Seroprevalence and preliminary treatment of toxoplasmosis of pregnant goats in Kalubia Governorate, Egypt

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ABSTRACT

Toxoplasmosis is a zoonotic disease that commonly infects goat in Egypt. For its diagnosis, sera form 48 pregnant does were tested for *Toxoplasma gondii* antibodies through indirect haemagglutination test (IHAT) and modified agglutination test (MAT). Samples were collected between the periods extended from November 2006 to March 2007. The seropositive rate of pregnant does (Meet Kenana, Kalubia Governorate, Egypt) was 35.42% (17/48). After using MAT test against *T. gondii*- IgM, 11 sera (22.9%) were found positive. The older animals were more frequently infected (50%) than the younger goats (26.3%). Higher prevalence in aged does was confirmed by MAT. Total *T.gondii* antibodies and IgM were gradually increased with the development of pregnancy. Two months post treatment with sulfademidine 33.3 %, for 5 successive days at a dose of 200 mg/kg, resulted in not only reduction of *T.gondii* total antibodies and IgM in all treated does, but re-modulation of *T.gondii* antibody titres moderately toward the mild titres, and prevention of congenital infection and abortion as well. Whereas IgM was not recorded in treated does, it was detected in the untreated ones. No abortion was recorded in all treated does, but an untreated doe delivered two dead fetuses. The delivered kids belonged to treated does were seropositive for *T.gondii* antibodies by using IHAT, yet they were seronegative for *T.gondii*– IgM by using MAT. In contrast, kids belonged to untreated does were seropositive by using both IHAT and MAT. Adequate management might be useful and essential to control toxoplasmosis in the goat herds of Kalubia Governorate.

Key words: *Toxoplasma gondii*, goat, pregnancy, treatment, Egypt.
INTRODUCTION

Goats are important popular food animals in Egypt. They are farmed for their minimal cost of rearing in addition to rapid and good production of flavored meat. Toxoplasmosis is a wide spread zoonosis caused by the coccidian protozoan Toxoplasma gondii [5,12], and it is a common infection of goat world wide [3,7,12,17,22,30,34,38]. It is recognized as one of the major causes of infectious reproductive failure [3,30,31].

Concerning public health importance, T. gondii infects about 30-60% of people worldwide. Not only meat but also milk from infected goat is a potential source for infection [31,40]. Moreover, goat’s milk is now being introduced as an alternative dairy food for infants allergic to cow’s milk [31]. Toxoplasmosis causes mental retardation and loss of vision in children [24]. Latent toxoplasmosis is known to induce behavioral changes because local inflammation in the brain increases dopamine, which could lead to schizophrenia [39].

Diagnosis of T. gondii depends mainly on serological tests. Indirect haemagglutination test (IHA T) is fast, accurate, and not expensive [2,20,30]. Intraperitoneal inoculation of bioassay of lymph node, liver, or spleen into mice is useful and accurate as is culture of parasite in fibroblast cells [36].

Although very few epidemiological studies concerning caprine toxoplasmosis in Egypt were available [15,17,21], none of them dealt with toxoplasmosis in pregnant does and its treatment.

The main objective of the present study was to determine, for the first time, the burden of T.gondii infection in pregnant does in Kalubyia Governorate, Egypt, along with its treatment at mid pregnancy by sulfadimidine 33.3%.

MATERIALS AND METHODS

Serum samples

Forty-eight blood samples were collected from 48 pregnant balady goats, belonged to private farms in Meet Kenana, Tokh, Kalubyia Governorate, Egypt. The animals were at various stages of pregnancy. Does were examined for toxoplasmosis during the period extended from November 2006 to March 2007. Concerning examination of newly born kids, blood samples were taken from their jugular veins.

Serological detection of T.gondii

The frozen serum samples were thawed and used for serologic indirect haemagglutination test (IHAT). The IHAT was performed by using commercial kits1, following the manufacture instruction.

Sera were diluted double folds from 1: 32 to 1: 512. The titre 1:32 was considered as a negative result, while the titre 1: 64 was considered as a positive titre. Titres from 1:128 to 1: 512 were suggested to be the strongest response to infection [14,17,34],

Detection of T. gondii IgM was carried out by modified agglutination test (MAT) through addition of 2 mercapto-ethanolamine [1,42].

Chemotherapy

Six pregnant does, at mid pregnancy, expressing the strongest T.gondii antibody titres, were divided into two groups (each group consisted of 3 animals). The first group (Gr I) was injected, slowly intravenous, with sulfadimidine 33.3%2 for 5 successive days (200 mg/ kg b.wt). After that, animals in the second group (Gr II) were injected with normal saline and considered as a control group. Throughout the studying period, all animals were kept indoors, received balanced ration and clean source of water, and protected from cats. Blood samples were taken from animals two months posttreatment, and serum samples were separated and stored at -20 ºC until they were used for serological studies [32].

Animal inoculations

Regarding laboratory animal inoculations, samples from brain, spleen, liver, and lymph nodes from stillborn kids were homogenized in 0.9% NaCl aqueous solution. The resulting suspension was allowed to settle for 2 hrs at laboratory temperature. Then, the supernatant was inoculated intraperitoneally into 10 adult mice. Afterwards, the peritoneal exudates were aspirated by sterile syringes after 10, 15, and 20 days postinoculation, and slide smears were stained by Giemsa stain to detect T.gondii tachyzoites [19].

RESULTS

The prevalence of T.gondii in pregnant does (Table 1) indicated that 17 samples (35.42%) out of 48 were seropositive for T. gondii by using IHAT, while IgM were detected only in 11(22.9%) samples by using MAT. Serodagnosis showed that goats were infected with T. gondii with acquisition of age. Older goats (50%) were more likely to be seropositive than younger goats (26.3 %). Such result was confirmed by MAT, as 37.3% of elder does were seropositive versus 15.9 % of younger does. Also, abortion occurred only in an aged doe.
Seroprevalence of *T. gondii* during different stages of pregnancy in goats (Table 2), revealed that the total *T. gondii* antibodies and IgM were gradually increased with the development of pregnancy. In early pregnancy (from conception to 50th days post conception), the higher IHA titres 1:128 and 1:256 were recorded in 2 samples (15.4%), and IgM was recorded in an infected doe (7.7%). During mid pregnancy (from 51st to 120th day post conception), higher IHA titres (1:128 and 1:256) were recorded in 6 samples (24%), and IgM was recorded in 5 samples (20%). Comparatively higher prevalences of IHA and IgM titres, (1:128, 1:256, and 1:512) were recorded in 5 samples (50 %) in late pregnancy (from 121st day till delivery).

Treatment with sulfademidine 33.3 %, contributed to reduction of *T. gondii* antibodies in all treated does (Table 3). No abortion was recorded in the treated group, whereas an untreated doe delivered two still-borns. All delivered kids belonged to treated does were seropositive for *T. gondii* antibodies by using IHA only. In contrast, the delivered kids related to untreated does were seropositive for *T. gondii* infection when tested by both IHAT and MAT. Diagnosis of toxoplasmosis also done by laboratory animal inoculation, and then tachyzoites were detected in Giemsa stained peritoneal exudates from all experimentally inoculated mice (Figure 1).

**DISCUSSION**

Goat is one of the most important animals for meat and milk production [34] which could be turned into potential sources of human toxoplasmosis. Cysts, frequently found in the brain and skeletal muscles, may remain for the remainder of the life of goat. In contrast, cattle and deer may eventually become clear of infection [5].

Toxoplasmosis not only lacks specific clinical signs or syndromes, but also can be transmitted from a recently infected mother to their fetus [29,33]. Therefore, application of laboratory diagnostic techniques is crucial for proper clinical management of pregnant does.

**Table 1.** Seroprevalence of *Toxoplasma gondii* at different periods of pregnant goats, from November 2006 to March 2007, in Meet Kenana, Kalubiyia Governorate, Egypt.

<table>
<thead>
<tr>
<th>Age</th>
<th>Examined samples (N)</th>
<th>Positive IHA</th>
<th>Positive MAT</th>
<th>Aborted does</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>N</td>
<td>%</td>
<td>N</td>
</tr>
<tr>
<td>12-16 months</td>
<td>19</td>
<td>5</td>
<td>26.3</td>
<td>3</td>
</tr>
<tr>
<td>17- 36 months</td>
<td>21</td>
<td>8</td>
<td>30.1</td>
<td>5</td>
</tr>
<tr>
<td>Over 36 months</td>
<td>8</td>
<td>4</td>
<td>50</td>
<td>3</td>
</tr>
<tr>
<td>Total</td>
<td>48</td>
<td>17</td>
<td>35.42</td>
<td>11</td>
</tr>
</tbody>
</table>

IHAT: indirect haemagglutination test. MAT: modified agglutination test.

**Table 2.** Seroprevalence of *Toxoplasma gondii* during different stages of goat’s pregnancy, during the period from November 2006 to March 2007, in Meet Kenana, Kalubiyia Governorate, Egypt.

<table>
<thead>
<tr>
<th>Titres</th>
<th>Early pregnancy (N=13)</th>
<th>Mid pregnancy (N=25)</th>
<th>Late pregnancy (N=10)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IHAT</td>
<td>MAT</td>
<td>IHAT</td>
</tr>
<tr>
<td>1 : 32</td>
<td>9 (69.2 %)</td>
<td>0</td>
<td>17 (68%)</td>
</tr>
<tr>
<td>1 : 64</td>
<td>2 (15.4 %)</td>
<td>0</td>
<td>2 (8%)</td>
</tr>
<tr>
<td>1 : 128</td>
<td>2 (15.4 %)</td>
<td>1 (7.7%)</td>
<td>3 (12%)</td>
</tr>
<tr>
<td>1 : 256</td>
<td>0</td>
<td>0</td>
<td>3 (12%)</td>
</tr>
<tr>
<td>1 : 512</td>
<td>0</td>
<td>0</td>
<td>None</td>
</tr>
<tr>
<td>Total (positive)</td>
<td>4 (30.77%)</td>
<td>1 (7.7%)</td>
<td>8 (32%)</td>
</tr>
</tbody>
</table>

IHAT: indirect haemagglutination test. MAT: modified agglutination test.
Caprine toxoplasmosis was reported worldwide [3,7,12,22,30,38]. Even though very few studies have been conducted in Egypt [15,17,21], nothing is known about treatment of pregnant does and the role of *T. gondii* in caprine abortion.

Our data indicated that 35.42% of the examined pregnant goats in Kalubia Governorate were infected and developed *T. gondii* antibody titres without clinical signs. Pyretic period in goats after experimental infection was recorded [10]. Nevertheless, such period was not observed by the goat farmers during natural infection.

The high prevalence of *T. gondii* infection indicated continuous exposure of goats to infection due to heavy environmental contamination with oocysts shed form the observed stray cats in the farms. Similar observation was also reported in Italy [30].

The Seroprevalence result was in accordance with another Egyptian study which recorded 18.75%, 28.12%, and 31.25% caprine toxoplasmosis by using

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**Table 3.** Effect of chemotherapy on *Toxoplasma gondii* antibodies in does and their fetuses, during the period from November 2006 to March 2007, in Meet Kenana, Kalubia Governorate, Egypt.

<table>
<thead>
<tr>
<th>Gr</th>
<th>State</th>
<th>IHAT (N= 6) B</th>
<th>IHAT (N= 6) A</th>
<th>MAT(N= 6) B</th>
<th>MAT(N= 6) A</th>
<th>Dead fetuses</th>
<th>Live fetuses (N = 7)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>B</td>
<td>A</td>
<td>B</td>
<td>A</td>
<td>None</td>
<td>1 : 32</td>
</tr>
<tr>
<td>2 years (treated)</td>
<td>1 : 128 1 : 64</td>
<td>+ve</td>
<td>-ve</td>
<td>None</td>
<td></td>
<td>1 : 32</td>
<td></td>
</tr>
<tr>
<td>3 years (treated)</td>
<td>1 : 256 1 : 128</td>
<td>+ve</td>
<td>-ve</td>
<td>None</td>
<td></td>
<td>1 : 32</td>
<td></td>
</tr>
<tr>
<td>2 years (treated)</td>
<td>1 : 256 1 : 128</td>
<td>+ve</td>
<td>-ve</td>
<td>None</td>
<td></td>
<td>2 : 32</td>
<td></td>
</tr>
<tr>
<td>2 years (untreated)</td>
<td>1 : 128 1 : 256</td>
<td>+ve</td>
<td>+ve</td>
<td>Non</td>
<td></td>
<td>1 : 128</td>
<td></td>
</tr>
<tr>
<td>3 years (untreated)</td>
<td>1 : 256 1 : 512</td>
<td>+ve</td>
<td>+ve</td>
<td>None</td>
<td></td>
<td>2 : 128</td>
<td></td>
</tr>
<tr>
<td>3 years (untreated)</td>
<td>1 : 256 1 : 512</td>
<td>+ve</td>
<td>+ve</td>
<td>2 (33.3%)</td>
<td></td>
<td>-</td>
<td></td>
</tr>
</tbody>
</table>

Gr = Group; B= before treatment; A= 2 months after treatment. IHAT: indirect haemagglutination test. MAT: modified agglutination test. +ve: positive result; -ve: negative result.

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**Figure 1.** Tackyzoites (arrow head) in Giemsa stained peritoneal exudates from experimentally inoculated mice (X 1000).
The extent of toxoplasmosis in goat populations varied widely from flock to flock around the world. Relatively similar results were recorded, 31% in Uganda [3], 30% in Botswana [38], 28.93% in Brazil (Bahia State) [34], 23% in Switzerland [7], and 27.9% in Thailand [22].

Unlike our result, lower prevalences were recorded, 12.9% in Bangladesh [37], 12.3% in Italy [30], 17% in Brazil (São Paulo State) [31], and 19.25% in Iran [19].

Such differences of the prevalences of caprine toxoplasmosis may be attributed to the diagnostic techniques or demographics of goat populations, breeding condition and management [30], geographic structure of *T. gondii* in relation to genetic variation [24], immune status, timing of infection, and genetic composition of the host and the organism [41], or distribution and behavior of cats [5].

In relation to age susceptibility, samples of old goats showed higher proportions of positive reactions. Such result may be attributed to prolonged exposure of elderly goats to infective oocysts throughout their life.

In the present study, we confirmed IHA T by application of MAT because IgM antibodies may fail to give information in the early diagnosis of congenital toxoplasmosis [8]. Antibodies are detectable 2 weeks post infection [26,28] and maintain a low level through the host’s life [27]. Accordingly, testing for IgM antibodies which appear about 1 week post infection permitted detection of recent infection of *T. gondii* [23]. IgM was detected by MAT in 10 (12.34%) out of 48 samples. This result also agreed with a similar study, for IgM was detected in a lower percentage (5.6%) among goats compared to 12.3% by IHA T in Italy [30]. Such values of IgM indicate true infection with *T. gondii* [6].

With regard to treatment of toxoplasmosis in pregnant does with sulfademidine 33.3%, it re-modulated titres of *T. gondii* antibody slightly toward the mild titre, after two months post treatment. Moreover, no abortion occurred in the treated group, whereas untreated group showed higher titres, and 2 dead kids were delivered by an untreated doe. Our results conformed with a similar study which revealed that treatment of a sporadic doe by sulfonamide decreased *T. gondii* antibodies and gave birth to 2 healthy kids with normal *Toxoplasma* titres, 1: 16 and 1: 64 [32]. Moreover, pyrimethamine and sulfonamides given together were widely used against *T. gondii* [25,36].

The detected IgM in apparently healthy kids belonged to untreated does indicated congenital toxoplasmosis, such result was in agreement with that reported by several authors [29,33].

As a consequence of toxoplasmosis, an untreated doe (33.3%) delivered 2 stillborn kids. Abortion may occur at any time from 9 days after goats have been inoculated by *T. gondii* due to focal necrosis with mineral deposits in the cotyledons [10]. In such respect, several authors explained what could happen after infection of a pregnant doe for the first time: parasitemia occur during the first week, and the placenta becomes infected in the second followed by the fetus in the third week. Therefore, toxoplasmosis may cause fetal death with subsequent resorption, abortion, mummification, stillbirth, or delivery of weak or normal kids [4,10,16]. Consequently, *T. gondii* is a major cause of reproductive failure, with significant economic losses to goat breeders [3,10,12,13,16,30,31].

Toxoplasmosis is a worldwide problem which indicated that *T. gondii* oocysts contaminate the environment due to presence of large number of infected stray cats [5,12]. Oocysts may be shed continuously in the cat’s feces from 4 until 14 days after infection, with an expected peak output of tens of millions at 6-8 days [11]. Thus, fifty grams of infected cat feces may contain as many as 10 million oocysts [11], which can remain after sporulation so for over a year [9].

Perhaps the most common source of infection is the contaminated pasture [5], and fields treated with manure and bedding from farm buildings where cats live [18]. Careless storage of farm feeds may also pose a risk [35]. Consequently, goats feed and water should be kept covered all times to prevent their contamination with cat’s feces.

**CONCLUSIONS**

The IHA T and MAT were practical tests for rapid diagnosis of asymptomatic caprine toxoplasmosis. Older goats were more likely to be seropositive than younger goats. Caprine toxoplasmosis could be transmitted congenitally and might cause abortion or delivery of dead kids along with great economic losses.
Treatment of infected does by sulfadimidine 33.3% for 5 successive days, resulted in re-modulation of *T. gondii* antibodies toward mild titres, as well as prevention of congenital infection and abortion. The present data recommend that prevention of toxoplasmosis could reduce its risk through confinement, proper management, and adequate hygiene measures.

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**REFERENCES**


**SOURCES AND MANUFACTURERS**

1. FAST ABC Diagnostic, Egypt.
2. Sulfadimidine sodium, Adwia Company, Egypt.

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