The updated prevalence of *Coxiella burnetii* in Central and Eastern European dairy herds – a short communication

Attila Dobos[1,2*], Mislav Kovacic[3], Goran Jakovljevic[4] and Marko Samardzija[5]

[1]*Large Animal Clinical Laboratory, University of Veterinary Sciences, Faculty of Veterinary Medicine, Brno, Czechia*
[2]*Ceva-Phylaxia Veterinary Biologicals Co. Ltd., Budapest, Hungary*
[3]*Department of Biology, University of Osijek, Osijek, Croatia*
[4]*Greenlab pet vet, Beograd, Serbia*
[5]*Faculty of Veterinary Medicine, University of Zagreb, Zagreb, Croatia*

ABSTRACT

This study aimed to extend our previous research, to collect more bulk tank milk samples from the Central and Eastern European region. Samples were collected between January 2020 and August 2023 from 126 dairy herds from four Central and Eastern European countries (Hungary, n=87; Czechia, n=24; Slovakia, n=10 and Croatia n=5). A positive correlation was found between herd size and the percentage of *C. burnetii* positive results (with PCR and/or ELISA tests) from bulk tank milk in dairy herds. On the basis of our results we can conclude that the growing number of farms managing a high number of dairy cows, with high stocking density, correlates with the increasing prevalence of *C. burnetii* in the region.

Keywords: dairy cattle; Central and Eastern Europe; Q fever; bulk tank milk

Introduction

The first description of the disease dates back to 1937, when it was observed in slaughterhouse workers in Australia, and named Q fever (query fever) because its etiology was unknown at the time. The causative agent, *Coxiella burnetii,* was identified later that year as an obligate intracellular Gram-negative bacterial pathogen. *C. burnetii* is ubiquitous and is able to infect and cause disease in both humans and animals. Research has revealed various manifestations of this disease and a wide host spectrum, including domestic animals, reptiles, ticks, birds, and even marine mammals. Bacteria are shed via birth products, urine, faeces and milk (ELDIN et al., 2017), contributing to its high zoonotic potential. *C. burnetii* infections in humans were often associated with disease outbreaks in domestic ruminants, which warrants monitoring of cattle, sheep, and goat populations as the main reservoirs of the disease and the most common source of human infections (MAURIN and RAULT, 1999). OIE guidelines recognize the combined use of serological and molecular methods as the most reliable way to diagnose Q fever – with ELISA and PCR being the preferred assays (OIE, 2018).

*Corresponding author:
Attila Dobos, Large Animal Clinical Laboratory, University of Veterinary Sciences, Faculty of Veterinary Medicine, Palackeho tr. 1946/1, Brno-61242, Czechia, Ceva-Phylaxia Veterinary Biologicals Co. Ltd., Szállás u. 5, Budapest H-1107, Hungary, e-mail: attila.dobos@ceva.com

Materials and methods

Our previous study was conducted to assess the prevalence of *Coxiella burnetii* in dairy herds in Central and Eastern European countries, on the basis of ELISA and PCR tests. In 2019, a total of 370 bulk tank milk samples were collected, originating from Croatia (n=13), Czechia (n=138), Hungary (n=126), Serbia (n=24), Slovakia (n=53) and Slovenia (n=16) (DOBOS at al., 2020).

We extended our research to collect more bulk tank milk samples from these countries. Samples were collected between January 2020 and August 2023 from 126 dairy herds from four Central and Eastern European countries (Hungary, n=87; Czechia, n=24; Slovakia, n=10 and Croatia n=5). Samples were also taken randomly from dairy herds of different sizes that were not vaccinated against Q fever. These were herds that were not investigated previously. No further samples were collected from Serbia or Slovenia.

Forty mL samples were taken from the bulk milk tanks. Lactosera were tested with commercial ELISA kits (ID Screen® Q Fever Indirect Multi-species, IDVet Inc., Grabels, France; IDEXX Q Fever Ab Test, IDEXX Europe B.V., Hoofddorp, the Netherlands) according to the manufacturer's instructions. A real-time PCR assay, specific to the IS1111 element, was used to detect the presence of *C. burnetii* in the milk samples. Spearman's rank correlation was applied to analyze the correlation between the percentage of all positive test results by the ELISA and/or PCR tests and herd size (ranked as follows: 1= herd size of 50-249 animals, 2= 250-499 animals, 3= 500-999 animals and 4= herd size of ≥1000 animals), using R software (R-CORE TEAM, 2013).

Results

One hundred twenty-three out of the 126 (97.91%) bulk tank milk samples showed positivity after testing with ELISA and/or PCR, and the IS1111 element of *C. burnetii* was detected in 74 out of the 126 samples (58.73%) by real-time polymerase chain reaction (RT-PCR). Detailed results of the ELISA tests and PCR assays of the 126 bulk tank milk samples are summarized in Table 1. According to our statistical analysis, we found a positive correlation between herd size and the percentage of *C. burnetii* positive results (Spearman's rank correlation, rho=0.736, P<0.001).

Discussion

C. burnetii specific ELISA revealed 100.00% positivity in all examined countries in herds that consisted of at least 250 milking cows, consistent with our previously published studies (DOBOS et al., 2020; DOBOS et al., 2022), but PCR positivity (58.73%) was higher than in our last results (44.05%). All PCR positive samples were also positive with ELISA. In the present study, the detection of *C. burnetii* specific antibodies showed higher positivity than the PCR assays in all countries, ranging between 91.6-100.0% (Croatia, Czechia, Hungary and Slovakia). DOBOS et al. (2020) reported lower positivity in bulk tank milk using ELISA in Europe, with the lowest results ranging between 25.0-37.9% for Greece, Ireland and Portugal, and the highest results between 45.5-78.6% for Belgium, Denmark, the Netherlands, Poland and Spain. The average herd size in the examined countries was 217 animals/herd in Slovakia, 138 in the Czechia and 76 in Hungary, making it the highest in Europe, with high cattle density also in the Slavonian-Baranja region of Croatia. These characteristics contribute to a higher risk of disease spread in the examined region compared to other regions of Europe (FAO, 2016; DOBOS, 2023).

A similar study was performed to determine the prevalence of *C. burnetii,* and the results indicated the widespread presence of the pathogen in dairy cattle in the region, with sheep and goats also contributing to the spread of the disease (DOBOS et al., 2021).

Conclusions

On the basis of our results we can conclude that the growing number of farms managing a high number of dairy cows, with high stocking density, correlates with the increasing prevalence of *C. burnetii* in the region. In our recent study, it was also

Table 1. Summary of *Coxiella burnetii* specific ELISA and PCR test results from bulk tank milk samples originating from Central and Eastern Europe

Country	Herd size[a]	Number of herds	ELISA[b]	PCR[c]	ELISA & PCR[d]	Positive results with ELISA and/or PCR tests[e]	
						Total number	Percentage
Croatia	250-499	2	0	0	2	2/2	100.00%
Croatia	500-999	3	0	0	3	3/3	100.00%
Croatia	Total CRO	5	0	0	5	5/5	100.00%
Czechia	50-249	3	1	0	0	1/3	33.33%
Czechia	250-499	10	5	0	5	10/10	100.00%
Czechia	500-999	9	2	0	7	7/7	100.00%
Czechia	≥1000	2	0	0	2	2/2	100.00%
Czechia	Total CZ	24	8	0	14	22/24	91.60%
Hungary	50-249	4	2	0	1	3/4	75.00%
Hungary	250-499	34	12	0	22	34/34	100.00%
Hungary	500-999	39	12	0	27	39/39	100.00%
Hungary	≥1000	10	2	0	8	10/10	100.00%
Hungary	Total HU	87	76	0	47	86/87	98.85%
Slovakia	250-499	3	1	0	2	3/3	100.00%
Slovakia	500-999	5	2	0	3	5/5	100.00%
Slovakia	≥1000	2	0	0	2	2/2	100.00%
Slovakia	Total SK	10	3	0	8	10/10	100.00%
Countries	Total	126	87	0	74	123/126	97.61%

[a]Ranges of number of animals in the examined herds are given according to country of origin. Abbreviations: CRO - Croatia, CZ - Czechia, HU – Hungary, SK – Slovakia
[b]Number of positive results using only ELISA tests
[c]Number of positive results using only PCR tests
[d]Number of results positive using both ELISA and PCR tests

demonstrated that a high *C. burnetii* seroprevalence among dairy farm workers correlated with a high prevalence of *C. burnetii* in dairy herds in Central and Eastern Europe (DOBOS and BALLA., 2021a). Moreover, our research found 100% seropositivity among dairy farm veterinarians, which was the highest of all percentages previously reported in international surveys (DOBOS and BALLA, 2021b).

Ethics statement

From an ethical perspective, the material collected and used as part of this study was outside the scope of Directive 2010/63. All methods were carried out in accordance with the relevant guidelines and regulations, and the manuscript adheres to ARRIVE guidelines.

Declaration of competing interest

The authors declared no potential conflicts of interest with respect to the research, authorship, or publication of this article.

References

FAO (2016): Food and Agriculture Organization of United nations. EU Dairy farms report 2013. European Commission. http://ec.europa.eu/agriculture/fadn/documents/dairy-report-2015_en.pdf

DOBOS, A., Z. KREIZINGER, Á. B. KOVÁCS, M. GYURANECZ (2020): Prevalence of *Coxiella burnetii* in Central and Eastern European dairy herds. Comp. Immunol. Microbiol. Infect. Dis. 72, 101489.
https://doi.org/10.1016/j.cimid.2020.101489

DOBOS, A., I. FODOR, G. KISS, M. GYURANECZ (2021): Serological survey of *Coxiella burnetii* infections in dairy cattle, sheep, goats and zoo animals in Hungary. Acta Vet. Hung. 69, 105-109.
https://doi.org/10.1556/004.2021.00016

DOBOS, A., E. BALLA (2021a): Industrial Dairy Cattle Farms in Hungary Source of *Coxiella burnetii* Infection in Humans. Vector Borne Zoonotic Dis. 21, 498-501.
https://doi.org/10.1089/vbz.2020.2749

DOBOS, A., E. BALLA (2021b): *Coxiella burnetii* infection rate among intensive dairy farm veterinarians. Magy. Allatorvosok 143, 11-16. (in Hungarian).

DOBOS, A., I. FODOR, T. TEKIN, D. ĐURIČIĆ, M. SAMARDZIJA (2022): Presence of *Coxiella burnetii* in dairy cattle and farms in the Czech Republic. Polish J. Vet. Sci. 25, 231-235.
https://doi.org/10.24425/pjvs.2022.141807

DOBOS, A. (2023): Presence of Coxiella burnetii as a causative agent of Q-fever in dairy cattle farms in Croatia. Zbornik radova znanstveno-stručnog skupa s međunarodnim sudjelovanjem - Veterinarski dani 2023, 26-29 October, Osijek, Croatia, p. 186 (in Croatian).

ELDIN, C., C. MÉLENOTTE, O. MEDIANNIKOV, E. GHIGO, M. MILLION, S. EDOUARD, J.-L. MEGE, M. MAURIN, D. RAOULT (2017): From Q fever to *Coxiella burnetii* infection: a paradigm change. Clin. Microbiol. Rev. 30, 115-190.
https://doi.org/10.1128/CMR.00045-16

MAURIN, M., D. RAOULT (1999): Q fever. Clin. Microbiol. Rev. 12, 518-553.
https://doi.org/10.1128/cmr.12.4.518

OIE (2018): World Organisation for Animal Health. Q fever. In: Manual of Diagnostic Tests and Vaccines for Terrestrial Animals. 8th ed., Office International des Epizooties, Vol. 1, Part 3, Section 3. Chapter 3.1.16.

R CORE TEAM (2013): A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna. Vol. 1. https://www.R-project.org

---

DOBOS, A., M. KOVACIC, G. JAKOVLJEVIĆ, M. SAMARDZIJA: Novije spoznaje o prevalenciji *Coxiella burnetii* u mliječnim stadima srednje i istočne Europe - kratko priopćenje: Vet. arhiv 94, 421-424, 2024.

SAŽETAK

Istraživanje je imalo za cilj proširiti naša prethodna istraživanja kako bismo prikupili više uzoraka mlijeka iz spremnika na području srednje i istočne Europe. Uzorci su prikupljeni između siječnja 2020. i kolovoza 2023. godine od 126 mliječnih krava iz četiri zemlje (Mađarska, n=87; Češka, n=24; Slovačka, n=10 i Hrvatska n=5). Primjenom PCR i/ili ELISA testova, u mlijeku iz spremnika istraženih mliječnih stada analizirana je prisutnost *C. burnetii*. Pronađena je pozitivna korelacija između veličine stada i postotka pozitivnih rezultata. Na temelju naših rezultata možemo zaključiti da je porast broja farmi koje drže veliki broj mliječnih krava, s povećanom gustoćom naseljenosti u objektima, u korelaciji s rastućom prevalencijom *C. burnetii* u regiji.

Ključne riječi: mliječne krave; centralna i istočna Europa; Q groznica; uzorci mlijeka iz spremnika

---