

**Comparison Between Enzyme Linked Immunosorbent Assay (ELISA)
and Modified Agglutination Test (MAT) for Detection of
Toxoplasma gondii Infection in Sheep and Goats Slaughtered
in an Export Abattoir at Debre-zeit, Ethiopia**

¹Endrias Zewdu Gebremedhin, ²Mukarim Abdurahaman,
³Tsehay Hadush and ⁴Tesfaye Sisay Tessema

¹Department of Veterinary Laboratory Technology, Faculty of Agriculture
and Veterinary Sciences Ambo University, P.O.Box 19, Ambo, Ethiopia

²College of Veterinary Medicine and Agriculture, Jimma University, P.O.Box 307, Jimma, Ethiopia

³Faculty of Veterinary Medicine, Samera University, P.O.Box 132, Samera, Ethiopia

⁴Institute of Biotechnology, College of Natural and computational Sciences,
Addis Ababa University, P.O. Box 1176, Ethiopia

Abstract: Toxoplasmosis is one of the most common parasitic zoonoses world-wide. Studies on prevalence and comparison of the serodiagnosis of *T. gondii* infection in small ruminants in African countries are limited. A cross-sectional study was carried out from September, 2011 to November, 2012 on 242 cardiac blood samples of sheep and goats collected from an export abattoir in Debre-Zeit, Central Ethiopia. Comparative study was done in diagnosis of *T. gondii* infection and revealed MAT = 27.7% (67/242) and ELISA 9.9% (24/242) seropositivity by Enzyme Linked Immunosorbent Assay (ELISA) and MAT (reference test) respectively with a moderate agreement ($\kappa = 0.42$, $P < 0.001$). The overall sensitivity, specificity, positive and negative predictive values of ELISA was 34.3%, 99.4%, 95.8% and 79.8% respectively. All ELISA positive samples were also positive by MAT. The performance of ELISA is better in sheep than goat samples and in adult sheep and goat samples than in younger. The high specificity and positive predictive value of ELISA indicate its potential as diagnostic tests, particularly in areas with a high prevalence of infection while its low sensitivity and negative predictive value limit its value as a screening test. Therefore, MAT is reliable test for population screening tests. Further detailed evaluation of performance of ELISA as diagnostic test in various body fluids, different animal species and breeds is suggested.

Key words: Comparison • ELISA • MAT • Sheep • Goat • Seropositivity • Kappa • *Toxoplasma Gondii*

INTRODUCTION

Toxoplasmosis is one of the most common parasitic zoonoses and establishes long-lasting infections in humans and animals [1, 2]. Humans get infections with *T. gondii* after ingesting raw or undercooked meat, sporulated oocysts via contaminated soil, food or water and congenitally by transplacental transmission of tachyzoites [1, 3].

Diagnosis of toxoplasmosis by demonstration of *T. gondii* in tissues of sheep and goats is difficult. Therefore, the detection of antibody response by serological screening of slaughtered animals appears to be an important tool for proper diagnosis concerning toxoplasmosis public health hazard [4, 5]. Serological techniques such as the Sabin-Feldman dye test, modified agglutination test (MAT), enzyme-linked immunosorbent assay (ELISA), indirect fluorescent antibody test (IFAT)

Corresponding Author: Endrias Zewdu, Department of Veterinary Laboratory Technology, Faculty of Agriculture and Veterinary Sciences Ambo University, P.O.Box 19, Ambo, Ethiopia. Tel: +251 911894802, Fax: +251 112362037.

and immunoblotting can be used to detect *T. gondii* specific antibodies. MAT is the major recommended test for diagnosis of *T. gondii* in several animals and humans [1] due to its highest sensitivity among all serological tests [6] and this was confirmed by Shaapan *et al.* [7]. Seroepidemiological assessment of *T. gondii* infection coupled with measures taken to reduce infection at farm level through hygienic farm management practice is best way to reduce the risk of *T. gondii* infection for consumers [8].

The performances of different serological tests have been evaluated for the diagnosis of *T. gondii* infection in sheep in Cairo [7] and Serbia [9] and in pigs in northeastern United States [8]. In view of the economic significance of small ruminants and their potential to spread toxoplasmosis to humans in Ethiopia, the present study was initiated with the objective of comparison of the diagnosis of *T. gondii* infection by ELISA and MAT tests.

MATERIALS AND METHODS

Study Animals: The study was conducted in sheep and goats brought to an export abattoir for slaughter from two purposively selected Zones of Central Ethiopia (East Shoa and West Shoa). The origin of the animals is mainly from Fentale, Ada'a-Liben (East Shoa Zone) and Ambo (West Shoa Zone) districts. Afar, Arsi-Bale and Western Highland breeds of goat predominate in Central Ethiopia. Similarly sheep breeds common in Central Ethiopia are Afar, Arsi-Bale and Horro. Small ruminants are kept for meat production in most parts of the country; however, pastoralists in Fentale district additionally use goats and rarely sheep for dairy purpose. Small ruminants of the study area are predominantly raised under extensive husbandry system. In this study, few females were sampled because females are normally kept for breeding purpose and those arriving at the abattoir are culls and/or infertile animals. Sheep and goats of different breeds aged over six months were included in this study. Age was determined by observation of the erupted permanent incisors [10]. Animals' = 1 year were considered as young while those above one year were considered adult.

Study Design: This cross-sectional study was carried out from September, 2011 to November, 2012. A total of 242 cardiac blood samples (104 sheep and 138 goats) were collected directly from heart chambers, properly labeled and the sera were separated by centrifugation at 3200 RPM for 10 minutes. The extracted sera were transferred to other sterile vials and kept at -20°C until serologically assayed.

Serological Tests

Modified Agglutination Test (MAT): *Toxoplasma gondii*-specific IgG antibodies were detected by the modified agglutination test (Toxo screen DA, bioMérieux®, France) following the procedure described by manufacturer of the kit. Sera were assayed at a screening dilution of 1/40 and 1/4000 in order to avoid the false negative results that might happen at low dilutions when using sera with high antibody titers. Sedimentation of antigen at the bottom of the well and clear agglutination above half of the well at either dilution were recorded as negative and positive results respectively.

Enzyme Linked Immunosorbent Assay (ELISA): Sera samples extracted from cardiac blood were analyzed for the presence of IgG antibodies against *Toxoplasma gondii* following the instructions of the manufacturer of the indirect enzyme linked immunosorbent assay (P-30 ELISA) kit (ID VET Innovative Diagnostic, ID Screen®, Montpellier, France). The kit uses non-species specific protein conjugate hence used to detect chronic *T. gondii* infection in multi-species.

For interpretation of the results S/P% was calculated as: $\text{S/P\%} = (\text{OD}_{450} \text{ value of the sample} - \text{OD}_{450} \text{ value of the negative control}) / (\text{mean OD}_{450} \text{ value of the positive control} - \text{OD}_{450} \text{ value of the negative control}) \times 100$. Any sample with $\text{S/P\%} \leq 40\%$, $\geq 50\%$ and $40\% - 50\%$ were considered as negative, positive and doubtful, respectively. The doubtful results were also retested. The test was validated if the mean value of the positive control O.D. (OD_{PC}) is greater than 0.350 ($\text{OD}_{\text{PC}} > 0.350$) and the ratio of the mean O.D. values of the positive and negative controls (OD_{PC} and OD_{NC}) is greater than 3.5 ($\text{OD}_{\text{PC}} / \text{OD}_{\text{NC}} > 3.5$).

Statistical Analysis: The data generated were stored in Microsoft excel spreadsheet (Microsoft Corporation) and analyzed using STATA version 11.0 for Windows (Stata Corp. College Station, USA). All the variables assessed were categorical. For test comparison the association of the origin, species, age, sex and breed of animals with *T. gondii* IgG seropositivity was analyzed by Chi-square test. Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) was calculated. Agreement between the two tests were calculated and interpreted following the recommendations of Dohoo *et al.* [11] which states Kappa values as: <0.2 : slight agreement, $0.2-0.4$: fair agreement, $0.4-0.6$: moderate agreement, $0.6-0.8$: substantial agreement and >0.8 : almost perfect agreement. The 95% confidence level was used and results were considered significant at $P \leq 0.05$.

Table 1: Comparative IgG seroprevalence of *T. gondii* infection in sheep and goats slaughtered at export abattoir in Debre-Zeit as detected by MAT and ELISA by host related factors

Factors		Number of tested	MAT Positive No. (%)	ELISA Positive No. (%)	P-value (MAT)	P-value (ELISA)
Origin	East Shoa Zone	130	32 (24.62)	10 (7.69)	0.250	0.210
	West Shoa Zone	112	35 (31.25)	14 (12.5)		
Species	Caprine	138	34 (24.6)	11 (8.0)	0.222	0.243
	Ovine	104	33 (31.7)	13 (12.5)		
Age	Young (=1 yr)	167	44 (26.4)	15 (9.0)	0.487	0.468
	Adult (> 1yr)	75	23 (30.7)	9 (12.0)		
Sex	Male	235	65 (27.7)	22 (9.4)	0.958	0.094
	Female	7	2 (28.6)	2 (28.6)		
Breed: sheep				0.122	0.173	
	BHS	6	0 (0.0)	0 (0.0)		
	Afar	34	9 (26.47)	2 (5.88)		
	Horro	64	24 (37.5)	11 (17.19)		
Breed: goat				0.047	0.805	
	Arsi-Bale	39	5 (12.82)	3 (7.69)		
	Western highland	48	11 (22.82)	3 (6.25)		
	Afar	51	18 (35.29)	5 (9.80)		

BHS = Blackhead Somali

Table 2: Evaluation of sensitivity, specificity, positive predictive value and negative predictive value of ELISA using MAT as gold standard for comparison of the MAT and ELISA to detect *T. gondii* antibodies

Sample	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Kappa	Interpretation
Sheep and goats ^a	34.3	99.4	95.8	79.8	0.42	Moderate agreement
Sheep only ^b	39.4	100	100	78.0	0.47	Moderate agreement
Goats only ^c	29.4	99.0	90.9	81.1	0.37	Fair agreement
Young sheep and goats ^d	31.8	99.2	93.3	80.3	0.39	Fair agreement
Adult sheep and goats ^e	39.1	100	100	78.8	0.47	Moderate agreement

^{a,b,c,d,e} P<0.0001

RESULTS

Out of 242 serum samples, 67 (27.7%) and 24 (9.9%) were positive by MAT and ELISA test respectively. There is statistically significant difference between the results of the two tests ($P \leq 0.001$). Comparative evaluation of the performance ELISA vs MAT for diagnosis of toxoplasmosis in relation to origin, sex, age, species and breed of animals was shown in Table 1. Out of 24 MAT positive Horro breeds of sheep only 11 reacted positive by ELISA. Out of 18 Afar breed of goat that reacted positive by MAT only 5 gave positive result by ELISA (Table 1).

Using the MAT as reference test, the sensitivity, specificity, positive predictive value and negative predictive value of ELISA along with kappa coefficient and interpretation are indicated in Table 2. The overall kappa coefficient was 0.42, indicating a moderate agreement between the results of MAT and ELISA (Table 2).

Repeated testing of those sera samples ($n = 43$) which produced discordant results by the two assays (i.e., positive for MAT but negative for ELISA) confirmed

the initial findings. Out of the 43 samples tested positive by MAT but negative by ELISA 2, 3, 27, 6, 2 and 3 had a MAT end titers of $\leq 1:60$, $1:180$, $1:540$, ≥ 1620 , $1:18000$ and $1:54000$ respectively.

DISCUSSION

In this study comparison of the performance of ELISA (it uses P30 antigen) to detect *T. gondii* antibodies was made with MAT (it uses whole killed tachyzoites as antigen) as reference test. Out of 67 samples positive by MAT only 24 samples reacted positively by ELISA giving sensitivity of 35.8%. ELISA is a widely used tool for the diagnosis of toxoplasmosis in both animals [12-15] and humans [16-18]. However, before an ELISA can be used as a routine diagnostic test or as a screening test in epidemiologic studies, performance of the assay must be validated in a specific target population. Validation is necessary because different tests might have different false-positive and false-negative rates in different populations, depending on the amount of cross-reactivity. The results of this study showed 35.8% and 100% of sensitivity and specificity, respectively, by ELISA.

In contrast, Negash *et al.* [19] reported 98.9% sensitivity and 90.9% specificity for ELISA using MAT as reference test on sera of sheep and goats.

The indirect ELISA kit currently used was designed by the manufacturer for the detection of *T. gondii* antibodies from sera, plasma and meat juice. However, we wanted to evaluate its performance using sample collected directly from cardiac chamber which is easily obtained sample from slaughtered animals compared to blood from jugular vein. Thus, weaker concentration of *T. gondii* antibodies in cardiac blood extracts [20, 21] or non-specific reaction at lower concentration [22] as compared to sera or factors unknown for us in the cardiac fluid might have lowered sensitivity of ELISA compared to MAT. Non-specific inhibitors present in contaminated and hemolysed cardiac samples [8, 23] and the different conditions under which the tests were done (ELISA test was done in different laboratory after thawing samples stored for up to 4 months while MAT was done on fresh samples within 24 hours of collection) might have additionally contributed for the higher false negative results by ELISA as compared to MAT. In agreement to our results Gamble *et al.* [8] reported reduced sensitivity of ELISA to an unacceptable level using naturally infected pig sera. Our result is comparable to the findings of Klun *et al.* [9] who showed moderate agreement of ELISA with MAT (at cut-off titer of 1:100). However, kappa agreement greater than 0.6 between ELISA and MAT has been reported earlier by Dubey *et al.* [24].

The variation between the results obtained using different serological tests might be due differences in sensitivity and specificity of the serological tests used. Differences in seropositivity between assays might also reflect the different antigen profiles presented in each test for antibody detection (P 30 antigen in ELISA and whole tachyzoites in MAT) [25]. Thus, IgG antibodies are likely to be detected against varieties of antigens in the whole tachyzoites thereby increasing the sensitivity of detection. In this study, the concordance of the two tests showed moderate agreement (kappa = 0.42). Those MAT negative serum samples were also negative in ELISA but only 24 out of 67 MAT positive serum samples were positive in ELISA and this has resulted in high specificity and positive predictive value and low sensitivity and negative predictive value of the ELISA test. This indicates that some MAT positive sera have been missed by ELISA. The high specificity (100%) and positive predictive value (100%) are at the expenses of sensitivity and negative predictive values and indicate absence of false-positive results and missing of some positive sera by ELISA that has been captured as positive by MAT.

In this study the performance of ELISA is affected by the species, the age and the breed of sheep and goats. The performance of ELISA is better in sheep than goat samples and in adult sheep and goat samples than in young. ELISA also performed poor for Afar sheep (5.88% vs 26.47%) and Afar goat (9.8% vs 35.29%) breeds compared to MAT (Table 1). The reason for this is not clear but it was hypothesized that it might be related to the genetics and/or nutritional conditions of the animals.

The high specificity and PPV of ELISA is also suggestive of its potential for diagnostic purposes, particularly in field conditions in case of, for instance, abortion storms [9] or in a population where the disease is highly prevalent. However, due to low sensitivity and negative predictive value, the results may be an underestimate. Therefore, MAT is reliable test for population screening tests. Nevertheless, both tests have their own advantages and limitations. ELISA demonstrates great specificity, is quantitative, low cost and may be automatically adopted. However, 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, easy to perform and does not require species specific conjugate or sophisticated equipment and can be used on any species [1]. However, longer time required to get MAT result, subjectivity in reading the results, the relatively higher price and limited availability particularly in laboratories in developing countries are limitations towards routine usage of MAT.

In conclusion, ELISA has low sensitivity and negative predictive values but high specificity and positive predictive value when compared with MAT. Therefore, MAT is reliable test for population screening tests. Further detailed evaluation of performance of ELISA as diagnostic test in various body fluids, different animal species and breeds is suggested.

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