Occurence of Coxiella burnetii in bulk tank milk from northwestern Italy

SHORT COMMUNICATION

Occurrence of *Coxiella burnetii* in bulk tank milk from northwestern Italy

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Coxiella burnetii, the aetiologic agent of Q fever (Query fever), is an intracellular Gram-negative bacterium belonging to the family of Coxiellaceae (Drancourt and Raoult 2005). Infections by *C burnetii* involve a wide range of susceptible hosts, ranging from mammals to arthropods (Angelakis and Raoult 2010). Abortion, stillbirth and non-viable neonates are the main clinical signs of infection in sheep and goats, while metritis, infertility and, rarely abortion, are typical for cattle (Arricau-Bouvery and Rodolakis 2005). *C burnetii* can produce spore-like forms, which are easily spread by wind and are able to survive for several months in the environment; for this reason the inhalation of contaminated aerosol and/or dust is considered the primary route of infection (Arricau-Bouvery and Rodolakis 2005). The consumption of contaminated raw milk does not seem to represent an efficient route of disease transmission (Rodolakis and others 2007), however, bulk tank milk (BTM) is an important specimen for epidemiological survey on dairy herds. In fact, even if *C burnetii* can be excreted in milk by infected animals continuously or intermittently (Guatteo and others 2007), milk is the most frequent way of shedding in asymptomatic cows (Rodolakis and others 2007). Many investigations have been performed among ruminant farms in European countries (Paiba and others 1999, Agger and others 2010, McCaughey and others 2010, Ruiz-Fons and others 2010, Muskens and others 2011, van den Brom and other 2012), also in Italy (Martini and others 1994, Masala and others 2004, Cabassi and others 2006, Parisi and others 2006, Natale and others 2009, Perugini and others 2009). Most of the above-mentioned Italian studies have focused on abortion as the major clinical problem in the livestock, while no data are available about the spread of *C burnetii* infection in dairy herds. The aim of this study was to determine the occurrence of *C burnetii* in a large number of dairy herds through the sampling of BTM in one of the Italian areas with the highest density of dairy farms A total of 780 BTM samples (one sample for each herd) were collected between 2007 and 2011 within different sanitary programmes from different provinces of Emilia-Romagna, Piedmont and Lombardy regions. Four to 10 months after the first analysis, a second round of BTM samples were collected from 287 randomly selected farms in three provinces of Lombardy region independently from their first test results (Table 2). All specimens were transported to the laboratories at 4°C, and stored at −24 ± 6°C until analysis. For PCR analysis, 1 ml of milk was centrifuged at 13,000 g for 20 minutes at 4°C. Cream and milk layers were removed and pellets were washed twice with sterile water. DNA was extracted and purified with DNeasy Blood and Tissue mini kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. A PCR assay, targeting a 687 bp segment of the transposon-like genomic region, was performed as described by Berri and others (2000). Comprehensively, 337 BTM samples, over the 780 analysed at the first sampling, were found positive for *C burnetii*. The corresponding overall occurrence was 43 per cent (Table 1). A second BTM sampling was collected from 287 herds in three provinces of Lombardy (Table 2). This second sampling, considering even the results of the first sampling, revealed an overall occurrence of 60 per cent (173 positive and 114 negative) showing a consistent number of new positive herds. Particularly, the occurrence reached 38 per cent in the Pavia's province, 80 per cent in Cremona's province, and 78 per cent in Lodi's province (Table 2). To the best of our knowledge, this is the first wide investigation about the occurrence of *C burnetii* infection in Italian dairy cattle herds. Our data showed a high occurrence of this bacterium in dairy cattle of the investigated area. Previous studies conducted in Belgium on dairy cattle herds reported 30 per cent of BTM-positive samples for *C burnetii* (Czaplicki and others 2012), while an epidemiological survey on BTM in the USA reported a prevalence of 94.3 per cent (Kim and others 2005). More recently, a BTM survey carried out in the UK showed a prevalence of 69.7 per cent (Valergakis and others 2012). Our study confirmed the usefulness of

PCR analysis of BTM for the detection of *C burnetii* and confirmed that a single BTM PCR-negative result is not sufficient to define a herd *C burnetii*-free status. In fact, the group of lactating cows present in farms varies during the year, and sporadic and persistent shedders can coexist in infected herds (Guatteo and others 2007, Rodolakis and others 2007). In this context, our data showed a high percentage of new infected herds (20 per cent) revealed after the second sampling, underlying the importance of more than one round for the definition of *C burnetii* status in each herd (Guatteo and others 2007, Rodolakis and others 2007). After a recent Q fever epidemic in The Netherlands involving sheep, goats and human beings, PCR-positive bulk milk is now one of defined criteria for veterinary notification of Q fever in The Netherlands (Coulombier 2010, Van der Hoek and others 2010). Risk evaluation of *C burnetii* infection for farmers, veterinarians and all people who have contact with infected dairy cattle herds, even if occasionally, should be taken into consideration (Monno and others 2009, Coulombier 2010). Despite the high occurrence in dairy herds detected in this study, only few cases of Q fever have been reported in human beings in the investigated area. Nevertheless, since notification to the Health Authorities is not mandatory for Q fever in Italy, this data could be underestimated. On the other hand, most of the outbreaks caused by *C burnetii* have been associated with isolates from goat or sheep and rarely with isolates from cattle (Coulombier and others 2010, Van der Hoek and others 2010). Meanwhile, preventive measures should be enhanced in order to contain spread of infection, in particular, regular cleaning and disinfection of calving area, manure management, prompt destruction of fetus or birth products and frequent bedding decontamination. Finally, the recent development of a new phase 1 vaccine for ruminants able to reduce clinical signs and shedding of *C burnetii* could represent an additional tool for the control of this zoonosis (Guatteo and others 2008).

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Table 1: Data referred to the first sampling of the study

Region	Province	Nr. of farms investigated	Nr. positive samples	% positive samples (I.C. 95%)
Lombardy	Pavia	135	32	24 (17-32)
	Cremona	152	83	55 (46-63)
	Mantova	113	46	41 (31-50)
	Lodi	101	56	55 (45-65)
Emilia Romagna	Piacenza	126	44	35 (27-44)
Piedmont	Cuneo	28	18	64 (44-81)
	Novara	13	2	15 (02-45)
	Torino	112	56	50 (40-59)
tot		780	337	43 (40-47)

Samples derived from different Italian regions

Table 2 : Data referred to the second sampling of the study

Region	Province	nr. of farms investigated	nr. positive at first sampling	nr. of new positive at second sampling	Total of positive samples
Lombardy	Pavia	130	29 (22,3%)	20 (15%)	49 (38%)
	Cremona	56	30 (53,6%)	15 (27%)	45 (80%)
	Lodi	101	56 (55,4%)	23 (23%)	79 (78%)
tot		287	115 (40%)	58 (20%)	173 (60%)

Samples derived from the same region (Lombardy) but from different provinces