Seroprevalence of Coxiella burnetii *in dairy cattle from Sicily*

Paola Galluzzo[1], Sara Villari[1], Francesco Geraci[2], Carmela Sciacca[1], Francesca Grippi[1], Vittoria Currò[3] and Giuseppina Chiarenza[1*]

[1]*Area Diagnostica Sierologica, Istituto Zooprofilattico Sperimentale della Sicilia "A. Mirri", Palermo, Italy.*
[2]*Area Sorveglianza epidemiologica, Istituto Zooprofilattico Sperimentale della Sicilia "A. Mirri", Palermo, Italy.*
[3]*Area territoriale Palermo, Istituto Zooprofilattico Sperimentale della Sicilia "A. Mirri", Palermo, Italy.*

[*]*Corresponding author at: Area Diagnostica Sierologica, Istituto Zooprofilattico Sperimentale della Sicilia "A. Mirri", Palermo, Italy. Tel.: +39 091 65 65 230, e-mail: giuseppina.chiarenza@izssicilia.it.*

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Summary
Q fever is a widespread zoonotic disease caused by *Coxiella burnetii*, an obligate intracellular bacterium with a wide range of hosts. The aim of this study was to estimate the seroprevalence of *C. burnetii* infection in cattle in Sicilian farms. A total of 4,661 serum samples, from cattle belonging to 198 Sicilian farms, were examined by ELISA test and 246 resulted positive. The average seroprevalence at the farm level was 38.8% (77/198) (95% CI), while at the animal level it was 5.28% (246/4,661) (95% CI). The present study highlights the need for continuous monitoring of *C. burnetii* spread as it represents a serious risk for human health.

Diffusione di Coxiella burnetii *nei bovini da latte in Sicilia*

Parole chiave
Bovino,
Sieroprevalenza,
Coxiella burnetii.

Riassunto
La febbre Q è una zoonosi diffusa causata da *Coxiella burnetii*, un batterio intracellulare obbligato capace di infettare diversi ospiti. Lo scopo di questo studio è stato quello di stimare la prevalenza di *C. burnetii* nei bovini presenti nelle aziende agricole siciliane, al fine di adottare misure preventive utili a ridurre la prevalenza della malattia nel territorio regionale, visti anche i potenziali rischi zoonotici. Sono stati esaminati mediante test ELISA nr. 4661 campioni di siero, provenienti da bovini appartenenti a 198 aziende siciliane; di questi, nr. 246 sono risultati positivi. La sieroprevalenza a livello aziendale è stata 38.8% (77/198) (95% CI), mentre a livello animale è risultata 5,28% (246/4661) (95% CI).

Introduction

Coxiella burnetii is an intracellular zoonotic bacterium able to cause Q fever in humans as well as several animal species: sheep, goats and cattle are the primary animal reservoirs. Moreover, ticks and rodents also are natural reservoirs of *C. burnetii* (OIE 2015). Q fever is a recognized occupational infection in workers having regular contact with ruminants or their products, such as farmers, veterinarians, laboratory technicians, slaughterhouses and cheese factories personnel, all categories at higher risk of infection (Schimmer *et al.* 2014).

Infected animals shed large numbers of organisms in their placenta, birth fluids and milk (Agerholm 2013). *C. burnetii* can also be excreted through vaginal mucous and feces post parturition (Roest *et al.* 2012). The main route of human exposure to *C. burnetii* is the inhalation of contaminated aerosols from excreta, especially birth products (Maurine and Raoult 1999). The role of raw milk and unpasteurized dairy products in the transmission of Q fever to humans is debated but, until now, not proven for either acute infection or clinical disease (Capuano *et al.* 2012, Eldin *et al.* 2013, Gale *et al.* 2015, OIE 2015). Moreover, low levels of *C. burnetii* were detected in sewage water (Schets *et al.* 2013).

C. burnetii is well equipped to resist to drought (Kazar 2005), and when infected animal excreta dry and turn to dust, the bacterium spreads to the environment. *C. burnetii* is extremely infectious; also a low dose can cause contamination (Madariaga *et al.* 2003).

Spreading of *C. burnetii* from contaminated farms to the environment may e.g. occur with soil, animal skin, wool or fur, non-pasteurized milk and wastewater. In fact, *C. burnetii* survives in the environment for months to years due to its resistance to heat, pressure and chemical stress (Kazar 2005), and the most likely route of dispersion of the bacterium is through air with aerosols and dust particles (Astobiza *et al.* 2011, Raoult *et al.* 2005).

Abortion is an important symptom of infection for dairy goats and sheep, while in cattle this is rarely observed and shedding of *C. burnetii* is of lower level (Rodolakis *et al.* 2007, Hansen *et al.* 2011). Infected cows shed the bacterium in feces, milk and birth products (Guatteo *et al.* 2012). The pathogen can be excreted for up to 13 months in cow's milk (Kargar *et al.* 2013).

Since the clinical symptoms are often generic and the infection could be asymptomatic, in most instances, the diagnosis of Q fever relies upon serology. Among the various techniques useful for animal serological diagnosis, the most common are the indirect immunofluorescence assay (IFA), the enzyme-linked immunosorbent assay (ELISA) and the complement fixation test (CFT). Currently, no IFA commercial kit is available for ruminants; therefore, ELISA is the preferred choice for seroepidemiological surveys, also due to practical reasons (easier and faster to perform than CFT) (Natale *et al.* 2012).

In Italy, Q fever surveys concerning seroprevalence in animals are very scarce, as reports have been mainly focused on reproductive disorders and, particularly, on abortion as the major clinical problems (Parisi *et al.* 2006, Natale *et al.* 2009). To our knowledge, the only extensive investigation conducted to date was carried out in Sardinia among flocks, revealing a seroprevalence of 38% and 47% on sheep and goat farms, respectively; furthermore, *C. burnetii* was also found by PCR in 10% and 6% of ovine and caprine fetuses (Masala *et al.* 2004).

Among the other studies, a survey carried out throughout the Campania region has shown a Q fever seroprevalence of 11.8% within sheep, 6.3% within goats, 14% in cattle and 7% in dogs (Capuano *et al.* 2001). A seroprevalence around 8% was found in cattle from an Apennines area of the Emilia-Romagna region (Martini *et al.* 1994). Data showed a high occurrence of *C. burnetii* in dairy cattle in the Pavia province (38%), in Cremona province (80%) and in Lodi province (78%) (Vicari *et al.* 2013). In Northern Italy, 44.9% of cattle that experienced abortion were seropositive for *C. burnetii* (Cabassi *et al.* 2006). In a serological survey in the province of Bologna, 0.87% of dogs were found to have antibodies to *C. burnetii* and 35% of dog owners were also found seropositive (Baldelli *et al.* 1992). The seroprevalence for *C. burnetii* in dogs was 31.5% in Sicily (Torina *et al.* 2006) and 7% in Southern Italy (Capuano *et al.* 2001). More recently, the prevalence of *C. burnetii* in cattle and sheep raw milk farms was determined in Central Italy, showing a higher value for cattle (50%) than sheep (21%) farms (Guidi *et al.* 2017).

Knowing Q fever prevalence in animals is necessary to prevent the human disease. In fact, the identification and removal of any head of cattle with intrauterine infection would prevent the shedding of large amounts of bacteria into the environment via placenta and birth fluids (after both abortion or normal delivery), thereby lowering the risk of spread of *C. burnetii* to animals and humans (Sánchez *et al.* 2006, Rousset *et al.*2009, Roest *et al.* 2012).

Concerning the human disease, seasonal agricultural workers were recently tested in Sicily and *Coxiella* antibodies were found in the 21.4% of serum samples from women and in the 25.0% of serum samples from men (Verso *et al.* 2016). The highest prevalence of antibodies was demonstrated in Trapani (45.0%), higher than that observed in Agrigento (22.7%) and Palermo (17.7%). None of the sampled individuals reported in the anamnesis

Table I. *N. of tested herds according to* Winepi *software (http://www.winepi.net/), and positive farms distribution for each province.*

Province	N. tot of farms per province (and distribution as %)	N. of farms to test according to *Winepi*	N. of examined farms	N. of farms with at least 1 positive sample	% of positive farms for each province
Agrigento	489 (5)	9	12	9	75
Caltanissetta	281 (3)	6	6	3	50
Catania	650 (6)	11	11	3	27
Enna	1,251 (12)	23	27	11	41
Messina	2,311 (22)	41	41	11	27
Palermo	2,503 (24)	45	46	13	28
Ragusa	1,724 (16)	30	31	20	64.5
Siracusa	985 (8)	17	18	7	39
Trapani	378 (4)	8	6	0	0
Total	10572	190	198	77	-

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Table II. *Number of serum samples examined in all Sicilian provinces and distribution of positive samples for each province.*

Province	N. of examined samples	N. of positive samples (%)
Agrigento	133	23/133 (17.3)
Caltanissetta	164	4/164 (2.4)
Catania	186	8/186 (4.3)
Enna	775	29/775 (3.7)
Messina	650	13/650 (2)
Palermo	1,260	28/1,260 (2.2)
Ragusa	1,011	114/1,011 (11.3)
Siracusa	389	27/389 (6.9)
Trapani	93	0/93 (0)
Total	4,661	246/4,661

risk factors like working in stables, or being in direct contact with animals; only one male worker in the province of Agrigento reported the occurrence of a tick bite in the past. This study compared data originating from human samples with those coming from animals raised in the same areas: cattle (14.6% in Agrigento, 1.9% in Palermo and no positive animal in Trapani) and sheep (17.4% in Agrigento, 15.1% in Palermo and 17.6% in Trapani).

Materials and methods

Study area

Sicily is an island located in the Mediterranean Basin and it is divided into nine provinces: Palermo (PA), Agrigento (AG), Enna (EN), Caltanissetta (CL), Catania (CT), Messina (ME), Ragusa (RG), Siracusa (SR) and Trapani (TP). The region is strongly devoted to animal productions and according to the Italian National Livestock registration database (www.vetinfo.sanita.it) in 2013 the regional cattle population was composed of 375,840 cattle belonging to 10,572 farms.

Table III. *Number of animals to test in each farm based on its size according to* Winepi *software(http://www.winepi.net/).*

	Farm size		
	≤ 30	≤ 40	≤ 50
N. of animals to test	≤ 28	≤ 37	≤ 45

Study design

Blood samples were collected in 2014 and 2015. All specimens examined in the study were randomly selected among those routinely conferred to the Istituto Zooprofilattico Sperimentale della Sicilia (IZSSI) for the Brucellosis National Eradication Program. . This program establishes to test twice per year all the animals older than 12 months present in each cattle herd within the regional territory. As the average number of animals present in Sicilian herds, according to the Italian National Livestock registration database (www.vetinfo.sanita.it) is around 50, only farms within this size were included in the present study.

The total number of cattle herds (n = 198) to be sampled was selected considering an expected prevalence of 50%, with 5% precision at the 95% confidence level (as no other epidemiological data were available), according to Winepi software (http://www.winepi.net/) (see Tables I-IV for all sampling details and for the descriptive of cattle farms in Sicily).A significant number of animals per herd was then selected by random sampling, based on farms' size and according to Winepi software.

Serological tests

A total of 4,661 blood samples were collected from cattle belonging to 198 Sicilian farms (Tables I-IV).

Table IV. *Distribution of the average farms' size within each Sicilian province according to the Italian National Livestock registration database (www.vetinfo.sanita.it).*

Province	Minimum	1st quartile	Median	3rd quartile	Maximum
Agrigento	1	3	10	23,25	266
Caltanissetta	1	13	33	41,2	250
Catania	1	8	23	61	231
Enna	1	9	25	47,7	264
Messina	1	8	20	39	425
Palermo	1	5	14	31	664
Ragusa	1	11	24,5	53	619
Siracusa	1	12	31	66	781
Trapani	1	1	2	5	14

Blood samples were taken from the coccygeal vein into a 10 ml vacuum tube, stored in a refrigerated bag and conferred to IZSSI. Sera were then removed by centrifugation and stored at -20 °C until tested by ELISA.

Antibodies to *C. burnetii* were detected by a commercial ELISA test (ID SCREEN® Q FEVER INDIRECT MULTI-SPECIES, IDVet, Grabels, FRANCE) according to the manufacturer's instructions. As recommended by the manufacturer, any sample was considered positive if the OD percent was over 50. If the OD percent was between 40 and 50, the result was considered as doubtful, while any sample with an OD percentage under 40 was considered as negative. Any farm with at least one positive result was considered as positive.

Epidemiologic analysis

Epidemiologic analysis was carried out using two different softwares: MapInfo (version 8.5) and Sat-scan (version 9.0).

MapInfo was used to analyze the spatial position of each farm identified by geographic coordinates (latitude and longitude), expressed in decimal degrees.

Sat-scan software was used in order to: check for the existence of statistically significant clusters of disease; verify if the disease was randomly distributed in space; get information about the areas identified as at higher disease prevalence.

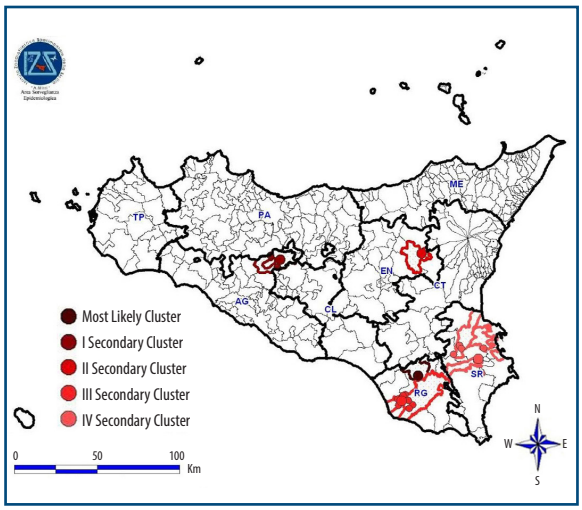

Figure 1. *Spatial distribution of* C. burnetii *seropositive herds in Sicily.* Each line represents the borders of the municipality involved in the cluster, with a color-scale descending intensity (from dark brown for the most likely cluster to light pink for the IV secondary cluster). Each dot represents the location of the farm(s) involved in the cluster. Furthermore, the bigger dots correspond to the farms with the higher seroprevalence (most Likely Cluster (80.4%), I Secondary cluster (48%), II Secondary cluster (44.4%), III Secondary cluster (36%) and IV Secondary cluster (34%); the smaller dots represent each positive farm within 10 km radius from the clusters.

Results

Seroprevalence and spatial distribution of *C. burnetii* seropositive herds

The seroprevalence at the farm level was 38.8% (77/198) (95% CI), while at the animal level it was 5.28% (246/4,661) (95% CI). Only nine samples resulted as 'doubtful'; they were all retested by ELISA confirmed either positive (2/9) or negative (7/9).

The serological results obtained in each province by ELISA are shown in Tables I and II.

Epidemiologic analysis

The territory of Chiaramonte Gulfi (RG), in particular with 41 positive samples out of 51 animals controlled in just one herd, was identified as the one with the highest prevalence of antibodies. Moreover, 5 farms in Cammarata (AG) fell into the I Secondary cluster, 1 farm in Regalbuto (EN) fell In the II Secondary group, 9 herds in Ragusa fell in the III Secondary group and 9 farms near Ferla, Carlentini, Melilli and Canicattini Bagni (all in SR province) fell in the IV Secondary cluster (Figure 1).

Discussion and conclusions

The present study shows that *C. burnetii* is widespread in Sicily.

The provinces of Agrigento and Ragusa showed the most intense serological prevalence, having the two highest rates of positive farms and animals (75% and 17.3% for Agrigento, 64.5% and 11.3% for Ragusa, respectively), and thus representing the areas where control measures should be particularly accurate. Furthermore, the territory of Chiaramonte Gulfi (RG), with 41 positive samples out of 51 tested in one herd, and Cammarata (AG), with 5 farms in the I Secondary cluster, showed the highest *C. burnetii* serological prevalence.

The high seroprevalence in Chiaramonte Gulfi involved a dairy farm with intensive management system, may suggest that animals in intensive breeding are at greater risk to contract the disease than those raised in extensive systems, as previosly reported (Paul *et al.* 2012). This is probably due to an indirect transmission from contamination with the barn environment, as cows in intensive management breeding usually spend more time inside the barns, thus being more exposed to the bacterium (Paul *et al.* 2012). Furthermore, dairy herds have a greater risk to develop the infection

than beef or mixed-breeding herds, in accordance with other studies (Paul *et al.* 2014, Mccaughey *et al.* 2010), maybe as beef cattle are maintained for a shorter management cycle than dairy cattle.

The high numbers of positive farms in the areas of Agrigento and Ragusa (75% and 64.5%, respectively) were also similar to what described in other European Countries by bulk milk ELISA, e.g. the Netherlands (Muskens *et al.* 2011), with 78.6% *C. burnetii* prevalence in dairy herds, Denmark (Agger *et al.* 2010) with 59% positive herds, Portugal with 61.1% positive herds (Pimenta *et al.* 2015).

When compared to the results from Verso and colleagues (Verso *et al.* 2016) describing human seroprevalence, these results confirm that *C. burnetii* is present in the territory of Western Sicily.

In light of the significant presence of specific *C. burnetii* antibodies, it appears quite essential to deepen the knowledge on local epidemiological situations for Q fever. A high seroprevalence in dairy cattle should lead to take preventive measures, including a control strategy to reduce the disease circulation.

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