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# Screening for *Coxiella burnetii* in dairy cattle herds in Poland

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## Abstract

**Introduction:** The intracellular bacterium *Coxiella burnetii* is the aetiological agent of Q fever, a zoonosis affecting many animal species worldwide. Cattle and small ruminants are considered the major reservoirs of the bacteria and they shed it through multiple routes. **Material and Methods:** A total of 2,180 sera samples from 801 cattle herds in all Polish voivodeships were tested by ELISA for the presence of specific antibodies. Milk samples were obtained from seropositive cows in 133 herds as part of a separate study. The milk samples were examined by ELISA and real-time PCR tests. **Results:** Seroprevalence at the animal level was 7.06% and true positive seroprevalence was 6.0% (95% confidence interval (CI) 1.1–9.4). Seroprevalence at the herd level was estimated at 11.1% and true positive seroprevalence was 10.5% (95% CI 3.2–15.8). Shedding of the pathogen in milk was detected by real-time PCR in 33 out of 133 tested herds (24.81%, 95% CI 17.74–33.04%) and the presence of *C. burnetii* antibodies was confirmed in 85 of them (63.9%, 95% CI 55.13–72.05%). The highest level of conformity between ELISA and real-time PCR results was obtained for bulk tank milk samples. **Conclusion:** *Coxiella burnetii* infections are quite common in cattle herds across the country, which emphasises the crucial roles of surveillance and adequate biosecurity measures in the prevention and limitation of Q fever spread in Poland.

**Keywords:** *Coxiella burnetii*, Q fever, cattle, seroprevalence, prevalence.

## Introduction

Q fever (coxiellosis) is a worldwide zoonosis caused by the intracellular bacterium *Coxiella burnetii*. The pathogen affects many animal species, with cattle, sheep and goats being the most important reservoirs (12). Animals shed the bacteria most heavily in placentas and reproductive discharges, but also in milk, vaginal secretions, faeces and urine, and this contaminates the environment (2). *Coxiella burnetii* is highly resistant to multiple physical and chemical agents, which enables its persistence in the environment for a long time. The main route of transmission for domestic animals and humans is inhalation of contaminated aerosols (29), but human infection by ingestion of contaminated milk or dairy products is also possible (4).

In cattle, infections are usually asymptomatic, although clinical manifestations such as abortions, infertility, stillbirths, endometritis and mastitis may occur and impact the herd's owner economically (38). Infected animals may shed bacteria intermittently or remain seronegative despite being active shedders (36). Considering these characteristics of *C. burnetii* infection the possible several-month duration of persistent shedding by asymptomatic animals *via* various routes (23), laboratory tests are crucial in Q fever diagnosis.

Serological screening is a well-known and important tool enabling the assessment of an animal's exposure to viruses and bacteria, including *C. burnetii.* For routine serological testing of animals for Q fever, the ELISA method is recommended by the World Organisation for Animal Health (46). The possibility of applying ELISAs not only in sera sample but also in milk screening facilitates sample collection and allows herd status to be evaluated by bulk-tank milk (BTM) testing. Real-time PCR assays are sensitive and rapid

molecular tools that enable the detection of shedders in herds by testing a variety of clinical samples.

According to the Commission Implementing Regulation (EU) 2018/1882 (14), Q fever is a category E disease, a listed disease for which there is a need for surveillance within the Union, as referred to in Article 9(1)(e) of Regulation (EU) 2016/429 (15). Based on the Act on Animal Health Protection and Fighting Infectious Animal Diseases, Q fever is a notifiable disease in Poland (28). Since 2010, serological surveillance of cattle and small ruminants has been implemented by the government (21). Serological screening of cattle herds has also been carried out within the framework of the "Protection of animal and public health" multiannual monitoring programme.

A previous seroprevalence study showed that 25.39% of Polish cattle tested between 2014 and 2017 had specific antibodies against *C. burnetii* (41). In other research, the presence of the pathogen was confirmed by real-time PCR in 31.54% (88/279) of the tested herds (42). These data indicate that infection with *C. burnetii* is a common problem in cattle herds in Poland. Therefore, epizootic surveillance is essential to monitor disease trends, facilitate the control of disease or infection, and provide data for use in risk analysis.

The aim of this study was to determine the prevalence of antibodies against *C. burnetii* in dairy cattle herds in Poland based on analyses of sera and milk samples by the ELISA technique. It also estimated the true seropositivity to *C. burnetii* at the animal and herd levels in individual Polish voivodeships using appropriate statistical methods. The investigation also evaluated the prevalence of the pathogen by molecular testing of milk samples. Lastly, it evaluated the conformity between the results of an ELISA and a real-time PCR for individual and bulk-tank milk samples statistically.

## Material and Methods

Sera samples were collected between 2018 and 2021 in the ambit of the "Protection of animal and public health" multiannual monitoring programme. Milk samples were obtained from seropositive cows between January 2019 and June 2022 as part of a separate study. All samples were collected from unvaccinated animals by authorised veterinarians, 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 collection.** Blood samples were randomly collected from 2,180 non-vaccinated cows in 801 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 tested.

**Milk collection.** Samples were obtained from animals in which the presence of specific antibodies in sera had been confirmed by ELISA test. A total of 133 cattle herds from all Polish voivodeships (Table 2) were included in the study. Individual milk samples were taken from 109 herds, BTM was obtained from 19 herds and individual milk samples plus BTM were collected from 5 herds. All collected samples were stored and transported to the laboratory at 5 ± 3°C. If the serological test and nucleic acid isolation were performed within 48 h, the temperature of the milk was maintained between 4°C and 8°C, otherwise the specimens were frozen at −20 ± 5°C until tested. The herd was classified as positive if at least one of the tested samples collected from the herd was positive.

**Serological analysis.** A Q-Fever (*Coxiella burnetii*) Antibody Test Kit (IDEXX, Liebefeld, Switzerland) was utilised to screen sera and milk for the presence of *C. burnetii* antibodies. Following the manufacturer's instructions, the optical density (OD) percentage was calculated as (OD sample − ODneg)/(ODpos − ODneg) × 100 after averaging the duplicate values. Sera were considered to be negative when %OD <30, dubious when %OD ≥30 and %OD ≤40 or positive when %OD >40.

**DNA extraction and real-time PCR.** DNA extraction from milk samples was performed using a QIAamp DNA Mini Kit (Qiagen, Hilden, Germany) according to the real-time PCR kit manufacturer's instructions. Aliquots of DNA were stored at −20°C until use. The qualitative real-time PCR test targeting the IS*1111* repetitive element was carried out using an Adiavet COX Real-Time PCR kit (Bio-X Diagnostics, Rochefort, Belgium). A panel of required positive and negative controls was included in each run. An analytical cut-off value of 36.0 was selected corresponding to the defined limit of detection of the test.

**Statistical analysis.** The Bayes approach was used to include the uncertainties resulting from the small number of positive herds detected in this study and the large unevenness of particular groups of animals that were considered in relation to the risk factor characteristics. Statistical calculations were performed using the R (ver. 4.1) free software environment with the prevalence software package (8). The apparent and true seroprevalence of infection with *C. burnetii* at both the animal and herd level was estimated using a statistical model based on the Bayesian approach (17, 20).

The true seropositivity to *C. burnetii* at the animal and herd levels in individual Polish voivodeships was evaluated using latent class analysis providing a unified framework for various methods found in a dispersed literature, characterising each by the number of

populations or subgroups in the data and the number of observations made of each individual (44). Typically, the true disease status is only latently observed, because most diagnostic tests have insufficient sensitivity and specificity. For many epidemiological agents, this estimation of sensitivity (Se) and specificity (Sp) is complicated because of the lack of a suitable reference test, *i.e.* a diagnostic test that has a known accuracy when applied to samples from a specific target population (*e.g.* a gold standard/error-free test or one of which the misclassification error is reliably known). The statistical models take into account the fact that the true classification of an individual outcome is not known, and are therefore sometimes referred to as the "latent class". There is a distinction between true prevalence (the proportion of a population that is actually infected) and apparent prevalence (the proportion of the population that has tested positive for the disease). Given estimates for Se, Sp, and apparent prevalence (AP), the true prevalence may be calculated using the following expression:

True prevalence = (AP + Sp − 1)/(Se + Sp − 1).

The evaluation of the true seroprevalence of animals and herds infected with *C. burnetii* allowed the range of the real number of infections of Q fever 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, while the apparent herd seroprevalence 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 ELISA test.

In this study, we used beta-binomial models to estimate both the animal and herd prevalence according to a commonly accepted formula (20). Estimation of the diagnostic sensitivity (DSe) and specificity (DSp) of ELISA was based on the available data from the test's manufacturer (DSe = DSp = 100%) and published data (45), with DSe = 97.9% (95% CI: 73.9–96.4) and DSp = 97.7% (93.2–99.7). These parameters were expressed as the mode and confidence intervals shown as the percentile values of a $\beta$-distribution with 0.025 for the lower and 0.975 for the upper limit.

Multivariate (MVA) statistical analysis (22) was used for the identification of any association between the presence of antibodies against *C. burnetii* in sera samples and different categories of variables. Analysis was undertaken using individual data from all 133 cattle herds that were explored for variables such as the presence of antibodies against *C. burnetii* in individual milk samples and BTM, geographical location of farms, and positive and negative conformity rates between results from the ELISA test and results from real-time PCR. Data from MVA were analysed using Statistica software, version 10.0 (StatSoft Inc, Tulsa, OK, USA) and $P \leq 0.05$ was considered statistically significant. For each dimension and row or column point, the software computed the statistical parameters of MVA such as inertia, quality, and eigenvalues (6, 27). Based on this analysis, the coordinates referred to were compiled in a two-dimensional graph. Distances between row points or column points reflect their similarity or dissimilarity. Points grouped around their respective coordinates formed a given cluster, which was marked on the graph. Statistically significant relationships between copy numbers and particular variables were calculated and expressed as point G (PG) values. When close to zero, PG values indicated the lack of statistically significant relationships, while values higher than 0.5 showed the presence of a statistically significant relationship.

## Results

The results of the serological testing of sera, including the overall seroprevalence and the true seroprevalence estimates, are shown in Table 1. Serological analyses of sera samples confirmed the presence of antibodies against *C. burnetii* in 154 out of 2,180 (7.06%) tested animals with a 95% confidence interval (CI) of 6.00–8.20% and in 89 out of the 801 herds investigated through blood samples (11.11% CI: 9.02–13.49%). Positive cattle were present in all tested regions and seropositivity varied from 0.70 to 15.69% among them. The highest percentages of seropositive cows were noted in the Warmia-Mazuria (15.69% CI: 10.30–22.40%) and Silesia (10.63% CI: 6.30–16.50%) voivodeships. The lowest seropositivity rate of the tested samples from individual cows was calculated for Subcarpathia (0.70% CI 0.10–4.00%), followed by the rates for Lesser Poland (2.00% CI: 0.10–10.60%) and Lower Silesia (3.00% CI: 1.10–6.40%). Świętokrzyskie and Mazovia were the voivodeships with the highest percentages of seropositive cattle herds (40.00%, 95% CI: 5.27–85.34% and 28.57%, 95% CI: 11.28–52.18%, respectively), while the lowest herd seroprevalence was observed in Subcarpathia (0.75%, 95% CI: 0.02–4.09%) and Lower Silesia (9.09%, 95% CI: 1.92–24.33%). Doubtful ELISA results were obtained for 10 animals (0.45%) in 10 out of the 801 (1.25%) tested herds. The true seroprevalence at the animal and herd levels in voivodeships is presented on the map in Fig. 1.

Next, the true prevalence 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 *C. burnetii* per voivodeship. A large variation in seroprevalence at the animal and herd levels between the voivodeships was observed. True seroprevalence at herd level varied from 1.0% (95% CI: 0.0–4.3) to 45.2% (95% CI: 10.9, 87.3), whereas true seroprevalence at animal level ranged from 1.0% (95% CI: 0.0–4.2) to 15.7% (95% CI: 6.7, 25.6). The highest values of true seroprevalence at herd level were noted in Świętokrzyskie voivodeship at 45.2% (95% CI: 10.9–87.3), Lesser Poland at 33.3%

(95% CI 4.1–80.7), Masovia at 31.2% (95% CI: 12.1–57.8), Kuyavia-Pomerania at 20.9% (95% CI: 9.8–36.4), and Warmia-Masuria at 19.6% (8.8–33.8), whereas the highest values of true prevalence at animal level were seen in Warmia-Masuria at 15.7% (95% CI: 6.7–25.6), Świętokrzyskie voivodeship at 12.1% (95% CI: 1.4–34.2) and Silesia at 10.1% (95% CI: 2.8–18.2). The lowest values of animal and herd true seroprevalence were noted in the Subcarpathian voivodeship (Table 1). A statistically significant difference was noted between the lowest value of herd-level true seroprevalence recorded in Subcarpathia and those in Świętokrzyskie, Warmia-Masuria, Mazovia and Kuyavia-Pomerania. At the animal level, a statistically significant difference was recorded only between Warmia-Masuria and Subcarpathia.

The presence of *C. burnetii* antibodies in the milk of seropositive animals was detected in 85 out of the 133 herds from which these samples were taken (63.91%, 95% CI: 55.13–72.05%). Seropositive herds were noted in all voivodeships except Lesser Poland. Specific immunoglobulins were identified in 68/109 (62.39%, 95% CI: 52.6–71.48%) herds where only individual milk samples were examined, in 12/19 (63.2%, 95% CI: 38.36–83.71%) where only BTM were obtained and in all herds (5/5, 95% CI: 47.82–100%) where both types of samples were tested. Detailed results of the real-time PCR and ELISA at the herd level are presented in Table 2.

Shedding of *C. burnetii* was confirmed by real-time PCR in 33 out of 133 tested herds (24.81%, 95% CI 17.74–33.04%). Positive herds were identified in the majority of tested voivodeships (13/16) and was not only in Lesser Poland, Lubusz and West Pomerania. Bacterial DNA was detected in 23/109 herds (21.1%, 95% CI: 13.87–29.96%) where only individual milk samples were examined, in 8/19 (42.1%, 95% CI: 20.25–66.5%) where only BTM samples were taken and in 2/5 (40%, 95% CI: 5.27–85.34%) where both types of samples were tested.

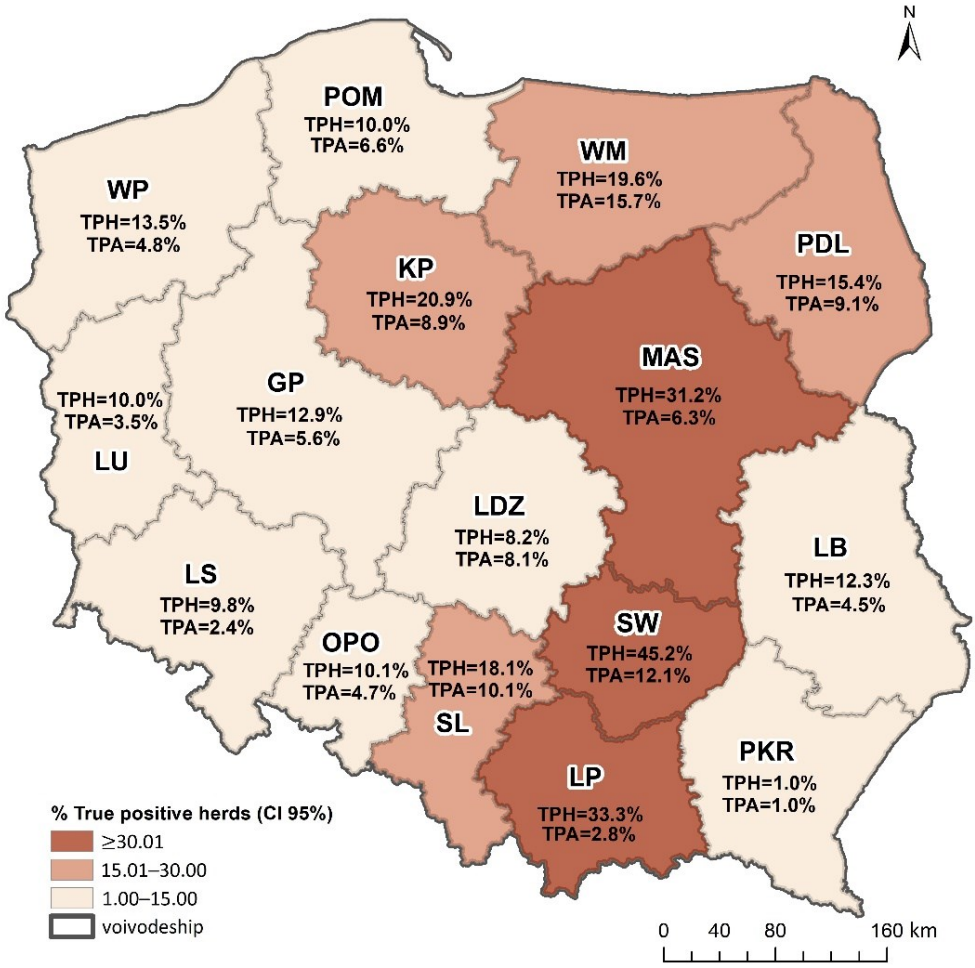


**Fig. 1.** True seroprevalence of *C. burnetii* at animal and herd level in cattle herds in the voivodeships of Poland based on sera analyses by ELISA. TPH – true positive herds; TPA – true positive animals; voivodeships: GP – Greater Poland, KP – Kuyavia-Pomerania, LP – Lesser Poland, LS – Lower Silesia,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

**Table 1.** Results of ELISA on sera samples at herd and animal level in each voivodeship

| Voivodeship | Animal level | | | | Herd level | | | |
|---|---|---|---|---|---|---|---|---|
| | Number of tested animals | Number of positive animals (%) | CI (95%) Clopper Pearson | % True positive animals (CI 95%) | Number of tested herds | Number of positive herds (%) | CI (95%) Clopper Pearson | % True positive herds (CI 95%) |
| Greater Poland | 173 | 11 (6.36) | 3.2–11.1% | 5.6 (0.7–11.7) | 26 | 3 (11.54) | 2.45–30.15% | 12.9 (2.1–32.8) |
| Kuyavia-Pomerania | 243 | 23 (9.47) | 6.1–13.9% | 8.9 (2.2–15.4) | 65 | 13 (20.0) | 11.1–31.77% | 20.9 (9.8–36.4)[c)] |
| Lesser Poland | 50 | 1 (2.0) | 0.1–10.6% | 2.8 (0.1–11.3) | 4 | 1 (25.0) | 0.63–80.59% | 33.3 (4.1–80.7) |
| Lower Silesia | 200 | 6 (3.0) | 1.1–6.4% | 2.4 (0.2–6.5) [a)] | 33 | 3 (9.09) | 1.92–24.33% | 9.8 (1.3–25.3) |
| Lublin | 103 | 5 (4.85) | 1.6–11.0% | 4.5 (0.4–11.4) | 35 | 4 (11.43) | 3.2–26.74% | 12.3 (2.2–29.4) |
| Lubusz | 106 | 4 (3.77) | 1.0–9.4% | 3.5 (0.3–10.0) | 42 | 4 (9.52) | 2.66–22.62% | 10.0 (1.6–23.9) |
| Łódź | 206 | 18 (8.74) | 5.3–13.5% | 8.1 (1.8–14.7) | 169 | 15 (8.88) | 5.05–14.22% | 8.2 (1.9–15.4) |
| Mazovia | 131 | 9 (6.87) | 3.2–12.6% | 6.3 (1.0–13.2) | 21 | 6 (28.57) | 11.28–52.18% | 31.2 (12.1–57.8)[d)] |
| Opole | 150 | 8 (5.30) | 2.3–10.2% | 4.7 (0.5–10.7) | 51 | 5 (9.8) | 3.26–21.41% | 10.1 (1.8–22.5) |
| Podlaskie | 116 | 11 (9.48) | 4.8–16.3% | 9.1 (2.1–17.6) | 41 | 6 (14.63) | 5.57–29.17% | 15.4 (4.1–32.1) |
| Pomerania | 87 | 6 (6.9) | 2.6–14.4% | 6.6 (0.8–15.4) | 51 | 5 (9.8) | 3.26–21.41% | 10.0 (1.7–23.0) |
| Silesia | 160 | 17 (10.63) | 6.3–16.5% | 10.1 (2.8–18.2) | 30 | 5 (16.67) | 5.64–34.72% | 18.1 (5.0–37.8)[e)] |
| Subcarpathia | 136 | 1 (0.7) | 0.1–4.0% | 1.0 (0.0–4.2) [b)] | 134 | 1 (0.75) | 0.02–4.09% | 1.0 (0.0–4.3)[c),d),e),f),g)] |
| Świętokrzyskie | 20 | 2 (10.0) | 1.2–31.7% | 12.1 (1.4–34.2) | 5 | 2 (40.0) | 5.27–85.34% | 45.2 (10.9–87.3)[f)] |
| Warmia-Masuria | 153 | 24 (15.69) | 10.3–22.4% | 15.7 (6.7–25.6) [a),b)] | 69 | 13 (18.84) | 10.43–30.06% | 19.6 (8.8–33.8)[g)] |
| West Pomerania | 146 | 8 (5.48) | 2.4–10.5% | 4.8 (0.5–10.8) | 25 | 3 (12.0) | 2.55–31.22% | 13.5 (2.1–33.7) |
| Total | 2,180 | 154 (7.06) | 6.0–8.2% | 6.0 (1.1–9.4) | 801 | 89 (11.11) | 9.02–13.49% | 10.5 (3.2–15.8) |

CI (95%) – 95% confidence interval; [a), b), c), d), e), f), g)] – statistically significant differences (P ≥ 0.05)

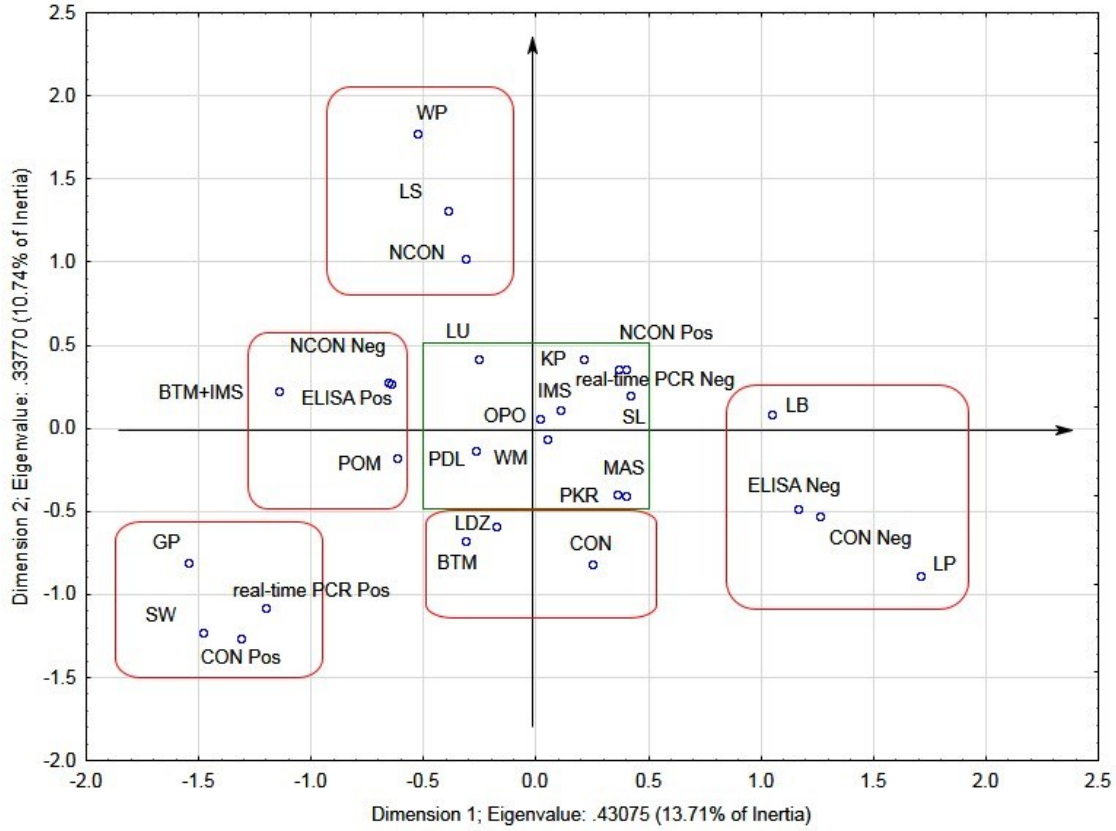


**Fig. 2.** Multivariate analysis of the presence of antibodies against *C. burnetii* in individual milk samples and bulk tank milk, geographical location of farms, positive and negative conformity rates between results from ELISA test and results from real-time PCR. Blue points represent each category of analysed variables. Blue points with similar profiles (low value of distances indicating a strong association between variables) are marked by the red rectangle without corners. Blue points located in the green rectangle in the graph's centre showed the points with similar profiles but representing eigenvalues indicating the lack of any association. Voivodeships: GP – Greater Poland, KP – Kuyavia-Pomerania, LP – Lesser Poland, LS – Lower Silesia, LB – Lublin, LB – Lubusz, LDZ – Łódź, MAS – Mazovia, OPO – Opole, PDL – Podlaskie, POM – Pomerania, SL – Silesia, PKR – Subcarpathia, SW – Świętokrzyskie, WM – Warmia-Masuria, WP – West Pomerania; BTM – bulk tank milk; IMS – individual milk sample; CON – conforming results between ELISA and real-time PCR; NCON – non-conforming results between ELISA and real-time PCR; CON Pos – positive conforming results in ELISA and real-time PCR tests; CON Neg – negative conforming results in ELISA and real-time PCR; ELISA Neg – negative ELISA results; ELISA Pos – positive ELISA results; real-time PCR Neg – negative real-time PCR results; real-time PCR Pos – positive real-time PCR results

**Table 2.** Results of real-time PCR and ELISA for milk samples at herd level in each voivodeship

| Voivodeship | Sample category** | Number of tested herds | Number of positive herds in real-time PCR* | Number of positive herds in ELISA* | Real-time PCR | | ELISA | |
|---|---|---|---|---|---|---|---|---|
| | | | | | Percentage of positive herds | CI (95%) | Percentage of positive herds | CI (95%) |
| Greater Poland | IMS | 1 | 1 | 1 | | | | |
| | BTM | 1 | 0 | 1 | | | | |
| | BTM + IMS | 1 | 1 | 1 | | | | |
| | Total | 3 | 2 | 3 | 66.67% | 9.43–99.16% | 100% | 29.24–100.00% |
| Kuyavia-Pomerania | IMS | 27 | 4 | 18 | | | | |
| | BTM | 1 | 0 | 0 | | | | |
| | BTM + IMS | 0 | 0 | 0 | | | | |
| | Total | 28 | 4 | 18 | 14.29% | 4.03–32.67% | 64.29% | 44.07–81.36% |
| Lesser Poland | IMS | 3 | 0 | 0 | | | | |
| | BTM | 1 | 0 | 0 | | | | |
| | BTM + IMS | 0 | 0 | 0 | | | | |
| | Total | 4 | 0 | 0 | 0.00% | 0.00–60.24% | 0.00% | 0.00–60.24% |
| Lower Silesia | IMS | 4 | 1 | 3 | | | | |
| | BTM | 1 | 0 | 1 | | | | |
| | BTM + IMS | 0 | 0 | 0 | | | | |
| | Total | 5 | 1 | 4 | 20% | 0.51–71.64% | 80.00% | 28.36–99.49% |
| Lublin | IMS | 4 | 1 | 3 | | | | |
| | BTM | 1 | 0 | 1 | | | | |
| | BTM + IMS | 0 | 0 | 0 | | | | |
| | Total | 5 | 1 | 4 | 20% | 0.51–71.64% | 80.00% | 28.36–99.49% |
| Lubusz | IMS | 3 | 0 | 1 | | | | |
| | BTM | 0 | 0 | 0 | | | | |
| | BTM + IMS | 0 | 0 | 0 | | | | |
| | Total | 3 | 0 | 1 | 0% | 0.00-70.76% | 33.33% | 0.84-90.57% |
| Łódź | IMS | 6 | 1 | 3 | | | | |
| | BTM | 1 | 1 | 1 | | | | |
| | BTM + IMS | 1 | 1 | 1 | | | | |
| | Total | 8 | 3 | 5 | 37.5% | 8.52–75.51% | 62.50% | 24.49–91.48% |
| Mazovia | IMS | 15 | 3 | 7 | | | | |
| | BTM | 3 | 1 | 1 | | | | |
| | BTM + IMS | 1 | 0 | 1 | | | | |
| | Total | 19 | 4 | 9 | 21.05% | 6.05–45.57% | 47.37% | 24.45–71.14% |
| Opole | IMS | 8 | 1 | 5 | | | | |
| | BTM | 1 | 1 | 1 | | | | |
| | BTM + IMS | 0 | 0 | 0 | | | | |
| | Total | 9 | 2 | 6 | 22.22% | 2.81–60.01% | 66.67% | 29.93–92.51% |
| Podlaskie | IMS | 16 | 6 | 13 | | | | |
| | BTM | 3 | 0 | 1 | | | | |
| | BTM + IMS | 0 | 0 | 0 | | | | |
| | Total | 19 | 6 | 14 | 31.58% | 12.58–56.55% | 73.68% | 48.8–90.85% |
| Pomerania | IMS | 7 | 3 | 5 | | | | |
| | BTM | 1 | 1 | 1 | | | | |
| | BTM + IMS | 0 | 0 | 0 | | | | |
| | Total | 8 | 4 | 6 | 50% | 15.70–84.3% | 75.00% | 34.91–96.81% |
| Silesia | IMS | 3 | 0 | 1 | | | | |
| | BTM | 2 | 1 | 1 | | | | |
| | BTM + IMS | 0 | 0 | 0 | | | | |
| | Total | 5 | 1 | 2 | 20% | 0.51–71.64% | 40.00% | 5.27–85.34% |
| Subcarpathia | IMS | 4 | 1 | 2 | | | | |
| | BTM | 0 | 0 | 0 | | | | |
| | BTM + IMS | 0 | 0 | 0 | | | | |
| | Total | 4 | 1 | 2 | 25% | 0.63–80.59% | 50.00% | 6.76–93.24% |
| Świętokrzyskie | IMS | 2 | 1 | 2 | | | | |
| | BTM | 2 | 2 | 2 | | | | |
| | BTM + IMS | 0 | 0 | 0 | | | | |
| | Total | 4 | 3 | 4 | 75% | 19.41–99.37% | 100.00% | 39.67–100% |
| Warmi-Masurian | IMS | 3 | 0 | 1 | | | | |
| | BTM | 1 | 1 | 1 | | | | |
| | BTM + IMS | 1 | 0 | 1 | | | | |
| | Total | 5 | 1 | 3 | 20% | 0.51–71.64% | 60.00% | 14.66–94.73% |
| West Pomerania | IMS | 3 | 0 | 3 | | | | |
| | BTM | 0 | 0 | 0 | | | | |
| | BTM + IMS | 1 | 0 | 1 | | | | |
| | Total | 4 | 0 | 4 | 0% | 0.00–60.24% | 100% | 39.76–100% |
| Total | IMS | 109 | 23 | 68 | 21.1% | 13.87–29.96% | 62.39% | 52.6–71.48% |
| | BTM | 19 | 8 | 12 | 42.11% | 20.25–66.5% | 63.16% | 38.36–83.71% |
| | BTM + IMS | 5 | 2 | 5 | 40% | 5.27–85.34% | 100% | 47.82–100% |
| | Total | 133 | 33 | 85 | 24.81% | 17.74–33.04% | 63.91% | 55.13–72.05% |

* – herd was classified as positive if at least one sample was positive in ELISA/real-time PCR test
** – sample category: IMS – individual milk samples, BTM – bulk tank milk, BTM + IMS – bulk tank milk and individual milk

Statistical analysis as MVA (Fig. 2) showed a high correlation between the ELISA and real-time PCR in the Lublin and Lesser Poland voivodeships and a lack of conformity between ELISA and real-time PCR results in Lower Silesia and West Pomerania. The divergences in the results of MVA analysis for Lublin and Lower Silesia voivodeships, despite identical percentages of seropositive and PCR-positive herds, arose from differences in the combinations of ELISA and real-time PCR results obtained for herds in both regions. The highest level of conformity between ELISA and real-time PCR results was obtained for BTM samples. In contrast, there was a lack of conformity of results obtained for the BTM+IMS and IMS herd sampling categories.

## Discussion

*Coxiella burnetii* infections occur in domestic ruminants worldwide. Usually infection is subclinical, but sometimes it causes reproductive problems and abortion storms, which are observed predominantly in small ruminants. The aforementioned factors cause a decrease in animal productivity, leading to economic losses. Moreover, the presence of the bacteria in the ruminant population poses a zoonotic threat to humans. Approximately 60% of human infections result only in seroconversion (3). In symptomatic patients, acute Q fever usually presents as a self-limiting, influenza-like febrile illness and less often as pneumonia or hepatitis. Recently it has been suggested that the term 'chronic Q fever' is inadequate because it artificially combines significantly different persistent foci of infection under serological criteria (30). The term "persistent focalised infection" has been proposed to describe clinical manifestations of chronic infections with the *C. burnetii* bacterium such as endocarditis, vascular infections, osteomyelitis, arthritis or hepatitis (13). People occupationally exposed to the pathogen, such as farmers or veterinarians, are in the most danger (16). The long persistence of *C. burnetii* in the environment facilitates its spread and the wind plays a crucial role in the transmission between farms and from ruminants to humans (32, 33). In this context, control of the status of *C. burnetii* in ruminant herds is essential for public and animal health.

This study was conducted to evaluate the epizootic situation of *C. burnetii* in dairy cattle herds in Poland *via* testing of sera and milk collected from cows in all voivodeships. In 2020, the cattle stock in Poland was estimated at 6,278,900 animals, including 2,125,694 dairy cows, and this large national herd proves that the cattle industry is an important branch of the Polish agricultural sector (35). An extrapolation was made from the obtained results to the total population of dairy cattle in Poland. Analysis at the animal level showed that 127,542 (6.00%) of the dairy cows were truly seropositive with *C. burnetii.* The prevalence of antibodies in sera of tested animals was estimated at 7.06% (true seroprevalence 6%) and was lower than the 14.85% reported by Saglam and Sahin (39) in Turkey, 47.2% recorded in Hungary (10) and 55.3% noted in Mali (9). In Latvia, 27% of cows which aborted tested positive for the presence of *C. burnetii* antibodies (7).

Serological studies conducted in other countries show high variability of herd-level seroprevalence among tested cattle herds – from 3% reported in Kenya (31), through 19.6% in Bosnia and Herzegovina (40) and 36% in France (18) to 38.8% in Sicily (19). In this research, the presence of specific antibodies was detected in sera from 11.1% (true seroprevalence 10.5%) of the tested herds and varied from 0.7% to 40% between voivodeships. The lowest risk of infection at both the animal and herd levels was in the Subcarpathian voivodeship. The herd-level seroprevalence determined in this study is much lower than that reported in a previous study (41), which showed 24.46% overall seroprevalence at the herd level and 2.5% to 61.4% seroprevalence in the voivodeships. Research published in 2015 (24) showed an even higher rate of serology-positive herds (40.41%), although this study was conducted on sera samples tested by the complement fixation test and collected from 14 voivodeships. Analysis of the available studies indicates a downward trend in the prevalence of *C. burnetii* antibodies in Polish cattle. It may be caused by the rising awareness among farmers and veterinarians, who are increasingly deciding to vaccinate animals. It should be noted that immunised animals are excluded from serological studies. Taking into consideration that vaccination does not eliminate shedding, but reduces its duration and level, it cannot be ruled out that some of the immunised individuals shed the bacteria (5).

A separate serological and molecular study was conducted on milk samples collected from 133 herds in all voivodeships. The presence of *C. burnetii* DNA was confirmed in 24.81% of the cattle herds, which is less than the 39.60% (40/101) estimated for BTM samples in previous research (40). An overview of the published data on the prevalence studies of *C. burnetii* in raw milk finds the presence to vary greatly between countries (34). Dobos *et al.* (11) tested 370 dairy cattle herds from six countries of the Central and Eastern European region and detected *C. burnetii* shedding in 44.05%. The study performed by Kalaitzakis *et al.* (26) in Greece showed 33.8% prevalence, whereas 10.7% of herds were PCR-positive in Latvia (7).

There are further studies also indicating that the prevalence of antibodies in milk samples is highly heterogeneous between countries. In Ireland 19.5% of dairy cattle herds were positive based on BTM analyses (37), in Portugal it was estimated at 37.8% (1) and in Quebec (Canada) at 43.2% (43). In this study, testing of milk samples by ELISA showed 63.9% seroprevalence at the herd level. This high rate was expected because samples were collected from suspected seropositive animals. Taking into account that dry cows are not included in screening tests on milk samples and may later shed a huge number of bacteria during parturition

and the postpartum period, additional laboratory tests of samples from dry animals should be carried out.

There was a notable discrepancy between seroprevalence in milk and seroprevalence in serum in the Lesser Poland voivodeship. This phenomenon was noted also by Joulié *et al.* (25) in sheep. They observed that the milk collected from some animals of which the sera were highly positive did not contain specific antibodies. In this study, cows from Lesser Poland presented low levels of immunoglobulins in blood, which might explain the negative results of the milk analysis.

In Q fever, seropositivity to *C. burnetii* is not strongly correlated with shedding of the pathogen. Therefore, serology may not reflect the real infection rate in the herds, but it is a useful tool for screening purposes. This misrepresentation of the infection rate was the outcome in the West Pomerania voivodeship, where all tested herds were negative in real-time PCR despite the presence of antibodies in milk samples. The lack of congruence between ELISA and real-time PCR can be caused by multiple factors, including the phenomenon of intermittent shedding (23). Significant conformity between the results of ELISA and those of real-time PCR was only noted for the BTM samples. A lack of conformity between ELISA and real-time PCR was observed for the IMS + BTM category.

The findings of the current study show a downward trend in seroprevalence and prevalence of *C. burnetii* in Polish cattle but indicate that infections are present in cattle herds across the country. Considering the importance of this pathogen as a public health threat, a permanent information campaign about Q fever should be initiated, especially among occupationally exposed people. These results emphasise the crucial role of surveillance and adequate biosecurity measures such as quarantine and identification of the infection status of newly purchased animals in the prevention and limitation of Q fever spread in Poland.

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