

## Induction of *Toxoplasma gondii* in Goats

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**T**HE PREVALENCE of anti-*Toxoplasma gondii* antibodies was investigated in goats slaughtered for food in various areas of the Giza province, Egypt. Two hundred and thirty blood samples collected from the slaughtered goats were used for the serologic diagnosis of *T. gondii* infection by the modified agglutination test (MAT) with cut-off value 1:25. The antibodies to *T. gondii* were found in 44.3 % (102/230) of goat sera. Experimental infection of goats with the infective stages of both locally isolated and RH strains of *T. gondii* was carried out. Persistence of *T. gondii* tissue cysts and lesions were demonstrated in the different tissues and organs of the experimentally infected goats by the histo-pathological examination. This study is the 1st assay of toxoplasmosis in Egyptian goats by MAT and is the unique successful determination of the *T. gondii* infective stages within the experimentally infected goat tissues by the histo-pathological examination. Consequently the finding results obtained scope the public health significance of goat meat as source of human infection.

**Keywords:** *Toxoplasma gondii*, Goats, MAT, Experimental infection, Histopathology.

*Toxoplasma gondii* is a single-celled parasite of all warm-blooded hosts that is distributed worldwide. The organism can infect a wide range of animal species and man as an intermediate hosts. Cats are the definitive hosts which are the main reservoirs of the infection because they are the only hosts that can excrete the resistant stage (oocysts) of the parasite in the feces. Humans become infected by eating undercooked meat from infected animals or ingestion of food and water contaminated with oocysts. The infection causes mental retardation and loss of vision in children, and abortion in pregnant women and livestock. (Sreekumar *et al.*, 2005).

Goats are economically important food animals due to the minimal cost of rearing and maintaining them, and act as an important source of both meat and sometimes milk for humans in many countries (Dubey, 1990). Infection with *T. gondii* in goats not only results in significant reproductive disorders as abortion or neonatal mortalities but also has implications for public health since

consumption of infected goat meat or milk can facilitate zoonotic transmission. (Waldeland and Loken, 1991).

Although the importance of *T. gondii* in worldwide goat breeding and public health, there is a lack of data on its seroprevalence and factors that contribute to spread the infection. The little surveys of *T. gondii* in goats from Egypt revealed that the rate of infection was 54% using Sabin Feldman Dye test (DT) in goats from Assiut Province (Fahmy *et al.*, 1979). Using indirect hemagglutination (IHAT) and indirect fluorescent antibody test (IFAT) the prevalence of infection was 38 and 40% respectively among slaughtered goats in Tanta abattoir (Ibrahim *et al.*, 1997). While by enzyme linked immune-sorbant assay (ELISA), the prevalence of infection was 39% in goats from private farm in Fayoum governorate (Kandil and Abou-Zeina, 2000).

In other hand a numerous studies have been reported in many countries for the detection of anti-*T. gondii* antibodies in goats. The prevalence rate was 19.6 and 3.9 % in Jordan using DT and complement fixation test (CFT), respectively, (Morsy *et al.*, 1979), 55 and 65% in USA using IHAT and modified agglutination test (MAT), respectively, (Patton *et al.*, 1990), 60 and 66% in Czech Republic using CFT and IFAT, respectively, (Slosarkova *et al.*, 1999), 26.8% in Ghanaian goats using ELISA (Puije *et al.*, 2000), 37% in barren-ground goats from northern Canada using MAT (Kutz *et al.*, 2001), 12.3% in goats from Sardinia island, Italy using IFAT (Masala *et al.*, 2003) and 30.6% in Brazil using IFAT (Neto *et al.*, 2008).

Among domestic food animals, *T. gondii* is most pathogenic for goats (Dubey, 1987). The presence of *T. gondii* cysts in the tissues of naturally and experimentally infected goats have been reported (McSporran *et al.*, 1985, Dubey, 1989; Engeland *et al.*, 1996 and Nishi *et al.*, 2001). *Toxoplasma gondii* has been detected from soft tissues such as brain, cardiac muscle, skeletal muscle, small intestine, liver, lungs, lymph node and diaphragm. The microscopical lesions were seen in tissues of infected goats consisted of multifocal necrotic areas in most of tissues, acute enteritis, necrosis of mesenteric lymph nodes and encephalomyelitis, pancreatic alterations, glomerulonephritis, interstitial pneumonia, necrotic placental lesions and infiltration of mononuclear inflammatory cells as diffuse infiltrations with round cells in the liver, brain and heart in addition of occasional groups of numerous *T. gondii* tachyzoites and sometimes tissue cysts. (Mehdi *et al.*, 1983, Dubey, 1989, Bari. *et al.*, 1993 and Pereira-Bueno *et al.*, 2004).

In view of the economic importance of goats in Egypt and their potential role in the zoonotic transmission of toxoplasmosis therefore, the main objectives of this study were to detect the Seroprevalence of antibodies to *T. gondii* in the domestic Egyptian goats and determine the distribution of the infective stages of the parasite and its histo-pathological effect on the goat tissues and thus to help assess their potential as reservoirs of infection.

## Materials and Methods

### *Blood samples*

A total of 230 blood samples of goats from different localities of the Giza province, Egypt were collected at the time of slaughtering. Sera were separated, labeled and kept at -20°C until use.

### *Serological assay*

The Modified Agglutination Test (MAT) was performed for the detection of anti-*T. gondii* antibodies in goat sera considering 1:25 dilution as the cut-off point. The formalized killed whole tachyzoites antigen was prepared using RH strain of *T. gondii* as described by Desmonts and Remington (1980). The procedures were carried out according to the method of Dubey and Desmonts (1987).

### *Experimental infection*

#### *Goats*

Three local Egyptian-breed goats of age about 12 to 18 month-old were used, they were kept under good hygienic conditions and fed with already prepared dry ration and provided with clean water. All goats were proved sero-negative for *T. gondii*-specific IgG by using MAT before the experimental infection and their feces were free from coccidian oocysts.

#### *Procedures of infection*

The three goats used in this experiment classified into one control remained as uninfected and the other two as experimental tests. The experimental infection with the *T. gondii* strains was done as the procedures described by Dubey and Beattie (1988), as follows, the 1<sup>st</sup> experimental goat was orally inoculation with  $5 \times 10^3$  sporulated oocysts of a *T. gondii* locally isolated strain (Shaapan and Ghazy, 2007). The 2<sup>nd</sup> was subcutaneously injected with  $3 \times 10^6$  tachyzoites of *T. gondii* RH strain freshly obtained from colony maintained in our laboratory through serial passage in mice.

### *Histopathological examination*

120 days post infection, all experimentally infected and non-infected goats were slaughtered. Portions from heart, tongue, diaphragm, and thigh muscles were individually collected as described by Anderson *et al.* (1994). Tissue specimens about 0.5 cm<sup>3</sup> were obtained from each sample, fixed in 10 % neutral buffered formal saline solution, sectioned and further processed according to conventional routine histological technique as described by Culling (1974), including cutting at 5  $\mu$ m and staining with haematoxylin and eosin (H & E) or periodic acid-Schiff (PAS) reagent. The stained tissue sections were examined by optical microscope at power magnification of x400 and x1000 and the protozoal associated lesions, pathological alterations and the *T. gondii* tissue cysts were scored.

## Results

### *Prevalence of anti- T. gondii antibodies in goats*

Examination of a total of 230 serum samples of slaughtered goats by MAT revealed that 102 had antibodies against *T. gondii*, resulting in a seroprevalence of 44.3 % in titers of 1: 25 using RH *T. gondii* as antigen.

### *Histopathological examination of the experimentally infected goat tissues*

#### *Heart (Fig.1)*

Grossly, the heart of experimentally infected goat showed edematous pericardium, severe dilatation and congestion of cardiac blood vessels. Microscopical examination of paraffin embedded sections stained with H & E revealed the presence of myocytes and multi-focal interstitial myocarditis with infiltration of plasma cells, lymphocytes, macrophages and few neutrophils. Free tachyzoites were seen in cardiac myocytes bordering the lesions and among inflammatory cells. Large numbers of *T. gondii* tissue cysts were present in between the muscle fibers often without inflammatory response. The tissue cysts were thin-walled ( $> 0.5 \mu\text{m}$ ), rounded or elongated and large in size (up to  $100 \mu\text{m}$ ) enclosing PAS-positive bradyzoites ( $3 \times 9 \mu\text{m}$ ). Infiltration of mononuclear cells among myocytes and foci of extravasated erythrocytes between the bundles of myofibers were easily recognized, in addition to scattered infiltration of eosinophils singly or in small clusters. Focal areas of mild degenerative muscular changes in the form of hyaline degeneration were also seen.

#### *Tongue (Fig. 2)*

The histopathological examination of H&E stained sections of tongue root revealed the presence of large number of *T. gondii* tissue cysts in the muscular and sub epithelial layers of the tongue, which characterized by thin wall, rounded or elongated shape cysts containing several numbers of terminal-nucleated bradyzoites. The tissue cysts measured ( $15 \mu\text{m}$ ) wide to ( $35 \mu\text{m}$ ) long. The minor inflammatory foci consisting of mononuclear cell infiltration particularly of eosinophils were seen between tongue muscle fibers, but squamous epithelial and glandular layers were free from inflammatory cells. Glossal myositis accompanied by necrosis in some areas of section was also observed.

#### *Diaphragm (Fig. 3)*

The histopathological findings in the diaphragm were expressed by the presence of large number of oval shaped *T. gondii* tissue cysts in between the bundles of the diaphragmatic muscles together with heavy infiltration of eosinophils. Also these lesions associated with multiple lympho-histocytic infiltrations of mononuclear inflammatory cells such as macrophages and neutrophils. Mild degenerative muscular changes in the form of hyaline detected between muscle fibers. The degenerative changes were also seen in fibrous layer close to muscular tissues.

*Skeletal muscles (Fig. 4)*

The histopathological changes of the skeletal muscles (thigh muscles) showed few numbers of *T. gondii* tissue cysts with a thin cyst wall and contained bradyzoites of terminal nuclei. Scattered eosinophils singly or in small clusters and many foci of extravasated erythrocytes were seen between bundles of muscles. In addition mild infiltrates of mononuclear inflammatory cells between the muscle fibers were observed surrounding focal areas of degenerative muscular changes.



Fig. 1. Heart muscles.

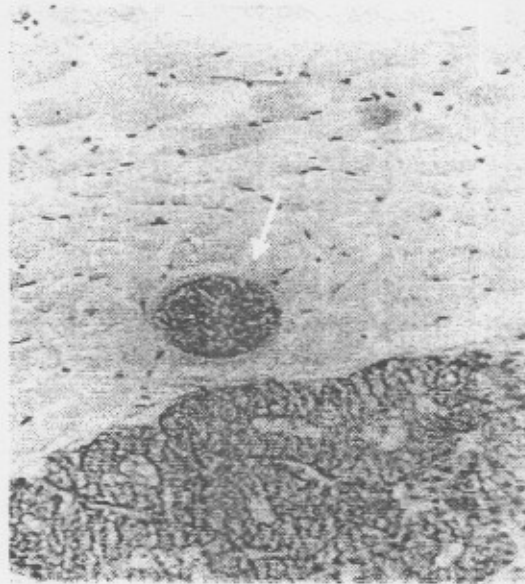


Fig. 2. Tongue muscles.

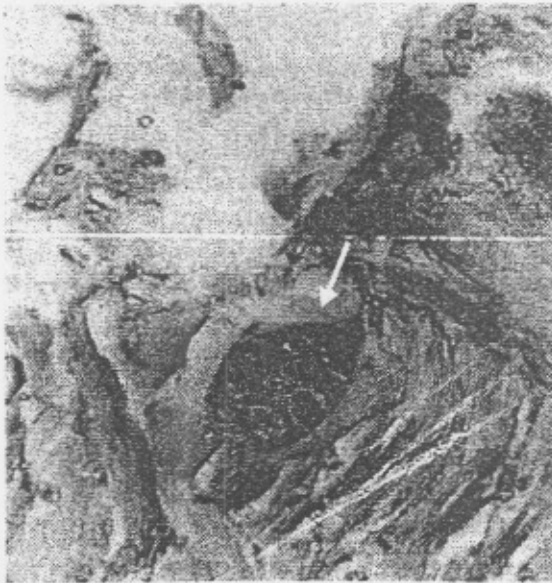


Fig. 3. Diaphragmatic muscles

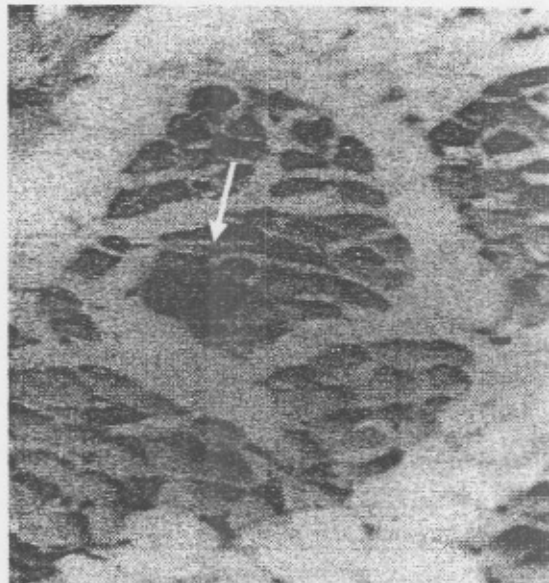


Fig. 4. Thigh muscles.

Focal mononuclear cell infiltrations and *T. gondii* tissue cyst (arrow) in the Heart muscles (Fig. 1), Tongue (Fig. 2), Diaphragmatic muscles (Fig. 3) and thigh muscles (Fig. 4) of the experimentally infected goats 120 DPI X400, stained with H& E.

## Discussion

Toxoplasmosis is an important zoonosis in humans because of the severe problems experienced in immune-suppressed persons (Holliman, 1988). The high prevalence of *T. gondii* antibodies using MAT with RH strain of *T. gondii* as antigens (44.32) % found in the goats of the our investigated study was nearly similar to 40%, 39% and 37% obtained, respectively, by Ibrahim *et al.* (1997) using IFAT, Kandil and Abou-Zeina (2000) using ELISA and Kutz *et al.* (2001) using MAT. Meanwhile lower incidence rates (12.3%, 19.6% and 26.8%) were recorded, respectively, by Masala *et al.* (2003) in Italy using IFAT, Morsy *et al.* (1979) in Egypt using DT and Puije *et al.* (2000) in Ghana using ELISA. However higher incidence rates (54%, 65% and 66%) were reported, respectively, by Fahmy *et al.* (1979) in Egypt using DT, Patton *et al.* (1990) in USA using MAT and Slosarkova *et al.* (1999) in Czech Republic using IFAT.

The differences in seroprevalence rates of *T. gondii* in goats obtained during the present study and those previously reported investigations are probably due the characteristics of the sampled animals; higher or lower degrees of feline presence in different locations shedding oocysts and/or different cut-off points in the serologic test used: initial serum dilution; the virulence and type of *T. gondii* strains which used in the Ag preparation; the immune status, age, climatic conditions and management investigated animals in different localities (Dubey & Beattie, 1988 and Bisson *et al.*, 2000).

The modified agglutination test (MAT) is the major recommended test for diagnose the *T. gondii* infection in several animals and man (Dubey & Adams, 1990 and Garcia *et al.*, 1999). Also Dubey (1997), found that MAT has the highest sensitivity among all serological tests and also the results obtained by Shaapan *et al.* (2008), demonstrated the benefits of using more sensitive and specific MAT for the detection of *T. gondii* antibodies in sheep sera which is cheaper, easier than other tests and does not need special sophisticated equipment.

The high prevalence (44.32%) of *T. gondii* in the goats of this study suggests that the oocysts and the reservoirs are widely dispersed in the environment and thus also represent a risk to human health. Also cooking methods are generally adequate for destroying the infectious stage of the organism in tissues, another possible source of infection for humans seems to be raw goat milk the presence of cats and the close contact with this species is important in the epidemiology of the disease (Jittapalapong *et al.*, 2005 and Neto *et al.*, 20008). This finding indicates that further studies should be performed with regard to *T. gondii* infections in cats from Egypt.

Lesions found by the gross and microscopical examination of the different tissues and organs of the experimentally infected goats in our study found were consistent to the ones demonstrated by other authors who studied lesions in animals and man (Morales *et al.*, 1996, Esteban-Redondo *et al.*, 1999 and Hill &

Dubey, 2002). Tissues harboring *T. gondii* tissue cysts in this study most frequently were the heart and tongue muscles, but organisms were detected less frequently in the diaphragmatic and thigh muscles, this in agreement with results obtained by Prickett *et al.* (1985) and Silva and Langoni (2001).

### Conclusion

This study is the first report that revealed the high prevalence of anti-*T. gondii* specific antibodies in Egyptian goats and confirm the presence infective stages of the parasite in their organs tissues. So it will be necessary to conduct further studies to determine the impact of toxoplasmosis on the animal industry, and the potential risk of its transmission to humans through consumption of meat contaminated with tissue cysts of *T. gondii*.

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## احداث عدوى بالتكسوبلازما جوندای فى الماعز

رأفت محمد شعبان ، محيى الدين عبد الحفيظ حسنين ، نوال عبد الحفيظ حسنين  
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القاهرة - مصر .

تم تقدير معدل انتشار الأجسام المضادة لطفيل التكسوبلازما جوندای فى الماعز المذبوحة للأكل وذلك بتجميع ٢٣٠ عينة دم من الماعز المذبوح من أماكن مختلفة فى محافظة الجيزة بجمهورية مصر العربية وتم عمل التشخيص السيرولوجى باستخدام اختبار التلازن المعدل (MAT) وكانت نسبة وجود الأجسام المضادة لطفيل هي ٤٤.٣ ٪. وتم اجراء تجربة عدوى الماعز بالأطوار المعدية للعترات المعزولة محليا وال RH ؛ وقد لوحظ تواجد الحويصلات النسيجية والاصابات فى الأعضاء والأنسجة المختلفة للماعز تحت الدراسة. وتعتبر هذه الدراسة هي الأولى لمرض التكسوبلازما فى الماعز المعدة تجريبيا بواسطة الفحص المرضى النسيجي ؛ وعلى ذلك فان النتائج المتحصل عليها تلقى الضوء على أهمية الصحة العامة للحم الماعز كمصدر لعدوى الانسان.