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SEROEPIDEMIOLOGY OF TOXOPLASMOSIS IN THE LUBLIN REGION

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Abstract: Reported are results of serologic examinations for the presence of anti-*Toxoplasma* antibodies by direct agglutination in 1,497 people: 1,327 forestry workers and 86 farmers occupationally exposed to *T. gondii* from the Lublin region (eastern Poland) and 84 inhabitants of the city of Lublin examined as the control group, including 50 blood donors and 34 workers from forestry headquarters. 58.5% positive results in forestry workers, 56.9% in farmers and 46.4% in the control group were obtained. The highest percentages of positive results were obtained in Sosnowica, Włodawa and Sobibór, all localities in the Chełm district. This finding and the prevalence of clinical cases may suggest that the Chełm district (easternmost area of the Lublin region, bordering Ukraine) is an endemic area of toxoplasmosis. A case of toxoplasmosis in a 39 year old farmer is described in whom reinfection was identified 20 years after primary diagnosis. Rapid increase in specific serologic titres and symptoms typical for toxoplasmosis were noted. The rest of the family and household animals were also found to be positive which supports the suggestion of a family-environmental case of toxoplasmosis. Survey for anti-*Toxoplasma* antibodies in various domestic and wild animals comprised sera from 262 cows, 120 pigs, 34 geese, 65 chickens, 3 roe deer and 10 sheep from the Lublin region. High percentages of positive results were found in cattle (53.8%) and in pigs (15%). Fowl were positive only in 0-5.9%. The cattle and pigs from the Chełm district are most probably the main sources of toxoplasmosis threatening humans in this area.

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INTRODUCTION

Toxoplasmosis - a common zoonosis found in humans and many animal species - may entail a serious epidemiologic problem, particularly in the agricultural environment. It is one of the most frequent parasitic infections, especially dangerous in congenital form and in people with immunologic deficiency [28]. Toxoplasmosis is very common among human population. It is estimated that every third person in the world is infected and the frequency differs depending on the geographical area: people from USA were found serologically positive from 20% to 30%, in Japan 25%, in Netherlands 60%, in Italy 60%, in France 50%, and in Finland 35% [21]. In Poland 50-60% people are infected [28]. Due to the data from the Polish Institute of Hygiene and the Ministry of Health, 1,638 clinical cases of toxoplasmosis were noted in the years 1994-1999, including 32 cases of congenital toxoplasmosis in the years 1997-1999.

Toxoplasma gondii is an obligate intracellular parasite living in many animal species and in man. This protozoan has a complex life cycle including definitive host, which is an animal from *Felidae* family (cat, bobcat, puma) and an intermediate host that may be any of the domestic animals, wild mammals, birds, and man [21]. Domestic and free living animals are the reservoirs of *T. gondii*. It is

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believed that the most important factors in the epidemiology of *T. gondii* are cats, pigs, sheep, cattle, fowl and wild game [4]. Serologic examination for toxoplasmosis in Poland showed a high percent of positive reactions in cattle (55%), pigs (21.2%-53%) and in sheep (up to 80%) [28].

There is much evidence that the habit of consumption of undercooked or raw meat is related to high toxoplasmosis morbidity. In the case of bad sanitary and consumption habits toxoplasmosis may concern more members of the family and thus have the family-environmental character [28].

Toxoplasmosis may result from consumption of undercooked or raw meat containing parasite cysts, or from contact with food, water or sand contaminated with oocysts spread by infected cats. There is also the possibility of transplacental transmission of the parasite to the foetus during pregnancy. The disease may also develop in the laboratory workers handling infectious material, after transplantation of organ containing cysts, and through transfusion of infected blood. In people with a well-functioning immunologic system the infection usually has no symptoms except for development of specific antibodies. In people with immunologic disorder primary invasion may develop or recurrence of chronic invasion with clinical signs. Most common is the lymphonodular form of toxoplasmosis involving mainly lymph glands in the neck and nape area. Changes may concern also liver, spleen, lungs and cardiac muscle, central nervous system and eyes. In the case of immunologic disorder generalized toxoplasmosis may develop, as in AIDS patients. Especially dangerous is the congenital form of toxoplasmosis; it develops in about half of pregnant women who were primarily infected during pregnancy. The parasites may cause intracranial calcifications, hydrocephalus and chorioretinitis [28].

In animals *T. gondii* rarely ignites symptoms. The most frequent is the latent form characterized by the presence of tissue cysts in muscles and organs of animals for prolonged periods.

The aim of the present study was an evaluation of the frequency of anti-*Toxoplasma gondii* antibodies in forestry workers and farmers in the Lublin region. Detection of infected animals as the potential sources of toxoplasmosis for humans and characterization of the foci of family-environmental toxoplasmosis were the secondary goals.

MATERIALS AND METHODS

Examined subjects

Seroepidemiologic examinations of humans. Examinations were carried out in 1,327 people employed in forestry from five districts and in 86 farmers from two districts of the Lublin region (Fig. 1). The administrative division before the reform introduced in 1998 is taken into consideration throughout the paper. 34 office workers employed in the forestry headquarters in Lublin and 50 blood donors (healthy inhabitants of Lublin) were examined as the control group.

Figure 1. Map showing area of study: five districts in the Lublin region.

The majority of forestry workers were people actually working in the forest. Age, gender, working time, contact with free living and domestic animals, and past diseases were taken into account. The mean age of 1,086 men and 241 women from the forestry group was 40 years, and the mean period of employment was 13 years. Epidemiologic anamnesis has shown that almost everyone from this group had contact with domestic and wild animals. The mean age of 54 women and 32 men from farmers group was 41 years and the mean age of 75 men and 9 women in the control group was 35 years.

Seroepidemiologic examination of animals. Sera from 494 animals were examined: 262 from cows, 120 from pigs, 34 from geese, 65 from 38-week old chickens from a breeding farm. Three roe deer and 10 sheep were also examined from areas where high percentages of positive reactions in people were found. All animals originated from the Lublin region.

Examination of the family-environmental case of toxoplasmosis. During the study a case of family-environmental character was evidenced in Potok Wielki village (Tarnobrzeg district). Detailed epidemiologic anamnesis was performed. Data about family members concerning age, gender, place and time of employment, past diseases, contact with domestic and wild animals, information about diet, origin of the food, type of food and method of cooking, and the presence of raw meat in the diet were collected. Data about animal diseases were also collected. The sera collected from men and animals were examined for toxoplasmosis.

Serological tests

The basic test used in serological examination of humans and animals was direct agglutination test with 2-mercaptoethanol (DA). Other tests, such as indirect fluorescence test (IFT), immunosorbent agglutination assay for IgM (ISAGA IgM, bioMérieux, France), enzyme linked fluorescent assay (ELFA, Vidas Toxo IgG and Vidas Toxo IgM, bioMérieux, France), were used additionally in the examination of clinical cases.

Most of the subjects: 1,327 forestry workers, 86 farmers, 84 persons of the control group, 5 persons from the case of family-environmental toxoplasmosis, a forest worker from Sosnowica with primary infection and all 502 animals were examined with DA. Other tests (IFT, ISAGA IgM, ELFA IgG and ELFA IgM) were used additionally for the examination of the case of family-environmental toxoplasmosis (5 persons), 10 clinical cases from Biała Podlaska and Chełm districts and one forestry worker with primary toxoplasmosis.

Direct agglutination test with modifications suggested by Desmonts and Remington was used to detect antibodies in IgG class [1]. Sera were treated with 2-mercaptoethanol to reduce IgM antibodies and then incubated with formalin-treated *Toxoplasma* antigen. The antigen was prepared in the Department of Occupational Biohazards of the Institute of Agricultural Medicine in Lublin. Tachyzoites from 3 day peritoneal exudate of mice inoculated with RH strain of *Toxoplasma gondii* were centrifugated at 3,000 rpm, washed with PBS (pH 7.6) twice and then mixed with trypsin for 20 min in an electromagnetic mixer. After washing with PBS twice, tachyzoites were formalinized (2%) for 24 hrs and after centrifugation and washing with PBS were dyed with 0.7% methylene blue for 20 min. After centrifugation and final washing, tachyzoites were suspended in 0.2% formalin. Antigen was added to examined sera and the test was incubated for 24 hrs at 37ºC. Agglutination of parasites took place if the serum contained antibodies. This test is easy to perform, specific and sensitive and can be used for testing human or animal sera [12, 25, 30].

Indirect fluorescence test (IFT) detects specific antibodies of IgG and IgM classes. The fresh antigen was prepared in our laboratory. Tachyzoites from 3 day peritoneal exudate of mice inoculated with RH strain of *Toxoplasma gondii* were centrifugated at 3,000 rpm and formalinized (1%) for 90 min. After centrifugation, sediment was suspended in PBS (pH 7.6). Then antigen was diluted with PBS (until 10-30 tachyzoites were visible in the field of view of the microscope at 400 × magnification) and dripped on glass slides, air-dried and stored at –20ºC until needed. Before use, antigen-coated slides were rinsed with water and repeatedly air-dried. Tested sera were placed on antigen spots (15 µl/spot) in two dilutions (1:32 and 1:128) and incubated at 37ºC for 30 min. The slides were then rinsed in water, washed in PBS (pH 7.6) three times for 5 min each, and dried. A drop of fluorescein isothiocyanate labelled goat anti-human immunoglobulin diluted in PBS (in proportion established by titration) was added to each spot. An addition of equal volume of 0.01% Evans blue to immunoglobulin helped in later visualisation of *Toxoplasma* fluorescence. Slides were incubated at 37ºC for 30 min, washed, dried and mounted with buffered glycerol. Slides were examined using a fluorescent microscope. Visible fluorescence of cytoplasmic membrane contrasting with red dyed cytoplasma of parasite was regarded as positive. Positive titres were greater or equal to 32. Serum positive in both dilutions was examined in higher dilutions to establish endpoint titre.

Immunosorbent agglutination assay IgM (ISAGA IgM, bioMérieux, France) detects immunoglobulins of IgM class. All human IgM immunoglobulins in examined sera are captured in the wells coated with mouse monoclonal antibodies. Specific IgM antibodies are then detected with the suspension of *Toxoplasma gondii* antigen in formalin. Every serum is examined with an increasing amount of antigen. Positive reaction evokes full agglutination at the bottom of the well (no sediment - 4 points); if antigen remains unbound it elicites intermediate reactions with visible sedimentation (1–3 points). Whole antigen sediments at the bottom in the case of negative reaction (0 points). The result of this test is expressed as an index (0–12 points) being the sum of the results from three consecutive wells filled with different amounts of antigen. The result of index from 9–12 points is regarded as positive, from 6–8 points as equivocal, and from 0–5 points as negative [10].

Enzyme linked fluorescent assay (ELFA, Vidas Toxo IgG, bioMérieux, France) with the use of MiniVidas. *Toxoplasma* antigens from membrane and cytoplasm with high content of P30 protein were placed on solid phase and covered with examined sera. Anti-*T. gondii* IgG antibodies were detected by mouse monoclonal anti-human IgG antibodies conjugated with alkaline phosphatase. Final reading was based on the fluorescence measurement, automatically compared to calibration curve and calculated to IU/ml. The results were considered as positive from 10 IU per ml and 8-9 IU/ml were regarded as equivocal.

Enzyme linked fluorescent assay (ELFA, Vidas Toxo IgM, bioMérieux, France) with the use of MiniVidas. This is an “immunocapture” method. Mouse antibodies anti-human µ chains were placed on solid phase. Human IgM immunoglobulins from tested sera were captured and their presence was detected with immunologic complex (*Toxoplasma* antigen + monoclonal antibodies anti-P30 conjugated with alkaline phosphatase). Final reading was based on the fluorescence measurement. The results were expressed as a ratio of examined serum

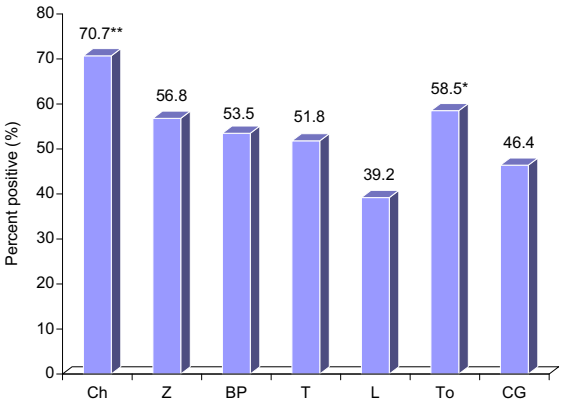

Figure 2. Positive results in DA for toxoplasmosis in forestry workers in five districts compared to the control group. Ch: Chełm district (n=530); Z: Zamość district (n=125); BP: Biała Podlaska district (n=226), T: Tarnobrzeg district (n=270); L: Lublin district (n=176); To: total (n=1327); CG: control group (n=84).
*-** Percent of positive reactions significantly higher compared to the control group: *p<0.05; **p<0.001

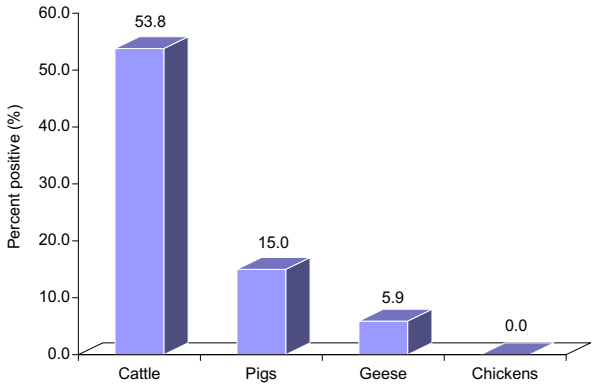

Figure 3. Positive results in DA for toxoplasmosis in some species of animals from the Lublin region. Cows (n=262); pigs (n=120); geese (n=34); chickens (n=65).

fluorescence to the calibrator signal. The results were considered as positive at 0.65. Equivocal results ranged from 0.55–0.65 [3].

Vidas Toxo IgG and Vidas Toxo IgM tests are highly sensitive and specific [11, 16]. Full automatization assures laboratory work safety and efficiency.

Statistical analysis was performed with chi-square test.

RESULTS AND DISCUSSION

Prevalence of serologic reactions in humans. The mean percentage of seropositive results in the 1,327 examined forestry workers amounted to 58.5% (776 persons). The persons from the control group reacted positively in 46.4% (Fig. 2). Statistical analysis has shown a significant difference between forestry workers and the control group (p = 0.03). The highest percentage of positive results among forestry workers was noted in Sosnowica area - 89.5%, Krasnystaw - 76.0%, Włodawa - 73.1% and Sobibór - 68.6% (all in Chełm district); the lowest in Bychawka and Firlej areas (Lublin district) - 28.3% and 42.8% respectively and in Buda Stalowska - 44.4% (Tarnobrzeg district) (Tab. 1). It is worth noting that in Parczew in Biała Podlaska district and in Urszulin in Chełm district many workers (22.8% and 17.8% respectively) had high titres (1,000 and above in DA). Among patients with both classes of antibodies (IgG and IgM) there were found six cases of clinical toxoplasmosis with obstetric anamnesis, one case of lymphonodular toxoplasmosis and one case of congenital toxoplasmosis. All these patients originated from Biała Podlaska district. Two cases of hydrocephalus caused by *Toxoplasma* infection were also noted in Chełm district.

During studies carried out in the Sobibór area, one case of primary infection was found. This was patient T.R., a 42 years old forester and hunter, managing his own farm where cattle and pigs were bred. Seropositive reactions in DA (titre 4,000), ELFA IgG (4,300 IU/ml) and ELFA IgM (0.83) were found.

Among 86 farmers, with mean age 41 years and in contact with domestic and wild animals, there were 49 persons with positive results (56.9%). In Buda Stalowska, out of 39 farmers investigated, 21 reacted positively with *T. gondii* antigen; 17 in the range of titers from 32–128 (80.9%) and 4 (19.1%) in the range 256-512. In Wohyń, 28 sera out of 47 gave positive results, 25 (89.3%) in the range 32–128 and three (10.7%) in the range 256–512.

The percentages of positive results obtained in forestry workers and farmers are higher compared to those obtained in the past in the people from the Lublin region examined with complement fixation test by Tość-Luty *et al.* (9.5%) [23] and Stroczyńska-Sikorska (17.7%) [20]. On the other side, they are lower compared to the results obtained by Umiński *et al.* with IFT in 1985 (63%) [24] and in 1986–1987 (75.6%) [27]. The results of our study conform to the results of these authors [17, 19, 28] who found that people occupationally exposed to contact with animals are more frequently reacting positively than the other groups, and that the prevalence of seropositive persons is higher in rural population than in urban population. Taylor *et al.* found a higher percentage of positive reactions in children from a rural area (16.6%) compared to children from an urban area (10.2%) [22]. Martinez Sanchez *et al.* investigating pregnant women in Cuba also found more positive cases in the rural population [8]. However, there are reports which do not confirm the effect of place of residence on the prevalence of serologic reactions to *Toxoplasma gondii*. Lebech *et al.*

Table 1. Serologic results in DA for toxoplasmosis in forestry workers from five districts in the Lublin region.

District	Place	N	% of positive
Chełm	Krasnystaw	50	76.0
	Sobibór	121	68.6
	Urszulin	86	52.3
	Włodawa	216	73.1
	Sosnowica	57	89.5
Subtotal		530	**70.7**
Zamość	Mircze	58	53.4
	Zwierzyniec	67	59.7
Subtotal		125	**56.8**
Biała Podlaska	Chotyłów	63	52.4
	Biała Podlaska	55	56.4
	Sarnaki	48	45.8
	Parczew	60	58.3
Subtotal		226	**53.5**
Tarnobrzeg	Janów Lubelski	144	47.9
	Buda Stalowska	45	44.4
	Gościeradów	81	63.0
Subtotal		270	**51.8**
Lublin	Puławy	57	45.6
	Świdnik	17	47.0
	Firlej	42	42.8
	Bychawka	60	28.3
Subtotal		176	**39.2**
Total		**1327**	**58.5**

Table 2. Serologic results in DA for toxoplasmosis in animals from the Lublin region.

Species	District	Place	N (% of positive)
Cows	Chełm	Klesztów	20 (0.0%)
		Krasnystaw	10 (10.0%)
		Włodawa	144 (73.6%)
		SUBTOTAL	174 (61.5%)
	Lublin	Dębina	16 (50.0%)
		Pszczela Wola	12 (0.0%)
		Pawłówek	10 (20.0%)
		Kijany	10 (80.0%)
		Stasin	10 (80.0%)
		Wesołówka	10 (30.0%)
		SUBTOTAL	68 (42.6%)
	Biała Podlaska	Krzesimów	4 (100%)
		Bereza	16 (6.3%)
		SUBTOTAL	20 (25.0%)
TOTAL			262 (53.8%)
Pigs	Chełm	Horodyszcze	57 (8.9%)
		Siedliszcze	31 (19.4%)
		Chełm surroundings	20 (25.0%)
		SUBTOTAL	108 (14.8%)
	Biała Podlaska	Dawidy	12 (16.6)
TOTAL			120 (15.0%)
Geese	Lublin	Bełżyce	34 (5.9%)
TOTAL			34 (5,9%)
Chickens	Lublin	Brzeźce	20 (0.0%)
		Niemce	45 (0.0%)
TOTAL			65 (0.0%)

[5] in Denmark and Ljungström *et al*. [6] in Sweden found no differences between rural and urban population in surveys of pregnant women.

Many authors reported high percentages of serologic reactions with *T. gondii* antigen among rural workers, animal breeders, inseminators, poultry slaughterhouse workers, hospital and diagnostic laboratory staff [24, 29]. There is no data in accessible bibliography concerning forestry workers and this study for the first time makes them the subject of such an extensive survey. High percentages of positive reactions and cases of clinical toxoplasmosis observed in this group may suggest an occupational background and requires further investigations.

Prevalence of serologic reactions in animals. Serological examination of animals showed a very high percentage of positive reactions in cows (53.8%, n = 262) and a high percentage in pigs (15%, n = 120). The percentage of seropositive reactions in fowl was low (0–5.9%) (Fig. 3). In cattle, the highest seroprevalence was found in Włodawa (73.6%) where also the highest percentage (9.5%) of high titers (above 1,000) was found. In all places low titres were dominant, in the range 32–64. In pigs, the highest percentage was found in the Chełm area - 25%. Among 34 geese investigated, two were found to be positive (5.9%) in titres 32–64. No anti-*Toxoplasma* antibodies were found in sera of chickens from Niemce and Brzeźce (Tab. 2). In three examined deer and in nine out of 10 examined sheep the presence of anti-*T. gondii* antibodies was found in low titres (32–64).

Toxoplasmosis in pigs has been described on most continents. The most frequent form is latent toxoplasmosis evidenced only by positive serological response. In the Sabin-Feldman dye test, the percentages of positive results were reported from 13% (Norway) to 62% (Denmark) [28]. In DA in Poland 28% of positive results were reported [26]. It is believed that pigs are an important factor in the life cycle of *Toxoplasma*. Sheep

Table 3. Seroepidemiologic examinations of family members from the familial-environmental case.

Initials		Age	Date of examination	Results of serologic tests				
				IFT	ELFA IgM	ISAGA IgM	ELFA IgG (IU/ml)	DA
H.W.	Patient	39	1996-08-07	512		(-)		512
			1996-11-27	256		(-)		2,000
			1997-04-04	128		(-)		512
			1997-04-17	128		(-)		512
			1997-04-24	128		(-)		1,000
			1998-07-08	>2,000	(-)		4,800	
			1998-08-14	16,000	(-)		>4,800	32,000
			1999-02-17	4,000	(-)		>4,800	
			1999-06-30	2,000	(-)		3,200	
M.W.	Husband	38	1998-08-14	128	(-)		21	32
K.W.	Son	10	1998-08-14	(-)	(-)		(-)	(-)
J.W.	Father	64	1998-08-14	128	(-)		23	64
A.W.	Mother	60	1998-08-14	32	(-)		20	32

are also a frequent host for *T. gondii* although clinical signs are rarely observed. High percentages of positive reactions were observed in sheep bred in Norway (53.6%), in Denmark (61%), in USA (65.5%) and in Poland (up to 80%) [28]. The characteristic symptom of toxoplasmosis in sheep is miscarriage.

In cattle, clinical manifestations are rarely observed although positive serologic results are frequent. The studies revealed 55.5% cows infected in Poland, 88.6% in Italy [28] and 25–61% in Sweden [2]. Dogs are often infected with *T. gondii* but their role in the epidemiology is not significant. Fowl play the main epidemiologic role among birds, particularly on small farms. Fowl bred on specialised farms, isolated from cats, are safe from infection [14]. Examinations of chickens from farms for the present study confirm this view.

Toxoplasmosis was also observed in wildlife animals: hares, roe deer, wild boars, red foxes and small mammals which play an important role in spreading the protozoans in nature [28]. Specific antibodies were found in 39 of 41 species of domestic and wild animals examined in Poland in the Institute of Agriculture Medicine [18].

The familial-environmental case of toxoplasmosis. Results of clinical and serological examinations of a 39 year-old farmer with ocular form of toxoplasmosis diagnosed at the age of 18, members of her family and animals from her household confirmed the existence of the family-environmental case. Due to anamnesis, the patient has been working on the farm for 25 years. After serologically confirmed ocular toxoplasmosis she was hospitalised and treated many times. In 1997 the patient was cured in the Department of Occupational Diseases of the Institute of Agricultural Medicine in Lublin. In July 1998 routine examinations showed a sudden increase in the level of IgG class antibodies compared to previous examinations. Further examinations of members of her family detected three of four persons as positive in IFT, ELFA IgG and DA and negative in ELFA IgM (Tab. 3). Among animals, positive results were obtained in a cow, a dog and a cat. Sera from a duck, a calf, a pig and a bull were negative. All members of the family had contact with bred animals. Meat and meat products derived from animals slaughtered on the farm. For many years the patient had eaten raw meat when preparing meals and had no habit of cleaning the surface of the table and kitchen equipment immediately after the preparation of meals. Patient and her mother had frequent contact with soil due to cultivation of vegetable garden. Past history of the health status of the animals from the household did not suggest clinical toxoplasmosis in any of them. Conditions of breeding enabled contact with a cat and its faeces or with small mammals.

The past history of the patient suggests that primary infection with *T. gondii* had been acquired many years ago. In 1998, routine test for toxoplasmosis revealed a rapid increase of specific antibodies level compared to previous years. General symptoms such as weakness, muscle pains, dizziness, recurrent slight increase of body temperature were caused by reinfection. Patient was treated for hyperthyroidism that might be responsible for immunosuppression and recurrence of parasitic disease. On the other hand, positive results in family members and in animals (cat, cow and dog) suggest the appearance of a new source of infection in this household. Similar circumstances were reported in many cases of family-environmental toxoplasmosis [7, 9, 13, 15, 27] where asymptomatic infection in the rest of the family accompanied the clinical case.

CONCLUSIONS

1. High percentages of positive results in serologic tests for toxoplasmosis in humans and animals in the area

of the Biała Podlaska and Chełm districts and new cases of the disease may suggest the endemic foci of toxoplasmosis in this area.

2. High prevalence of seropositive results in forestry workers may suggest an occupational exposure of this group to *Toxoplasma gondii*.

3. High percentages of positive results in serologic tests for toxoplasmosis in cattle and pigs from the Chełm district suggests that these species may constitute the main reservoir of *T. gondii* among domestic animals.

4. Described case of familial toxoplasmosis confirms frequent coexistence of infection in family members which may suggest a common source of infection and indicates the need for the examination of the whole family in similar cases.

REFERENCES

1. Desmonts G, Remington JS: Direct agglutination test for diagnosis of Toxoplasma infection: method for increasing sensitivity and specificity. *J Clin Microbiol* 1980, **11**, 562-568.

2. Dubey JP, Beattie CP: *Toxoplasmosis of Animals and Man*. CRC Press Inc, Boca Raton 1988.

3. Grzeszczuk A, Bobrowska E: Ocena serologicznej diagnostyki toksoplazmozy przy użyciu metody immunoenzymatyczno-fluorescencyjnej (ELFA) i immunofluorescencji pośredniej (IF). *Diagn Lab* 1995, **31**, 245-249.

4. Krupa K, Bartoszcze M: Rezerwuary toksoplazmozy. *Przegl Epid* 1990, **44**, 317-321.

5. Lebech M, Larsen SO, Petersen E: Occurrence of toxoplasmosis in pregnant women in Denmark. A study of 5402 pregnant women. *Ugeskr Laeger* 1995, **157**, 5242-5245.

6. Ljungström I, Gille E, Nokes J, Linder E, Forsgren M: Seroepidemiology of *Toxoplasma gondii* among pregnant women in different parts of Sweden. *Eur J Epidemiol* 1995, **11**, 149-156.

7. Luft BJ, Remington JS: Acute toxoplasma infection among family members of patients with acute lymphadenopathic toxoplasmosis. *Arch Intern Med* 1984, **144**, 53-56.

8. Martinez Sanchez R, Bacallao Gordo R, Alberti Amador E, Alfonso Berrio L: Prevalence of toxoplasmosis in pregnant women of the province of La Habana. *Rev Inst Med Trop Sao Paulo* 1994, **36**, 445-450.

9. Masur H, Jones TC, Lempert JA, Cherubini TD: Outbreak of toxoplasmosis in a family and documentation of acquired retinochorioiditis. *Amer J Med* 1978, **64**, 396-402.

10. Paul M: Zastosowanie metody ISAGA w wykrywaniu swoistych przeciwciał IgM, IgA, IgE w nabytej i wrodzonej toksoplazmozie. *Wiad Parazyt* 1997, **43**, 39-51.

11. Pelloux H, Ciapa P, Gouher-Fleuret A, Ambrois-Thomas P: Évaluation du system Vidas pour le diagnostic sérologique de la toxoplasmose. *Ann Biol Clin* 1993, **50,** 875-878.

12. Petithory JC, Reiter-Owona I, Berthelot F, Milgram M, De Loye J, Petersen E: Performance of European laboratories testing serum samples for *Toxoplasma gondii*. *Eur J Clin Microbiol Infect Dis* 1996, **15**, 45-49.

13. Roudaut P, Colin J, Renord G: Toxoplasmose oculaire „familiale": discussion pathogenique. *Bull Soc Ophtal France* 1992, **82**, 1575-1576.

14. Rzedzicki J, Boś M: Ptaki jako potencjalne źródło zakażeń ludzi pierwotniakami *Toxoplasma gondii*. *Medycyna Wet* 1999, **55**, 351-355.

15. Saks JJ, Roberto RR, Brooks NF: Toxoplasmosis infection associated with raw goat's milk. *JAMA* 1982, **248**, 1728-1732.

16. Sandin RL, Knapp CC, Hall GS, Washington JA, Rutherford I: Comparision of the Vitek Immunodiagnostic Assay System with an indirect immunoassay (Toxostat Test Kit) for detection of immunoglobin G antibodies to *Toxoplasma gondii* in clinical specimens. *J Clin Microbiol* 1991, **29,** 2763-2767.

17. Souza WJS, Coutinho SG, Lopes CWG, Dos Santos CS, Neves NM, Cruz AM: Epidemiological aspects of toxoplasmosis in school children residing in localities in the urban or rural characteristics within the city of Rio de Janeiro, Brazil. *Mem Inst Oswaldo Cruz* 1987, **82**, 475-482.

18. Sroka J, Umiński J, Cisak E, Chmielewska-Badora J, Zwoliński J: Problem toksoplazmozy jako choroby zawodowej u pracowników leśnictwa. **In:** *Materiały Sympozjum "Zagrożenia Zdrowotne w Środowisku Pracy," Wrocław, November 3-5, 1995*, 15.

19. Stray-Pedersen B, Pedersen JO, Omland T: Estimations of the incidence of *Toxoplasma* infections among pregnant women from different areas in Norway. *Scand J Infect Dis* 1979, **11**, 247-252.

20. Stroczyńska-Sikorska M: Badania nad zarażeniem toksoplazmowym ludności wiejskiej. *Wiad Parazyt* 1979, **25**, 647-653.

21. Śpiewak E, Małafiej E: Toksoplazmoza – wybrane zagadnienia epidemiologii, kliniki i diagnostyki. *Mikrobiol Med* 1996, **1,** 14-28.

22. Taylor MR, Lennon B, Holland CV, Cafferkey M: Community study of *Toxoplasma* antibodies in urban and rural school children aged 4 to 18 years. *Arch Dis Child* 1997, **77**, 406-409.

23. Toś-Luty S, Umiński J, Stroczyńska M: Badania nad toksoplazmozą pracowników rzeźni, garbarni i u ludności wiejskiej. *Wiad Parazyt* 1961, **7**, 445-447.

24. Umiński J: Toksoplazmoza jako choroba zawodowa. *Wiad Parazyt* 1985, **31**,105-112.

25. Umiński J, Cisak E: Badania porównawcze nad testem ELISA, odczynem immunofluorescencji pośredniej i odczynem aglutynacji bezpośredniej w diagnostyce toksoplazmozy u ludzi. **In:** *The XVth Meeting of the Polish Parasitological Society. Katowice, September 24-26, 1987. Abstracts of Communications.*

26. Umiński J, Cisak E, Badora J: Toxoplasmosis in rural environment of Poland. XXII International Congress of Agricultural Medicine and Rural Health, Stockholm, Sweden, July 10-13, 1994. *Ann Agric Environ Med* 1994, **1**, 178.

27. Umiński J, Cisak E, Chmielewska-Badora J, Skomra S: Poszukiwanie ognisk toksoplazmozy rodzinnej w środowisku wiejskim. *Wiad Parazyt* 1989, **35**, 289-297.

28. Umiński J, Cisak E, Chmielewska-Badora J, Zwoliński J: Toksoplazmoza u ludzi i zwierząt. *Medycyna Wet* 1994, **50**, 589-591.

29. Wildführ G, Wildführ W: *Toxoplasmose. Ratgeber für Ärzte und Tierärzte*. VEB G. Fischer Verlag, Jena 1975.

30. Wilson M, Ware DA, Juranek DD: Serologic aspects of toxoplasmosis. *JAVMA* 1990, **196**, 277-281.