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Epidemiological study of toxocariasis using ELISA and PCR techniques in some areas of Salah Al-Deen Governorate/ Iraq

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ABSTRACT

Toxocariasis is one of the most important zoontic diseases resulting from *Toxocara canis* and *Toxocara cati*, and they are among the most important parasitic nematodes in the small intestine of dogs and cats and other canidae and felidae, which accidentally infect humans. This study designed to diagnose Toxocariasis in human in some areas of Salah El-Din Governorate using ELISA- TEST IgG and PCR techniques, and finding the relationship of toxocariasis with age, gender, residential location, profession**.** In the current study 277 blood samples of people living in different areas of Salah Al-Deen Governorate, of both sexes were teasted, their ages ranged between 15-60 years, to investigate toxocariasis, using IgG test-ELISA techniques and PCR, from November to June 2019. The number of positive serum samples for ELISA test was 22%. The infection in Tikrit and Baiji district reached 23.9%, and in Al-Alam district 17.9%, by ELISA test. The highest infection was recorded in 37-47 years old with percentage was 23.94%, and the highest rate of infection was within males group and 36-26 years old and in females The highest rate was among 15-25 years and 37-47 years, and it reached 21.8%, and the infection among the uneducated group was 26.6%, and for those who owned dogs and cats, was 26.31%. The prevalence of the nematode eggs in stool samples was 23.3% in Baiji district, while the prevalence rate in soil samples was 30% , 45% in Baiji , and Al-Alam district, respectively. The molecular study was carried out to distinguish between *Toxocara canis* and *T. cati* eggs using ITS gene marker of helminthes eggs after DNA samples were extracted, which could serve as an effective genetic marker for identifying *Toxocara* spp. Which are closely related formally so that they cannot be distinguished from each other. The results of current research were absence of diagnostic gene in studied samples indicating that they are different types of worms and contain different diagnostic genes.

Keywords: Toxocara canis, Toxocara cati, Epidemiology, and diagnosis.

Introduction

Toxocara canis(Werner, 1782) Johnston, 1991 and T. cati (Schrank, 1788) Brumpt, 1927 belong to Nematoda phylum, Chromadorea class, Ascarididae family. Toxocariasis is among the most important zoonotic diseases resulting from *T. canis* and *T. cati* [1].

Humans are accidentally infected with toxocariasis, especially children, by eating food contaminated with worm eggs, work in soil and contact with dogs and cats. As these eggs hatch in the front part of small intestine the hatched larvae penetrates the mucosa of intestinal wall and are transported by blood or lymph to visceral organs such as liver, heart, lungs and kidneys cause Visceral Larva Migrans (VLM) and sometimes migrate to eyes causing Ocular Larva Migrans (OLM), and the migration of these larvae into different organs causes pathological symptoms such as fever, increase in acidophilus, and other symptoms similar to asthma [2].

Due to lack of sufficient information on epidemiology of human toxocariasis in Iraq [3,4] or diagnosis of worm eggs in soil [5] [6]. this study was designed to diagnose toxocariasis in human in some areas of Salah El-Din Governorate using ELISA- test IgG and PCR techniques, and finding the relationship of toxocariasis with age, gender, residential location, occupation.

Materials and methods

A- Epidemiological study

1- Location and duration of study: The study was conducted in the district of Tikrit, Baiji and al-Alam in Salah El-Din governorate from the beginning of November to June2019, information was collected from people according to a questionnaire that included name, gender, age, it was carried out in Baiji General Hospital, Al-Alam Hospital, and private laboratories in Tikrit.

2- Blood samples Collection: 277 Blood samples were collected by withdrawing 5-4 ml of venous blood using a tourniquet. samples were divided and placed in two types of laboratory tubes, where 2 ml of blood sample were placed in tubes containing anticoagulant for purpose of completing the blood tests, and 3 ml of remaining sample was placed in a tube devoid of any substance and left for 10 - 15 minutes at room temperature for blood to coagulate, then centrifuged for 3500 rpm, serum was isolated and transferred to Abendorf tubes and kept frozen at a temperature of -20 ° C until serological tests performed.

3-Collection of dog and cat feces: 30 samples of dog and cat feces were collected from different areas of Baiji district. 20 grams of stool were taken, and they were placed in clean, sealed plastic bottles.

4-Soil samples collection: 40 soil samples were collected, and more than 30 gm were taken from a depth of 1-10 cm, and placed in clean plastic bottles, and this was done during the period from April to June from Baiji and Al-Alam regions.

B-Laboratory tests:

1- Precipitation and Flotation technique:

The survey tests consisted of examining dog faeces and soil, which was conducted in Parasites Research Laboratory at College of Education for women at Tikrit University.

The sedimentation method was used in the examination of faeces, according to [7] . The flotation technique was used to determine worms eggs, which depend on difference in specific density according to[8] . In The method of flocculation with a solution of pivot sheather: soil samples were examined according to the method [9] .

ELISA test: The test was performed in Central Research Laboratory at Tikrit University, using a test kit provided by Demeditec Company with a sensitivity of> 95% and a specificity of> 95%.

DNA Extraction

A-Extraction of stool DNA:

DNA Electrophoresis was carried out to identify the DNA fragments after extraction or to detect the result of PCR reaction while the standard DNA was present to distinguish the size of the result of PCR reaction on agarose gel.

According to Sambrook *et al*., 1989 Aquarose gel was made at a concentration of 1.5g from agarose in 100 ml from SB 1X buffer.

3 µl of loading solution (Intron / Korea) was mixed with 5 µl of the assumed DNA, the samples were carried to the gel holes, and then exposed to an electrical current of 7 volts for a period of one to two hours until the dye reached to the other side.

Determination of DNA concentration: 200 micro-liters of Tris-EDTA (TE) was added to 3,800 µl distilled water and mix, withdraw 10 micro-liters of it and discard it, then added 10 micro-liters of DNA Di. And 200 micro liters of the mixture for each sample. The following set of tubes were prepared as follows: blank 200 µl , sample 200 µl and standard 200 µl that's all mix with DNA extraction 2 µl from sample and 2 µl from standard, then a second mixing of samples was carried out using a mixer. Samples were left at room temperature for 5 minutes, And extracted the mix immediately.

The gene was not found in our samples because we touch these samples from deferent places from original study.

Statistical analysis: Statistical analysis was performed using Chi-square test (X2) and level of significance of differences between the rates was estimated using one way ANOVA followed by Duncan test.

Results and discussion

The study was conducted to investigate the spread of toxocariasis in some areas of Salah al-Din governorate from November to June in district of Baiji, Tikrit and al-Alam. The results showed that the rate of toxocariasis was 22%.

This result agrees with study of [10] who recorded the incidence of toxocariasis in adults 7.3% in Iraq, In Egypt a study was conducted in the adult group in which the infection rate was 6.6% [11] , as for the studies that were conducted in Salah El-Din governorate. the infection rate was 6.98% [12] .

The highest percentage of infections was in age group 47-37 years old which was 23.943%, and lowest percentage in 26-36 age group which was 20.932%, Table (1).

Table (1): The percentages of toxocariasis according to age group using ELISA technique.

Age group	No.of samples tested	No.of Positive samples	%
15 -25	81	17	20.987
26 –36	86	18	20.932
37 -47	71	17	23.943
48 -58	39	9	23.076
total	277	61	22
Statistical analysis	Chi-Square = 0.288 P-Value = 0.962		

This result indicates that this age group continues to be exposed to infection with toxocara worms, which work to secrete their antigens in body and thus means spread of toxemia on a large scale.

Our current study showed that the highest unit value extracted in the ELISA test is in 37-47 years, and 58-48 years old, and this is consistent with a study [13] which confirmed that the seroprevalence of antibodies to Toxocara increase with age increases, and perhaps Because of recurrence of infection that leads to elevation of the antibody.

The highest percentage of infection was recorded in females in age group 15-25, 47-37 and 48-58 years, the percentage reached 21.8%, 21.8% and 21.7% respectively, and lowest percentage was within age group 26-36 years was 16.9%. while in males. The highest percentage was recorded within age group 36-26 years, it reached 27.2, and lowest percentage was recorded within age group 15-25 years, which was 19.2%. No significant differences between the sexes were recorded according to the statistical analysis (Table 2).

Table (2): The percentage of toxocariasis according to age groups by using ELISA technique.

Age group	male			Female		
	No. of sample tested	Positive no.	%	No. of sample tested	Positive no.	%
15 -25	26	5	19.2	55	12	21.8
26 –36	33	9	27.2	53	9	16.9
37 -47	39	10	25.6	32	7	21.8
48 -58	16	4	25	23	5	21.7
total	114	28	24.5	163	33	20.2
Statistical analysis	Chi-Square = 0.556 P-Value = 0.906			Chi-Square = 0.518 P-Value = 0.915		

The results of this study agree to the findings by [12] according to its results, the rate of the infection was higher within female 8.37% compared to males 6.58%, and the reason may be due to the differences in playing behavior between sexes, because they live under the same conditions in addition to similarity of studied areas and the pollutants that surrounding them, and this is what exposes both sexes to developing toxocariasis.

The results of the current study do not agree with results of [12] , which through her study showed that there is no relationship between the infection and dogs and or cats keepers. A team of researchers believes that keeping a dog as a pet without comparing with vaccinations pets a risk factor for toxocariasis [14].

The level of soil pollution with toxocariasis was correlated with the numbers of dogs and cats of all ages, as well as with social behavior, cultural level, religious belief in addition to health awareness, which may lead to exposure to infection, especially for children [9].

DNA was extracted from the parasite samples found in the feces of 30 samples from infected animals after confirming their microscopic infection, as eggs were completely purified by special laboratory techniques, and then DNA was extracted from them as samples of parasite's DNA and after confirming the presence of DNA as in picture (1) Duplication and amplification processes were performed for genes under diagnostic study, but results were negative. Then the same experiments were conducted using RT-PCR technique to ensure correctness of the work and the accuracy of the results, then the results were sent outside the country to ensure the presence of genes in samples under study and diagnostic experiment, and they gave same results, which indicated presence of DNA and absence of diagnostic genes and this is an indicator, that the types of genes used to detect and diagnose parasite in the current study are different types of *Toxocara* spp. For several reasons, including difference in environment and occurrence of genetic variation in species to adapt to environment to which parasite belongs, and new receptions have a gene expression that enables them to change parasite type according to its functional and adaptive need for that.

Diagnosis of toxocariasis relies heavily on conventional microscopy and serological techniques, however these techniques are largely unreliable due to the low specificity. It may be difficult to diagnose the presence of *toxocara* eggs depending on morphological characteristics by conventional methods [15]. Therefore, techniques based on PCR have been developed and used to accurately identify and diagnose toxocariasis due to its high sensitivity, specificity, speed and usefulness[16,17] .

Several studies have been conducted using molecular scales based on PCR and RFLP techniques to characterize the eggs of *T. canis* and *T. cati* worms. According to recent research, there are 11 species of *Toxocara* spp. [18].

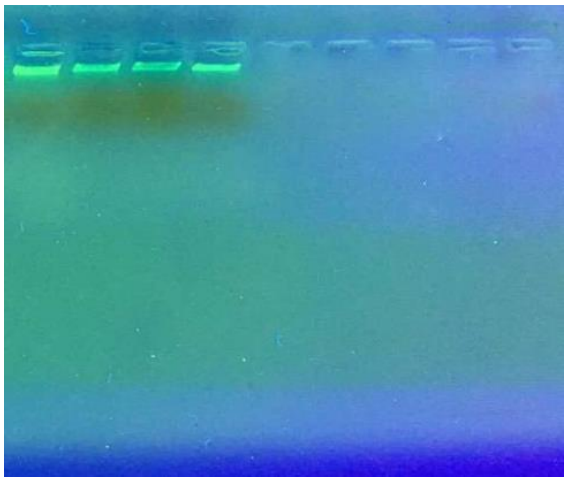
Previous studies showed that ITS2 gene sequence transcribed from rDNA provides a good genetic marker for molecular study in parasitic populations [19] and that ITS is located between 18S, 5.8S, and 28S coding regions of nuclear ribosome, which has proven useful for diagnostic purposes. At the species level [20]. ITS sequence of other parasitic worms has been demonstrated that can serve as an effective genetic marker for determining T.canis from other parasitic species[21,22].

The ITS1 and ITS2 gene segments from rDNA have been used in several studies to distinguish between Toxocara spp. These are closely related morphologically so that they are indistinguishable from each other as species[23]. [24] the qPCR method targeting ITS sequences to identify *Toxocara* egg species in stool samples, The use of the newly developed qPCR could be considered a useful tool for detection. of *T.canis* and *T.cati* eggs in faecal samples. Recent studies have shown that mtDNA is a genetic marker that can be useful in diagnosis and verification of nematodes in general [17] .

The results of current research confirmed results of a lot of research in that diagnostic genes differ from one region to another, as[25,17] who confirmed that genetic information on *Toxocara* parasites is very little and limited, and there is still a need for a target region in DNA confirmed by research. to know accurate diagnosis of *T.canis* by PCR techniques.

It has been demonstrated in various studies to provide reliable genetic markers for identifying and distinguishing *Toxocara* through the ITS1 and ITS2 gene[26,27] , and in a study conducted by Bekir in 2018 in which the gene ITS2 was used and it was shown that it is best option to identify and distinguish

nematodes , and reported[28] that mitochondrial ATPase 6 genes are well preserved in *Toxocara* species and can be used to distinguish between species and in molecular evolution of genes. Therefore, there is a need to know appropriate DNA target region (genetic marker) in order to accurately identify nematodes by PCR technique.



picture1: Electrophoresis of DNA extract products, agarose gel by concentration 1% samples is a representation of DNA.

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