

Study on *Toxoplasma gondii*, *Leptospira* spp., *Coxiella burnetii*, and *Echinococcus granulosus* infection in veterinarians from Poland

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Abstract

Introduction: Exposure to zoonotic factors in veterinary practice is closely related to the nature of the work. The main aim of the study was to determine the risk of selected zoonotic infections among the occupational group of veterinarians in Poland. **Material and Methods:** Blood samples of 373 veterinarians (162 males and 211 females) from 12 provinces of Poland were collected by the venipuncture of a forearm for serological tests. Commercial immunoenzymatic tests (ELISA) were used for detection of specific IgG antibodies to *Echinococcus granulosus*, IgM and IgG to *Leptospira* spp., and IgM, IgA, and I and II phase IgG to *Coxiella burnetii*. Enzyme-linked fluorescence assays (ELFA) were used to detect IgM and IgG antibodies to *Toxoplasma gondii*. **Results:** Positive results were found in 209 (56.0%) veterinarians for at least one of the examined diseases. The overall proportion of participants found to have specific *Toxoplasma gondii* antibodies in the IgM and/or IgG assays amounted to 44.5%. The presence of *Coxiella burnetii* antibodies was found in 16 (4.3%) subjects, while *Leptospira* spp. antibodies were detected in 63 (16.9%) veterinarians. Among the 373 veterinarians examined, no *Echinococcus granulosus* antibodies were found. **Conclusion:** Results of the study seem to indicate a slightly elevated risk of *Toxoplasma gondii* infection and a moderate risk of infection with *Leptospira* spp. and *Coxiella burnetii* in veterinarians.

Keywords: veterinarians, *Toxoplasma gondii*, *Leptospira* spp., *Coxiella burnetii*, *Echinococcus granulosus*.

Introduction

The risk of exposure to zoonotic factors in veterinary practice is closely related to the nature of the work. Infectious diseases occurring among veterinarians are underreported, hence practicing veterinarians should effectively protect their own health as well as that of staff. Many aetiological factors of infectious diseases are so far still poorly known, both concerning their occurrence in the environment and the transmission paths of diseases which jeopardise health.

Toxoplasma gondii (Nicolle and Manceaux, 1908) counts among the parasitic protozoa widespread in the environment and is the aetiological factor of toxoplasmosis. The course of toxoplasmosis in healthy people is usually subclinical, manifesting only with the presence of specific antibodies. *T. gondii* infection can

be particularly dangerous in the case of primary invasion in pregnant women, due to the risk of invading the foetus. It can also have serious consequences for people with weakened immunity. Toxoplasmosis can also manifest in the form of inflammation of the eyes or neurological disorders, diagnosed even many years after the time of infection (5). Human infection is most often caused by ingestion of raw meat containing cysts of the parasite or consumption of food or water contaminated with oocysts (5, 26). An invasion may also result from direct contact with the faeces of an infected cat. The frequency of human infection in Poland based on serological studies is estimated at 40%–60%. Results of studies on the animal reservoir in Poland show a significant level of infection (pigs 10%–15%, cattle up to 20%, and cats up to 75%) and indicate the risk of invasion for consumers of raw meat

and for people who have direct contact with infected animals such as veterinarians (2, 23, 26).

The aetiological factor of Q fever is the Gram-negative *Coxiella burnetii*. The main bacterial reservoirs are domestic animals (sheep, goats, and cattle) usually latently infected (18). Ticks are an important factor in the circulation of *C. burnetii* in nature, but human *Coxiella* infections are rarely caused by tick bites. The most common sources of infection are animals, their secretions, meat, skin products, and definitely less often the dust-like faeces of an infected tick which can get into the body through the nose, mouth, or eyes. *C. burnetii* usually infects humans by inhalation or by the intestinal route (as a result of infected milk consumption). Infected animals can excrete the bacteria with urine, faeces, and milk, and the pathogen may be present in placenta, amniotic fluid, and aborted fetuses (19). The first symptoms of infection are high fever, chills, weakness, and muscle and head pain. In the later phase of the disease, pneumonia, liver damage, or endocarditis may occur. The people at the highest risk level are farmers of dairy animals and veterinarians (9).

Leptospirosis is a worldwide zoonotic disease caused by various species of *Leptospira*. The reservoir of *Leptospira* spp. is mainly dogs, cats, pigs, cattle, horses, and wild animals (31). These bacteria penetrate the body through damaged skin, conjunctiva, and mucous membranes (34). People can also be infected by the consumption of contaminated water or inhalation of an aerosol containing *Leptospira* (4). Leptospirosis may have different courses, from an asymptomatic form, through a mild influenza-like manifestation, to a serious multisystemic disease. In the severest cases multi-organ failure and death may occur (7).

Echinococcosis is a zoonotic parasitic disease that is caused by eggs of a tapeworm from the genus *Echinococcus*. Definitive hosts which have mature tapeworms are the most common species of canids: foxes, wolves, dogs, and jackals (35). Man can become an intermediate host due to infection with eggs released to the environment by infected animals. The invasion occurs after eating food or drinking contaminated water (12). Symptoms of echinococcosis in intermediate hosts may be different, depending on the affected organ and size and number of blisters located in it (28). Blisters can cause pressure on blood vessels and bile ducts, induce jaundice, congestive cholangitis, and also cause coughing, shortness of breath, haemorrhage, chest pain, and even death (35). The clinical course of echinococcosis involving the liver and lungs, is usually chronic (from ingestion of eggs to the onset of the first clinical symptoms may take from several to a dozen or more years), while the location of the parasite in the brain or eyeball manifests early and usually inflicts diseases with a severe course. There are two species of this genus: single-chambered (*Echinococcus granulosus*) and multi-chambered (*Echinococcus*

multilocularis). The last one is usually harder to treat due to lack of a definite border between host and parasite tissues.

Many zoonoses (such as Q fever and leptospirosis) are regarded as neglected diseases which are properly diagnosed in Poland only in a small number of cases. However, they may be an important cause of occupational diseases, occurring mostly in farmers, veterinarians, butchers, and dairy workers. The main aim of the study was to determine the risk of selected zoonosis infections among occupational group of veterinarians in Poland.

Material and Methods

A total of 373 veterinarians working in district veterinary inspectorates and private veterinary clinics located in 12 provinces of Poland (Fig. 1) were examined from June to December 2017. The examined veterinarians comprised 162 males (43.4%) and 211 females (56.6%), and their age ranged from ≤ 30 to ≥ 61 years. Blood samples of the veterinarians were collected by the puncture of a forearm vein for later serological tests. Each of the persons signed an informed consent form, and the research was approved by the Bioethical Commission of the Institute of Rural Health (Permit No. 9/2017).

Commercial immunoenzymatic tests (ELISAs from Bordier Affinity Products SA, Switzerland) were used for testing serum samples for the presence of specific IgG antibodies to *Echinococcus granulosus*. The result was positive when the absorbance of the analysed sample was higher than the absorbance of weak positive serum. For II phase IgM, I phase IgG, and IgA to *Coxiella burnetii* assays (Institut Virion/Serion, Germany), the results were determined compared to absorbance of cut-off serum. Results were considered borderline when they fell in the range of 90%–110% of cut-off serum absorbance. In the IgM and IgG to *Leptospira* spp. and II phase IgG to *Coxiella burnetii* assays (Institut Virion/Serion, Germany), the mathematical curve fitting antibody quantification based on the 4-parameter logistic function was plotted with results expressed in U/mL. Borderline results of IgM antibodies to *Leptospira* ranged from 15 to 20 U/mL, and to *C. burnetii* they ranged from 10 to 15 U/mL for IgG and from 20 to 30 U/mL for II phase IgG. Enzyme-linked fluorescence assays (ELFA) (Vidas Toxo IgG and Vidas Toxo IgM, bioMérieux, France) were used to detect IgM and IgG *Toxoplasma gondii* antibodies. A Mini Vidas immunoanalyser device facilitated the tests (bioMérieux). An IgM index value < 0.55 was considered a negative, ≥ 0.55 and < 0.65 a borderline, and ≥ 0.65 a positive result. IgG class was given in IU/mL units where a result below 4 was considered a negative, ≥ 4 and < 8 a borderline and ≥ 8 a positive result.

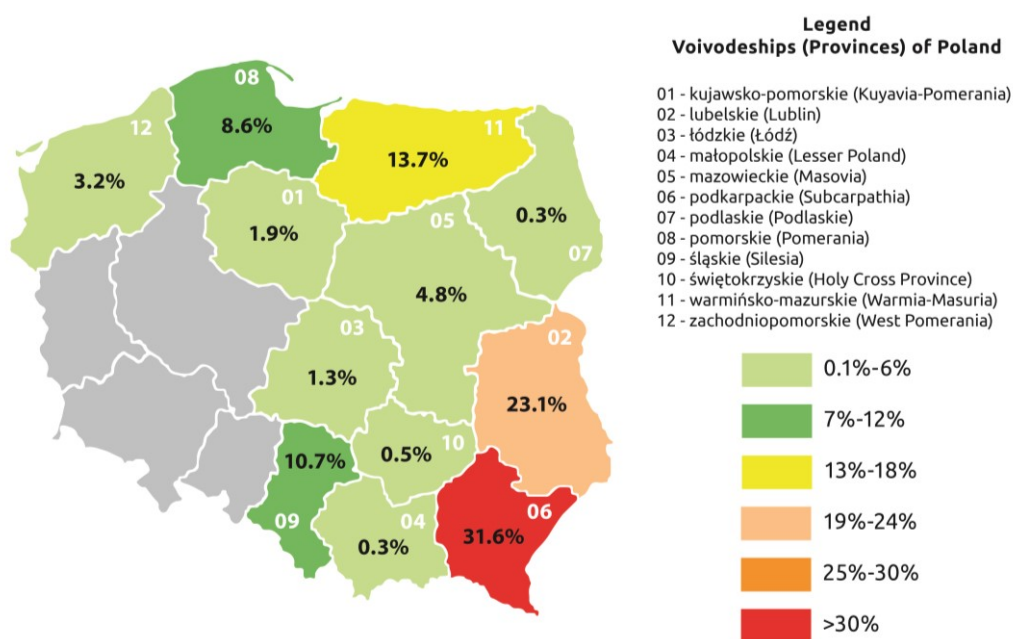


Fig. 1. Percentages of veterinarians from particular provinces participating in the study

The performance of all tests and interpretation of results were carried out according to the manufacturer's instructions.

The obtained qualitative data was subjected to statistical analysis with chi-squared (χ^2) and Spearman's tests using the Statistica 8.0 package (Statsoft Inc., USA).

Results

Results of serological tests for the detection of *Toxoplasma gondii* antibodies. The overall proportion of participants found to have specific antibodies in IgM and/or IgG anti-*Toxoplasma gondii* amounted to 44.5%. A statistically significant increase in the proportion of seropositive individuals was observed with age increase (from 25.42% in the age group below 30 to 78.85% in the group of people over 61) (Spearman's rank correlation test =0.39; $P < 0.001$). Considering only positive results, they were most commonly found in people aged 51–60, in both the IgM (7.25%) and IgG (68.12%) classes. Significantly more frequent positive results occurred in the group of men (55.21%) than in the group of women (36.19%) ($\chi^2 = 13.4$; $P < 0.001$). All persons who were positive in the IgM class (eight participants) also had positive results in the IgG class (Table 1).

Results of serological tests for the detection of *Coxiella burnetii* antibodies. The presence of specific *Coxiella burnetii* antibodies was found in 16 (4.29%) subjects. Most people with positive results were in the 51–60-year-old age group (8.7%). The highest percentage of affected veterinarians was diagnosed with II phase IgG (3.22%). In turn, the lowest

percentage was people with positive results in the IgM class (1.07%), which may indicate a recent infection. *C. burnetii* antibodies were more frequently detected in the group of men than women (6.13% and 2.86%, respectively), but it was not statistically significant ($P = 0.13$). Similarly, there was no statistically significant relationship between occurrence of seropositive results and age (Table 1).

Results of serological tests for the detection of *Leptospira* spp. antibodies. The presence of specific *Leptospira* spp. antibodies was detected in 63 (16.89%) practitioners. Antibodies in the IgM class were found in 9.65% of veterinary workers, while positive results in the IgG class were detected in 8.04% of participants. The highest percentages of seropositive persons were found in the 31–40 (21.6%) and 51–60 age groups (20.29%). The lowest percentages of positive results were found in people over 61 years old (7.69%). There were no statistically significant correlations between the age of respondents and the incidence of positive results. Positive results were found more frequently in women than in men (20.48% and 12.27%, respectively) ($\chi^2 = 4.4$; $P < 0.05$) (Table 1).

Results of serological tests for the detection of *Echinococcus granulosus* antibodies. Among 373 examined veterinarians, no *Echinococcus granulosus* antibodies were found. However, ELISA results (OD value) in the case of 6 (1.6%) people were close to the border value for a positive result (cut-off). It was recommended that the test should be repeated approximately four weeks after the first blood sample was collected.

Among the investigated veterinarians, in 209 (56.0%) cases positive results were found for at least one of the examined diseases. Two samples (0.5%) had

antibodies to *Toxoplasma gondii*, *Leptospira* spp., and *Coxiella burnetii*. In 24 (6.4%) participants the presence of *T. gondii* and *Leptospira* spp. antibodies was confirmed and in 8 (2.1%) cases the copresence of *T. gondii* and *C. burnetii* antibodies was found. Only in

one case the co-occurrence of *C. burnetii* and *Leptospira* spp. antibodies (1.6%) was detected and the result was statistically significant ($\chi^2 = 3.6$; $P < 0.05$) (Fig. 2).

Table 1. Seropositive results for toxoplasmosis, Q fever, and leptospirosis among veterinarians from Poland

Parameter	No. ^a (%)	Toxoplasmosis No. ^b /(%)			Q fever No. ^b /(%)				Leptospirosis No. ^b /(%)			
		IgM	IgG	Total	IgM phase II	IgA phase I	IgG phase II	IgG phase I	Total	IgM	IgG	Total
Age (years)												
≤ 30	59 (15.82)	0 (0.00)	15 (25.42)	15** (25.42)	1 (1.69)	0 (0.0)	2 (3.39)	0 (0.0)	2 (3.39)	7 (11.86)	4 (6.78)	9 (15.25)
31–40	125 (33.51)	0 (0.0)	35 (28.0)	35** (28.0)	2 (1.6)	0 (0.0)	2 (1.6)	1 (0.8)	3 (2.4)	17 (13.6)	11 (8.80)	27 (21.6)
41–50	68 (18.23)	1 (1.47)	28 (41.18)	28** (41.18)	1 (1.47)	0 (0.0)	1 (1.47)	0 (0.0)	2 (2.94)	6 (8.82)	3 (4.41)	9 (13.24)
51–60	69 (18.5)	5 (7.25)	47 (68.12)	47** (68.12)	0 (0.0)	5 (7.25)	4 (5.8)	3 (4.35)	6 (8.7)	5 (7.25)	9 (13.04)	14 (20.29)
≥ 61	52 (13.94)	2 (3.85)	41 (78.85)	41** (78.85)	0 (0.0)	1 (1.92)	3 (5.77)	1 (1.92)	3 (5.77)	1 (1.92)	3 (5.77)	4 (7.69)
Gender												
Women	210 (56.6)	3 (1.43)	76 (36.19)	76** (36.19)	4 (1.9)	1 (0.48)	3 (1.43)	1 (0.48)	6 (2.86)	31 (14.76)	15 (7.14)	43* (20.48)
Men	163 (43.4)	5 (3.07)	90 (55.21)	90** (55.21)	0 (0.0)	5 (3.07)	9 (5.52)	4 (2.45)	10 (6.13)	5 (3.07)	15 (9.2)	20* (12.27)
Total	373 (100.0)	8 (2.14)	166 (44.5)	166 (44.5)	4 (1.07)	6 (1.61)	12 (3.22)	5 (1.34)	16 (4.29)	36 (9.65)	30 (8.04)	63 (16.89)

* $P < 0.05$; ** $P < 0.001$; No.^a – number of examined veterinarians; No.^b – number of positive results.

Spearman's rank correlation test (seroprevalence of *T. gondii* vs age), chi-squared test (seroprevalence of *T. gondii* vs gender, seroprevalence of *Leptospira* spp. vs gender)

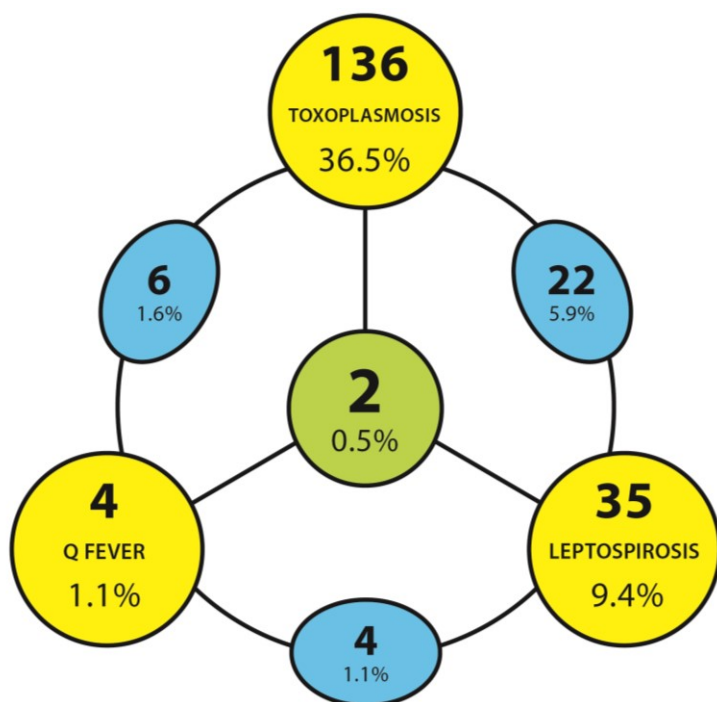


Fig. 2. Co-infection positive results in studied veterinarians

Discussion

In Poland, seroprevalence of toxoplasmosis in people lies in the 40%–60% range, depending on the examined group (17). The present study showed an overall 44.5% seroprevalence of *T. gondii* infection among veterinarians, and this infection rate does not appear to be very high against the known range for the general population. To date, numerous studies have suggested preventive strategies against toxoplasmosis for people having close contact with animals, in mitigation of the unavoidable high risk such workers are. Unfortunately, data reported on toxoplasmosis among this group of employees are limited. Previous studies suggested that veterinary personnel have a higher risk of *T. gondii* infection since they frequently interact with cats (25). However, other authors reported no significant difference in the prevalence of *T. gondii* antibodies counterposing veterinary school students (5.6%) with undergraduate students of non-veterinary disciplines (2.4%) (21). Another study also observed no statistically significant difference in the infection rate between the veterinary laboratory science student group and the control group (22), and our results seem to correspond to this trend. In the present study, age was the only risk factor to have a significant impact on *Toxoplasma* seropositive status in tested persons, and those who were older were more likely to have *T. gondii* antibodies. Age was also considered a significant factor for seropositivity of vets in a study by Brandon-Mong *et al.* (2). Due to the high percentage of *Toxoplasma gondii* antibodies determined in the present study in veterinarians (about 45%), where this percentage reaches 25% in the group of people below 30 years of age, veterinary service workers should be periodically subject to precautionary serological tests for toxoplasmosis. In particular, prophylactic examinations should cover women of childbearing age due to the risk of congenital toxoplasmosis.

On the basis of the conducted research, it can be stated that the awareness of veterinarians of the risks associated with some of the zoonoses is quite high, which is evidenced by negative results in echinococcosis test. According to the annual bulletin “Infectious diseases and poisonings in Poland”, echinococcosis is recorded in Poland as one entity, and several dozen cases are registered annually (64 cases were reported in 2016 and 75 in 2017) (16). In Estonia, *Echinococcus* spp. seroprevalence in the general population was 3.3% and 0.5% of the cases were confirmed by Western blot test. Among veterinarians the prevalence of positive results was 2.0% and among animal caretakers it was 1.1% (11). In Turkey, sera from two out of 93 veterinary surgeons (2.15%) were found to be positive for *Echinococcus* IgG antibody (10). The negative results of diagnostic tests for *Echinococcus granulosus* obtained in the present study could be the effect of the awareness of veterinarians or should be correlated with environmental study results

regarding the occurrence of the above-mentioned pathogen.

Higher seroprevalence of leptospirosis and Q fever has been determined in veterinarians where the percentages of seropositive individuals were 16.89% and 4.29%, respectively. In one case, the entire family of a seropositive veterinarian was examined, and one child aged 10 was found to have *Coxiella burnetii* antibodies, and the infection was most likely caused by ingestion (consumption of sheep’s milk cheeses). In Poland, the last epidemics occurred in 1983 in the Lublin region, and according to the annual report of the National Institute of Public Health – National Institute of Hygiene, only a single case of Q fever has been recognised since 2010. In the study of Fiecek *et al.* (8) on *Coxiella burnetii* infection, 4.4% seropositivity was found among cattle breeders and veterinarians. Q fever cases in Poland are statutorily recorded, and the zero figures for 2015, 2016, and 2017 indicate that there were no incidents of this disease in the country. The seroprevalence in veterinarians in this study (4.3%) is lower than in farm workers in Poland, reported between 15.23% and 39.07% by Szymańska-Czerwińska *et al.* (27). Results presented in this study are also much lower than obtained by Monno *et al.* (15) in Italy, concerning subjects exposed to farm animals (73.4%) and a control group (13.6%). Lower results can be explained by the risk factor of Q fever associated with *C. burnetii* infection rising with the number of hours of animal contact per week (30). Veterinarians examined in this study probably had less contact with cattle, sheep, and goats than did farmers. The prevalence of antibodies to *C. burnetii* among Dutch livestock veterinarians reached 65.1% in 2012, which could be explained by the Q fever epidemics in the Netherlands between 2007 and 2010 (30). A higher risk among veterinarians, people associated with veterinary medicine, and veterinary students has been also demonstrated in other countries and is shown in seroprevalences: 22.2% in the United States (33), 13.5% in Japan (1), 7% in Turkey (6), 26.3% in Taiwan (3), and 11.02% in Spain (29). The low seroprevalence among veterinarians from Poland in relation to other countries could be also explained by the use of animal vaccination since 2013.

The seroprevalence of *Leptospira* spp. in veterinarians from Poland (16.89%) is much higher than the 2.5% rate of leptospiral infection detected in veterinarians in the United States (32) or the 5.1% in New Zealand (24). A low frequency of leptospirosis was reported among veterinarians with a large animal practice (0.6%) in Argentina (14). However, in Mexico seropositivity for *Leptospira* spp. among swine-specialist veterinarians was 38.8% (20). No extensive epidemics of leptospirosis have occurred in Poland since the 1970s, and only a few cases of infection have been noted in the country. The prevalence of seropositive farmers noted in the study of Wasiński *et al.* (31) with field work conducted after a flood was

low (3%), and insignificantly small compared to farmers from areas not affected by the flood (9.2%). Such high prevalence of *Leptospira* antibodies detected among Polish veterinarians is therefore unexpected. To minimise the risk of human leptospirosis, vaccination of farm livestock could be considered, as is New Zealand practice with a cattle vaccine (13).

Due to detection of anti *Leptospira* spp. and *Coxiella burnetii* antibodies at the levels of 17% and 4%, respectively, there is a need to implement educational and prophylactic activities to counter threats from these pathogens. It seems particularly important to broaden the knowledge of veterinarians regarding potential sources of infection both in the veterinarian's work environment and outside it.

In conclusion, our results seem to indicate a slightly elevated risk of *Toxoplasma gondii* infection in veterinarians, although this hazard remaining close to the level of risk for the general population. The results also indicate a moderate risk of infection with *Leptospira* spp. and *Coxiella burnetii*, while low or no risk seems to occur with regard to *Echinococcus granulosus*.

Human Rights Statement: All procedures performed in studies involving human participants were in accordance with the ethical standards approved by the Bioethical Commission of the Institute of Rural Health (Permit No. 9/2017).

Conflict of Interest Statement: The authors declare that there is no conflict of interests regarding the publication of this article.

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