

Seroprevalence of Selected Zoonotic Agents among Hunters from Eastern Poland

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Abstract

The aim of our study was the collection of seroprevalence data for *Toxoplasma gondii*, *Coxiella burnetii*, *Trichinella* spp., and *Francisella tularensis* from hunters in Lublin Province. The antibodies against *T. gondii* and *C. burnetii* were recorded in 38.5% and 16.2% of the sera, respectively. 4.05% of the sera were seropositive for both *T. gondii* and *C. burnetii*. None of the sera tested reacted positively with *F. tularensis* or *Trichinella* spp. Seroprevalence of *T. gondii* and *C. burnetii* is common among the hunters from Lublin Province. It seems reasonable to undertake similar research among hunters from other regions of eastern Poland.

Key words: *Coxiella burnetii*, *Francisella tularensis*, *Toxoplasma gondii*, *Trichinella* spp., hunters

Zoonotic agents are distributed widely throughout the world and are noted both in livestock and wild animals (Ciszewski *et al.*, 2014; Richard and Oppliger, 2015; EFSA, 2016; Eliášová *et al.*, 2017). The high risk of zoonotic transmission often occurs in individuals with occupational exposure to animals, such as veterinarians, farmers, and hunters. Human infection is usually acquired through direct contact with infected animals, inhalation of contaminated aerosols or close contact with contaminated environment through secretions and excretions from infected animals. Moreover, the transmission of zoonoses is possible via alimentary route after the consumption of raw or undercooked meat as well as drinking raw milk or water. Hunters are extremely exposed to direct contact with wild-living animals as well as dead animals, contaminated water, soil, and tick bites, which are vectors of many pathogens. Therefore, the risk of zoonotic infections is increased in this group (Richard and Oppliger, 2015; Tokarska-Rodak *et al.*, 2016). In 2016, the European Food Safety Authority and the European Centre for Disease Preven-

tion and Control published a report on zoonoses, zoonotic agents, and food-borne outbreaks noted in 2015 in 32 European countries. Among the important zoonotic factors that may pose a threat to public health are: *Toxoplasma gondii*, *Coxiella burnetii*, *Francisella tularensis* and *Trichinella spiralis* (EFSA, 2016). Generally, the prevalence of zoonotic diseases in humans is underestimated in Europe, including Poland. There are only a few reports in available databases about the prevalence of zoonotic agents in hunters. The data about zoonotic diseases in humans are underestimated mainly due to nonspecific symptoms and low awareness of physicians. Therefore, the aim of our study was to collect the seroprevalence data for *T. gondii*, *C. burnetii*, *Trichinella* sp., *F. tularensis* from hunters in eastern Poland.

The blood samples from hunters belonging to the hunting associations from Lublin Province were taken from October 2014 to April 2015 by venipuncture. The Bioethics Committee at the Medical University of Lublin approved the sampling and laboratory testing of the specimens obtained, decision No. KE-0254/177/2014.

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A total of 148 hunters aged 23–80 (average 53, SD 11.13) including 123 men and 25 women were examined. Sera were separated by centrifugation (10 min at 1400 × g) and stored at –20°C until analysis.

The serum samples were examined for the presence of IgG antibodies against *T. gondii* with the direct agglutination test (DAT), using a commercial kit (Toxo-Screen DA, bioMérieux, France). The test was performed according to the manufacturer's instruction.

Antibodies against of *C. burnetii* antigens (specific for phase I and II) were analyzed by the complement fixation test (CFT; GmbH, Germany and Biomed, France). The dilution of the samples ranged from 1:5 to 1:80. Partial inhibition of hemolysis in a 1:10 dilution was regarded as a positive result.

For the detection of anti-*F. tularensis* antibodies, the serum agglutination test (SAT) was performed. A commercial antigenic preparation Francisella Tularensis Antigen (Becton Dickinson) was used. The test was carried out with the microagglutination method, following the manufacturer's instruction. For each tested serum, two-fold dilutions between 1/10 and 1/80 were tested. The controls (commercial *F. tularensis* antigen, control antigen and negative serum), were used in each plate. The reaction was interpreted as positive at dilutions of 1:40 and higher.

The serum samples taken from the hunters were preserved in the National Reference Laboratory for Trichinellosis in the National Veterinary Research Institute in Pulawy (NRL) and examined for the presence

of anti-*Trichinella* antibodies according to accredited protocol in the Istituto Superiore di Sanita (Rome, Italy), as a part of the services that EURLP (European Union Reference Laboratory for Parasites) provides to NRLs (LAB N° 0689 MI-03 Rev. 3 2014 accredited by the Accredia). Microtiter plates coated with *T. spiralis* excretory/secretory (E/S) antigens were used (Gómez-Morales *et al.*, 2008). OD value was measured at 450 nm. Test results were provided in report No. 56/2015.

The data obtained were analyzed statistically using Statistica v.10 software. Chi-square test was performed for nominal features to detect statistically significant dependence. The assumptive level of significance was $p = 0.05$.

Serological screening revealed that 54.7% (81/148) of the samples tested were seropositive, 16.9% (25/148) were classified as doubtful and 35.8% (53/148) were negative. The antibodies were recorded against *T. gondii* and *C. burnetii* in 38.5% and 16.2% of the sera investigated, respectively. None of the tested sera reacted positively to *F. tularensis* and *Trichinella* spp. The anti-*T. gondii* IgG antibodies were recorded in 60% (15/25) women and 34.1% (42/123) men ($p = 0.042$, χ^2 Pearson's 6.3). The anti-*C. burnetii* antibodies were recorded in 12% (3/25) women and 17.1% (21/123) men ($p = 0.769$, χ^2 Pearson 0.52) (Table I). Interestingly, antibodies against *C. burnetii* and *T. gondii* were found simultaneously in 7.4% (11/148) of the tested sera. Moreover, 4.05% (6/148) the sera were seropositive for *T. gondii* and *C. burnetii* (Table II).

Table I
Results of serological tests for all four microorganisms.

Sex	Serological results			Result of statistical analysis
	Number of sera (%)			
	Seronegative	Seronegative	Doubtful	
<i>T. gondii</i>				
M	76 (61.8)	42 (34.1)	5 (4.1)	$p = 0.042$ $\chi^2 = 6.31$
F	10 (40)	15 (60.0)	0 (0)	
Total	86 (58.1)	57 (38.5)	5 (3.4)	
<i>C. burnetii</i>				
M	85 (69.1)	21 (17.1)	17 (13.8)	$p = 0.769$ $\chi^2 = 0.52$
F	19 (76.0)	3 (12.0)	3 (12.0)	
Total	104 (70.3)	24 (16.2)	20 (13.5)	
<i>F. tularensis</i>				
M	123 (83.1)	–	–	–
F	25 (16.9)	–	–	
Total	148 (100)	–	–	
<i>Trichinella</i> sp.				
M	123 (83.1)	–	–	–
F	25 (16.9)	–	–	
Total	148 (100)	–	–	

Table II
Results of serological tests for *T. gondii* and *C. burnetii*.

<i>T. gondii</i>	<i>C. burnetii</i>		
	Number of sera (%)		
	Seronegative	Seropositive	Doubtful
Seronegative sera	53 (35.8)	17 (11.4)	16 (10.8)
Seropositive sera	47 (31.7)	6 (4.05)	4 (2.7)
Doubtful sera	4 (2.7)	1 (0.67)	0 (0)
Total	104 (70.3)	24 (16.2)	20 (13.5)

There was no statistically significant relationship between the age of the subjects and the presence of antibodies among the pathogens tested.

Hunters are one of the groups occupationally exposed to infection with tick borne disease and other zoonotic agents. Hunters may be exposed to contact with live or dead animals as well as their excretions and secretions. Moreover, they are exposed to tick bites, so the transmission of zoonotic agents and tick-borne diseases, e.g., Q fever caused by *C. burnetii* cannot be ruled out. The most common zoonotic disease is toxoplasmosis. It is estimated that approximately 25% – 30% of the global population is infected with *T. gondii*; however, the incidence varies between countries and regions or between different communities within a region. Seroprevalence at the level of 10–30% was noted in North America and northern Europe, while in the countries of central and southern Europe (Gangneux and Dardé, 2012) the percentage ranged from 30 to 50%. The previous reports from Poland revealed that seroprevalence of *T. gondii* were close to the world's average (Kapka *et al.*, 2010; Sroka *et al.*, 2010; Milewska-Bobula *et al.*, 2015). Our investigation showed a very similar level of seroprevalence for *T. gondii* among hunters (41.9%). Parallely, in 37.5% of the sera antibodies against *C. burnetii* were detected. The previous reports showed anti-*T. gondii* IgG antibodies in employees of forest inspectorates and their family members in eastern Poland (61.4%) (Sroka and Szymańska, 2012), farmers (66.9%) (Sroka *et al.*, 2010), and meat processing industry workers (65.4%) (Sroka *et al.*, 2003). People living in rural households are infected with *T. gondii* more frequently (66.9%) than city dwellers (41%), and the incidence of the infection increases with age (Sroka *et al.*, 2010). Szymańska-Czerwińska *et al.* (2015) estimated seropositivity for *C. burnetii* among humans occupationally exposed to zoonoses at 31.12%, 39.07%, and 15.23% in IFA, ELISA, and CF methods, respectively.

Q fever outbreaks caused by *C. burnetii* are very common in the world, but in many countries human data are very limited. In the report from 2015, 833 confirmed cases of Q fever were reported in the EU (the notification rate was 0.16 per 100 000 of the popula-

tion). The highest notification rate was observed in Spain (0.54), Croatia (0.49), Cyprus (0.47), France and Germany (both 0.38), and Hungary (0.35), while Estonia, Iceland, Lithuania, Malta, and Slovakia reported no human cases in 2015 (EFSA, 2016). In Poland, one case of Q fever was reported in 2014 (incidence 0.003/100 000), and no cases were reported in 2015 (Czarkowski *et al.*, 2016). Taking into consideration that Q fever outbreaks have been noted in cattle and small ruminants in recent years, it is very probable that these data are underestimated. Our results are comparable to that reported by Szymańska-Czerwińska *et al.* (2015).

Another subject of our survey was tularemia. In European countries, the highest prevalence of tularemia in 2001–2010 was noted in Kosovo (incidence 5.2/100 000), Sweden (incidence 2.80/100 000), and Finland (incidence 1.19/100 000) (Gürcan, 2014). As reported by the National Institute of Public Health – National Institute of Hygiene, in Poland there were 11 tularemia cases (incidence 0.029/100 000) in 2014 and 9 cases (incidence 0.023/100 000) in 2015 (Czarkowski *et al.*, 2016). No anti-*F. tularensis* antibodies were detected in the serum samples from hunters in this study. The number of tularemia cases in Poland may be underrated due to the widespread use of aminoglycoside antibiotics and fluoroquinolones as second-line drugs used in adults to treat soft tissue and lymph node infections, which eliminate the tularemia symptoms without diagnosing the disease (Weiner and Kubajka, 2015). In the field of food safety, nematode parasites of the genus *Trichinella* still represent a concern for the public health due to hundreds of human infections documented yearly as the outcome of the consumption of wild boar meat (Murrell and Pozio, 2011; EFSA, 2012). *T. spiralis* foci have been present in Poland in domestic and wild animals, but other *Trichinella* species, such as *Trichinella britovi*, *Trichinella pseudospiralis* and *Trichinella nativa* have been detected in wildlife in the last decade (Chmurzyńska *et al.*, 2013; Bilska-Zajac *et al.*, 2016; Bilska-Zajac *et al.*, 2017). The consumption of unexamined pork causes average incidence of 0.9 cases per million persons per year (Mpy). The incidence caused by the consumption of wild boar meat is twice as high

and is estimated at 1.97 Mpy. Since wild boar meat is the most important source for trichinellosis outbreaks in humans, this study was aimed at evaluating the seroprevalence within the group at high risk. Hunters are recognized as the group of high risk due to their hobby.

Seroprevalence of *T. gondii* and *C. burnetii* is common among the hunters from Lubelskie Province while antibodies against *F. tularensis* and *T. spiralis* are absent. It seems reasonable to undertake similar research among hunters from other regions of eastern Poland. Knowledge in this field might be of importance for public health.

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