

Serodiagnosis of Toxocariasis by ELISA test using anti- *T. canis* IgG antibodies in stray dogs compared to PCR

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Abstract

Toxocara (T.) canis is a nematode parasite of canines; belong to the *Ascarididae* family, which accidentally infected humans. Puppies expel the eggs with the feces from the fourth week of the life cycle. This study is the first study in Iraq for detection seroprevalence in stray dogs and extended from January to September 2017. Our study was aimed to investigate the seroprevalence of *T. canis* infection in stray dogs from different areas in the Al-Diwaniya province, Iraq to detection of specific IgG antibodies to *T. canis* compared to Conventional PCR technique with the effect of the risk factor. One hundred of the blood sample and one hundred of a faecal sample of same dogs after shooting were studied using indirect ELISA test and PCR. The result revealed that 71% of the dogs had a seropositive result for this parasite by ELISA test. Dog age is an important factor and affects seroprevalence, were shown that positive rate in adult dogs was more 83.05% than the young dogs 53.65%, while no significant between dogs according to sex. PCR technique showed 58% of dogs were positive for internal transcribed spacer 1 (ITS1) ribosomal RNA. The sensitivity and specificity of ELISA test was 79 and 40% respectively.

Keyword: Dogs, *Toxocara canis*, ELISA, PCR
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التشخيص المصلي لمرض داء السهميات بواسطة تقنية الاليزا باستخدام مضادات IgG للسهمية الكلبية في الكلاب السائبة مقارنة بتقنية تفاعل سلسلة البلمرة

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الخلاصة

السهمية الكلبية هو طفيلي الديدان الخيطية في الكلاب تنتمي إلى عائلة *Ascarididae*. السهمية الكلبية التي تصيب البشر بطريق الخطأ. الجراء تطرح البيض بالبراز من الأسبوع الرابع من دورة الحياة. هذه الدراسة هي أول دراسة في العراق لتحديد نسبة الإصابة المصلية في الكلاب السائبة وتمتد الدراسة من تشرين الثاني ولغاية تشرين الأول ٢٠١٧. كان الهدف من هذه الدراسة تحديد انتشار الإصابة المصلي لطفيلي السهمية الكلبية في الكلاب السائبة من مناطق مختلفة في مدينة الديوانية، العراق، لقياس مضادات IgG مقارنة مع تقنية تفاعل سلسلة البلمرة مع دراسة تأثير عوامل الخطر باستخدام تقنية الاليزا الغير مباشر وتفاعل سلسلة البلمرة. بينت النتائج أن ٧١% من الكلاب كانت لها نتيجة إيجابية بواسطة اختبار الاليزا. عمر الكلاب هو عامل مهم يؤثر على نسبة الإصابة المصلية حيث كانت نسبة الإصابة في الكلاب البالغة ٨٣,٠٥% أكثر من الكلاب الصغيرة ٥٣,٦٥% في حين لا يوجد فرق معنوي بين الكلاب وفقاً للجنس. أظهرت تقنية تفاعل سلسلة البلمرة ٥٨% من الكلاب إيجابية للفصل لجين Internal Transcribed Spacer 1 (ITS1) كانت حساسية وخصوصية اختبار الاليزا ٧٩ و ٤٠% على التوالي.

Introduction

Toxocara canis is parasite causes a disease called toxocariasis. *Toxocara canis* is causing parasitic intestinal infection of domestic animals (1). In humans, the roundworms are an etiological factor of ocular and visceral larva migrans (2). *T. canis* have an oral-faecal transmission cycle, infective eggs from soil that contain on (playgrounds, sandpits and gardens) from direct contact with dogs, or raw vegetables or infected unwashed hands (3). The infected young puppies usually show acute clinical signs of toxocariasis. The typical clinical signs are a loss of condition, poor growth and sometimes show potbelly. The worms may be passed in the faeces or vomited. Also, may be appearing clinical sign as constipation, vomiting, diarrhea, and flatulence (4). Infected dogs by *Toxocara canis* can be detected from clinical symptoms and case history. The final diagnosis occurs by finding eggs (characterized by pitted shells, dark brown in color and thick) in feces (5). Indirect ELISA methods can detect IgG antibodies against TES Ag (IgG TES-ELISA) that could be considered diagnosis tool for toxocariasis (6). However, cross-reaction is common among the helminths (7).

Materials and methods

Collection of samples

A total of 100 blood sample that collected from the heart and jugular vein of stray dogs after shooting , and 100 fecal samples were collected from the same animal. A blood sample was put in gel tube , while fecal samples put in a container with information include date, age and sex.

Serum preparation and ELISA test

Centrifugation separated the serum at 3000 round per minute for 10 minutes, the serum aspirated carefully by pipette into dry, sterile and labelled test tubes, storage at -20°C until used. Indirect ELISA kit was purchased from MyBioSource company in America country were used for detection anti- *T. canis* IgG antibodies in serum samples.

Molecular study

For polymerase chain reaction, the primer was designed in this study using complete sequence *Toxocara canis* isolate, internal transcribed spacer 1, 5.8S ribosomal RNA gene from GenBank: KF577856.1. These primers were provided by Bioneer Company, Korea showing in the table (1).

Genomic DNA was extracted from 100 fecal samples using AccuPrep®Stoolused kit from Bioneer company in Korea, and performed based on company directions. The extracted DNA were tested by apparatus called Nanodrop spectrophotometer (THERMO, made in the USA). These apparatus measures the DNA concentration and purity by

reading the absorbance at (260 /280 nm). This technique was carried out according to company instructions as in table (2). Then PCR products were analyzed by agarose gel electrophoresis.

Table 1: Gene, primer, sequence and PCR product size

Primer	Sequence	PCR product size
<i>T. canis</i>	F CTCACCTAGCTATTGCCCGG R CCTTGGCAAGGTACGCTGTA	516 bp

Table 2: The PCR thermocycler constructs reaction conditions

PCR Step	Temp.	Time	Repeat
Initial denaturation	95 °C	5 minutes	1
Denaturation	95 °C	30 seconds	30 cycle
Annealing	58 °C	30seconds	
Extension	72 °C	60 seconds	
Final extension	72 °C	5 minutes	1
Hold	4 °C	forever	-

Sensitivity and specificity calculation

In this study, sensitivity and specificity of ELISA for *T. canis* infection versus PCR result were calculated according to (8) (Table 3).

Table 3: Explained sensitivity and specificity calculation

	PCR		Total
	Positive	Negative	
ELISA	Positive true +ve	false +ve	test +ve
	Negative false -ve	true -ve	test -ve
Total			

$$*Sensitivity = \frac{True+}{(True+)+(False-)} \times 100$$

$$* Specificity = \frac{True-}{(True-)+(False+)} \times 100$$

Statistical analysis

It was done by social science for the statistical package (SPSS) version 17 for Windows software and Microsoft Excel 2010. Differences between groups were used test of the chi-square at (P≤0.05).

Results

One hundred blood samples were analysis revealed that 71% of the dogs had seropositive result for this parasite. The infection rate in males was 79.16% higher than the females 63.46% (Table 4).

Table 4: Show rates of *Toxocariasis* infection in dogs by ELISA according to sex

Sex	No. of examined samples	No. of Positive samples	%
Males	48	38	79.16
Females	52	33	63.46
Total	100	71	71

χ^2 : 0.165 p value: 0.684 (non-significant difference ($p > 0.05$)).

Our study founded the percentage of infected adult dogs by toxocariasis was (83.05%) while the young dogs were (53.65%) as in Table (5).

Table 5: Rates of *Toxocariasis* infection in dogs by ELISA according to ages.

Age	No. of examined samples	No. of Positive samples	%
Young	41	22	53.65
Adult	59	49	83.05
Total	100	71	71

χ^2 : 10.150 p value: 0.0014 (significant difference $p < 0.05$)

The total results of PCR technique showed that out of 100 dogs fecal samples 58% were positive for the gene of the ribosomal RNA of the ITS1 (Figure 1).



Figure 1: Agarose gel electrophoresis image show the PCR product analysis of internal transcribed spacer 1 (ITS1) ribosomal RNA gene in *Toxocara canis* of DNA extracted from faecal samples of stray dogs , where Lane (M) ladder (2000-100bp), lane 1, 2, 4, 5, 6, 8, 10, 11, 12, 13, 15, 16, 18, 21, 27, 30, 31 shown positive *Toxocara canis* at (516bp) PCR product size from 33 sample.

The specificity and sensitivity of serodiagnosis of toxocariasis in dogs are compared to PCR was 40 and 79% respectively as in table (6).

Table 6: Represented Sensitivity and Specificity of ELISA for *T. canis* infection versus PCR result

		PCR		Total
		Positive	Negative	
ELISA	Positive	46	25	71
	Negative	12	17	29
Total		58	42	100

$$*Sensitivity = \frac{46}{46+12} \times 100 = 79\%$$

$$* Specificity = \frac{17}{17+25} \times 100 = 40\%$$

Discussion

In many reports that related to toxocariasis in dogs are diagnosed by the detection of the parasite eggs (9). In the present study investigated the *T. canis* parasite in stray dogs serologically using the indirect ELISA test to detect IgG-ELISA.

This study is the first time that studies the seroprevalence of *Toxocara canis* in stray dogs from the different region of the Al-Diwaniya province, south of Iraq. The seroprevalence of *Toxocara* in stray dogs was 71%. The seroprevalence was higher than those reported by Rubel *et al.* (10) who recorded 22% in the middle-income region and 40% in a low-income region out of 105 in Argentina. Also in Mexico by Garcia *et al.* (11) recorded 56.1%. Serological of anti-*T. canis* IgG recorded in Brazil was 82.7% (12) which is higher than that recorded in the present study. In Argentina, the seroprevalence was reported high rate 86.95% had positive serology for this parasite (13). The indirect ELISA test in only measured antibodies against *T. canis* and the presence of IgG antibodies does not mean the animal was harbor the infection at that specific time. A study of (11) mentioned that the anti- *Toxocara* antibodies (IgG) may remain for a long time, even in the absence of the disease, therefore, a positive result by ELISA-IgG cannot distinguish between past and current.

According to sex, no differences in seroprevalence were observed between males and female dogs, similar results previously reported by Garcia *et al.* (11). However, there is data that showed higher seroprevalence in male dogs (12). Moreover, it is important to consider that female dogs (bitch) keep larval stages in their bodies' tissues during pregnancy periods which infect their puppies after birth (14).

The rate of infection in the present study varied according to ages. It was significantly higher among adult dogs 83.05%, and the lowest rate in young dogs 53.65%. These results may be because dogs are constantly getting infected with *T canis* eggs from the contaminated

environment as they grow up. Also, the ELISA test in this study only measured antibodies against *T. canis*, and the presence of antibodies does not mean the animal was sick at that specific time. Also, in the adult dogs the most common the worms developed in the small intestine to the adult stage. Wherever, the larvae become latent in the host tissue (15). The findings agree with previous studies in Mexico by Garcia *et al.* (11) who recorded high titers of antibodies of the anti-*T. canis* in adult dogs' more than young dogs. A study of Silva *et al.* (12) recorded that adult dogs are positive serologically more than the young.

The serological technique does not need a long time and more sensitive as compared with a classical parasite examination of faeces of *T. canis*. There only the one preventive factor in using ELISA is not easy for clinical laboratories and small-scale research (16).

In this study, the sensitivity and specificity for toxocariasis in dogs serological compared to PCR were recorded 79% and 40% respectively. A study of Jin *et al.* (17) recorded sensitivity and specificity of the ELISA were 79% and 40%, respectively. A study of (18) compared sensitivity of dot-ELISA, and the standard ELISA were Sensitivity 100% for both, while specificity 95 % and 90 % for dot-ELISA and the standard ELISA respectively. A study of (19) compared between using of IgG-ELISA and IgG4-ELISA, the specificities were 36 and 78.6% respectively, the sensitivity of the IgG-ELISA was 97.1%, while that of the IgG4-ELISA was 45.7%.

Detection of the toxocariasis serologically has cross-reactions with other helminthiases, that make it less specific (20), such as geohelminthes, *Fasciola*, and *Strongyloides* (20,21). Furthermore, *Echinococcus*, *Schistosoma* and *Taenia* (22). The serodiagnosis is considered a problem in tropical areas because of the most common mixed parasitic infections.

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