Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 6, 2021, Pages. 4120 - 4125
Received 25 April 2021; Accepted 08 May 2021.

Genotyping of *Cryptosporidium Spp*. in Domestic Dogs and Humans in Karbala Province Iraq.

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Abstract

In this study, *Cryptosporidium sp.* was diagnosed in Domestic dogs and humans in Karbala province in Iraq by molecular detection by using a nested polymerase chain reaction (nPCR) by outer primer 18s (9005bp) and inner primer 18s (830bp) were used. our study was beginning of December (2019) to September (2020). A total of 100 fecal samples were collected from adult and young and from both sexes of dogs and 100 stool samples were collected from adult and young and from both sexes humans. The sequence study for 10 samples from the positive samples recorded at the National Center for Biotechnology Information (NCBI) was found 7 species *C. parvum* under accession number (MT329015.1 - MT329013.1) and 3 samples *C. canis* under accession number (MT329014.1, MT329016.1, and MT329018.1). The result of the sequence analysis In humans for 10 samples from the positive samples recorded at the (NCBI) was indicate 10 isolate *Cryptosporidium parvum.* under the accession numbers (MW186198 - MW186207)).

1-Introduction

Cryptosporidium, a protozoan parasite belonging to the Phylum Apicomplexa and Family Cryptosporiidae, is a common cause of diarrhea in humans, domestic animals and wild vertebrates (**Bouzid*et al*., 2018).**. Cryptosporidium genus contains over 30 so far recognized species and numerous genotypes may be infected dogs with C. canis, C. parvum, C. ubiquitum and C. andersoni, about twenty *Cryptosporidium spp*. have been reported but only a few have been found to infect both humans and animals and these include; *Cryptosporidium parvum, Cryptosporidium felis, Cryptosporidium ubiquitum,Cryptosporidium muris, Cryptosporidium meleagridis, Cryptosporidium suis*and *Cryptosporidium cani*s. The occurrence of animal *Cryptosporidium spp*. in humans indicates that humans are constantly at risk of contracting cryptosporidiosis from these reservoir hosts **(Bialek*et al*., 2002).** *C.parvum* was thought to infect all animals. However, it is generally accepted that *C. parvum* infects primarily ruminants(cattle, sheep and goat) and humansand also have been found occasionally in other mammals such as mice and dogs, although companion animals are most often infected with host-specific Cryptosporidium spp. **(Xiao and Fayer, 2008) and (Al- Zubaidei and Kawan, 2020).**The infection can be transmitted to human through close contact with dogs **(Hunter and Thompson 2005).** The close relationship between man and his domestic pets, particularly dogs, makes transmission of zoonotic diseases very easy, especially those that could be acquired through environmental contamination such as cryptosporidiosis. *C. parvum*is the most frequently

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identified species of *Cryptosporidium spp*. in dogs and its isolation in human patients from both developed and under-developed countries **(Jian*et al*., 2014).** There were many studies in Iraq about *Cryptosporidum spp*. In humans **(Ali *et al*., 2015) (Maysoon*et al* ., 2018)** Due to no reports between the molecular analysis of the various *Cryptosporidium* species in human and dogs, the study design for this aim.

2- Material and methods

Collected of 100 hundred fecal samples from domestic dogs and 100 hundred stool samples from humans from different sex and age from several area of Karbala province, during the study period from start of December (2019), to end of September 2020

The kit used for D NA extraction from (Geneaid, Korea) as per the manufacturer's instruction. The samples were stored at -80˚C until use. To identify Cryptosporidium spp. in the faecal samples, fragments covering 18S rDNA gene were amplified by nested PCR. First, amplification of the 900 bp Crypto18S1 (FTTCTAGAGCTAATACATGCG.And.Crypto18S1.R.CCCATTTCCTTCGAAACAGGA) region was carried out, and next, for secondary PCR, the 830 bp (Crypto.18S2F GGAAGGGTTGTATTTATTAGATAAAG.AndCrypto18S2R.CTCATAAGGTGCTGAAGGA GTA) fragment was amplified using 3 µl of the first PCR product. Primers, mixture composition and PCR conditions have been described by (18). The secondary PCR products were examined electrophoretically in 1% agarose gels and visualized after staining with Advance DNA stain. The identified species typing analysis was done by phylogenetic tree analysis in comparison with NCBI-Blast known sequences.

3-**Results**

The result of present study showed the amplification conditions were optimized for the PCR assay, using specific primers sequences of 18s RNA (900bp. According to nested-PCR examination, samples were subjected to molecular analysis by using small subunit ribosomal RNA gene specific primers(830bp), in order to identify the species of *Cryptosporidium*.

Prevalence of Cryptosporidium spp. In dogs and humans by using PCR Technique.the infection rates in dogs in DNA samples showed 47% (47/100) positive that among 100 examined the fecal samples that were collected dogs in Karbala city. In humans DNA samples showed the infection rate of *Cryptosporidium spp*. by using PCR Technique. showed 34%(34/100) were given positive result.

Ten samples from each dogs and humans selected randomly for the sequences to indicated the*Cryptosporidium species*, which had already been identified by nested PCR. The presentstudy indicated Sequences obtained by genotyping were compared with sequences deposited in Gen Bank base found of two *Cryptosporidium* species in dogs: 7 samples *C.*

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*parvum*that have been deposited in the gene bank under the accession numbers (MT329015.1, MT329017., MT329019.1, MT329020.1, MT329021.1, MT329022.1, and MT329013.1). and3 samples identified *Cryptosporidium canis* gene (18S) that have been deposited in the gene bank under the accession numbers (MT329014.1, MT329016.1, and MT329018.1). while in humans all samples the identified *Cryptosporidium parvum* genes (18S) that have been deposited in the gene bank under the accession numbers (MW186198, MW186199, MW186200, MW186201, MW186202, MW186203, MW186204, MW186205, MW186206, MW186207).

4-Discussion

The frequency of infection rates in dogs in DNA samples showed 47% (47/100) positive that among 100 examined the fecal samples of dogs in Karbala city. In Iraq only one previous study was by **(Azhar,2020),** she found the prevalence of *Cryptosporidium spp*. In dogs by using PCR technique was 28.6% .Infection rate of *Cryptosporidium spp.* By using molecular technique Iran 12.3% was done by **(Mehdi *et al*., 2017).**The prevalence of *Cryptosporidium spp*. In dogs in Egypt by Molecular Screening 24% was found by **(Rasha*et al*., 2018),** disagree with them. Also our result different In China was recorded by **(Jiayu*et al*.,2019)** and in Japan by **(Naoyuki*et a*l., 2019),** they found infection rates of *Cryptosporidium spp*. in dogs by using PCR were 6.9% and 21.0% respectively. The global prevalence of *Cryptosporidium spp.* in dogs could vary depending on the sensitivity of used diagnostic techniques, geographic locations, and the tested population, In our study, the higher prevalence of *Cryptosporidium* in dogs compared to the prevalence in previous studies might be attributed to contact with stray dogs, poor feeding such as ingestion of dog feces (dog coprophagia) and water, contaminated soil, poor sanitation with infected animals agree with **(Rasha*et al*., 2018).**

In humans DNA samples showed the infection rate of *Cryptosporidium spp*. by using PCR Technique. showed 34%(34/100) were given positive result. In Iraq the previous study about the infection rate in human by using molecular technique was conducted by **(Maysoon*et al*.,2018),** They found infection rate was 47.33%, agree with them. In Neighboring countries such as Iran the prevalence of infection rate by using PCR was 4.94% is done by **(Mitra*et al*.,2015),** and in Jordon the frequency of *Cryptosporidium* infection in humans by using molecular examination was 8.3% recorded by **(Hijjawi*et al*., 2016).** Disagree with study was done in Turkey that recorded prevalence of *cryptosporidium spp.* In humans was 8.93% **(Nadim and Şadiye. 2017).** In Egypt **(Rasha*et al*., 2018),** find frequency of distribution of *Cryptosporidium spp*. in 100 stool samples of children was 14% by nested PCR, it is dos not identical to our study.The variation in prevalence of *Cryptosporidium spp* in humans among different studies may by attributed to samples size, large or small population, hygiene application, level of contamination of food and water, improper disposal of garbage and dog feces, and contact with other livestock **(Rasha*et al*., 2018).**

The present Results of molecular study showed Sequences obtained by genotyping were compared with sequences deposited in GenBank base found of two *Cryptosporidium* species in

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dogs: *C. parvum* and *C. canis*Genotyping analysis of the PCR-positive samples from dogs identified *C.parvum* in Iraq by **(Azhar,2020).**Our results were in agreement with results recorded by **(Piekara*et al*.,2020).**In Poland he recorded 3 cases of *C. canis* and 2 cases of *C.parvum* in dogs were detected.Thesequence analysis of the *Cryptosporidium*-positive canine samples in Chania by **(Shenquan*et al*., 2020),** revealed that presence of *C. canis*and *C. parvum.*While**(Jian*et al*., 2014),** he was only recorded *C. canis* in chain's dogs. Also in Chain **(Jiayu*et al*.,2019),** Whorecorded three species were identified as positive for *C. canis, C. muris* and the *Cryptosporidium* rat genotype IV. Genotyping of nested PCR positive dog samples showed the presence of only genotype *C. parvum* in five samples and the restriction fragments banding patterns of this genotype in Egyptian dogs by **(Rasha*et al*., 2018).** In IranMolecular identification of *Cryptosporidium* species showed that the species in dogs is *C. parvum* by **(Reza *et al*.,2017).** A sequencing analysis in Japans by **(Naoyuki*et al*., 2019),** demonstrated that species infected dogs with *C. canis*. As in current study, *C. parvum*and C. canis has been identified as the most common Cryptosporidium species in dogs around the world, although a few cases of *C. parvum, C. muris,* and *C. meleagridis* infection have also been reported **(Jian*et al*., 2014).** The variation of *Cryptosporidium spp.*In dogs could be due to their close relationship with animals particularly cattle and sheep. Given that dogs with cryptosporidiosis lack clinical symptoms, as they are companion animals for humans. The existence of *C. parvum* in stool specimens of dogs in this study can probably be due to the close relationship between dogs with sheep and with cattle, and then, these relationships are likely to be a potential risk factor for infecting dogs, agree with **(Reza *et al*.,2017).**

DNA sequences of the 18S rRNA gene of ten samples selected randomly from infected human were 99.31% to 99.45% identical to sequences in the GenBank of *C. parvum* isolates from humans, similar to the results of studies conducted in Iraq by **(Maysoon*et al*.,2018) and (Haider. 2019).**record that (*C parvum*and *C hominis)* and (*C. parvum ,C. andersoni*and *C. hominis*) respectivelyisolated from humans samples. Also agree with study conducted in Iran that amplification of small subunit ribosomal RNA gene was performed using a nested polymerase chain reaction protocol and its products were digested using two restriction enzymes for *Cryptosporidium* species and genotype differentiation and restriction pattern revealed the presence of *C.parvum* was done by **(Mitra*et al*., 2015).** In Jordan **(Hijjawi*et al*., 2016),** demonstrated that species infected humans with *C. hominis and C. parvum*. In Kuwait the DNA sequencing done by **(Jamshaid*et al*., 2011),** was used to confirm *Cryptosporidium* species infect humans genotypes. RFLP analyses of the secondary PCR product and identified*C. parvum and C. hominis.* In TurkeyGenetic analysis of the *Cryptosporidium spp*. Infected humans Turkey indicated that *C. hominis and C. parvum* are species infected humans, that recorded by **(Nadim and Şadiye. 2017).** Genotyping analysis of the PCR-positive samples from humans. In Egypt genotyping of the nested PCR positive human samples revealed the existence of human genotype *C. hominis* and genotype *C. parvum***(Rasha*et al*.,2018).** DNA sequence analysis confirming and identifying the current *Cryptosporidium* isolates as *C. parvum*, indicates infect humans in Sudan by **(Adam *et al*., 2019).** Current study explained that *C. parvum* is the main

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Cryptosporidium spp. Infecting humans and animals in Iraq and thought these isolates to be adaptable for both zoonotic and anthroponotic transmissions, by direct or indirect contact. Our findings are supported by previous studies **(Mitra*et al*., 2015),** (**Chen *et al*.,2017) and (Adam *et al*., 2019).** Our results investigate that humans could acquire *Cryptosporidium* infection through anthroponotic and zoonotic transmission. the nested PCR products of (18S) gene demonstrate five *C. parvum* genotypes from humans samples identical to *C. parvum* genotypes from dogs samples. These findings indicated that household dogs are potential reservoirs for *C. parvum* (bovine genotype) with the possibility of direct or indirect zoonotic transmission to humans in close contact with dogs and other domestic livestock agree with **(Rasha*et al*.,2018).**

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