DETECTION OF TOXOPLASMOSIS BY USING LATEX AGGLUTINATION TEST AND ENZYME LINKED IMMUNOSORBENT ASSAY IN MAYSSAN PROVINCE EWES

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ABSTRACT

Venous blood samples were collected 283 ewes observed in nine different regions of Maysan province during the period from October 2012 to March 2013 for detecting toxoplasmosis by using Enzyme linked immunosorbent assay (ELISA-IgG) and latex agglutination test (LAT). Three groups of ewes pregnant, non-pregnant and aborted were selected for both tests, and the results showed that the rates of *Toxoplasma* seropositivity in aborted group is the highest, followed by pregnant group using the LAT test (73.33% and 65.38%) respectively, while the proportion of ewes aborted were the top (40%) in the ELISA test.

The results of LAT and ELISA tests showed that Al –Musharah subdistrict is the highest in the seropositivity against *Toxoplasma* (78.26% and 39.13%) respectively. As for the relationship between age and *Toxoplasma* seropositivity, the results showed that the highest seropositivity rate in the age groups more than a year and two years (65.30% and 65.57%) respectively by using LAT test , and high seropositivity rate (32.65) in the age group more than a year with ELISA test.

The statistical analysis showed no significant differences ($p \ge 0.05$) between the LAT and ELISA tests with regard to *Toxoplasma* seropositivity.

INTRODUCTION

Toxoplasmosis is a zoonotic disease is caused by the apicomplexan protozoan *Toxoplasma gondii*, an obligate intracellular parasite which is one of the most common parasitic infections of warm-blooded animals, including human(1). Toxoplasmosis is of major medical and veterinary importance, being a cause of congenital disease in human and abortion in all kinds of livestock, leading to serious economic losses worldwide(2). The three means by which it is mainly spread are transplacental transmission, ingestion of infective tissues, and ingestion of food or water contaminated with infective feces(3).

Sheep are important in the epidemiology of *Toxoplasma gondii* infection; asymptomatic sheep can serve as a source of infection for humans(4). Severity of infection is associated with the stage of pregnancy at which the ewe becomes infected. Infection during the early stage of gestation can result in fetal death, resorption and abortion, while infection in the latter stage of gestation (fetal immunity is relatively well developed), may have no clinical effect and lambs are usually born normal but infected and immune(3,5).

Many studies were done in Iraq deal with detection of toxoplasmosis in sheep by using different immunological tests(6,7,8,9).

The present study was designed to achieve the following aims:

1-Detection of anti *toxoplasma* IgG antibody in the serum of infected ewes by using two serological tests.

2- Estimation of the prevalence of toxoplasmosis in different regions of maysan province.

3 - Evaluation the efficiency of two serological methods in the detection of toxoplasmosis statistically.

MATERIALS AND METHODS

From October 2012 to March 2013, 283 venous blood samples were collected from sheep selected randomly from nine regions of Maysan province (Ali Al-Sharqi , Kumeit , Al- Teeb, AL-Musharah, Al-kahlaa, AL-Magar Al-kabeer , AL-Maymona , Missan center, slaughter house) . Blood samples were drawn by 10ml disposable syringe, 5-8 ml of blood was collected in sterile and labeled tubes without anti-coagulant or preservatives (plain tubes), Samples were transported on ice box to laboratory, and left for 30 minutes at room temperature then samples were centrifuged at 3000 r.p.m for 15 min. Subsequently, serums were separated in Eppendorf tubes about 1.5-2 ml and each sample was divided into two parts one part stored at -20 C and other part directly used in latex agglutination test. The ewes age ranged from < 1 year to > 3 year. The age, regions, presence and absence of abortion and number of abortions were reviewed from each selected ewes.

Two serological tests Has been applied to investigate the toxoplasmosis; latex agglutination test (LAT) and IgG based Enzyme Linked Immunosorbent Assay (ELISA)

Test. ELISA test procedure was carried out according to the method described by (10). while LAT test procedure was carried out according to the method described by (11).

The prevalence for *T. gondii* was statistically analyzed by the Chi-square test (12).

RESULTS

According to the case history and clinical pictures of 283 ewes , the results showed a significant differences ($p \le 0.05$) between the aborted and non pregnant sheep , and the higher rate (73.33 %) of seropositivity was observed in ewes who were previously aborted for one or more times (table1)

Table (1) : distribution of *T. gondii* based on latex positive results in ewes according to the clinical picture .

Animal	Examin. NO	Sero-positive	percentage
aborted ewes	15	11	73.33%
pregnant ewes	78	51	65.38%
on pregnant ewes	190	92	48.42%
Total	283	154	54.41%

 $X^2 = 9.443$, df = 2 , $p \le 0.05$

There were deferent seropositivity rate against *T. gondii* among the studied ewes of different Maysan province regions ($p \le 0.05$), and there were higher seropositivity rate (78.26%) observed in Al- Musharah subdistrict, while the overall rate of latex seropositivity was (54.41%) (table 2).

Region	Examin. No	-positive	Percentage %
l- Sharqi		21	52.50
strict	40	21	
ait subdistrict	27	13	48.14
ahlaa district	38	25	65.78
aymona district	22	14	63.63
lajar district	43	27	62.79
rah center	21	8	38.09
lusharah subdistrict	23	18	78.26
ib subdistrict	33	19	57.57
ghterhouse	36	9	25
	283	154	54.41

Table (2) :The distribution of *T. gondii* latex agglutination test positive results in ewes of different regions in missan province .

The relationship between clinical pictures and the seropositivity in deferent studied regions was displayed in table (3). In case of pregnant ewes; the higher rate (77.78%) of LAT seropositivity against *T. gondii* was observed in ewes of Al-Musharah subdistrict . While high rate (72.73 %) of seropositive non pregnant ewes was observed in AL–Musharah subdistrict. The aborted seropositive ewes appear in higher rate (100%) in AL- Kumait subdistrict, AL-Majar district, AL-Musharah subdistrict and AL-Teeb subdistrict, and there were significant differences ($p \le 0$. 0 5) between the clinical pictures and studied regions.

Tabte (3) :	distribution	of <i>T</i> .	gondii	based	on latex	agglutination	test positive	results in
ewes accord	ing to the cli	nical p	oictures	s in eac	h studie	d regions .		

Ew	ves	egnant ewes	v Pregnant ewes	borted ewes	total	
l Sharqi	Total	15	23	2	40	
istrict	-positive	9	11	1	21	
	entage%	60	47.82	50	52.5	
ait subdistrict	Total	7	19	1	27	
	-positive	4	8	1	13	
	entage%	57.14	42.11	.00.00	48.14	
ahlaa district	Total	16	18	4	38	
	-positive	12	10	3	25	
	entage%	75.00	55.56	75.00	65.78	
путопа	Total	7	14	1	22	
ict	-positive	5	9	0	14	
	entage%	71.43	64.29	-	63.63	
ajar district	Total	12	29	2	43	
	-positive	8	17	2	27	
	entage%	66.67	58.62	100	62.79	
rah center	Total	4	16	1	21	
	-positive	1	7	0	8	
	entage%	25.00	43.75	-	38.09	
usharah	Total	9	11	3	23	
istrict	-positive	7	8	3	18	
	entage%	77.78	72.73	100	78.26	
eeb subdistrict	Total	8	24	1	33	
	-positive	5	13	1	19	
	entage%	62.50	54.17	100	57.57	
ghterhuose	Total	0	36	0	36	
	-positive	0	9	0	9	
	entage%	-	25	-	25	
X ² =222.203,	df=16,	p≤0.01				

According to the age groups (table 4), the results of latex agglutination among tested ewes sera were revealed a high significant prevalence ($P \le 0.05$) in age group >1 and > 2 years (65.30% and 65.57%) respectively when compared with other two age groups < 1 and > 3 years (34.66% and 48.97%) respectively.

 Table (4) : distribution of T. gondii based on latex positively in ewes of different age groups .

Age grou	2	Ewes no . (%)					
ars	Examin. No	sero-positive	Percentage				
< 1	75	26	34.66				
>1	98	64	65.30				
>2	61	40	65.57				
>3	49	24	48.97				
total	283	154	54.41				

 $X^2 = 12.17871$, df = 3, $p \le 0.05$

ELISA result :

Application of ELISA test on 283 head of ewes explained significant differences ($p \le 0.05$) between the aborted and pregnant sheep ,and the higher rate (40 %) of seropositivity was observed in previously aborted ewes for one or more times (table 5).

Table (5) : Distribution of *T*.gondii based on ELISA positive results in ewes according to

the clinical pictures.

Animal	Examin. No.	Sero-positive	Percentage%
aborted ewes	15	6	40
on pregnant ewes	190	49	25.78
pregnant ewes	78	18	23.07
Total	283	73	52.79

$X^2 = 33.65036 \;, \;\; df = 2 \quad , \, p \le 0.05$

In table (6) there was high rate (39.13%) of seropositive ewes observed in Al-Mmusharah subdistrict, and there were variable significant seropositivity against *T.gondii* among the studied ewes of different Maysan province regions ($p \le 0.05$), and the overall rate of ELISA seropositivity was (25.79%).

Tab	ole (6):	The	distr	ibuti	ion of	f T .	gondii	based	EL	JSA	positive in	n ewes	of	different	region	of
	· (- / -						0				P		~ -			

Region	Examin. No	ero-positive	Percentage%
li Al Sharqi subdistrict	40	7	17.50
Kumait subdistrict	27	5	18.51
Al Kahlaa district	38	13	34.21
Al-Maymona district	22	4	18.18
Al-Majar district	43	10	23.25
Amarah center	21	6	28.57
l-Musharah subdistrict	23	9	39.13
Al-Tib subdistrict	33	11	33.33
Slaughterhouse	36	8	22.22
Total	283	73	25.79

maysan province.

 $X^2 = 19.85827$, df = 8, $p \le 0.05$

The seroposivity rate in concern to the relationship between ewes clinical pictures and the studied regions was displayed in table (7), in case of pregnant ewes; the higher rate (62.5%) of ELISA seropositivity against *T. gondii* was observed in ewes living in Al-Teeb subdistrict . while high rate (33.33%) of seropositive non pregnant ewes was observed in Al-Kahlaa district. while the aborted seropositive ewes appear in higher rate (100%) in both Al-Musharah subdistrict and Al-Majar district. There were significant differences ($p \le 0.05$) among the studies ewes concerning their clinical pictures and living regions.

Table (7): Distribution of *T.gondii* based on ELISA positive results in ewes according to

Ewes		ant ewes	Pregnant	ed ewes	otal
Reegion			ewes		
I Sharqi subdistrict		15	23	2	40
•	sitive	2	4	1	7
	age%	3.33	17.39	50	7.5
umait subdistrict		7	19	1	27
	sitive	1	4	0	5
	age%	4.28	21.05	-	8.51
l Kahlaa district		16	18	4	38
	sitive	4	6	3	13
	age%	25	33.33	75	4.21
AL- mymona		7	14	1	22
District	sitive	1	3	0	4
	age%	4.28	21.42	-	8.18
l-Majar district		12	29	2	43
	sitive	3	5	2	10
	age%	25	17.24	00	3.25
Amarah center		4	16	1	21
	sitive	1	5	0	6
	age%	25	31.25	-	8.57
lusharah subdistrict		9	11	3	23
	sitive	4	2	3	9
	age%	4.44	18.18	00	9.13
-Teeb subdistrict		8	24	1	33
	sitive	5	6	0	11
	age%	62.5	25	-	3.33
Slaughterhuose		0	36	0	36
	sitive	0	8	0	8
	age%	-	22.22	-	2.22
$X^2 = 349.4$	df = 16		$p \leq 0.0$)5	

their clinical pictures in each studied regions .

According to the age groups (table 8), the results of ELISA test among tested ewes sera revealed no significant differences ($P \le 0.05$) between the age groups ,and a high prevalence (32.65%) observed in > 1 year old ewe.

X²

Table (8) : Distribution of *T. gondii* based on ELISA positive result in ewes of different

age groups.

Age	group			
ars		Examin. N	sero-positive	Percentage %
	< 1	75	16	21.33
	< 1	15	10	32.65
	> 1	98	32	02.00
	> 2	61	17	27.86
	> 3	49	8	16.32
				25.79
	Total	283	73	
5.236618,		df = 3	$, p \le 0.03$	5

The percentage of toxoplasmosis and the results of both tests were compared, and there was no significant differences (P>0.05) between the two tests as shown in table (9).

Table (9): compartion of toxoplasma seropositivity by LAT and ELISA tests in studied ewes.

	L	positive	entage %
A			þ
X ² =0.353	•	df = 1	P≤0.05

DISCUSSION

Toxoplasmosis causes heavy economic losses to sheep and goat industry and is considered as one of the main causes of infectious ovine and caprine abortion, The diagnosis of toxoplasmosis in sheep by clinical signs or demonstration of *T.gondii* in tissues or body fluids is much difficult (5).

The results obtained in the present study showed that there were antibodies against *T. gondii* in the sera of tested sheep in ewes of many regions of Maysan province, and it was considered as

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the first record in this province. almost sample taken from rural region in which the ewes and cats live together in the same area and in warm and moist climates; this help parasite to reproduct and transmission and this fact conformed by (13) who suggest that *Toxoplasma* seroprevalence is variable, higher prevalence being observed in warm and moist areas than in cold or hot dry areas , Apart from this, variation may also be related to the age of the animals sampled and husbandry practices, and the obtained results agreed with results of (9) in his study on sheep of basrah province .

The present results showed that the percentage of *T. gondii* antibodies for sheep 54.41% by LAT and 25.79 % by indirect IgG based ELISA test, and these results were in agreement with (9), who recorded seropositivity rate (60.84% by LAT and 51.11% by indirect IgG based ELISA test) in ewes of five different regions in Basrah province, while (14) in study in Mosul province showed that by using LAT the positive sera percentage was 79% which is not in line with the present study.

The results of LAT and ELISA IgG in this study showed higher prevalence in Maysan province than in middle and north of Iraq these results disagreed with that of (6) who found that the prevalence in sheep in Nineveh province was 29.1% by LAT. In Baghdad province (7) mentioned that the prevalence in sheep was 30.04% by LAT and 16.66% by using IgG based ELISA test also this results disagreed with results of the present study. The high prevalence reported in this study may comes from the fact that most recorded cases were taken from regions originally known to have high predisposition to infection because low health awareness of animal , raising animals inside houses , and lack of hygiene.

The data obtained from table (1) showed that the aborted ewes (73.33%) was higher than the pregnant and non-pregnant (65.38%,48.42%) respectively by using LAT test and data obtained from table (5) showed that the aborted ewes (40%) was higher than the pregnant and non-pregnant (23.07% and 25.78%) respectively by using ELISA test and these resuls agreed with

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(9) in Basrah province who recorded the higher percentage in pregnant (79.03%) than aborted ewes (71.40%) and non- pregnant (56.75) respectively by using LAT test, and higher percentage in aborted ewes (83.33%) than pregnant (56.52%) and non- pregnant (51.11%) respectively by using ELISA test

From the data in tables (2,3) which showed higher percentage in AL-Musharah subdistrict (78.26%) by using LAT test ,and data from tables (6,7) also showed higher percentage in AL-Musharah subdistrict(39.13%) by using ELISA test, than other regions of Maysan province ,and this may relate to climate difference,raising animals inside houses , lack of hygiene and weather in this region which is usually warm and moist because it is located close to marshes region. These result could be supported by two studied ,one of them (15) refer to the epidemiology of toxoplasmosis lead us to believe that herbivores acquire infection by ingestion of contaminated grass and water with *T. gondii* oocysts which shed by cats, While second study (16) mention that the higher prevalence rates of toxoplasmosis were in warm and moist areas compared to that in cold areas.

The age groups (>1 and >2) were higher in seropositivity (65.30, 60. 57) respectively by LAT test (table 4) than the other age groups, and the age group (> 1 year) was higher in seropositivity (32.65) by ELISA test (table 8) than the other groups ,these results agreed with (17) who showed that the prevalence was highest (38.88%) in age group of 16-28 months and lowest (8.51%) in age group of 68-80 months the reason for these results may be explained on the basis that the animals included in this age group were less resistant to *T. gondii*. These results are supported by (18,19,20,21) who reported that the system of management and health practices have a significant effect on the incidence of blood born parasites.

The statistical analysis affirmed that there were no significant differences ($p \ge 0.05$) between latex and ELISA tests, and this result was supported by (22) who found that the low correlation indicated different test sensitivity which reflected the ability of the test applied to detect

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antibodies produced by the disease therefore any decrease in the test sensitivity reflected the false negative results ,which might occur due to, selection of unsuitable time for sampling . In conclusion the immunological test which were used in this study for detection of antibodies seropositivity in the sera of studied ewes and estimation of toxoplasmosis distribution reveal non significant differences in the seropositivity against *toxoplasma* , so we can considered these test as good means for the diagnosis of toxoplasmosis as the results of these tests was nearly similar and had strong correlation with the clinical picture of the studied ewes.

الكشف عن داء المقوسات باستخدام اختباري التلازن وإنزيم الادمصاص المناعي في نعاج محافظة ميسان

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الخلاصة

تم جمع عينات من الدم الوريدي من 283 نعجة من تسع مناطق مختلفة في محافظة ميسان للفترة من تشرين الثاني 2012وحتى آذار 2013 للكشف عن داء المقوسات باستخدام اختباري إنزيم الادمصاص المناعى وفحص التلازن.

تم اختيار ثلاث مجاميع من النعاج الحوامل , غير الحوامل والمجهضة لكلا الاختبارين، وأظهرت النتائج أن نسبة الايجابية المصلية ضد المقوسات الإصابة في المجهضات هي الأعلى وتليها الحوامل باستخدام اختبار التلازن 73.33٪، 65.38٪ على التوالي، في حين أن نسبة النعاج المجهضة هي الأعلى 40٪ في اختبار إنزيم الادمصاص المناعي.

أظهرت نتائج اختباري إنزيم الادمصاص المناعي والتلازن أن ناحية المشرح هي الأعلى في نسبة انتشار داء المقوسات 78.26٪، 39.13٪على التوالي.أما بالنسبة للعلاقة بين العمر والايجابية المصلية، فأظهرت النتائج أن أعلى نسبة في الفئات العمرية لأكثر من سنة وسنتين 65.30، 65.57 على التوالي باستخدام اختبار التلازن، و (32.65)في الفئة العمرية اكبر من سنه في اختبار إنزيم الادمصاص المناعي.

أظهر التحليل الإحصائي عدم وجود فروق معنوية (P ≥ 0.05) بين اختباري التلازن و إنزيم الادمصاص المناعى فيما يتعلق بالكشف الايجابية المصلية للمقوسات.

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