Seroprevalence of Coxiella burnetii *in dairy cattle and buffalo from Southern Italy*

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Summary
A cross-sectional survey was carried out in dairy cattle and buffalo herds from the Southern Italy to detect antibodies against *Coxiella burnetii*. From 2014 to 2018, 402 herds were monitored and 50 mL of bulk-tank milk (BTM) per farm was analyzed by indirect ELISA. Blood samples of animals from positive farms were also taken and analyzed with the same ELISA test. The overall seroprevalence was 35% [95% Confidence interval (CI):30-39] at herd level and 13% (95%CI:13-14) at animal level. Herd province seroprevalences ranged from 17% to 75%. The provinces of Matera (71%, 95%CI:38-105) and Agrigento (75%, 95%CI:51-100) showed the highest percentage of infected farms. These results describe the widespread distribution of *C. burnetii* in livestock from Southern Italy, highlighting the need to implement a monitoring program for Q fever.

Q fever is a zoonotic disease caused by *Coxiella burnetii*, a bacterium developing spore-like forms that are highly resistant in the environment. Cattle, sheep, and goats are the main reservoirs of the bacteria. They can shed *C. burnetii* in urine, feces, milk, and birth products, and humans usually acquire the infection through inhalation of contaminated aerosols (Arricau-Bouvery and Rodolakis 2005). Q fever is considered mainly as an occupational zoonosis, being farmers, laboratory workers, veterinarian at high risk of infection (Schimmer *et al.* 2014). Consumption of raw/unpasteurized milk and tick bites have also been claimed as possible routes of transmission, but they are probably far less frequent than the airborne one (Duron *et al.* 2015, Gale *et al.* 2015).

Q fever is frequently misdiagnosed and underreported. In human, the disease has been associated with a wide clinical spectrum, from asymptomatic to fatal disease. However, in most of the cases, it is characterized by flu-like symptoms (Arricau-Bouvery and Rodolakis 2005). In livestock, the infection can cause significant economic losses due to abortion, infertility and subclinical mastitis (Van Asseldonk *et al.* 2013).

Q fever is listed in the OIE Terrestrial Animal Health Code. Therefore, Member Countries have the legal obligation to report information on the disease to the OIE. National regulations exist also at country level. In Italy, Q fever is a notifiable disease in humans and it has been listed in the Occupational disease list (Italian Republic, Ministry of Works Decree 14th January 2008). However, the epidemiological situation is not well-known because of the scarce monitoring in both humans and livestock, as it has been demonstrated for other notifiable animal diseases (Fanelli *et al.* 2020, Fanelli and Tizzani 2020).

Indeed, the occurrence of *C. burnetii* has been investigated in flocks and herds only in limited areas of the country (Masala *et al.* 2004, Rizzo *et al.* 2016, Guidi *et al.* 2017, Galluzzo *et al.* 2019). The major constraint is represented by the poor knowledge and awareness of Q fever in both farmers and veterinarians.

Considering that monitoring and reporting the infection in livestock is crucial for the prevention of human disease, the objective of this study is to estimate the seroprevalence of *C. burnetii* in dairy cattle and buffalo herds from Southern Italy, an area characterized by a closed and interconnected farms network.

From 2014 to 2018 402 semi-intensive farms (herd

size ranges from 20 to 50 heads for cattle and 230 to 800 for buffalo) were sampled in 18 Italian provinces (Figure 1). The sample collection does not represent a random sampling, as it is represented by farms in which abortions due to *C. burnetii* were detected by RT-PCR or farms located in the surrounding area (within a radius of 5 km from an infected farm).

A multiple sampling strategy was applied to assess the circulation of *C. burnetii* in the study area. Firstly, an ELISA test [LSI Qfever ruminant serum/milk ELISA kit (LSI, France] was performed on 50 mL of bulk tank milk (BTM). The use of ELISA to test BTM has been proved to a be cost-effective and valuable tool to monitor herds (Ryan *et al.* 2011). The test was repeated in the herds tested negative after 10 months, in order to consider also animals that were in dry period during the first sampling. Ten mL of serum per animal were assessed with the same ELISA test in each positive herd. The number of animals to be tested was determined considering the herd size, the expected seroprevalence (20%), the absolute precision desired (5%) and the confidence interval (CI) (95%) (Cannon and Roe 1982). The assay was performed according to the manufacturer's instructions. For BTM, a sample was considered positive if the OD percent was over 40, doubtful if it was between 30 and 40, and negative if it was under 30. For serum, a sample was considered positive if the OD percent was over 50, doubtful if it was between 40 and 50, and negative if it was under 40.

The overall seroprevalence was 35% at herd level (95%CI:30-39) and 13% (95%CI:13-14) at animal level, with differences among provinces (Table I, Figure 2). Province seroprevalences ranged from 17% to 75%.

When compared to the results from other Mediterranean regions, the majority of the provinces present a seroprevalence at the herd level in line with those reported in Spain (43%) and Portugal (61.1%) (Ruiz-Fons *et al.* 2010, Pimenta *et al.* 2015). The high numbers of positive farms in the province of Matera (Basilicata) and Agrigento (Sicily) are similar to what described in cattle from central Italy (68.5%) (Barlozzari *et al.* 2020), and it confirms the significant presence of *C. burnetii* in the territory of Western Sicily (Galluzzo *et al.* 2019).

Some authors have reported a higher risk of being seropositive for animals originating from larger herds (Agger *et al.* 2013, Paul *et al.* 2014). In this study, the risk factors of *C. burnetii* infection on each herd were not assessed, however, the differences detected are unlikely to depend on the herd-size or management system, considering the similar features of the sampled farms (with the exception of the size of buffalo herds).

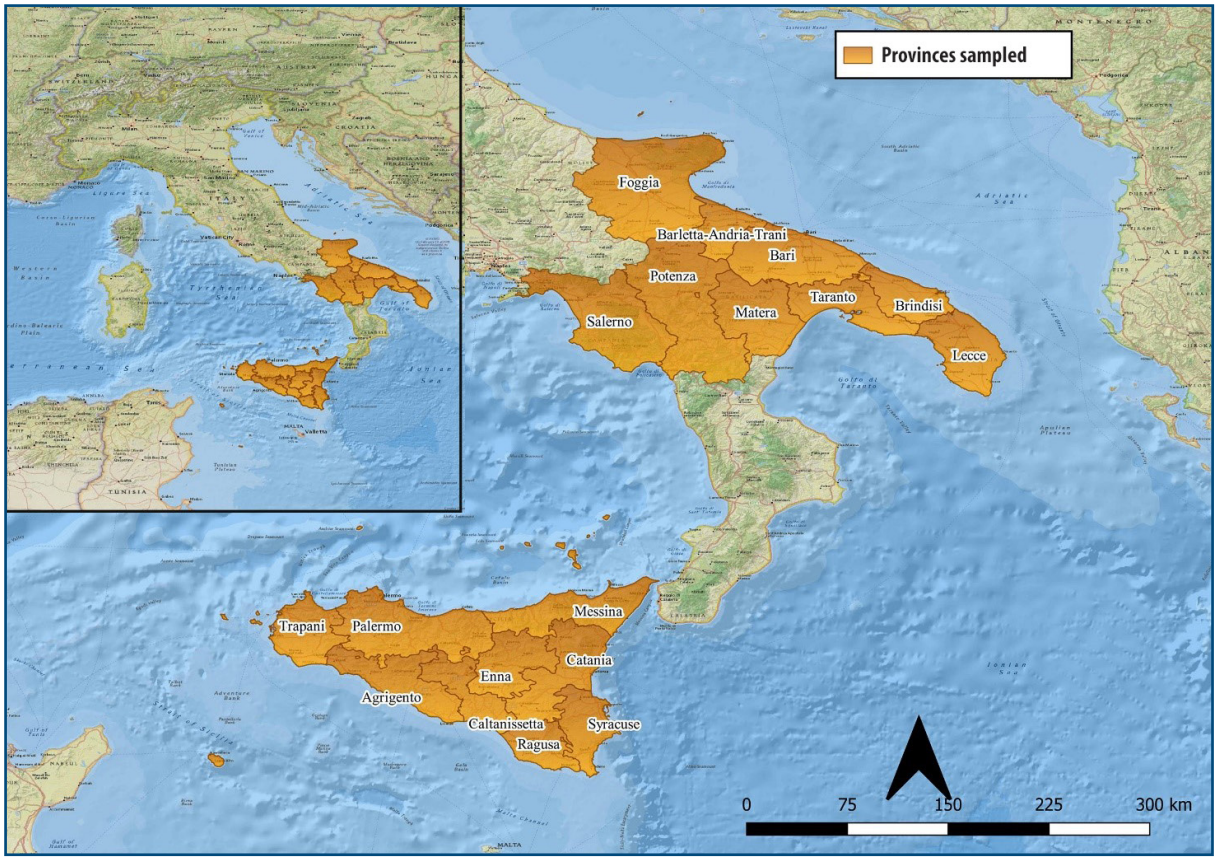

Figure 1. *Map showing the provinces sampled to assess the seroprevalence of* C. burnetii.

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Table I. C. burnetii *seroprevalence at both herd and animal level according to provinces.*

Region	Province	Herds sampled	Positive herds	Prevalence % (95%CI)	Number of animals tested	Positive Animals	Prevalence % (95%CI)
Apulia	Bari	24	13	54 (34-74)	340	153	45 (40-50)
	Barletta-Andria-Trani[1]	1	1	NA*	22	9	41 (20-61)
	Brindisi[1]	1	0	NA*	-	-	-
	Foggia	11	5	45 (16-75)	204	91	45 (38-51)
	Lecce[1]	1	1	NA*	16	11	69 (46-91)
	Taranto	46	21	46 (31-60)	560	246	44 (40-48)
Basilicata	Matera	7	5	71 (38-105)	60	13	22 (11-32)
	Potenza	11	6	55 (25-84)	62	18	29 (18-40)
Campania	Salerno[2]	104	19	18 (11-26)	247	77	31 (25-37)
Sicily	Agrigento	12	9	75 (51-100)	151	22	15 (9-20)
	Caltanissetta	6	2	33 (-4-71)	163	3	2 (0-4)
	Catania	11	2	18 (-5-41)	201	6	3 (1-5)
	Enna	26	11	42 (23-61)	784	27	3 (2-5)
	Messina	41	11	27 (13-40)	531	15	3 (1-4)
	Palermo	46	11	24 (12-36)	1259	24	2 (1-3)
	Ragusa	30	14	47 (29-65)	1092	101	9 (8-11)
	Syracuse	18	7	39 (16-61)	389	21	5 (3-8)
	Trapani	6	1	17 (-13-46)	99	1	1 (-1-3)

[1]Only one herd was sampled for the provinces of Barletta-Andria-Trani, Lecce and Brindisi, thus it was no possible to compute the seroprevalence at herd level.
[2]The buffalo farms included in this study are all located in Salerno province. *Not applicable.

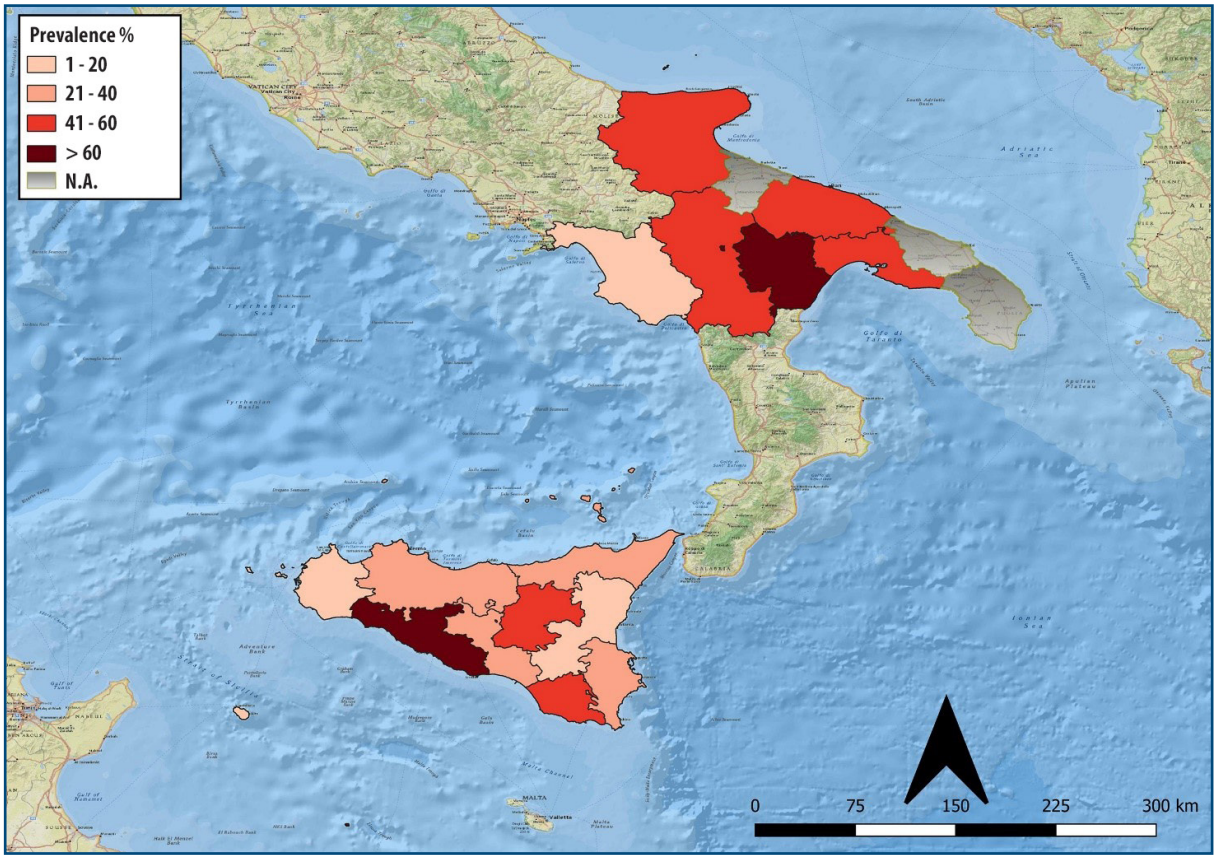

Figure 2. *Choropleth map displaying* C. burnetii *seroprevalence at herd-level in connection with the first level administrative boundaries.*

We focused on semi-intensive farms, where animals are housed in winter and graze during spring and summer. Because of that, livestock is at high risk of *C. burnetii* infection due to both the transmission by infected aerosols derived from contaminated materials left in the environment (such as birth fluids) or by fomites and the exposure to large numbers of infected ticks by grazing pastures during spring and summer.

We do believe that the rural reality and the cultural tradition of the livestock farming characterizing our study area have a great influence on the epidemiological framework of the disease. Indeed, local farms tend to perform little to no biosecurity prevention practices, and adjacent farms are most likely to share equipment and have human and livestock movements among them. All these factors may contribute to the spread of *C. burnetii* in the study area.

Recently, Agger and colleagues (Agger *et al.* 2013) demonstrated that biosecurity is crucial for the prevention of *C. burnetii* infections. The authors suggested that the veterinarian might bring the bacterium into the farm, and that the hygiene precautions (i.e. changing boots and/or clothes) significantly reduce the risk of the infection.

Data from this study made an important contribution from both public and animal health perspectives. Baseline epidemiological data presented herein will be useful for comparative purposes during future studies in areas with circulation of the pathogen.

A One Health approach, with an integrated surveillance system involving the systematic notification of cases in both humans and animals, is needed to better understand the spread of *C. burnetii* in the study area.

As regards livestock, further investigations should be performed to assess the potential risk factors that could influence the exposure of *C. burnetii*. This information is crucial to improve the management of biosecurity at farm level, and to prevent the introduction into susceptible populations.

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