SEROPREVALENCE OF *Toxoplasma gondii* IN FARM AND WILD ANIMALS FROM THE AREA OF LUBLIN PROVINCE

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**Abstract**

Using a direct agglutination test with 2-mercaptoethanol, 971 sera from farm animals and 71 sera from wild animals living in the Lublin province (Eastern Poland) were examined for IgG *Toxoplasma gondii* antibodies. Seropositive results were found in 27.5% of farm animals and in 19.7% of wild animals. Antibodies were more prevalent in cattle (53.7%), cats (50.9%), and dogs (42.2%) and less prevalent in pigs (10.5%) and rabbits (7.4%). The majority of positive results were found at low titres. Seroprevalence in animals from the areas where toxoplasmosis was more prevalent in humans was statistically higher compared to the rest of the Lublin province (34.8% vs. 17.9%, P<0.0001).

**Key words:** farm animals, wild animals, *Toxoplasma gondii*, antibodies, agglutination test, Eastern Poland.

Toxoplasmosis is one of the most common parasitic infections in humans and animals. Beside its socioeconomic impact, toxoplasmosis may constitute important health problem for pregnant women, because of the threat of foetus infection, and for immunocompromised patients in whom the parasite may cause serious pathological changes. *T. gondii* infection in animals is mainly considered as a public health problem, but the infection may cause also notable economic losses in animal productivity by reproductive disorders.

Farm animals, wild game animals, and rodents form the reservoir for *T. gondii*. The parasite in these animals occurs in the form of tissue cysts. The most important species in *Toxoplasma* epidemiology are pigs because of the common pork consumption. Usually, the infection is asymptomatic and may be found only by the detection of specific antibodies in serum. Thus, constant serological monitoring of farm animals is important, in particular of animals for slaughter.

There were areas of high seropositivity in humans (68.6-89.5%) found in the Lublin province in earlier studies on toxoplasmosis prevalence (1994-1999) compared to the rest of the province (51.9% in average). Moreover, in these areas the clinical cases of human toxoplasmosis were detected (13, 14).

In order to explain the relationship between the proportion of animals infected with *T. gondii* and the toxoplasmosis morbidity in humans in the Lublin province, serological examinations in farm and wild animals were carried out.

**Material and Methods**

**Study area.** Investigations were carried out in two regions of the Lublin province (Eastern Poland): region I - the areas of endemic (more frequent) prevalence of toxoplasmosis in humans; and region II - the areas of less frequent prevalence of toxoplasmosis in humans (Fig. 1).

**Animals.** A total of 971 sera collected from farm animals were examined (259 of cattle, 398 of pigs, 53 of cats, 71 of dogs, 27 of rabbits, and 163 of poultry). Additionally, 71 sera from wild animals were tested (19 of roe deer and 52 of wild boars) (Table 1).

**Serological examination.** The blood samples were collected in cattle from the jugular vein, in cats and dogs from the saphenous vein, in poultry from the brachial wing vein, and in rabbits from the marginal ear vein. In pigs examined in slaughterhouse, the blood was collected from carotid artery cut at the slaughter and in those living on farms from the marginal ear vein. The blood from wild animals was collected from the heart of the dead animal. After allowing blood samples to clot they were centrifuged (at 800 × g) and then the serum was transferred to test-tubes and stored at 4°C until examination.
Fig. 1. Map of Poland with marked area of the study in the Lublin province.

Table 1
Examined animals

<table>
<thead>
<tr>
<th>Species</th>
<th>Number of examined animals</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Region I</td>
<td>Region II</td>
</tr>
<tr>
<td>Cattle</td>
<td>181</td>
<td>78</td>
</tr>
<tr>
<td>Pigs</td>
<td>281</td>
<td>117</td>
</tr>
<tr>
<td>Cats</td>
<td>16</td>
<td>37</td>
</tr>
<tr>
<td>Dogs</td>
<td>17</td>
<td>54</td>
</tr>
<tr>
<td>Rabbits</td>
<td>12</td>
<td>15</td>
</tr>
<tr>
<td>Poultry</td>
<td>41</td>
<td>122</td>
</tr>
<tr>
<td>Subtotal</td>
<td>548</td>
<td>423</td>
</tr>
<tr>
<td>Roe deer</td>
<td>19</td>
<td>0</td>
</tr>
<tr>
<td>Wild boars</td>
<td>10</td>
<td>42</td>
</tr>
<tr>
<td>Subtotal</td>
<td>29</td>
<td>42</td>
</tr>
<tr>
<td>Total</td>
<td>577</td>
<td>465</td>
</tr>
</tbody>
</table>

Antibodies of IgG class were detected using direct agglutination test with 2-mercaptoethanol (DA-2ME) according to Desmonts and Remington (2), with *T. gondii* antigen prepared in the Institute of Agricultural Medicine. The titre was determined on the basis of agglutination of antigen forming a mat covering no less than half of the well base at possibly greatest dilution. Sera showing titres greater or equal to 32 were regarded as positive. In a part of the studies commercial kit Toxo-Screen DA (bioMérieux, France) was used accordingly to the manufacturer's instructions. The minimal titre for a positive result in this kit was established as greater or equal to 40 (12). To compare the titres of both tests, control studies were carried out (13).

**Statistical analysis.** The data were analysed by chi-square ($\chi^2$) test with the use of Statistica for Windows v. 5.0 package (StatSoft Inc., Tulsa, OK, USA).

**Results**

**Farm animals.** Among 971 animals examined for the presence of *T. gondii* antibodies, positive results were found in 267 animals (27.5%). The species with most positive results were cattle (53.7%), cats (50.9%), and dogs (42.2%). The lesser percentages of seropositive reactions were found in poultry, pigs, and rabbits – 16.6%, 10.5%, and 7.4%, respectively.
### Table 2
Seroprevalence of *Toxoplasma gondii* in farm animals from two regions located in the Lublin province

| Species | N   | N (%) positive | Quantitative distribution of titre ranges (%) |  |  |  |  | Region II |
|---------|-----|----------------|--------------------------------------------|--|---|---|---|-----------------|---|---|---|---|
|         |     |                | 40-60 32-128 180-520 256-512 ≥1620*   | N   | N (%) positive | 40-60 32-128 180-520 256-512 ≥1620* |
|         |     |                |     |               |     |               |     |               |
| Cattle  | 181 | 113 (62.4%)ab*** | 99 (87.6%) 4 (3.5%) 10 (8.8%) | 78 | 26 (33.3%)b** | 26 (100.0%) 0 0 |
| Pigs    | 281 | 35 (12.4%)b***  | 23 (65.7%) 5 (14.3%) 7 (20.0%) | 117 | 7 (5.9%)b*** | 6 (85.7%) 1 (14.3%) 0 |
| Cats    | 16  | 13 (81.2%)a*, b** | 0 2 (15.4%) 11 (84.6%) | 37 | 14 (37.8%)b* | 3 (21.4%) 6 (42.8%) 5 (35.7%) |
| Dogs    | 17  | 9 (52.9%)        | 4 (44.4%) 3 (33.3%) 2 (22.2%) | 54 | 21 (38.9%)b*** | 7 (33.3%) 10 (47.6%) 4 (19.0%) |
| Rabbits | 12  | 2 (16.7%)        | 0 0 2 (100.0%) | 15 | 0             | - - - |
| Poultry | 41  | 19 (46.3%)v***   | 11 (57.9%) 2 (10.5%) 6 (31.6%) | 122 | 8 (6.5%)b*** | 3 (37.5%) 3 (37.5%) 2 (25.0%) |
| Total   | 548 | 191 (34.8%)y***  | 137 (71.7%) 16 (8.4%) 38 (19.9%) | 423 | 76 (17.9%)   | 45 (59.2%) 20 (26.3%) 11 (14.5%) |

* P<0.05, ** P<0.001, *** P<0.0001 (test $\chi^2$), a - significantly different when comparing region I to region II, b – significantly different when given species was compared to all other animals examined in the same region,  
  - Toxo-Screen DA, v - OA-2ME
Among 548 farm animals belonging to 6 species from region I, the presence of antibodies against *T. gondii* was found in 191 of them (34.8%). The highest percentages of positive results were revealed in cats (81.2%) and cattle (62.4%), whereas in pigs only 12.4% of positive results were found.

Among 423 farm animals belonging to 6 species from region II, seropositive results were found in 76 of them (17.9%). Dogs (38.9%), cats (37.8%), and cattle (33.3%) were the species with the highest frequency of seropositive reactions in this region. The results are shown in Table 2.

Analysing *Toxoplasma gondii* seroprevalence in two regions, significantly more animals showed the presence of specific antibodies in region I (with high rate of human toxoplasmosis) than in region II (the remaining part of Lublin province) – respectively 34.8% and 17.9% (P<0.0001). Significant differences in the frequency of high titres (≥ (52.9%) were found more often in region I than in region II (Table 2).

Out of 71 serum specimens collected from wild animals (wild boars and roe deer) the presence of *T. gondii* antibodies was found in 14 (19.7%), including 11 wild boars (21.1%) and 3 roe deer (15.8%). The majority of positive results (64.3%) had low titres and the rest had moderately high titres, whereas no high titres were found. There was no significant difference between the frequency of seropositive results in wild animals from region I and region II.

**Wild animals.** Out of 71 serum specimens from wild animal species (wild boars and roe deer) the presence of *T. gondii* antibodies was found in 14 (19.7%), including 11 wild boars (21.1%) and 3 roe deer (15.8%). The majority of positive results (64.3%) had low titres and the rest had moderately high titres, whereas no high titres were found. There was no significant difference between the frequency of seropositive results in wild animals from region I and region II.

**Discussion**

Serological examinations showed that *Toxoplasma gondii* is widespread in farm and wild animals in Eastern Poland. The highest rates of seroprevalence were found in cattle (53.7%), cats (50.9%), and dogs (42.2%). The lowest rates were detected in pigs (10.5%) and in rabbits (7.4%). The survey proved that seropositive reactions in animals are significantly higher in these areas of the Lublin province (region I), where in the course of earlier studies a high prevalence of toxoplasmosis in humans had been found (13, 14). In the remaining part of the Lublin province with lower prevalence of toxoplasmosis in humans (region II), the prevalence of seropositive reactions to *T. gondii* in animals was also lower.

According to earlier works, the extensiveness of *T. gondii* infection in different species of animals varies and depends on many factors (type of breeding, zoohygienic status, geographical region, and place in the food chain). The special role is attributed to cats, which are definitive hosts of this parasite and when infected, they excrete oocysts with faeces to the environment. The rate of the infection in cats varies and depends on the age, environment (rural or urban), and type of keeping (inside or outside the house). Feral cats preying on small rodents (which pose a main source of infection for this species except raw meat of farm animals) are particularly exposed to *T. gondii* infection. Similarly, a majority of cats examined in this study were kept in a mixed environment (inside and outside) and were fed raw meat. A comparison of cats' seropositivity from the regions I and II shows a significantly higher frequency in region I (81.2% vs. 37.8%), and resembling a similar relationship in other examined species. This suggests that tissues of intermediate hosts may play an important role in the infection. Cats are infected primarily by ingestion of *T. gondii*-pampered oocysts and by the fact that cats from region I were older and exposed for a longer period to the parasite. Compared to earlier Polish studies, the total seroprevalence of *T. gondii* in cats found in the present work (50.9%) is similar to that reported by Smieleswka-Loś and Pacoń – 52.5% (16). Lower result was reported by Gabryś and Liberski – 14% (7), while higher results were reported by Ramisz and Żemburowa - 86.4% (11), by Wasiańczyk - 70.6% (20), and by Michalski and Platta-Samoraj – 70.6% (9). Compared to the results reported from other European countries, the stated by us percentage of seropositive reactions in cats is higher than that noted in France (43.0%), Italy (9.0-33.0%), Sweden (42.0%), and Turkey (43.0%) (18). It is similar to the results obtained in Germany (34.0-51.0%), and lower compared to those found in the Czech Republic (59.0%) and Slovenia (57.0%) (18).

Serological examinations in pigs revealed a significant difference in the seropositivity to *T. gondii* between regions (12.4% in region I vs. 5.9% in region II). Pigs from region I were kept mainly in individual farms, where hygienic status was often poor. This probably led to greater exposure to the parasite. The obtained results suggest a higher rate of environmental contamination of these farms with *T. gondii* oocysts. In region II pigs were younger and were kept in breeding farms making the probability of the contact with sources of infection lower. This was confirmed by the lower seroprevalence of *T. gondii* in this group. The total rate of infected pigs found by us (10.3%) is close to the result obtained by Pawłowski - 13.2% (10) and lower compared to the results reported by other Polish authors: Ramisz and Żemburowa (26.3-46.0%) (11), Umński et al. (21.2-53.0%) (19), and Krupa and Bartoszcześ (35.2%) (8). Compared to the results obtained in other European countries, the percentage of seropositive reactions found by us in pigs is higher than that noted in Austria (1.0-3.0%), Finland (3.0%), Norway (3.0%) and Portugal (5.0%), and lower than that noted in the Czech Republic (35.0%), Italy (64.0%), the Netherlands (31.0%), and Germany (18.0%) (18).

The total percentage of positive results found in this study in cattle was rather high and amounted to 53.7%. However, most of the positive results were noted at low serological titres. No clinical symptoms...
suggesting the infection with \textit{T. gondii} were found in the examined animals. Significantly higher frequency of seropositive results was detected in cattle from region I (62.4\%) as compared to region II (33.3\%). Animals from region I were kept mostly on individual farms, raised on pasture, and in part of the farms proper hygienic conditions were not respected. In the majority of these farms, cats had a constant entry to farming rooms. The source of infection for cattle could be grass from pasture and other feeds contaminated with cats' faeces. Earlier studies conducted in Poland on serological prevalence of toxoplasmosis in cattle showed the results similar to our study: Ramisz and Zemburowa reported 22.5-54.6\% of positive results (11) and Umiński et al. - 55.5\% (19). Compared to the results reported from other European countries, the percentage of seropositive reactions obtained in our study is higher than those noted in the Czech Republic (22.0\%), Greece (40.0\%), the Netherlands (13.0-43.0\%), Norway (5.0\%), Portugal (43.0\%), Spain (40.0\%), and Switzerland (14.0\%). It is lower compared to seroprevalence noted in France (69.0\%), Italy (92.0\%), and Turkey (66.0\%) (18).

The role of poultry in spreading of toxoplasmosis was considered by many authors (1, 3, 12, 18). In this study, the total percentage of seropositive results in poultry was 16.6\%. The seroprevalence in region I (46.3\%) was much higher than in region II (6.5\%), which may be partly due to a high proportion of young birds originating from breeding farms in region II. All birds from region I were kept in a free-range backyard and were more exposed to \textit{T. gondii} infection. The seroprevalence found in the present study in poultry is higher compared to the earlier Polish authors’ studies (1.3-6.9\%) (13). It may be, at least in part, due to a frequent use in the past of the complement fixation test, which is known to deliver false negative results with birds sera (4). Compared to the results obtained in other countries, the percentage of seropositive reactions stated by us in poultry is higher than that found in the Czech Republic (1.0-5.0\%) and Turkey (0-4.0\%) (18).

The role of dogs in \textit{Toxoplasma gondii} epidemiology is not important as the dogs are not directly linked to the chain leading from cats to humans (6), although there does exist the opinion that they can mechanically carry oocytes (5). A total percentage of seropositive reactions in dogs found in this study (42.2\%) are close to that reported by Śmielewska-Łoś et al. (8.6-44.2\%) (17) and are lower compared to that reported by Ramisz and Zemburowa (65.5\%) (11). When compared to the results obtained in other European countries, the percentage of seropositive reactions observed by us in dogs is higher than that found in Italy (17.0\%) and Sweden (30.0\%), similar to those obtained in Czech Republic (33.0-39.0\%) and France (39.0\%), and lower than that found in Spain (47.0\%) and Turkey (75.0\%) (18).

Toxoplasmosis in rabbits has not been described in Poland until recently. Our results show that from 7.4\% up to 22.2\% of rabbits reveal the presence of specific antibodies. A significant role of rabbits in the epidemiology of toxoplasmosis has been demonstrated by the isolation of the virulent strain of \textit{T. gondii} from the brain of one of the rabbits seropositive in this study, which showed disease symptoms (13, 15).

In the literature, no data on \textit{T. gondii} seroprevalence in wild animals in Poland is found. The results of this study support a view that raw meat consumption or direct contact with the tissues of hunted animals during bleeding out, skinning, or evisceration may pose a risk of infection for venison consumers and hunters or for workers of a venison store. A total percentage of seropositive reactions found in this study in wild boars (21.1\%) are higher compared to that obtained in the Czech Republic (15.0\%) and Austria (19\%), and lower compared to those obtained in the USA (44\%) and Germany (25\%) (18).

References

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