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THE OCCURRENCE OF *TOXOPLASMA GONDII* INFECTION IN PEOPLE AND ANIMALS FROM RURAL ENVIRONMENT OF LUBLIN REGION – ESTIMATE OF POTENTIAL ROLE OF WATER AS A SOURCE OF INFECTION

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Abstract: A total of 254 humans and 489 domestic animals living on farms in the Lublin province (eastern Poland) were examined for the presence of antibodies against *Toxoplasma gondii* using, respectively, the ELFA and direct agglutination tests. In parallel, 182 samples of potable water, mostly from shallow household wells, were taken on farms and examined for the presence of *T. gondii* by microscopy and PCR. The frequency of seropositive reactions in farm inhabitants (66.9%) was significantly greater ($p<0.01$) compared to the reference group of 39 healthy urban dwellers (41.0%). A highly significant positive correlation was found between the age of examined farm inhabitants and the rate of positive reactions with *Toxoplasma* antigen ($p<0.0001$). Among domestic animals, the greatest frequency of seropositive reactions to *T. gondii* occurred in cats (75.0%) and dogs (53.6%), less frequent in cattle (33.8%) and hens (33.5%) and the least frequent in pigs (17.9%) and ducks (21.2%). The presence of *T. gondii* was found in potable water samples taken from water intakes on farms: in 12.6% of samples by microscopy, and in 22.5% of samples by PCR. Among 19 water samples taken from bathing places on the territory of the Lublin province, 2 samples positive for *T. gondii* (10.5%) were found by microscopic examination and confirmed by PCR. The presence of live parasites in water samples was demonstrated by the isolation of *Toxoplasma gondii* strains in mice. By use of RFLP-PCR it was found that the majority of isolated *Toxoplasma* strains (78.0%) belonged to clonal type I which is most virulent for humans and animals. Although no statistically significant relationship between the presence of *T. gondii* in water and occurrence of seropositive reactions in farm inhabitants and/or domestic animals could be found, the above-mentioned data suggest a potential role of potable water in the spread of toxoplasmosis in the rural environment.

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Key words: *Toxoplasma gondii*, epidemiology, serological examinations, farm inhabitants, domestic animals, potable water, PCR.

INTRODUCTION

Infection with the parasitic protozoan *Toxoplasma gondii* still represents an actual public health problem. Prevalence of the positive serologic reactions in humans varies depending on geographical location, age, nutritional habits, and keeping of hygienic standards [10, 34]. In Poland, it is estimated at 50–60% [24, 28]. The *T. gondii* invasion presents a particular hazard for pregnant women, with respect to the possibility of foetus malformations, and for

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debilitated persons, in whom pathological changes may occur in many organs or tissues. Recently, it was shown that the invasion may cause distant effects in the form of disturbances of sight and neural functions [35].

According to current views, humans contract toxoplasmosis mostly via the alimentary route, by eating raw meat (mainly pork) containing cysts of *T. gondii*, or by consumption other foods or water contaminated with oocysts of the parasite [10, 11, 35]. However, at the assessment of concrete clinical cases it is still not possible to determine which form of the parasite have caused the infection. Hence, at the identification of the source of infection in human clinical cases, it could be important to determine the clonal type of parasite isolated.

The results obtained hitherto by our group and other authors clearly indicate that toxoplasmosis may constitute a serious epidemiological problem, particularly in the rural environment where there are many cross routes of *Toxoplasma* transmission [10, 11, 28, 30]. Cats play a special role in the spread of *T. gondii*, contaminating the environment with oocysts, the dispersive forms of this parasite. Due to natural phenomena, oocysts are spread over a considerable area which may also lead to the contamination of surface and ground water. In this context, shallow household wells which are often the source of water supply in rural areas of eastern Poland and are not covered by examinations for pathogenic protozoans, pose a potential risk of infection.

Hence, the objectives of this study were following:

• To determine the prevalence of seropositive reactions to *Toxoplasma gondii* in farm inhabitants and domestic animals.

• To determine the frequency of occurrence of *Toxoplasma gondii* in drinking water from wells and water supply systems, and in recreational waters in the rural areas of the Lublin province, with respect to a potential health risk for humans and domestic animals.

• To evaluate the role of water in the transmission of *Toxoplasma* infection in the rural environment.

The present study is a continuation of the previous one from 2006 [30], and concerns the next areas of the Lublin province.

MATERIALS AND METHODS

Study area

Examined farms. Studies of drinking water were conducted on a total number of 164 farms in 35 villages located in 12 districts of the Lublin province (eastern Poland), with special consideration of the areas where high rates of seropositive reactions were noted in earlier examinations of humans and animals (districts of Włodawa, Parczew, and Chełm). A total number of 182 samples of drinking water was examined from the following intakes: 121 household wells, 33 drilled wells, and 28 water supply system intakes. Among household wells, 88 intakes were shallow wells with a surrounding casing of concrete (depth up to 7 m) and a manual winch, while the 33 remaining wells were 7–19 m deep. The majority of wells were secured only by a provisional wooden cover. 33 intakes were deeper (below 20 m) drilled wells, where water was pumped by means of a manual or electric pump. The samples from 28 intakes of the water supply system were taken from taps inside houses.

Recreational waters. Water samples were taken from 19 bathing places on the territory of the Łęczna-Włodawa Lakeland. The sites were located at 17 lakes (Piaseczno, Rogóźno, Łukcze, Krasne, Białe, Białe Uścimowskie, Maśluchowskie, Skomelno, Tomaszcze, Czarne, Zagłębocze, Wytyckie, Miejskie, Bialskie, Czarne, Firlej, Jezioro), and at 2 ponds (Wyklik, Siemień).

Questionnaire survey. A survey was conducted among 254 inhabitants of farms, aged 8–75 yrs, mean age 45.2 ± 20.4 yrs. The group constituted 154 women (60.6%) and 100 men (39.4%). The mean age of women was 44.4 ± 20.0 yrs and of men 46.5 ± 21.0 yrs.

The survey concerned: a) people living on a farm, economic status, occurring diseases, nutritional habits; b) animals kept on farm, occurring diseases; c) water intakes, ways of using water, frequency of its use.

Serologic studies of people and animals living on farms

Serologic examination of people living on farms. A total of 254 farm inhabitants subjected to questionnaire study and characterized above were examined. Reference group consisted of 39 healthy persons living in the city of Lublin, aged 23–59 yrs, mean age 37.7 ± 10.2 yrs. The reference group constituted 30 women (mean age 37.6 ± 11.0 yrs) and 9 men (mean age 38.1 ± 6.8 yrs).

Blood serum samples taken from the inhabitants of farms and members of the reference group were examined by immunoenzymatic tests ELFA (Enzyme Linked Fluorescent Assay) for the presence of IgG and IgM antibodies against *Toxoplasma gondii* (Vidas Toxo IgG and Vidas Toxo IgM, bioMérieux, Marcy l'Etoile, France). To perform the tests by the ELFA technique, a Mini VIDAS device was used.

Serologic examination of animals kept on farms. A total of 489 domestic animals belonging to 11 species were examined. The group constituted of: 74 cows, 67 pigs, 44 cats, 69 dogs, 14 horses, 7 goats, 5 rabbits and 209 specimens of poultry, comprising 173 hens, 33 ducks, 2 turkeys and 1 goose (Tab. 2).

Blood serum samples taken from the animals were examined by the direct agglutination test for the presence and level of IgG antibodies against *Toxoplasma gondii*, using commercial kit (Toxo-Screen DA, bioMérieux, Marcy l'Etoile, France).

Study of water

Preparation of water samples. A total of 182 water samples were taken into plastic cans with a volume of 50 litres. *T. gondii* isolation trials were performed based on the method described by Isaac-Renton *et al.* [19]. The samples of water were filtered through cellulose filters with a pore diameter of 0.45 μm. The filters were washed with phosphate buffer (PBS) of pH 7.8, with the addition of 0.01% Tween 80, which was centrifuged for 4 min at 1,050 × g. The pellet obtained was suspended in 20 ml of distilled water. Further isolation was conducted by the flotation method, with the use of sugar solution of the following composition: 53 g saccharose, 0.8 ml phenol, 100 ml distilled water (specific gravity – 1.15 g). The pellet suspension was first added to 30 ml of sugar solution and centrifuged for 4 min at 1,050 × g, after which 25 ml of the liquid was sampled from the surface, transferred to a new test-tube, and 75 ml PBS containing 0.01% Tween 80 was added. After final centrifugation for 10 min at 1,050 × g, the supernatant was removed, and the sediment obtained was preserved for further studies.

Microscopic examination of water sediment. Sediment samples were suspended in a small amount of 0.9% NaCl (in the proportion 1:2) and examined under a microscope for the presence of *T. gondii* oocysts, using 200 × and 400 × magnifications. From each sample 5 preparations were made.

Bioassay on mice. Swiss-Webster females at the age of 3 weeks were used. Before testing of each batch, circa 10% mice were randomly selected and their sera were examined by the direct agglutination test for the presence of anti-*Toxoplasma* antibodies. All the examined mice proved to be seronegative.

Samples of water sediments in which the presence of *T. gondii* was stated by PCR test and/or microscopic examination were selected for testing. Each sample was suspended in the proportion 1:5 in 0.9% NaCl containing antibiotics (1,000 U of penicillin and 100 μg of streptomycin per 1 ml). The suspension was given orally by stomach tube to 3 mice in the volume of 0.5 ml per mouse. Mice were observed daily, and in the case of appearance of clinical symptoms (apathy, depression, diarrhea), they were killed and sectioned. The specimens which died were also subjected to section. During sections, samples of peritoneal exudate, internal organs (liver, spleen, lungs) and brain were taken for PCR test and histopathological examination, and blood was drawn for serologic examination (direct agglutination test). The remaining, healthy mice were killed and sectioned 6 weeks after inoculation, and the samples of peritoneal exudates and internal organs were examined as described above. Everywhere, several blind passages were performed, in which the next batch of mice were injected intraperitoneally with the suspension of homogenized organs from sectioned mice, after addition of antibiotics.

Polymerase Chain Reaction test (PCR). DNA isolation from water sediment samples was performed using a commercial kit (QIAmp DNA Mini Kit, Qiagen, Syngen Biotech, Wrocław, Poland), according to manufacturer's instruction.

Detection of *T. gondii* DNA based on amplification of 35-fold-repetitive gene B1 fragment in 2 following nested PCR reactions was performed with the method described by Grigg and Boothroyd [14]. The reaction mixture (50 μl) contained: 1.5 U Tag DNA polymerase (Qiagen, Syngen Biotech, Wrocław, Poland), 5 μl of the 10 × PCR Buffer containing 1.5 mM $MgCl_2$, 0.2 mM dNTPs (Polgen, Łódź, Poland), 1 μl of each of the primers 10 mM: in the first reaction Pml/S1 (5'-TGTTCTGTCCTATCGCAACG) and Pml/AS1 (5'-ACGGATGCAGTTCCTTTCTG), and in the second nested-PCR reaction – Pml/S2 (5'-TCTTCCCAGACGTGGATTTC) and Pml/AS2 (5'-CTCGACAATACGCTGCTTGA) (Eurogentec, Seraing, Belgium), nuclease-free water (Applied Biosystems, Warsaw, Poland) and 5 μl matrix DNA.

Two-stage PCR reaction consisted of 30 and 20 cycles. Each cycle included: the proper denaturation at 94°C for 30 sec, primers annealing at 60°C for 30 sec, and elongation at 72°C for 90 sec. The reaction products of stage I amplifications (5 μl) were used at stage II PCR. Additionally, at each stage, the initial denaturation (2 min at 94°C), and the final elongation (2 min at 72°C) were performed. The 531 bp-long products of the second amplification were detected in 2% agarose gel (Prona, Basica L.E.) after staining with a water solution of ethidium bromide. The *Toxoplasma gondii* strains: RH (type I), ME49 (type II), and C56 (type III) were used as positive control, while nuclease-free water (Applied Biosystems, Warsaw, Poland) was used as a negative control.

To identify the clonal type (I or II/III) of the isolated *T. gondii* strains, RFLP PCR was performed. The PCR amplification products were treated with restriction enzymes: Eco 721 and Xhol (Fermentas, Vilnius, Lithuania), and the reaction products detected in 2% agarose gel.

DNA sequencing was performed at the DNA Sequencing and Oligonucleotides Laboratory (Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Warsaw, Poland) and at the Institute of Agricultural Medicine in Lublin, Poland. The results were compared to published sequences in the GenBank database using the BLAST server at the National Center for Biotechnology Information (Bethesda, Maryland, USA).

Statistical analysis. The data was analysed with chi-square (χ^2) test and t-Student's test using Statistica 8.0 package (Statsoft Inc., Tulsa, OK, USA).

RESULTS

Questionnaire study

All the examined 254 people lived on farms and had everyday contact with animals, and all adults reported

the agricultural profession. 10 individuals had in the past symptoms suggesting the nodal form of toxoplasmosis (one person was treated for this disease), and 7 women had disorders during pregnancy. All 17 persons had anti-*Toxoplasma* antibodies of IgG class. The inhabitants of 73 out of 164 farms reported drinking unboiled water, while inhabitants of 54 farms reported consumption of raw meat. The inhabitants of 30 farms reported biting by ticks in the past.

The majority of examined farms were small, with free-run rearing of poultry and seasonal grazing of cattle and goats near farm buildings. On these farms, the animals were kept in improper zoohygienic conditions and fed with self-produced fodder. It was stated that cats kept on farms had free access to human residential areas, as well as to animal houses and fodder stores. In 14 farms there occurred in the past cases of disease in animals, associated with abortions and/or deaths of cows, calves, piglets, horses and cats. On the majority of farms, the household wells were used not only for consumption by people and animals, but also for other purposes, such as watering of gardens.

Serologic response of people and animals to *Toxoplasma gondii*

Serologic response of people. 170 out of 254 examined farm inhabitants (66.9%) showed the presence of anti-*Toxoplasma* antibodies of IgG class, while only 5 (2.0%) showed the presence of antibodies of IgM class (Tab. 1). Most of the IgG-dependent positive results were weakly or mediocre positive (50.6% and 47.6%, respectively), while only a small proportion (1.8%) was strongly positive (Tab. 1). Prevalence of seropositive results in men and women was similar (66.0% *vs* 67.5%). Rates of positive reactions in women living on farms and total farm inhabitants were significantly greater compared to the reference group (respectively, 40.0% and 41.0%, $p<0.01$). The difference was not significant in the case of men from the reference group (44.4%, $p=0.19$).

Table 1. Prevalence of seropositive reactions to *Toxoplasma gondii* in farm inhabitants *vs* reference group assessed by ELFA test.

Group	IgG-dependent reactions number of positive (percent)				IgM-dependent reactions number of positive (percent)
	Weak <60 IU	Mediocre 60–300 IU	Strong >300 IU	Total	Total
Farm inhabitants					
Women N=154	57 (37.0)	46 (29.9)	1 (0.6)	104 (67.5)*	3 (1.9)
Men N=100	28 (28.0)	36 (36.0)	2 (2.0)	66 (66.0)	2 (2.0)
Total N=254	86 (50.6)	81 (47.6)	3 (1.8)	170 (66.9)*	5 (2.0)
Reference group					
Women N=30	6 (20.0)	6 (20.0)	0	12 (40.0)	0
Men N=9	2 (22.2)	2 (22.2)	0	4 (44.4)	0
Total N=39	8 (20.5)	8 (20.5)	0	16 (41.0)	0

IU – International Units; N – number of examined persons; *significantly greater compared to reference group ($p<0.01$).

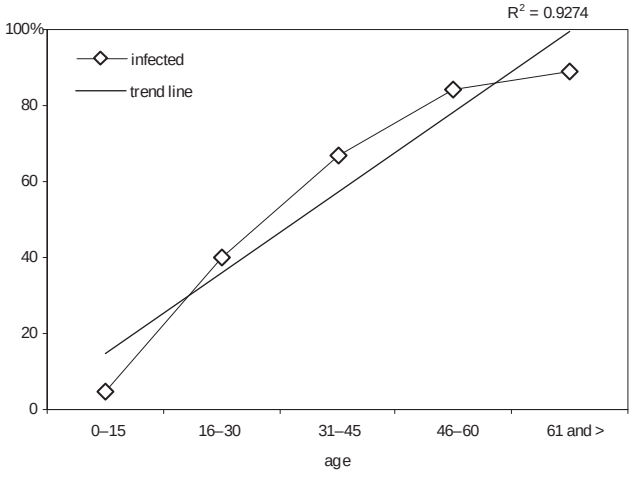

Figure 1. Correlation between age of examined farm inhabitants and prevalence of positive serologic reactions to *Toxoplasma gondii*.

A highly significant positive correlation was found between the age of examined farm inhabitants and the rate of positive reactions with *Toxoplasma* antigen (Fig. 1). The mean age of seropositive farm inhabitants was significantly greater compared to that of seronegative ones: in the case of women it was 50.8 *vs* 30.9 yrs ($p<0.0001$), in the case of men – 54.5 *vs* 31.0 yrs ($p<0.0001$), and in the case of the total examined – 52.3 *vs* 30.9 yrs ($p<0.0001$).

Moderate higher rates of seropositive results occurred among the people on these farms, where cases of disease in animals (associated with deaths and/or abortions) were reported in the past, but difference was not significant (69.3% *vs* 66.9%, $p=0.83$).

No significant difference was found between the rates of positive reactions to *Toxoplasma* in the inhabitants of these farms in which *T. gondii* DNA was found in water, and the remainder (57.4% *vs* 69.2%, $p>0.05$). Similarly, the rates of positive reactions to *Toxoplasma* in the group of farm inhabitants using wells compared to those using water supply system did not show a significant difference (66.0% *vs* 67.8%, $p>0.05$). In farm inhabitants reporting consumption of unboiled water the rate of positive anti-*Toxoplasma* reactions was greater compared to those drinking boiled water, but the difference did not attain significance level (67.9% *vs* 61.3%, $p>0.05$).

Serologic response of animals. A total of 177 out of 489 examined animals (36.2%) showed seropositive reactions to *Toxoplasma gondii* (Tab. 2). The highest rates

Table 2. Prevalence of seropositive reactions to *Toxoplasma gondii* in domestic animals assessed by direct agglutination test.

Animal species		Titers of positive reactions number of positive (percent)			
	N	Low 40–60	Mediocre 180–540	High >1620	Total
Pigs	67	3 (4.5)	4 (6.0)	5 (7.4)	12 (17.9)
Cows	74	22 (29.7)	0	3 (4.1)	25 (33.8)
Cats	44	0	3 (6.8)	30 (68.2)	33 (75.0)
Dogs	69	16 (23.2)	12 (17.4)	9 (13.0)	37 (53.6)
Horses	14	0	0	0	0
Goats	7	1 (14.3)	0	4 (57.1)	5 (71.4)
Rabbits	5	0	0	0	0
Hens	173	17 (9.8)	26 (15.0)	15 (8.7)	58 (33.5)
Ducks	33	5 (15.2)	0	2 (6.0)	7 (21.2)
Turkeys	2	0	0	0	0
Geese	1	0	0	0	0
Total	489	64 (13.1)	45 (9.2)	68 (13.9)	177 (36.2)

N – number of examined animals.

Table 3. Occurrence of *Toxoplasma gondii* in water samples taken from water intakes located on farms.

Method of detection	Positive samples: number (percent)			
	Shallow wells	Deep wells	Water supply system	Total
N	121	33	28	182
Microscopic examination	21 (17.4)	2 (6.1)	0	23 (12.6)
PCR	31 (25.6)	5 (15.2)	5 (17.9)	41 (22.5)
Bioassay on mice*	4 (28.6)	3 (21.4)	2 (14.3)	9 (64.3)**

N – number of examined samples.
* Only 14 samples positive in PCR test were examined.
** Result of PCR testing of tissues and/or peritoneal exudate of mice inoculated with sediments of positive water samples. By microscopy, toxoplasmas were found in mice tissues and/or peritoneal exudate only in 5 out of 14 water samples (35.7%).

of positive results were noted in cats (75.0%) and goats (71.4%). The lower response rates were found in dogs (53.6%), cows (33.8%), hens (33.5%), ducks (21.2%), and pigs (17.9%). No positive results were noted in 14 horses, 5 rabbits, 2 turkeys and 1 goose. Most of the animals reacted in high titers (13.9%), less in mediocre (9.2%) and low titers (13.1%) (Tab. 2).

No significant difference was found between the frequencies of positive results in animals on farms where *T. gondii* DNA was detected in water by PCR, compared to farms with negative results of water examination (38.1% *vs* 35.7%, $p>0.05$). Similarly, no significant difference was noted between the frequencies of positive results in animals on farms using well water, compared to those using water supply system (35.0% *vs* 39.3%, $p>0.05$).

Studies of water

Microscopic examination. Among a total number of 182 drinking water samples, in 23 samples (12.6%) the presence of individual oocysts of the size 9–10 × 12–13 µm was noted, which were considered as *Toxoplasma gondii* oocysts. These oocysts possessed a double wall and variable internal structure: from homogenous, granular protoplasm to visible outlines of 2 sporocysts. 21 positive water samples came from shallow wells, 2 from deep drilled wells and none from intakes of the water supply system (Tab. 3). The prevalence of *Toxoplasma* in shallow wells was significantly greater than in intakes of the water supply system ($p<0.05$).

PCR test. Among 182 water samples examined, the presence of *T. gondii* DNA was detected in 41 samples (22.5%), including: 31 samples from shallow wells (25.6%), 5 samples from deep wells (15.2%), and 5 samples from intakes of the water supply system (17.9%). No significant differences were found between the frequencies of *T. gondii* in water from different sources.

The presence of *T. gondii* DNA in the PCR-positive samples was confirmed by sequencing. Using RFLP-PCR, the clonal types of 41 positive samples were determined. Most of the *T. gondii* DNA isolates (32 samples) were classified into the virulent for mice type I, and 3 isolates into type II/III. The remaining 6 isolates were classified as "atypical" (Tab. 4).

Among 19 water samples taken from bathing places on the territory of the Lublin province, 2 samples positive for *T. gondii* (10.5%) were found by microscopic examination, and confirmed by PCR. Using the RFLP-PCR analysis,

Table 4. Prevalence of clonal types of *T. gondii* (determined by RFLP-PCR) in water samples from water intakes located on farms, depending on type of intake.

Clonal type of *T. gondii*	Water intake: number of positive samples (percent)				
	Shallow wells <7 m deep	Shallow wells 7–19 m deep	Deep wells >20 m deep	Water supply system intakes	Total
N	26	5	5	5	41
Type I	20 (77.0)	4 (80.0)	4 (80.0)	4 (80.0)	32 (78.0)
Type II/III	3 (11.5)	0	0	0	3 (7.3)
Atypical (not determined)	3 (11.5)	1 (20.0)	1 (20.0)	1 (20.0)	6 (14.7)
Total	26 (100)	5 (100)	5 (100)	5 (100)	41 (100)

N – number of examined samples.

it was determined that one *T. gondii* isolate (from Białe Lake) pertained to the clonal type II/III while the other (from Maśluchowskie Lake) pertained to type I.

Bioassay on mice. Toxoplasmas (tachyzoites or bradyzoites) were found microscopically in tissues and/or peritoneal exudate in mice inoculated with 5 out of 14 sediments of water samples found earlier to be positive in PCR test for *T. gondii*. All microscopy findings were confirmed by PCR test, which was also positive in mice inoculated with further 4 sediments, so altogether, bioassay on mice proved to be positive in 9 out of 14 PCR-positive water samples (64.3%). The positive result of PCR test for mice tissues and/or peritoneal exudates was obtained in 8 samples only in the first passage, and in 1 sample also in the second passage. All results for the third passage were negative. The results of serological examination were positive only in mice inoculated with 3 out of 14 water samples, in titers ranging from 64–540.

The strains of *T. gondii* isolated from 2 water sediment samples pertained to clonal type I (virulent for mice) and an atypical one, causing quick deaths of mice after inoculation. The results of serological tests in these mice were negative or doubtful. The mice inoculated with water sediments from which *T. gondii* of II/III type was isolated, survived in good condition 6 weeks until the section, and the presence of *T. gondii* was confirmed by PCR and/or microscopy only in brain tissue.

DISCUSSION

The results of this study show that people living on farms are infected with *Toxoplasma gondii* significantly more often than urban dwellers, and that the infection rate in farm inhabitants increases significantly with age. Similar relationships were demonstrated by Studeničová *et al.* [33], Nash *et al.* [23], Bibic *et al.* [4] and Sroka [29]. The lack of these correlations were observed by Studeničová *et al.* [32] and Sousa *et al.* [27]. Other results of the present work indicate that one of the reasons for the common occurrence of *Toxoplasma* infection among farm inhabitants may be the close contact with domestic animals (and with their tissues) which could be infected with *T. gondii* at high rates.

Because of the common meat consumption, slaughter animals are regarded as an important source of *T. gondii* infection in Europe [8]. The results obtained in this study confirmed the significant role of pigs in the epidemiology of toxoplasmosis (infection rate amounted to 17.9%). The percentage of positive results detected in pigs is higher than previously obtained in the same areas (10.5%) [31] and in Wielkopolska region (Poland) [25]. The high rates of seropositive results obtained in this study among cats and goats populations (respectively, 75.0% and 71.4%) showed that these species may also play an important role in the transmission of this parasite in the rural environment of Lublin region. As poultry meat is usually frozen, it does not constitute a frequent source of infection for humans. Similarly, cattle, despite the high seropositivity noted, are regarded as a rare source of infection [35].

Considering the potential sources of toxoplasmic infection both for the people and animals living on farms, special attention must be paid to potable water. On the world scale, a number of waterborne cases of toxoplasmosis in humans have been described [2, 3, 6, 16]. However, a relatively small number of studies have been devoted to the transmission of *Toxoplasma* by water, largely because of methodological difficulties. The application of PCR, as a method more sensitive compared to microscopy, increased the effectiveness of detection of parasites in water [20, 21, 36]. Nevertheless, most researchers are of opinion that people contract toxoplasmosis mainly by eating raw or undercooked meat containing cysts of *Toxoplasma*, or by consumption of products contaminated with faeces of sick cats, containing oocysts [10, 34, 35].

In the earlier work [30] we found a significant correlation between the consumption of unboiled well water and the presence of specific anti-*Toxoplasma* antibodies in farm inhabitants, particularly on farms of poor hygienic state, including shallow wells. Similarly, other authors analyzing cases of *T. gondii* infection in various populations, also noted a significant correlation between the drinking of unboiled water and occurrence of *Toxoplasma* invasion in examined people [9, 12, 22, 24]. The demonstration in the present work of the considerable frequency of *T. gondii* in the samples of potable water taken on farms (12.6% by microscopy and 22.5% by PCR) suggests that water may play an important role in spreading of toxoplasmosis in the rural environment. These findings were confirmed by the isolation from water samples of the virulent *T. gondii* strains in mice. However, in this work we were not able to find a significant relationship between the presence of *T. gondii* in water and the occurrence of seropositive reactions in farm inhabitants and domestic animals. This suggests that for the majority of examined people and animals the sources of *Toxoplasma* invasion could probably be different from potable water.

Depending on variability in genetic material and virulence for mice, 3 main clonal types are distinguished among the *T. gondii* strains: type I (virulent for mice), and types II and III (avirulent for mice) [14, 18]. Strains of type II are responsible mainly for invasions in animals, whereas strains isolated from human clinical cases often belong to type I or type III [5, 17, 18]. More recently, also so called "atypical" strains have been distinguished [1, 13, 15] which could be found as dominant, mostly in studies of the environment [7, 11]. In the present study, genotyping of the *T. gondii* DNA isolates based on the detection of the differences in the fragment of B1 gene, allowed for the differentiation of type I from type II and/or III [14]. The majority of the isolates from water samples (78.0%) were classified into type I, while strains II/III and atypical were less common (7.3% and 14.7%, respectively). The prevalence of virulent type I in water samples could be recognized as hazardous from

the epidemiological point of view. Nevertheless, for the full characteristics of the isolated *T. gondii* strains an analysis based on the greater number of markers is recommended, for example: B1, SAG1-SAG3, GRA6, L363. Genotyping of *Toxoplasma* strains is regarded by leading researchers as a valuable part of epidemiological investigation and an important marker of health hazard for the people [37].

In spite of the lack of a significant relationship in this study between the presence of *T. gondii* in water and occurrence of seropositive reactions in farm inhabitants and domestic animals, the role of potable water in the spreading of toxoplasmosis in rural areas cannot be excluded. On most of the farms, simultaneous infections occurred in several farm inhabitants and/or in the majority of domestic animals, which indicates the existence of the infection sources, including water intakes.

To summarize, the results of the present study and those by other authors justify the necessity to implement the sanitary monitoring of rural household wells and to supplement the scope of routine examinations of water from water supply systems by studies for parasitic protozoans. The implementation of preventive measures on farms at risk would also be important.

CONCLUSIONS

1. People living on farms showed a significantly greater serological response to *Toxoplasma gondii* than healthy urban dwellers constituting the reference group.

2. Among examined species of animals the greatest frequency of seropositive reactions to *T. gondii* occurred in cats, goats and dogs, less so in cattle, poultry and pigs.

3. The presence of *T. gondii* was found in nearly one quarter of potable water samples taken from water intakes on farms, and the majority of isolated *Toxoplasma* strains belonged to clonal type I, which is most virulent for humans and animals. The lack of a significant relationship between the presence of *T. gondii* in water and the occurrence of seropositive reactions in farm inhabitants and/or domestic animals suggest that apart from potable water, other important sources of infection also exist in the surroundings of examined people and animals.

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