Seroprevalance of Toxoplasmosis in sheep and goat: Iraq/ Sulaimania

Lazem H. Al-Taie [1] **Shadan H. Abdulla** [2]
[1] College of medicine-Department of microbiology /Alnihrine university
[2] College of Vet. Medicine / Sulaimania university
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Summary

Back ground: Toxoplasmosis is an important zoonosis that causes economic losses in animal herds due to abortion and stillbirth as well as changes in the reproductive and neural system of susceptible animals .

Objective: The aims of the present study is to determination the prevalence of *T. gondii* in farm animals (sheep& goat)of both genders and different ages in Sulaimani province by using two serological tests (ELISA and LAT).

Methods: Blood samples were collected from farm animals ,142 sheep and 46 goats , of different sexes and ages. Tow different serological tests ,ELISA and LAT for qualitative determination of T. gondii antibody titer in sheep and goats serum samples.

Results: The prevalence rate in sheep was 73 (51.7 %) and 82 (57 %) , and 21 (54.6 %) and 25 (54.35 %) in goats ,by ELISA and LAT respectively. The prevalence of toxoplasmosis was highest in age group 7-9 (66.6%) in sheep in compares' with other age groups. There was no significant differences between both spp.and tow test.

Conclusion: Statistical results show no significant differences between both tests (ELISA &LAT) at ($P \geq 0.05$).The prevalence of toxoplasmosis was increased proportionally with the age of animals, while gender has no effect on the prevalent rate .

Key words: *Toxoplasma gondii*; seroprevalence; ELISA;LAT; Sulaimani

الانتشار المصلي لداء المقوسات في الأغنام و الماعز:العراق – السليمانية

د .لازم حميد الطائي **شادان حسن عبدالله**

الخلاصة

يعد داء المقوسات من الامراض المشتركة المهمة في العراق والتي تسبب خسائر اقتصادية كبيرة بسبب الاجهاض والتأثيرات العصبية في الحيوانات المصابة .هدفت الدراسة الى تحديد نسب الاصابة في الحيوانات الحقلية)الاغنام والماعز (والتي لها علاقة مباشرة بالاستهلاك البشري من الذكور والاناث وبالاعمار المختلفة في منطقة السليمانية باستخدام الفحص المصلي بطريقتي تلازن اللاتكس والايلازا لتحديد معيار الاجسام المضادة كميا .استخدمت 142عينة دم عشوائية للاغنلم و 46للماعز من كلا الجنسين ولاعمار مختلفة .بلغت نسبة الاصابة في الاغنام (73) 51.7% و (82) 57%والماعز (21) 54.6%و (25) 54.35%باستخدام اختبار الايلايزا وتلازن اللاتكس على التوالي اظهرت الدراسة عدم وجود فرق معنوي في نسبة الاصابة بين الاغنام والماعز .تزداد نسبة الاصابة مع تقدم العمر وكانت اعلى نسبة اصابة في الفئة العمرية 9 -7سنوات (66.6%)في الاغنام بالمقارنة مع الفئات العمرية الاخرى بينما لم يكن للجنس تأثيرا على نسبة الاصابة .لم تظهر نتائج الدراسة فرقا معنويابمستوى (P> 0.05)بين طريقتي الفحص .

Introduction

Toxoplasmosis is an important zoonosis that causes abortion in human and congenital abnormality in children , it also causes economic losses in animal herds due to abortion and stillbirth as well as changes in the reproductive and neural system of susceptible animals (1). Toxoplasmosis is a world wide distribution, approximately half a billion humans have antibody to *T. gondii* (2), occurring naturally in man, domesticated, wild animals and birds and a high incidence may occur in particular areas (3), Intermediate hosts become infected by ingestion of sporulated oocysts -contaminated meats, contact with free tachyzoites ,or congenitally via placenta (4). During the acute stage of infection, tachyzoites invade all organs especially the muscles including the heart, liver, spleen, lymph nodes and CNS. During latent infection bradyzoites are present in tissue cysts, and the sporozoites are found in environmentally resistant oocysts formed after the sexual stage of the life cycle (5). Serodiagnosis for antibody detection has been a more full and adequate tool to diagnose *Toxoplasma* infection in both human and animals, such as the latex agglutination test (6), and Enzyme Linked Immuno Sorbent Assay (ELISA) (7). The incidence of infection in humans and animals may vary in different parts of a country (8), toxoplasmosis is more prevalent in worm, moist areas of the world than in cold or hot dry areas (9). Toxoplasmosis has been reported to be widespread in animals throughout the world (10) and data collected from serological studies in many countries surrounding Iraq have shown different prevalence rates of toxoplasmosis , in Ankara of Turkey the seroprevalence of toxoplasmosis in sheep was 69.8% (11). (12) reported that seroprevalence with *Toxoplasma* infection in sheep and goats of dry land is significantly lower, than from animals of damp and humid areas . Also (3) reported that the high rate of infection has been related to a high rainfall which allows longer survival of oocysts on pasture. Cats are the key in the transmission of *T. gondii* to humans and other animals because they are the only hosts that excrete the environmentally resistant oocysts (13). Toxoplasmosis was also reported in other parts of Iraq, and in different animals, in sheep and goats (14), in cat (15), in rodent (16) and in cattle (17). About the neighboring countries toxoplasmosis was also a prevalent infection in animals like in Turkey (2), in Iran (18), in Saudi Arabia (19), in Syria (%52%%%52 20) and in Jordan (21).

The aims of the present study is to determination the prevalence of *T. gondii* in farm animals (sheep& goat)of both genders and different ages in Sulaimani province bya using two serological tests (ELISA and LAT).

Materials and Methods

Blood Samples : Blood samples were collected randomly from 188 animals, comprising 142 sheep and 46 goats, during September to December of 2006 , they were in different ages, were ranged from (6 months - 9 years), and clssified in to three age groups, regarding the gender, sample were collected from both females and males, out of 152 female samples 118 were from sheep, and 34 were from goats, the remaining 36 samples were collected from males, of which 23 were from sheep, and 13 were from goats.

Serological Tests : Latex Agglutination Test (LAT) was performed by using a comer tail kit received from (Bio kit, S.A.) Spain and used for diagnosing all the sera samples including , sheep and goats. A qualitative test performed on a undiluted serum sample can be used to estimate the immunologic response of the individual. A negative reaction means the absence of *Toxoplasma* antibodies or titers lower than 10 IU/ml. A positive reaction means presence of *Toxoplasma* antibodies which may reflect chronic or an acute *Toxoplasma* infection.

ELISA: Two types of ELISA kits were used for antibody detection in this study:-

1 – The MONO BIND, INC. Costa Mesa, CA 92627 (USA), kit for qualitative determination of *Toxoplasma gondii* IgG antibody in serum samples. The IgG value of anti-Toxo in each animales is obtained as follows:-

$$\text{EU/ml of Sample} = \frac{\text{O. D. of Samples}}{\text{O. D. of Calibrator}} \times \text{EU /ml of Calibrator}$$

According to the manufacturers instruction, results were reported in (IU); and samples contain anti-toxo IgG less than 10 IU/ml were considered negative, samples contain anti-toxo IgG between 10 - 20 IU/ml were considered equivocal and samples contain anti-toxo IgG above 20 IU/ml were considered positive.

2 – The Evlonline *Toxoplasma* test kit, by B.V. EUROPEAN VETERINARY LABORATORY. (B.V. EVL), Cat. no. B 1011 - AB 01, for detecting of total antibodies against *Toxoplasma gondii* infections in serum and plasma samples of sheep and goat .Test samples are considered *Toxoplasma* positive if the absorbency is above two times the absorbency of negative control.

Statistical Analysis: The chi-square test (X^2) was used for the analytic assessment between proportions, the differences were considered to be statistically significant when the P value less than (0.05) .

Results

The prevalence rate of seropositivity was 73 (51.4%) and 82 (57.75 %) in sheep by ELISA and LAT respectively. While the rate was 21 (45.6 %) and 25 (54.35 %) in goats by ELISA and LAT respectively. No significant

differences found between both species at ($P \geq 0.05$) by both tests, as illustrated in Table (1).

The prevalence rate of toxoplasmosis was recorded in different age groups of sheep and goats by the tow test ELISA and LAT, as illustrated in Table (2 &3). ELISA test results reveled that in sheep the highest rates were 6 (4.2 %) in the age group (7 - 9) years ; 20 (14.0 %) in the age group (≤ 3) years ; 46 (32.3 %) in the age group (4 - 6) years , by LAT 7 (4.9 %) in the age group (7 - 9) years ; 22 (15.4 %) in the age group (≤ 3) years ; 53 (37.3 %) in the age group (4 - 6) years . Of goat the the highest 14 (13.08 %) and 17 (36.9 %) in the age group (4 - 6) years ; 8 (17.3 %) in the age group (≤ 3) years by both tests ELISA and LAT, and no rate had recorded in the age group (7 - 9) years by both tests.

Levels of antibody titers of *T. gondii* in sera of sheep and goats which estimated by semiquantitative method of LAT, and it was illustrated in table (4). The highest titer 1: 128 was recorded in 3 sheep and the lowest titer was 1: 4 in 8 sheep, also there were 1: 8, 1: 16, 1: 32 and 1: 64 in 25, 24, 14 and 8 sheep respectively. While the highest titer 1: 32 recorded in 6 goats and the lowest 1: 4 in 4 goats, also there were 1: 8 and 1: 16 in 8 and 7 goats respectively. The prevalent titers in females of the both species were 1:8 and 1:16 as they were recorded in 21 sheep and 5 goats , respectivly. The distribution of titers in sheep male were as follows:- 1: 8, 1: 16 and 1: 4 recorded in 4, 3 and 2 sera respectively, while in goats male the titer 1: 8 recorded in 3 sera followed by the titers 1: 16 and 1: 32 in 2 sera.

The results of the present study illustrated in table(5) revealed that the titer 1: 8 was the highest in the age group of (≤ 3) years in sheep recorded in 8 sera, then the titer 1: 16 recorded in 7 sera and the titers 1: 4 and 1: 32 recorded in 5 and 2 sera respectively. While in goats all the presence titers 1: 2, 1: 4, 1: 8 and 1: 16 recorded in 2 sera. About the age group of (4 - 6) years in sheep, 1: 8 and 1:1 6 were the prevalent titers recorded in 16 sera, followed by the titer 1: 32 reported in 10 sera then the titers 1: 4 and 1: 64 recorded in 8 and 3 sera respectively. While the prevalent titer in goats of the same age was 1: 8 recorded in 6 sera followed by 1: 16 reported in 5 sera, then the titers 1: 4 and 1: 32 recorded in 2 and 4 sera respectively. Among the age group of (7 - 9) years, which recorded only in sheep the titer 1: 128 was the prevalent titer recorded in 3 sera, followed by the titer 1: 32 recorded in 2 sera then the titers 1: 8 and 1: 16 recorded only in 1 serum sample.

Discussion

In the present study, the prevalence rate of toxoplasmosis by LAT and ELISA were 50 % and 56.91 % respectively. Statistically, no significant differences were found between ELISA and LAT at ($P \geq 0.05$), as it was shown in Table (1). So the role of animals in transmitting of toxoplasmosis in Sulaimani province should not be ignored, as found with other countries (8).

Table (1) shows no significant differences between both species of sheep and goat at ($P \geq 0.05$) by ELISA and LAT for toxoplasmosis, although the higher prevalence rate were reported in sheep 51.4 % and 57.7 % by ELISA and LAT respectively, and in goats the prevalence rate by both tests were 45.6 % by ELISA and 54.3 % by LAT. Similarly (22) reported 52.2 % in sheep which was higher than in goats 51.7 % by IFAT in Saudi Arabia without significant differences ($P \geq 0.05$), (23) found 22.9 % in sheep and 11.6 % in goats by IHT in Ethiopia, (24) found 24.50 % in sheep and 19.25 % in goats by LAT in Iran, and (25) reported the same results by LAT in Bangladesh, which were 17.65 % in sheep and 12.09 % in goats, while (26) found higher prevalence rate in goats 28.93 % than in sheep 18.75 % by LAT n Brazil. Goats had a higher prevalence (25.4 %) as compared to the sheep (11.2 %); and higher in the female (24 %) than in the males (19%) for both species. The prevalence was significantly higher in adult sheep and goats than younger animals.(27)

The rates between sexes of both species shown in Table (2), 52.54 % and 47.06 % for female of both sheep and goats by ELISA and 58.47 % and 55.88 % by LAT respectively, and in male of both species were 45.83 % and 41.67 % by ELISA, and 54.17 % and 50 % by LAT respectively for both sheep and goats. Also (28) in India reported higher prevalence rate of seropositivity between females in both sheep and goats which were 10.5 % and 10.2 % respectively than in males 6.6 % in sheep and 6.7 % in goats. Similar results were reported by (29) in Sir Lanka, and (30) in Zimbabwe. While results of the study differ from that of (20) in Baghdad, Iraq as she reported higher prevalence rate in male of both species of sheep and goats which were 81.3 % and 80.4 % compared to that of female 61.1 % in sheep and 60 % in goats by IHAT.

Tables (3&4) represented the significant differences between the age groups of animals at ($P \leq 0.05$) by both tests ELISA and LAT, the prevalence rate increased with the age ofanimals. Similarly (31) in Venzuela. And in Brazil reported higher prevalence rate of *T. gondii* antibodies in older animal, and such fact is due to increasing opportunities of exposure to several sources of *Toxoplasma* infection with age (32). Delayed activation of macrophage in old individuals may be another reason (33). Also (34) in Turkey reported that the seropositivity rates differ among geographical regions and factors including age, sex, and breed were significant.

Regarding the incidence of toxoplasmosis, the result in table (5) show the distribution of antibody titers in both species of animals by LAT, the highest reported titer was 1: 128 and only seen in sheep, while in goats the highest reported titer was 1: 32 and the prevalent titer in both species was 1:8. There is a gradual decrease in the total number of positive sera from the titer 1: 8 as the reciprocal of the antibody titers increased till the titer 1: 128. (35) reported that high values of antibody titers can be ascribed to active toxoplasmosis infection as well as reactivation of infection due to

immunosuppresser conditions. Concerning the possible causes of toxoplasmosis in animals (36) attributed the prevalence of antibodies against *T. gondii* in a particular animal species to the feeding habits of that species.

While seroprevalence studies indicate relatively higher prevalence rates of toxoplasmosis in farm animals, the infection is sub-clinical and *T. gondii* has virtually no importance as a cause of clinical disease in farm animals with the exception of that associated with abortion and neonatal disease (3). Also (23) reported that toxoplasmosis may be an important cause of reproductive wastage in small ruminants. The best time to examine ewe sera to assist diagnosis of *Toxoplasma* abortion is one week after abortion (36). Improved hygiene and management should be applied to reduce the prevalence rate, and the major importance of toxoplasmosis in farm animals is it's zoonotic potential (3)

Table (1) Prevalence of toxoplasmosis in both species of sheep and goats by ELISA and LAT.

Animal Species	Animal Sex	Total Tested animal No.	ELISA		LAT	
			No. of + ve	(%)	No. of + ve	(%)
Sheep	Male	24	11	45.83	13	54.17
	Female	118	62	52.54	69	58.47
	Total inf.rate	142	73	51.4	82	57.7
Goat	Male	12	5	41.67	6	50
	Female	34	16	47.06	19	39.1
	Total inf.rate	46	21	45.6	25	54.3

Table (2) prevalence of toxoplasmosis in different ages between sheep and goats by ELISA.

Age (y)	Total Tested sheep No.	Sheep				Goat			
		No. of – ve	(%)	No. of + ve	(%)	No. of goats/ – ve	(%)	No. of + ve	(%)
≤ 3	53	33	23.2	20	14.0	19/11	23.9	8	17.3
4 – 6	81	35	24.6	46	32.3	26/12	26.0	14	30.4
7 – 9	8	2	1.4	6	4.2	1/1	2.1	--	--
Total	142	70	49.2	72	50.7	46/24	52.1	22	47.8

Table (3) prevalence of toxoplasmosis in different ages between sheep and goats by LAT

Age (y)	Total Tested animal No.	Sheep				Goat			
		No. of – ve	(%)	No. of + ve	(%)	No.of tested ans./ – ve	(%)	No. of + ve	(%)
≤ 3	53	31	21.8	22	15.4	19/11	23.9	8	17.3
4 - 6	61	28	19.7	53	37.3	26/9	19.5	17	36.9
7 - 9	8	1	0.7	7	4.9	1/1	2.1	--	--
Total	142	60	42.3	82	57.7	46/21	45.6	25	54.3

Table (4) Quantities of *T. gondii* antibody titers by LAT in animals sera between sexes.

Animal Sex	Animal Species	Total No. of Sera	No. of – ve (%)	No. of + ve (%)	Antibody titers						
					1:2	1:4	1:8	1:16	1:32	1:64	1:128
Female	Sheep	118	48 (33.8)	70 (47.2)	--	6	21	21	14	6	2
Male total No.	142	24	12 (8.1)	12 (8.1)	--	2	4	3	--	2	1
Female	Goat	34	16 (34.7)	18 (39.1)	--	4	5	5	4	--	--
Male total NO.	46	12	5 (10.8)	7 (15.2)	--	--	3	2	2	--	--

Table (5) Quantities of *T. gondii* antibody titers by LAT in animals sera of different ages.

Age (year)	Animal Species	Total No. of Sera	No. of – ve (%)	No. of + ve (%)	Antibody titers						
					1:2	1:4	1:8	1:16	1:32	1:64	1:128
≤ 3	Sheep	53	31 (58.49)	22 (41.51)	--	5	8	7	2	--	--
4 - 6		81	28 (34.57)	53 (65.43)	--	3	16	16	10	8	--
7 - 9		8	1 (12.5)	7 (87.5)	--	--	1	1	2	--	3
≤ 3	Goat	19	11 (57.89)	8 (42.11)	--	2	2	2	2	--	--
4 - 6		26	9 (34.62)	17 (65.38)	--	2	6	5	4	--	--
7 - 9		1	1 (100)	-- --	--	--	- -	--	--	--	--

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