



Potential sources of infection with selected zoonotic agents in the veterinary work environment – pilot studies

Angelina Wójcik-Fatla^{1,A-F}, Jacek Sroka^{2,A-D}, Violetta Zając^{1,A-D},
Anna Sawczyn-Domańska^{1,B-C}, Anna Kloc^{1,B-C}, Jacek Zwoliński^{1,C-D}, Teresa Kłapeć^{1,B},
Maria Bernadeta Studzińska^{3,B}, Robert Chmura^{4,B}, Jacek Dutkiewicz^{1,A,C-F}

¹ Department of Health Biohazards and Parasitology, Institute of Rural Health, Lublin, Poland

² Department of Parasitology, National Veterinary Research Institute, Pulawy, Poland

³ Department of Parasitology and Invasive Diseases, Faculty of Veterinary Medicine, University of Life Sciences in Lublin, Poland

⁴ Department of Epidemiology and Biostatistics, Institute of Rural Health, Lublin, Poland

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Abstract

Introduction and Objective. The problem of occupational biohazards is very important, especially in the field of agriculture and in human and veterinary medicine. The aim of the study was to determine the potential sources of infection in veterinary professionals with selected zoonotic agents, including: *Toxoplasma gondii*, *Giardia duodenalis*, *Leptospira* spp., *Cryptosporidium* spp. and *Coxiella burnetii*.

Materials and Method. A total of 50 air samples from barns, piggeries and veterinary surgeries were examined for the presence of *Leptospira* spp. and *C. burnetii* DNA. Serum samples of 86 pigs and 80 cows were tested for the presence of antibodies to *Leptospira* spp. and to phase I and II *C. burnetii* antigens. Serum of 70 cats were tested for the presence of antibodies to *T. gondii* and 65 samples of cat faeces for the presence of *T. gondii* oocysts. The presence of *G. duodenalis* and *Cryptosporidium* spp. were examined in 50 of dog faeces and 50 of bovine faeces samples.

Results. DNA of *Leptospira* spp. was detected in 2 air samples from the piggeries (4%). *C. burnetii* DNA was not found in any sample. Anti-*Leptospira* spp. antibodies were detected in 51 (59.3%) of examined pigs. Neither anti-*Leptospira* spp. nor anti-*C. burnetii* antibodies were found among samples of bovine serum. Anti-*T. gondii* antibodies were found in 52 cat serum samples (74.3%). Among samples of cat faeces, no *T. gondii* oocysts were detected. In one sample of cattle stool (2%), *G. duodenalis* was detected and in another (2%) – *Cryptosporidium* spp. *G. duodenalis* was detected in 7 samples (14%) and *Cryptosporidium* spp. in 2 samples (2%) of dog faeces.

Conclusions. The results of this study demonstrate the potential risk of infection with *Leptospira* spp. in veterinarians working with pigs. Veterinarians could be also be at risk of infection with *T. gondii* and *G. duodenalis*.

Key words

environment, *Coxiella burnetii*, *Toxoplasma gondii*, veterinarians, *Leptospira* spp, *Cryptosporidium* spp, *Giardia duodenalis*

INTRODUCTION

In the environment, the major sources of biohazards that pose a threat to humans and animals include sewage, waste, animal and plant products, dusts, human and animal excreta, clinical material, soil, water and bioaerosols. Biological agents are often transmitted by air droplet, air dust, through the skin and mucous membranes, or by some arthropods (ticks, fleas) [1]. The problem of occupational biological risk factors seem to be very important, although not fully understood, especially in the field of occupational medicine and public health. Veterinarians are among the occupations with the greatest exposure to extremely dangerous biological hazards, in this case zoonotic microorganisms causing severe infectious diseases. Thus, it is most important to take measures to learn

and, consequently, reduce the exposure of veterinarians to biohazard agents by taking preventive actions.

Leptospirosis is a zoonotic disease caused by various species of *Leptospira* spirochetes. Dogs, cats, pigs, domestic cattle, horses and wild animals may serve as the reservoir of *Leptospira* spp. and the bacteria reproduce in the kidneys [2]. Leptospirosis is one of the most widespread worldwide, and the number of cases is estimated at approximately 1.03 million annually (including 58,900 fatal cases) [3]. Primarily, leptospirosis outbreaks appear in regions with a tropical climate (such as the Pacific Islands region), where frequent floods and heavy rains contribute to the development of infection [4].

Toxoplasma gondii is a widely-distributed protozoan among animals and humans. It has sources in the wild environment into which oocysts are excreted by infected cats [5]. The occurrence of *T. gondii* in the natural environment is often associated with particular animal species occurring in a given area. For example, in Brazil, distribution of this parasite

Address for correspondence: Angelina Wójcik-Fatla Department of Health Biohazards and Parasitology, Institute of Rural Health, Poland
E-mail: afatla@poczta.onet.pl

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was identified as high among population of horses, mules and donkeys, where seropositive animals were observed on 95% of examined farms [6]. The main source of human infection, with the exception of contact with an infected cat, remains the ingestion of raw or undercooked meat. Research from Brazil confirmed the wide distribution and viability of *Toxoplasma gondii* tissue cysts in pig organs and commercial meat cuts [7]; *T. gondii* was also isolated from pigs destined for human consumption [8]. In Poland, *T. gondii* DNA was detected in 5.4% of examined retail raw meat products [9]. Another transmission route is water contaminated with oocysts [10]. Consumption of the unpasteurized milk of infected animals (mainly sheep and goats) is a less common route for acquiring toxoplasmosis [11, 12].

Cryptosporidium spp. and *Giardia duodenalis* are defined as waterborne pathogens [13]. In China, both *Cryptosporidium* and *Giardia* have been detected in sewage and river waters, which confirms that both species circulate through the aqueous environment [14]. Human infection occurs through the digestive tract by consumption of food or water contaminated with the faeces of infected animals containing *Cryptosporidium* oocysts or *Giardia* cysts [15]. The prevalence of *Cryptosporidium parvum* in dairy calves has been confirmed in Argentina [16] and in Germany [15], and *Giardia duodenalis* among livestock and pets in Poland [17], and in pigs in China [18]. Therefore, the foodborne transmission of both parasites may be more common than until recently recognized.

Coxiella burnetii is a Gram-negative intracellular bacterium – etiological factor of Q fever – usually transmitted to humans by inhalation of contaminated aerosol [1] or by ticks bites. The pathogen has been detected not only in ticks from vegetation but also in ticks collected from livestock [19]. Domestic animals, such as sheep, goats and cattle, remain the main reservoir of *Coxiella burnetii* [20]. The animal source of infection could be products of abortion, faeces, urine, raw milk or cheeses made from unpasteurized milk [21]. In a recent study [22], the entire family of a seropositive veterinarian was examined, and one child aged 10 years was diagnosed with anti-*Coxiella burnetii* antibodies. The infection was most likely caused by consumption of sheep cheeses; thus, foodborne infection may not be excluded.

OBJECTIVE

The main aim of the study was to determine the potential sources of infection of veterinary professionals with selected zoonotic agents, including: *Leptospira* spp., *Coxiella burnetii*, *Toxoplasma gondii*, *Giardia duodenalis* and *Cryptosporidium* spp.

Leptospira spp. and *Coxiella burnetii* were isolated from the air of farms and veterinary surgeries and identified by PCR. *Coxiella burnetii* was also isolated from bovine placenta and likewise identified by PCR. The presence of antibodies against *Leptospira* spp. and *Coxiella burnetii* was additionally examined in pigs and cows.

Toxoplasma gondii was isolated from bovine placenta and identified by PCR. The stool samples of cats were tested for the presence of *T. gondii* oocysts. The presence of antibodies against *Toxoplasma gondii* was also examined in cats.

The stool samples of cows and dogs were tested for the

presence of parasitic protozoans *Giardia duodenalis* and *Cryptosporidium* spp.

MATERIALS AND METHOD

A total of 50 air samples collected in cow barns (32 samples), piggeries (10 samples) and veterinary surgeries (8 samples) were examined for the presence of *Leptospira* spp. and *Coxiella burnetii*. Air samples were taken for 30 minutes on polypropylene and cellulose filters using an AS-50 sampler at the flow rate of 50 l/min. DNA from the filter was isolated using Qiamp DNA Mini Kit (Qiagen, USA), according to the manufacturer's instructions. The detection of *Coxiella burnetii* DNA was based on amplification of the insertion sequence with primers IS1111f and IS1111r, described by Rolain and Raoult [23] and Subramanian et al. [24]. Detection of *Leptospira* spp. was performed according to Amutha et al. [25] using amplification by semi-nested PCR based on the fragment of *LipL32* gene [26].

Serum samples of pigs and cattle from the Lublin province were supplied by veterinarians who collected blood from livestock as a part of statutory monitoring. A total of 86 pig and 80 bovine blood samples were obtained for this study. The commercial immunoenzymatic tests (ELISA) were used for testing serum samples for the presence of specific antibodies to *Leptospira* spp. antigens (VetLine Leptospira, NovaTec Immundiagnostica GmbH, Germany), and to phase I and phase II *Coxiella burnetii* antigens (VetLine Coxiella Phase 1 and Phase 2 NovaTec Immundiagnostica GmbH, Germany).

Serum samples of 70 cats were supplied by veterinarians and were taken during other veterinary-medical activities requiring blood collection from the cat. A questionnaire containing information about the cats' living style was completed and supplied together with the serum samples. The commercial indirect immunofluorescence test (IFA) was used for testing the serum samples for the presence of specific antibodies to *Toxoplasma gondii* (Fuller Laboratories, USA) according to the manufacturer's instructions. Simultaneously, 65 samples of cat faeces were tested for the presence of *Toxoplasma gondii* oocysts [27].

Fragments of placenta after the miscarriage of cows were provided by veterinarians from Lublin province. A total of 27 placenta were examined for the presence of *Toxoplasma gondii* DNA. From each placenta, 10 fragments were acquired. DNA from 270 samples were isolated by Qiamp DNA Mini Kit, according to the tissue protocol. Detection of *Toxoplasma gondii* DNA was based on the amplification of the B1 fragment gene, according to Grigg and Boothroyd [28]. Detection of *Coxiella burnetii* DNA was performed as described previously.

A total of 50 samples of dog faeces and 50 samples of bovine faeces were examined for the presence of *Giardia duodenalis* cysts and *Cryptosporidium* spp. oocysts using direct fluorescent antibody (DFA) commercial test (Aqua-Glo™ G/C Direct Comprehensive Kit, Waterborne Inc., USA). Samples of bovine faeces were collected in cowsheds located in villages in the Lublin microregion., and samples of dog faeces collected in the urban and rural areas of the Lublin microregion.

The results were subjected to statistical analysis with Student's t-test and Pearson test for correlation, using Statistica v. 6.0 package (Statsoft, Tulsa, OK, USA). P-value <0.05 was assumed as significant.

RESULTS

Prevalence of *Leptospira* spp. and *Coxiella burnetii* in farm air. DNA of *Leptospira* spp. was detected in two air samples from the piggeries (4.0% of total samples and 33.3% of the samples taken in the piggery). *Coxiella burnetii* DNA was not found in the examined samples.

Serological response of pigs and cows to *Leptospira* spp. and *Coxiella burnetii*. Anti-*Leptospira* spp. antibodies were detected in 51 of 86 (59.3%) examined pigs. Neither anti-*Leptospira* spp. nor anti-*Coxiella burnetii* antibodies were detected in 80 samples of bovine sera.

Serological response of cats to *Toxoplasma gondii* – occurrence of *T. gondii* oocysts in cat faeces. Most of examined cats belonged to the European breed (76.5%). Specific anti-*Toxoplasma gondii* antibodies were found in 52 of 70 examined cat serum samples (74.3%). Antibodies of IgG class were detected in 71.4% samples, whereas those of the IgM class – only in 31.4% samples ($P < 0.001$). In 20 cats (28.6%) antibodies of both IgM and IgG class. Positive results were more frequent in females (82.1%) than in males (60.7%) and the difference was nearly significant ($P = 0.06$). An increase was observed in the percentage of seropositive animals with increase in their age, from 50.0% for cats below 1 year old, up to 74.4% for those between 1 – 5-years-old, 76.9% for those between 5 – 10-years-old, and finally, 100% for those above 10-years-old. The correlation between age and seropositivity proved to be significant ($P < 0.05$). A similar distinct increase in seropositivity was also observed with relation to cats' habits (animals kept indoors reacted positively in 53.8%, freely moving in 73.7% and feral in 91.7%); the correlation in this case was also significant ($P < 0.05$). A higher percentage of positive samples was found among cats living in the countryside (83.8%) than in the city (52.2%), and the difference proved to be significant ($P < 0.01$). Positive results were found insignificantly more often in sterilized cats (76.5%) compared to those not sterilized (66.7%; $P > 0.05$). Anti-*T. gondii* antibodies were significantly ($P < 0.05$) more common in unvaccinated animals (86.8%) than in those vaccinated against rabies and/or other viral diseases (58.6%). Nevertheless, in 86.5% of seropositive cats the owners and veterinarians did not report any symptoms.

Among 65 examined samples of cat faeces, no *Toxoplasma gondii* oocysts were detected.

Prevalence of *Toxoplasma gondii* and *Coxiella burnetii* in bovine placenta. In 270 examined samples of bovine placenta, no *Toxoplasma gondii* or *Coxiella burnetii* DNA were detected.

Prevalence of *Giardia duodenalis* and *Cryptosporidium* spp. in bovine stool samples. Among 50 cattle stool samples examined, in one sample (2.0%) *Giardia duodenalis* cysts were detected and in another (2.0%) the presence of *Cryptosporidium* spp. oocysts. A positive *Giardia duodenalis* sample was collected in a barn housing 60 dairy cows, 5 – 7-years-old, which were not moved to pasture. A *Cryptosporidium* spp. positive sample was collected in a cowshed with 20 dairy cows and calves.

Prevalence of *Giardia duodenalis* and *Cryptosporidium* spp. in canine stool samples. Among 50 samples of dog faeces examined, in 7 (14.0%) *Giardia duodenalis* cysts were detected, and in 2 (4.0%) *Cryptosporidium* spp. oocysts. In one stool sample (2.0%), *Cryptosporidium* spp. and *Giardia duodenalis* parasites were found simultaneously. All dogs in which parasites were detected came from an urban area, in which 23 samples (46% of the total) were collected. The prevalence of parasites in urban area was significantly higher compared to rural area (16% vs. 0%; $p < 0.04$).

DISCUSSION

In the presented study, no *Coxiella burnetii* DNA was found in the air samples, while in two samples of air collected on pig farms, the presence of *Leptospira* spp. was detected. Due to the fear of farm owners of African swine fever, it was not possible to collect air samples from large pig farms. The samples were taken only in several small piggeries, where animals were kept for breeders' own use. The percentage of positive air samples in these piggeries was high (33.3%) but because of small number of total samples examined this result needs confirmation by further studies. Nevertheless, the results obtained in the current study which

indicate the possibility of airborne infection of veterinarians with *Leptospira* in home piggeries are supported by the high prevalence of anti-*Leptospira* antibodies found in pigs in this study, as well as by a considerable percentage of seropositive results among veterinary surgeons (16.9%), as stated by Wójcik-Fatla et al. [22]. Infection with *Leptospira* may occur by the penetration of spirochetes through damaged skin, conjunctiva and mucous membranes [29], by the inhalation of bacteria-containing aerosol [30], by consumption of contaminated water [26], and probably also by tick bite [31].

In the current study, anti-*Leptospira* spp. antibodies were detected in 59.3% of the pigs tested, which is a high prevalence, 2–3 times higher compared to similar studies performed in Italy [32] and Germany [33], and close to that reported by Cruz-Romero et al. from small households in Mexico [34].

In contrast, in the current study, no antibodies were found against *Leptospira* spp. in cattle, unlike in other countries, such as Italy [35] and France [36]. Thus, the presented pilot study seems to indicate that in Poland exposure to pigs is mainly associated with the threat of veterinarians contracting leptospirosis. It would be therefore reasonable to introduce prophylactic diagnostic tests to detect anti-*Leptospira* spp. antibodies in veterinarians, both in the initial phase of employment and during the course of their professional work. Vaccination may be another solution to prevent leptospirosis which, in the case of cattle, is already used in New Zealand [37].

In the serological study of cattle, the authors of the current study did not find antibodies against *Coxiella burnetii*, which corresponds with the negative results of air sampling. To-date, seropositive results in the study of Q fever in cattle have been obtained mainly in Asian countries and in South America [38, 39].

In the study of cat sera for the presence of antibodies against *T. gondii*, positive results were obtained in almost 75% of cases. This result is in agreement with earlier serological studies [40] and with the well-established view on the main role of the cat as a definitive host of *T. gondii*, excreting

infective oocysts into soil and water [5]. In the presented study, a distinct increase of seroprevalence was found with cats' age in up to 100% in animals more than 10-years-old. Similar results were obtained by Esteves et al. [41]. In the presented study, no oocysts of *T. gondii* were found in the samples of cat faeces. This may be related to the fact that cats expel oocysts into the environment, on average, for a period of 1–2 weeks, most often only once during their entire life. Nabi et al. demonstrated that the percentage of tested cats that expel oocysts does not usually exceed 2% [42].

The current study on the occurrence of *Toxoplasma gondii* and *Coxiella burnetii* in samples of bovine placenta produced negative results. However, there were limitations to the study: the relatively low number of samples, and the fact that the placenta of sheep and goats – well-known hosts of both pathogens, in particular *C. burnetii* – were not examined. Thus, despite the negative results obtained, the possibility of veterinarians' infection at birth reception cannot be ruled out, especially when working with other farm animals (sheep, goats).

The authors' coproscopic studies on the prevalence of *Giardia duodenalis* cysts and *Cryptosporidium* spp. oocysts in the faeces of cows and dogs, showed a low incidence of parasites in cows and a distinctly greater incidence in dogs, especially with regard to the incidence of *Giardia duodenalis* in dogs living in an urban environment.

CONCLUSION

This study on the occurrence of five zoonotic pathogens (*Leptospira* spp., *Coxiella burnetii*, *Toxoplasma gondii*, *Giardia duodenalis*, *Cryptosporidium* spp.) in various elements of veterinarians' working environment demonstrated the potential risk of infection with *Leptospira* spp. in veterinarians working with pigs. Thus, the application of preventive measures against leptospirosis, such as serological monitoring and/or vaccination, seems to be reasonable. Veterinarians could also be at risk of infection with *Toxoplasma gondii* on contact with cats and cat faeces, as well as at risk of infection with *Giardia duodenalis* on contact with dog faeces.

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