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Research Article

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# Serological Studied On Toxoplasma Gondii Infection in Sheep and Goats in Ismailia Province

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## Abstract

*Blood serum samples from 100 sheep and 100 goats were collected and examined for Toxoplasma gondii antibodies by Enzyme Linked Immunosorbent Assay and Modified Agglutination Test. The seroprevalences of Toxoplasma gondii in sheep were 34% and 33% and in goats were 32%, 31% by ELISA and MAT respectively. The prevalence in the females of sheep and goats were higher than males. The seroprevalences were higher in adult animals than young in both sheep and goats. Using the MAT as a reference test, the sensitivity and specificity of the ELISA Test were 100% and 98.5% respectively.*

## Introduction

*Toxoplasma gondii* is an apicomplexan parasite of warm-blooded animals, with cats as definitive and other animals and humans as intermediate hosts [1].The most important intermediate hosts among farm animals are sheep, goats and pigs. In sheep, infection may cause foetal death and abortion following a primary infection during pregnancy, recently, it has been suggested that there is a possible recrudescence of infection during pregnancy [2]. *T. gondii* infection in sheep is distributed worldwide, with seroprevalences of 20–91% in different countries [3].

Infection is more common in warm climates and in low-lying areas than in cold climates and mountainous regions, where conditions for sporulation and survival of oocytes are less favorable [4].

Among the large food animal, goats are one of the most susceptible hosts to Toxoplasma gondii, moreover, undercooked meat of sheep and goats containing tissue cyst of T. gondii represent a potential source of human toxoplasmosis [5].

Since direct observation of cysts in tissues is not a suitable diagnostic method to be carried out on live animals, the serological techniques appear to be the methods of choice. In veterinary laboratories, Enzyme Linked Immunosorbent Assay (ELISA) is very useful to diagnose pathogens like T. gondii, which infect various animals, because its format allows testing many species. On the other hand, several other serological tests are available and considered by several authors as reference tests for the diagnosis of toxoplasmosis, a Modified Agglutination Test (MAT) is the major recommended test for this purpose [6].

The aim of this study is to determine the Seroprevalence of Toxoplasma gondii among sheep and goats by using ELISA and MAT.

## Material and methods

### Serological Techniques

Collections of serum samples: Two hundreds blood samples, each of 10 ml, were collected (100 from sheep and other 100 from goats) from Ismailia abattoir into a sterile screw capped tube. Each sample identified and the tube left in a sloping position at room temperature for about one hour and centrifuged at 3000 R.P.M. for 15 min. Sera were aspirated by pasture pipette in other clean dry crocked bottle which labeled in a serial number and stored at -20ºC in the refrigerator until used. The study were done in the period extend from November till December 2010.

**A-Enzyme linked immunosorbent assay (ELIZA) according to Voller et al. B-Modified agglutination test** [9]: Formalized whole tachyzoites antigen and incorporating 2 mercaptoethanol as described by ***Desmonts and Remington*** and modified by ***Dubey and Desmonts*** were used [7, 8].

**Statistical analysis:** The results are expressed in percentages. The prevalence for *T. gondii* was statistically analyzed by the Chi-square test ($\chi 2$) considering the variables sex, age. The differences were considered statistically significant at $P \leq 0.05$. [10]. Also Kappa statistic test was used to test the agreement between the two serological tests. It is defined as the excess agreement that expected by chance, divided by the potential excess. Kappa values of greater than 0.81: almost perfect agreement, 0.6 - 0.80: substantial agreement 0.41 - 0.06 moderate agreements, 0.21 - 0.09: fair agreement; 0 - 0.2 slight agreement and 0: poor agreement [11].

**Using the MAT as a reference test, the sensitivity and specificity, of the ELISA Test were calculated according to Savela et al.** The differences between ELISA and MAT in detection of T. gondii antibody were made by McNemar's test of MedCalc program for non-independent samples which used to assess the statistical

differences between the sensitivity, specificity, predictive values (positive and negative) of ELISA and MAT [12].

## Results

[Table 1] showed that, the seroprevalence of *Toxoplasma gondii* in sheep were 34% and 33% by ELISA and MAT respectively. Test agreement beyond chance between the two tests was K=0.90 and indicates almost a perfect agreement. Using the MAT as a reference test, the sensitivity and specificity of the ELISA Test were 100% and 98.5% respectively.

Also [Table1] showed that, the seroprevalence of *Toxoplasma gondii* in goats were 32% and 31% by ELISA and MAT respectively. By Test agreement beyond chance between the two tests was K=0.90 and indicates almost a perfect agreement. Using the MAT as a reference test, the sensitivity and specificity of the ELISA Test were 100% and 98.5% respectively.

[Table 2] revealed that of the examined 100 sheep's sera (59 females and 41 males), the prevalence of Toxoplasma gondii was significantly higher in females (42& 42%) than males (19& 22%) as assayed by MAT and ELISA respectively ($P < 0.05$).

[Table 3] revealed that of the examined 100 goat's sera (69 females and 31 males), the prevalence of T. gondii was higher in females (36 & 38%) than males (19& 19%) as assayed by MAT and ELISA respectively. No significant differences in sex were found in goat ($P>0.05$).

[Table 4] denoted that the prevalence of T. gondii was significantly higher in adult sheep (43% & 44%) than the lambs (22 & 22%) as determined by MAT and ELISA respectively ($P < 0.05$).

[Table 5] denoted that the prevalence of T. gondii was higher in adult goats (36& 360%) the kids (18 & 21%) as determined by MAT and ELISA respectively. No significant differences in age were found in goat ($P>0.05$).

**Table 1: Seroprevalence of *Toxoplasma gondii* antibodies in examined sheep and goats**

| | No. of sheep | +ve | % | No. of goats | +ve | % |
|---|---|---|---|---|---|---|
| ELISA | 100 | 34 | 34 | 100 | 32 | 32 |
| MAT | 100 | 33 | 33 | 100 | 31 | 31 |

**Table 2: Seroprevalence of *Toxoplasma gondii* in examined sheep in relation to sex by MAT and ELISA**

| | Ex. No. | MAT | | ELISA | |
|---|---|---|---|---|---|
| | | +ve | % | +ve | % |
| Male | 41 | 8 | 19 | 9 | 22 |
| Female | 59 | 25 | 42 | 25 | 42 |
| Total | 100 | 33 | 33 | 34 | 34 |

**Table 3: Seroprevalence of *Toxoplasma gondii* in examined goats in relation to sex by MAT and ELISA**

| | Ex. No. | MAT | | ELISA | |
|---|---|---|---|---|---|
| | | +ve | % | +ve | % |
| Male | 31 | 6 | 19 | 6 | 19 |
| Female | 69 | 25 | 36 | 26 | 38 |
| Total | 100 | 31 | 31 | 32 | 32 |

**Table 4: Seroprevalence of *Toxoplasma gondii* in examined sheep in relation to age by MAT and ELISA**

| | Ex. No. | MAT | | ELISA | |
|---|---|---|---|---|---|
| | | +ve | % | +ve | % |
| Older than one year (adults) | 54 | 23 | 43 | 24 | 44 |
| Less than one year (lambs) | 46 | 10 | 22 | 10 | 22 |
| Total | 100 | 33 | 33 | 34 | 34 |

**Table 5: Seroprevalence of *Toxoplasma gondii* in examined goats in relation to age by MAT and ELISA**

| | Ex. No. | MAT | | ELISA | |
|---|---|---|---|---|---|
| | | +ve | % | +ve | % |
| Older than one year (adults) | 54 | 23 | 43 | 24 | 44 |
| Less than one year (lambs) | 46 | 10 | 22 | 10 | 22 |
| Total | 100 | 33 | 33 | 34 | 34 |

## Discussion

The present study showed that, the overall prevalence of *T. gondii* antibodies in sheep were 34% and 33% as assayed by ELISA and Mat respectively. Our results seem close to that reported by ***Puijo et al***. in Ghana (33.2%), ***Oncel and Vural*** (31%) in Turkey ***Marca et al.*** (35.2%) in Spain and ***Ghazaei*** (31%) [13-16].

The present results were lower than those of ***Malik et al.*** (62.64%) in USA and Waltner-Toews et al. (57.6%) in Canada, Vesco G, et al (49.9%) in Italy, Hamidinejat et al. (72.6%) in Iran and Ivana et al. (84.5%) in Serbia [17-21]. On the other hand, our results were higher than those reported by Sawadogo et al. (23%) in Morocco and Samra et al. (4.3%) in South Africa, Dubey and Foreyt (3.6%) in USA and Silva et al. (27%) in Brazil The obtained results showed that, the overall prevalence of T. gondii antibodies in goats's sera was 32% and 31% as assayed by ELISA and MAT respectively. The present results seem close to that reported by Fahmy and Ayouib (2002) (31%) in Alexandria, Egypt, Sharma et al. (30%) in Botswana, Kutz et al. (37%) in Canada and Ragozo, et al. (32.2%) in Brazil [22-29].

Other studies showed lower prevalence of Toxoplasma antibodies in goats like, ***Sathaporn et al.*** (27.9%) in Thailand and ***Negash et al.*** (25.9%) in Ghana, ***Bahrieni et al.*** in Iran (15.8%) and ***Ramadan et al.*** (22.9%) in Kalubyia, Egypt [30-33].

On the other hand, other studies showed higher prevalence of Toxoplasma antibodies in goats like Chiari et al. (92.4%) in Brazil, Rodriguez-Ponce et al. (63.3%) in Canary Islands Patton et al. (65%) in USA and Shaapan et al. (44.3%) in Giza, Egypt [34-37].

However in the present study, the overall prevalence of *T. gondii* antibodies in goats's sera was 32% and 31% as assayed by ELISA and MAT respectively and that was higher than the estimated worldwide seroprevalence of toxoplasmosis in livestock that has been reported as 15% in goats [38].

The observed variation in prevalence of *T. gondii* could be due to the diagnostic techniques used in the different regions, frequency of felines on the farms, age of the animals, and the climatic variations from one region to another [39]. On the other hand, the reason of this difference may be due to the poor management conditions in related goat flocks [40].

The MAT and ELISA Tests detected almost similar proportion of Toxoplasma positive serum samples. Therefore, both are reliable for population 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 does not require species specific conjugate and can be used on any species [41].

In our opinion, the perfect agreement between the two tests as explained by good k-value, suggests the use of one procedure over the other depending on the choice of the investigator and availability of equipments.

The present results confirmed that the prevalence of T. gondii was significantly higher in female (42 &42%) than male (22 & 19%) sheep as assayed by both ELISA and MAT respectively, which is compatible with Alexander and Stinson (1988)who reported that female animals were more susceptible than males to infection with protozoan parasites. However, Oncel and Vural in turkey reported that, no significant difference was observed between male and female groups in the prevalence of T. gondii [42]. On the other hand, ***Lashari and Tasawar***, in Iran reported that the prevalence was higher in male (30.15%) than in female sheep (18.46%) [43]. Also the present results showed that the prevalence of *T. gondii* was higher in female (38&36%) than male (19& 19%) goats as assayed by both ELISA and MAT respectively, in this regard, ***Shaapan et al.*** in Giza, Egypt reported that, the prevalence of *T. gondii* was higher (63.3%) in female than (32.1 %) in male goats. However, ***Bahrieni et al***. reported that no significant difference was observed in the presence of *T. gondii* antibodies in females as compared with males. his discrepancy in the results could be explained as that females have more immunity than males, which may be due to the presence of estrogen in females which normally increases the immunity, while androgen in males decreases the immunity [37, 44-46]. But there are various other factors which may break down the immunity in females e.g., changes in sex associated hormones, environmental factors, age, nutrition and pregnancy [47].The obtained results showed that, the prevalence of *T. gondii* was higher in adult sheep (43 & 44%) than the lambs (22 & 22%) as determined by MAT and ELISA respectively. This was agreed with ***Dubey and Welcome*** who indicated that the seropositivity increased with age; as (40.2%) of 1-year-old ewes had detectable antibody vs. (89.2%) of 2-year-old ewes [48]. Also the obtained results showed that the prevalence of T. gondii was higher in adult goats (36 & 36%) than kids (18 & 21%) as determined by MAT and ELISA respectively. This result agreed with that of Shaapan et al. in Giza, Egypt who detected higher prevalence (58 %) in aged goats (>1.5 years) than (32.8 %) in younger goats (≤1.5 years) [37]. Such results may be attributed to prolonged exposure of olderly goats to infective T. gondiioocysts throughout their life [33].

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