

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

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Summary

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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

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 formalinized and tanninized 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 1. Seroprevalence of *Toxoplasma gondii* in sheep using IHAT

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

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 и 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 world-wide 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 (Górecki *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 than percentages reported in Brazil – 92.4% (Gondim *et al.*, 1999) and the Canaries – 63.3% (Rodriguez-Ponce *et al.*, 1995), and higher than 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 (Weiland & Dalchow, 1970; Dubey, 1994). Infected cats shed *T. gondii* oocysts that after spo-

Table 2. Seroprevalence of *Toxoplasma gondii* in goats using IHAT

No	Settlement	Samples, total number	Positive (%)	Antibody titres									
				1:10	1:20	1:40	1:80	1:160	1:320	1:1280	1:2560		
1	Pavel banya	37	26 (70.2)	1	3	5	4	5	4	4	2	2	2
2	Enina	40	29 (72.5)	2	1	6	5	5	4	4	3	3	3
3	Vetren	37	24 (64.8)	-	-	3	3	6	5	5	5	2	2
4	Maglzh	45	31 (68.8)	-	-	2	2	5	5	5	10	7	7
5	Rozovo	33	19 (57.5)	-	-	-	1	2	4	4	2	10	10
6	Zagore	35	15 (42.8)	1	-	-	6	2	3	3	2	1	1
7	Kaloyanovets	40	21 (52.5)	2	1	3	5	5	4	4	1	-	-
8	Preslaven	30	18 (60.0)	2	2	3	3	4	2	2	2	-	-
9	Samevo	38	23 (60.5)	-	-	-	5	5	3	3	6	4	4
10	Tihomirovo	29	12 (41.3)	-	1	1	3	4	3	3	-	-	-
Total		364	218 (59.8)	8	8	23	37	43	37	37	33	29	29

ulation, become infective for men and animals, preserving this capability for up to 18 months in the environment (Gorbani *et al.*, 1983).

Comparing the observed results in sheep and goats, it should be emphasized that both the seroprevalence rates and antibody titres were higher in goats compared to sheep. In the latter, the highest numbers of animals (67 and 46) reacted with titres of 1:40 and 1:80 respectively. Titres of 1:320 and 1:1280 were detected in single animals only.

In goats, antibody titres were more elevated – 43 of seropositive goats exhibited titres of 1:160 and 37, 33 and 29 – titres of 1:320, 1:1280 and 1:2560, respectively.

The higher antibody titres could be explained with an active toxoplasmic infection as well as with reactivation of a latent infection following immunosuppressive states (Robert *et al.*, 1981).

In the data of Arnaudov (1971, 1973) positive antibody titres observed in sheep blood sera ranged from 1:10 to 1:1280. In a serological survey on the prevalence of toxoplasmosis among sheep in Egypt done by IHAT, Marca *et al.* (1996) observed antibody titres from 1:40 to 1:320. Abd El-Rahman *et al.* (1996) reported antibody titres in sheep against *T. gondii* by means of IHAT ranging from 1:64 to 1:2048. Mirdha *et al.* (1999) provided evidence that sheep and goats in India, seropositive for *T. gondii* reacted with antibody titres of 1:10 to 1:2560. The relatively low antibody titres indicated that in most cases, the infection was chronic.

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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