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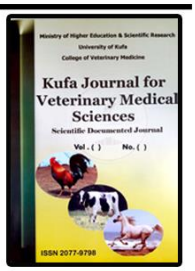

Prevalence of seropositive toxoplasma cases in association with the frequency of abortion in sheep and goat

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Abstract

Toxoplasmosis are a serious public-health problem inhuman and animals especially in developing countries, it appear approximately thirty percent from all cases and have higher level of morbidity .At every stage of life the *Toxoplasma gondii* infections are a significant cause .The epidemiology and handling of this condition are various in the developing world, where infectious agents became predominate .This study was proceed during the period from March 2017 to August 2017 to detect *Toxoplasma gondii* in sheep and goat serum by characterize them using two assays Latex agglutination test and IgG and IgM Toxoplasmosis ELISA kits.

A total of 74 sera samples were collected from sheep and goat from different ages ranged 6 month to 2.5 years old were achieved in different reign in AL- Najaf city / Iraq ,they suffered from endemic abortion and still birth. The overall prevalence of *Toxoplasma gondii* in were32(62%),18(75%) by Latex agglutination test for sheep and goat, respectively, with non-significant variation (P>0.05) between the two them ,The serum are arranged as follows in a total of 23 (71.8%) and 9 (28.2%)abortion and still birth of sheep ,respectively, and17(94.4%) 1(5.6%) aborted and still birth of goat ,respectively, with significant differences (P≤0.05) between the two them. Out of 32 serum positive of Toxoplasmosis in sheep by using Latex agglutination test, there were 23(71,9%) and 11(43,4%) positive serum by using IgG and IgM ELISA kits, respectively, On the other hand out of 18 toxoplasmosis parasite in goat have positive result in Latex agglutination test, there were 11(61,1%) and 6(33,3%) by using IgG and IgM ELISA kits, respectively, our study was accelerated due to endemic clinical abortion and instill birth for sheep and goat and narrowing functional range of molecular assays by time consumption, in this regard the aim of our study used two functional assay diagnosis Latex agglutination assay and ELISA techniques with increase the accuracy for the detection of the Toxoplasmosis in sheep and goat.

Key Words: Toxoplasmosis, Latex agglutination assay, IgG and IgM Toxoplasmosis ELISA kits.

انتشار الإصابة المصلية الموجبة لداء القطط وعلاقته مع تعدد حالات الإجهاض في الأغنام والماعز

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الخلاصة:

تعد داء المقوسات مشكلة خطيرة في الصحة العامة في الإنسان والحيوان وخاصة في البلدان المتقدمة، وتظهر حوالي ثلاثين في المائة من جميع حالات ارتفاع مستوى الاعتلال و في كل مرحلة من مراحل الحياة عدوى توكسوبلازما غونديي هي سبب مهم. يختلف التعامل وبائيا" مع هذه الحالة في الدول المتقدمة خصوصا عندما تصبح العوامل المعدية هي السائدة. بينت الدراسة

خلال الفترة من مارس 2017 إلى أغسطس 2017 للكشف عن توكسوبلازما غونديي في مصل الأغنام والماعز من خلال وصفها باستخدام اختبارين من المقايسات المناعية وهي اللاتكس اختبار التراص و فحص المقايسة المناعية المرتبطة بالأنزيم لداء المقوسات في جسم المضاد IgG وIgM .
تم جمع ما مجموعه 74 عينة من الأغنام والماعز من مختلف الأعمار التي تراوحت بين 4-8.5 شهرا في مناطق مختلف في مدينة النجف / العراق، عانت من الإجهاض المتوطن ولادة عقيمة. وكان معدل انتشار التكسوبلازما غوندي الكلي 32 (62٪) و 18 (75٪) عن طريق اختبار تراص اللاتكس للأغنام والماعز، على التوالي، مع عدم وجود اختلاف معنوي (P> 0.05) بين الحيوانين و رتب الامصال على النحو الاتي لكل مجموعه 23 (71.8 ٪) و 9 (28.2٪) مجهضة وولادة عقيمة في الأغنام على التوالي، و 17 (94.4٪) 1 (5.6٪) مجهضة وولادة عقيمة في الماعز على التوالي ، مع وجود فروق معنوية (P≤0.05) بين الاثنين. من أصل 32 مصل إيجابي من داء المقوسات في الأغنام باستخدام اختبار تراص اللاتكس، كان هناك 23 (71،9٪) و 11 (43،4٪) مصل إيجابي باستخدام فحص المقايسة المناعية المرتبطة بالأنزيم لكل من جسمي المضاد IgGوIgM على التوالي ، من ناحية اخرى ما مجموع 18 طفيلي داء المقوسات في الماعز لها نتيجة إيجابية في اختبار تراص اللاتكس، كان هناك 11 (61،1٪) و 6 (33،3٪) باستخدام فحص المقايسة المناعية المرتبطة بالأنزيم لكل من جسمي المضاد IgGوIgM على التوالي ، دعت الدراسة إلى اتخاذ إجراءات سريعة لمعالجة مشكلة الإجهاض السريري المتوطن و الولادة العقيمة لكل من الأغنام والماعز وبسبب تضييق نطاق وظيفي من الفحوصات الجزيئية عن طريق استهلاك الوقت، في هذا الصدد الهدف من دراستنا تستخدم اثنين من فحص الفحص الوظيفي اللاتكس تراص الفحص وتقنيات إليسا مع زيادة دقة للكشف عن توكسوبلازموسيس في الأغنام والماعز.

الكلمات المفتاحية : داء المقوسات، اختبار اللاتكس والتراص، وفحص المقايسة المناعية المرتبطة بالأنزيم للجسم المضاد IgGوIgM.

Introduction

Toxoplasma gondii parasite is the cause of toxoplasmosis, which is a cosmopolitan intracellular parasitic infection .The intermediate host for infectious agent is Mammals while domestic and wild felines are the definitive hosts. The most symptoms of the disease in meat-producing animals like sheep and goats and human beings is abortion (1).

Humans infected either by horizontal or vertical routes; the most important horizontal routes are consumption of undercooked meat and raw which containing *T. gondii* tissue cysts or ingestion of, food, vegetables and water or soil have oocysts produced in cat feces. Trans placental transmission could occur if infection happens during pregnancy, the parasite can infect the fetus leading to congenital abnormalities , still birth and abortion (2).

Sheep and goat constitute the principal source of meat to human consumption in Iraq. Toxoplasmosis causes losses of economic to goat and sheep industry as a results of abortion, embryonic damage and maternal infertility (3 and 4).The major source of infection in sheep and goat is ingestion of contaminated pasture with cat feces. In addition, many researcher have proposed that goat and sheep persistently infected with *T. gondii* can pass infection to the their fetus in subsequent pregnancy more constantly than previously thought (5 and 6)

Serological tests usually used for the identification of causative agent with *T. gondii* in animals. There were no gold standard tests for the detecting of the most diversity of Toxoplasmosis in many host species. The specificity and sensitivity of the assays depend on species of the animal (2).in the present time, the direct latex agglutination test (DLAT) seems to be the best adapted tests to a large number of species (7), Otherwise ,the specific enzyme-linked immune sorbent assays (ELISAs) had been developed for some domestic animal species. The latex agglutination test is relatively rapid and neither requires time consumption and complex laboratory facilities .While the enzyme immunoassay requires complex laboratory equipment's but on the same time its take large numbers of

samples and doesn't depend on human interpretation for the result (8).

Although, domestic cats are uncommonly reared in our homes, Iraqi researchers have demonstrated an increased prevalence of toxoplasma antibodies in women and men as well. Therefore, the present study was aimed to collect new data on the scope of infection, and to determine the prevalence of toxoplasma antibodies in blood samples from goat and sheep by using different serological methods.

Materials and Methods

Collection of serum

A cross-sectional study (point-prevalence) was conducted to investigate the prevalence of toxoplasma antibodies in sheep and goats. A total of 74 blood samples were collected from sheep and goat from different reign in AL- Najaf city ,during the period from March to August 2017.The serum were collected from eight different localities. Precisely, 50 sheep sera samples from mathlom, Razav, Albuhawwatm, Shalal and Malha

Otherwise, 24 goat sera samples collected from Old airport road, Qodus, and karbala farms. The blood samples were collected aseptically from the jugular vein using vacutainer tubes, and delivered to the Department of Microbiology in ice box .All tube collection was centrifuged for 5 minutes at 5000 rpm, and then kept serum at – 20 °C (9). History of each case examined and herd , were collected and data documented which include age of examined animal, cases of abortion and stillbirth.

Toxoplasma latex Agglutination Test (LAT):this kits was used as instructed by the manufacturer's instructions (PLASMATEC LABORATORY PRODUCTS LTD, UK) and the experiments were designed in to following routes :

Qualitative Toxoplasma Latex Agglutination Test (LAT):

The Plasmatic Toxoplasmosis Latex Test kit was used for the detection of antibodies against *Toxoplasma gondii* in serum slide agglutination. Positive and negative controls were used for each run .The naked-eye visible of any degree of agglutination indicated the positive reaction ,while smooth suspension with no visible agglutination regarded as negative reaction.

IgM and IgG-Enzyme Linked Immuno-Assay (IgM & IgG-ELISA) :

This test was done by kits of omega diagnostics Scotland (united kingdom) which includes:

1-Microtitration plate which contains wells coated with specific antibody.
2-Diluted Buffer
3-Control sera
a-Negative control serum
b-high positive control serum
c-low positive control serum
4-Wash Buffer
5-Conjugates :
a-HRP –labeled toxoplasma conjugate .
b-IgG &IgM-conjugate dilution buffer.
6-Control antigen with substrate solution.
7-Stop solution.

The technique was took place according to the following points:

1-.One ml of dilute buffer was mixed with both 10µL horse reddish peroxides (HRP)-labeled Toxoplasma conjugate (100x) and the diluted IgM & IgG-conjugate was stabled for four hours at room temperature 20°C or 24 hours at 4°C.

2-Patient serum was diluted by mixing 10µL of serum with one ml of diluted buffer (1/100).

3-100µL of each control or dilated sample was placed on each wall of the microtitre plate, which was incubated in humid chamber at 37°C for one hour.

4-The well contents was discarded by absorbent paper and washed 5 times by an automatic microplate washer. After washing

by wash buffer the contents were discarded by absorbent paper as striking the plate upside down.

5-100µL of diluted conjugate was added to each well then incubated for one hour at 37°C in humid chamber.

6-The well contents was discarded and washed 5 times.

7-100µL substrate solutions were added to each well then incubated at room temperature in the dark room for 30 minutes.

8-100µL of stop solutions were added to each well, then gently shake for ten seconds and incubated the plate in the darkness for 30minutes.The color changed from blue to yellow,

The absorbance of each wall was measured immediately with an ELISA Reader at 450 nm filter to obtain the optical density of test and control .The averages of optical density of each test were calculated, through the average OD of the low positive control, this is the cut-off value of the assay. A ratio lower than cut-off indicates a negative sample while the ration greater than cut-off which indicates a positive sample, otherwise, the ratio between values indicates an equivocal result and must be tested with afresh new sample and must be repeated.

Statistical analysis

All produced results were analyzed by Chi square statistic at the level of significant when p- value < 0.05. the statistical analysis was performed using SPSS program (10).

Results

Results of the present study indicated that serological diagnosis of Toxoplasmosis in (74) suspected sheep and goats are reached to 32(62%),18(75%) respectively ,the present study was illustrated that out of 74 suspected sheep & goat with *T.gondii* , 50 are positive in LAT, as in table 1 , 2and figure 1.

Table 1: The prevalence of Toxoplasmosis in Sheep from different reign in AL- Najaf city by LAT*

Locality	No. of sample	No. of positive(%)
L1	12	9(75%)
L2	8	5(62,5%)
L3	17	11 (64,7%)
L4	4	0(0%)
L5	9	7(77,8%)
Total	50	32(62%)
Statistical analysis	X^2= 2.84, Degree of freedom 4, Non-significant , P = 0.58	

*L1=mathlom ,L2=Razav L3=Albuhawwatm L4=Shalal, L5= Malha

Table2: The prevalence of Toxoplasmosis in Goats from different reign in AL- Najaf city by LAT*.

Locality	No. of sample	No. of positive(%)
L_1	8	8(100%)
L_2	9	6(66,7%)
L_3	7	4(57,1%)
Total	24	18(75%)
Statistical analysis	X^2= 0.57, Degree of freedom 2, Non-significant, P= 0.75	

*L1 =Old airport road,L2= Qodus,L3 =karbala farms

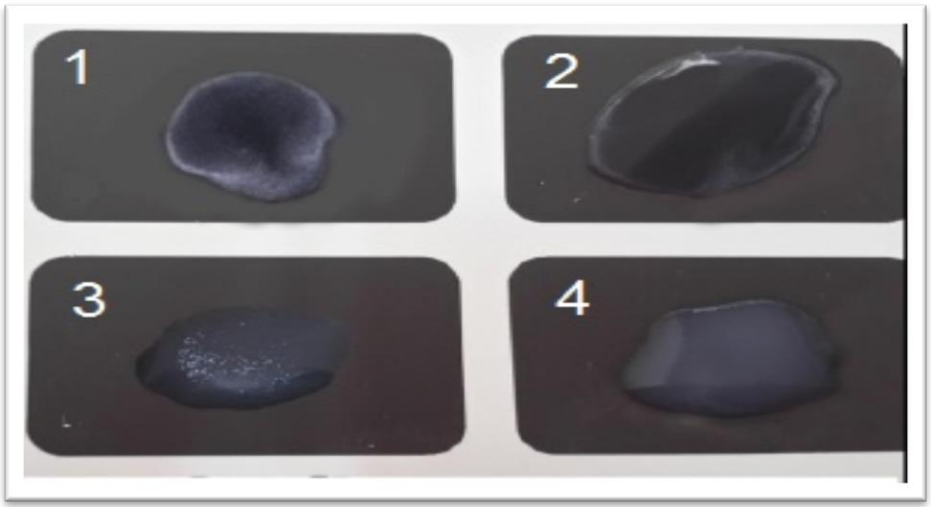

Figure 1: latex agglutination test for identification Toxoplasmosis, well 1: control positive, well 2: control negative, well 3: positive test apparent aggregation of particles in the well, well 4: negative test clear surface without any agglutination.

According to the number of abortions .

The total number of the aborted sheep & goat were 40(31%) explained the higher than still birth 10 (13%),as in table 3 and figure -1 .

Table3: The incidence of abortion & non aborted in sheep & goat.

animal	No. of positive samples	No. of aborted	No. of still birth
sheep	32	23 (71.8%)	9 (28.2%)
goat	18	17(94.4%)	1(5.6%)
Total	50	40	10
Statistical analysis	X^2= 3.66 , Degrees of freedom= 1, highly significant		

According to Age:

It has been indicated that age would be associated with the seroprevalence of Toxoplasmosis, as(1-2 year) of sheep and goat had a higher rate of infection compared to other ages , as in table 4 and figure 2.

Table4: The prevalence of Toxoplasmosis in sheep & goat in different ages.

Age	Sheep	No. of Toxoplasmosis	Goat	No. of Toxoplasmosis
Less than 1 year	12	8	7	3
1-2 years	18	13	9	8
More than 2 years	20	11	8	7
Total	50	32	24	18
Statistical analysis	X^2= 0.282, Degree of free dome = 2, P= 0.86, Non significant		X^2= 0.88, Degree of freedom = 2, P= 0.64, Non significant	

From an analysis of the ELISA data, imposing that 32 total number of positive Toxoplasmosis in sheep there were 23(71,9%) and 11(43,4%) by using IgG and IgM ELISA kits ,respectively. On the other hand out of 18 toxoplasmosis parasite in goat have positive result in Latex agglutination test, there were 11(61,1%) and 6(33,3%) by using IgG and IgM ELISA kits, respectively table 5 figure 2.

Table 5:IgG and IgM of ELISA in positive LAT in sheep & goat.

Type of animals	*LAT* *	*ElISA** IgG*	*Percentage*	*ELISA** IgM*	*Percentage*
sheep	32	23	71,9	11	34,4
Goat	18	11	61,1	6	33,3
Total	50	34	68	17	34

*LAT = Latex Agglutination Test

**ELISA = Enzyme Linked ImmunoAssay

Table 6: number of samples ordered by ELISA reader

Date of Assay	1	2	3	4	5	6	7	8	9	10	11	12
A	-	-	-	-	-	-	+					
B	-	-	-	-	-	-	+					
C	+	+	-	-	-	-	+					
D	-	+	-	-	-	-	+					
E	+	-	+	+	-	-	-					
F	-	-	-	-	-	-	-					
G	+	-	-	-	+	+	+					
H	-	-	-	-	+	+	+					

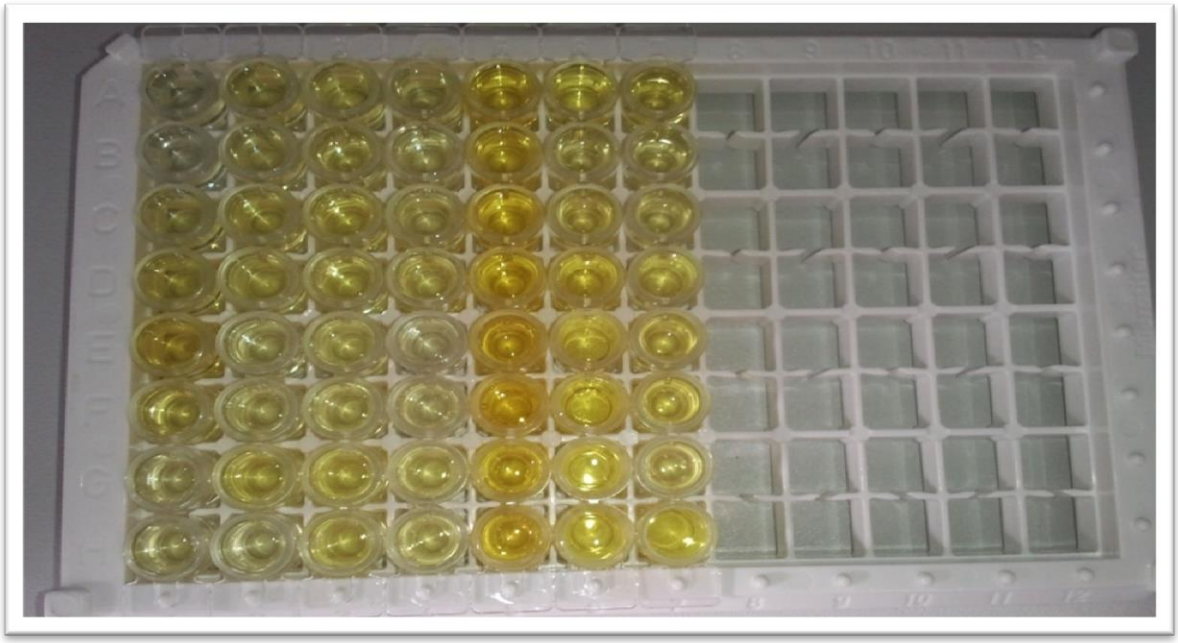

Figure 2: Standards with samples ELISA micro titer plate

Discussion

Toxoplasma is an infectious disease that affects many mammalian species including human. Infections are caused by the parasites protozoa as *Toxoplasma gondii* . Infections are usually acquired by taking inadequately cooked meat or from feces of infected cats(11).The conventional slide agglutination assays of Toxoplasmosis Latex Test kit for identification of Toxoplasmosis involve the preparing of animal sera , following by screened on polystyrene particles sensitized with *Toxoplasma gondii* antigens (12) the results showed that formation of antigen-antibody complexes, figure (1), one of the most important point for interpretation of these results its depend on the presence of *Toxoplasma gondii* antibodies which reflect either an evolving infection or a past infection, therefore the

results must be confirmed and determined by preparing serial two-fold dilutions. From an analysis of the group classifications of ages the results showed that 6-6.5 years are more prevalence of Toxoplasmosis rather than other groups table(4),these results were considered by (13) who was recorded that as older sheep and goat had a higher prevalence of Toxoplasmosis infection compared to younger sheep. Table (1 and 2) showed that there were no significant differences (P= 0.58 and P =0.75) for sheep and goat, respectively. The Differences in seroprevalence between regions may be due to same climatic in one country, and our study not excluded the other risk factors which decrease the health of the animals ,The prevalence in sheep and goats, has not changed over time, because the source of infection of these herbivorous animals kept on pastures has remained unchanged. In farmed sheep, the seroprevalence in Europe is logically correlated with age, increasing from lambs (17 to 22%) to adult (65 to 89%) (14).Viable *T. gondii* organisms have been recovered from as many as 67% of sheep samples. Sheep, rather than pigs, are the main source of infected meat in Southern European countries. Rates of seropositivity reported for goats vary from 4 to 77% (15). An ELISA assays were used for the measurement of toxoplasma IgG antibodies in sheep and goat serum using *toxoplasma gondii* antigen-coated polystyrene beads as a solid phase and anti rabbit IgG-horse radish peroxidase conjugate as an enzymatic carrier (16),. Modern studies have been appeared that enzyme immunoassay (EIA) may be more sensitive and precise than classical latex agglutination assays because the apparatus have able to visualize the private antigen serotypes from serum animals(17).These results were done to evaluate the comparison between Latex Agglutination assays compared with ELISA assays. A laboratory techniques were carried out on 74 sera of sheep and goat at Al-najaf governorate /Iraq, the study determined that there were some evaluation in the comparison table 5, the study had 32(62%), 18(75%) seropositive to *T.gondii* with LA method for sheep and goat respectively, and68% and34%seropositive with ELISA among sheep and goat by using IgG and IgM Toxoplasma ELISA kits, respectively. And then the same evaluation was carried out with the same animal but in the case abortion history only. Since the direct detections of *Toxoplasma gondii* were rarely successful, serological methods play an important part in diagnosing a toxoplasmosis infection, The antibodies of the IgG and IgM antibodies can be detected in about 8 days after infection, IgM antibodies disappear after a few months, while IgG antibodies continue for lifelong of animals(18) In most cases IgG and IgM are identified as antibodies can prove a new infection that can pose a risk to pregnancy, Levels of anti-Toxoplasma gondii antibodies were analyzed in a group of 50 sheep and goat using the IgM and IgG-Enzyme Linked Immuno-Assay Anti-Toxoplasma gondii ELISA (IgG). With a cut-off value, 71.9 % and 34.4% of the sheep and goat, respectively, were anti-Toxoplasma gondii positive (tabel2). None of the many tests used mostly for diagnosis of toxoplasmosis seems quite adequate for the purposes of group examination as far as they are analytical reliability, ease of pilot, and speed response is concerned. In fact some of the them need special equipment not widely available, others cannot be used as individual tests because of this information is not complete available due to poor association with the clinical situation (19)And poor association with the clinical situation.

In conclusion our results might make a useful contribution two assays, Latex agglutination test and ElISA kits for

detection of small ruminant have infected by Toxoplasmosis, towards preventing *Toxoplasma gondii* in animals and decreasing losses in the livestock especial abortion and stillbirth, so it's very important to progress in control through monitored serologically and estimated epidemiologically.

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