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Prevalence of chlamydiae in dairy cattle herds and factors contributing to the spread of infections

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Abstract

Introduction: Different *Chlamydia* species affect cattle and contribute to economic losses. One of them, *C. pecorum*, is a globally endemic livestock pathogen. Despite its endemicity, prevalence data from Poland have so far been limited. The present study aimed to obtain insight into the chlamydiae prevalence in Polish dairy cattle. **Material and Methods:** A screening of chlamydial seroprevalence in dairy cattle was initially performed, followed by *Chlamydiaceae*- and species-specific real-time qPCR. Vaginal swabs (n = 239) and placenta samples (n = 2) from seropositive animals in 142 herds were collected to detect shedders. The study population consisted of cows (n = 2,780) from dairy herds (n = 1,153) located in all Polish voivodeships. **Results:** The true animal prevalence was determined to be 33.3%, while the true herd prevalence was 42.7%. Five groups of Polish voivodeships were identified using appropriate statistical tools, highlighting differences that may arise from various factors impacting the spread of chlamydial infections. The only detected chlamydia species was *C. pecorum*, the presence of which was confirmed in two herds. **Conclusion:** This study revealed that chlamydial infections are commonly present in Polish dairy cattle across the country.

Keywords: chlamydiae, prevalence, dairy cattle.

Introduction

Chlamydiae are Gram-negative obligate intracellular bacteria belonging to the *Chlamydiaceae* family. *Chlamydia* spp. are widely distributed throughout the world and cause a variety of diseases both in humans and animals, including zoonotic infections (8). Currently, 15 *Chlamydia* species are recognised and four are *Candidatus* species (31). The most common chlamydial agents in cattle are *C. pecorum* and *C. abortus*. However, because of their ability to cross natural host barriers, *C. suis* (endemic in swine) and *C. gallinacea* (endemic in chickens) have sporadically been recorded as causing asymptomatic infections in cattle, while *C. psittaci* (of which birds are the reservoir) has also been noted to cause an infection associated with clinical signs (12, 13). Moreover *C. abortus* co-infections with other chlamydiae species are also possible.

Chlamydiosis is a multifactorial disease that involves interactions among low-virulence pathogens, nutritional deficiencies, poor management and hygiene and host genetics (17). *Chlamydia abortus* and *C. pecorum* affect cattle, causing abortions and reproductive problems that may lead to significant economic losses. The disease has a nonspecific clinical presentation, and clinical symptoms such as pneumonia, conjunctivitis, enteritis, polyarthritis, sporadic encephalomyelitis, abortion, vaginitis, endometritis, repeat breeding, weak calf syndrome, perinatal mortality and fertility disorders have been reported around the world (13). Infection with *Chlamydia* spp. could also have a subclinical course. *Chlamydia abortus* has been identified in suboptimal production of livestock in the absence of other pathogens (21). It is commonly known as zoonotic agent (21), and pregnant women may develop life-threatening *C. abortus* infections, resulting in septic abortion and stillbirth (27). For a long time *C. pecorum* was considered non-pathogenic to humans,

but recently the first case in a man with pneumonia and respiratory failure was reported (4).

Taking into account the zoonotic risk and negative impact of chlamydiae on breeding cattle, surveys monitoring their prevalence are essential. Relevant data applying to Polish cattle herds are very limited. The studies conducted in the last decades showed that chlamydial infections are present in Polish bovine herds (16, 25), but current epizootic data from Poland are lacking.

Coxiella burnetii is the second intracellular and zoonotic agent that commonly occurs in the dairy cattle population worldwide, including Poland (24). Although *Coxiella burnetii* and chlamydiae belong to phylogenetically unrelated species, they show some similarities in their interaction with the host and pathogenesis of the infection (14). The clinical features of abortion caused by *Chlamydia* spp. and *Coxiella burnetii* are very similar, and such mixed infections have been suggested to be a common occurrence in sheep and goats (1).

The aim of this study was to establish the prevalence of chlamydiae in dairy cattle herds in Poland based on analyses of sera by the complement fixation test (CFT). It also estimated the true prevalence (TP) to chlamydiae at the animal and herd levels in individual Polish voivodeships using appropriate statistical methods. The investigation also evaluated the shedding of the pathogen by molecular testing of vaginal swabs and placentas collected from seropositive animals. Lastly, it statistically evaluated the conformity between seroprevalence of *Chlamydia* spp. and *Coxiella burnetii* in sera.

Material and Methods

Sera samples were collected between 2019 and 2023 in the ambit of the "Protection of animal and public health" multiannual monitoring programme. Depending on which material was available, swabs and placentas were obtained from seropositive cows. All samples were collected from random animals by authorised veterinarians (veterinary inspectorate employees), following standard procedures and with farmers' consent. According to the Local Ethical Committee on Animal Testing at the University of Life Sciences in Lublin (Poland), formal ethical approval is not required for this kind of study.

Blood and biological samples collection. Blood samples were randomly collected from 2,780 cows in 1,153 herds in all Polish voivodeships (Table 1). The samples were then stored at room temperature for 30–45 min to allow clotting. Serum was obtained by centrifugation of blood samples at 1,000 × *g* for 10 min. If the serological test was performed within 48 h, the temperature of the sample was maintained between 4°C and 8°C, otherwise the sera were stored at −20 ± 5°C until they were tested. Vaginal swabs (n = 239) and placenta samples (n = 2) were collected from seropositive animals in 142 herds. Until DNA extraction, dry swabs and placenta samples were stored at −20ºC.

Serological tests. Institut Virion/Serion (Wurzburg, Germany) reagents were used for CFTs. Before each examination, an intra-laboratory evaluation with antigen titration against a positive control serum and verification of the activity of other reagents used in the reaction was 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 (30). The starting dilution of the examined samples was 1:4 and the final dilution was 1:64. Serum was considered positive when at least a partial inhibition of haemolysis was observed at the dilution of 1:32. All sera were also tested in parallel for the presence of antibodies against *Coxiella burnetii* using an IDEXX Q Fever Ab Test (IDEXX, Baar, Switzerland). These results were previously partially published and were used for statistical analysis in this study (24).

DNA isolation and real-time PCR. Extraction of DNA from swabs and placenta specimens was performed using a QIAamp DNA Mini Kit and DNeasy Blood & Tissue Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. DNA extracts were stored at −20°C before analysis. A real-time PCR was performed using a 7500 Fast Real-Time PCR system v2.3 (Applied Biosystems, Foster City, CA, USA) to detect a *Chlamydiaceae*-specific 23S rRNA gene fragment in swabs and placentas (6). Furthermore, species-specific real-time PCRs were conducted on all *Chlamydiaceae*-positives to identify which it was of *C. gallinacea* (11), *C. psittaci* (15), *C. abortus*, *C. pecorum* or *C. suis* (18). A panel of required positive and negative controls was used in each run, including TaqMan Exogenous Internal Positive Control (Thermo Fisher Scientific Baltics, Vilnius, Lithuania) as a commercial internal amplification control to monitor PCR inhibitors. All samples with a cycle-threshold (Ct) value above 36 were considered negative. The cut-off value was selected based on the limit of detection determined in the validation process. These real-time PCR procedures were validated under laboratory conditions.

Statistical analysis. The apparent and true prevalence of infection with *Chlamydia* spp. at both the animal and herd levels were estimated using a statistical model based on the Bayesian approach as previously published (24). The true positivity to *Chlamydia* spp. at the animal and herd levels in individual Polish voivodeships was evaluated using latent class analysis as previously described (24). The data on the number of dairy cows and herds in voivodeships used for the calculations were obtained from the 2022 annual report published by Statistics Poland (23).

The evaluation of the true prevalence of animals and herds infected with *Chlamydia* spp. allowed the range of the real number of infections of *Chlamydi*a spp. in Poland to be defined. The apparent animal prevalence

was calculated as the number of test-positive animals among the total number of animals tested, and the apparent herd prevalence was calculated as the number of test-positive herds among the total number of herds tested. A herd was considered positive when at least one animal showed the presence of antibodies in the CFT test.

In this study, we used beta-binomial models to estimate both the animal and herd prevalence according to a commonly accepted formula. Estimation of the diagnostic sensitivity (DSe) and specificity (DSp) of the CFT was based on the available published data (22) and depended on the voivodeship. The DSe was from 62.4% (95% confidence interval (CI): 38.0–97.0) to 65.9% (38.1–97.8%) and the DSp was from 91.2% (84.3–99.5) to 91.6 (84.3–99.5%). These parameters were expressed as the mode and confidence intervals shown as the percentile values of a *β*-distribution with 0.025 for the lower and 0.975 for the upper limit.

Multivariate (MVA) statistical analysis was used for the identification of any association between the presence of antibodies against *Chlamydia* spp. in sera samples and different categories of variables. Analysis was undertaken using individual data from all 1,153 cattle herds that were explored for variables such as the presence of antibodies against *Chlamydia* spp. in sera at herd and animal level, the geographical location of farms and dairy cow stock in the voivodeship, positive and negative conformity rates between results for *Chlamydia* spp. and results for *Coxiella burnetii* in the same sera samples from a previously published study (24). Data from the MVA were analysed using Statistica software version 10.0 (StatSoft Inc, Tulsa, OK, USA) and a P-value ≤ 0.05 was considered statistically significant, as it was in the previously published research (24).

Results

Serological analyses of sera samples confirmed the presence of antibodies against chlamydiae in 866 out of 2,780 (31.2%) tested animals with a 95% confidence interval (CI) of 29.4–32.9%. The overall herd-level prevalence was 42.7% (492/1,153) with a 95 % CI of 39.8–45.6%. Positive cattle herds were present in all tested voivodeships and seropositivity varied from 23.7% (CI: 17.6–30.7%) to 80.0% (44.4–97.5%) among them. Lesser Poland and Greater Poland were the provinces with the highest percentage of seropositive cattle herds (80%; CI: 44.4–97.5% and 73.7%; CI: 48.8–90.9%, respectively), while the lowest herd prevalence was observed in Subcarpathia (23.7%; CI: 17.6–30.7%). The highest percentages of positive cows were noted in Świętokrzyskie (38.3%; CI: 29.6–47.6%), Greater Poland (37%; CI: 29.2–45.4%), Podlaskie (36.6%; CI: 29.4–44.2%), Łódź (36.2%; CI: 29.5–43.4%) and Kuyavia-Pomerania (36%; CI: 30.0–42.3%) provinces. The lowest seropositivity rates of tested specimens were calculated for Warmia-Masuria (23.3%; CI: 16.8–30.9%), Subcarpathia (24.2%; CI: 18.1–31.1%) and Lesser Poland (25.6%; CI: 16.9–35.8%). The results of the serological testing of sera, including the overall prevalence and the true prevalence estimates, are shown in Table 1.

Next, the TP was estimated using the Bayesian framework. The posterior median and 95% confidence intervals were displayed. By including the voivodeship as a covariate in the model, it was possible to estimate the seroprevalence of *Chlamydia* spp. per voivodeship. A large variation in seroprevalence at the animal and herd levels between the voivodeships was observed. The TP values at herd level were the same as for apparent prevalence, but the Clopper–Pearson 95% CIs were different. The mean TP at animal level was slightly higher and was 33.3%, with a CI of 21.2–76.8%, individual voivodeship TPs ranging from 23.9% (CI: 8.5–63.0%) to 43.3% (CI: 28.3–95.9%). The highest values of TP at herd level were noted in the Lesser Poland voivodeship at 80.0% (CI: 49.7–95.6%), Greater Poland at 73.7% (CI: 51.6–89.2%), and West Pomerania at 67.4% (CI: 52.7–80.0%), whereas the highest values of TP at animal level were seen in the Świętokrzyskie voivodeship (43.3%; CI: 28.3–95.9%), Łódź (41%; CI: 27.0–95.1%) and Greater Poland (40.3%; CI: 26.9–95.5%). The lowest values of TP at herd level were noted in Subcarpathia (23.7%; CI: 17.8–30.6%), and at animal level in Warmia-Masuria (23.9%; CI: 8.5–63.0%). The true prevalence at the animal and herd levels in voivodeships is presented on the map in Fig. 1.

Statistically significant differences were found between the lowest TP value at herd level noted in the Subcarpathia voivodeship and the equivalents in the Kuyavia-Pomerania, Warmia-Masuria, Lesser Poland, Masovia, Opole, Podlaskie, Pomerania, Świętokrzyskie, Greater Poland and West Pomerania voivodeships. A statistically significant difference was also observed between the highest TP value recorded at herd level in the Lesser Poland voivodeship and same statistics in the Lublin, Łódź, Opole, Greater Poland and West Pomerania voivodeships. Significant differences were also noted between the herd-level average TP value recorded in the West Pomerania voivodeship and these values in the Warmia-Masuria, Silesia, and Kuyavia-Pomerania voivodeships. There were no significant differences in average TP at animal level across the various voivodeships, which is related to the high uncertainty of TP resulting from the poor diagnostic parameters of the CFT method.

The results of MVA statistical analysis include the following factors: the presence of antibodies against *Chlamydia* spp. in sera at herd and animal level, the geographical location of the farms and the dairy cow stock in the voivodeship and positive and negative conformity rates between results of serological screening for *Chlamydia* spp. and *Coxiella burnetii* in the same sera samples. The analysis identified five groups of voivodeships with noteworthy differences (Fig. 2).

Table 1. Results of complement fixation tests for chlamydiae on dairy cow serum samples at herd and animal level in each Polish voivodeship

Group	Voivodeship	Animals				Herds			
		Number tested	% positive	% positive (Clopper–Pearson 95% CI)	% true positive (95% CI)	Number tested	% positive	% positive (Clopper–Pearson 95% CI)	% true positive (95% CI)
G1, G5	Greater Poland	146	54	37.0 (29.2–45.4)	40.3 (26.9–95.5)	19	14	73.7 (48.8–90.9)	73.7 (51.6–89.2) [c, d, l, o]
G1	Podlaskie	175	64	36.6 (29.4–44.2)	39.9 (26.8–94.5)	97	43	44.3 (34.2–54.8)	44.3 (34.7–54.2) [i]
G1	Świętokrzyskie	120	46	38.3 (29.6–47.6)	43.3 (28.3–95.9)	60	29	48.3 (35.2–61.6)	48.3 (36.0–60.8) [k]
G1	Łódź	196	71	36.2 (29.5–43.4)	41.0 (27.0–95.1)	160	64	40 (32.3–48.0)	40 (32.6–47.7) [d, e]
G1	Kuyavia-Pomerania	250	90	36.0 (30.0–42.3)	37.8 (26.4–93.5)	152	68	44.7 (36.7–53.0)	44.7 (37.0–52.7) [a, b]
G2, G5	Masovia	164	46	28.0 (21.3–35.6)	30.0 (15.3–76.8)	27	16	59.3 (38.8–77.6)	59.3 (40.6–76.1) [g]
G2	Opole	200	54	27.0 (21.0–33.7)	28.8 (14.4–71.9)	54	33	61.1 (46.9–74.1)	61.1 (47.8–73.3) [d, h]
G2	Lesser Poland	90	23	25.6 (16.9–35.8)	26.7 (10.4–73.4)	10	8	80.0 (44.4–97.5)	80 (49.7–95.6) [c, d, f]
G2	Subcarpathia	178	43	24.2 (18.1–31.1)	24.6 (10.2–63.3)	173	41	23.7 (17.6–30.7)	23.7 (17.8–30.6) [a, e, f, g, h, i, j, k, l, m]
G2	West Pomerania	245	83	33.9 (23.8–37.2)	35.6 (24.0–90.7)	43	29	67.4 (51.5–80.9)	67.4 (52.7–80.0) [b, c, d, m, n, p]
G3	Lublin	112	32	28.3 (20.2–37.6)	31.3 (15.4–78.8)	60	22	36.7 (24.6–50.1)	36.7 (25.3–49.3) [c]
G3	Silesia	220	66	30.0 (24.0–36.5)	32.0 (19.3–80.2)	58	23	39.7 (27.0–53.6)	39.7 (27.8–52.5) [h]
G3	Pomerania	192	58	30.2 (23.8–37.2)	33.9 (18.8–80.1)	87	42	48.3 (37.4–59.2)	48.3 (38.0–58.7) [j]
G3	Lower Silesia	250	73	29.2 (23.6–35.3)	30.7 (18.0–77.1)	39	17	43.6 (27.8–60.3)	43.6 (28.9–59.1)
G3	Lubusz	91	28	30.8 (21.5–41.3)	34.2 (17.0–87.3)	48	19	39.6 (25.8–54.7)	39.6 (26.7–53.7)
G4	Warmia-Masuria	150	35	23.3 (16.8–30.9)	23.9 (8.5–63.0)	66	24	36.4 (24.9–49.1)	36.4 (25.5–48.4) [c, o, p]
Total/mean		2,780	866	31.2 (29.4–32.9)	33.3 (21.2–76.8)	1,153	492	42.7 (39.8–45.6)	42.7 (39.8–45.5)

95% CI – 95% confidence interval; [a, b, c, d, e, f, g, h, i, j, k, l, m, h, n, o, p] – statistically significant differences ($P \leq 0.05$). The assignment of the same letter to voivodeships signifies a statistically significant difference between them

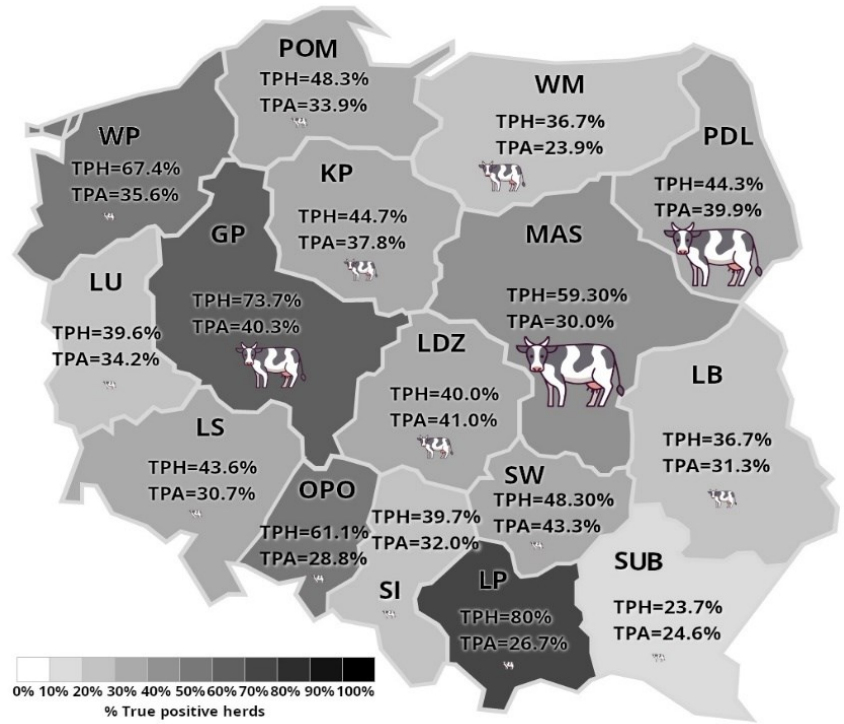

Fig 1. True seroprevalence of chlamydiae at animal and herd level in cattle herds in the voivodeships of Poland based on serum analyses by complement fixation test. TPH – true-positive herds; TPA – true-positive animals; Voivodeships: GP – Greater Poland; PDL – Podlaskie; SW – Świętokrzyskie, LDZ – Łódź; KP – Kuyavia-Pomerania; MAS – Masovia; OPO – Opole; LP – Lesser Poland; SUB – Subcarpathia; WP – West Pomerania; LB – Lublin; SL – Silesia; POM – Pomerania; LS – Lower Silesia; LU – Lubusz; WM – Warmia-Masuria

– the size of the symbol corresponds to the number of cows in the voivodeship

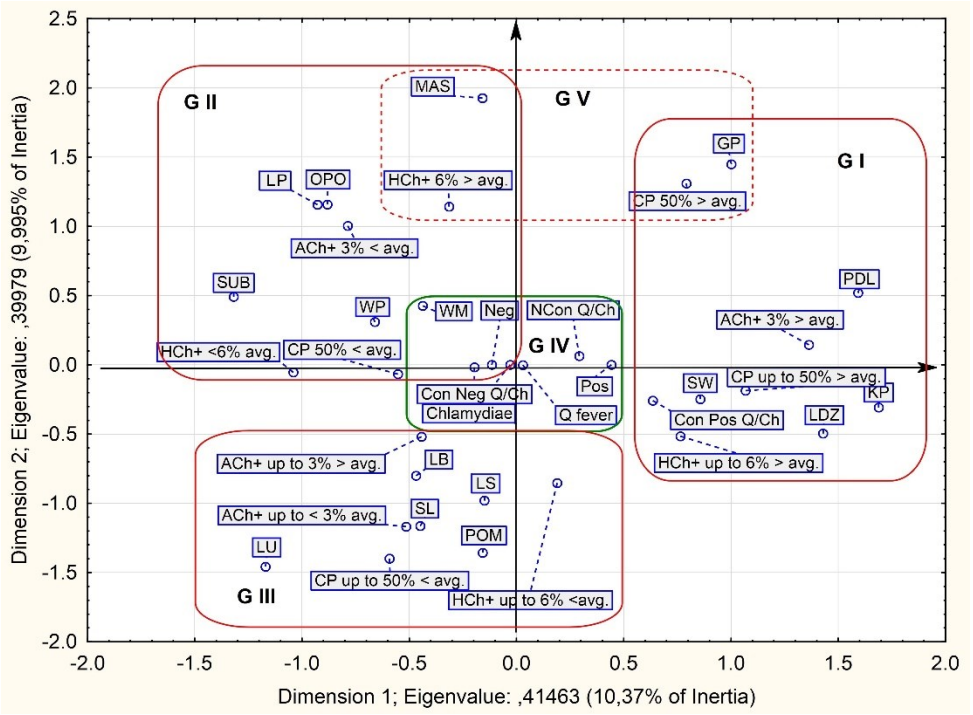

Fig. 2. Multivariate analysis of the presence of antibodies against chlamydiae in sera at herd and animal level, the geographical location of the farms and the dairy cow stock in the voivodeship and the positive and negative conformity rates between the results of serological screening for chlamydiae and *Coxiella burnetii* in the same sera samples. Blue points represent each category of analysed variables. Blue points with similar profiles (low value of distances indicating a strong association between variables) are marked by the red rectangles. Blue points located in the green rectangle in the graph's centre show the points with similar profiles but representing eigenvalues indicating the lack of any association. Blue points located in the dashed rectangle shows the points with similar profiles but representing eigenvalues indicating the association with more than one groups
ACh+ – seroprevalence of chlamydiae at animal level; HCh+ – seroprevalence of chlamydiae at herd level; CP – dairy cattle population; Con Pos Q/Ch – conformity between positive results of presence of *Chlamydia* spp. and *Coxiella burnetii* antibodies in the same sera samples; Con Neg Q/Ch – conformity between negative results of presence of *Chlamydia* spp. and *Coxiella burnetii* antibodies in the same sera samples. Voivodeships: GP – Greater Poland; PDL – Podlaskie; SW – Świętokrzyskie, LDZ – Łódź; KP – Kuyavia-Pomerania; MAS – Masovia; OPO – Opole; LP – Lesser Poland; SUB – Subcarpathia; WP – West Pomerania; LB – Lublin; SL – Silesia; POM – Pomerania; LS – Lower Silesia; LU – Lubusz; WM – Warmia-Masuria

The first group (G I) consisted of the Podlaskie, Świętokrzyskie, Łódź, Greater Poland and Kuyavia-Pomerania voivodeships, where the cattle population is moderately higher (up to 50%) than the national average except for the Greater Poland voivodeship, where it exceeds 300% of the national average (23). In these voivodeships, the seroprevalence of chlamydiosis at animal level was over 3% higher than the national average obtained in this study and it was a predominant factor that determined assignment to this group. Moreover, the herd-level seroprevalence in this group was up to 6% higher than the national average. In this group, congruence between the results for the presence of antibodies against *Chlamydia* spp. and *Coxiella burnetii* in the individual cow was a factor of statistical significance.

The second group (G II) consisted of the Opole, Lesser Poland, Subcarpathia and West Pomerania voivodeships, where the dairy cattle population is significantly lower (<50%) than the national average, and the Masovia voivodeship, where dairy cow stock exceeds 300% of the national average. The low seroprevalence level among animals (at least 3% lower than the national average) was a predominant factor that determined assignment to this group. The seroprevalence of *Chlamydia* spp. at herd level was not uniform and was up to 6% higher than or up to 6% lower than the national average, depending on the voivodeship.

The third group (G III) consisted of the Lublin, Silesia, Pomerania, Lower Silesia and Lubusz voivodeships, which have a moderately lower dairy cattle population (up to 50% below the national average) (23) and these lower stock numbers were a factor that determined assignment to the group. In this group, herd-level seroprevalence was up to 6% lower than the national average, and the seroprevalence among animals ranged from 3% below to 3% above the average.

The fourth group (G IV) consisted of the Warmia-Masuria voivodeship, where none of the analysed factors showed any correlation.

The fifth group (G V) consisted of the Masovia and Greater Poland voivodeships, where the dairy cow population is over 50% higher than the national average (23), and the seroprevalence at herd level exceeded the national average by over 6%. The predominant factor that determined assignment to this group was the size of the population of dairy cows.

A statistically significant correlation was found only between positive results for the presence of *Chlamydia* spp.

and *Coxiella burnetii* antibodies in the same sera samples in G I. This group consisted of voivodeships where herd seroprevalence was up to 6% higher than the national average obtained in this study, and where there are 50% more head of cattle than the national average.

Analyses conducted for all other factors for G I as well as all factors for G II to G V did not show any statistically significant results.

The presence of *Chlamydiaceae* DNA in vaginal swabs from seropositive animals was detected only in 2 (1.4%) out of the 142 tested herds. *Chlamydiaceae*-positive herds were located in Kutnowski county in the Łódź voivodeship. *Chlamydiaceae*-positive samples were positive in the *C. pecorum*-specific real-time PCR and yielded threshold values of 31.87 and 35.59. All tested placentas were *Chlamydiaceae* negative.

Discussion

The cattle industry in Poland has long been an important branch of the national agricultural sector. In 2022, Poland's cattle stock was estimated at over 6 million animals, of which over 2 million were dairy cows (20). The highest densities of dairy cattle were noted in Masovia, Great Poland and Podlasie (23). Many infectious agents both viral and bacterial, including intracellular bacteria such as *Coxiella burnetii* and chlamydiae, might cause economic losses in cattle breeding. Numerous European studies based on serology indicated an endemic distribution of different *Chlamydia* species in cattle, with prevalence ranging from 5 to 100%, the key investigations being in Ireland 4.75% (29), Germany 19.6% (7), Italy 24% (5), Sweden 28% (9), Austria 45% (19), Switzerland 47% (26) and Germany 100% (2). Data on cattle chlamydiosis in Poland are very limited. This study was conducted to evaluate the epizootic situation of chlamydiosis in dairy cattle herds in Poland *via* serological testing of sera collected from dairy cows in all voivodeships. A study conducted in the past decade indicated that prevalence of antibodies was quite low, averaging 4.15% in asymptomatic cattle and 7.20% in animals with reproductive disorders (25). Other research including only pregnant and imported cows showed 19.3% seroprevalence (16). The seroprevalence in this study was significantly higher than in previous research, as average TP at herd level being 42.7% (CI 39.8–45.5%) and at animal level 33.3% (CI 21.2 –76.8%). It was nevertheless comparable with the seroprevalence in other European countries, *e.g.* Switzerland or Austria (19, 26). Differences in seroprevalence between individual voivodeships were revealed in this study (Table 1). Statistical correlations for herd TP were noted, while there was a lack of correlation for TP at animal level (Table 1). According to the OIE Terrestrial Manual, the CFT is suitable only in very limited circumstances for assessing the prevalence of chlamydial infection; therefore, the confidence intervals for TP determined with CFT are very wide, which impacted the statistical analysis. Moreover, chlamydiae share common antigens with other Gram-negative bacteria, making the CFT test or crude ELISAs non-specific (30). Unfortunately, the ELISA test is specific only for *C. abortus*, and there are no commercially available ELISA kits enabling identification of antibodies to other chlamydial agents. Therefore, only the CFT enables serological screening tests for *Chlamydia* spp.

A detailed statistical analysis identified five groups of voivodeships, highlighting differences that may have arisen from various factors (Fig. 2). As is commonly known, high stocking density facilitates pathogen transmission, and that was confirmed by the results of the performed analyses. Almost all voivodeships in G I (LDZ, KP) have high (Łódź and Kuyavia-Pomerania) or very high (Greater Poland and Podlaskie) numbers of cattle per 100 ha of agricultural land (23). Those voivodeships as well as Masovia (G II and G V), which also has a very high number of cattle per 100 ha of agricultural land, presented the highest seroprevalence at herd level, up to or over 6% higher than the national average. Moreover, group I was the only group where congruence between positive results for presence of *Chlamydia* spp. and *Coxiella burnetii* antibodies in the same sera samples was statistically significant. The lack of a clear correlation between factors in many voivodeships suggests the influence of random factors or other variables that require further investigation. To better assess the impact of potentially significant factors, *e.g.* herd size, farm management practices, livestock sanitation conditions, intensity of trade and movement of animals, and the impact of other factors *e.g.* stressors, which may influence disease epidemiology in individual regions, additional studies should be conducted.

A high level of seroprevalence may be the result of cattle exposure to chlamydiae during their lives due to the common circulation of *C. pecorum* in calves. As cattle grow, they develop a competent immune response, as already reported in sheep, and it might be the reason why chlamydiae shedders were identified only in 2 out of 142 (1.4%) tested herds in this study (3). In contrast to other European studies (18), but similarly to a study from Switzerland (13), we only confirmed the presence of *C. pecorum* infections and none with other chlamydial species or mixed infections. The single presence of *C. pecorum* is in accordance with the common hypothesis that the pathogen is endemic in ruminants worldwide (28). *Chlamydia pecorum* was more commonly detected in Austrian dairy cows than *C. abortus*, a while German study showed that *C. psittaci* was the most frequently detected species followed by *C. abortus*, and *C. pecorum* was found the most rarely (10, 19). In a previous study from Poland, *C. pecorum* and *C. psittaci* were completely absent, whereas *C. abortus* and *C. suis* were detected (25). The absence of *C. abortus* in tested seropositive animals in the present research was surprising, taking into account the type of tested specimens (*i.e.* vaginal swabs and placentas). The low percentage of *Chlamydia* spp. shedders is congruent

with reports from other European countries, where prevalence of *C. pecorum* in dairy cows range between 0.7 and 8.9% (10, 19), but notably incongruent with China's high percentage of 57% (12). Based on Swiss studies, it is known that that *C. pecorum* loads were higher in younger animals than in older ones (13). In this study only adult dairy cattle were tested, and this might be the explanation for the low number of shedders. However, it is not possible to directly compare this research with other publications because differences abound in many factors, *e.g.* the animal age and breed, the kind of specimens and the time point of sampling. Comparison of serological results with PCR test results and species identification is impossible because no commercial *C. pecorum*-specific ELISA tests are available currently. Taking into account the high level of seroprevalence based on CFT results in individual regions in parallel with the rare detection of chlamydial agents, it might be assumed that this pathogen is rarely shed *via* the tested routes or that the phenomenon of intermittent shedding might occur.

Conclusion

The findings of this research are congruent with previous data on the epizootic situation in cattle worldwide, including Europe and Poland. These data indicate that chlamydial infections are commonly present in Polish dairy cattle across the country but also that there is a much higher level of seroprevalence than in previous years. *Chlamydia pecorum* was the only detected species, but presence of others cannot be excluded. Further longitudinal studies are necessary on chlamydiae shedding both in dairy and beef cattle herds and also in calves, including different kinds of samples taken from animals in different age categories.

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References

1. Aitken I.D., Clarkson M.J., Linklater K.: Enzootic abortion of ewes. Vet Rec 1990, 126, 136–138, doi: 10.1136/vr.126.6.136.
2. Biesenkamp-Uhe C., Li Y., Hehnen H.R., Sachse K., Kaltenboeck B.: Therapeutic *Chlamydophila abortus* and *C. pecorum* vaccination transiently reduces bovine mastitis associated with *Chlamydophila* infection. Infect Immun 2007, 75, 870–877, doi: 10.1128/IAI.00691-06.
3. Bommana S., Walker E., Desclozeaux M., Jelocnik M., Timms P., Polkinghorne A., Carver S.: Molecular and serological dynamics of *Chlamydia pecorum* infection in a longitudinal study of prime lamb production. Peer J 2018, 1–15, doi: 10.7717/peerj.4296.
4. Cao L., He L., Wang S., Xu L., Zhuang S.: Severe community-acquired pneumonia caused by *Chlamydia pecorum.* Int J Infect Dis 2022, 124, 171–173, doi: 10.1016/j.ijid.2022.09.03.
5. Cavirani S., Cabassi C.S., Donofrio G., De Iaco B., Taddei S., Flammini C.F.: Association between *Chlamydia psittaci* seropositivity and abortion in Italian dairy cows. Prev Vet Med 2001, 50, 145–151, doi: 10.1016/S0167-5877(01)00197-0.
6. Ehricht R., Slickers P., Goellner S., Hotzel H., Sachse K.: Optimized DNA microarray assay allows detection and genotyping of single PCR-amplifiable target copies. Mol Cell Probe 2006, 20, 60–63, doi: 10.1016/j.mcp.2005.09.003.
7. El-Rahim I.A.: Serological investigations for determination of the spreading of Chlamydia infection in cattle in northeast Germany (in German). Prakt Tierarzt 2002, 82, 268–273.
8. Elwell C., Mirrashidi K., Engel J.: *Chlamydia* cell biology and pathogenesis. Nat Rev Microbiol 2016, 14, 385–400, doi: 10.1038/nrmicro.2016.30.
9. Godin A.C., Bjorkman C., Englund S., Johansson K.E., Niskanen R., Alenius S.: Investigation of *Chlamydophila* spp. in dairy cows with reproductive disorders. Acta Vet Scand 2008, 50, 2–7, doi: 10.1186/1751-0147-50-39.
10. Kemmerling K., Muller U., Mielenz M., Sauerwein H.: *Chlamydophila* species in dairy farms: Polymerase chain reaction prevalence, disease association, and risk factors identified in a cross-sectional study in western Germany. J Dairy Sci 2009, 92, 4347–4354, doi: 10.3168/jds.2009-2051.
11. Laroucau K., Aaziz R., Meurice L., Servas V., Chossat I., Royer H., De Barbeyrac B., Vaillant V., Moyen J., Meziani F., Sachse K., Rolland P.: Outbreak of psittacosis in a group of women exposed to *Chlamydia psittaci*-infected chickens. Euro Surveill 2015, 20, 1–6, doi: 10.2807/1560-7917.es2015.20.24.21155.
12. Li J., Guo W., Kaltenboeck B., Sachse K., Yang Y., Lu G., Zhang J., Luan L., You J., Huang K., Qiu X., Wang Y., Li M., Yang Z., Wang C.: *Chlamydia pecorum* is the endemic intestinal species in cattle while *C. gallinacea*, *C. psittaci* and *C. pneumoniae* associate with sporadic systemic infection. Vet Microbiol 2016, 193, 93–99, doi: 10.1016/j.vetmic.2016.08.008.
13. Loehrer S., Hagenbuch F., Marti H., Pesch T., Hassig M., Borel N.: Longitudinal study of *Chlamydia pecorum* in a healthy Swiss cattle population. PLoS One 2023, 18, e0292509, doi: 10.1371/journal.pone.0292509.
14. Lukacova M.: Are *Coxiella burnetii* and Chlamydia related? Antigenic properties of *Coxiella burnetii* and *Chlamydiae*. Alpe Adria Microbiol J 1996, 5, 3–13.
15. Menard A., Clerc M., Subtil A., Megraud F., Bebear C., de Barbeyrac B.: Development of a real-time PCR for the detection of *Chlamydia psittaci*. J Med Microbiol 2006, 55, 471–473, doi: 10.1099/jmm.0.46335-0.
16. Niemczuk K.: Occurrence of antibodies anti-*Chlamydophila* sp. in selected cattle population in Poland. Med Weter 2006, 6, 804–806.
17. Orjuela A.G., Reyes Castañeda L.J., Tobón J.C., Parra Arango J.L., Guzmán-Barragán B.: Seroprevalence of antibodies to *Chlamydia abortus* and risk factors in cattle from Villavicencio, Colombia. Heliyon 2022, 18, 8, e09481, doi: 10.1016/j.heliyon.2022.e09481.
18. Pantchev A., Sting R., Bauerfeind R., Tyczka J., Sachse K.: Detection of all *Chlamydophila* and *Chlamydia* spp. of veterinary interest using species-specific real-time PCR assays. Comp Immunol Microbiol Infect Dis 2010, 33, 473–484, doi: 10.1016/j.cimid.2009.08.002.
19. Petit T., Spergser J., Aurich J., Rosengarten R.: Prevalence of *Chlamydiaceae* and Mollicutes on the genital mucosa and

serological findings in dairy cattle. Vet Microbiol 2008, 127, 325–333, doi: 10. 1016/j.vetmic.2007.08.022.
20. Polish Federation of Cattle Breeders and Dairy Farmers: Ocena i Hodowla Bydła Mlecznego – Dane za rok 2022 (Evaluation and Breeding of Dairy Cattle, Statistical Report for 2022 – in Polish). Polska Federacja Hodowców Bydła i Producentów Mleka, Warsaw, 2023, https://pfhb.pl/fileadmin/user_upload/OCENA/publikacje/publikacje_2023/wyniki_oceny/Wyniki_oceny_za_rok_2022_PFHBiPM_Polska.pdf.
21. Reinhold P., Sachse K., Kaltenboeck B.: Chlamydiaceae in cattle: commensals, trigger organisms, or pathogens? Vet J 2011, 189, 257–267, doi: 10.1016/j.tvjl.2010.09.003.
22. Szeredi L., Schiller I., Sydler T., Guscetti F., Heinen E., Corboz L., Eggenberger E., Jones G.E., Pospischil A.: Intestinal Chlamydia in Finishing Pigs. Vet Pathol 1996, 33, 369–374, doi: 10.1177/030098589603300401.
23. Statistics Poland: Farm animals in 2022. Tables in XLSX format. Table 6, 8 and 37. Statistics Poland, Warsaw, 2023, https://stat.gov.pl/download/gfx/portalinformacyjny/en/defaultaktualnosci/3608/1/5/1/tables_farm_animals_in_2022.xlsx.
24. Szymańska-Czerwińska M., Jodełko A., Osiński Z., Niemczuk K.: Screening for *Coxiella burnetii* in dairy cattle herds in Poland. J Vet Res 2022, 66, doi: 10.2478/jvetres-2022-0070.
25. Szymańska-Czerwińska M., Niemczuk K., Galińska E.M.: Serological and nested PCR survey to determine the occurrence of chlamydia infections in the Polish cattle population. Ann Agric Environ Med 2013, 20, 682–686.
26. Teankum K., Pospischil A., Janett F., Brugnera E., Hoelzle L.E., Hoelzle K., Weilenmann R., Zimmermann D.R., Gerber A., Polkinghorne A., Borel N.: Prevalence of chlamydiae in semen and genital tracts of bulls, rams and bucks. Theriogenology 2007, 67, 303–310, doi: 10.1016/j.theriogenology.2006.07.012.
27. Turin L., Surini S., Wheelhouse N., Rocchi M.S.: Recent advances and public health implications for environmental exposure to *Chlamydia abortus*: from enzootic to zoonotic disease. Vet Res 2022, 53, 37, doi: 10.1186/s13567-022-01052-x.
28. Walker E., Lee E.J., Timms P., Polkinghorne A.: *Chlamydia pecorum* infections in sheep and cattle: A common and under-recognised infectious disease with significant impact on animal health. Vet J 2015, 206, 252–260, doi: 10.1016/j.tvjl.2015.09.022.
29. Wilson K., Sammin D., Harmeyer S., Nath M., Livingstone M., Longbottom D.: Seroprevalence of chlamydial infection in cattle in Ireland. Vet J 2012, 193, 583–585, doi: 10.1016/j.tvjl.
30. World Organisation for Animal Health: Part 3, Chapter 2.3.1, *Avian Chlamydiosis* (Version adopted in May 2018) In: *Manual of Diagnostic Tests and Vaccines for Terrestrial Animals* Seventh Edition, World Organisation for Animal Health (OIE), Paris, France, 2018, https://www.oie.int/standard-setting/terrestrial-manual/access-online/.
31. Zarębа-Marchewka K., Bomba A., Scharf S., Niemczuk K., Schnee Ch., Szymańska-Czerwińska M.: Whole genome sequencing and comparative genomic analysis of *Chlamydia gallinacea* field strains isolated from poultry in Poland. Pathogens 2023, 12, 891, doi: 10.3390/pathogens12070891.