DETECTION OF TOXOPLASMOSIS AND SOME RISK FACTORS WITH THE INFECTION RATE IN LOCAL AND SHAMI GOATS

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ABSTRACT

This study was aimed to evaluate detection of *T. gondii* in blood DNA based on the B1 gene and serologically, also to investigate the effect of some risk factors (sex, ages, breeds and flocks). A total of 190 goats were included. The blood samples were obtained from the jugular vein and subjected to nested PCR (nPCR) and Elisa for detection of *T. gondii*. The results obtained that the prevalence was 24.21% by nPCR and 46.31% by ELISA with significant differences. The degree of agreement between nPCR and ELISA was a slight (Kappa=0.212). The sensitivity and specificity of ELISA was 67.39 and 60.42 respectively. According to results of nPCR, there was a significant difference between sex, with a higher infection rate in males (40.42%) than females (18.88%). Males are more at risk to infection than that of female (The crude odds ratio (COR)=2.91, (95% CI 1.41-5.97) and adjusted odds ratio (AOR)=4.66, (95% CI 2.01-10.79). The infection rate in the Al-Dibuni flock (36.36%) was significantly higher than that of Abu-Gharib (19.25%). The goats in Al-Dibuni are more at risk to infection than that in Abu-Ghraib flock COR=2.39, (95%CI 1.19-4.80) and AOR=2.31, (95%CI 0.97-5.50).

Keywords: Toxoplasmosis, nPCR, ELISA, risk factors, detection, infection

تشخيص داء المقوسات وبعض عوامل الخطورة بمعدل الإصابة في الماعز المحلي والشامي

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المستخلص

هدفت الدراسة إلى تقييم الكشف عن *T. gondii* في الحامض النووي للدم بناءً على الجين B1 والمصلي ، وكذلك لمعرفة تأثير بعض عوامل الخطر (الجنس ، والعمر ، والسلالات ، والقطعان). تم تضمين ما مجموعه 190 ماعز. تم الحصول على عينات الدم من الوريد الوداجي وخضعت لـ nPCR و Elisa للكشف عن .T. gondii أظهرت النتائج أن معدل الاصابة كان 24.21٪ بواسطة nPCR و 46.31٪ بواسطة ELISA مع وجود فروق إحصائية .كانت درجة التوافق بين nPCR و ELISAطفيفة (Kappa = 0.212) كانت حساسية وخصوصيةELISA 67.39 و 60.42 على التوالي. وفقًا لنتائج nPCR، كان هناك فرق معنوي بين الجنس ، حيث كان معدل الإصابة أعلى لدى الذكور (40.42٪) مقارنة بالإناث (18.88٪). الذكور أكثر عرضة للإصابة من الإناث (نسبة الأرجحية الخام2.91 = (COR) ،95٪) CI 1.41-5.97) ونسبة الأرجحية المعدلة 4.66 = (AOR) 95٪) CI 2.01-10.79). أشارت النتائج أيضا إلى أن نسبة الإصابة في قطيع الديبوني (36.36٪) كانت أعلى معنويا (P <0.05) من مثيلتها في أبو غريب (19.25٪).الماعز في الدبوني كانت اعلى خطورة للاصابة من مثيلاتها في قطيع أبو غريب COR= 2.39 و AOR=31.2.

الكلمات المفتاحية: داء المقوسات، nPCR، ELISA، عوامل الخطر، التشخيص، الاصابه

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INTRODUCTION

Toxoplasmosis in goats and sheep has great importance because it leads to many economic and production losses, consequently transmitted to humans (10). In addition, goats are regarded to be more susceptible to toxoplasmosis than sheep because of their higher activity and mobility, which increases the chances of coming into contact with polluted sources (2). Even in older goats, toxoplasmosis can cause miscarriage and death (12). The greater part of *T. gondii's* disease burden is among the highest of all foodborne parasitic illnesses globally (42), third among all foodborne diseases in the United States, and second amongst foodborne parasitic infections in Europe (9). The ELISA and molecular tests were used to detect *Toxoplasma gondii* in the blood (29; 3). In general, many studies have been conducted using a serological and molecular diagnosis of *T. gondii* for camels, sheep, horse, quail and goats in Iraq **(8; 9;** 26; 29). In goats in Iraq Kader and Al-Khayat (24) found via latex, MAT, ELISA 25 (28.4%), 21 (84%), 4 (16%), 3 (12%), and 22 (88%) positive and negative results, respectively. Qazaz and Faraj (34) reported a significant difference between male (71.42%) and female (87.32%) in goats. Also, the similar study found that the age significantly (P<0.05) affects the infection rate. Gazzonis *et al*. (19) found infected goats from 74 Crossbreed (48.7%), (30.7%) of 31 Saanen, (and 38.9%) of 37 Alpine. Flock size, and especially when it is small, is the most significant risk factor, particularly particular to the regions under investigation, this finding may have its roots in the methods of farm management that are commonly used there (1). Small herds are the ones managed traditionally because (a) the livestock's aliment is easily accessible to cats; (b) the animals' grazing is frequent, and the transition from intensive to extensive and vice versa was done daily; and (c) there are no zoo-hygienic measures in place, such as feeding organization, cleaning, etc. (44; 25). How a herd or flock is managed and produced affects its overall size. Larger herds are more likely to be subject to intense management practices. Smaller herd farms are likely to have less specialization and less intense confinement. Farms that prioritize animal welfare in their production methods typically have smaller herds or flocks because raising animals requires more room. Exposure of livestock to *T. gondii* can occur by oocyst contamination of feed, water, or farmland and through contact to other infected intermediate hosts, such as rodents; however, the relationship between herd or flock size and these factors is not well understood (42). In Iraq one study described risk factors flock Al-Hamada *et al* (8). This study aimed to evaluate the detection of *T. gondii* in blood DNA based on the B1 gene and serologically, also to investigate the effect of some genetic and non-genetic factors on the infection rate.

MATERIALS AND METHODS

Samples collection: Blood samples (20 ml) were drawn from the jugular veins of 190 goats (Shami and local), both female and male and with different ages, at two flocks (Ruminant Research Station of the General Authority for Agricultural Research /Ministry of Agriculture Abu Ghraib / Baghdad and AL-Dibuni Research Station for Researches / Wasit). The disposable needles were used and simple vacutainer tubes (gel tubes), then samples were brought to the laboratory in a cooler box with ice. Serum samples were extracted using a 2,000 g centrifuge for 10 minutes and kept at - 20 °C in labelled Eppendorf tubes until ELISA testing. One hundred ninety blood samples were obtained from the jugular vein using a medical syringe with a capacity of 10 ml (Vacum Tube Needle) with EDTA (Ethylene Diamine Tetra Acetic Acid) and kept at - 20 °C for the DNA extraction.

Nested PCR: This technique was performed to detect *Toxoplasma gondii* based on the B1 gene from blood samples. This method was carried out according to the method described by Halleyantoro *et al*. (21) as following steps: These primers were provided by Scientific Researchers. Co. Ltd / Iraq. B1 gene PCR primer and B1 gene Nested primer Primers Sequence 5'-3' PCR product size B1 gene PCR primer F GGAACTGCATCCGTTCATGAG 230 bp R GGCGACCAATCTGCGAATACACC B1 gene Nested primer F TGCATAGGTTGCAGTCACTG 131 bp R TCTTTAAAGCGTTCGTGGTC

Statistical analysis

Data were collected and subjected to the Elisa test and nPCR. The comparison was made between the results to obtain sensitivity, specificity, and kappa coefficient to evaluate the Elisa test (27). The odds ratios were estimated to identify the risk of some factors. Two types of odds ratios were estimated; the crude odds ratio directly and the adjusted odds ratio using logistic regression, Chi-square test was used to assess the differences among unpaired proportions whereas the McNemar test was used for paired proportions. P<0.05 is considered a significant.

RESULTS AND DISCUSSION

Total infection by indirect ELISA and Npcr: Blood: Out of 190 goats' blood examined by using nPCR positive sample was 46(24.2%). while, in the indirect IgG ELISA, the positive sample was 88(46.3%) for serum, indicating a significant difference (p<0.0001) between the two tests **(Table 1).**

Table 1. Comparison between infection rate using ELISA and PCR for blood

Type of test	No	Infect No	%	McNemar test (Paired proportions)	P-value
nPCR	190	46	24.21	32.88	<0.0001
ELISA	190	88	46.31		

A study on other microorganisms, including *Toxoplasma gondii*, *Histomonas meleagridis* and *Strongyloides stercoralis*, reported higher sensitivity of nested PCR compared with real-time (39). As the estimation of prevalence by nPCR is more accurate than ELISA and lower accurate compared with PCR technique with high resolution melting mechanism (nested-qPCR-HRM), the estimation of true prevalence was (30.92 %), 95% CI (23.72-39.28). Halleyantoro *et al* (19) found that the human B1 gene did not amplify DNA from any other bacterial or fungal species expect *Toxoplasma gondii* and that its sensitivity was unaffected by changes in DNA count or protein levels. Furthermore, the B1 gene was the most commonly utilized in toxoplasmosis molecular research (6). Also, the results, according to Saied Jassam and Salih (37), may have occurred as a result of the B1 primers' high sensitivity and the two primers' extreme specificity for the *T. gondii* strain found in Iraq. Additionally, it is peculiar to the *T. gondii* magnification DNA utilized in the PCR procedure (6). Nested PCR was compared with PCR technique with high resolution melting mechanism (nested-qPCR-HRM) for detection of toxoplasmosis for the B1 gene and found that the sensitivity and specificity were 78.31 and 100% also, the nested-qPCR-HRM and nested-PCR findings had a 0.49 kappa coefficient of correlation, which indicates that the two techniques for analyzing the B1 gene were quite agreeable (10). So, we estimated true prevalence using the sensitivity and specificity mentioned in this research, as shown in Table (2). Using the following equation (34):

True prevalence= (Apparent prevalence + Sensitivity-1) / (Sensitivity + Specificity-1)

Table 2. Estimation of apparent and actual prevalence by nPCR

	Prevalence %	95%CI
Apparent prevalence	24.21	18.67-30.77
True prevalence	30.92	23.72-39.28

The result of the kappa (0.212) indicated a slight agreement between ELISA and nPCR in blood with sensitivity (67.39) and specificity (60.42) **Table (3).**

Table 3. Some parameters for evaluating ELISA compared with nPCR in blood

	Blood nPCR		
Blood ELISA	-	+	
-	87	15	102 (53.7%)
+	57	31	88 (46.3%)
	144 (75.8%)	46 (24.2%)	190
Weighted Kappa[a]			0.212
Standard error			0.064
95% CI			0.0868 to 0.337
Sensitivity			67.39
Specificity			60.42

PCR makes early toxoplasmosis diagnosis is much easier (41). The adoption of PCR for the detection included different pathogens in animal farms (30; 31) Increased antibody titers might suggest an ongoing *T. gondii* and my point out the conversion of status from chronic to acute as a result of reinfection due to immunosuppressive circumstances. Or occurrence of a new infection could be happened for several reasons, explosion to contaminated water, pasture, or feed containing *T. gondii* oocysts or the introduction of recently acquired animals into a herd without previous knowledge of toxoplasmosis incidence at the animal's origin site (16). Qazaz and Faraj (32) found *toxoplasma gondii* in goats using ELISA 83.69 %. Zhou *et al*. (46) found that *T.gondii* in blood ELISA and nPCR were (42.5%), and 37.6%), respectively. As a result, serology is insufficient since it depends on antibody formation, which either absent or delayed. In contrast, Hade *et al*. (18) stated that the nPCR is dependent on the existence of parasite genetic material. However, the difference between ELISA and RT PCR in sheep blood samples was not significant (4).

Effect of some genetic and non-genetic factors on the infection rate by using nPCR

Total infection rates of toxoplasmosis in goats according to the breed: Results illustrated that the infection rate in local 17(30.90%) did not differ significantly compared with Shami 29(21.48%) the COR=1.63 whereas the corresponding AOR=2.31 **Table (4).**

Table 4. Effect of breed on infection rate with toxoplasmosis

Breed	Total No	Infected	P-value	COR (95%CI)	AOR (95%CI)
Local	135	29(21.48 %)	0.16	Ref. (1)	Ref.(1)
Shami	55	17(30.90 %)		1.63(0.80-3.30)	2.31(0.97-5.50)
Total No	190				

COR=Crude odds ratio
AOR=Adjusted odds ratio

Results showed that local goats have a higher risk of about 2-fold. These results could be attributed to non-significant genetic resistance difference between the breeds. Mavrogenis *et al*. (28) mentioned that Shami goats are a dual-purpose type of endemic to the Middle East noted for their outstanding milk and meat output when compared to other local goats' varieties and considered one of the favourite breeds because it is relatively resistant to many infectious and non-infectious diseases. Also, Damascus goats are dairy goats that are more adaptive to their surroundings (45). In Iraq, studies were done on Shami goats (20)

Total infection rates of toxoplasmosis in goats according to sex.

The results of sex showed that the infection rate in males 47(40.42%) was significantly ($p<0.01$) higher than in females 27(18.88%), with only significant differences **in Table (5).**

Table 5. Effect of sex in the infection rate of toxoplasmosis

Sex	No	Infect	P-value	COR (95%CI)	AOR (95%CI)
Female	143	27(18.88)	<0.01	Ref. (1)	Ref. (1)
Male	47	19(40.42)		2.91(1.42-5.97)	4.66(2.01-10.79)
Total No	190				

COR=Crude odds ratio
AOR=Adjusted odds ratio

The results were identical to what was found by Bahreh *et al*. (11) using nPCR. The incidence of males was (19.5%) higher than females (3.4%). This is attributed to the fact that females reared for milk's purpose. These differences could be resulted from the management style as the males are not subjected to culling, and they stay in the herd for a long time, which means they will be more exposed to the pathogen for a long time. Also, the males increased the risk by mating with several females. Males have a much greater frequency than females, as previously observed (25). Female animals were shown to be more resistant to protozoan parasite infection than male animals females and this could be attributed to high immunity in females. This is likely related to estrogen in females, which boosts immunity, whereas testosterone lowers immunity in males (38). However, a number of additional variables might compromise female immunity, including changes in sex-related hormones, environmental factors, age, diet, and pregnancy (35). In contrast, Qazaz and Faraj (32) found that females were more likely than males to contract toxoplasmosis. However, some studies that used serological tests for detection confirmed that females are more at risk compared with males and contributed to the policy of management which slaughtered the males at an early age (16).

Total infection rates of toxoplasmosis in goats according to age.

The findings showed a high rate of infection (28.57%) in the 2 years age group, followed by ≥4 years (27.41%) ≤1 year (24.13%), then the 3 years age group (16.67) without significant difference **Table (6).**

Table 6. Total infection rates of toxoplasmosis in goats according to age

Age/years	Total No	Infected n (%)	P-value	COR (95%CI)	AOR (95%CI)
≤1	58	14(24.13)	0.58	Ref 1	Ref. 1
2	28	8(28.57)		1.25 (0.45-3.47)	1.31 (0.95-2.79)
3	42	7(16.67)		0.63 (0.23-1.72)	0.57 (0.19-1.23)
≥4	62	17(27.41)		1.56 (0.68-3.59)	1.66 (0.37-2.92)
Total	190				

COR=Crude odds ratio
AOR=Adjusted odds ratio

These results agree with results obtained by Bahreh *et al*. (11) who demonstrated no significant differences in ages. However, some researcheres mentioned that age has a significant effect on infection rate such as Tilahun *et al*. (43) who found that >1 year old were 3.45 times more infected than those ≤1 year old. Qazaz and Faraj (32) found that goats older than two years and younger than two years had different infection rates in the ELISA, 75% and 86.76%, respectively. Rahman *et al*. (33) indicated that colostrum-learnt antibodies in kids were diminishing by the second month of life which leads to high risk of infection. The eating habits of the goat may lead to the greater infection rate in adult goats compared to young goats, infection with *T. gondii* oocysts can occur in goats because they frequently graze on short grasses and lick the soil nearby (32). Karthika *et al*. (22) found a higher prevalence in goats over four years of age.

Total infection rates of toxoplasmosis in goats according to the flock

Out of 190 goats from two regions in AL-Dibuni (55) infection was, 20 (36.36%) while in Abu Ghraib (135) infection was 26 (19.25%) with significant differences (P<0.05), COR=2.39 (95%CI; 1.19-4.80) AOR=2.31 (%95CI; 0.97-5.50) **Table (7).**

Table 7. Effect of the flock on the infection rate of toxoplasmosis

Flock	No	Infec	P- *	COR (95%CI)	AOR (95%CI)
Abu-Ghraib	135	26(19.25 %)	<0.05	Ref. 1	Ref. 1
AL-Dibuni	55	20(36.36 %)		2.39 (1.19-4.80)	2.31 (0.97-5.50)
Total	190				

COR=Crude odds ratio
AOR=Adjusted odds ratio (Logistic regression)
***Chi-square**

These differences may belong to the existence of more cats in AL-Dibuni than in Abu Ghraib. The presence of many wandering cats capable of contaminating the pipe water supply increased the infective oocysts, as found by Deng *et al*. (14). These results confirmed the results obtained by Tilahun *et al*. (43), who explained the higher risk of infection in cattle, sheep and goats drink pipe water. Additionally, in the fields, rear sheep and goats in a semi-intensive system, the opportunity to ingest oocysts by these browsing ruminants is also high (38). A higher prevalence of seropositivity is observed as the number of animals in a herd or flock decreases (40). In Iraq according to Al-Hamada *et al*. (7) there were large amounts of cat feces on bags and in loose feed, possibly the result of cats hunting rodents in these areas, thus, it would be anticipated that more intense flock management that involves feeding animals may increase the probability of infected small ruminants.

Nested PCR product analysis

PCR analysis of the B1 gene in *Toxoplasma gondii* from goat's blood samples are shown in Figure (1), and (2)

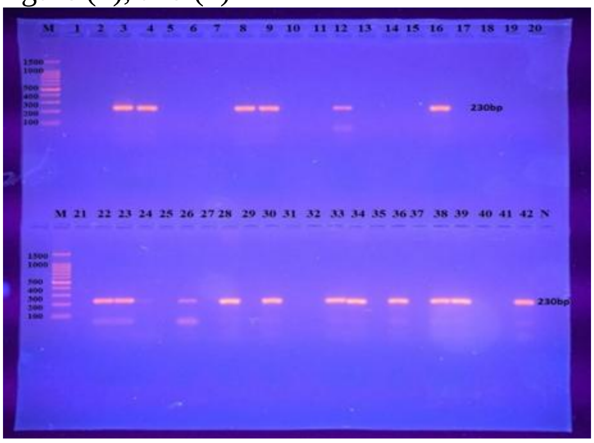

Figure 1. Agarose gel (1%) electrophoresis image showed PCR analysis of the B1 gene in *Toxoplasma gondii* from goat's blood samples. Where M: marker (1500-100 bp) Lanes (1-42) showed some *Toxoplasma gondii* were showed at (230 bp) PCR and N: non-DNA template negative control samples

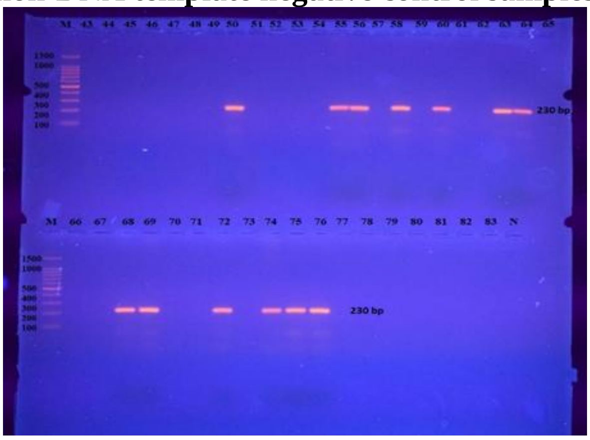

Figure 2. Agarose gel (1%) electrophoresis image showed PCR analysis of the B1 gene in *Toxoplasma gondii* from goat's blood samples. Where M: marker (1500-100 bp) Lanes (43-83) showed some *Toxoplasma gondii* were showed at (230 bp) PCR and N: non-DNA template negative control samples

CONCLUSION

In conclusion, our results indicated that the agreement between nPCR and ELISA is a slight. Also, among the studied risk factors the sex and flocks should be taken in our consideration when we planning to control the disease.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

DECLARATION OF FUND

The authors declare that they have not received a fund.

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