Part 1. Veterinary medicine

UDC 619:616.98:579.882.11:636.22/.28(438)

PRELIMINARY STUDY IN RANGE OF PREVALENCE *CHLAMYDIA* SPP. IN THE POLISH CATTLE POPULATION

Szymańska-Czerwińska M., Niemczuk K.
Laboratory for Serological Diagnosis, Department of Cattle and Sheep Diseases,
National Veterinary Research Institute, Puławy, Poland, e-mail: monika.szymanska@piwet.pulawy.pl

Summary. *Chlamydia* spp. is an obligate intracellular agent that causes chlamydiosis in animals and humans. The aim of the presented study was to investigate the prevalence of *Chlamydia* infection in the Polish cattle population, both asymptomatic and having reproductive disorders. The study was performed on 900 serum samples collected from 14 Polish provinces at the turn of 2014–2015. The samples were tested by complement fixation test (CFT) and ELISA. Moreover, 40 samples of biological materials from cattle were tested by real time PCR. The study indicates that infections with *Chlamydia* spp. are present in Polish cattle. The study demonstrated that average percentage of seropositives herds is 26.40%. The real-time PCR gave the positive results for Chlamydiaceae into vaginal swabs in 10 tested herds. Moreover, the molecular studies showed that infection *Chlamydia pecorum* and *Chlamydia abortus* is more spread than with *Chlamydia pisttaci*.

Keywords: cattle, *Chlamydia* spp., chlamydiosis, complement fixation test, ELISA, PCR

Introduction. *Chlamydia* are obligate, intracellular, gram-negative bacteria that infect a wide variety of host species including vertebrates, arthropods, and amoeba (Wheelhouse and Longbottom, 2012). Chlamydiaceae only has a single genus *Chlamydia* that comprises eleven species according to the new taxonomy. Firstly, the division of Chlamydia was divided into genera *Chlamydia* and *Chlamydophila*. As their general importance is based on two aspects: economic losses to the animal owners and potential zoonotic transmission to humans. The most prominent *Chlamydia* agent in cattle is *Chlamydia abortus* (Markey, 2011). Moreover, recent evidence suggested that infection of cattle with *Chlamydia suis* and *Chlamydia psittaci* is also possible (Jee et al., 2004; Pantchev et al. 2010). Chlamydial infection in cattle has been associated with reproductive disorders including abortion, endometritis, repeat breeding, vaginitis, seminal vesiculitis, weak calves, and perinatal mortality (Reinhold, Sachse and Kaltenboeck, 2011). Moreover, cause bovine infertility and epizootic bovine abortion. In cattle *Chlamydia* such as *Chlamydia pecorum, Chlamydia abortus*, and *Chlamydia psittaci* are found in connection with infection of the respiratory tract as well (Sachse et al., 2009). *Chlamydia abortus* is a recognised cause of epizootic bovine abortion and a cause of bovine infertility worldwide. In bulls, the infection can cause epididymitis, seminal vesiculitis, and testicular atrophy, and affects semen quality. The organism has also been shown to be shed in semen, and multiplication of *Chlamydia abortus* via contaminated semen can result in local infections and inflammatory reactions in the uterus, which can subsequently lead to infertility in heifers (Livingstone and Longbottom, 2006). In dairy cows, the presence of *Chlamydia* was significantly associated with subclinical inflammation of the mammary gland, and while vaccination significantly lowered somatic cell counts in milk and increased antibody levels against *Chlamydia*, it failed to reduce shedding levels of these bacteria in milk. It is conceivable that chlamydial infections of the respiratory and/or intestinal tracts in calves originating from cows with subclinical chlamydia-associated mastitis may have an impact on developing calves, even in the absence of clinical illness. However, there are no data in the literature to verify this hypothesis (Uhe et al., 2005). Chlamydial infection in cattle has been reported in many countries including Poland but the data about prevalence are limited.

The aim of the study was to investigate the seroprevalence of the chlamydial infection in Polish cattle population in the individual region of the country. Moreover, the aim of the studies was identification of *Chlamydia* species and evaluation which one was the most common. The results may provide base-line data for the implementation of integrated strategies to prevent and control of chlamydial infection in dairy cattle in the future.

Materials nad methods. ***Blood samples and vaginal swabs.*** The study was performed at the turn of 2014–2015 on 900 serum samples collected from different region (voivodeships) of Poland. The samples were collected from randomly selected cattle herds. Totally, samples were taken from 317 herds. The number of samples from individual herd depended on availability for sampling. The blood samples was taken from coccygeal vein into a 10 ml vacuum tube, stored in a refrigerated bag, brought to the laboratory and centrifuged at 2500 g for 10 min within 24 h. Serum was then removed and stored at – 20°C. Moreover, from 40 seropositive herds the vaginal swabs were taken for confirmation the presence of active *Chlamydia* infection by real-time PCR. The samples were not taken from all herds because the farmers did not accept further research. Totally, we tested 400 swabs. The swabs were stored in conservation buffer SPG at – 80°C until subjected to DNA extraction.

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) formal ethical approval is not required for this kind of study. We were using guidelines published by this ethics committee (Anon, 2006), which confirm that this work is acceptable without specific ethical approval.

Serological examinations. The serological examinations were performed using CFT, a diagnostic technique recommended by the world Organisation for Animal Health (OIE). For CFT, Institut Virion/ Serion GmbH (Germany) and Sera and Vaccines Manufacturing (Biomed-Krakow, Poland) reagents were used. Before each examination, an intralaboratory evaluation including antigen titration against a positive control serum and checking the activity of other reagents used in the reaction, were carried out to find the actual titre versus activity ratio in relation to that declared by the manufacturers. The specific reaction of CFT, its consecutive steps, and result interpretation, were performed according to the 'Manual of diagnostic tests and vaccines for terrestrial animals (mammals, birds and bees)' (OIE, 2012). The starting dilution of the examined samples was 1:4, and the ending dilution was 1:64. Serum was considered as positive when a partial inhibition of haemolysis was observed at the dilution of 1:32. Moreover, all seropositive samples were tested by ELISA (*Chlamydophila abortus* Antibody test Kit, IDEXX). This test detects antibodies against *C. abortus* in serum and plasma samples from ruminants. The sera, positive, and negative controls were tested in a dilution of 1:400 in duplicate. According to the manufacturer's instructions, the percentage of optical density (OD) was calculated as (sample OD-negative OD)/(positive OD-negative OD)×100 after averaging the duplicate values. Sera were considered to be negative when % OD < 30, dubious when % OD ≥ 30 and % OD ≤ 40, and positive when % OD > 40.

PCR. DNA extracts from swabs, were performed using the commercial QIAamp DNA Mini Kit (Qiagen), following the manufacturer's instructions. A Chlamydiacae-specyfic real-time PCR targeting the 23S rRNA gen (described previously by Pantchev et al., 2010) was used in this study. All samples with a cycle threshold (Ct) > 38 were considered negative. Normalization of target genes was performed using glycerinaldehyde-3-phosphate (GAPDH) as an endogenous standard. The positive control was DNA extracted from reference strain of *Chlamydia abortus* B-577 (ATCC-VR-656). The samples positives for Chlamydiaceae were tested by real-time test for species-specific detection of *Chlamydia psittaci* and *Chlamydia abortus* previously described by Panchev et al. (2009). The samples negative for *Chlamydia abortus* and *Chlamydia psittaci* were tested by nested PCR for *Chlamydia pecorum* according the protocol described previously by Kaltenböeck, Schmeer and Schneider (1997). The DNA form reference strain of *Chlamydia pecorum* obtained from Reference Laboratory for chlamydiosis in FLI, Jena, Germany.

Results. Serological results are presented in Table 1. Taking into account all CFT and ELISA results as positive, the seroprevalence of *Chlamydia* spp. infections in the Polish cattle population is presented. The average percentage of seropositives herds is 26.40% and ranges from 91.67% in the Mazovia Province to 0.00% in the Lubuskie Province. Whereas average the percentage the seropositive animals was 30.11%.

Based on the study, it was possible to observe that the highest level of the *Chlamydia* spp. seropositivity was in cattle herds localized in more industrialized regions of Poland, which have more intensive levels of agricultural and cattle breeding production. The highest percentage of positive samples was noted in the Mazovia Province (96.67%), the Pomerania Province (85.71%), the Silesia Province (50%), the Podlasie Province (43.48%), the Wielkopolska Province (30.77%), Świętokrzyskie Province (33.30%). The lower percentage seropositive herd was noted in Warmia-Mazuria Province (26.32%) and West Pomerania Province (21.43%). The lower percentage cattle herds with presence of antibodies against *Chlamydia* spp. was in Łódź Province (2.5%). While any seropositive herd was detected in Lubuskie Province. From three region Lower Silesia, Opole and Małopolska Province samples were not tested.

The results of molecular survey are presented in Table 2. The results showed that *Chlamydia* spp. organisms were present in the cattle. The real-time PCR gave the positive results for Chlamydiaceae to vaginal mucous in 10 tested herds. Both species of *Chlamydia abortus* and *Chlamydia pecorum* were detected in the tested samples but the most prominent was *Chlamydia pecorum*. However, the presence of other Chlamydia species (e.g. *Chlamydia psittaci*) was not confirmed. The positive herds for *Chlamydiacae* were in Mazovia Provine (two herds), Wielkopolska Province (one herds), Podlasie Province (three herds), Pomerania Province (three herds), Świętokrzyskie Province (one herds). *Chlamydia pecorum* was detected in 7 herds.

Table 1 – Seroprevalence of *Chlamydia* spp. in the Polish cattle in the individual region

Province	Number of tested herds	Number of seropositive herds (percentage)	Number of tested animals	Number of seropositive animals
Lower Silesia *	-	-	-	-
Kujawy-Pomerania	29	4 (13.79%)	61	25 (40.98%)
Lubelskie	12	3 (25%)	40	3 (7.50%)
Lubuskie	1	-	1	-
Łódź	40	1 (2.5%)	40	1 (27.50%)
Mazovia	12	11 (91.67%)	60	25 (41.67%)
Małopolska *	-	-	-	-
Opole *	-	-	-	-
Podkarpacie	106	21 (19.81%)	106	21 (19.81%)
Podlasie	23	10 (43.48%)	253	123 (48.62%)
Pomerania	7	6 (5.71%)	40	15 (37.50%)
Silesia	16	8 (50%)	102	32 (31.37%)
Świętokrzyskie	12	4 (33.33%)	40	4 (10%)
Warmia-Mazuria	19	5 (26.32%)	68	7 (10.29%)
Wielkopolska	26	8 (30.77%)	38	9 (23.68%)
West Pomerania	14	3 (21.43%)	51	6 (11.76%)
Total	317	84 (26.50%)	900	271 (30.11)

* from this region sampling was impossible

Discussion. The serological results showed that cattle revealed positive results of serological survey in all tested provinces of Poland excluding Lubuskie Province, and the presence of *Chlamydia* antibodies was noted. There is preliminary study and it is difficult to evaluate current epidemiological situation because the real-time PCR test was not performed in all provinces. These data show that the problem of *Chlamydia* spp. infection in cattle in Poland is presented and the pathogen is country-wide, and the control investigations have to be performed. these results compared with published data from Poland from several past years, indicate that the percentage of seropositive samples in cattle is now higher (Niemczuk 2006, 2005) although, the previous studies were performed on the selected population of cattle including cattle herds with reproductive disorders and the seroprevalence was not evaluated for herds but for all tested animals in individual provinces. To our knowledge, the present survey is the first epidemiological evaluation of the prevalence of *Chlamydia* in randomly selected cattle herds in a country.

Table 2 – Results of molecular studies (real-time PCR and nested PCR)

Province	Number of tested herds	Number of seropositive herds
Lower Silesia	-	-
Kujawy-Pomerania	-	-
Lubelskie	2	-
Lubuskie	1	-
Łódź	2	-
Mazovia	2 *	2
Małopolska	-	-
Opole	-	-
Podkarpacie	-	-
Podlasie	3^	3
Pomerania	3^	3
Silesia	5	-
Świętokrzyskie	1^	1
Warmia-Mazuria	2	-
Wielkopolska	1*	1
West Pomerania	-	-
total	40	10

* herds with presence of *Chlamydia abortus* infection confirmed by PCR and real-time PCR

^ herds with presence of *Chlamydia pecorum* infection confirmed by PCR and real-time PCR

Antibodies against *Chlamydia* were found in sera of cattle in several European countries (Borel et al., 2006; Kauffold et al., 2007; Wang et al., 2001; Wilson et al., 2012). Most of the surveys were performed by ELISA, so it is very difficult to compare them with our results because CFT is less sensitive but is still recommended by the OIE to detect antibodies against *Chlamydia*. The complement fixation test detects only IgG1 immunoglobulins persisting for 3–4 weeks after

infection, ELISA also detects other antibody subclasses. Moreover, most of the serological tests cannot detect the infection with these pathogens in the first phase because, generally, the titres are very low (Niemczuk and Truszczyński, 2012).

Pantchev et al. (2010) performed the examinations of cattle in Germany, and observed cases of mixed chlamydial infection as well. Combinations of *Chlamydia abortus* and *Chlamydia psittaci* or *Chlamydia suis* and less *Chlamydia pecorum* and *Chlamydia suis* in cattle were described. Recent data have demonstrated that subclinical chlamydial infections by both species, *Chlamydia abortus* and *Chlamydia pecorum*, are ubiquitous in cattle and often not detected due to low sensitivity of diagnostic techniques (Pantchev et al., 2010; Reinhold et al., 2008). However, frequently there is no correlation between the results of PCR or real-time PCR detecting the presence of DNA and serological tests determining the level of specific antibodies. The recent studies of bulls where *Chlamydia* antibodies were detected in 50.8% of bulls, while the PCR confirmed the presence of *Chlamydia* in semen in 9.2%, preputial washing in 10.7%, and faecal samples in 18.0% (Kauffold et al., 2007). PCR-positive but serologically negative bulls might have not exhibited a systemic immune response, possibly due to the obligate intracellular lifestyle of *Chlamydia* that might hide them from the systemic immune response. In contrast, other data showed that naturally infected calves were in 60% seropositive, while all of them were the shedders of *Chlamydia* (Reinhold et al., 2008).

Generally, based on the literature data it is known that chlamydial infections occur in cattle breeding. However, the epizootic situation varies in different European countries. For example in Sweden, there are 0.4% of seropositive cows, and performed studies suggested that *Chlamydia abortus* infection is absent or rare, whereas *Chlamydia pecorum* is probably more spread. Our results showed that both *Chlamydia pecorum* and *Chlamydia abortus* are present. The same researcher suggests that *Chlamydia* spp. are not related to reproductive disorders in the Swedish cattle (Godin et al., 2008). In Italy, serological investigation of cattle in different areas detected seroprevalence for *Chlamydia* ranging from 2 to 28% but association between seropositivity and abortion has not been examined (Cavirani et al., 2001). In Ireland, the percentage of seropositive cattle in ELISA was 4.44, in Taiwan — 51.3% for asymptomatic animals and 71.4% for aborted cows (Wang et al., 2001; Wilson et al., 2012).

The occurrence of *Chlamydia abortus* and *Chlamydia pecorum* in the Polish cattle can suggest that these pathogens can be one of the many factors responsible for these disorders in bovine, and can have impact on economic losses. Economic losses caused by late-term *Chlamydia abortus* infection, and the subsequent epizootic bovine abortion are readily apparent. However, infection may result in unrecognised economic losses as the consequence of subclinical infertility (Kaltenböeck, Hehnen and Vaglenov, 2005).

Conclusion. The study indicates that infections with *Chlamydia* spp. are present in Polish cattle. The study demonstrated that average percentage of seropositives herds is 26.40%. Moreover, the molecular studies show that infection *Chlamydia pecorum* and *Chlamydia abortus* is more spread than with *Chlamydia pisttaci*. It should be noted that if the *Chlamydia* agent is present in the Polish cattle population, there is a real risk to transmission of the infection to humans (Pospischil et al., 2002) but there is no information in the available literature about epidemiological situation in humans exposed to chlamydiosis in Poland.

References

Borel, N., Thoma, R., Spaeni, P., Weilenmann, R., Teankum, K., Brugnera, E., Zimmermann, D. R., Vaughan, L. and Pospischil, A. (2006) 'Chlamydia-related abortions in cattle from Graubunden, Switzerland', *Veterinary Pathology*, 43(5), pp. 702–708. doi: 10.1354/vp.43-5-702.

Cavirani, S., Cabassi, C. S., Donofrio, G., De Iaco, B., Taddei, S. and Flammini, C. F. (2001) 'Association between *Chlamydia psittaci* seropositivity and abortion in Italian dairy cows', *Preventive Veterinary Medicine*, 50(1–2), pp. 145–151. doi: 10.1016/s0167-5877(01)00197-0.

Godin, A.-C., Björkman, C., Englund, S., Johansson, K.-E., Niskanen, R. and Alenius, S. (2008) 'Investigation of *Chlamydophila* spp. in dairy cows with reproductive disorders', *Acta Veterinaria Scandinavica*, 50(1). doi: 10.1186/1751-0147-50-39.

Jee, J., Degraves, F. J., Kim, T. and Kaltenboeck, B. (2004) 'High prevalence of natural *Chlamydophila* species infection in calves', *Journal of Clinical Microbiology*, 42(12), pp. 5664–5672. doi: 10.1128/jcm.42.12.5664-5672.2004.

Kaltenböck, B., Schmeer, N. and Schneider, R. (1997) 'Evidence for numerous omp1 alleles of porcine *Chlamydia trachomatis* and novel chlamydial species obtained by PCR.', *Journal of Clinical Microbiology*, 35(7), pp. 1835–1841. Available at: http://jcm.asm.org/content/35/7/1835.

Kaltenboeck, B., Hehnen, H.-R. and Vaglenov, A. (2005) 'Bovine *Chlamydophila* spp. infection: do we underestimate the impact on fertility?', *Veterinary Research Communications*, 29(S1), pp. 1–15. doi: 10.1007/s11259-005-0832-4.

Kauffold, J., Henning, K., Bachmann, R., Hotzel, H. and Melzer, F. (2007) 'The prevalence of chlamydiae of bulls from six bull studs in Germany', *Animal Reproduction Science*, 102(1–2), pp. 111–121. doi: 10.1016/j.anireprosci.2006.10.013.

Livingstone, M. and Longbottom, D. (2006) 'What is the prevalence and economic impact of chlamydial infections in cattle? The need to validate and harmonise existing methods of detection', *The Veterinary Journal*, 172(1), pp. 3–5. doi: 10.1016/j.tvjl.2005.05.001.

Markey, B. (2011) 'Chlamydial infection in cattle: the burden of proof?', *The Veterinary Journal*, 189(3), pp. 246–247. doi: 10.1016/j.tvjl.2010.10.012.

Niemczuk, K. (2005) 'Prevalence of antibodies against *Chlamydophila psittaci* and *Chlamydophila abortus* in cattle in Poland. A preliminary report', *The Bulletin of the Veterinary Institute in Pulawy*, 49(3), pp. 293–297. Available at: http://bulletin.piwet.pulawy.pl/images/stories/pdf/20053/20053293298.pdf.

Niemczuk, K. (2006) 'Occurrence of antibodies anti-*Chlamydophila* sp. in selected cattle's population in Poland' [Występowanie przeciwciał anty-*Chlamydophila* spp. w wybranej populacji bydła w Polsce], *Medycyna Weterynaryjna*, 62(7), pp. 804–806. Available at: http://medycynawet.edu.pl/images/stories/pdf/pdf2006/07/20067s08040806.pdf. [in Polish].

Niemczuk, K and Truszczyński, M. (2012) 'Pathogenicity of Chlamydiaceae with reference to cattle' [Chorobotwórczość Chlamydiaceae ze szczególnym uwzględnieniem bydła], *Medycyna Weterynaryjna*, 68(6), pp. 328–332. Available at: http://medycynawet.edu.pl/images/stories/pdf/pdf2012/062012/201206328332.pdf. [in Polish].

OIE. (2012) Chapter 2.7.7. Enzootic abortion ewes. In: *Manual of diagnostic tests and vaccines for terrestrial animals (mammals, birds and bees)*. Vol. 2. 7th ed. Available at: http://www.oie.int/fileadmin/home/eng/health_standards/tahm/2.07.07_enz_abor.pdf.

Pantchev, A., Sting, R., Bauerfeind, R., Tyczka, J. and Sachse, K. (2010) 'Detection of all *Chlamydophila* and *Chlamydia* spp. of veterinary interest using species-specific real-time PCR assays', *Comparative Immunology, Microbiology and Infectious Diseases*, 33(6), pp. 473–484. doi: 10.1016/j.cimid.2009.08.002.

Pospischil, A., Thoma, R., Hilbe, M., Grest, P., Zimmermann, D. and Gebbers, J.-O. (2002) 'Abortion in humans caused by *Chlamydophila abortus* (*Chlamydia psittaci* serovar 1)' [Abort beim Menschen durch *Chlamydophila abortus* (*Chlamydia psittaci* serovar 1)], *Schweizer Archiv für Tierheilkunde*, 144(9), pp. 463–466. doi: 10.1024/0036-7281.144.9.463. [in German].

Reinhold, P., Jaeger, J., Liebler-Tenorio, E., Berndt, A., Bachmann, R., Schubert, E., Melzer, F., Elschner, M. and Sachse, K. (2008) 'Impact of latent infections with *Chlamydophila species* in young cattle', *The Veterinary Journal*, 175(2), pp. 202–211. doi: 10.1016/j.tvjl.2007.01.004.

Reinhold, P., Sachse, K. and Kaltenboeck, B. (2011) 'Chlamydiaceae in cattle: commensals, trigger organisms, or pathogens?', *The Veterinary Journal*, 189(3), pp. 257–267. doi: 10.1016/j.tvjl.2010.09.003.

Sachse, K., Vretou, E., Livingstone, M., Borel, N., Pospischil, A. and Longbottom, D. (2009) 'Recent developments in the laboratory diagnosis of chlamydial infections', *Veterinary Microbiology*, 135(1–2), pp. 2–21. doi: 10.1016/j.vetmic.2008.09.040.

Uhe, C., Li, Y., Sachse, K., Hehnen, H.-R. and Kaltenboeck, B. (2005) Therapeutic vaccination against *Chlamydophila abortus/pecorum* reduces subclinical mastitis in dairy cows. In: Cevenini, R. and Sambri, V. (eds.) *Diagnosis and pathogenesis of animal chlamydioses: proceedings of the 3rd Workshop, Siena, Italy, September 22–23, 2005*. Bononia University Press. ISBN 978-88-7395-090-5. pp. 31–32. Available at: http://www.cost.eu/media/publications/05-08-Diagnosis-and-Pathogenesis-of-Animal-Chlamydioses-Proceedings-of-the-3rd-Workshop.

Wang, F.-I., Shieh, H. and Liao, Y.-K. (2001) 'Prevalence of *Chlamydophila abortus* infection in domesticated ruminants in Taiwan', *Journal of Veterinary Medical Science*, 63(11), pp. 1215–1220. doi: 10.1292/jvms.63.1215.

Wheelhouse, N. and Longbottom, D. (2011) 'Endemic and emerging chlamydial infections of animals and their zoonotic implications', *Transboundary and Emerging Diseases*, 59(4), pp. 283–291. doi: 10.1111/j.1865-1682.2011.01274.x.

Wilson, K., Sammin, D., Harmeyer, S., Nath, M., Livingstone, M. and Longbottom, D. (2012) 'Seroprevalence of chlamydial infection in cattle in Ireland', *The Veterinary Journal*, 193(2), pp. 583–585. doi: 10.1016/j.tvjl.2011.12.018.