SEROPREVALENCE OF *TOXOPLASMA GONDII* INFECTION AMONG SHEEP AND GOATS IN THE STARA ZAGORA REGION

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Summary


By means of the indirect haemagglutination test (IHAT), blood serum antibody titres against *Toxoplasma gondii* were analyzed in 380 sheep and 364 goats originating from 10 settlements in the Stara Zagora region, Bulgaria. Positive samples (titres > 1:80) were investigated one more time after treatment with 2-mercaptoethanol to detect IgM antibodies. In sheep, 183 samples (48.2 %) were positive, with antibody titres ranging from 1:10 to 1:1280, whereas in goats seropositive samples were 218 (59.8 %) with titres between 1:10 and 1:2560. IgM antibodies were detected in blood sera from 5 sheep and 11 goats.

Key words: Bulgaria, epidemiology, indirect haemagglutination test, goats, seroprevalence, sheep, *Toxoplasma gondii*

INTRODUCTION

Toxoplasmosis is a widely prevalent zoonosis, caused by the facultative two-host protozoan *Toxoplasma gondii*. The definitive hosts of the parasite are domestic and wild cats (Frenkel *et al.*, 1970). Intermediate hosts (all mammals including man) are infected by ingestion of sporulated oocysts, cyst-contaminated meat, milk contaminated by tachyzoites or transplacentarily (Pepin *et al.*, 1997). Meat from *T. gondii*-infected pigs and sheep and goat milk are shown to be primary sources of infection for men (Smith, 1991; 1993). The results from an epidemiological study revealed a statistically significant correlation between seropositivity against *T. gondii* in humans and goat milk consumption (Chiari *et al.*, 1987). The infection with *T. gondii* is an important cause for abortions, delivery of dead or debilitated offspring (Dubey & Beattie, 1988).

In the diagnostics of toxoplasmosis in animals and men, a number of serological tests are used: indirect haemagglutination test (IHAT), indirect immunofluorescence assay test (IFAT), enzyme-linked immunosorbent assay (ELISA). Serology is widely used in epidemiological surveys, the detected prevalence being various in the different countries (Hashemi-Fesharki, 1996; Gondim *et al.*, 1999; Tenter *et al.*, 2000; Hove *et al.*, 2005).

The literature data about the prevalence of *T. gondii* infection among sheep and goats in Bulgaria are scarce. Angelov *et al.* (1956, 1958) detected for the first
Seroprevalence of Toxoplasma gondii infection among sheep and goats in the Stara Zagora region

time toxoplasmosis in men by means of serological and allergological tests. Arnaudov (1971, 1973) has investigated the prevalence of toxoplasmosis among domestic animals by complement binding reaction, passive haemagglutination test, agar gel microprecipitation test etc.

The lack of studies during the last 30−40 years in Bulgaria, the structural reorganization of animal husbandry, the increased risk of infection from consumption of goat milk and meat because of the increased goat population entails the necessity of introducing highly specific and sensitive tests for diagnosis of this zoonosis.

The purpose of the present study was to investigate the seroprevalence of Toxoplasma gondii infection among sheep and goats from different settlements in the Stara Zagora region.

MATERIALS AND METHODS

Animal blood sera

A total of 380 sheep originating from 10 farms and 364 goats from 10 settlements in the Stara Zagora region were tested. From each animal, individual blood samples were obtained (5 mL) from the jugular vein. After clotting, sera were centrifuged at 500×g for 10 min and stored at −20 °C.

Serological test

Blood sera were tested in the indirect haemagglutination test (IHAT). In the preparation of toxoplasmic erythrocytic diagnosticum, somatic antigens from T. gondii strain RH trophozoites and formalized and tanned chicken erythrocytes were used.

All positive samples that reacted with titres > 1:80 were tested once again after treatment with 2-mercaptoethanol (2-ME) to detect IgM antibodies (Camargo et al., 1978).

RESULTS

The results from the serological tests of 380 sheep blood samples are shown in Table 1.

Out of all 380 ovine blood samples, 183 have reacted positively (48.2%). This

<table>
<thead>
<tr>
<th>Farm</th>
<th>Samples, total number</th>
<th>Positive (%)</th>
<th>Antibody titres</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>1:10 1:20 1:40 1:80 1:160 1:320 1:1280</td>
</tr>
<tr>
<td>1</td>
<td>37</td>
<td>18 (48.6%)</td>
<td>3 4 8 2 1 – –</td>
</tr>
<tr>
<td>2</td>
<td>38</td>
<td>16 (42.1%)</td>
<td>– 2 6 5 2 1 –</td>
</tr>
<tr>
<td>3</td>
<td>42</td>
<td>27 (64.3%)</td>
<td>– 5 11 8 2 1 –</td>
</tr>
<tr>
<td>4</td>
<td>35</td>
<td>11 (31.4%)</td>
<td>1 3 5 2 – – –</td>
</tr>
<tr>
<td>5</td>
<td>36</td>
<td>21 (58.3%)</td>
<td>2 4 8 5 1 1 –</td>
</tr>
<tr>
<td>6</td>
<td>38</td>
<td>19 (50.0%)</td>
<td>– 1 7 7 3 1 –</td>
</tr>
<tr>
<td>7</td>
<td>40</td>
<td>24 (60.0%)</td>
<td>1 2 6 6 4 3 2</td>
</tr>
<tr>
<td>8</td>
<td>37</td>
<td>17 (45.9%)</td>
<td>1 3 5 4 2 2 –</td>
</tr>
<tr>
<td>9</td>
<td>39</td>
<td>9 (23.1%)</td>
<td>– 2 3 2 2 – –</td>
</tr>
<tr>
<td>10</td>
<td>38</td>
<td>21 (55.3%)</td>
<td>2 5 8 5 1 – –</td>
</tr>
<tr>
<td>Total</td>
<td>380</td>
<td>183 (48.2%)</td>
<td>10 31 67 46 18 9 2</td>
</tr>
</tbody>
</table>

Table 1. Seroprevalence of Toxoplasma gondii in sheep using IHAT
percentage was the highest in farms No 3 and 7, 64.3% and 60.0% respectively. The performed serological test demonstrated antibody titres from 1:10 to 1:1280. The most prevalent titres in all 183 positive samples were 1:40 (67 samples).

The repeated tests of 75 positive samples with antibody titres > 1:80 with 2-ME detected IgM antibodies only in 5 samples from farms No 3 and 7.

The data from the serological tests of 364 caprine blood samples are presented in Table 2. In 218 (59.8%), positive titres against T. gondii antibodies were detected. Antibody titres of 1:40 were present in 23 samples, 1:80 – in 37, 1:160 – in 43, 1:320 – in 37 sera. Low titres (1:10 to 1:20) were observed in only 16 samples. The considerable number of sera with high titres: 33 with 1:1280 and 29 with 1:2560 should be noted.

The repeated analysis of 179 positive samples with titres higher than 1:80, showed IgM antibodies in 11 samples.

DISCUSSION

The performed serological survey exhibited a higher prevalence of T. gondii among sheep and goats in the region of Stara Zagora, 48.2% and 59.8%, respectively. Seropositive sheep and goats were detected in all investigated farms and settlements.

Smith (1991) reported a different prevalence of toxoplasmosis among countries. According to Dubey & Beattie (1988) and Tenter et al. (2000), T. gondii infection is widely distributed at a worldwide scale, with incidences from 0% to 100% in the different countries.

The 48.2% prevalence of toxoplasmosis observed in this study is higher than that reported in Turkey – 31% and 34.6% (Oncel & Vural, 2006; Tutuncu et al., 2003), Greece – 23% (Stefanakes et al., 1995) and Morocco – 27.6% (Sawadogo et al., 2005) but lower than that found out in Canada – 57% (Waltner-Toews et al., 1991), 53.65% in Poland (Görecki et al., 2005), 55.66% in Turkey (Sevgili et al., 2005) and 49.9% in Sicily (Vesco et al., 2007).

The observed T. gondii seroprevalence among goats in the region of Stara Zagora was lower that percentages reported in Brazil – 92.4% (Gondim et al., 1999) and the Canaries – 63.3% (Rodríguez-Ponce et al., 1995), and higher that the respective rates of 31% in Uganda (Bisson et al., 2000), 19.3% in Iran (Hashemi-Fesharki, 1996) and Venezuela – 5.9% and 33% (Nieto & Melendez, 1998; Figueiredo et al., 2001).

These variations could be attributed to the different geographic areas, but the results show convincingly that toxoplasmosis is a widely prevalent protozoosis and that a considerable part of animals were in contact with the infective agent.

Our data from the IHAT in blood sera of sheep and goats are different from those of Arnaudov (1971, 1973). This author observed that 32.65% of studied sheep and 27.16% of goats were positive for toxoplasmosis. Our percentages of seropositivity were higher, 48.2% and 59.8%, respectively. The differences are probably due to the long period of time between both studies (about 35 years), the structural reorganization in animal husbandry practices and the use of chicken erythrocytes in the diagnosticum prepared by us.

The high seroprevalence of T. gondii among sheep and goats could be attributed to cats residing in farms, probably young animals shedding oocysts (Weilland & Dalchow, 1970; Dubey, 1994). Infected cats shed T. gondii oocysts that after spo-
Table 2. Seroprevalence of *Toxoplasma gondii* in goats using IHAT

<table>
<thead>
<tr>
<th>No</th>
<th>Settlement</th>
<th>Samples, total number</th>
<th>Positive (%)</th>
<th>Antibody titres</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1:10</td>
</tr>
<tr>
<td>1</td>
<td>Pavel banya</td>
<td>37</td>
<td>26 (70.2)</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>Enina</td>
<td>40</td>
<td>29 (72.5)</td>
<td>2</td>
</tr>
<tr>
<td>3</td>
<td>Vetren</td>
<td>37</td>
<td>24 (64.8)</td>
<td>–</td>
</tr>
<tr>
<td>4</td>
<td>Maglizh</td>
<td>45</td>
<td>31 (68.8)</td>
<td>–</td>
</tr>
<tr>
<td>5</td>
<td>Rozovo</td>
<td>33</td>
<td>19 (57.5)</td>
<td>–</td>
</tr>
<tr>
<td>6</td>
<td>Zagore</td>
<td>35</td>
<td>15 (42.8)</td>
<td>1</td>
</tr>
<tr>
<td>7</td>
<td>Kaloyanovets</td>
<td>40</td>
<td>21 (52.5)</td>
<td>2</td>
</tr>
<tr>
<td>8</td>
<td>Preslaven</td>
<td>30</td>
<td>18 (60.0)</td>
<td>2</td>
</tr>
<tr>
<td>9</td>
<td>Samevo</td>
<td>38</td>
<td>23 (60.5)</td>
<td>–</td>
</tr>
<tr>
<td>10</td>
<td>Tihomirovo</td>
<td>29</td>
<td>12 (41.3)</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td><strong>Total</strong></td>
<td><strong>364</strong></td>
<td><strong>218 (59.8)</strong></td>
<td><strong>8</strong></td>
</tr>
</tbody>
</table>
The presence of IgM-positive antibodies in some samples exhibited a recent or active infection. Such animals could be an important source of transmission of the infection to men, as in the acute stage of the disease they are shedding *T. gondii* tachyzoites in all body fluids, including milk. Similar views have expressed Chiari & Neves (1984) and Dubey (1994). Chiari & Neves (1984) proved the release of tachyzoites in the milk of naturally infected goats.

Toxoplasmosis in goats is more extensively studied because of its importance for human health, as the consumption of goat milk is recommended to children with allergy to cow milk.

The results of the present investigation showed the presence of antibodies against *T. gondii* in the tested sheep and goat sera in different settlement of the Stara Zagora region, Bulgaria. Toxoplasmosis could be one of the causes for reproductive disorders in sheep and goats in these areas. The observed high seroprevalence of *T. gondii* in sheep and goats is an evidence for environmental contamination with infective oocysts. The only definitive hosts and transmitters of toxoplasmosis in the environment are the representatives of the Felidae family. In a study, Kostova et al. (1999) did not detect *T. gondii* oocysts in any of tested 120 domestic cats, so it could be assumed that semi-domesticated and synanthropic cats, as well as wild felids play a more essential role in the epidemiology of toxoplasmosis rather than domestic cats.

Our data showed that the performance of serological surveys using adequate method to detect the *T. gondii* prevalence among sheep and goats was useful, as it would permit to take adequate measures to control the infection in the farms. In case of abortions, *T. gondii* should be considered as one of the possible agents.

The studies on toxoplasmosis are important with regard to the fact that being a zoonosis, infected animals are a potential source of infection to people.
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