Comparative serological diagnosis of toxoplasmosis in sheep using a crude antigen and commercial antigen

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Abstract: In the present study, a crude antigen of the locally isolated *Toxoplasma gondii* tachyzoites from sheep's meat (LA) was used for the first time in Riyadh to detect *T. gondii* antibodies in sheep's. We compared the sensitivity of standard ELISA assay using local antigen and a commercial kit (ELISA and IFAT) for detecting antibodies of *T. gondii* in 200 sheep serum samples collected from suspected cases of toxoplasmosis. Results showed that ELISA assay developed in our laboratory using give better result 50% compared with the commercial kit (46%) and IFAT 45%. So, this study recommended utilization of the bound fraction of sheep origin in diagnosis of toxoplasmosis in sheep's using ELISA (50%) which proved better diagnostic potency compared with commercial (IFAT (45%) & ELISA (46%) in Saudi Arabia .
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Key Words: Toxoplasmosis, Serological diagnosis, Commercial ELISA kit, Standard ELISA, Sheep and Saudi Arabia.

1. Introduction:

Toxoplasmosis is a widespread zoonosis caused by the coccidian protozoan *T. gondii.* It is an important cause of abortion, stillbirth and neonatal mortality in sheep **(Dubey and Beattle, 1988).** The definitive hosts of the parasites are domestic cats and other felines, the sexual cycle occurring only in these species **(Frenkel *et al*., 1970).** Human toxoplasmosis can be acquired both through ingestion of sporulated oocysts and via ingestion of bradyzoites in the tissues of numerous food animals. The infection is also transmitted transplacentaly **(Dubey, 1994).** Transmission of *T.gondii* tachyzoites in unpasteurised sheep or goat milk and blood transfusions can occur, but are probably not important epidemiologicaly **(Schirley, 1995)**.

It is an Apicomplexan protozoan. The definitive host is domestic and wild cats. The lifecycle of this parasite is both sexual and asexual. The sexual cycle occurs only in definitive host, the result is the non-infectious oocyst that is shed into the environment via cat feces **(Dubey and Frenkel, 1972).** Within 1-3 days, they undergo sporulation to become infectious sporozoites. Although cats are the exclusive final hosts but the transmission of oocysts from cat to cat is not efficiently, so, its transmition via intermediate host (human, bird, rodent) takes place. Asexual stage start when intermediate host of the parasite become infected by ingestion of sporulated oocysts. Cyst-contaminated meats, contact with free tachyzoites. It can invade almost any cells and multiplies rapidly to form bradyzoite. Tissue cysts are formed that contain thousand of bradyzoites **(Dubey, 1977).** Cysts are found mainly in skeletal muscle, heart muscle, and CNS tissue, but bradyzoites can invade anywhere. The cyst will remain unexposed to the immune ystem, for the life of host, but, it becomes reactive at immunosuppressed states. The tissue cyst or bradyzoites are highly infective and can be transmitted through eating raw or undercook meets. All *Toxoplasma* strains are morphologically and serologically similar, leading to their designation as a single, globally distributed species **(Dubey and Beattle, 1988).**

So, this study aimed to evaluate the performance of a new local antigen and comprises with commercial antigen for detection of antibodies in serum sheep's

2- Material and Methods:

- Evaluate the performance of a new local antigen (sheep derived *T. gondii* as antigen), which comprises with commercial antigen for detection of antibodies in serum sheep's:

Comparative serological diagnosis of toxoplasmosis in sheeps using a crude antigen of the locally isolated *T. gondii* tachyzoites (LA) from sheeps tissues and commercial antigen were used for the detection of *T. gondii* antibodies in sheeps.

-Antigen

T. gondii antig was obtained from Kingdom of Saudi Arabia, King Abdulaziz City for Science and Technology, General Directorate of Research Grants Programs (AT-28-72).

- Serum samples

Twenty of 3-months-years-old lambs, clinically healthy and presenting negative serologically for *T. gondii*. 10 of lambs infected with $3X10^3$ of tachyzoite *T. gondii* and tested positive in the present of the cyst tissue as a the positive control serum samples and negative control serum samples were obtained from the control animals (n = 10). Also 100 serum samples were

obtained from sheep's were collected, labeled in serial numbers and stored at -20 C until use. These sheep's randomly chosen from a serosurvey accomplished in Riyadh. Sera were tested to evaluate the analytical and diagnostic performance of developed ELISA local unbound fraction (LAunb) compeer with commercial ELISA and IFAT.

-Serological tests:

A: Eliza using locally isolated antigen (antigen preparation):

The ELISA was performed in flat-bottomed 96-well microplates (Nunce, UK) Were coated with 2 ug of crude tachyzoite antigen was diluted starting from 1:100 to 1:1000 per well and incubated for one 1h at 37°C or overnight at 4°C. The plates were then aspirated, blocked with 1-3% bovine serum albumine in PBS in phosphate buffer saline 37°C for 1 h. Unbound antigen was removed by washed four times with PBS-T PBS containing 0.05%Tween 20 (PBS-T). 50 ul each serum controls (negative and positive) and serum samples were added.

There were used serial dilutions of sera ranging from 1:100 to 1:500 in PBS. After serum incubation (37°C for 1h) and washing, Anti-Sheep IgG (whole molecule) peroxidase conjugates (Sigma) was used diluted 1:100,000 in PBS was added to the wells and incubated at 37°C, 1h. Plate was washed and bound antibodies were detected by incubation at room temperature with 3, 30, 5, 50-tetramethylbenzidine liquid substrate 0.2%. After 10 min, 2N sulfuric acid was used to stop the reaction, Reading was carried out at 492 nm using Titertek Multiskan Spectrophotometer.

B: ELISA kit:

It was performed by a commercial kit Toxoplasma IgG ELISA Kit **(**GenWay**)** , according to the manufacturer's instructions for detection of anti *T. gondii* antibodies.

C: Indirect fluorescent antibody test (IFAT)

The IFAT was adopted according to the technique described by **Sulzer *et al* (1971)** at a dilution Of 1:64. Antigen slides were incubated with sheep sera diluted 1:64 in PBS and then with a Fluorescein isothiocyanate-labeled rabbit anti-sheep IgG (ICN-Immunobiologicals) diluted 1:100 in incubation the slides were washed 3 times with PBS. Positive and negative control sera were included in each slide. Slides were examined under fluorescence microscopy and only a bright, linear peripheral fluorescence of the *T. gondii* tachyzoites was considered positive

-Study area

A total of 600 sheep's serum samples were tested for the presence of anti-*T. gondii* antibodies using Elisa. Were randomly select from four districts of Riyadh, (pasture (Sheep graze in the pasture)**, cattle** marketplace in Al-Nassim, Riyadh Modern Slaughterhouse and Ibrahim Abdel Aziz al-fold for the sale of sheep). All sheep were females, From these sheep approximately 3 ml of blood was drawn from the jugulare vein from each sheep, sera were obtained by centrifugation at 2300 g for10 min and transferred in eppendorf tubes and were kept in a freezer at -20 ° C until tested for antibodies to *T. gondii* using a commercial ELISA test kit (kit Toxoplasma IgG ELISA Kit **(**GenWay**)** and ELISA assay with local *T. gondii* strain antigen.

3- Results

The results obtained between comparison test by IFAT, ELISA (commercial) and ELISA using local unbound fraction (LAunb) for *T. gondii* antibody detection in 100 sheep sera are shown in Table 1. Evaluation of the diagnostic efficiency of ELISA using local antigen in comparison to IFAT and ELISA (commercial) revealed that LAunb-ELISA gave the highest diagnostic efficiency (50%) followed by ELISA (commercial) (46%) and IFAT (45%). Also there not statistically significant differences between tests, P value ≥ 0.05 (Table 1).

Generally, ELISA assay using local unbound fraction (LAunb) showed higher prevalence of anti- *T. gondii* antibodies than that obtained by ELISA using the commercial kit. However, the difference in the detection of anti- *T. gondii* antibodies between the two ELISA assays was statistically significant within group A and very statistically significant within group B, it was not statistically significant within group C and D (table 2& figure 1).

The prevalence of anti- *T. gondii* antibodies in sera samples collected from Pasture was higher than that detected in sera samples from the other locations by both assays (Table 2). The difference in the prevalence of anti- *T. gondii* antibodies between sheep sampled from different location using both ELISA assays was statistically significant ($P \leq 0.05$) except the difference between group A and D was not statistically significant ($p=0.764$ and 0.1897) either by ELISA using commercial kit or ELISA using local unbound fraction (LAunb) respectively.

Moreover, anti- *T. gondii* antibodies levels as detected by mean OD reading were ingeneral higher in all groups by ELISA assay using local unbound fraction than that detected by ELISA using the commercial kit. Sera samples collected from Pasturehad higher mean OD reading by both ELISA assays (Figure 2).

Table 1: Comparison between results obtained by IFAT, ELISA (commercial) and ELISA using local unbound fraction (LAunb) for *T. gondii* antibody detection in sheep sera

Test*	**Test Results			
	positive		Negative	
	#	%	#	%
IFAT using commercial kit	45	45	55	55
ELISA using commercial kit	46	46	54	54
ELISA using local unbound fraction (LAunb)	50	50	50	50

*Same 100 sheep sera samples were tested in each test

** The association between tests and results is considered to be not statistically significant by Fisher's exact test, the two-tailed (P value ≥ 0.05)

Table 2: Prevalence of anti- *T. gondii* antibodies in sheep sampled from different location in Riyadh as determined by two ELISA assays

Group*	ELISA using commercial kit		ELISA using local unbound fraction (LAunb)		**Fisher's exact test The two-tailed (P value)
	# positive	%	# positive	%	
A	98	49	120	60	(0.0348) statistically significant
B	50	25	80	40	(0.0019) very statistically significant
C	22	11	32	16	(0.1875) not statistically significant
D	94	47	106	53	(0.2713) not statistically significant

*200 sheep samples were tested from each group

**Fisher's exact test, the two-tailed (P value) was used to calculate the statistical difference in the detection of anti- *T. gondii* antibodies between the two ELISA assyas within each group.

A- Pasture
B- Cattle marketplace in Al-Nassim
C- Riyadh Modern Slaughterhouse
D- Ibrahim Abdel Aziz al-fold for the sale of sheep

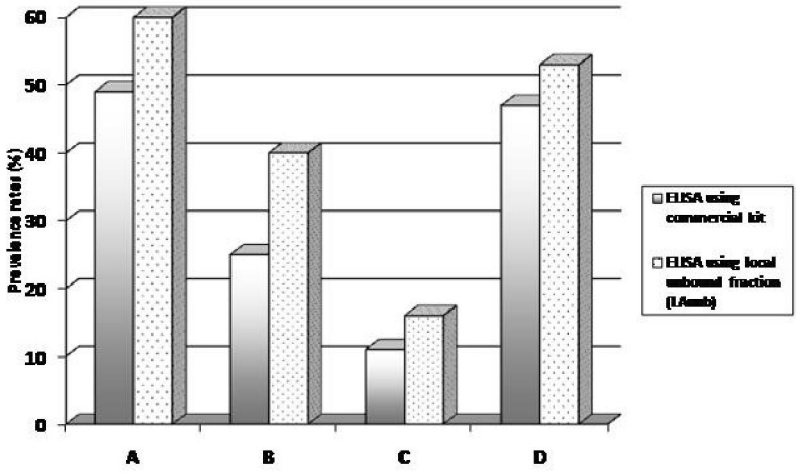

Fig 1: Comparison between prevalence rates (%) of anti- *T. gondii* antibodies in sheep sampled from different location in Riyadh using two ELISA assays.

A- Pasture
B- Cattle marketplace in Al-Nassim
C- Riyadh Modern Slaughterhouse
D- Ibrahim Abdel Aziz al-fold for the sale of sheep

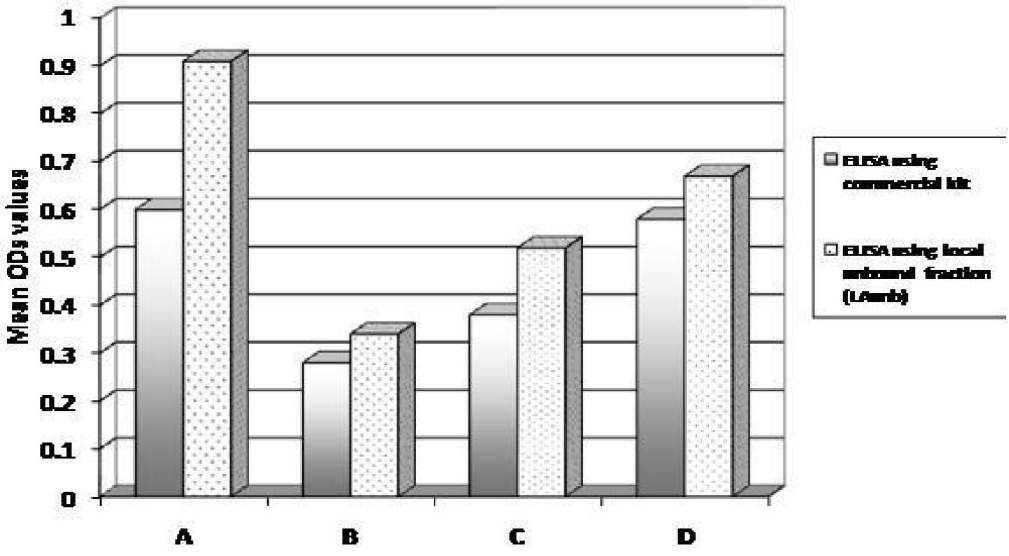

Fig 2: Anti- *T. gondii* antibodies levels as detected by mean OD reading in sheep sera sampled from different location in Riyadh by two ELISA assays.

A- Pasture
B- Cattle marketplace in Al-Nassim
C- Riyadh Modern Slaughterhouse
D- Ibrahim Abdel Aziz al-fold for the sale of sheep

4-Discussion:

In the present study, a crude antigen of the locally isolated *T. gondii* tachyzoites from sheep's meat (LA) was used for the first time in Riyadh to detect *T. gondii* antibodies in sheep's. We compared the sensitivity of standard ELISA assay using local antigen and a commercial kit (ELISA and IFAT) for detecting antibodies of *T. gondii* in 200 sheep serum samples collected from suspected cases of toxoplasmosis. Results showed that ELISA assay developed in our laboratory using give better result 50% compared with the commercial kit (46%) and IFAT 45%. So, this study can suggest that the ELISA assay with local *T. gondii* strain antigen is more sensitive than the commercial ELISA kit. **Malik *et al.* (1990)** found that ELISA gave better results (62.5%) than IFAT (55%) in sheep toxoplasmosis,also at Tabouk Official Abattoir showed the result of sera from 397 sheep were examined for anti-Toxoplasma IgG by IFAT were infection rates 52.2% ($P < 0.001$). Our result agreed with that previously obtained in Egyptian sheep (50.4% and 61.4% by LAT and ELISA) **(Hassanain, *et al.*, 2011),** While **Gamble *et al.* (2005)** reported that ELISA performed slightly better than MAT in detecting *T. gondii* antibodies in naturally infected pigs.

In addition, the serological tests used in this work depended on Ag prepared from locally isolated strains of *T. gondii*, which were much cheaper when compared with the expensive patented kits in the previous studies in King Saudi Arabia. However, ELISAs based on tachyzoite crude antigens have a higher sensitivity rates **(Aduriz *et al.*, 2007).** ELISA is the most widely used test for screening toxoplasmosis in sheep and other animals.

This study recommended utilization of the crude and bound fraction obtained from the locally isolated tachyzoites (sheep's origin) diagnosis of toxoplasmosis in sheep's using ELISA which proved better diagnostic potency and sensitive compared with commercial ELISA kit and IFAT. ELISA based on a crude tachyzoite antigen instead of a commercial kit reduce the cost of the analysis.

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