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Prevalence of *Coxiella burnetii* in cows’ and ewes’ bulk tank milk samples from selected dairy farms of Central Italy

Fabrizia Guidi, Annalisa Petruzzelli, Floriana Ciarrocchia, Anna Duranti, Andrea Valiani, Giulia Amagliani and Giuliana Blasi

Istituto Zooprofilattico Sperimentale dell’Umbria e delle Marche, Perugia, Italy; Dipartimento di Scienze Biomolecolari, University of Urbino “Carlo Bo”, Urbino, Italy

**ABSTRACT**

The prevalence of *Coxiella burnetii*, the causative agent of Q fever, in cattle and sheep raw milk farms was determined in Central Italy, an area in which dairy production plays an important economic role. Milk samples (n. 189), collected from 66 dairy farms in 2012–2013, were tested by a commercial real-time PCR assay. Seventeen dairy farms had at least one positive milk sample; percent positive was higher for cattle (50%) than sheep (21%) farms. Concerning milk, 15% of samples tested overall gave a positive result, with the highest percentage of positivity observed for bovine milk compared with sheep milk (41% and 12%, respectively). In the only bovine farm repeatedly sampled during the study, *C. burnetii* contamination was persistently found for almost a year. The prevalence calculated for the sheep farms showed a discontinuous trend with a maximum peak in February. The results obtained underline the widespread presence of the pathogen in the considered geographical area, giving new epidemiological information. Since the milk route of elimination is a potential vehicle of infection for farmers, veterinarians, and for dairy stakeholders in general, BTM screening by real-time PCR can be applied as a useful surveillance tool both for the identification of infected flocks and implementation of control programmes.

**Introduction, materials and methods**

According to the European Food Safety Authority (EFSA 2015), animal Q fever has been described in almost every country in Europe, but the epidemiological situation is not well-known. Considerable variation in monitoring methods, and the lack of definitively harmonised guidelines and specific surveillance systems make both animal and human *C. burnetii* infections underdiagnosed and under-reported (EFSA 2010). However, the occurrence of two recent and large outbreaks of human infection (Netherlands, 2007–2010 and Hungary, 2013) highlights how this zoonosis may also represent a public health threat and emphasise the need for strengthening surveillance (EFSA 2015).

Diagnoses at herd/flock level, rather than at individual animal level, to monitor the circulation of *C. burnetii* have been suggested (EFSA 2010) as more significant in terms of risk to public health. An active monitoring system has been proposed for countries that may wish to evaluate the prevalence of Q fever in their animal population and includes PCR testing of bulk tank milk (BTM). Indeed, although vaginal swabs and birth products are indicated for abortion diagnosis, BTM samples are more appropriate to investigate the sanitary condition of dairy cattle and goat herds.

In Italy, Q fever is a notifiable disease both in animals and humans, and it has been included in the Occupational disease list (Italian Republic, Ministry of Works Decree 14th January 2008). National regulations have also been produced about *C. burnetii* in raw milk. Nevertheless, information about *C. burnetii* presence in small ruminants is currently incomplete and largely based on reports of reproductive disorders on livestock farms (Rizzo et al. 2016). Therefore, the aim of the present study was to estimate the prevalence rate of *C. burnetii* in cattle and sheep raw milk in Marche and Umbria, regions of Central Italy in which dairy production plays an important economic role and the data on the spread of this pathogen are scarce.

BTM samples (n. 189, n. 70 from Marche and n. 119 from Umbria) from cattle (*Bos taurus*) and sheep...
(Ovis aries) dairy farms (n. 66) in Central Italy, all authorised to produce raw milk for direct human consumption and dairy products from unpasteurised milk, were collected during the 2012–2013 biennium (Table 1). The sample collection does not represent a random sampling as tests were done only on aliquots submitted to Istituto Zooprofilattico Sperimentale dell’Umbria e delle Marche for official and self-monitoring analysis.

DNA was extracted from 200 µL of each milk sample by the QIAamp DNA Mini Kit (Qiagen, Hilden, Germany) and subsequently amplified by the real-time PCR test ADIAVET COX REALTIME (Adiagene, Saint Brieuc, France), following manufacturer’s instructions. The sequence targeted by the assay is the C. burnetii specific insertion sequence IS1111. Real-time PCR results were elaborated by the 4.10 version of MxProQPCR Software (Agilent Technologies, Santa Clara, CA).

Dairy farms in which at least one milk sample tested positive for the presence of C. burnetii were considered as positive. The prevalence of C. burnetii in BTM was calculated as the number of samples testing positive, divided by the total number of samples tested (Galiero et al. 2016). The prevalence associated 95% confidence limits (95%CI) were calculated to estimate the accuracy of the obtained values. Statistical analysis of the prevalence independent data was based on the ‘chi-square’ test and p values of <.05 were considered statistically significant.

Results and discussion

Six (6/12, 50%) cattle and 11 (11/52, 21%) sheep dairy farms showed evidence of infection with C. burnetii, having at least one positive milk sample (Table 1). The percentage of positive samples was comparable between the two regions of Central Italy under investigation. Results of milk analysis from positive farms are shown in Table 2. Positive results were obtained from 15% of the overall raw milk samples tested (95%CI: 10–20%), irrespective of the animal species. The percentage of bovine milk samples testing positive in this study (41%; 95%CI: 20–62%), falls within the range of recent studies in Italy (27–60%) (Petruzelli et al. 2013; Vicari et al. 2013), which used a touchdown PCR targeting the same IS1111 transposon-like repetitive region of the commercial kit used here. Investigations in other countries showed, however, quite variable positivity percentages, ranging from 83.8% in France (Guateo et al. 2007) to 66.7% in Hungary (Gyuranecz et al. 2012), 57.1% in the United States (Loftis et al. 2010), 53.7% in Japan (Hirai et al. 2005) and 6.2% in Iran (Rahimi et al. 2010).

Regarding sheep milk, the percentage of samples testing positive was 12% (95%CI: 7–17%). Unfortunately, a comparison of the prevalence obtained in this study with other national data about sheep is not possible, due to the lack of previous studies in Italy using real-time PCR. A comparison between data from real-time PCR and serology is not appropriate. Indeed, as antibodies can persist for variable intervals after a pathogen is immunologically or therapeutically eliminated, serology does not confirm active or persistent infection. On the other hand, C. burnetii shedding can also occurs in seronegative and asymptomatic animals (Eldin et al. 2017), thus a DNA-based test appears to be the most sensitive and rapid means for the direct detection of C. burnetii and the identification of shedding animals. However, a sero-prevalence of 38.7% and 19.5% in sheep and goat farms, respectively, was recently described (Rizzo et al. 2016), and C. burnetii was found in 34% of goat and ewe unpasteurised cheeses in Tuscany (Galiero et al. 2016).

Higher positivity values found for cattle compared with sheep farms (p < .05) and milk (p < .01) were consistent with data previously reported (Guateo et al. 2007, 2011; Proroga et al. 2011). This difference could be explained by considering that, while in cattle the main route for C. burnetii shedding is milk, in sheep the release of this pathogen occurs primarily through faeces, vaginal secretions and products of birth (Rahimi et al. 2010). Moreover, although the real-time PCR assay was used in this study as a qualitative test, the Ct values obtained in 75% of the ovine positive samples would suggest low pathogen concentrations. Average Cts were 32.5 ± 4.06 for sheep and 30.7 ± 3.71 for cattle BTM samples.

Considering seasonal prevalence, in only one bovine farm all samples (taken in December 2012,
April, September and November 2013) tested positive, revealing the persistence of *C. burnetii* for almost a year. Following real-time PCR analysis, cycle thresholds showed a characteristic cyclic trend, with values of about 34.2 ± 0.45 in December 2012, 26.6 ± 0.55 in April 2013, raising again to 33 ± 1.41 in September and November 2013. Regarding sheep, shedding showed a maximum peak in February, suggesting a possible association with the season of birth, with percentages of positive samples ranging from 33% in the first quarter to 13%, 6% and 5%, respectively, in the second, third and fourth.

### Conclusions

The present results show the widespread circulation of *C. burnetii* both in the cattle and in the sheep populations of Central Italy. The convenience sampling used in this study does not represent all the local herds and it causes a not uniform distribution of milk samplings among farms. However, the high numbers of sampled farms and BTM samples collected give considerable information about pathogen presence which is useful for data collection for epidemiological surveillance.

Shedding of *C. burnetii* in milk, although different in amount and duration between animal species, still has to be considered an important vehicle of infection for farmers, veterinarians and for dairy stakeholders in general (Mangili et al. 2016). Moreover, although the oral transmission of Q fever to humans through ingestion of infected milk is controversial (Eldin et al. 2017), the infection risk cannot be excluded, especially if considering the increasing trend towards consumption of unpasteurised dairy products.

Active monitoring, along with vaccination and standard hygiene measures, remain key preventive veterinary actions to prevent dissemination of *C. burnetii* in the environment and between animals. Thus, the identification of infected animals, at the herd/flock level, even if asymptomatic, should be adopted as an essential preventive measure to control the risk of animal and human infection. BTM screening by real-time PCR can be applied as useful surveillance tool not only for the identification of infected flocks, but also for the management of product safety, as well as for the implementation of prophylaxis and control programmes.

### Disclosure statement

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

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### ORCID

Fabrizia Guidi [http://orcid.org/0000-0002-5329-6613](http://orcid.org/0000-0002-5329-6613)
Anna Duranti [http://orcid.org/0000-0001-7392-9193](http://orcid.org/0000-0001-7392-9193)
Andrea Valiani [http://orcid.org/0000-0003-1306-8066](http://orcid.org/0000-0003-1306-8066)

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### Table 2. Results of *C. burnetii* detection in milk samples and distribution of positive milk samples obtained from positive farms.

| Animal species | Region | District | Positive/total milk samples per district | Positive farms | Positive/total milk samples in each positive farm |
|---------------|--------|----------|------------------------------------------|---------------|-----------------------------------------------|
| Cattle        | Marche | AN       | 1/2                                      | 33            | 1/1                                           |
|               |        | AP       | 5/5                                      | 4             | 4/4                                           |
|               |        | FM       | 2/2                                      | 3             | 1/1                                           |
|               | Umbria | PG       | 1/8                                      | 37            | 1/1                                           |
| totalb        |        |          | 9/17                                     |               |                                               |
| Sheep         | Marche | AP       | 8/12                                     | 8             | 3/5                                           |
|               |        | FM       | 1/14                                     | 17            | 1/1                                           |
|               |        | MC       | 2/10                                     | 20            | 2/2                                           |
|               |        | PU       | 2/16                                     | 14            | 2/2                                           |
| Umbria        | PG     | 4/81     | 49                                       | 1/6           |                                               |
| totalb        |        |          | 20/162                                   |               |                                               |

| Animal species | Region | District | Positive/total milk samples per district | Positive farms | Positive/total milk samples in each positive farm |
|---------------|--------|----------|------------------------------------------|---------------|-----------------------------------------------|
| Sheeps        | Marche | AP       | 8/12                                     | 8             | 3/5                                           |
|               |        | FM       | 1/14                                     | 17            | 1/1                                           |
|               |        | MC       | 2/10                                     | 20            | 2/2                                           |
|               |        | PU       | 2/16                                     | 14            | 2/2                                           |
| Umbria        | PG     | 4/81     | 49                                       | 1/6           |                                               |
| totalb        |        |          | 20/162                                   |               |                                               |

\*AN: Ancona; AP: Ascoli Piceno; FM: Fermo; MC: Macerata; PU: Pesaro-Urbino; PG: Perugia; TR: Terni.\n
\*Total number of BTM samples analysed in positive farms. It does not match with the total number of BTM samples tested overall (positive + negative farms).
References

Eldin C, Mélenotte C, Medianiokv O, Ghigo E, Million M, Edouard S, Mege JL, Maurin M, Raoult D. 2017. From Q fever to Coxiella burnetii infection: a paradigm change. Clin Microbiol Rev. 30:115–190.

EFSA. 2010. Prepared by Sidi-Boumedine K, Rousset E, Henning K, Ziller M, Niemczuck K, Roest HIJ, Thiéry R. 2010. SCIENTIFIC REPORT submitted to EFSA. Development of harmonized schemes for the monitoring and reporting of Q-fever in animals in the European Union. Question No EFSA-Q-2009-00511.

EFSA. 2015. The European Union Summary Report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2013. EFSA J. 13:3991.

Galiero A, Fratini F, Cammà C, Di Domenico M, Curini V, Baronti I, Turchi B, Cerri D. 2016. Occurrence of Coxiella burnetii in goat and ewe unpasteurized cheeses: screening and genotyping. J Food Microbiol. 237:47–54.

Guatteo R, Beadeau F, Joly A, Seegers H. 2007. Assessing the within-herd prevalence of Coxiella burnetii milk-shedder cows using a real-time PCR applied to bulk tank milk. Zoonoses Public Hlth. 54:191–194.

Guatteo R, Seegers H, Taurel AF, Joly A, Beadeau F. 2011. Prevalence of Coxiella burnetii infection in domestic ruminants: a critical review. Vet Microbiol. 149:1–16.

Gyuranecz M, Dénes B, Homok S, Kovács P, Horváth G, Jurkovich V, Varga T, Hajtós I, Szabó R, Magyar T, et al. 2012. Prevalence of Coxiella burnetii in Hungary: screening of dairy cows, sheep, commercial milk samples, and ticks. Vector-Borne Zoonot. 12:650–653.

Hirai A, Kaneko S, Nakama A, Ishizaki N, Odagiri M, Kai A, Sadamasu K, Shinkai T, Yano K, Morozumi S. 2005. Investigation of Coxiella burnetii contamination in commercial milk and PCR method for the detection of C. burnetii from egg. J Food Hyg Soc Jpn. 46:86–92.

Loftis AD, Priestley RA, Massung RF. 2010. Detection of Coxiella burnetii in commercially available raw milk from the United States. Foodborne Pathog Dis. 7:1453–1456.

Mangili P, Amagliani G, Micci E, Brandi G, Foglini M, Cinti B, Tonucci F, Schiavino GF. 2016. A model for Coxiella burnetii monitoring in a sheep dairy farm. JSM Biotechnol Bioeng. 3:1050.

Petruzzelli A, Amagliani G, Micci E, Foglini M, Di Renzo E, Brandi G, Tonucci F. 2013. Prevalence of Coxiella burnetii and verocytotoxin-producing Escherichia coli in bovine raw milk trough molecular identification. Food Control. 32:532–536.

Proroga YTR, Casalinuovo F, Mancusi A, Gagliardi R, Rippa P, Albano F, Damiani V, Squillaro D, Guarino A, Capuano F. 2011. Preliminary study on the prevalence of Coxiella burnetii in cheeses produced in southern Italy. It J Food Safety. 1:71–74.

Rahimi E, Doosti A, Ameri M, Kabiri E, Sharifian B. 2010. Detection of Coxiella burnetii by nested PCR in bulk milk samples from dairy bovine, ovine, and caprine herds in Iran. Zoonoses Public Hlth. 57:38–41.

Rizzo F, Vitale N, Ballardin M, Borromeo V, Luzzago C, Chiavacci L, Mandola ML. 2016. Q fever seroprevalence and risk factors in sheep and goats in northwest Italy. Prev Vet Med. 130:10–17.

Vicari N, Faccini S, Ricchi M, Garbarino C, Decastelli L, Boldini M, Rosignoli C, Dalmaso A, Bronzo V, Fabbri M. 2013. Occurrence of Coxiella burnetii in bulk tank milk from northwestern Italy. Vet Rec. 172:687.