Sci. 2014;2(11):153-158. [available at]
3. Mahdi NK, Ali N H. Cryptosporidiosis among animal handlers and their livestock in Basrah, Iraq. East Afr Med J. 2002;79(7):62-65. DOI: 10.4314/eamj.v79i10.8820
4. Jarad NI. Molecular detection of *Cryptosporidium parvum* in chicken in Al-Diwaniya province. Iraqi J Vet Sci. 2020;34(2):441-445. DOI: 10.33899/ijvs.2019.126159
5. Matty HN, Hassan AA. The relationship between HSP70 and level of leptin and luteinizing hormones in female rats exposed to chronic and acute heat stress. Iraqi J Vet Sci. 2020;34(1):37-43. DOI: 10.33899/ijvs.2019.125565.1082
6. Habib NH. Molecular characterization of heat shock protein 70 gene in Iraqi buffalo. Iraqi J Vet Sci. 2020;34(1):139-143. DOI: 10.33899/ijvs.2019.125633.1116
7. Hassan AA, Asim RA. Effect of vitamin C and acetylsalicylic acid supplementation on some hematological value, heat shock protein 70 concentration and growth hormone level in broiler exposed to heat stress. Iraqi J Vet Sci. 2020;34(2):357-363. DOI: 10.33899/ijvs.2019.125950.1195
8. Rahmouni I, Essid R, Aoun K, Bouratbine A. Glycoprotein 60 diversity in *Cryptosporidium parvum* causing human and cattle cryptosporidiosis in the rural region of northern Tunisia. Am J Trop Med Hyg. 2014;90(2):346-350. DOI: 10.4269/ajtmh.13-0522
9. Mead JR, Arrowood M. Cryptosporidium Methods and Protocols: Methods and Protocols. New York: Humana Press; 2020. 407 p. [available at]
10. Ghaffari S, Kalantari N, Hart C. A multi-locus study for detection of cryptosporidium species isolated from calves population, Liverpool. Int J Mol Cell Med. 2014;3(1):35-42. [available at]

11. Zhou L, Singh A, Jiang J, Xiao L. Molecular surveillance of Cryptosporidium spp. in raw wastewater in Milwaukee: Implications for understanding outbreak occurrence and transmission dynamics. J Clin Microbiol. 2004;42(4):5254-5257. DOI: 10.1128/JCM.41.11.5254-5257.2003
12. Alkhaled MA, Hamad WA. Molecular characterization of Cryptosporidium spp. in sheep and goat in Al-Qadisiyah province, Iraq. Iraqi J Vet Sci. 2017;41(2):31-37. DOI: 10.30539/iraqijvm.v41i2.44
13. Al-Jabbar HA. Epidemiological and Molecular study of Cryptosporidium genotypes in sheep in Al-Diwaniyah province [master's thesis]. Al-Qadisiyah: College of Veterinary Medicine, University of Al-Qadisiyah; 2021. 107 p.
14. Alves M, Xiao L, Antunes F, Matos O. Distribution of Cryptosporidium subtypes in humans and domestic and wild ruminants in Portugal. Parasitol Res. 2006;99(3):287-292. DOI: 10.1007/s00436-006-0164-5
15. Wang L, Zhang H, Zhao X, Zhang L, Zhang G, Guo M, Liu L, Feng Y. Xiao L. Zoonotic Cryptosporidium species and *Enterocytozoon bieneusi* genotypes in HIV-positive patients on antiretroviral therapy. J Clin Microbiol. 2013;51(2):557-563. DOI: 10.1128/JCM.02758-12
16. Peng X Q, Tian G R, Ren G J, Yu ZQ, Lok JB, Zhang LX, Wang XT, Song JK, Zhao GH. Infection rate of *Giardia duodenalis*, Cryptosporidium spp., and *Enterocytozoon bieneusi* in Cashmere, dairy, and meat goats in China. Infect Genet Evol. 2016;41:26-31. DOI: 10.1016/j.meegid.2016.03.021
17. Taha S, Elmalik K, Bangoura B, Lendner M, Mossaad E, Daugschies A. Molecular characterization of bovine Cryptosporidium isolated from diarrheic calves in the Sudan. Parasitol Res. 2017;116(11):2971-2979. DOI: 10.1007/s00436-017-5606-8
18. Taghipour N, Nazemalhosseini-Mojarad E, Haghighi A, Rostami-Nejad M, Romani S, Keshavarz A Alebouyeh M. Zali M. Molecular epidemiology of cryptosporidiosis in Iranian children, Tehran, Iran. Iran J Parasitol. 2011;6(4):41-45. [available at]
19. Feng Y, Li N, Duan L, Xiao L. Cryptosporidium genotype and subtype distribution in raw wastewater in Shanghai, China: Evidence for possible unique *Cryptosporidium hominis* transmission. J Clin Microbiol. 2009;47(1):153-157. DOI: 10.1128/JCM.01777-08
20. Wang R, Wang H, Sun Y, Zhang L, Jian F, Qi M, Ning C. Xiao L. Characteristics of Cryptosporidium transmission in preweaned dairy cattle in Henan, China. J Clin Microbiol. 2011;49(3):1077-1082. DOI: 10.1128/JCM.02194-10
21. Cama VA, Bern C, Roberts J, Cabrera L, Sterling CR, Ortega Y, Gilman RH, Xiao L. Cryptosporidium species and subtypes and clinical manifestations in children, Peru. Emerg Infect Dis. 2008;14(10):1567-1574. DOI: 10.3201/eid1410.071273
22. Molloy SF, Smith, HV, Kirwan P, Nichols RA, Asaolu SO, Connelly L, Holland CV. Identification of a high diversity of Cryptosporidium species genotypes and subtypes in a pediatric population in Nigeria. Am J Trop Med Hyg. 2010;82(4):608-613. DOI: 10.4269/ajtmh.2010.09-0624
23. Razakandrainibe R, Diawara E, Costa D, Le Goff L, Lemeteil D, Ballet JJ, Gargala G, Favennec L. Common occurrence of *Cryptosporidium hominis* in asymptomatic and symptomatic calves in France. PLoS Negl Trop Dis. 2018;12(3):e0006355. DOI: 10.1371/journal.pntd.0006355

التحليل الجزيئي لأنواع خفية الأبواغ في الماعز المحلي وسط العراق

عقيل محمد كاظم الموسوي[١]، عبد الحسين حبش عواد[٢] و منصور جدعان علي الخالد[٣]

[١]فرع الطفيليات، كلية الطب البيطري، جامعة القاسم الخضراء، بابل، [٢]قسم علوم الحياة، كلية التربية للعلوم الصرفة، جامعة البصرة، البصرة، [٣]فرع الطفيليات، كلية الطب البيطري، جامعة القادسية، القادسية، العراق

الخلاصة

تهدف الدراسة الحالية إلى دراسة التنوع الجيني لأنواع طفيلي خفية الأبواغ في الماعز المحلي في مدينة بابل وسط العراق. تم جمع ١٠٠ عينة براز من أربعة مواقع. جميع العينات تم فحصها مظهريا باستخدام طريقة صبغة زيل نلسن المعدلة وباستخدام طريقة الاحيائي الجزيئي معتمدا على تسلسل جزئي لجين بروتين الصدمة الحرارية ٧٠. تم تحديد خمسة أنواع من طفيلي خفية الأبواغ لتشمل *C. parvum* و *C. hominis* و *C. ryana* و *C. xiaoi* و *C. bovis*. بعد التحليل الوراثي الجزيئي للتسلسل الجيني gp60 ١٠ عزلات من *C. parvum* و ١٠ عزلات من *C. hominis*. سجل نوعين فرعيين هما IIdA21G1 و IIdA19G1 ينتميان إلى *C. parvum*. سجل على ثلاثة أنواع فرعية من *C. hominis* وهي: IbA21G2 و IbA13G3 و IbA19G2. أظهرت هذه الدراسة أن *Cryptosporidium parvum* (الحيوانية المنشأ) أكثر انتشارا من أنواع الأبواغ الخبيئة الأخرى في الماعز من هذه المنطقة. يشير هذا إلى أن انتقال العدوى حيواني المنشأ هو الطريقة الرئيسية لانتقال عدوى خفية الأبواغ في محافظة بابل.