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Q FEVER IN POLAND – THE CURRENT EPIDEMIOLOGICAL SITUATION AND CONTROL

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Summary. The aim of the study was to assess the prevalence of Coxiella burnetii in small ruminants and cattle herds in different regions of Poland. Complement fixation test was performed on 1,200 serum samples collected from 449 cattle herds from 158 counties. Moreover, 1,287 samples of biological material from 180 cattle herds and 79 small ruminants herds were tested using real-time PCR. Molecular analysis by revealed that 320 from 1,287 tested samples (24.87%) were positive. The average rate of seropositive herds was 43.66%.

Keywords: Q fever, Coxiella burnetii, abortion, ruminants

Introduction. Q fever is a zoonosis caused by *Coxiella burnetii*. The etiological agent is a gram-negative, intracellular bacterium with a complex life-cycle. Sequencing of 16S rRNA has shown that the *Coxiella* genus belongs to the gamma subdivision of Proteobacteria, order Legionellales (Drancourt and Raoult, 2005). The pathogen can infect a wide range of mammals and non-mammalian species, including birds and arthropods (Babudieri and Moscovici, 1952).

In ruminants, which are considered as a main source of infection in humans, the bacteria caused non-specific reproductive disorders such as subfertility, abortion, stillbirth, delivery of weak offspring. Although, some infected animals remain asymptomatic and they constitute potential bacterial reservoirs capable of transmitting the disease. Ruminants shed a huge amount of the pathogen in birth products and a smaller number of bacteria in milk, urine, faeces, and semen.

Coxiella burnetii is transmitted to humans mainly by inhalation of contaminated dust or by direct contact with infected animals or contaminated wool, bedding, manure and birth products (ECDC, 2010). Therefore, cases of Q fever are usually notified among people occupationally exposed to the pathogen but the number of infected persons living in urban areas has been observed. A possibility of infection by alimentary route remains contradictory, but cannot be excluded (Masala et al., 2004; Angelakis and Raoult 2010, Signs et al., 2012).

The studies conducted by research from Central Europe have demonstrated that the prevalence of *Coxiella burnetii* infection in ruminants has been increasing in recent years (Astobiza et al., 2012, Czaplicki et al., 2009, Ryan et al., 2011). Detailed data about Q fever prevalence in Polish ruminants are limited and are mainly related to the endemic region in South-Eastern Poland (Recent monitoring studies showed a lot of seropositive results in milk cattle herd in Poland). Therefore, the aim of this study was to assess the prevalence of *Coxiella burnetii* in variety biological samples obtained from cattle and small ruminants herds from different regions of Poland.

Control of Q fever in Poland. In accordance with the regulation of Minister of Agriculture and Rural Development, Q fever is a notifiable disease in Poland. Moreover, since 2010, it has been included in the serological monitoring program performed by the Veterinary Inspection. Its results indicate that *Coxiella burnetii* has been consistently presented in a population of ruminants in Poland. At the beginning of this year, Veterinary Chief Officer has published the guidelines for veterinarians, which contains a surveillance and workflow schemes.

In the case of suspicion of the Q fever outbreak on the basis of clinical symptoms, it is recommended to perform serological tests. Samples should be collected from animals with clinical symptoms and from randomly selected. The age structure of the herd must be also taken into account and it is advisable to take samples from animals in each age category. When the result of a serological test performed by an official laboratory is doubtful or positive, information about the test result must be submitted to the appropriate District Veterinary Officer and the sample must be sent to National Reference Laboratory (NRL) for confirmation. If the sample, which was originally taken cannot be delivered to the NRL, the appropriate District Veterinary Officer should collect material from suspected animal. Along with the samples, information about the localization of the herd, its ID number, a number of ear tag and contact information to the District Veterinarian Officer should be given to NRL. If the serologic test performed in NRL is negative, withdraw from further proceedings. If, despite the negative serological test result, Q fever infection is still suspected, it is advisable to continue the diagnostic

procedure, as in the case of a positive result in a serological test.

In the case of positive result of a confirmatory serological test, it is recommended to collect the material from a seropositive animal for molecular testing using real-time PCR (qPCR). Depending on the availability it could be: bulk tank milk or individual milk sample and swab of the genital tract (if possible, collected in the perinatal period — up to 8 days after birth), placenta (fragment comprising of minimum three cotyledons) or sections from the internal organs of aborted fetuses (spleen, lungs, heart, liver). It is crucial to take a section from all of the mentioned organs because a lack of *Coxiella burnetii* in one of them does not exclude the presence of the bacteria in other tissues. If abortions occur in the herd, placenta, vaginal swabs and/or sections from the internal organs of aborted fetuses are the most suitable samples for molecular analysis.

A positive result in the qPCR test is the ultimate result confirming an outbreak of Q fever in the herd. The District Veterinary Officer is obliged to inform the Province Veterinary Officer and Sanitary Inspection about the outbreak. In this case, District Veterinary Officer should recommend the owner to isolate and treat/vaccinate or eliminate positive individuals. After isolation/culling of animals in the dairy cattle herds, it is recommended to test BTM and/or vaginal swabs using real-time PCR technique. If the herd was subjected to the treatment and/or vaccination, in order to evaluate the effectiveness of therapy, molecular tests should be performed but not earlier than 14 days after ending of therapy or vaccination. Type of material to study should be depended on its availability: bulk tank milk (from dried animals vaginal swabs in perinatal period should be collected), individual milk samples and/or vaginal swabs, placenta. According to the Manual of Diagnostic Tests and Vaccines for Terrestrial Animals 2015 published by OIE, if the abortions were noted in the herd, the real-time PCR tests (BTM and/or vaginal swabs — allowed the pooling of 9 swabs) should be performed every two months for a year.

If there is no possibility to test milk samples (beef cattle), a further diagnosis should be performed in the case of abortion. Then, to exclude *Coxiella burnetii* infection, placenta, and internal organs from an aborted fetus or vaginal swabs collected up to 8 days after birth should be tested by real-time PCR. If the bulk tank milk sample is positive in real-time PCR, it is recommended to test individual milk samples in order to separate the shedders. Non-shedding animals should be vaccinated. Infected cows should be treated and/or vaccinated or culled. Real-time PCR tests on BTM samples should be performed every two months for one year after treatment or vaccination.

Herd, which obtained positive serological test results in cases other than surveillance of animals' infections, could be typed for screening by District Veterinary Officer after analysis of an epizootic situation.

When the District Veterinary Officer receives information from the State Sanitary Inspector of the suspicion or diagnosis of Q fever in humans, then he undertakes actions ex officio to determine or exclude the disease.

Ethic statement. The samples were collected from animals by authorized veterinarians during clinical studies following standard procedures. The samples were collected specifically for this study with the agreement of the farmers. According to the Local Ethical Committee on animal Testing at University of Life Sciences in Lublin (Poland) from ethical approval is not required for this kind of study. We were using guidelines published by this ethic committee (Resolution No. 22/2006, November 7, 2006), which confirm that this work is acceptable without specific ethical approval.

Materials and methods. The serum samples were tested during the first part of the 3rd edition Multiannual Research Programme, the study was performed at the turn of 2014 and 2015 on 1,200 serum samples collected from 449 cattle herds from 158 counties. Most tested animals were milk cattle. Materials for diagnostic assay were collected from non-vaccinated animals. The complement fixation test using Siemens Healthcare Diagnostic Products (Germany), detecting both phase I and II antibodies, was done in agreement with the Manual of Diagnostic Tests and Vaccines according to the manufacturer's instructions. The temperature of inactivation of sera was 57±1 °C and 62±1 °C, respectively for cattle and small ruminants.

Moreover, a total of 1,287 samples of biological material from 79 small ruminants and 180 cattle herds were obtained for molecular analysis by real-time PCR. The material was sent to National Reference Laboratory for the confirmatory, research and service tests. 385 milk, 335 vaginal swabs, 539 blood, 2 tissues, 5 semen and 21 placenta samples were tested. DNA isolation was performed with commercially available DNA Mini Kit (Qiagen). The qualitative real-time PCR assay, detecting the IS*1111* element, was performed on blood and semen samples and for the other types of samples Adiavet COX RealTime PCR (Adiagene, Biomerieux Company) kit was used. PCR was performed according to the manufacturer's instructions. Only the samples presenting a typical amplification curve with a thresh hold value (Ct) values below 36 were considered positive.

Results and discussion. In Poland both human and animal cases of Q fever are notifiable. Cases of Q fever in animals are confirmed by the National Reference

Laboratories for Q fever. The information on animal cases or outbreaks is sent by the regional state veterinary officer to the National Sanitary Inspectorate. Elimination of the source of infection is achieved through established cooperation between veterinary and health services. Moreover, there is a monitoring program for Q fever in Poland for cattle and small ruminants. Outbreaks of Q fever in both humans and animals have been noted in Poland since 1956 (Lutyński et al., 1956). The largest epidemic of Q fever among humans and animals was recognized near Zamość (in the Lublin voivodeship in eastern Poland) in 1983 (Cygan et al., 1983, Mikołajczyk et al., 1986). More than 1,300 people fell ill in this epidemic centered around the area of Hrubieszów and Tomaszów Lubelski. Until 2007 when the large Q fever epidemic in the Netherlands broke out it had been considered the biggest Q fever epidemic in humans in the world. According to literature data from this time, anti-*C. burnetii* antibodies were found in cattle from this area (Cisak et al., 2003, Galińska et al., 2011, Niemczuk et al., 2011). Moreover, the serological studies performed by Cisak et al. (2003) among the farmers living in villages located in Lublin voivodeship showed the presence of specific antibodies to *C. burnetii* in 17.8% of 90 tested subjects for comparison in our studies the percentage of seroprevalence in tested farming population was higher in IFA (31.12%) and ELISA 39.07%.

The results of serological tests show a high percentage of seropositive cattle herds in Poland: a total of 524 serum samples were positive and the average rate of seropositive herds was 43.66%. Generally, the level of seroprevalence in Poland is similar to other European countries. The highest seroprevalence was noted in Mazowieckie and Lubelskie provinces. The lowest seropositive herds, below 20%, were in Warmińsko-Mazurskie and Łódzkie provinces.

Molecular analysis by real-time PCR revealed that 320 from 1,287 tested samples (24.87%) were positive. The percentage of positive cattle herds was 24.44%, for flocks of sheep it stood at 2.86% and for goat herds 22.22%. Goats seem to be more sensitive to acquire the pathogen because in many types of research prevalence in this species are higher than in sheep (Van den Brom et al., 2015). In this survey, a high percentage of positive goat herds cannot be representative of the whole goat population due to a small number of tested samples. The percentage of herds excreting *Coxiella. burnetii* in milk was 33.77% whereas presence in blood was noted only in 11.61% of cattle herds. *Coxiella burnetii* DNA in vaginal swabs was detected in 3 out of 15 tested herds. Molecular analysis showed a lower percentage of positive herds than serological test but it could be caused by high amount of blood samples, where bacteria are detectable in a short period during the infection.

Diagnosis of Q fever in animals is difficult because both the ELISA and the CFT test have some limitations. First antibodies appear about 14–21 days after infection and serological test performed in this period can give negative results. The literature data indicate that ELISA test loses positive results when the serum samples contained antibodies specific for phase II, and particularly when sera had low titer 1:10 (+ and ++) (Emery et al., 2012, Szymańska-Czerwińska et al., in press). It is due to a fact that ELISA is able to detect the IgG antibodies while CFT detects both IgG and IgM antibodies. On the other hand, some researches show that CFT has lower sensitivity compared with ELISA. Moreover, animals which shed *Coxiella burnetii* intermittently may remain seronegative but serological tests could be a reliable tool to identify heavy shedder, which are usually persistently highly-seropositive.

Real-time PCR is thought to be a fast and sensitive tool for detection of *Coxiella burnetii* shedders. Guatteo et al. (2006) observed that cattle shed the bacteria mainly by one route, but some animals excreted pathogen simultaneously in milk, faeces, and vaginal discharge. The data about predominant shedding route remain ambiguous, although it is thought that ewes shed more and longer in vaginal mucus than goats. The latter and cattle excrete bacteria more frequent in milk. (Arricau-Bouvery and Rodolakis 2005). What's interesting, most of the exanimated cows in this survey shed a huge amount of bacteria without any clinical signs, what is in agreement with results obtained by scientists in Europe. (Rodolakis et al., 2007)

Since 2013, the vaccine contains inactivated *Coxiella burnetti* bacteria, has been available for cattle and goats in Poland. It can reduce shedding of bacteria in these animals via milk and vaginal mucus but a drop in milk production is common in goats after the administration of a vaccine. Furthermore, the manufacturer informs that the immunization reduces the level of shedding only in non-infected animals. This fact was confirmed by many surveys (Hogerwerf et al., 2011; Van den Brom et al., 2013, Guatteo et al., 2008). Vaccinated ruminants cannot be distinguished from seropositive individuals by serological examinations, necessitating molecular techniques to confirm the infection.

Conclusion. The results of the survey performed in Poland show that *Coxiella burnetii* is widespread in ruminants population, mainly in cattle. Due to very low infectious dose and zoonotic character of this pathogen, further surveillance is crucial. Data about genotypes variations of *Coxiella burnetii* are limited in Poland, so the detailed genetic characterization of field strains will be valuable knowledge for epidemiological investigation in Q fever outbreaks.

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