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ORIGINAL ARTICLE

First report of seroprevalence of *Toxoplasma gondii* infection in sheep in Pomerania, northern Poland

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Abstract

Introduction and objective. Toxoplasmosis is parasitic disease which has economic relevance for both veterinary and human medicine. In sheep, toxoplasmosis is a major cause of abortion and can thus cause reproductive problems. The current study aimed to determine the occurrence of anti-*Toxoplasma gondii* IgG antibodies in sheep from 13 districts of northern Poland and thereby obtain actual data about *T. gondii* seroprevalence in this population of animals.

Materials and methods. Blood samples from 1,646 animals from 99 herds were collected, and an in-house enzyme-linked immunosorbent assay (ELISA) based on native *Toxoplasma* lysate antigen (TLA) was used for serological testing. The diagnostic sensitivity of diagnostic test used in this study was 98.6%, and specificity 94.9% for the group of 113 sheep sera (74 seropositive and 39 seronegative) previously characterized by using an commercial agglutination test.

Results. Antibodies against *T. gondii* were found in 921 (55.9%) of all tested animals. The percentage of infected sheep was the highest (67.6%) for older animals (>6 years), whereas for younger ones it was significantly lower (50.1% – 57.2% for 1–5-year-old animals, respectively). Furthermore, a higher percentage of seropositive animals was noted among males (63%) than females (55.5%). The results also showed that the size of the herd is not a factor which may affect the seroprevalence of *T. gondii* infection in the examined population of sheep.

Conclusion. The results of this study indicate that *T. gondii* infection in sheep from region of northern Poland is relatively high, and consumption of ovine meat and milk can be regarded as a significant source of infection for humans.

Key words

sheep, *Toxoplasma gondii,* toxoplasmosis, ELISA, seroprevalence, northern Poland

INTRODUCTION

Toxoplasma gondii (phylum Apicomplexa) is an obligate intracellular coccidian protozoan parasite that infects all warm-blooded animals, including mammals, birds and humans, and has substantial medical and veterinary significance [1]. The parasite has a worldwide distribution and is mainly transmitted by food contaminated by oocysts excreted in the faeces of infected cat, by undercooked or raw meat (mainly pork and lamb) containing tissue cysts, or unpasteurized milk of infected animals, and transplacentally [2]. Although the invasion is mostly asymptomatic and self-limiting in immunocompetent individuals, it may lead to a life threatening disease in humans with impaired immune response or immune system immaturity. Furthermore, intrauterine transmission of the parasite from the mother to the foetus during gestation can result in severe foetal and neonatal complications. Toxoplasmosis is also an economically important disease of livestock, especially sheep. *T. gondii* infection in sheep is distributed worldwide, with seroprevalence of 20–91% in different countries [3]. These differences may occur due to the study area, associated factors, and criteria for animal selection, as well as the technique used for the epidemiological studies. Ovine toxoplasmosis may give rise to various disorders and lead to lower reproductive yields, especially during pregnancy [4]. Already in the 1950s, *T. gondii* was reported as a significant cause of abortion in this population of animals [5]. In 1987, Blewett and Trees [6] suggested that the *T. gondii* parasite may be responsible for 1–2% of neonatal losses annually. Thus, these losses may translate to over 1.5 million lambs lost in Europe per year, representing a significant loss to producers and national economies [7]. In addition, the tissue cysts of *T. gondii* in meat of infected sheep are an important source of infection for humans [1].

The great importance of *T. gondii* as a causative agent of zoonosis indicate the need for epidemiological studies in animals that can be used as a source of food. In Poland, *T. gondii* infection is relatively widely prevalent in swine and cattle populations [8, 9, 10, 11, 12]. However, the presence of infection in the sheep population is scarcely documented. Considering the importance of toxoplasmosis in sheep reproduction and the lack of epidemiological information of ovine toxoplasmosis in Poland, the presented study aimed to determine the prevalence of *T. gondii* infection in sheep and the probable role of lamb consumption in human toxoplasmosis.

MATERIALS AND METHOD

Study area and serum samples. Investigations were carried out in 13 districts in northern Poland (Fig. 1). A total of 1,646

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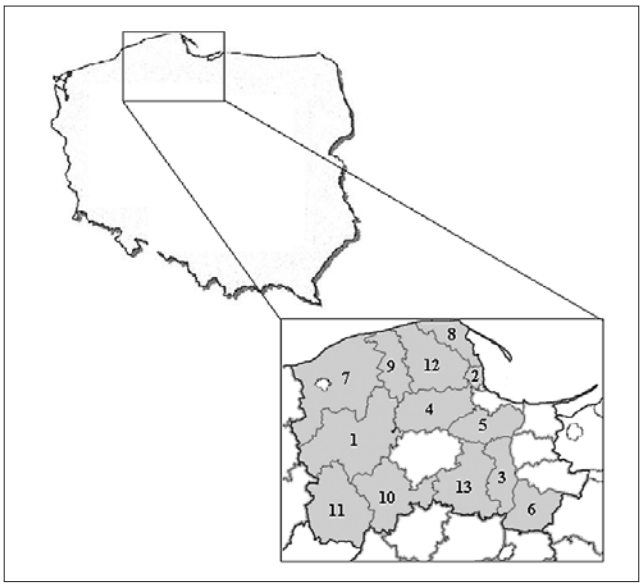

Figure 1. Sampled areas in Pomerania (grey, 1–13): 1) Bytowski, 2) Gdynia, 3) Tczewski, 4) Kartuski, 5) Gdański, 6) Kwidzyński, 7) Słupski, 8) Pucki, 9) Lęborski, 10) Chojnicki, 11) Człuchowski, 12) Wejherowski, 13) Starogardzki

blood samples were collected from sheep of various ages (1–9 years; mean age 3.014±1.834), most of them females (94%). Next, these samples were centrifuged and sera stored at -20 °C until assayed by IgG ELISA technique. The tested animals came from different farms (small or large and automated). The largest group of examined farms (70 out of 99) had herds of more than 20 head of sheep. Less than one-third of examined farms (26 out of 99) had more head of sheep (21–60 animals); however, the largest (more than 60 head of sheep), represented only 3.03% of all farms (3 out of 99).

In order to determine the cut-off value of the in-house IgG ELISA assay, a group of 20 control sheep sera previously characterized (seronegative for *T. gondii* and seropositive for *Neospora caninum*), collected in New Zealand, was used. This group consisted of sera for which negative results for specific anti-*T. gondii* antibodies were obtained with the use of agglutination test (Toxo-Screen DA, BioMérieux), whereas the positive results for specific anti-*N. caninum* antibodies were confirmed with the use of a commercially available competitive-inhibition enzyme-linked immunosorbent assay (cELISA) (VMRD, Inc.), and an immunofluorescence test (IFAT), using cut-off point of 1:50 [13], and as antigen, tachyzoites of *N. caninum* (Nc-1 strain), maintained by continuous passages in culture of VERO cells.

Furthermore, to assess the specificity and sensitivity of the in-house IgG ELISA assay used for epidemiological studies, a pool of previously characterized sheep serum samples was applied. A total of 113 sera were analyzed and divided into 2 groups in accordance with the results obtained using commercial tests (Toxo-Screen DA, BioMérieux): group I (IgG anti-*T. gondii* positive) – 74 sera from infected animals, and group II (IgG anti-*T. gondii* negative) – 39 sera from seronegative animals.

Serological examinations. The preparation of TLA has been described previously [8, 9]. MaxiSorp multiwells plates (Nunc, Denmark) were coated with 0.1 ml TLA at the final concentration of 1 μg/ml in a coating buffer (0.05 M carbonate buffer, pH 9.6). The plates were then incubated overnight at 4 °C, followed by washing 3 times in PBS-0,1% Triton X-100, and blocked for 2 h at 37 °C in blocking solution (1% bovine serum albumin, 0,5% Triton X-100 in PBS). After washing, the plates were incubated for 1 h at 37 °C with sheep sera diluted 1:100 in blocking solution. Next, the plates were washed 3 times with washing buffer and incubated with anti-sheep IgG peroxidase-labeled conjugates (Sigma) diluted 1:8,000 in blocking solution for 1 h at 37 °C. Then, o-phenylenediamine dihydrochloride (Sigma) chromogenic substrate was added. After 30 min at 37 °C incubation in darkness, the reaction was stopped by adding 2 M sulfuric acid. OD was measured at 490 nm using a microtiter plate reader (Multiscan FC; Thermo Scientific).

Each serum sample was examined twice and the results determined for each serum by calculating the mean value of the optical density (OD) reading for duplicate wells. Reference sera (positive and negative from groups I and II) on each ELISA plate were used in all experiments as controls.

Statistical analysis. The data were analyzed by chi-square (χ^2) test using the Microsoft Excel 2007 programme.

RESULTS

The pool of 1,646 sheep serum samples was examined for the presence of anti-*T. gondii* immunoglobulin G (IgG) antibodies using in-house enzyme-linked immunosorbent assay (ELISA), based on a native *T. gondii* antigen. The cut-off value was set as the mean value obtained for 20 seronegative serum samples from the control group plus 2 standard deviations, resulting in 0.474. A positive result of the IgG ELISA test was regarded as any absorbance value higher than the calculated value of the cut-off. The sensitivity and specificity of the IgG ELISA test used in this study was estimated using a pool of 73 seropositive sera (group I) and 39 seronegative sera (group II) from sheep. One serum samples from group I reacted below the cut-off value, resulting in a sensitivity of 98.6%, whereas for group II sera, 2 results above the cut-off were obtained (specificity 94.9%). Therefore, the accuracy of the test used in this study was estimated as 97.3%.

The mean absorbance of IgG ELISA obtained for all seropositive serum samples (921 out of 1,646) was 0.794 (range 0.479–1.891), whereas for all seronegative sera (425 out of 1646) – 0.338 (range 0.114–0.470). The prevalence of *T. gondii* infection in each district ranged from 30% – 100% (Tab. 1). The statistical differences between the seroprevalence among the districts were also found (χ^2=58.726; p<0.001). Seroprevalence in the examined animals increased progressively with age (Tab. 2), and the prevalence of *T. gondii* infection was significantly higher in older sheep (>6 years) than in animals aged less than 1.5 years. Additionally, the presented study did not find any influence of the size of a herd of sheep on the percentage of *T. gondii* infected animals. For both small herds of less than 20 head of sheep, as well as for larger herds, the percentage of infected animals was above 55% (Tab. 2). Furthermore, a higher percentage of seropositive animals was observed among males than females.

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Table 1. Seroprevalence of *T. gondii* infection among sheep in Pomerania, northern Poland (13 districts)

Districts	No. of examined samples	No. of positive samples	Seroprevalence (%)
Bytowski	620	319	51.5
Gdynia	50	38	76.0
Tczewski	81	53	65.4
Kartuski	385	215	55.8
Gdański	46	26	56.5
Kwidzyński	13	13	100
Słupski	10	3	30.0
Pucki	57	19	33.3
Lęborski	78	51	65.4
Chojnicki	113	82	72.6
Człuchowski	40	28	70.0
Wejherowski	141	70	49.6
Starogardzki	12	4	30.0
Total	1,646	921	55.9

χ^2=58.726, p<0.001

Table 2. Multivariate analysis for risk factors associated or not with infection caused by *T. gondii* in sheep from Pomerania in northern Poland

Variable	Category	Total No. of sheep		No. of sheep with *T. gondii* antibodies	
		n	%	n	%
Age	1–1.5 year	433	26.3	217	50.1
	2–3 years	656	39.9	357	54.4
	4–5 years	446	27.1	255	57.2
	6–11 years	111	6.7	75	67.6
Gender	females	1554	94.4	863	55.5
	males	92	5.6	58	63.0
Size of herd	1–20 sheep (70 herds)	517	31.4	294	56.9
	21–60 sheep (26 herds)	840	51.0	467	55.6
	more than 60 sheep (3 herds)	289	17.6	160	55.4

DISCUSSION

Sheep are highly susceptible to infections with *T. gondii* and may play a major role in the transmission of toxoplasmosis to humans. Furthermore, ovine toxoplasmosis has important veterinary implications because it is a significant cause of foetal loss in sheep worldwide. Considering the strong relevance of ovine toxoplasmosis for human health is very important to perform epidemiological studies which show the seroprevalence of this infection in the sheep population.

In the current study, the occurrence of antibodies against *T. gondii* in 1,646 sheep from northern Poland was investigated by the IgG ELISA test developed in our laboratory. In-house IgG ELISA assay was selected for epidemiological studies because this serodiagnostic method is a sensitive test that is able to detect low antibody titers even in recent infections; therefore, this technique is extremely efficient for the detection of IgG antibodies against *T. gondii* in animals. An IgG ELISA test based on TLA preparation was also previously used in epidemiological studies of the population of pigs and cattle [8, 9]. The seroprevalence of toxoplasmosis in the total sheep population examined in this study was 55.9%; thus, specific antibodies against *T. gondii* were found in 921 sera from the 1,646 tested samples. The literature date about the seroprevalence of toxoplasmosis among the sheep population in Poland are scarce.

To the best of the knowledge of the authors of the presented study, this is the first report about the occurrence of anti-*T. gondii* IgG antibodies in sheep in the Pomeranian Province of northern Poland, which also includes the largest population of animals that have been studied in our country. Furthermore, the prevalence obtained in this study is very similar to that reported earlier by Michalski and Platt-Samoraj [14] and by Górecki et al. [15] in Poland. These authors found the specific antibodies for *T. gondii* in 55% (11/20) [14] and 53.65% (22/41) [15, 16] of investigated sheep with the use of direct agglutination assay (Toxo-Screen DA, bioMérieux) and indirect fluorescent antibody test (IFAT), respectively. A similarly high percentage of infected animals has been observed in other European countries, e.g. Bulgaria – 48.2% [17], Czech Republic – 59% [18], France – 65.6% [19] and Lithuania – 42.1% [20]. This high percentage of serum-reactive sheep to *T. gondii* could be related to environmental contamination by the parasite. In Poland, most sheep breeding on farms is traditional, and the animals have direct contact with cats. Oocyst-contaminated pastures, fodder, and drinking water are regarded as potential sources of postnatal infection in sheep. Therefore, it is important to know which feeding practices pose an increased infection risk. Unfortunately, in this study, information about feeding practices of the examined sheep population was not available. However, this information would be very valuable.

The results of this study also show that the prevalence of toxoplasmosis among sheep depends on the age and gender of the animals. A progressive increase in anti-*T. gondii* antibodies with age (50.1% – 67.6% for the annual sheep and those older than 6 years, respectively) suggests a continuous exposure to the organisms in the environment. Moreover, these results indicate that postnatal acquisition of infection is an important route of transmission of the parasite in sheep. In the case of the gender of the examined animals, it was found that the prevalence in males (63%) was slightly higher than in females (55.5%). However, these results may differ from actual seroprevalence because the male and female population consisted of a different number of animals. The group of males represented only 5.6% of the tested population of animals. Furthermore, according to some authors, females are considered a risk factor for toxoplasmosis due to hormonal and physiological differences [21, 22].

The presented study also found that the size of herd had no impact on the percentage of animals infected with *T. gondii.* A similar percentage of infected animals was observed in both small household farms with up to 20 pieces of sheep (56.9%), as well as in larger herds (at a level above 55%). These results therefore indicate that sheep are highly susceptible to infections with *T. gondii.*

In conclusion, according to the high percentage of seropositive sheep and the importance of the meat in transmission of toxoplasmosis to human, investigation of sheep and other domestic and meat production animals is indicated and necessary. The monitoring of *T. gondii* infection in animals destined for human consumption is a great challenge for human toxoplasmosis prevention.

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This study documents for the first time the epidemiology of *T. gondii* infection in sheep in the Pomeranian Region of northern Poland. Further studies are needed to elucidate the epidemiology in the whole country.

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