Serodiagnosis of toxoplasmosis in sheep and goats in Erbil city, Iraq

J.M. Kader[1] and Z.A.Y. Al-Khayat*[2]

[1] Central veterinary laboratory, [2] Department of Microbiology, College of Medicine, Hawler Medical University, Erbil, Iraq
*Corresponding author: dr_zakarea@yahoo.co.uk

(Received December 15, 2011; Accepted February 28, 2013)

Abstract

Sera from (259) sheep and (88) goats from Erbil city were examined for antibodies to *Toxoplasma gondii* by using latex, MAT, and ELISA. By using latex test, 75 (25.4%) sheep and 25 (28.4%) goats were seropositive. By testing the latex seropositive sheep serum by both MAT and ELISA the distribution of both positive and negative results were as such respectively: 63 (84%), 12 (16%), 11 (14.7%), 64 (85.3%). Using both MAT and ELISA for testing the latex seropositive goats serum, the distribution of both positive and negative results were as such respectively: 21 (84%), 4 (16%), 3 (12%), 22 (88%). There was no significant difference between the results of ELISA vs. MAT.

Keywords: *Toxoplasma gondii*; Toxoplasmosis; ELISA; Latex; MAT.
Available online at http://www.vetmedmosul.org/ijvs

تشخيص مصلي لداء المقوسات في الضأن والماعز في مدينة اربيل، العراق

ژيان محمد قادر[١] و زكريا عبد الله ياسين الخياط[٢]

[١] المختبر البيطري المركزي، [٢] فرع الاحياء المجهرية، كلية الطب، جامعة هولير الطبية، اربيل، العراق

الخلاصة

تم فحص ٢٥٩ عينة مصل من الضأن و ٨٨ عينة مصل من الماعز للكشف عن الاجسام المضادة لداء المقوسات الكونيدية في مدينة اربيل باستخدام عدة اختبارات شملت التلازن، والتلازن المطور، والاليزا وكانت النتائج موجبة في اختبار التلازن ٧٥ (٢٥,٤ %) في الضأن و ٢٥ (٢٨,٤%) في الماعز. ثم فحصت النماذج الموجبة للضأن باستخدام اختباري التلازن المطور والاليزا وكانت النتائج الموجبة والسالبة لكلا الاختبارين ٦٣ (٨٤%)، ١٢ (١٦%)، ١١ (١٤,٧%)، ٦٤ (٨٥,٣%) على التوالي، اما عند استخدام اختباري التلازن المطور والاليزا لفحص النماذج الموجبة لمصول الماعز فكانت النتائج الموجبة والسالبة لكلا الاختبارين هي ٢١ (٨٤%)، ٤ (١٦%)، ٣ (١٢%)، ٢٢ (٨٨%) على التوالي، ولم يكن هناك فرق احصائي معنوي بين نتائج اختباري الاليزا والتلازن المطور.

Introduction

Toxoplasma gondii is an intracellular protozoan organism with a large numbers of intermediate hosts, including all warm-blooded animals, and humans. Felids, particularly the domestic cats, are definitive hosts and the only animal species in which oocyst develop (1,2). Serological surveys done in various parts of the world show that in some countries more than a third of the human population have antibodies against *T. gondii* (3). This high prevalence of infection in human proves the importance of toxoplasmosis as a zoonotic disease, particularly in pregnant women and immune-compromised patient (4). Toxoplasmosis also causes heavy economic losses to sheep industry worldwide (5). Toxoplasmosis is recognized as one of major cause of infectious reproductive failure in Great Britain, New Zealand, Australia, USA, Uruguay, Norway and other countries (1,2,4,5). Toxoplasmosis was also reported in other parts of Iraq, and in different animals, in sheep and goats (6), in cat (7), in rodent (8) and in cattle (9). About the neighboring countries toxoplasmosis was also a prevalent infection in animals like in Iran (10), in

Saudi Arabia (11), and in Jordan (8). Toxoplasmosis causes fetal resorption, abortion at any stage of pregnancy, fetal mummification, stillbirth or birth of weak live offspring in sheeps and goats (12).

The objectives of this study was to determine the prevalence of IgG and IgM in the latex positive sheep and goats sera by using ELISA and MAT (modified agglutination test).

Materials and methods

Study protocol and design

This study was carried out during the period November 2009 to April 2010 at the central veterinary laboratory in Erbil city (Iraq). This study was planned by using two stages of simple random sampling procedure (10,11). In the first step, randomly selection of owner or butchers who had given their acceptance, followed by random selection of animals in second step. Five ml blood was taken from jugular vein from each animal (sheep, goat) at the slaughter house. Three hundred eighty three (383) blood samples were collected which were included (295 sheep and 88 goats serum). Each serum was divided into three parts primarily. The first part was examined serologically for detection of antibody to *Toxoplasma gondii* by (LAT). In the case of the positive latex result, the second part of the serum was tested by MAT while the third part was tested by ELISA method.

Serological tests

Latex agglutination test

Determination of *Toxoplasma gondii* antibody by latex agglutination test, using the kit (Toxocell latex, BIOKIT, S. A. BARCELONA –SPAIN). The principle of the test as follows: If specific anti-*Toxoplasma* antibodies are present in sera tested they will react with soluble *Toxoplasma* antigen in the latex reagent and will be visualized by latex particles as whitish granules after five minutes of rotation of the slide.

Modified Agglutination test (MAT)

Treatment of latex positive serum with 2- Mercapto ethanol, (2ME SIGMA-ALDRIC –US A**,**Bach. 125 K 0165) for diagnosis of acute (IgM) or chronic phases of toxoplasmosis (IgG). A stock solution of (0.2) molarities of 2ME in phosphate buffer saline is prepared initially and stored at 4°C in an opaque bottle. It is further diluted to 0.05M with normal saline for use as the diluted in the test. Equal amount of 50 µl of the positive serum with LAT was treated with 50 µl of 2ME then incubated at 37°c for 15 minutes (13).

ELISA

Indirect ELISA (indirect Multi-species, IDVET innovative diagnostics - FRANCE**)**. This kit is used to detect anti-*Toxoplasma gondii* antibodies (IgM) in ruminant, cat or pig sera, plasma or meat juice. The test is validated if: -the mean value of the positive control O.D is greater than 0.350. -the ratio of the mean O.D values of the positive and negative controls (ODPC and ODNC) is greater than 3.5. All the serological tests above were done in the central veterinary laboratory in Erbil city.

The data analysis was performed by Chi-Square test using Statistical Analysis System (SAS) software program. The differences were considered statistically significant when $P \leq 0.05$.

Results

The prevalence of seropositive sheep was (75/295, 25.4%). By testing this 75 positive sera by MAT 63 (84%) were positive (IgG) and 12 (16%) were negative (IgM). While results of examination of the 75 latex positive sera by ELISA revealed that 11 (14.7%) were positive (IgM), and 64 (85.3%) were negative (IgG). Statistical analysis showed no significant difference was noted between (MAT vs. ELISA) at $P \leq 0.05$. (Table 1). It was found that from 88 goats sera tested by LAT, 25 (28.4%) were positive and 63 (71.6%) were negative. By testing this 25 positive sera by MAT, 21 (84%) were positive (IgG) and 4 (16%) were negative (IgM). While 3 (12%) were positive by ELISA (IgM) and 22 (88%) were negative (IgG). There was no significant difference between (MAT vs. ELISA at $P \leq 0.05$. (Table 2). No significant differences were seen between MAT and ELISA.

Table (1) Seroprevalence of IgG, IgM antitoxoplasma antibodies in sheep

Test	IgG	Ig M	Total
Modified agglutination test	63	12	75
ELISA	64	11	75
Total	127	23	150

No significant difference was noted between (MAT vs. ELISA) at $P \leq 0.05$, Cal. $X^2 = 0.06^{N.S.}$ Tab. $X^2 = 3.84$, $P \leq 0.05$ d.f=1.

Table (2) Seroprevalence of IgG, IgM antitoxoplasma antibodies in goats

Test	IgG	Ig M	Total
MAT	21	4	25
ELISA	22	3	25
Total	43	7	50

Discussion

This study is an attempt for serodiagnosis of *Toxoplasma gondii* infection in Erbil, using serological methods including latex agglutination test, modified agglutination test and ELISA techniques in slaughtered animals used for human consumption. The procedures used were LAT, MAT and ELISA. The choice of ELISA technique for this study is justified since it is considered to be one of the most sensitive immunological technique (14). A study followed the kinetics of IgG and IgM antibodies in 20 sheep fed oocysts, *T. gondii* IgM antibodies peaked at 3 weeks and precedes IgG response (15). In sheep inoculated intravenously with tachyzoites, IgM was detected by one month and persisted for three months (16). A study had reported that two sheep fed 5000, 50000 oocysts develop IgM antibodies detected by ELISA by 14 days (17).

The MAT and ELISA tests detected similar proportion of *Toxoplasma* positive serum samples. Therefore, both are reliable for screening tests. However, both tests have their own advantages and limitations. The need for species specific conjugates, and automatic processor to increase the efficacy and spectrophotometer for quantifying the activity of antibodies by ELISA test may limit its use. On the other hand, the MAT is safe and does not require species specific conjugate and can be used on any species (18). It was noted that that standardization of ELISA is a major problem because only a few kits are commercially available for use in animals (19). The diagnostic accuracy of these two tests (MAT, ELISA) were superior when performed on sheep (20). Unlike ELISA, which usually is developed to be used on particular species, MAT is commonly used to test sera from any species because it does not require species specific conjugates. The agreement between both tests (MAT and ELISA) was almost perfect and the accuracy would not justify by themselves the selection of either MAT or ELISA for its use in *toxoplasma* control schemes. Other aspects such as possibility of standardization, rapidity, economy should be considered as well (19). MAT is simpler to perform but involves more subjectivity when interpreting the results than the ELISA. ELISA can be easily automated, enabling the rapid screening of a large number of animals. However, they should be valuable tools for prevalence studies at flock or population levels (21).

Acknowledgment

The authors would like to thank the staff of public slaughter house and the central veterinary laboratory in Erbil city for their support during this study.

References

1. Dubey JPA. Review of toxoplasmosis in cattle. Vet Parasit. 1986; 22:177-202.
2. Dubey JP. Toxoplasmosis – a waterborne zoonosis. Vet Parasit. 2004; 126: 57-72.
3. Bhigjee A I, Naidoo K, Patel V B. Govender D. Intracranial mass lesions in HIV-positive patient the KwaZulu-Natal experience. South African Med J. 1999 ;89:1284-1288.
4. Tenter AM, Vietmeyer C, Johnson A M. Development of ELISAs based on recombinant antigens for the detection of *Toxoplasma gondii* specific antibodies in sheep and cats. Vet Parasitol. 1992;43:189–201.
5. Dubey J P. Strategies to reduce transmission of *Toxoplasma gondii* to animals and human. J Vet Parasitol. 1996; 64: 65 – 70.
6. Al-Taie LH, Abdulla SH. Seroprevalance of toxoplasmosis in sheep and goat: Iraq/ Sulaimania. IQ J Vet Med. 2011; 35: 16 – 24.
7. Fatohi FAM. Detection of toxoplasmosis among different group of population in Mosul city by using IHAT and CFT. [master's thesis] College of Med. Univ. Mosul, Iraq. 1985.
8. Al-Bakal A A S. Assessment of the knowledge and practice of women with toxoplasmosis in Erbil city. Msc thesis, College of Nursing. Salahaddin Univ. Erbil, Iraq. 2005.
9. Al-Dujaily KYO.Toxoplasmosis among abortion women in Baghdad. Msc thesis, College of Science, Univ. Baghdad,Iraq 1998.
10. Hashemi-Fesharki R. Seroprevalence of *Toxoplasma gondii* in cattle, sheep and goats. Iran Vet Parasitol. 1996;61: 1–3.
11. Gondim LF, Barbosa HV, Filho CHA, Sqeki H. Serological survey of antibodies to *Toxoplasma gondii* in goats, sheep, cattle and water buffaloes in Bahia state. Brazil Vet Parasitol.1999; 82: 273–276.
12. Dubey J P. Toxoplasmosis in sheep –The last 20 years. Vet Parasitol.. 2009;163:1-14.
13. Trees AJ, Crozier SJ, Buxton D, Blewett DA. Serodiagnosis of ovine toxoplasmosis: an assessment of the latex agglutination-test and the value of IgM specific titres after experimental oocyst-induced infections. Res Vet Sci.1989;46: 67–72.
14. Sawadogo P,Hafid J, Bellete B,TranMS, Chakdi M. Sero prevalence of *Toxoplasma gondii* in sheep from Marrakech, Morocco Vet Parasit. 2005;130:89-92.
15. McColgan C, Buxton D, Blewett D A. Titration of *Toxoplasma gondii* oocysts in non-pregnant sheep and the effects of subsequent challenge during pregnancy. Vet Rec 1988;123:467–470.
16. Tenter AM, Vietmeyer C, Johnson A M. Development of ELISAs based on recombinant antigens for the detection of *Toxoplasma gondii* specific antibodies in sheep and cats. Vet Parasitol. 1992; 43:189–201.
17. Negash T, Tilahun G, Patton S, Prevot F. and Dorchies P H. Serological survey on toxoplasmosis in sheep and goats in Nazareth, Ethiopia. Revue de Med Vet. 2004;155:486-487.
18. Mainar -Jaime R C. and Barberán M. Evaluation of the diagnostic accuracy of the modified agglutination test (MAT) and an indirect ELISA for the detection of serum antibodies against *Toxoplasma gondii* in sheep through Bayesian approaches. Vet. Parasitology. 2007;148:122-129.
19. Seefeldt S L, Kirkbride C A, Dubey J P. Comparison of enzyme-linked immunosorbent assay, indirect fluorescent antibody test, and direct agglutination test for detecting *Toxoplasma gondii* antibodies in naturally aborted ovine fetuses. J Vet Dia Invest. 1989; 1: 124-127.
20. Gamble HR, Dubey JP, Lambillotte D N. Comparision of a comerical ELISA with the modified agglutination test for detection of toxoplasma infection in the domestic pig. Vet Parasitol. 2005;128: 177-181.
21. De Paschale M, Agrappi C, Belvisi L, Cagnin D, Cerulli T, Clerici P, Mirri P, Manco MT, Cavallari S and Vigano E F. Revision of the positive predictive value of IgM anti- *Toxoplasma* antibodies as an index of recent infection. New Microbiol. 2008; 31:105-111.