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Seroprevalence of toxoplasmosis in aborted ewes by using different immunologic tests in Duhok governorate, Kurdistan region, Iraq

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Abstract

This study was conducted to investigate the anti-toxoplasma antibodies titer (IgG and IgM), in aborted ewes in different localities of Duhok Governorate. The study was carried out in the laboratory of post graduated study, Faculty of Veterinary Medicine, Duhok University from October 2010 to April 2011. Four hundred and ninety-six (496) serum samples of aborted ewes (98, 144, 152 and 102 were obtained from Aqra, Duhok center, Shikhan and Zakho, respectively). Latex Agglutination test (LAT), Modified agglutination test (MAT) and Enzyme Linked Immunosorbent Assay (ELISA) were used for the aforementioned purpose. The over all prevalence of toxoplasmosis was (83.3%), and the rate was different in different localities such as Aqra (86.7%), Duhok (89.6%), Shikhan (74.3%) and Zakho (84.3%) using LAT. Out of 413 sera, sample which were confirmed positively LAT (67.31%) were positive for Toxoplasma by MAT (have had IgG type of antibody) and 149 (36.08%) sera gave positive reaction by IgM ELISA test (have had IgM type of antibody). The result revealed that the rate in different age groups was statistically different and the rate was 90.38% in age group <4 year and 75.42% in age ≥ 4 years applying LAT.

Keywords: *Toxoplasma gondii*; seroprevalence; LAT; MAT; ELISA; Duhok governorate
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التشخيص المصلي لاجسام المضادة ضد داء المقوسات في النعاج المجهضة في بعض مناطق محافظة دهوك

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الخلاصة

أجريت هذه الدراسة للكشف عن وجود الاجسام المضادة (IgM and IgG) في مصل النعاج المصابة بالاجهاض نتيجة الاصابة بداء المقوسات (toxoplasmosis) وذلك في مناطق مختلفة في محافظة دهوك. تمت هذه الدراسة في مختبر الدراسات العليا، كلية الطب البيطري جامعة دهوك وذلك للفترة من شهر تشرين الثاني اوكتوبر ٢٠١٠ ولغاية شهر نيسان ابريل ٢٠١١. تم جمع ٤٩٦ عينة مصل من النعاج المجهضة كالتالي (٩٦، ١٤٤، ١٥٢ و ١٠٢) من كل من عقرة، دهوك، شيخان و زاخو على التوالي. ولهذا الغرض تم اجراء الفحوصات المصلية التالية وهي فحص التلازن السريع (LAT) و فحص التلازن المحور (MAT) واختبار الامتصاص المناعي للانزيم المقترن (ELISA). نسبة الاصابة بالمرض كانت ٨٣,٣% (اي ان عدد الاصابات كانت ٤١٣) و اختلفت نسبة الاصابة باختلاف الموقع الجغرافي حيث كانت الاصابة ٨٥(٨٦,٧%) في عقرة، ١٢٩ (٨٩,٦%) في دهوك، ١١٣ (٧٤,٣%) في شيخان و ٨٦ (٨٤,٣%) في زاخو. اعطت ٢٧٨ (٦٧,٣١%) عينة مصل نتيجة ايجابية لاختبار MAT و ١٤٩ (٣٦,٠٨%) عينة اعطت نتيجة ايجابية لاختبار ELISA. اظهرت النتائج أختلافاً أحصائياً باستخدام LAT في نسبة الاصابة حسب الفئات العمرية والتي كانت ٩٠,٣٨% في الفئة العمرية < ٤ سنوات و ٧٥,٤٢% في الفئة العمرية ≥ ٤سنوات.

Introduction

Toxoplasmosis is a zoonotic infection caused by the intracellular protozoan parasite *Toxoplasma gondii*. Infecting all warm-blooded animals with acute life-threatening consequences (1), in sheep and goats, it may manifest itself as a disease of pregnancy reflecting by multiplying of the etiology in the placenta and fetuses of infected animals. In the latter animals, it can terminate in abortion, stillbirth, neonatal motility or mummified fetus as well as pacentatitis (2). *T. gondii* has been recognized as a significant cause of lambing loss (3) and food hazard [4] which act as intermediate host. Cats serve as final hosts (5).

In sheep, the prevalence of *T. gondii* within the worldwide range is between 3–96% (6-8). Sheep raring constitute an important agricultural practice having great economy in Kurdistan region.

Natural infection with *T. gondii* generally leads to a state of long-lasting protective immunity (9). It is well established that both humoral and cell-mediated immune responses, include T-cell mediated immune response that involves both CD4+ and CD8+ T lymphocytes, and antibodies, are important in conferring immunity to *T. gondii* infection (10). However the infection is generally diagnosed by the presence of these specific antibodies (IgM, IgA, IgE and IgG) to *T. gondii* antigens in the sera of infected mammals. IgM antibodies usually become detectable within days after infection, while IgG antibodies become detectable after 1-2 weeks and may have lifelong persistence. The presence of IgM antibodies indicates a recent infection (11).

The aims of this study were to investigate the prevalence of *T. gondii* antibodies as well as to compare different serological for immunodiagnosis of toxoplasmosis in aborted ewes of Duhok province.

Materials and methods

Animals (aborted ewes)

The sheep of this study were of local breeding (Karadi) which were raised for different purposes by villagers and farmers. Only female aborted ewes were involved which were subdivided into two groups according to their ages by dentition (12). Age of the aborted fetuses and other subsequent clinical manifestation were not mentioned in the work

Sampling

A total of 496 sera were taken from aborted ewes of different localities including Aqra, Dohuk district, Shikhan and Zakho. Data on characteristics of animals were noted through questionnaires involving the age, frequency of previous abortion, type of managements whether the animals raised with cats in the field or not (Table 1).

Table 1: Total number of animals assigned according to the questionnaire list of the study

Locations	Total No.	Age		Frequency of Abortion			Co- presence of cats with animals in field	
		< 4 yrs	≥ 4 yrs	1	2	3	shared	Not shared
Aqra	98	50	48	89	8	1	68	30
Duhok district	144	91	53	126	15	3	81	63
Shikhan	152	47	105	140	10	2	54	98
Zakho	102	48	54	90	9	3	65	37
Total	496	236	260	445	42	9	268	228

Blood Collection

Ten milliliters (10 ml) of blood was drained by special disposable vacutainer tubes. The sera obtained were spinned by ordinary centrifuge 5000 rpm for 5 minutes, and the separated sera were kept at -20°C until being used.

Serological Tests

Two serological tests were followed in the current study *i.e*. Latex Agglutination Test (LAT) and Enzyme Linked Immunosorbent Assay (ELISA) to define seroprevalence of toxoplasmosis as mentioned by (13).

Statistical Analysis

The data were analyzed performed by Chi-square test using Statistical Analysis System (SPSS) software program. Chi-square was used to analyze the associations between seropositivity and influence of risk factors such as age of animals. The differences were considered statistically significant when $P \leq 0.05$ or $P \leq 0.01$ (14).

Results

Table 2 showed the findings of latex agglutination test in a total of 496 aborted ewes collected from different localities of Duhok Governorate. Out of 496 sera, 413 (83.3%) were positive. The percentage of positive cases ranged between 74% -89% among examined samples.

Table 2: Prevalence of antibodies against *T. gondii* in aborted ewes by LAT in different localities of Duhok governorate

Location	Total	Positive	%	Negative	%
Aqra	98	85	86.70%	13	13.30%
Duhok district	144	129	89.60%	15	10.40%
Shikhan	152	113	74.30%	39	25.70%
Zakho	102	86	84.30%	16	15.70%
Total	496	413	83.30%	83	16.70%

Table 3 shows the prevalence rate of toxoplasmosis which was statistically different at $P \geq 0.05$ between age groups of aborted ewes using LAT. The rate was higher in age group <4 years which was 235(90.38%) as compared with age group ≥ 4 years which was 178 (75.42%).

From a total of 413 sera, MAT was carried out on sera which were aforementioned positive by LAT, 135 (32.69%) sera had negative reaction to 2ME, and 278 (67.31%) sera were positive (Table 4). It means that those sera that reacted negatively had IgM type of antibody against *T. gondii*, while those sera that reacted positively had IgG type of antibody.

Another serological test (ELISA) was introduced to detect IgM type of antibody in sera of aborted ewes, which had previously positive reaction with LAT in different regions of Duhok (Table 5). Out of 413 sera, 149 (36.08%) positively reacted. The reading of positive findings were 33 (38.82%), 51 (39.54%), 32 (28.32) and 33 (38.37%) in Aqra, Duhok district, Shikhan and Zakho, respectively.

The results of the two tests (MAT and ELISA) are shown in Table 6, for estimation of IgM in two age groups which were fairly similar. The rates were 104 (58.43%) and 109 (61.24%) in animals age <4 years by using 2ME and ELISA respectively, while 31 (13.19%) and 40 (17.02%) by using MAT and ELISA in age ≥4 years, respectively.

Table 3: Prevalence of toxoplasmosis in different age groups by LAT

Age groups	Total	Positive No.	%	Negative No.	%
< 4 years	260	235 a	90.38%	25	9.62%
≥ 4 years	236	178 b	75.42%	58	24.58%
Total	496	413	83.3%	83	16.7%

Value: 19.873, p = 0.0001.

Table 4: Reaction of serum with MAT in aborted ewes from different localities of Duhok governorate

Location	Total no.	2ME			
		Positive**	%	Negative*	%
Aqra	85	57	67.06%	28	32.94%
Duhok district	129	83	64.34%	46	35.66%
Shikhan	113	81	71.68%	32	28.32%
Zakho	86	57	66.28%	29	33.72%
Overall	413	278	67.31%	135	32.69%

*Animals in acute infection, ** Animals in chronic infection.

Table 5: Prevalence of *T. gondii* IgM antibodies in sera of ewes of different localities by using ELISA test

Location	Total No.	ELISA			
		Positive	%	Negative	%
Aqra	85	33	38.82%	52	61.18%
Duhok center	129	51	39.54%	78	60.46%
Shikhan	113	32	28.32%	81	71.68%
Zakho	86	33	38.37%	53	61.63%
Total	413	149	36.08%	264	63.92%

Table 6: Results of IgM levels of two tests (MAT and ELISA) according to age in aborted ewes

No. of abortion	Total samples tested	MAT		ELISA	
		Positive No.	%	Positive No.	%
< 4years	178	104	58.43%	109	61.24%
≥ 4 years	235	31	13.19%	40	17.02%
Total	413	135	32.69%	149	36.07%

Discussion

The results of the current study were by LAT 83.30%, 2ME 67.31% and ELISA 36.08%, reveled high incidence of toxoplasmosis in Dohuk governorate (as compared with other regions of Iraq using different immunodiagnostic technique. Following latex agglutination test, (15) in Mosul and (16) in Sulaimani found the prevalence rate were 42.7% and 57.75%, respectively. However, using the same technique, the prevalence rate in Erbil was 100% (17), which is somewhat similar to our findings. On the other hand, using ELISA for determination of toxoplasmosis the results of this study were higher than those reported in other places of Iraq such as Misan, Thiqar, Al-Muthanna, Al-Basra and Erbil which were 25, 12.71, 16.6, 18.63 and 15.94%, respectively (17,18). However, the present result had similarity with other records in the different countries, as those of Turkey and Iran where the prevalence of toxoplasmosis were 31.4% and 35% by using ELISA and IHAT, respectively (19,20). In Brazil (21) found that the infection rate of goat toxoplasmosis were 39.4% Applying ELISA and were 35.95% applying IFAT, which were quite close to our results. On the other hand, lower prevalence rate were recorded elsewhere. In Mexico, the prevalence of toxoplasmosis of domestic sheep using MAT was 15.1% in Durango State (22) and was 23.1% in Oaxaca State (23). The differences observed could be due to the diagnostic techniques used in the different regions, frequency of felines on the farms, age, sex and breed of the animals (24), and the climatic variations and management conditions (5,25). Those findings were lower than ours and may be due to variation in geographical regions, system of breeding and grazing. However, our observations were in accordance with those (22) regarding the relationship between infection rate and age. This note was explained and interpreted the high incidence of toxoplasmosis in older animals due to the nature of older sheep to the prolonged exposure to the oocysts present in the environment (22).

The current finding are higher than those stated in South Africa which were 4.3% using ELISA (27) and higher than those reported in Pakistan which was 3% using LAT (28) and higher than those mentioned in Saudi Arabia which was 22% using ELISA (29). Application of IHAT, the results of the present study were lower than those observed in Saudi Arabia and Syria which were 39% (30) and 44.56% (31), respectively. Accordingly, the variability of rates might be due to methods of diagnosis applied for detection of Toxoplasma organism. Our results were in agreement with several authors ***i.e.*** (13,18,32,33) who stated that such variation of prevalence in different countries was due to methods of diagnosis followed.

In this study the rate of toxoplasam was higher in age group < 4 years which was 235 (90.38%) than ≥ 4years which were 178 (75.42%). The higher prevalence may be due to the fact that younger ewes have less resistance and are actively exposed to oocyst with consequent higher chances of acquiring infection. This phenomenon was early proved by (34,35). Furthermore (24) stressed that the prevalence decreases as age of sheep increases from 28 months onwards. Also, (9) reported that natural infection with *T.gondii* leads to a state of long lasting protective immunity. The variations of antibody titers had been recorded in this study from the lowest (1:2) to the highest (1:128). This change might significantly differ in terms of exposures, dose of infection, virulence of infectious agent and body response to developing immunity. These explanations were previously mentioned by (36).

In animals with recently acquired infection, IgM of *T.gondii* were detected initially and in most cases these titers became negative within a few months. However in some animals, positive IgM of *T.gondii* with specific titer can be observed during chronic stag of the infection. IgM antibodies had been recorded to persist as long as 12 years after the acute infection. The actual persistence IgM antibodies dose not appear to have any clinical relevance and those animals should be considered as chronic infected carriers (37).

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