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Original article

Presence of *Coxiella burnetii* in dairy cattle and farms in the Czech Republic

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Abstract

The aims of this study were to evaluate the prevalence of *Coxiella burnetii* on both herd and animal level based on ELISA and PCR tests. Antibodies to *C. burnetii* were detected in 22 out of the 24 bulk tank milk samples (91.6%) tested by ELISA and the IS*1111* element of *C. burnetii* was detected in 10 out of the 24 samples (41.6%) by real-time polymerase chain reaction (PCR). ELISA testing showed individual seropositivity in 67 out of the 165 cows (40.6%) examined in 24 dairy cattle farms in different parts of the Czech Republic. Our study revealed that the prevalence of *C. burnetii* has increased substantially in the Czech Republic over the past 30 years, and that the causative agent is a potential risk factor for some reproductive problems in dairy farms and a possible risk factor for human infection.

Key words: Q fever, surveillance, ELISA, real-time PCR, bulk tank milk

Introduction

Q fever is a zoonotic disease of worldwide distribution, caused by an obligate intracellular bacterium, *Coxiella burnetii* (Maurin and Raoult 1999). Although Q fever is an OIE-listed notifiable disease (OIE 2018), the interest in its causative agent and the disease itself is rather low in many countries. The main reservoirs of the bacterium are cattle, sheep and goats, but *C. burnetii* has been identified in many different host species including marine mammals, ticks, birds and reptiles (Eldin et al. 2017) Ticks are competent vectors but they may inefficiently transmit the disease in nature. The observed percentage of *C. burnetii*-positive ticks is lower than 5% (Duron et al. 2015). The highest concentration of the bacterium is contained in the placenta and other birth products, but *C. burnetii* is also shed in the urine, faeces, and milk of infected animals (Rodolakis et al. 2007). Recently, the Q fever paradigm has changed due to improved diagnosis in both humans and animals, as well as increased awareness of the disease (Eldin et al. 2017). Furthermore, a large out-

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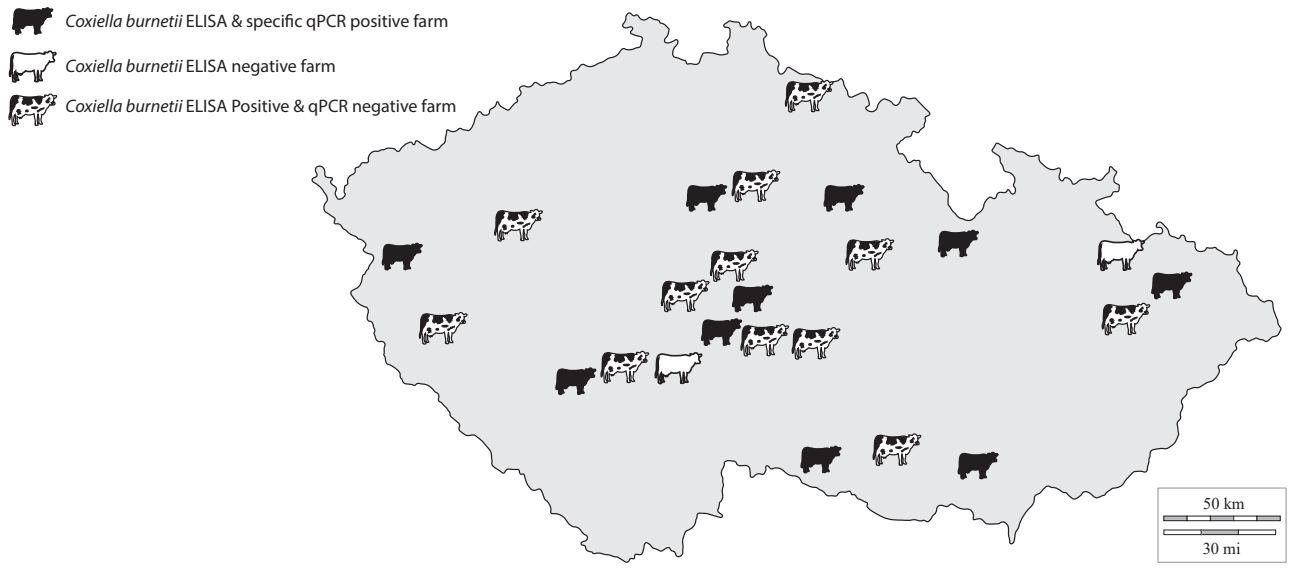

Fig. 1. Geographical distribution of the dairy farms positive by *Coxiella burnetii* ELISA and PCR in the Czech Republic.

break in the Netherlands, where thousands of people were hospitalised with severe clinical signs of Q fever and a huge number of animals had to be culled to control the disease, has shown that this epidemic could become a major public health problem (van der Hoek et al. 2012). Nowadays, there is also increased awareness of bovine coxiellosis as an economically important disease (Dobos et al. 2020a). Although infected animals often do not show clinical signs of disease, many reproductive disorders have been associated with the presence of the pathogenic bacteria (Agerholm 2013). The economic impact of Q fever in industrial dairy cattle farms can be attributed to impaired reproductive performance due to the abortion, premature delivery, stillbirth and weak offspring (APSW) complex, early pregnancy loss, metritis and retained placenta (Enserink 2010, Agerholm 2013, Dobos et al. 2020a). The aims of this study were to evaluate the prevalence of *C. burnetii* on both herd and animal level by ELISA and PCR tests and to reveal the possible sources of human infection in the Czech Republic.

Materials and Methods

Bulk tank milk and individual cow blood samples were collected between November 2019 and December 2020 from 24 dairy cattle farms in different geographic regions of the Czech Republic (Fig. 1). Blood samples were collected from 165 cows from the same farms where the bulk tank milk was sampled. Dairy farms were included in the survey based on the following criteria: farm size above 250 milking cows, use of regularly updated farm records, and willingness to provide data to the authors. Participation in the study was voluntary, and we encouraged veterinarians and herd managers to sample animals with suspected coxiellosis because of the APSW complex, early pregnancy loss, metritis or repeat breeding syndrome. Forty-ml samples were collected from the bulk tank milk, and milk sera were further 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 manufacturers' instructions. Somatic cells from milk samples were concentrated using low-speed centrifugation (3000×g at 4°C for 20 min), then 1-mL cell pellets were further centrifuged at 12,000×g at 4°C for 10 min. DNA extraction from 200 µL of the obtained cell pellets was performed using the Qiagen DNA Mini kit (Qiagen Inc., Valencia, CA) according to the manufacturer's instructions. Real-time PCR assay specific for the IS*1111* element was used to detect the presence of *C. burnetii* in the milk samples (Loftis et al. 2006). All 165 blood samples were tested with commercial enzyme-linked immunosorbent assay (ELISA) kits (ID Screen® Q Fever Indirect Multispecies IDVet Inc., Grabels, France) used according to the manufacturer's instructions. The percentage of ELISA positivity among the blood sampled animals on bulk tank milk PCR positive vs. negative farms was compared using Wilcoxon rank sum test in R version 4.0.2. (R Core Team, 2020). The level of significance was set to 0.05.

Results

Test results of the bulk tank milk (ELISA, PCR) and blood samples (ELISA) are summarised in Table 1. Antibodies to *C. burnetii* were detected in 22 out of the

Table 1. *Coxiella burnetii* seropositivity of bulk tank milk samples by PCR and ELISA and seropositivity of blood samples from dairy cattle by ELISA.

	Bulk Tank Milk Sample		Blood Sample	
	PCR positivity	ELISA positivity	Tested animals	ELISA positive
FARM 1	negative	positive	5	1(20%)
FARM 2	positive	positive	5	4(80%)
FARM 3	positive	positive	7	4(57.1%)
FARM 4	negative	positive	5	1(20%)
FARM 5	negative	positive	10	1(10%)
FARM 6	positive	positive	12	9(75%)
FARM 7	positive	positive	10	7(70%)
FARM 8	negative	negative	5	0(0%)
FARM 9	negative	positive	5	1(20%)
FARM 10	negative	positive	5	1(20%)
FARM 11	negative	positive	5	1(20%)
FARM 12	positive	positive	10	2(20%)
FARM 13	negative	positive	5	2(40%)
FARM 14	positive	positive	8	3(37.5%)
FARM 15	positive	positive	10	6(60%)
FARM 16	negative	negative	5	0(0%)
FARM 17	negative	positive	8	1(12.5%)
FARM 18	negative	positive	2	1(50%)
FARM 19	positive	positive	9	5(55.5%)
FARM 20	negative	positive	9	4(44.4%)
FARM 21	positive	positive	10	3(30%)
FARM 22	positive	positive	9	6(66.6%)
FARM 23	negative	positive	2	1(50%)
FARM 24	negative	positive	4	3(75%)
TOTAL	10(41.6%)	22(91.6%)	165	67(40.6%)

24 bulk tank milk samples (91.6%) tested by ELISA and the IS*1111* element of *C. burnetii* was detected in 10 out of 24 samples (41.6%) by real-time polymerase chain reaction (PCR). ELISA testing showed individual seropositivity in 67 out of the 165 cows (40.6%) examined. The seropositivity rate showed wide farm-to-farm variation between 10% and 80%. On average, 27.3% of the sampled animals was seropositive (median: 20.0%, interquartile range [IQR]: 14.4-43.3%) on the bulk tank milk PCR negative farms, compared to 55.2% (median: 58.6%, IQR: 42.0-69.2%) on the PCR positive farms. The percentage of ELISA positive blood samples was significantly higher on bulk tank milk PCR positive farms (p=0.006).

Discussion

Information about Q fever is limited in the Czech Republic, where the disease was first reported in 1953 (Patocka and Kubelka 1953). Some studies were published about this topic in the early 1990s. Herd-level seroprevalence of *C. burnetii* was reported from Northern Moravia by Literák in 1997 (Literak and Kroupa 1998). That study found a seroprevalence rate between 4% and 19% in 14 different herds by the complement fixation test (CFT). A *C. burnetii* Nine Mile Phase II strain was used for the serological tests (Literák and Kroupa 1998). Another study found 14.1% and 15.2% seropositivity rates by CFT in cows in 1991

and 1992, respectively (Literák and Calvo Rodríguez 1994). Two dairy farms were tested in the Karlovy Vary area between 1987 and 1989 in different seasons. The *C. burnetii* seroprevalence rates obtained by CFT ranged from 0.3 to 4.3% and from 0.3 to 10.6% in the two years, respectively. The highest seroprevalence rates were detected in July 1987 on both farms (Literák 1990). Two sheep flocks were tested in that region at the same time, but no antibodies to *C. burnetii* were detected. A serological survey conducted in game animals found 29% prevalence of *C. burnetii* antibodies among red and fallow deer, and some mouflons were also seropositive (Hubálek et al. 1993). At that time CFT was the only diagnostic method available for the detection of antibodies to *C. burnetii*. According to the Official Veterinary Report of the Czech Republic (2018), many ELISA-positive cases were found by testing blood samples from aborted cows between 2011 and 2018. In that period, 3,886 and 4,882 blood samples were tested by ELISA and CFT, respectively. In 2018, the ELISA test detected seropositivity in 1,110 out of 3,886 blood samples (28.5%), while the CFT demonstrated positivity in only 437 out of 3,886 samples (11.2%) (Statni Veterinarni Sprava 2018). Recent large-scale studies have found 98.55% prevalence of *C. burnetii* in Czech dairy farms based on ELISA and PCR tests of bulk tank milk samples. This study found that the prevalence of *C. burnetii* infection is higher in dairy farms of the Central and Eastern European countries including the Czech Republic than in the Western European countries, due to the growing number of animals kept in large industrial dairies and the shift of farm structure towards concentration (Dobos et al. 2020b). Some studies have reported that the CFT has lower sensitivity compared to ELISA; however, at present there is no clear evidence as to whether the lower seropositivity can be attributed to the different diagnostic methods or the seroprevalence has increased dramatically over the past twenty or thirty years in the Czech Republic (Niemczuk et al. 2011, Szymańska-Czerwińska et al. 2013). Serological surveys using the ELISA method are suitable for evaluating the prevalence of *C. burnetii* in herds (OIE 2018). Between 2014 and 2018 just one or two confirmed human Q fever cases were reported in the Czech Republic (ECDC 2019). The disease is probably underdiagnosed and underreported in both humans and animals. The biggest epidemic of Q fever was identified among Czech soldiers who served in the UN Protection Force in Bosnia and Herzegovina in 1997. Fourteen cases of febrile illness were diagnosed and further twelve subclinical infections were confirmed (Splino et al. 2003).

Rehácek summarised the epidemiology and significance of Q fever in Czechoslovakia in a review article. Between 1952 and 1987 the predominant sources of these epidemics in the country were cattle. However, some human Q fever outbreaks were noted in factories processing imported cotton, and they were linked also to small ruminant and livestock transport (Rehácek 1987).

Many international studies reported that dairy farm veterinarians are the occupational group most exposed to *C. burnetii* infection, although other farm workers are at a similarly high risk of infection in industrial dairy farms (Schimmer et al. 2014, Ghaoui et al. 2019, Dobos et al. 2021). As a higher seroprevalence of *Coxiella* could be linked to some reproductive performance problems in dairy cows, all relevant reproductive parameters need to be monitored in infected dairy farms (Lopez-Gatius et al. 2012).

In conclusion, the present study has demonstrated the importance of Q fever, which is widespread in dairy cattle and farms in the Czech Republic. The 91.6% herd-level seropositivity and 40.6% animal-level seropositivity for *C. burnetii* antibodies in cattle may pose a major risk as a source of human infection. More focus is needed on the highly infected herds where seroprevalence on the level of individual animals exceeds 50%. As the prevalence of *C. burnetii* has increased substantially in the Czech Republic over the past 30 years, the causative agent is a potential risk factor for some reproductive problems in dairy farms. Further studies are needed to determine the virulence and pathomechanism of *C. burnetii* in cattle.

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