Molecular analysis of Cryptosporidium species in domestic goat in central Iraq


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Article information

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.

Keywords: Cryptosporidium spp., Genotype, Subtypes, Domestic goat, Iraq

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. xiao, 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. hominis and C. parvum by targeting the (gp60) gene. Various primers were used for each process, as shown in (Table 1).

<table>
<thead>
<tr>
<th>Gene</th>
<th>Target</th>
<th>PCR</th>
<th>Oligonucleotide primer 5’- 3’</th>
<th>size (bp)</th>
<th>°C</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>HSP-70</td>
<td>Universal</td>
<td>conventional</td>
<td>F: GGTGGTGTTACTTTTGATGTAT</td>
<td>448</td>
<td>52</td>
<td>(10)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>nested</td>
<td>R: GCCGAACCTTTTGGATACCG</td>
<td>325</td>
<td>52</td>
<td>(10)</td>
</tr>
<tr>
<td>GP60</td>
<td>C. parvum</td>
<td>conventional</td>
<td>F: ATAGTCTGCCGTGATTTGTCC</td>
<td>1400</td>
<td>50</td>
<td>(11)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>nested</td>
<td>R: GGAAGGAACGGATGATCT</td>
<td>800</td>
<td>51</td>
<td>(11)</td>
</tr>
<tr>
<td>GP60</td>
<td>C. hominis</td>
<td>conventional</td>
<td>F: ATAGTCTCGCGGTATTGC</td>
<td>1400</td>
<td>50</td>
<td>(11)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>nested</td>
<td>R: GCAGAGGAACGGCATC</td>
<td>800</td>
<td>53</td>
<td>(11)</td>
</tr>
</tbody>
</table>

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.

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 IldA19G1 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).

**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. xiao, 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 IldA21G1 subtype isolate was the same as the C. parvum isolate from Italy under accession no. ALH22624.1 and the C. parvum IldA19G1 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 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).

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 Alkhaleed 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 Alkhaleed 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 IIdA21G1 and 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. xiao*, and *C. bovis*) and two subtypes of *C. parvum* IIdA21G1 and IIdA19G1 and three subtypes of *C. hominis* IbA21G2, IbA13G3 and IbA19G2.

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Conflict of interest

The authors declare that no conflict of interest exists.

References


