Comparative Immunology, Microbiology and Infectious Diseases 67 (2019) 101377

Contents lists available at ScienceDirect

Comparative Immunology, Microbiology and Infectious Diseases

journal homepage: www.elsevier.com/locate/cimid

Occurrence of *Coxiella burnetii* in Polish dairy cattle herds based on serological and PCR tests

Monika Szymańska-Czerwińska[a,b], Agnieszka Jodełko[a,*], Krzysztof Niemczuk[a,b]

[a] Department of Cattle and Sheep Diseases, National Veterinary Research Institute, 24-100, Puławy, Poland

[b] Laboratory of Serological Diagnosis, National Veterinary Research Institute, 24-100, Puławy, Poland

ARTICLE INFO

Keywords:
Coxiella burnetii
Q fever
Serology
Real-time PCR
Dairy cattle

ABSTRACT

The aim of the research was to assess the prevalence of antibodies to *Coxiella burnetii* in dairy cattle herds in Poland and to compare the results of real-time PCR and ELISA tests performed on bulk tank milk (BTM) samples. In total, 2635 serum samples collected from 969 dairy cattle herds from all provinces were tested using ELISA. Additionally, BTM specimens from 101 herds were analysed by ELISA and real-time PCR targeting IS*1111* element. Presence of anti-*C. burnetii* antibodies was confirmed in 25.39% of serum samples in 237 herds (24.46%) and the herd-level seroprevalence in Voivodeships varied from 2.5% to 61.4%. Moreover, 46 (45.5%) of analysed bulk tank milk samples gave postive result in ELISA and microbial DNA was detected in 40 (39.6%) of tested herds. The comparative analysis of ELISA and real-time PCR results obtained for BTM samples using the chi-square test showed statistically significant relationship between results of both methods.

1. Introduction

Coxiella (*C.*) *burnetii* is an obligate intracellular γ-proteobacterium and the etiological agent of zoonotic disease Q fever [1]. This pathogen was first described in 1930s [2,3] and currently is distributed almost worldwide, affecting a wide range of animal species [4]. Domestic ruminants (i.e. goats, sheep, cattle) are considered to be a major reservoir of *C. burnetii*, moreover, the wildlife fauna and ticks may also be an important sources of the pathogen. Taking into account that the clinical signs of coxiellosis in animals are non-specific and infection may be asymptomatic, especially in cattle, laboratory tests are crucial in Q fever diagnosis. Noteworthy is the fact that asymptomatic individuals and intermittent cattle shedders may remain negative in serological tests and unnoticeably shed the pathogen into the environment for several months or years [5,6]. The highest amounts of *C. burnetii* are shed during abortion and parturition in birth products (placenta, birth fluids), whereas lower levels are detected in milk, vaginal discharges, feaces, urine and semen [7]. In humans and animals inhalation of contaminated aerosols is the most common route of infection [8]. Sporadic cases of Q fever transmission by sexual contact, blood transfusion and transplantation were also reported [9,10]. The phenomenon of *C. burnetti* shedding in milk may pose a potential threat for public health, not only for occupationally exposed people but also for raw milk consumers [11,12].

Serological screenings of ruminant herds have been performed in many countries to assess exposure to the pathogen and the zoonotic risk [13–15]. The complement fixation test (CFT), indirect immunofluorescence assay (IFA) or enzyme-linked immunosorbent assay (ELISA) may be used, but the latter is thought to be the most robust and has good specificity and high sensitivity [16–18]. Moreover, the testing of BTM samples using ELISA allows a preliminary evaluation of the herd status, ease of sampling and reduces costs. In Poland Q fever is a notifiable disease, additionally serological monitoring survey of cattle and small ruminants has been implemented since 2010. The previous study showed that percentage of *C. burnetii* seropositive cattle herds in Poland is significant [19].

The objectives of this research were to estimate the prevalence of antibodies against *C. burnetii* in dairy cattle herds based on sera and bulk tank milk samples (BTM) analysis and to compare the results of real-time PCR and ELISA tests performed on BTM specimens.

2. Materials and methods

This study was a part of larger research project and was carried out between January 2014 and December 2017. Samples were collected specifically for this investigation from non-vaccinated dairy cattle by authorized veterinarians following standard procedures and with farmers' consent. According to the Local Ethical Committee on Animal

* Corresponding author.
E-mail address: agnieszka.jodelko@piwet.pulawy.pl (A. Jodełko).

https://doi.org/10.1016/j.cimid.2019.101377
Received 20 August 2019; Received in revised form 23 October 2019; Accepted 25 October 2019

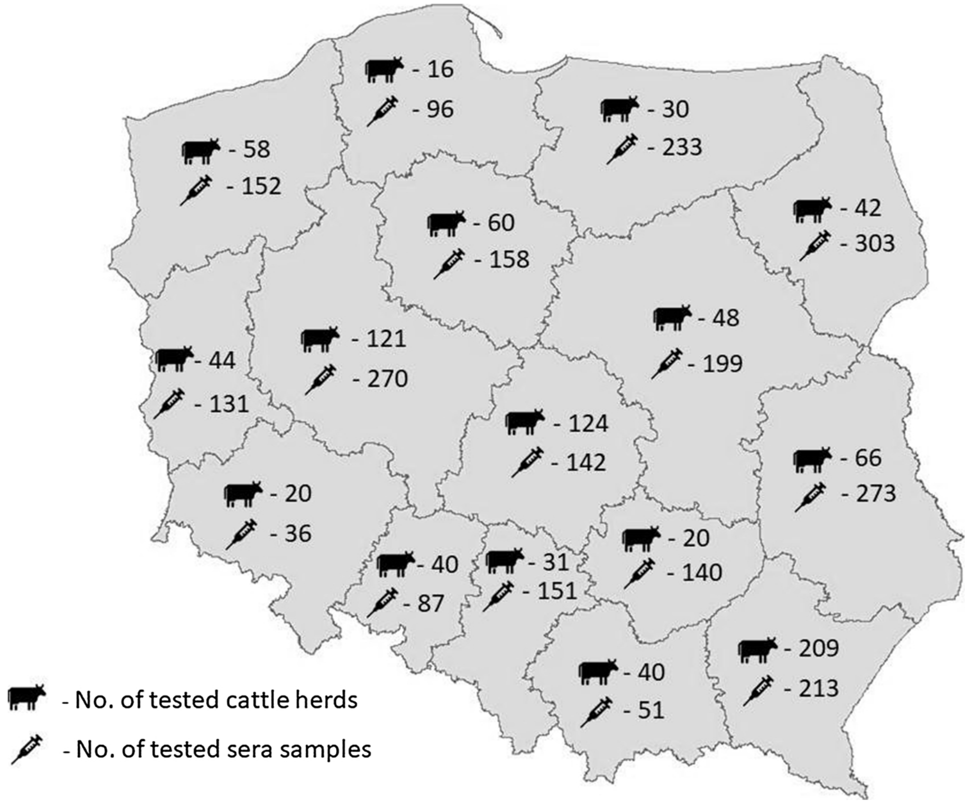

Fig. 1. Number of cattle herds and sera samples tested by ELISA in the Voivodeships of Poland.

Testing at the University of Life Sciences in Lublin (Poland), formal ethical approval is not required for this kind of study.

2.1. Blood sampling

The number of blood specimens and herds examined in different Voivodeships of Poland is shown in Fig. 1. In total, 2635 blood samples were collected from 969 dairy cattle herds by venipuncture of cranial vena cava. Specimens were then stored at room temperature for 30–45 min to allow clotting. Nextly they were centrifuged under $1000 \times g$ for 10 min to obtain serum. If the serological test was performed within 48 h, the temperature of the sample was maintained between 4 °C and 8 °C, otherwise sera were stored at -20 ± 5 °C until tested.

2.2. Bulk-tank milk sampling

Overall, 101 dairy cattle herds were randomly selected in separated cross-sectional survey and BTM specimens collected from the herds were tested for the presence of both anti-*C. burnetii* antibodies and pathogen's DNA. The BTM samples were collected in 14 out of 16 Voivodeships of Poland, excluding Lesser Poland and Silesia. If the serological test and nucleic acid isolation were performed within 48 h, the temperature of the BTM samples was maintained between 4 °C and 8 °C, otherwise specimens were frozen at -20 ± 5 °C until tested.

2.3. Serology

The Q-Fever (*Coxiella burnetii*) Antibody Test Kit (IDEXX, Switzerland) detecting IgG antibodies against *C. burnetii* phase I and phase II antigen was utilized to screen sera and BTM specimens. Following the manufacturer's instruction, the value %OD was calculated as (OD sample − ODneg)/(ODpos − ODneg) x 100 after averaging the duplicate values. Sera were considered to be negative when %OD $<$ 30, dubious when %OD $\geq$ 30 and %OD $\leq$ 40, positive when %OD $>$ 40.

2.4. DNA extraction and real-time PCR

Nucleic acid extraction from milk samples was performed using QIAamp DNA Mini Kit (Qiagen, Germany) according to the real-time PCR kit manufacturer's instructions. A qualitative real-time PCR test was performed on bulk tank milk samples using commercial kit Adiavet COX Real Time PCR (Bio-X Diagnostics, Belgium), targeting the IS*1111* gene.

2.5. Statistical analyses

All statistical data analyses were conducted using STATISTICA software, version 10.0 (StatSoft. Inc., USA). A comparative analysis of the results of ELISA and real-time PCR tests obtained for bulk tank milk samples was made using the chi-square test with a significance level set to alpha $<$ 0.05. Analysis was carried out with and without considering doubtful ELISA's results. Moreover, the percentages of seropositive herds determined based on testing of sera and BTM samples were comparatively analysed at Voivodeship level. The analysis included also comparison of percentages of herds positive in real-time PCR with percentages of herds in which presence of specific antibodies in sera was confirmed.The analyses were conducted utilizing chi-square test with Yates, Fisher or V-square corrections, depending on subgroup size. Differences were considered significant at a p-value less than 0.05.

3. Results

The presence of anti-*C. burnetii* antibodies was confirmed in serum samples obtained from 669 cows (25.39%) and seropositive animals were identified in 237 of 969 tested cattle herds. Seroprevalence at herd-level was calculated at 24.46%. Detailed data about the

Table 1
Summary of the prevalence of cattle herds and animals demonstrating antibodies to *C. burnetii* in blood in particular voivodeships of Poland.

Voivodeship	No. of tested herds	No.of seropositive herds (%)	No. of tested animals	No. of seropositive animals (%)
Lower Silesian	20	4 (20.0)	36	8 (22.2)
Kuyavian-Pomeranian	60	23 (38.3)	158	40 (25.3)
Lublin	66	20 (30.3)	273	47 (17.2)
Lubusz	44	27 (61.4)	131	78 (59.5)
Łódź	124	11 (8.9)	142	12 (8.5)
Lesser Poland	40	1 (2.5)	51	1 (2.0)
Masovian	48	22 (45.8)	199	51 (25.6)
Opole	40	5 (12.5)	87	13 (14.9)
Subcarpathian	209	34 (16.3)	213	34 (16)
Podlaskie	42	20 (47.6)	303	187 (61.7)
Pomeranian	16	8 (50.0)	96	13 (13.5)
Silesian	31	11 (35.5)	151	24 (15.9)
Świętokrzyskie	20	7 (35.0)	140	20 (14.3)
Warmian-Masurian	30	9 (30.0)	233	76 (32.6)
Greater Poland	121	31 (25.6)	270	60 (22.2)
West Pomeranian	58	4 (6.9)	152	5 (3.3)
total	969	237 (24.46)	2635	669 (25.39)

prevalence of seropositive herds and animals in Polish Voivodeships are summarized in Table 1. The percentages of seropositive herds in Voivodeships varied from 2.5% in Lesser Poland to 61.4% in Lubusz Voivodeship. The highest prevalence of *C. burnetii* seropositive cattle herds was noted in Lubusz (61.4%), Pomerania (50.0%), Podlaskie (47.6%) and Masovia (45.8%) Voivodeships. In contrast regions with the lowest seroprevalence were Lesser Poland (2.5%), West Pomeranian (6.9%) and Łódź (8.9%).

Serological test of bulk tank milk samples using ELISA revealed higher percentage of positive cattle herds. Presence of specific antibodies was confirmed in 46 of 101 BTM specimens (45.5%), while five samples (4.85%) were recorded as doubtful. Moreover, positive results of real-time PCR were obtained for 40/101 (39.60%) of BTM specimens (Table 2). The 47.5% of these samples were negative in ELISA and real-time PCR tests. In 34.65% (35/101) of samples both anti-*C. burnetii* immunoglobulins and DNA of the pathogen were detected, while in nearly 11% of specimens only the presence of antibodies was confirmed. In 2 of 101 (1.94%) samples, despite the positive result of real-time PCR test, specific immunoglobulins were not present. Moreover, a doubtful result of the ELISA test was obtained for 5 BTM specimens, in three of which *C. burnetii* DNA was detected. Summary of the results of bulk tank milk samples testing by ELISA and real-time PCR shows Table 3.

Comparative analysis of ELISA and real-time PCR results obtained for bulk tank milk samples was conducted using the chi-square test. It showed statistically significant relationship between results of both methods (p-value < 0,0001) with and without considering doubtful ELISA's results. Without taking into account samples doubtful in the ELISA test, the 87% of serological and molecular tests results were congruent. The calculated odds ratio (OR = 79.55) indicates that the chance of receiving a negative ELISA test result, when the real-time PCR test gave a negative result, is almost 80 times greater than receiving a negative ELISA result for samples positive in real-time PCR test.

Table 4 presents the results of comparative analyses of the percentages of cattle herds positive in ELISA (serum samples) and real-time PCR (BTM) also percentages of seropositive herds determined based on testing of sera and BTM at Voivodeship level. It should be noted that sample size in some of provinces e.g Opole, West Pomeranian was quite small. Differences ($P < 0.05$) between results of ELISA test of sera and real-time PCR test of BTM were observed in three Voivodeships (Opole, Warmian-Masurian, West Pomeranian). Comparison of the results of ELISA test of BTM and serum specimens revealed differences in

Table 2
Detailed results of ELISA and real-time PCR tests performed on bulk tank milk samples collected from 101 dairy cattle herds.

Voivodeship	Herd ID	Real-time PCR result	Ct value	ELISA result	S/P value
Lower Silesian	A1	positive	35.51	positive	49.952
	A2	negative	–	negative	0.355
Kuyavian-Pomeranian	B1	positive	30.97	positive	192.192
	B4	negative	–	negative	−0.824
	B7	positive	30.88	positive	91.74
	B10	positive	32.13	positive	148.99
	B11	positive	31.28	positive	195.73
	B14	positive	33.74	positive	52.983
	B15	positive	29.3	positive	120.191
	B24	negative	–	positive	50.724
	B27	negative	–	negative	3.424
	B29	positive	35.81	positive	108.613
	B31	negative	–	negative	22.344
	B32	negative	–	negative	2.729
	B33	positive	35.98	doubtful	34.221
	B34	negative	–	negative	0.447
	B35	negative	–	positive	55.916
	B36	positive	27.16	doubtful	39.991
	B37	negative	–	negative	0.447
	B38	negative	–	negative	28.316
Lublin	C1	positive	35.17	positive	50.573
	C10	negative	–	negative	3.08
	C11	negative	–	negative	2.149
	C16	positive	31.33	positive	117.243
	C20	negative	–	positive	138.758
	C21	positive	32.26	positive	139.252
	C34	negative	–	positive	66.199
	C35	negative	–	negative	−1.271
	C36	negative	–	negative	−0.033
	C39	negative	–	negative	0.573
	C40	negative	–	negative	6.668
	C43	negative	–	negative	8.169
	C45	negative	–	negative	−1.518
	C46	negative	–	negative	−0.882
	C47	negative	–	negative	−0.671
	C48	negative	–	negative	−0.953
	C51	negative	–	negative	3.848
	C52	negative	–	negative	−2.294
	C53	negative	–	negative	0.85
	C54	negative	–	negative	0.85
	C55	positive	28.89	positive	112.141
	C56	negative	–	negative	−1.234
Lubusz	D3	positive	27.73	positive	76.245
Łódź	E1	negative	–	negative	13.914
	E2	negative	–	negative	12.093
	E3	negative	–	negative	−0.424
	E7	negative	–	negative	−0.287
	E8	negative	–	negative	1.862
	E9	negative	–	negative	4.37
	E11	negative	–	doubtful	31.889
	E12	positive	29.82	positive	96.794
	E13	negative	–	negative	−1.453
Lesser Poland	G1	positive	33.71	positive	69.72
	G2	negative	–	negative	−0.033
	G3	negative	–	positive	56.877
	G4	negative	–	doubtful	34.185
	G5	negative	–	negative	4.226
	G6	negative	–	negative	1.433
	G7	negative	–	negative	3.94
	G12	positive	33.71	positive	71.26
	G13	positive	31.51	positive	160.458
	G19	negative	–	negative	27.983
	G20	negative	–	negative	3.868
	G25	negative	–	positive	83.095
	G26	negative	–	negative	−0.573
	G53	negative	–	negative	−2.577
	G56	negative	–	positive	64.549

(*continued on next page*)

Table 2 (*continued*)

Voivodeship	Herd ID	Real-time PCR result	Ct value	ELISA result	S/P value
Opole	H1	positive	35.72	negative	8.238
	H7	positive	28.33	positive	190.544
	H8	positive	34.34	positive	94.977
Subcarpathian	I17	negative	–	positive	144.546
Podlaskie	J1	positive	35.43	positive	41.34
	J2	positive	32.11	positive	173.232
	J3	positive	31.74	positive	71.903
	J5	negative	–	positive	43.025
	J6	positive	34.67	positive	242.908
	J15	positive	26.96	positive	95.989
	J16	negative	–	positive	143.417
	J21	negative	–	negative	0.358
	J22	positive	31.07	positive	139.542
	J26	negative	–	negative	3.424
	J28	positive	32.29	doubtful	38.47
Pomeranian	K9	negative	–	negative	−2.083
	K10	negative	–	negative	−1.235
	K12	negative	–	negative	4.115
	K14	negative	–	positive	50.951
Świętokrzyskie	L3	negative	–	negative	−3.301
	L4	positive	35.3	positive	72,108
	L5	positive	33.17	positive	94.178
Warmian-Masurian	M2	negative	–	negative	1.521
	M4	positive	31.57	positive	227.937
	M11	positive	30.52	positive	56.877
	M12	positive	30.38	positive	144.546
	M16	positive	31.74	positive	71.903
	M17	positive	30.38	positive	144.546
Greater Poland	N10	positive	33	positive	94.127
	N11	negative	–	negative	4.831
	N12	negative	–	negative	1.518
West Pomeranian	O1	positive	28.77	positive	186.891
	O2	positive	32.92	positive	91.167
	O3	positive	35.85	negative	27.913

Hyphen means that Ct value could not be detemined due to the absence of amplification curve.

Table 4
P-values calculated using chi-square test with with Yates, Fisher or V-square corrections, depending on subgroup size.

Voivodeship	p-value*	
	serum ELISA vs. BTM qPCR	serum ELISA vs. BTM ELISA
Lower Silesian	0.411	0.411
Kuyavian-Pomeranian	0.381	0.20
Lublin	0.272	0.789
Lubusz	0.799	0.799
Łódź	0.707	0.773
Lesser Poland	0.10	0.001
Opole	0.003	0.101
Subcarpathian	0.358	0.37
Podlaskie	0.349	0.135
Pomeranian	0.117	0.591
Świętokrzyskie	0.538	0.538
Warmian-Masurian	0.024	0.024
Greater Poland	0.714	0.714
West Pomeranian	0.0001	0.017

* Significance at $P < 0.05$.

Warmian-Masurian, West Pomeranian, and Lesser Poland.

4. Discussion

C. burnetii infections are noted in many animal species all over the world. In recent years number of reports about shedding of the pathogen in domestic ruminants, including dairy cattle, have been increased. Infected livestock may experience reproductive disorders including late abortion, stillbirth, premature delivery and delivery of weak offspring, reduction of conception rate or metritis [20], which can cause significant economic losses. Moreover, financial losses may also occur due to shedding of *C. burnetii* in milk. According to current legislation, raw milk from animals with clinical symptoms of an infectious diseases communicable to humans through milk, should be regarded as not meeting the requirements of point 1a Chapter I of Section IX of Annex III to Regulation (EC) No 853/2004. As a consequence it is considered as unsuitable for production of foodstuffs for humans. The phenomenon of *C. burnetii* shedding in milk is common in many countries [21], therefore monitoring survey of dairy cattle herds based on BTM testing is crucial. Despite the availability of PCR tests, serological assays are consider to be suitable to assess the status of herds at relatively low cost [22].

This research was performed to evaluate the epidemiological

Table 3
Summary of the results of bulk tank milk samples testing by ELISA and real-time PCR methods.

Voivodeship	No. of tested herds	No. of ELISA positive and qPCR positive herds	No. of ELISA positive and qPCR negative herds	No. of doubtful ELISA and qPCR positive herds	No. of doubtful ELISA and qPCR negative herds	No. of negative ELISA and positive qPCR herds	No. of negative ELISA and negative qPCR herds
Lower Silesian	2	1	–	–	–	–	1
Kuyavian-Pomeranian	18	7	2	2	–	–	7
Lublin	22	4	2	–	–	–	16
Lubusz	1	1	–	–	–	–	–
Łódź	9	1	–	–	1	–	7
Lesser Poland	15	3	3	–	1	–	8
Opole	3	2	–	–	–	1	–
Subcarpathian	1	–	1	–	–	–	–
Podlaskie	11	6	2	1	–	–	2
Pomeranian	4	–	1	–	–	–	3
Świętokrzyskie	3	2	–	–	–	–	1
Warmian-Masurian	6	5	–	–	–	–	1
Greater Poland	3	1	–	–	–	–	2
West Pomeranian	3	2	–	–	–	1	–
total	101	35	11	3	2	2	48

Hyphen means zero herds.

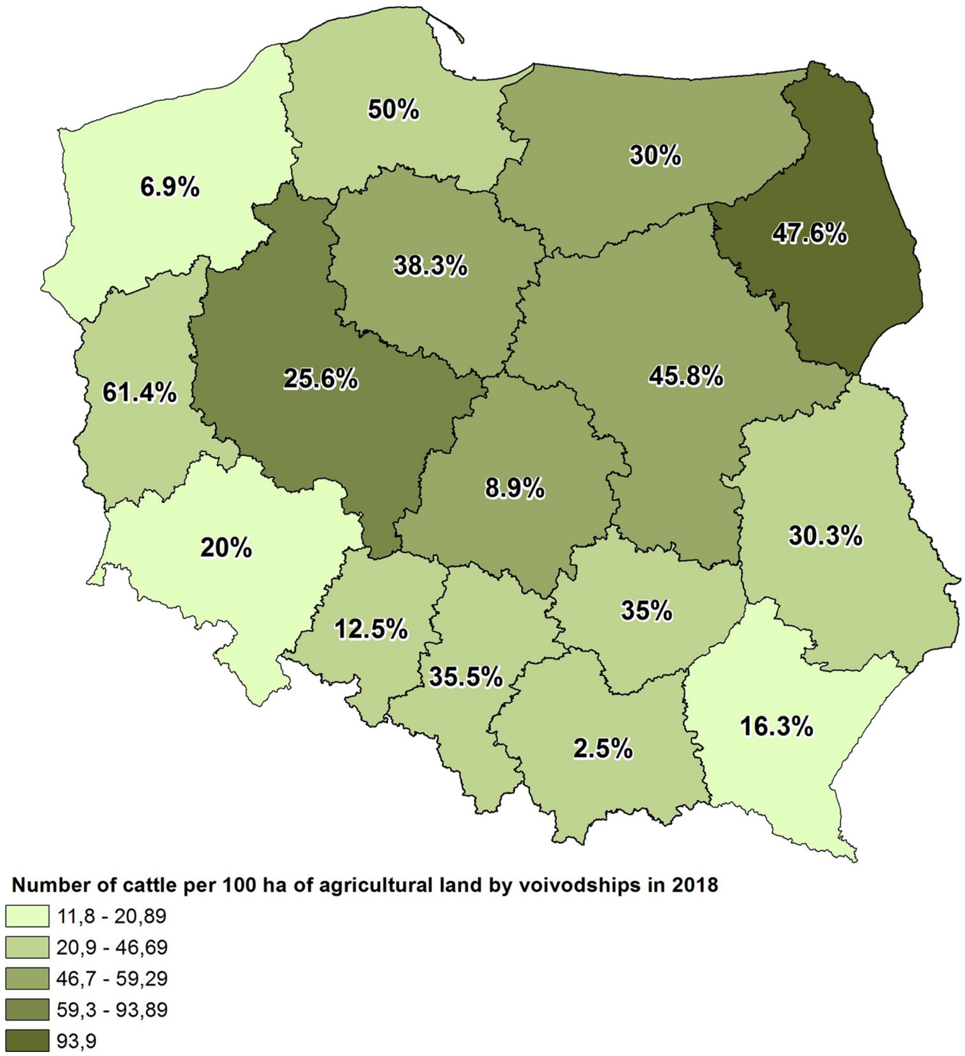

Fig. 2. Cattle density and prevalence of *C. burnetii* antibodies in serum samples at Voivodeship level.

situation of *C. burnetii* in dairy cattle in Poland *via* testing of sera and BTM samples by both ELISA and PCR techniques. Collected sera were analysed by ELISA test and average percentage of seropositive herds was estimated at 24.46%. This percentage falls within the range noted in other countries and is similar to 19.6% reported in Bosnia and Herzegovina [23], but is lower than reported in China (84%) [24] or in Northern Ireland (484%) [25]. It is also lower than 40.41% seroprevalence level determined in Poland in the previous study [19]. Samples were then tested using complement fixation test (CFT) detecting both IgG and IgM class antibodies, while ELISA identifies only IgG. The difference may also results from preventive activities and surveillance performed in the meantime by Veterinary Inspection. In this research high prevalence of seropositive herds was noted in Masovian and Podlaskie Voivodeships, which are the regions with high number and population density of dairy cows (Fig. 2) according to report of Statistics Poland [26]. The latter factor may facilitate transmission of infection between herds.

The herd-level prevalence of specific antibodies was additionally calculated based on BTM analysis. It was higher than obtained for sera samples and stood at 45.5%, while presence of *C. burnetii* DNA was confirmed in 39.6% of these specimens. Estimated percentage of BTM seropositive herds was higher than those reported in Sweden and Northern Ireland, which amounted to 8.2% and 21.5%, respectively [27,28]. It was comparable to 37.8% received by Anastácio et al. [29], who examined BTM samples collected from cattle in central Portugal. A much higher percentage of seropositive herds was recorded in Denmark (79.2%) or in the Netherlands (81.6%) [30,31]. When screening tests are performed on BTM specimens, particular attention needs to be paid to dry animals, which are excluded from BTM tests and may later shed huge amount of bacteria during parturition and the postpartum period. Therefore, if epidemiological data and anamnesis indicates that *C. burnetii* infection may be present within a herd, additional laboratory tests of samples from dried animals should be carried out.

Large percentage of BTM samples postive in ELISA and real-time PCR results from high affinity of *C. burnetii* for mammary gland, where bacteria can persist for long time. As a consequence the pathogen stimulates an immune response and is commonly shed in milk. Unexpectedly, difference between seroprevalence on herd-level estimated by testing of serum and BTM was quite high. Bulk tank milk samples derived from all lactating cows in the herd, while sera samples were collected from represantative, but not total, number of animals in herd. It could be one of the reasons of the deviation, although this issue requires further analyses. Higher prevalence of bacterial DNA in BTM samples than seroprevalence in sera at herd level might also result from aforementioned cause. In addition, it is generally known that some of *C. burnetii* shedders remain seronegative, especially if they shed pathogen intermittently or sporadically [6,32].

The popularity of healthy, ecological lifestyle has been increasing in Poland over recent years. As a consequence, more consumers prefer to purchase minimally-processed food, including raw milk and artisanal products made from unpasteurized milk. Moreover, numerous people spend holidays on agrotourism farms, where they can interact with animals and consume their products. The alimentary route of *C. burnetii* transmission remains controversial, but direct contact with infected animals or inhalation of contaminated aerosols pose a high risk of infection.

The statistical analysis revealed 87% concordance of the results of ELISA and real-time PCR obtained for BTM specimens that confirmed the usefulness of serological test of BTM for screening purposes. Comparative analyses of results of ELISA test of sera and PCRs of BTM as well as ELISA for milk and for serum showed that differences were statistically significant only for some of the comparisons.

5. Conclusion

This study was carried out to provide an insight into current epidemiological situation of *C. burnetii* in Polish dairy cattle. Analyses of BTM and serum samples revealed that the presence of specific immunoglobulins in tested dairy cattle herds is common and correlated with shedding of *C. burnetii* and recorded in different regions of the country. These findings underscore the importance of surveillance, application of adequate biosecurity measures and increasing the awareness of Q fever, especially among high-risk groups in Poland.

CRediT authorship contribution statement

Monika Szymańska-Czerwińska: Conceptualization, Methodology, Validation, Formal analysis, Writing - original draft, Writing - review & editing, Visualization, Project administration, Funding acquisition. **Agnieszka Jodełko:** Investigation, Writing - original draft, Writing - review & editing, Visualization. **Krzysztof Niemczuk:** Conceptualization, Writing - review & editing, Visualization, Supervision.

Declaration of Competing Interest

All authors declare no competing interests.

Acknowledgments

This work was funded by the National Science Centre, Poland (Grant No. 2015/17/D/NZ7/00816). The funder of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report.

References

[1] A. Stein, N.A. Saunders, A.G. Taylor, D. Raoult, Phylogenic homogeneity of *Coxiella burnetii* strains as determinated by 16S ribosomal RNA sequencing, FEMS Microbiol. Lett. 113 (1993) 339–344, https://doi.org/10.1111/j.1574-6968.1993.tb06537.x.

[2] G.E. Davis, H.R. Cox, R.R. Parker, R.E. Dyer, A filter-passing infectious agent isolated from ticks. I. Isolation from *Dermacentor andersoni*, reactions in animals, and filtration experiments, Publ. Health Rep. 53 (1938) 2259–2267.

[3] E. Derrick, Q. Fever, A new fever entity: clinical features, diagnosis and laboratory investigation, Med. J. Aust. 21 (1937) 281–299.

[4] M. Maurin, D. Raoult, Q. fever, Clin. Microbiol. Rev. 12 (1999) 518–553.

[5] R. de Cremoux, E. Rousset, A. Touratier, G. Audusseau, P. Nicollet, D. Ribaud, V. David, M. Le Pape, Assessment of vaccination by a phase I *Coxiella burnetii*-inactivated vaccine in goat herds in clinical Q fever situation, FEMS Immunol. Med. Microbiol. 64 (2012) 104–106, https://doi.org/10.1111/j.1574-695X.2011.00893.x.

[6] R. Guatteo, F. Beaudeau, A. Joly, H. Seegers, *Coxiella burnetii* shedding by dairy cows, Vet. Res. 38 (2007) 849–860, https://doi.org/10.1051/vetres:2007038.

[7] P.J. Plummer, J.T. McClure, P. Menzies, P.S. Morley, R. Van den Brom, D.C. Van Metre, Management of *Coxiella burnetii* infection in livestock populations and the associated zoonotic risk: a consensus statement, J. Vet. Intern. Med. 32 (2018) 1481–1494, https://doi.org/10.1111/jvim.15229.

[8] S.R. Porter, G. Czaplicki, J. Mainil, R. Guattéo, C. Saegerman, Q fever: current state of knowledge and perspectives of research of a neglected zoonosis, Int. J. Microbiol. (2011) 248418–248422, https://doi.org/10.1155/2011/248418.

[9] A. Milazzo, R. Hall, P.A. Storm, R.J. Harris, W. Winslow, B.P. Marmion, Sexually transmitted Q fever, Clin. Infect. Dis. 33 (2001) 399–402.

[10] L.A. Petty, H.S. Te, K. Pursell, A case of Q fever after liver transplantation, Transpl. Infect. Dis. 19 (2017) 1–9, https://doi.org/10.1111/tid.12737.

[11] A.D. Loftis, R.A. Priestley, R.F. Massung, Detection of *Coxiella burnetii* in commercially available raw milk from the United States, Foodborne Pathog. Dis. 7 (2010) 1453–1456, https://doi.org/10.1089/fpd.2010.0579.

[12] K.A. Signs, M.G. Stobierski, T.N. Gandhi, Q fever cluster among raw milk drinkers in Michigan, 2011, Clin. Infect. Dis. 55 (2012) 1387–1389, https://doi.org/10.1093/cid/cis690.

[13] E. Dovolou, T. Tsiligianni, D. Vouzaras, G. Amiridis, Prevalence of *Coxiella burnetii* antibodies in bulk milk and blood serum and associations with reproductive indices in cow dairy herds of Central and Northern Greece, J. Hellenic Vet. Med. Soc. 62 (2017) 314–319, https://doi.org/10.12681/jhvms.14861.

[14] F. Ruiz-Fons, I. Astobiza, J.F. Barandika, A. Hurtado, R. Atxaerandio, R.A. Juste, A.L. García-Pérez, Seroepidemiological study of Q fever in domestic ruminants in semi-extensive grazing systems, BMC Vet. Res. 20 (6) (2010) 3, https://doi.org/10.1186/1746-6148-6-3.

[15] M.-G. Seo, I.-O. Ouh, S.-H. Lee, J.W. Kim, M.H. Rhee, O.-D. Kwon, T.-H. Kim, D. Kwak, Prevalence of *Coxiella burnetii* in cattle at South Korean national breeding stock farms, PLoS One 12 (5) (2017) e0177478, , https://doi.org/10.1371/journal.pone.0177478.

[16] M.P. Emery, E.N. Ostlund, M. Ait Ichou, J.D. Ballin, D. McFarling, L. McGonigle, *Coxiella burnetii* serology assays in goat abortion storm, J. Vet. Diagn. Invest. 26 (2014) 141–145, https://doi.org/10.1177/1040638713517233.

[17] M.W. Horigan, M.M. Bell, T.R. Pollard, A.R. Sayers, G.C. Pritchard, Q fever diagnosis in domestic ruminants: comparison between complement fixation and commercial enzyme-linked immunosorbent assays, J. Vet. Diagn. Invest. 23 (2011) 924–931, https://doi.org/10.1177/1040638711416971.

[18] K. Niemczuk, M. Szymańska-Czerwińska, K. Śmietanka, Ł. Bocian, Comparison of diagnostic potential of serological, molecular and cell culture methods for detection of Q fever in ruminants, Vet. Microbiol. 171 (2014) 147–152, https://doi.org/10.1016/j.vetmic.2014.03.015.

[19] A. Jodełko, K. Niemczuk, M. Szymańska-Czerwińska, Seroprevalence of *Coxiella burnetii* in Polish cattle herds, Bull. Vet. Inst. Pulawy 59 (2015) 479–482, https://doi.org/10.1515/bvip-2015-0071.

[20] J.S. Agerholm, *Coxiella burnetii* associated reproductive disorders in domestic animals-a critical review, Acta Vet. Scand. 55 (2013) 13, https://doi.org/10.1186/1751-0147-55-13.

[21] A. Pexara, N. Solomakos, A. Govaris, Q fever and prevalence of *Coxiella burnetii* in milk, Trends Food Sci. Technol. 71 (2018) 65–72, https://doi.org/10.1016/j.tifs.2017.11.004.

[22] OIE, Manual of Diagnostic Tests and Vaccines for Terrestrial Animals, Part 3 Section 3.1, Chapter 3.1.16, (2019).

[23] A. Softic, K. Asmare, E. Granquist, J. Godfroid, N. Fejzic, E. Skjerve, The serostatus of *Brucella* spp., *Chlamydia abortus*, *Coxiella burnetii* and *Neospora caninum* in cattle in three cantons in Bosnia and Herzegovina, BMC Vet. Res. 14 (2018) 40, https://doi.org/10.1186/s12917-018-1361-z.

[24] H. El-Mahallawy, P. Kelly, J. Zhang, Y. Yang, L. Wei, L. Tian, W. Fan, Z. Zhang, C. Wang, Serological and molecular evidence of *Coxiella burnetii* in samples from humans and animals in China, Ann. Agric. Environ. Med. 23 (2016) 87–91, https://doi.org/10.5604/12321966.1196859.

[25] C. McCaughey, L. Murray, J. Mckenna, F. Menzies, S. Mccullough, H. O'Neill, D. Wyatt, C. Cardwell, P. Coyle, *Coxiella burnetii* (Q fever) seroprevalence in cattle, Epidemiol. Infect. 138 (2010) 21–27, https://doi.org/10.1017/S0950268809002854.

[26] Statistics Poland. Executive summary: Cattle, in Statistical Information. Farm Animals in 2018, Warszawa, (2018), pp. 16–18.

[27] A. Ohlson, J. Malmsten, J. Frössling, G. Bölske, A. Aspán, A. Dalin, A. Lindberg, Surveys on *Coxiella burnetii* infections in Swedish cattle, sheep, goats and moose, Acta Vet. Scand. 56 (2014) 39, https://doi.org/10.1186/1751-0147-56-39.

[28] E.D. Ryan, K. Wrigley, A. Hallinan, G. McGrath, T.A. Clegg, Antibodies to *Coxiella burnetii* in Irish bulk tank milk samples, Vet. Rec. 182 (2018) 550, https://doi.org/10.1136/vr.104663.

[29] S. Anastácio, N. Carolino, K. Sidi-Boumedine, G. da Silva, Q fever dairy herd status determination based on serological and molecular analysis of bulk tank milk, Transbound. Emerg. Dis. 63 (2016) 293–300, https://doi.org/10.1111/tbed.12275.

[30] J. Agger, S. Paul, Increasing prevalence of *Coxiella burnetii* seropositive Danish dairy cattle herds, Acta Vet. Scand. 56 (2014) 46, https://doi.org/10.1186/s13028-014-0046-2.

[31] E. van Engelen, N. Schotten, B. Schimmer, J. Hautvast, G. van Schaik, Y. van Duijnhoven, Prevalence and risk factors for *Coxiella burnetii* (Q fever) in Dutch dairy cattle herds based on bulk tank milk testing, Prev. Vet. Med. 117 (2014) 103–109, https://doi.org/10.1016/j.prevetmed.2014.08.016.

[32] E. Rousset, M. Berri, B. Durand, P. Dufour, M. Prigent, T. Delcroix, A. Touratier, A. Rodolakis, *Coxiella burnetii* shedding routes and antibody response after outbreaks of Q fever-induced abortion in dairy goat herds, Appl. Environ. Microbiol. 75 (2009) 428–433, https://doi.org/10.1128/AEM.00690-08.