Summary of Ph.D. Thesis

# Prevalence of *Coxiella burnetii* in dairy cattle and farm workers and associated bovine reproductive disorders in the Central European region

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**Introduction**

Q fever is a zoonotic disease present worldwide*,* which was first described in 1937 in slaughterhouse workers in Australia. *Coxiella burnetii*, an obligate intracellular Gram-negative bacterium was described as the causative agent of the infectious disease associated with high fever and flu-like symptoms. Knowledge about *C. burnetii* and the disease it causes has expanded intensely since its first description, regarding both host spectrum and epidemiology. The bacteria have been isolated from a number of mammal species as well as reptiles and marine mammals. Bacteria are shed several ways, including via urine, faeces, milk and placentas. Infected animals often remain asymptomatic, but various reproductive disorders such as abortion, retained placenta, metritis or early loss of pregnancy can also be associated with the pathogen. Domestic ruminants are the main sources of human infections. The ﬁrst diagnosis and report of Q fever in cattle and sheep in Hungary took place in 1956 (Romváry et al., 1957). Human cases were first described in 1977, with further case reports between 1977 and 1980 in two cattle farms in Bács-Kiskun county, revealing epidemics with prolonged respiratory symptoms among the farm workers (EPINFO, 2014). Multiple publications have described abortions caused by *C. burnetii* in Hungary in the past few decades (Rády et al.,1985; Rády et al., 1987; Szeredi, 2004). The latest major outbreak, registered in 2013, originated from a sheep flock in Southern Hungary, with 70 laboratory-confirmed human cases reported (Gyuranecz et al., 2014). Pathogenicity and virulence of *C. burnetii* depends on the infected animal species, the route of infection, the *C. burnetii* strain, and the size of the inoculum. Since epidemiological and pathogenicity characteristics depend mainly upon the genetic characteristics of the *C. burnetii* strain involved, comparative genomics are an essential part of surveys and epidemiological investigations. Continuous monitoring of domestic ruminants for *C. burnetii* infection is important, as cattle, sheep and goats are the main sources of human infections.

**Aims of the study**

The aims of the study were:

1. To assess the prevalence of *C. burnetii* in dairy cattle herds of different sizes in six countries in Central and Eastern Europe by examining bulk tank milk samples with enzyme-linked immunosorbent assay (ELISA) and real-time polymerase chain reaction (PCR) tests.
2. To evaluate the prevalence of *C. burnetii* antibodies in different hosts (dairy cattle, sheep, goats, and zoo animals) by testing individual blood samples.
3. To determine the seroprevalence of *C. burnetii* in different occupational groups of farm workers (with focus on veterinarians) based on the presence of IgG antibodies to Phase I and Phase II antigens of *C. burnetii*.
4. To determine the role of C. *burnetii* in pregnancy loss in dairy cows between days 29 and 70 of gestation by ELISA and complement fixation test (CFT).
5. To reveal the importance of C*. burnetii* in the retention of fetal membranes (RFM) in dairy cattle by comparing the occurrence of *C. burnetii* in retained fetal membranes and normally separated placentas with PCR tests.
6. To perform genotyping of *C. burnetii* strains isolated from retained placentas of cattle using the multispacer sequence typing (MST) assay.

**Materials and Methods**

Bulk tank milk samples were collected from dairy herds of various sizes from 6 countries in the Central-Eastern European region (Croatia, Czech Republic, Hungary, Serbia, Slovakia and Slovenia). Individual blood samples were collected from cattle, sheep, goats and zoo ruminants from different regions in Hungary. Human blood samples were collected from veterinarians in 19 Hungarian and 5 Slovakian large dairy herds and farm workers in Hungary. 167 cotyledon samples (70 from normally separated and 90 from retained placentas) were collected from different regions in Hungary and Slovakia to perform genotype testing of the isolated *C. burnetii* strains. Various laboratory methods (ELISA, CFT, immunofluorescent assay /IFA/) were used to determine seroconversion to *C. burnetii*. Based on international recommendations, actual prevalence rates were determined based on ELISA results for both bulk tank milk samples and individual blood samples (OIE, 2018; Guatteo et al., 2011). ELISA positive serum samples were examined by CFT to differentiate acute infections from chronic cases based on titers (OIE, 2018). Human blood samples were tested for antibodies against Phase I and Phase II antigens of *C. burnetii* by usinga commercial indirect immunofluorescent assay kit (Focus Diagnostics, Cypress, CA, USA) according to the manufacturer’s instructions. We considered the dilution of 1:16 as the threshold for both types of IgG. For genomic testing the IS*1111* fragment of the transposase gene was amplified using a TaqMan type real-time PCR system (Loftis et al., 2006). Validation with a commercial positive control (Adiavet Cox; Aes Chemunex Inc., Cranbury, NJ) revealed a sensitivity of 0.1 TFE for the PCR method.

Genotyping of the PCR-positive samples was performed by MST, amplifying and sequencing ten selected spacer regions (Cox 2, 5, 6, 18, 20, 22, 37, 51, 56 and 57) of the *C. burnetii* genome (Glazunova et al. 2005).

**Results**

**Results of ELISA tests and real time PCR assays of bulk tank milk samples:**

We found that *C. burnetii* seroprevalence varies among the countries (Croatia 100.00%, Czech Republic 98.55%, Hungary 97.61%, Serbia 70.83%, Slovakia 90.56%, and Slovenia showing 62.50%). *C. burnetii* specific ELISA revealed 100.00% positivity in all examined countries in herds of 250 or more milking cows.

**Results of ELISA tests of blood samples in different host species:**

ELISA testing revealed different *C. burnetii* seroprevalences in the examined ruminant host species. Seroprevalence in cattle was 47.2%, in small ruminants 25.5% (23.5% in sheep and 31% in goats). *C. burnetii* antibodies were not found in zoo animals.

**IgG Phase I and Phase II *C. burnetii* antibodies in human blood samples:**

Serum samples of 70 farm workers were tested, with IgG Phase I antibodies detected in 53 samples (75.7%) and anti-*C. burnetii* IgG Phase II in 59 samples (84.3%). Both IgG Phase I and Phase II antibodies were detected in 8 out of the 8 veterinarians (100%) working on intensive dairy farms. 100% seropositivity was determined for inseminators and animal caretakers also, while seroconversion was lower in the case of parlour workers (47%) and herd managers (71.4%).

***C. burnetii* seropositivity rate in cows that lost pregnancy in early stage:**

A higher percentage of *C. burnetii* positivity was noted in cows that had lost their pregnancy (80.5%) compared to cows that remained pregnant. ELISA positivity was greatly increased in cows which had lost pregnancy after the first breeding (94.4%). ELISA positive cows were tested with CFT and we found higher seropositivity regarding anti-Phase I antibodies in cows that lost pregnancy (50%) compared to cows that remained pregnant (38.5%).

**Real-time PCR results of bovine cotyledons from retained and normally separated placentas:**

We compared *C. burnetii* prevalence in retained and normally separated placentas. Eighty (88.9%) out of the 90 cotyledons from retained placentas and 31 (40.3%) out of the 77 cotyledons from normally separated placentas tested positive by IS*1111* real-time PCR. 21.3% of the positive samples from retained placentas were highly loaded with *C. burnetii,* with cycle threshold (Ct) values below 27.08. The samples showing the strongest positivity (Ct 11.92–18.28) were genotyped by multispacer sequence typing (MST). Genotyping of the 5 cotyledon samples revealed a new (ST61) sequence type (ST), a type that had not been detected in Hungary or Slovakia previously.

**Discussion**

Testing bulk tank milk in dairy herds of Central and Eastern European countries revealed that the prevalence of *C. burnetii* was higher in larger herds with higher animal density. We found that the prevalence of Q fever varies among the countries, but our studies revealed 100% positivity with ELISA in all examined countries in herds with 250 or more milking cows.

We found different *C. burnetii* seroprevalence rates in the host animal species tested. The present study has proven that the causative agent of Q fever is most widely spread in cattle in Hungary, although both sheep and goat herds may pose a human health risk as well. Zoo ruminants probably do not play a role in spreading the disease.

It has been demonstrated that seropositivity to *C. burnetii* is higher in Hungarian dairy farm workers than those described in international seroepidemiological studies of various occupational groups in other countries. Veterinarians are the occupational group most exposed to infection, but inseminators and animal caretakers are at a similarly high risk of infection in industrial dairy farms. The high prevalence of *C. burnetii* in dairy farms underlines the importance of disease control, since this study found high seroprevalence rates in farm workers also, not just the animals.

Multiple studies have found various reproductive disorders such as infertility, premature birth, abortion and early loss of pregnancy to be associated with the pathogen. In our study we found significantly higher seroprevalence rates in animals that had lost their pregnancy at an early stage (80.5%) than the rate found in pregnant cows. In conclusion we can say that the presence of *C. burnetii* on dairy farms probably increases the risk of early pregnancy loss and contributes to the deterioration of fertility indices.

The results of the present study indicate that the prevalence of *C. burnetii* is significantly higher in retained fetal membranes than in normally separated placentas, and this may act as a possible risk factor for human infection mostly in workers and veterinarians treating cows with retained placentas. The higher prevalence of the pathogen in retained placentas might also indicate that they play a role in the pathogenesis of this disorder. Genotyping by MST revealed a new *C. burnetii* sequence type ST61, which had not been found previously in Hungary and Slovakia. The new sequence type (ST61) and the ST20 genotype previously found in Hungary are the primary causes of bovine coxiellosis in the region

**Overview of the new scientific results**

**Ad 1.** The prevalence of *C. burnetii* was 100% in Central and Eastern European countries in dairy herds of 250 or more milking cows. This is significantly higher compared to other regions of Europe, probably due to the concentration of the sector (large scale farms).

**Ad 2.** *C. burnetii* is mostly widespread in dairy cattle, but sheep and goats also appear to pose a major risk among the different host species in Hungary.

**Ad 3.** *C. burnetii* seropositivity was 100% in veterinarians, inseminators and animal caretakers working on dairy farms. These occupational groups are highly exposed to *C. burnetii* infection. These seroprevalence rates are the highest in global comparison and most likely due to the high prevalence of *C. burnetii* in the dairy herds.

**Ad 4.** *C. burnetii* seropositivity was higher in cows that had lost their pregnancy than cows which were pregnant. The presence of *C. burnetii* in dairy farms might contribute to an increased risk of early pregnancy loss.

**Ad 5.** The prevalence of *C. burnetii* in retained fetal membranes is significantly higher than in normally separated placentas. The pathogen might play a role in the pathogenesis of this disorder. This may also increase the risk of human infections in workers - mostly veterinarians – who are exposed to retained placentas.

**Ad 6.**. The new ST61 and the older ST20 genotypes found in retained placentas in Hungary and Slovakia are the primary causes of bovine coxiellosis in the region.

**Scientific publications**

**Publications on the topic of the thesis in peer reviewed journals**

* Dobos, A., Kreizinger, Z., Kovács, A., Gyuranecz, M.: **Prevalence of *Coxiella burnetii* in Central and Eastern European dairy herds*,***Comparative Immunology Microbiology & Infectious Disease, 72, 101489, 2020.
* Dobos, A., Gábor, G., Wehmann, E., Dénes, B., Póth-Szebenyi, B., Kovács, Á. B. and Gyuranecz, M. : **Serological screening for *Coxiella burnetii* in the context of early pregnancy loss in dairy cows,** Acta Veterinaria Hungarica, 68, 305–309, 2020.
* Dobos, A., Balla, E.: **Industrial dairy cattle farms in Hungary as a source of *Coxiella burnetii* infection in humans.** Vector Borne and Zoonotic Disease, 21, 498-501, 2021.
* Dobos,A., Fodor, I., Kiss, G., Gyuranecz, M.: **Serological survey of *Coxiella burnetii* infections in dairy cattle, sheep, goats and zoo animals in Hungary*,***Acta Veterinaria Hungarica, 69, 105-109 , 2021.
* Dobos,A., Gyuranecz, M., Albert, M.,: **Incidence rate of *Coxiella burnetii* in the retention of fetal membranes in dairy herds,** Magyar Állatorvosok Lapja, 142, 593–597, 2020.
* Dobos, A., Balla, E.: ***Coxiella burnetii* infection rate among intensive dairy farm veterinarians** Magyar Állatorvosok Lapja, 143, 11–16, 2021.
* Dobos, A., Fodor, I.: **Prevalence of *Coxiella burnetii* in bovine placentas in Hungary and Slovakia; detection of a novel sequence type,**Acta Veterinaria Hungarica, 69, 2021 online
* Dobos, A., Fodor,I., Tekin,T., Đuričić, D., Samardzija, M.,**: Presence of *Coxiella burnetii* in dairy cattle and farms in the Czech Republic**, Polish Journal of veterinary sciences [accepted] 2022

**Publications on other topics in peer-reviewed journals**

* Battay, M., Dobos, A., Illés Cs., Ózsvári L. : **Az afrikai sertéspestis gazdasági hatásai Észak-Kelet Pest és Nógrád megye vadgazdálkodására, különös tekintettel a klasszikus sertéspestissel kapcsolatos korábbi tapasztalatokra,** Magyar Allatorvosok Lapja, 141, 39-46 , 2019.
* Dobos, A., Fodor, I., Kreizinger, Z., Makrai, L., Dénes,B., Kiss,I., Đuričić,D., Kovačić, M., Szeredi.: **Infertility in dairy cows – Possible bacterial and viral causes,** Veterinarska stanica, 53, 2021
* Đuričić, D., Dobos, A. , Grbavac, J., Stiles, C. , Bacan, I., Vidas, Željko, Marković, F., Kočila, P., & Samardžija, M.: **Climate impacts on reproductive performance of Romanov sheep in the moderate climate,** Journal of Animal Behaviour and Biometeorology, 10, 2021.

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