Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 3, 2021, Pages. 3000 - 3007
Received 16 February 2021; Accepted 08 March 2021.

Detection of Cryptosporidium Spp. in Domestic Dogs by Conventional and Molecular Method in Karbala Province Iraq.

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Abstract

Cryptosporidium spp was detected and identified in Domestic dogs in Karbala province in Iraq by conventional methods and confirmed by molecular diagnosis by using a nested polymerase chain reaction (nPCR) technique by outer primer 18s (9005bp) and inner primer 18s (830bp) were used. This was conducted during the period from beginning of December (2019) to September (2020). A total of 200 samples were collected from adult and young and from both sexes of dogs.result was record infection rate of *Cryptosporidium spp* in dogs 34.5 % (69/200). The higher infection rate 34.76% (44/79), was recorded in young's dogs than adult dogs 30.25% (25/121), and the higher infection rate according to sex was found in females than males 48.27% (42/87) and 23.89% (27/113) respectively. The various infection rates were recorded during the months of the study, A higher infection rates 56% (14/25) in December (2019) and the lower infection rate 16% (4/25) infection rate in July.

The infection rates in dogs in DNA samples showed 47% (47/100) that were collected randomly from total 200 fecal samples of dogs in Karbala city. In dogs Phylogenetic analysis was done by use outer 18s (900bp) primers and outer (830bp). The result of the sequence analysis for 10 isolates randomly from the positive nested PCR samples recorded at the National Center for Biotechnology Information (NCBI) was indicate 7 isolate *Cryptosporidium parvum* and their accession number (MT329015.1 - MT329013.1) and 3 isolate *Cryptosporidium canis* and their accession number (MT329014.1, MT329016.1, and MT329018.1).

1.Introduction

Cryptosporidiosis is an important zoonotic parasitic disease caused by *Cryptosporidium spp*. Are apicomplexan parasites that have a wide occurrence in developed and developing countries and have major Public and Veterinary concern. The disease affects humans and a variety of animal species including the stray and domestic dogs **(9)(23).** *Cryptosporidium* infects a wide range of animal species. Studies on *Cryptosporidium* isolates obtained from cattle, sheep, pigs, cats, dogs, kangaroos, squirrels,Ostriches and other mammals, have shown that most

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species are infected with a restricted host-adapted *Cryptosporidium spp* or genotype **(24).(25).(1).**

Cryptosporidium parvum was thought to infect all animals. However, it is generally accepted that *C. parvum* infects primarily ruminants and humans **(12).** *C. parvum* infections have been found occasionally in other mammals such as mice and dogs, although companion animals are most often infected with host-specific Cryptosporidium spp. **(25).**Then,cryptosporidiosis in dogs has been reported worldwide, involving both asymptomatic and diarrheic dogs **(21).** Although Cryptosporidium oocysts are frequently detected in dog faeces, most infected animals have normal stools **(10)(26).** Due to few reports on the molecular analysis of the various *Cryptosporidium* species in domestic dogs, the study design to including the following aims.Detection of *Cryptospridium spp*. In domestic dogs by traditional methods and effect of age, sex and months on infection rate, Identification of *Cryptosporidium* oocysts by Nested-PCR and genotyping by sequences and phylogenetic tree.

2. Materials and Methods

2.1.Microscopic examination

Collected of 200 hundred fecal samples fromdomestic dogs from different sex and age from severalarea of Karbala province, during the study period from start of December (2019), to end of September 2020, each sample used for Flotation Methodsby Sheather's sugar solution**(5)**and stained with modified Ziehl-Neelsen staining technique **(4).**

2.2.Molecular examination.

DNA extraction, the kit used for DNA extraction from (Geneaid, Korea)as per the manufacturer'sinstruction. The DNA samples were stored at -80˚C untilfurther use. To identify Cryptosporidium spp. in the faecalsamples, fragments covering 18S rDNA gene were amplifiedby nested PCR. First, amplification of the 900bpCrypto18S1(FTTCTAGAGCTAATACATGCG And Crypto18S1 R CCCATTTCCTTCG AAACAGGA) regionwas carried out, and next, for secondary PCR, the 830bp (Crypto18S2F GGAAGGGTTGTATTTATTAGATAAAG and Crypto18S2R CTCATAAGGTGCTGAAGGA GTA)fragment was amplified using 3 µl of the first PCR product.Primers, mixture composition and PCR conditions have beendescribed by**(18).** The secondary PCR productswere examined electrophoretically in 1% agarose gels andvisualized after staining with Advance DNAstain. The identified species typing analysis was done by phylogenetic tree analysis in comparison with NCBI-Blast known sequences.The Statistical Analysis System according to **(20).**

3. RESULT .

3.1. Prevalence of *Cryptosporidium spp.* in dogs by using microscopic examination

The morphological appearance and measurement of oocyst by microscopic examination showed the Cryptosporidium spp by using floatation sheathers sugar solution and

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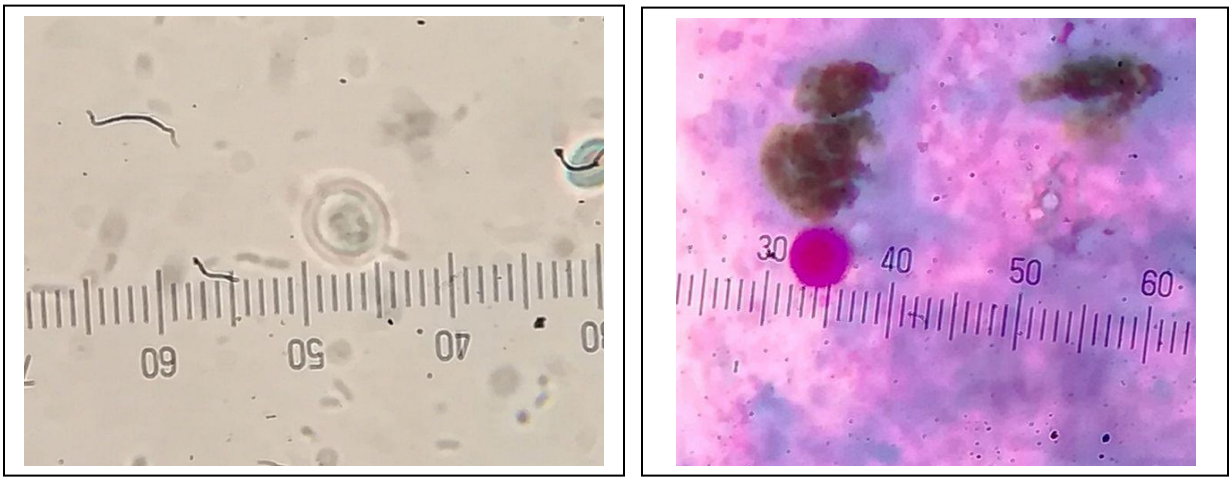
modified ZehielNeelsine stain, the oocyst appear spherical to oval shapes surrounded by thin membrane and contain undistinguished four sporozoites and purple with blue background and its size by using ocular micrometer was ±4µm × ±5.2µ (Fig.1)

Figure (1). Morphology of *Cryptosporidium spp.* by using Sheather's sugar solution and modified Ziehl-Neelsen stain



A total infection rate in dogs 34.5 % (69/200)were positive for *Cryptosporidium* oocysts and Prevalence of Cryptosporidium spp. according to age of animals, the young dogs, which recorded the high infection rate were 34.76% (44/79), while the lower infection rate 30.25% (25/121) was among the adult dogs, as in (Table.1).and prevalence of Cryptosporidium spp. according to Sex, a significant difference between the rate of infection and the major cases were among females, which recorded the highest percentage 48.27% (42/87), while the lows percentage 23.89% (27/113) was found in the males (Table. 2). prevalence of *Cryptosporidium* spp. in dogs according to months, higher infection rate with *Cryptosporidium* 56% (14/25) in December (2019) and the lowest 16% (4/25) infection rate in July. (Table.3).

Table (1): Total of infection rate Cryptosporidium spp in dogs according to age.

Age stage	Total number	Positive	Percentage (%)
Young's	79	44	55.69
Adults	121	25	20.66
Total	200	69	34.5%
Chi-Square (χ^2)	---	---	9.163 **
** (P≤0.01).			

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Table(2): Total of infection rate of Cryptosporidium spp. according to sex of Dogs.

Sex	Total number	Positive	Percentage (%)
Males	113	27	23.89
Females	87	42	48.27
Total	200	69	34.5%
Chi-Square (χ^2)	---	---	8.663 **
** (P≤0.01).			

Table(3): Total of infection rate of *Cryptosporidium spp.* in dogs according to months.

Months	Number of samples	Positive	Percentage (%)
December (2019)	25	14	56
January (2020)	25	11	44
February	25	9	36
March	0	0	0
April	0	0	0
May	25	8	32
June	25	6	24
July	25	4	16
August	25	6	24
September	25	11	44
Total	200	69	34.5
Chi-Square (χ^2)	---	---	14.882 **
** (P≤0.01).			

3.2. infection rate of*Cryptosporidium spp*. In dogs by using nested PCR Technique.

the total infection rates in dogs in DNA samples showed 47% (47/100) positive that among 100 examined the fecal samplesthat were collected randomly from total 200 fecal samples of dogs in Karbala city. In dogs Phylogenetic analysis was done by use outer 18s (900bp) primers and outer (830bp). The result of the sequence analysis for 10 isolates randomly from the positive nested PCR samples recorded at the NationalCenter for Biotechnology Information (NCBI) was indicate 7 isolate *C.parvum* and their accession number(MT329015.1-MT329013.1) and 3 isolate *C.canis*and their accession number(MT329014.1 - MT329018.1).**(Figure.4) and (Figure.5) .**

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Discussion.

Prevalence of *Cryptosporidium spp.* In dogs by Microscopic Examination the total infection rate with *Cryptosporidium spp.* In dogs that was 34.5 % (69/200), which was in agreementwith previous study in Iraq by**(6),** he was recorded the rate of *Cryptosporidium spp.*in dogs was 42%. Also agree with **(17)** he was found the rate of infection in Egypt by using microscopic examination was 35%. Alsoin Iraq our result didn't identical to result of**(3),** she was record the rate of infection in dogs was 15%. In Iran **(11),**Zambia (**14)** and Poland**(16).**They find the infection rate was 8% , 5.9 % and 2.7% respectively our result disagreement with them.

With regard to the effects of age on Cryptosporidiosis,the results ofpresent study found highest rate of infection in young's dogs were 34.76% (44/79),while the lower infection rate 30.25% (25/121) in the adult dogs.the results are in agreement with the studies that in Iraq were done by**(6)(3),** their studies recorded an infected rate highest in young's were 61.11% and 20% respectively and in adult were 31.25% and 10% respectively. The results are in agreement with study inEgypt done by **(17)**, who recorded 50% prevalence infectionin young's dogs compared with adults animals, it was 10%. In china **(8).** Whorecorded 12 % prevalence infection in young's dogs compared with adults animals, it was 5 % also agree with them. In South Africa prevalence of Cryptosporidium spp in adult higher than Puppies were 19% and 6% respectively **(1),** disagree with them.

The result showed A significant difference between the rate of infection and the major of *Cryptosporidium* cases were among females, which recorded the highest percentage 48.27% (42/87), while the lows percentage 23.89% (27/113) was found in the males.The results were inline agreed withthe study that in Iraq was done by**(3),**she was find the high prevalence of infection in females 21.42% and males were 8.5%. Agree with study conducted in Iran the higher infection rate recorded in females were 11/135 (8.14%) and the lower in males were 17/215 (7.9%) conduct by **(11).** In China the higher infection rate showed in female than male was 22 (7.6) and 9 (4.7) respectively by **(8).** Our result disagree with **(17)**in Egypt,the distribution of *Cryptosporidium* infection was higher inmale household dogs than females 38.9% and 21.4% respectively.

Prevalence of *Cryptosporidium* spp. in dogs According to Months,*Cryptosporidium*infection was showed highest rate in December (2019),January(2020), September,February and May were 56%, 44%, 44%, 36% and 32% respectively, then the infection rate was decrease during ,June,August and July were 24%,24% and 16 % respectively.These results are in line with study in Thailand conducted by **(19).**Who was recorded higher prevalence in winter was 9.7% and lower in rainy session was 6%. Also our result don't similar the results obtained by **(13).** In Iran, he was recorded the highest rate in autumn was 3.9% and lower rate in winter was 0.8%.

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Infection rate and phylogenetic of *Cryptosporidium spp*. In dogs by using PCR Technique.

The frequency of infection rates in dogs in DNA samples showed 47% (47/100) positive that among 100 examined the fecal samplesthat were collected randomly from total 200 fecal samples of dogs in Karbala city. In Iraq only one previous study was by **(3),** 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 **(11).**The prevalence of *Cryptosporidium spp*. In dogs in Egypt by Molecular Screening 24% was found by **(17),** disagree with them. Also our result different In China was recorded by **(8)**and in Japan by **(15),**they found infection rates of *Cryptosporidium spp*. in dogs by using PCR were 6.9% and 21.0% respectively. In Poland *Cryptosporidium spp.* were identified by nested PCR in canine stool samples 3.4% was done by **(16).**

BLAST analysis of ten samples selected randomlyfor the sequences to confirmed the species of*Cryptosporidium* strains, which had already been identified by nested PCR. The present Results of molecular study showedSequences obtainedby genotyping were compared with sequences depositedin GenBank basefound of two *Cryptosporidium* species in dogs: *C. parvum* and *C. canis*Genotyping analysis of the PCR-positive samples from dogs identified *C.parvum* in Iraq by**(15).**Our results were in agreement with results recorded by **(9).**In Poland he recorded 3 cases of *C. canis* and 2 cases of *C.parvum* in dogs were detected.In Italy the sequence analysis revealed that seven dogs harboured*C. parvum* and one dog was infected with C. canis**(2).**Thesequence analysis of the *Cryptosporidium*-positive canine samples in Chania by **(22),** revealed that presence of *C. canis*and *C. parvum.*While**(7),** he was only recorded *C. canis* in chain's dogs. Also in Chain **(8),** Whorecorded three species were identified as positive for *C. canis, C. muris* and the *Cryptosporidium* rat genotype IV.

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