

Epidemiological study of *Toxoplasma gondii* infection among cattle in Northern Poland

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Abstract

Toxoplasmosis, caused by *Toxoplasma gondii*, is a significant disease in livestock and humans. Because of medical and veterinary importance it is essential to study the prevalence of *T. gondii* infection among human and animals in various parts of the world. In this study, 4033 cattle from eight provinces of Northern Poland (belonging to 190 herds) were tested for IgG antibodies against *T. gondii* by an in-house ELISA technique based on native *Toxoplasma* lysate antigen. The diagnostic sensitivity of test used in this study was 96.3%, and specificity was 98% for the group of 77 cattle sera (27 seropositive and 50 seronegative) previously characterized with the use of agglutination and immunofluorescence methods. A 127 (3.15%) out of all tested animals belonging to 72 (37.9%) out of 190 herds were founded as positive. Furthermore, our results showed that the way of feeding and farming, the size of the herd and the age of animals have the influence on the prevalence of toxoplasmosis among cattle. The percentage of infected cattle was the highest for old animals which belongs to the small herds with the traditional way of farming. These results indicate that *T. gondii* infection in cattle from Northern Poland is relatively low and consumption of beef and milk can be regarded as a poor source of infection for humans.

Key words

cattle, *Toxoplasma gondii*, toxoplasmosis, ELISA, seroprevalence, Northern Poland

INTRODUCTION

Toxoplasma gondii is a protozoan parasite of both medical and veterinary importance, because it may cause abortion or congenital diseases in its intermediate hosts (all warm-blooded animals including humans). Toxoplasmosis is one of the more common parasitic zoonoses worldwide. People can become infected with *T. gondii* by ingesting raw or undercooked meat containing tissue cysts or by ingesting cat-shed oocysts via contaminated soil, food or water. The presence of the parasite in tissues among livestock is most common in pigs, sheep and goats, followed by rabbits and poultry. In cattle, natural *T. gondii* infection does not appear to cause clinical disease or abortion [1]. Therefore interest in *T. gondii* in cattle stems mainly from a public health perspective; if cattle carry infectious tissue cysts they may be an important source of human infections since beef is often consumed undercooked, especially in Europe. The role of beef in human *T. gondii* infection is unclear. Eating raw beef has been reported as one of the risk factors that predicts acute infection [2, 3, 4]. On the other hand, it has been suggested that cattle do not readily acquire infection [5], and that tissue cysts are not very persistent in cattle. Tissue cysts are only infrequently recovered from experimentally-inoculated cattle [1, 6, 7], and there are few successful recoveries from naturally infected cattle [1] or beef samples [8, 9]. Taking these aspects into consideration, large scale screening of cattle for the presence of *T. gondii* is necessary to clarify the role of cattle as a source of infection.

This study was undertaken to determine the frequency of anti-*T. gondii* antibodies in cattle by indirect ELISA based

on *Toxoplasma* Lysate Antigen (TLA) and the probable role of beef consumption in human toxoplasmosis.

MATERIALS AND METHOD

Study area and serum samples. Investigations were carried out in 8 provinces in Northern Poland (Tab. 1). The tested animals came from different farms (small or large and automated). The largest group of examined farms (92 out of 190) was small corrals to 5 head of cattle. More than half of studied farms (98 out of 190) had more head of cattle (from 6 to more than a hundred pieces), however the largest ones (more than 60 head of cattle) represented only 8.95% of all farms (17 out of 190) (Tab. 2). A total of 4033 blood samples were collected from cattle of various ages (1 – 14 years; mean age 4.15 ± 2.37) and the most of them constituted females (99.85%). Next, these samples were centrifuged and sera stored at -20°C until assayed by IgG ELISA technique.

In order to determining of the cutoff value of the in-house IgG ELISA assay has been used a group of 15 control cattle sera previously characterized (seronegative for *T. gondii* and seropositive for *Neospora caninum*) collected in New Zealand. 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), and latex agglutination test (LAT) (Pastorex™ Toxo, BIO-RAD), whereas the positive results for specific anti-*N. caninum* antibodies were confirmed with the use of cELISA test kit (VMRD, Inc.), and immunofluorescence test (IFAT), using acetone-fixed *N. caninum* tachyzoites slides (VMRD, Inc.). All commercial tests were performed according to the procedures recommended by producers.

Furthermore, to assess the specificity and sensitivity of in-house IgG ELISA assay used for epidemiological studies, pool of previously characterized serum samples from cattle

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was applied. A total of 77 sera were analyzed and divided into two groups in accordance with the results obtained using the agglutination test (Toxo-Screen DA, bioMérieux) and immunofluorescence test, with the use of slides coated with *T. gondii* antigen (bioMérieux), and specific conjugates (Jackson ImmunoResearch): group I (IgG anti-*T. gondii* positive) – 27 sera from infected animals, and group II (IgG anti-*T. gondii* negative) – 50 sera from seronegative animals.

Serological examinations. The preparation of TLA was as previously described [10]. 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). Then, plates were incubated overnight at 4 °C. After that, they were washed three 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, plates were incubated for 1 h at 37 °C with cattle sera diluted 1:100 in blocking solution. Next, the plates were washed three times with washing buffer and incubated with anti-bovine IgG peroxidase-labeled conjugates (Sigma) diluted 1:1000 in blocking solution for 1 h at 37 °C. Then, o-phenylenediamine dihydrochloride (Sigma) chromogenic substrate was added. After 45 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 were determined for each serum by calculating the mean value of the optical density (OD) reading for duplicate wells.

Moreover, reference sera (positive and negative from group 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 with the use of the Microsoft Excel 2007 program.

RESULTS

The pool of 4033 cattle 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 cutoff value was set as the mean value obtained for fifteen seronegative serum samples from the control group plus two standard deviations, resulting 0.323. A positive result of IgG ELISA test was regarded as any absorbance value higher than the calculated value of the cutoff. The sensitivity and specificity of the IgG ELISA test used in this study was estimated using a pool of 27 seropositive sera (group I) and 50 seronegative sera (group II) from cattle. One serum samples from group I reacted below the cutoff value, resulting in a sensitivity of 96.3%, whereas for group II sera one results above the cutoff was received (specificity of 98%). Therefore, the accuracy of the test used in this study was estimated as 97.4%. The mean absorbance of IgG ELISA obtained for all seropositive serum samples (127 out of 4033) was 0.536 ± 0.154 (range 0.333–1.013), whereas for all seronegative sera (3096 out of 4033) was 0.188 ± 0.052 (range 0.115–0.303).

The prevalence of *T. gondii* infection in each province ranged from 1.5% to 10.0% (Tab. 1). Furthermore, the statistical differences between the seroprevalence among the

Table 1. Seroprevalence of *T. gondii* infection among cattle in Northern Poland (8 provinces).

Province	No. of examined samples	No. of positive samples	Seroprevalence (%)
Kolbudy	80	8	10.0
Lichnowy	493	17	3.5
Malbork	750	28	3.5
Malbork (city)	14	1	7.1
Miłoradz	1260	43	3.4
Nowy Staw	639	17	2.7
Stare Pole	757	12	1.5
Starogard- Gdański	40	1	2.5
Total	4033	127	3.1

$\chi^2 = 20.941, p > 0.005$.

provinces was found ($\chi^2=20.941, p > 0.005$). Moreover, the obtained results showed that the size of the herd has a big influence on the number of animals infected by *T. gondii* (Tab. 2). It was the highest for small farms (counting below 5 animals) and the lowest for big, automated ones (counting over 100 animals). Additionally, the age of the animals had also an impact on the percentage of infected cattle and it increased with the age of examined animals (Fig. 1) ranging from 2.06% to 20.0%.

Table 2. Seroprevalence of *T. gondii* infection according to various sizes of herds.

No. of cattle in herd	1–5	6–20	21–60	61–100	>100
No. of all herds	92	45	36	8	9
No. of all cattle in herds	173	524	1166	630	1540
No. of infected animals by <i>T. gondii</i>	27	33	49	6	12
% of infected animals occurring in one herd	0.17	0.14	0.11	0.11	0.09

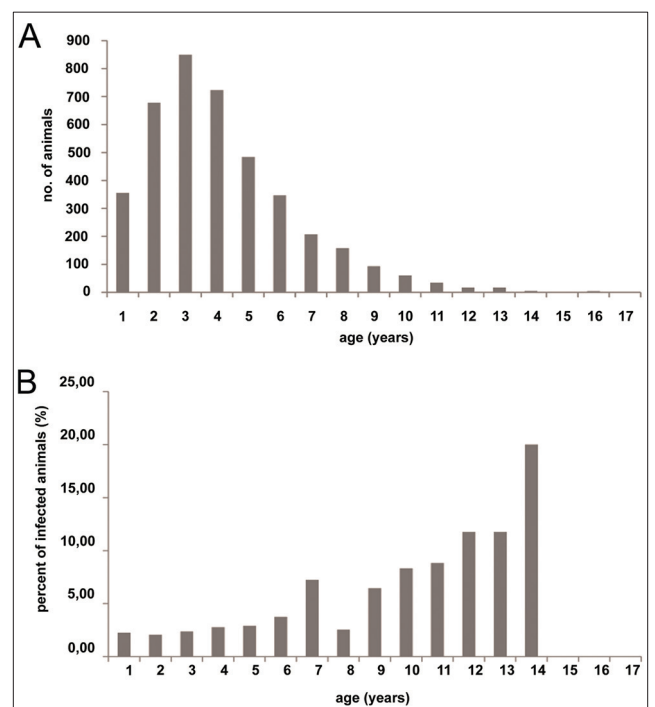


Figure 1. Number of examined animals at the various age (A) and percent of infected animals according to the age (B).



DISCUSSION

The prevalence and impact of *T. gondii* on human health are highly variable geographically. Sources of infection are different in human populations and depended on the differences in culture and eating habits [11, 12]. The source by which an individual has become infected with *T. gondii* cannot be discriminated by diagnostic tests. In humans, several epidemiological studies found a significant association between toxoplasmosis and consumption of cattle meat [2]. In Europe, up to 63% of human infections are attributable to the consumption of undercooked or cured meat products [3]. Three large case-control studies have pinpointed uncooked meat as the most important risk factor for pregnant women [2, 3, 13]. This prompted us to conduct a survey in cattle from Northern Poland to determine the seroprevalence of *T. gondii* infection in this animal. Since direct observation of cysts in tissues is not a suitable diagnostic method to be carried out on live animals and the fact that symptomatic toxoplasmosis is rare in cattle, the serological techniques appear to be the methods of choice [14]. The epidemiology of bovine toxoplasmosis in Poland has been little studied [15, 16, 17]. Examination of serum samples from cattle of Lublin region by the direct agglutination test (DA) for the presence and level of IgG antibodies against *T. gondii* showed a very high percentage of positive reactions: 53.8% (for 262 cows), 53.7% (for 259 cows) and 33.8% (for 74 cows). Compared to the results reported by Sroka et al, the percentage of animals with toxoplasmosis assessed in our study is lower (3.15%) than those noted in the Lublin region of Poland, and this prevalence increased statistically in the group of cattle more than 2 years old. The current literature presents values range from 0 to 92% for the presence of anti-*T. gondii* antibodies in this group of livestock. High seroprevalence values were found in Spain (41%) [18], Serbia (76.3%) [19], Greece (40%) [20], Italy (92%) [21], and Brazil (71%) [22], suggesting that a high proportion of cattle is exposed to the parasite, although pigs, sheep, and goats are the species most often infected with *T. gondii* [23]. Lower prevalence of infection in cattle also reported in some countries such as Montana in USA (3.2%) [24], Brazil (1%) [25], Tanzania (3.6%) [26], France (7.8%) [12], and Malaysia (4%) [27]. This difference in seroprevalence between various countries depend on many factors: type of breeding and management practices, zoohygienic status, age of examined animals and geographical region. Furthermore, it is possible that some part of seropositive results obtained in cattle with the use of serodiagnostic tests (such as ELISA assay based on *T. gondii* native antigens) could be due to cross reaction with antibodies specific for other related parasites (for example *N. caninum*). For this reason, in this study to determine the cutoff value of Ig ELISA test, the well-characterized pool of sera (seropositive for *N. caninum* and seronegative for *T. gondii*) was applied.

This study also showed that the prevalence of toxoplasmosis among cattle depends on the way of farming, size of herds and the age of the animals. According to the size of herd, the highest percentage of animals infected with *T. gondii* was received for small farms and the lowest for the biggest ones. These results may be explained by various ways of farming, conditions and hygiene. In small farms, the contact of cats (definitive hosts of *T. gondii*) and rodents (which may transmit the parasite) with the cattle is much more probable. They may go to the areas where food for farm animals is stored

and have a constant entry to farming rooms. Furthermore, the source of infection for this group of cattle could be grass form pasture. The way of feeding in big farms is much more different. It is automated with the high emphasis on the hygiene. No animals, which could transmit the parasite, are able to go there.

In our opinion, also the difference may results from the number of examined animals, and the method used for detection of specific antibodies against *T. gondii*. Serological assays allow high through-put testing at low costs and are therefore preferred for screening. Although parasites are not detected directly, the seroprevalence can give an indication of the risk of human infection by eating meat from a certain species if the detection of antibodies against *T. gondii* and the presence of tissue cysts have a strong correlation. A strong correlation has been shown in pigs [28] and sheep [29, 30], but for cattle the correlation is unclear. It was shown that it is difficult to develop a serological assay that is sufficiently discriminatory to reliably detect the presence of antibodies against *T. gondii* in cattle. This is probably caused by the fact that cattle, in contrast to other animals, do not show high titers against *T. gondii*. This could be an effect of the alleged capability of cattle to clear the infection or reduced within a few weeks. The present study also suggested that the antibody response to *T. gondii* in cattle does not persist for life. This hypothesis should be further tested.

In conclusion, although our results cannot provide an estimate of the percentage of infected cattle meats, consumption of beef, especially from young cattle, may be regarded as a poor source of infection for humans in Poland. It is also worth to add that the seroprevalence of toxoplasmosis in the pig population in Poland was 19.2%, using the same as in this study in-house ELISA method [10]. This indicates that the consumption of uncooked pork probably poses a much higher risk of infection to the consumer than beef meat.

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