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OCCURRENCE OF *TOXOPLASMA GONDII* IN WATER FROM WELLS LOCATED ON FARMS

Jacek Sroka, Angelina Wójcik-Fatla, Jacek Dutkiewicz

Department of Occupational Biohazards, Institute of Agricultural Medicine, Lublin, Poland

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Abstract: Among various species of parasitic protozoans which may contaminate drinking water, *Toxoplasma gondii* is of a special importance due to the high incidence of infections with this parasite noted in animals and humans. The objective of this study was to determine the frequency of occurrence of *T. gondii* in drinking water on farms in the area of the Lublin province (eastern Poland) with respect to health risk among the inhabitants, and to assess the role of water in the transmission of *Toxoplasma* infections in the rural environment. Studies were conducted on 87 farms located in the Lublin province, 14 of which were classified as possessing a good hygienic state, and 73 as possessing a poor hygienic state. A total number of 114 drinking water samples were taken, 80 samples from shallow household wells with a windlass, 16 from deep wells with a pump, and 18 from the water supply system. In microscopic and PCR examinations of 114 water samples, *T. gondii* was found in 15 (13.2%) and 31 (27.2%) of samples, respectively. The presence of *T. gondii* DNA detected by PCR test was found significantly more frequently in water samples from the shallow windlass-operated wells than in those from deep wells ($p<0.05$) and water supply system ($p<0.01$). Water samples collected from shallow wells located on farms of poor hygienic state contained significantly more frequently DNA of *T. gondii* than samples from shallow wells located on farms of good hygienic state (43.1% *vs.* 13.3%, $p<0.05$). In 26.3% of water samples, oocysts of other protozoans were found belonging to *Isospora*, *Eimeria*, and *Cryptosporidium*. Serologic examinations for the presence of anti-*Toxoplasma* antibodies conducted among 99 inhabitants of the farms where household wells were used showed 64.6% of seropositive results in IgG class antibodies and 1.0% in IgM class antibodies. Clinical cases of toxoplasmosis were also noted. In the total population examined, a positive correlation was observed between the consumption of unboiled well water and the presence of antibodies against *T. gondii* ($p<0.05$), this correlation being especially strong on farms of poor hygienic state enclosing shallow wells ($p<0.001$). In conclusion, the recorded presence of *T. gondii* in well water provides an evidence of the potential risk of waterborne infection for humans and animals. Therefore, it seems necessary to implement prophylactic actions on the endangered farms.

Address for correspondence: Jacek Sroka, DVM, Department of Occupational Biohazards, Institute of Agricultural Medicine, P.O. Box 185, 20-950 Lublin, Poland.
E-mail: jack@galen.imw.lublin.pl

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INTRODUCTION

In recent years, a number of waterborne epidemics caused by parasitic protozoans, e.g. giardiasis, cryptosporidiosis and toxoplasmosis, were noted worldwide [3, 4, 10, 12]. In Poland, little is known to date about the contamination of drinking water (water supply system and well water) with dispersive forms of these parasites.

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Studies conducted during the period 2000-2001 in Poznań and vicinity showed the presence of pathogenic protozoa in surface water [13]. In recent years, other Polish researchers have also paid attention to this scope of problems [15, 16].

Among various species of parasitic protozoans which may contaminate drinking water, *Toxoplasma gondii* is of special importance due to the considerable frequency of infections with this parasite noted among humans and animals. *T. gondii* invasion may be especially dangerous for pregnant women with respect to the possibility of occurrence of congenital defects in the foetus, as well as for people with decreased immunity, in whom it may lead to severe systemic changes or even death.

The results of own studies, as well as data from literature [18, 19], indicate that *Toxoplasma gondii* infection may constitute a serious epidemiological problem, especially in the rural environment where there are many cross routes of spreading of this protozoan. Cats play a special role in the spread of *T. gondii* infection, 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 waters. In eastern Poland, shallow household wells, into which may penetrate chemical and biological contaminants from the nearest surroundings, are frequently the source of water supply in rural areas. Such wells are not covered by constant monitoring, which is applied at the intakes of water supply systems; however, these intakes are also not covered by examinations for pathogenic protozoans. Well water is neither treated nor filtered, hence the presence of invasive forms of the parasite in it may constitute a serious health risk for the inhabitants of farms and animals bred on these farms.

The objective of this study was to determine the frequency of occurrence of *T. gondii* in drinking water on farms with respect to health risk for inhabitants, and to evaluate the role of drinking water in the transmission of *Toxoplasma* infection in the rural environment.

MATERIALS AND METHODS

Study area

Examined farms. Studies of drinking water were conducted on a total number of 87 farms in 32 villages located in the area of 10 districts of the Lublin province (eastern Poland) (Fig. 1).

On 14 farms (F1), studies of water were carried out in the years 2001-2003 within the comprehensive study concerning humans, animals, and elements of the environment associated with the occurrence on these farms of toxoplasmosis of a family character. The hygienic conditions on these farms were satisfactory, and household wells were usually enclosed and properly secured.

On the remaining 73 farms (F2), studies of water were performed in the years 2004-2005. These farms were selected for the study on the basis of the following criteria: poor hygienic state of the farm, lack of protection of well and presence of a cat.

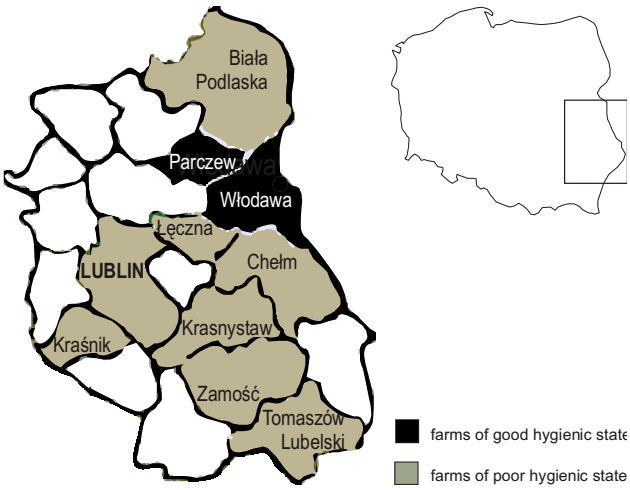

Figure 1. Map showing area of study comprising 10 districts in Lublin province.

A total number of 114 samples of drinking water were examined. The samples were taken from: 96 wells and 18 water supply system intakes; 80 intakes were shallow household wells with a surrounding casing of concrete (depth up to 7 m) and a manual winch (F1 – 15, F2 – 65). From these wells water is taken from the first, the shallowest water-bearing level, located directly under the soil layer and not covered by impermeable layers. The majority of wells were secured only by a provisional wooden cover. On some farms, the water taken was contaminated with leaves, fragments of branches and sand. 16 intakes (F2) were deeper (below 10 m) drilled wells, where water was pumped by means of a manual or electric pump. The samples from 18 intakes of water supply system (F1 – 10, F2 – 8) were taken from the tap inside houses.

Questionnaire survey. A survey was conducted among 147 inhabitants of farms concerning water usage, animals kept on the farms and diseases occurring in animals and humans living on the farms. Information was gathered concerning ways of using well water, frequency of its use and the use of other sources of water, e.g. water supply system. Special attention was paid to the issue whether inhabitants of a farm drank (consumed) unboiled well water or not. 76 farms in the study (F1 – 10, F2 – 66) were connected to the water supply system; however, household wells were still used for watering animals and garden, as well as for consumption purposes. On 11 farms (F1 – 4, F2 – 7) the only source of water was a household well.

Study of water

Preparation of water samples. Water samples were taken into plastic cans of the volume of 5 litres. *T. gondii*

isolation trials were performed based on the method described by Isaac-Renton *et al.* [8]. 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 then centrifuged for 10 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 10 min at 1,050 × g, then 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 PBS and examined under the microscope for the presence of *T. gondii* oocysts, using 200 × and 400 × magnifications. From each sample 5 preparations were made.

Polymerase Chain Reaction test (PCR). DNA isolation was performed using the commercial kit (Genomic Mini, A&A Biotechnology, Gdynia, Poland), according to manufacturer's instruction. DNA was isolated from water sediment by lysis in the lysing LT buffer containing proteinase K (15 hrs at 50ºC). Then the material was placed on a mini-column with a silica bed. The isolated DNA was washed out by means of low-ion buffers.

Amplification of *T. gondii* DNA was performed using PCR kit obtained from DNA-GDAŃSK II s.c. (Gdańsk, Poland). Detection of *T. gondii* DNA was based on amplification of gene fragment coding 65 kDa antigen protein in two subsequent reactions with the same pair of primers. Primers, polymerase Delta 2, deoxynucleotides and other ingredients of reaction mixture, positive control (genomic DNA of the RH strain of *Toxoplasma gondii*), and a marker M100-500 were included in the kit.

The size of the amplified fragment was 262 base pairs. Amplification and reamplification reactions were carried out in the PTC-150 thermal cycler (MJ Research, Inc., Waltham, MA, USA). Samples were initially denatured for 2 min at 94ºC. Subsequent cycles were at 94ºC for 30 sec (denaturation), 64ºC for 1 min (annealing), and 72ºC for 30 sec (extension). 35 amplification cycles and 30 reamplification cycles were performed. Then, final extension was applied for 2 min at 72ºC.

For the analysis of PCR amplification products, 13 µl aliquots of reaction mixtures, marker, positive control and negative control (redistilled water) were applied to 2.0% agarose gels (Basica LE, Prona, EU) with Tris-Borate-EDTA (pH 8.2) as running buffer and electrophoresis was performed for 55 min at 110 V. DNA bands were stained with ethidium bromide and visualised by UV transillumination. Achieved specific products of 262 base pairs were considered as a positive result.

Serologic studies of inhabitants of farms

Population examined. In order to evaluate the effect of drinking water on the health of rural population with respect to risk of *T. gondii*, 39 farms were selected (F1 – 14, F2 – 25) with 40 wells (F1 – 15, F2 – 25), and the inhabitants of these farms were subjected to serologic tests for the presence of antibodies against *T. gondii*. A total number of 99 people were examined (F1 – 57, F2 – 42), among which females constituted 59.6% (F1 – 57.9%, F2 – 61.9%). People in the study were aged 8-87, mean age was 44.8 ± 22.5 (F1 – 36.6 ± 19.6, F2 – 55.9 ± 21.6). 26 people (F1 – 2, F2 –24) lived on 15 farms (F1 – 1, F2 – 14) where the result of microscopic and/or PCR examination of water for the presence of *T. gondii* was positive (further marked as W+), and 73 people (F1 – 55, F2 – 18) came from 24 farms (F1 – 13, F2 – 11) where the parasite was not found in well water (further marked as W-). All 99 people were subjected to questionnaire study.

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

Statistical analysis. The data were analysed by χ^2 test, t-Student test, Spearman test, and Mann-Whitney test, with the use of STATISTICA for Windows v. 5.0 package (StatSoft Inc., Tulsa, Oklahoma, USA).

RESULTS

Studies of water

Microscopic examination. Among a total number of 114 drinking water samples, in 15 samples (13.2%) the presence of individual oocysts of the size 9-10 × 12-13 µm was noted, which were considered as *T. gondii* oocysts (Fig. 2). These oocysts possessed a double wall and variable internal structure: from homogenous, granular protoplasm to visible outlines of 2 sporocysts. All positive water samples came from shallow wells (SW) (Tab. 1). In microscopic examination of water samples from deep wells (DW) and water supply system (WSS) the presence of *Toxoplasma gondii* oocysts was not observed. In 30 (26.3%) water samples (29 SW and 1 DW) oocysts of *Isospora*, *Eimeria* and *Cryptosporidium* genera were noted (Tab. 1). The oocysts were found significantly more frequently in water samples from shallow wells than in those from deep wells and water supply system ($p<0.05$) (Tab. 1).

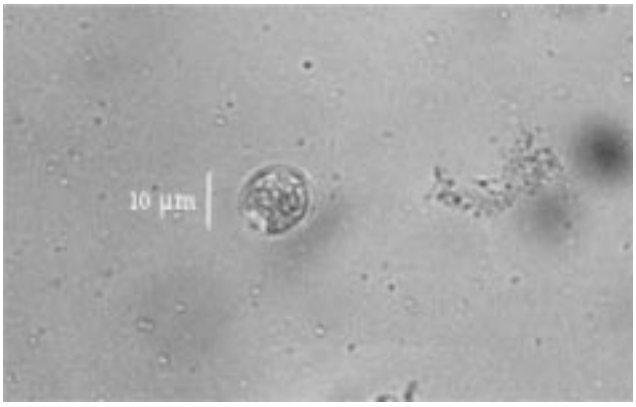

Figure 2. Structure corresponding to *Toxoplasma gondii* oocyst in the sediment of well water, sample F2/7, × 700.

Among 25 water samples from the farms F1, in 2 (8.0%) samples (SW) individual oocysts, were found which were considered as *T. gondii* oocysts on the basis of their morphometric features. In 8 (32.0%) water samples (SW) the presence of oocysts of *Isospora* spp. and *Eimeria* spp. was noted.

Among 89 water samples from farms F2, in 13 samples (14.6%) the presence of individual oocysts was observed, which were considered as *T. gondii*. All positive samples of water came from shallow wells. In 22 (24.7%) water samples (21 SW and 1 DW) the oocysts of *Cryptosporidium* spp., *Isospora* spp. and *Eimeria* spp. were found (Tab. 1).

PCR test. Among 114 water samples examined, the presence of *T. gondii* DNA was noted in 31 samples (27.2%) (30 SW and 1 DW). All samples of water from a water supply system were negative. All 15 samples of well water, positive in microscopic examination for the presence of *T. gondii* oocysts, were also positive in PCR test. The presence of *T. gondii* DNA, with a simultaneous negative result in microscopic examination, was observed in 16 samples (15 SW and 1 DW); in only 2 of these samples other species of protozoans were found. In 5 samples of well water with positive results of both PCR test and microscopic examination for the presence of *T. gondii* oocysts, also oocysts of other protozoan species were noted. In the case of 23 samples (22 SW and 1 DW), where only oocysts of other genera of protozoans were found in microscopic examination, the result of PCR test for the presence of *T. gondii* DNA was negative.

The presence of *T. gondii* DNA was found significantly more frequently in water samples from the shallow windlass-operated wells than in those from deep wells ($p<0.05$) and water supply system ($p<0.01$) (Tab. 1).

Among 25 water samples from farms F1 examined by PCR, the presence of *T. gondii* DNA was observed in 2 (8.0%) samples (SW). In these 2 samples, the presence of the oocysts of *T. gondii* and of other protozoans (*Isospora* spp. and/or *Cryptosporidium* spp.) was noted in the microscopic examination. In the case of 6 samples from SW, in which only oocysts of other protozoans were found in microscopic examination, the PCR test result for the presence of *T. gondii* DNA was negative.

Among 89 water samples from farms F2 examined by means of the PCR test, the presence of *T. gondii* DNA was noted in 29 samples (32.6%) (28 SW and 1 DW). The parallel positive results of PCR test and microscopic examination for the presence of *T. gondii* were obtained in the case of 13 samples SW. In 3 of them, also oocysts of *Isospora* spp. and/or *Cryptosporidium* spp. were found. The presence of *T. gondii* DNA, with a simultaneous negative result of microscopic examination, was observed in 16 samples (15 SW and 1 DW); in 2 of them (SW) the presence of other protozoans was noted. In 17 samples (16 SW and 1 DW), where only oocysts of other protozoans were found in the microscopic examination, the PCR test result for the presence of *T. gondii* DNA was

Table 1. Occurrence of *Toxoplasma gondii* in samples of water taken from water intakes located on farms.

	Type of farm	Shallow, windlass-operated wells (SW) N=80	Deep, pump-operated wells (DW) N=16	Water supply system (WSS) N=18	Total N=114
Toxoplasma gondii oocysts Detected microscopically (positive, percent)	F1	2/15 (13.3%)	0	0/10 (0)	2/25 (8.0%)
	F2	13/65 (20.0%)	0/16 (0)	0/8 (0)	13/89 (14.6%)
	F1 + F2	15/80 (18.7%)$^{\#}$	0/16 (0)	0/18 (0)	15/114 (13.2%)
Cysts of other protozoans Detected microscopically (positive, percent)	F1	8/15 (53.3%)$^{\#\#}$	0	0/10 (0)	8/25 (32.0%)
	F2	21/65 (33.8%)*	1/16 (6.2%)	0/8 (0)	22/89 (24.7%)
	F1 + F2	29/80 (36.2%)$^{*\#\#}$	1/16 (6.2%)	0/18 (0)	30/114 (26.3%)
Toxoplasma gondii DNA Detected by PCR (positive, percent)	F1	2/15 (13.3%)	0	0/10 (0)	2/25 (8.0%)
	F2	28/65 (43.1%)$^{**\#}$	1/16 (6.2%)	0/8 (0)	29/89 (32.6%)
	F1 + F2	30/80 (37.5%)$^{*\#\#}$	1/16 (6.2%)	0/18 (0)	31/114 (27.2%)

F1 – farms of good sanitary state, F2 – farms of poor sanitary state.
$^{*-**}$ significantly greater compared to deep wells (DW): $^{*}p<0.05$, $^{**}p<0.01$.
$^{\#-\#\#}$ significantly greater compared to water supply system (WSS): $^{\#}p<0.05$, $^{\#\#}p<0.01$.

Table 2. Occurrence of anti-*Toxoplasma* antibodies in farmers and their families owning wells, related to water consumption and results of water examination for the presence of *Toxoplasma*.

		Persons consuming well water	Persons not consuming well water	Persons exposed to *Toxoplasma* in well water (microscopic examination)		Persons exposed to *Toxoplasma* in well water (PCR examination)		Total
				Positive	Negative	Positive	Negative	
F1	ELFA test for IgG antibodies	N=20	N=37	N=2	N=55	N=2	N=55	N=57
	Positive, percent	13 (65.0%)	22 (59.5%)	1 (50.0%)	34 (61.8%)	1 (50.0%)	34 (61.8%)	35 (61.4%)
	IU/ml (median)	38.5	20.0	40.0	30.0	40.0	30.0	30.0
	ELFA test for IgM antibodies	0	0	0	0	0	0	0
	Positive, percent	0	0	0	0	0	0	0
F2	ELFA test for IgG antibodies	N=29	N=13	N=11	N=31	N=24	N=18	N=42
	Positive, percent	24 (82.8%)***	5 (38.5%)	9 (81.8%)	20 (64.5%)	17 (70.8%)	12 (66.7%)	29 (69.0%)
	IU/ml (median)	51.0**	0.0	99.0	15.0	19.0	25.0	22.0
	ELFA test for IgM antibodies							
	Positive, percent	1 (3.4%)	0	1 (9.1%)	0	1 (4.2%)	0	1 (2.4%)
Total	ELFA test for IgG antibodies	N=49	N=50	N=13	N=86	N=26	N=73	N=99
	Positive, percent	37 (75.5%)*	27 (54.0%)	10 (76.9%)	54 (62.8%)	18 (69.2%)	46 (63.0%)	64 (64.6%)
	IU/ml (median)	43.0	12.0	80.0	21.0	19.0	30.0	27.0
	ELFA test for IgM antibodies							
	Positive, percent	1 (2.0%)	0	1 (7.7%)	0	1 (3.8%)	0	1 (1.0%)

F1 – farms of good sanitary state, F2 – farms of poor sanitary state. IU - International Units.
*-*** Significantly greater compared to persons not consuming well water: *p<0.05, **p<0.01, ***p<0.001.

negative (Tab. 1). Altogether, the presence of *T. gondii* DNA in the water samples was found significantly more frequently in farms F2 then in farms F1 (32.6% *vs.* 8.0%, p<0.05). The difference was smaller in the case of microscopic examination (14.6% *vs.* 8.0%, p>0.05). In compared occurrence on farms, the presence of *T. gondii* in well water was found on 1 out of 14 farms F1 (7.1%) and on 28 out of 73 farms F2 (38.4%) (p<0.05).

Correlation between the results of microscopic examination and PCR test. A highly significant correlation was observed between the detection of *T. gondii* oocysts in the microscopic examination and a positive result of PCR test (r=0.629; p<0.0000001). This correlation was much lower in the case of oocysts of other protozoans (r=0.280, p<0.01).

Seroepidemiological studies of inhabitants of selected farms

Questionnaire survey on water usage. Altogether, 50 inhabitants of farms examined by serologic tests in the survey, confirmed drinking unboiled well water, whereas 49 denied this fact. Water from 23 shallow wells (F1 – 6, F2 – 17) was used by the inhabitants for consumption and watering of animals, while water from the remaining 17 wells (F1 – 9, F2 – 8) served only for watering animals and garden. On these farms, the inhabitants used water from the water supply system.

Serologic tests. Among the total number of 99 people from 87 farms, seropositive results in IgG class antibodies were found in 64 inhabitants (64.6%), within the range from 9–4,200 IU/ml. Mean age of seropositive people (55.6 ± 24.2) was significantly higher compared to those who were seronegative (29.6 ± 19.2) (p<0.001). Among seropositive results, 42 (65.6%) were within the range from 10–100 IU/ml, 15 (23.5%) from 101-300 IU/ml, and 7 (10.9%) of highly positive results were over 300 IU/ml. In 1 person IgM class antibodies were noted.

Among 26 inhabitants of farms with positive PCR result for the presence of *T. gondii* in water (farms W+), IgG antibodies were observed in 18 people (69.2%) (Tab. 2). Among 73 inhabitants of farms on which the result of water examination was negative (farms W-), in 46 people (63.0%) serologic tests showed the presence of IgG antibodies. The difference between the percentages of seropositive results noted in the 2 groups examined (69.2% *vs.* 63.0%) was not statistically significant. On 15 farms W+ seropositive result was significantly more often observed among people who reported the consumption of well water in the questionnaire survey (17/19, 89.5%) than among people drinking water only from the water supply system (2/7, 28.6%) (p<0.001). No significant relationship was noted between drinking well water and seropositive results among inhabitants of 24 farms W- (p>0.05). In the total population, seropositive results were significantly more frequently observed in people consuming well water (75.5 *vs.* 54.0%, p<0.05%) (Tab. 2).

Farms F1. Among the total number of 57 people in the study, seropositive results in IgG antibodies class were noted in 35 (61.4%) within the range 10-4,200 IU/ml (Tab. 2). Mean age of seropositive people (49.5 ± 27.1) was significantly higher than the mean age of seronegative people (23.3 ± 15.2) ($p<0.001$). Among positive results, 24 (68.6%) were within the range 10-100 IU/ml, 6 (17.1%) were within the range 101-300 IU/ml, and 5 (14.3%) were over 300 IU/ml.

In 1 of 2 people living on farm W+ the presence of IgG class antibodies was observed. Among the remaining 55 inhabitants of farms, where the result of the examination of water for the presence of *T. gondii* was negative, serologic tests revealed the presence of IgG class antibodies in 34 people (61.8%). In the population examined no statistically significant relationship was found between drinking well water and seropositive results (Tab. 2).

Farms F2. Among the total number of 42 people examined, seropositive results in IgG class antibodies were observed in 29 (69.0%), within the range 9-724 IU/ml. Mean age of seropositive people (63.0 ± 18.1) was significantly higher, compared to the mean age of those who were seronegative (40.2 ± 21.1) ($p=0.001$). Among positive results, 18 (62.1%) were within the range 10-100 IU/ml, 9 (31.0%) were within the range 101-300 IU/ml, and 2 (6.9%) were over 300 IU/ml. In 1 person, IgM class antibodies were present.

Among 24 inhabitants of farms where positive PCR result was noted for the presence of *T. gondii* in water (farms W+), the IgG class antibodies were observed in 17 people (70.8%) (Tab. 2). Among 18 inhabitants of farms where the result of water examination was negative (farms W-), serologic tests revealed the presence of IgG class antibodies in 12 people (66.7%). The difference between the percentages of seropositive results noted in these 2 groups examined (70.8% *vs.* 66.7%) was not statistically significant. On farms W+ seropositive results were noted more often among people who reported in the survey the consumption of unboiled well water, compared to people drinking water from the water supply system ($p<0.001$). No significant relationship was observed between drinking well water and seropositive results among the inhabitants of farms W-, where *T. gondii* was not detected in water. Among the total number of inhabitants of farms F2, seropositive results were significantly more often noted in people who consumed unboiled well water (82.8% *vs.* 38.5%, $p<0.001$) (Tab. 2). Also, the median concentration of anti-*Toxoplasma* antibodies was significantly greater in inhabitants of farms F2 who reported drinking unboiled well water compared to those who did not (51.0 IU/ml *vs.* 0.0 IU/ml, $p<0.01$).

Clinical cases. In 12 people from farms F1, in the past there occurred symptoms suggesting *T. gondii* infection – the enlargement of lymph nodes with accompanying flu-like symptoms was observed (in 9 people), and disorders in the course of pregnancy (in 2 people). In 3 cases, toxoplasmosis was diagnosed and proper treatment applied. The remaining people did not seek medical advice. The present study revealed the presence of *T. gondii* IgG class antibodies in all these people.

Among the inhabitants of farms F2, 1 case of a lymphonodular form of toxoplasmosis was noted. This was the case of a 31-year-old female farmer living in the village Liśnik Duży. The woman was hospitalized. The serologic tests performed confirmed the diagnosis by detecting the presence of IgM class antibodies (ELFA IgM: +) and IgG class antibodies (ELFA IgG: 724 IU/ml). The microscopic examination of a water sample from the well on this farm showed the presence of *T. gondii* oocysts, which was also confirmed by a positive result of PCR test. Water on this farm was used both for consumption by the members of the household and for watering animals and the garden. Based on the results obtained, it may be presumed that in this case water might have been the source of *T. gondii* infection.

Among the population living in the remaining farms F2, the occurrence in the past of the symptoms characteristic of toxoplasmosis was not observed. None of the people examined were treated due to toxoplasmosis, nor had serologic tests for this disease been performed.

DISCUSSION

According to general opinion, *T. gondii* infection in humans most often takes place as a result of the consumption of raw or undercooked meat containing cysts of the protozoan, or food contaminated with cat's faeces [5]. To date, only few reports have been devoted to the waterborne route of infection, which was probably caused by the lack of effective research methods. Worldwide in recent years, cases of contracting toxoplasmosis have been noted, water being the source of infection. Among other countries, in Panama in the year 1979 *T. gondii* infection was observed among soldiers stationed in the jungle. Epidemiological investigation showed that the source of infection was water from a stream contaminated with oocysts excreted by jungle cats [2]. In British Columbia (Canada), 110 acute cases of toxoplasmosis were noted, associated with the contamination of water from the water supply system with *T. gondii* oocysts washed from soil contaminated with wild cats' faeces during heavy rains. It was estimated that as many as 8,000 people contracted the infection [3]. The largest focus of infection (290 cases) was noted in Brazil. It was demonstrated that the disease was associated with the drinking of unfiltered water from a well [1]. Cases of toxoplamosis were also confirmed among a group of vegetarians in India, which were related to the contamination of drinking water with the oocysts of the parasite [7]. These events attracted public attention to the problem and resulted in the development of increasingly more perfect research methods, including those in the area of molecular biology. The application of the PCR techniques

specially contributed to the increase in the effectiveness of detection of the parasite in water [9, 10, 20].

The presence of oocysts and DNA of *T. gondii* in a considerable number of water samples examined in the present study (13.2% and 27.2% respectively), as well as the significantly more frequent occurrence of seropositive results among people who consumed unboiled well water, provide an evidence that water may play an important role in the spread of *T. gondii* infection, especially in the rural environment. The division of farms into two groups in the present study revealed differences in the results obtained. Among 14 farms F1, where the hygienic conditions were correct and wells were surrounded and protected, the presence of *T. gondii* in well water was noted only on 1 farm (7.1%). Among 73 farms F2, however, characterised by incorrect hygienic conditions and lack of proper protection of wells, the presence of *T. gondii* was observed on as many as 28 farms (38.4%). In both groups, the mean age of seropositive people was significantly higher than that of seronegative, which was associated with different time of exposure to the parasite.

The lack of significant relationship between drinking well water and seropositive results noted on farms F1 suggests that in the majority of people from this population the cause of *T. gondii* infection were sources of parasite other than water [17]. Only in one case of clinical toxoplasmosis in an inhabitant of farm F2, where *T. gondii* was found in drinking water, the waterborne route of parasitic infection seems most probable.

A significant correlation between drinking unboiled water from a well and the presence of specific antibodies against *T. gondii* was noted among inhabitants of farms F2 which, in combination with the results of the water examination, may indicate that on neglected farms where wells could be easily contaminated, well water may be an important source of the parasite. Other authors, while analysing cases of *T. gondii* infection in various populations, also indicated a significant correlation between drinking unboiled water and the occurrence of invasion in the populations examined [4, 6, 11, 14].

It seems that the results of the present study and those by other authors justify the necessity to implement a system of monitoring household wells and the supplementation of the scope of routine examinations of water from water supply systems by studies for parasitic protozoans. The implementation of prevention measures on farms at risk would also be important.

CONCLUSIONS

1. The presence of oocysts and DNA of *Toxoplasma gondii* in well water provides an evidence of the contamination of the rural environment with dispersive forms of this protozoan and creates a potential risk of waterborne toxoplasmosis for humans and animals.

2. Among inhabitants of farms where the hygienic conditions are poor, a significant relationship is observed between the consumption of unboiled well water and *T. gondii* infection.

3. It is desirable to carry out prophylactic actions in the areas of farms at risk in order to protect the health of humans and animals living on these farms.

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