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SHORT COMMUNICATION

Serological survey of *Coxiella burnetii* infections in dairy cattle, sheep, goats and zoo animals in Hungary – Short communication

ATTILA DOBOS[1]*, ISTVÁN FODOR[1], GERDA KISS[2] and MIKLÓS GYURANECZ[3]

[1] CEVA-Phylaxia Veterinary Biologicals Co. Ltd., Szállás u. 5, Budapest H-1107, Hungary

[2] Department of Animal Hygiene, Herd Health and Mobile Clinic, University of Veterinary Medicine, Budapest, Hungary

[3] Institute for Veterinary Medical Research, Centre for Agricultural Research, Budapest, Hungary

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ABSTRACT

Q fever is a disease of high zoonotic potential, but interest in its causative agent is rather low although it causes some public health problems in Hungary. The prevalence of Q fever is highly variable by country. The main reservoirs of the disease are the same domestic ruminant species everywhere, but the epidemiological profile depends on the features of the specific reservoir. The aim of this large-scale study was to demonstrate the importance of Q fever in different species as a possible source for human infection in most regions of Hungary. A total of 851 serum samples from 44 dairy farms, 16 sheep flocks, 4 goat farms and 3 zoos located in different parts of Hungary were tested. The presence of antibodies to *Coxiella burnetii* was surveyed in dairy cattle (n = 547), goats (n = 71), sheep (n = 200) and zoo animals (n = 33). The animal species tested in Hungary showed different seroprevalence values of *C. burnetii* infection. Seropositivity by the enzyme-linked immunosorbent assay was found in 258 out of 547 (47.2%) cows and in 69 out of 271 (25.5%) small ruminants, among them in 47 out of 200 (23.5%) sheep and in 22 out of 71 (31.0%) goats. Antibodies to *C. burnetii* were not detected in zoo animals. Seropositivity was demonstrated in 44 out of 44 (100%) dairy cattle farms, with at least one serum sample found to be positive on each farm. The seropositivity rate of small ruminant farms was 55.0% (11 positive out of 20 tested), with 9 out of 16 (56.3%) sheep flocks and 2 out of 4 (50.0%) goat herds showing seropositivity.

KEYWORDS

Q fever, *Coxiella burnetii*, dairy cattle, sheep, goat, zoo animals

Q fever is a zoonosis of worldwide occurrence and an OIE-listed disease (OIE, 2018), caused by *Coxiella burnetii*. The agent is a strictly intracellular, Gram-negative bacterium, which has two cell variants. The large-cell variant (LCV) is sensitive to environmental stress. The small-cell variant (SCV) characterised by high environmental stability can remain infectious in the extracellular environment for more than a year in highly resistant spore-like forms (McCaul and Williams, 1981; Howe and Mallavia, 2000). The agent has a broad reservoir range including many domestic and wild mammals, but the main reservoirs are cattle, sheep and goats (Maurin and Rault, 1999). Many seroepidemiological studies have been conducted in these three species, and some authors have also reported *C. burnetii* infection in zoo and wild animals (Clemente et al., 2008; Porter et al., 2011). Cattle, sheep and goats are the main sources of human infections: *C. burnetii* is mainly shed by infected domestic ruminants via birth products, vaginal secretions, faeces, and milk (Eldin et al., 2017), but dust particles contaminated with *C. burnetii* may also remain infectious for long periods after shedding (Joulié et al., 2015). Q fever outbreaks in humans have been generally associated with small ruminants (Tissot-Dupont et al., 1999; Van den Brom et al., 2013), but there are several reports of sporadic human disease cases closely linked to cattle (Dobos and Balla, 2021).

*Corresponding author. Tel.: +36 30 541 4483.
E-mail: attila.dobos@ceva.com

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Serological surveys are suitable for evaluating the prevalence of *C. burnetii* in herds or flocks or other groups of animals (OIE, 2018), although some authors have noted that infected animals may be found seronegative while shedding the bacteria (Rousset et al., 2009; Roest et al., 2012). However, Guatteo et al. (2007) established that persistent shedder cows were mostly highly seropositive. The aims of this study were to evaluate the prevalence of *C. burnetii* antibodies in different host species and reveal the possible sources of human infection in Hungary.

Blood samples were collected between May 2019 and December 2020 from two large statistical geographic regions of Hungary (Transdanubia, Great Plain and North) (Fig. 1). A total of 851 serum samples were tested from 44 dairy farms, 16 sheep flocks, four goat farms and three zoos. Samples from zoo animals were also collected in the Central region but not from other species as that region is industrial. Herds and flocks were included in the study based on the following criteria: farm size above 350 animals, use of regularly updated farm records, and willingness to provide data to the authors. Participation in the study was voluntary and we encouraged farmers and veterinarians to sample animals with suspected Q fever because of infertility or a previous diagnosis of abortion, premature delivery or stillbirth. There were no special inclusion criteria for zoo animals, and the objective was to include as many ungulate species as possible. Antibodies to *C. burnetii* were surveyed in dairy cattle (n = 547), goats (n = 71), sheep (n = 200) and zoo animals (n = 33), among them different wild ungulate species including camels, alpacas, bison, Cameroon goats, fallow deer, giraffes, antelopes, reindeer, and buffaloes. The 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. Cattle, goat and sheep farms were considered positive when at least one animal tested ELISA positive. The occurrence of seropositivity on animal level was compared among cattle, small ruminants (i.e. sheep and goats grouped together), and zoo animals using Fisher's exact test. P values were corrected for multiple comparisons using False Discovery Rate correction. Furthermore, the odds of seropositivity on animal level were modelled, taking the geographical region into account, in those groups of animals where at least one positive animal was found. For this purpose, a logistic mixed model was built with seropositivity as a binary dependent variable, animal type and geographic region as fixed factors, and farm as random effect, using the glmmTMB package (Brooks et al., 2017). Statistical analysis was performed in R 4.0.3. (R Core Team, 2020).

The test results obtained for the different animal groups and their geographical distribution are summarised in Table 1. ELISA testing showed individual seropositivity in 258 out of the 547 (47.2%) cows examined and in 69 out of the 271 (25.5%) small ruminants tested, among them in 47 out of 200 sheep (23.5%) and in 22 out of 71 goats (31.0%). Antibodies to *C. burnetii* were not found in zoo animals. Cattle were more likely to be seropositive than small ruminants ($P < 0.0001$) and zoo animals ($P < 0.0001$), as were small ruminants compared to zoo animals ($P = 0.0002$). After adjustment for geographical region, cattle were 4.32 times more likely (95% confidence interval of odds ratio: 2.13–8.75, $P < 0.0001$) to be seropositive than small ruminants. No significant difference in animal-level seropositivity was found between regions ($P = 0.697$). Seropositivity was demonstrated in 44 out of 44 (100%) dairy cattle farms, with at least one serum sample found to be positive on each farm. The seropositivity rate of small ruminant farms was 55.0%

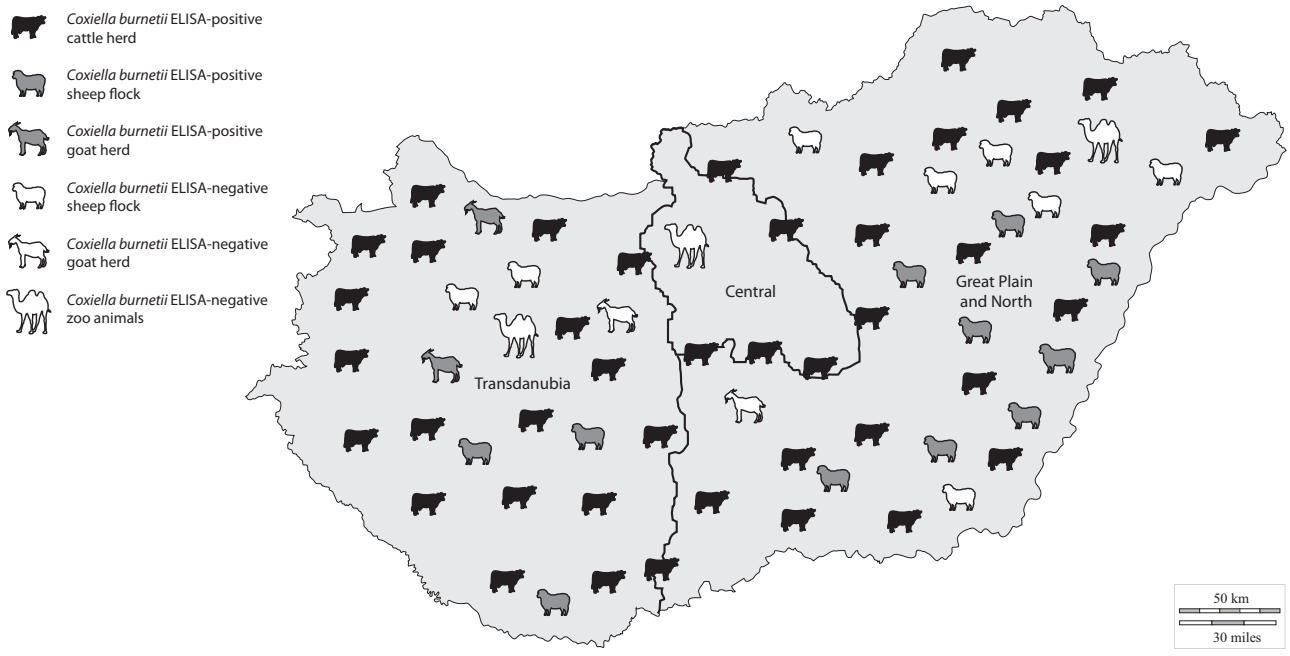

Fig. 1. Geographical distribution of the dairy cattle herds, sheep flocks, goat herds and zoos surveyed in Hungary

Table 1. Seropositivity to *Coxiella burnetii* in dairy cattle and small ruminants in Hungary

Statistical Large Region	Planning and Statistical Region	Tested cattle herds	Positive herds, %	Tested animals	Seropositive animals, %
Transdanubia	Western Transdanubia	6	6 (100%)	88	41 (46.6%)
	Central Transdanubia	7	7 (100%)	97	46 (47.4%)
	Southern Transdanubia	6	6 (100%)	76	38 (50%)
Great Plain and North	Northern Hungary	7	7 (100%)	80	30 (37.5%)
	Northern Great Plain	9	9 (100%)	107	56 (52.3%)
	Southern Great Plain	9	9 (100%)	99	47 (47.5%)
Total dairy cattle		44	44 (100%)	547	258 (47.2%)
Statistical Large Region	–	Tested sheep flocks and goat herds	Positive flocks/ herds, %	Tested animals	Seropositive animals, %
Transdanubia	–	8	4 (50%)	106	33 (31.1%)
Great Plain and North	–	12	7 (58.3%)	165	36 (21.8%)
Total small ruminants	–	20	11 (55%)	271	69 (25.5%)

(11 positive out of 20 tested), with 9 out of 16 (56.3%) sheep flocks and 2 out of 4 (50.0%) goat herds showing seropositivity. There are several similar surveys from many countries. This research found different *C. burnetii* infection rates in the different animal species tested. Most seroepidemiological studies indicate that the seroprevalence of antibodies to *C. burnetii* is higher in cattle than it was 20–30 years ago (Maurin and Raoult, 1999). The present study found 47.2% seropositivity in cattle, which is higher than that reported previously (38%) in Hungary (Gyuranecz et al., 2012). A recent study, which found 52% *C. burnetii* seropositivity, only focused on early pregnancy loss in three Hungarian dairy farms, and it was not as large-scale and representative as the present research (Dobos et al., 2020). The seroprevalence found by the present study in cattle is much higher than the European average (20%) (Guatteo et al., 2011). Cattle usually shed the bacteria without showing any clinical signs (Guatteo et al., 2007). According to a recent survey, seroprevalence among sheep in Hungary was 6% by ELISA (Gyuranecz et al., 2012). The present study found 23.5% seropositivity in sheep, which is also higher (15%) than the European average (Guatteo et al., 2011). However, *C. burnetii* seropositivity on individual animal level in sheep shows huge differences among countries. Animal-level seroprevalence was 1.8% for sheep in Switzerland (Magouras et al., 2017) and 16.3% in Italy (Rizzo et al., 2016). Sheep-level seroprevalence was found to be 14.7% in Canada, and it was higher in dairy sheep (24.3%) than in meat sheep (10.2%) (Meadows et al., 2015). Hungary has a relatively small national goat population (63,000 goats; https://www.ksh.hu/docs/hun/agrar/), which is usually kept in herds of 1–50 animals per farm. No previous serological survey on *C. burnetii* infection was available on Hungarian goat farms. Only a single caprine *C. burnetii* abortion case was diagnosed and reported in 2006 (Szeredi et al., 2006). In this study, the four biggest Hungarian goat farms (herd size: 300–500 animals) were tested and found to have 31.0% seropositivity by ELISA. There is a correlation between the incidence of Q fever and goat density. In The Netherlands there was a 75-fold increase in the goat population between 1985 and 2009, and the country faced one of the largest Q fever outbreaks in the world (Eldin et al., 2017). According to a large-scale study conducted in The Netherlands in 2008, 21.4% of the goats were seropositive for antibodies to *C. burnetii*, while the farm prevalence was 43.1% (Schimmer et al., 2011). However, wildlife can also constitute a reservoir and *C. burnetii* infection was confirmed in some zoos (Kruse et al., 2004; Clemente et al., 2008). We could not find seropositive animals among different species at the three biggest zoos in Hungary. In Africa, some animal species such as camels are significant reservoirs of the disease. Schelling et al. (2003) reported 80% *C. burnetii* seropositivity among camels in Chad, Bellabidi et al. (2020) found 75.5% seroprevalence of *C. burnetii* antibodies in Algeria, but *C. burnetii*-specific antibodies were detected in 40.7% of camels in Egypt as well (Klemmer et al., 2018). The first diagnosis and report of Q fever in Hungary in cattle and sheep took place in 1956 (Romváry et al., 1957). Two large outbreaks were recorded in dairy cattle farms with several human cases in 1977 (EPINFO, 2014). The latest major outbreak, registered in 2013, originated from a sheep flock in Southern Hungary, where 70 laboratory-confirmed human cases were reported (Gyuranecz et al., 2014). Seropositivity to *C. burnetii* was found to be 44.6% in this affected flock by ELISA (EPINFO, 2014). A recent study has found 100% seropositivity among dairy farm veterinarians, which is the highest of all figures previously reported by international surveys (Dobos and Balla, 2021).

In conclusion, the present study has demonstrated the importance of Q fever, which is widespread in dairy cattle, but sheep and goats also appear to pose a major risk as the sources of human infection. Preventive veterinary and standard hygiene measures are a key point in the control of Q fever. Vaccination is an available option to decrease the spread of infection, and it is essential according to the recommendations of the OIE Manual of Diagnostic Tests and

Vaccines for Terrestrial Animals (OIE, 2018). It is highly recommended to vaccinate cows, which are the main reservoir of the disease in Hungary. Proper manure management is also of key importance to avoid spreading of the bacteria from infected farms to the environment.

Declaration of competing interests: The first two authors work for a company which is the marketing authorisation holder of a vaccine against the bacterium studied.

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