**Seroprevalence of Coxiella burnetii in dairy cattle from Sicily**

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Keywords

Bovine, Coxiella burnetii, Seroprevalence.

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

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% (246/4,661) (95% CI). Il presente studio evidenzia la necessità di un monitoraggio continuo della diffusione di C. burnetii che rappresenta un serio rischio per la salute umana.

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, Raoul 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

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**Table 1. N. of tested herds according to Winepi software (http://www.winepi.net/), and positive farms distribution for each province.**

<table>
<thead>
<tr>
<th>Province</th>
<th>N. tot of farms per province (and distribution as %)</th>
<th>N. of farms to test according to Winepi</th>
<th>N. of examined farms</th>
<th>N. of farms with at least 1 positive sample</th>
<th>% of positive farms for each province</th>
</tr>
</thead>
<tbody>
<tr>
<td>Agrigento</td>
<td>489 (5)</td>
<td>9</td>
<td>12</td>
<td>9</td>
<td>75</td>
</tr>
<tr>
<td>Caltanissetta</td>
<td>281 (3)</td>
<td>6</td>
<td>6</td>
<td>3</td>
<td>50</td>
</tr>
<tr>
<td>Catania</td>
<td>650 (6)</td>
<td>11</td>
<td>11</td>
<td>3</td>
<td>27</td>
</tr>
<tr>
<td>Enna</td>
<td>1,251 (12)</td>
<td>23</td>
<td>27</td>
<td>11</td>
<td>41</td>
</tr>
<tr>
<td>Messina</td>
<td>2,311 (22)</td>
<td>41</td>
<td>41</td>
<td>11</td>
<td>27</td>
</tr>
<tr>
<td>Palermo</td>
<td>2,503 (24)</td>
<td>45</td>
<td>46</td>
<td>13</td>
<td>28</td>
</tr>
<tr>
<td>Ragusa</td>
<td>1,724 (16)</td>
<td>30</td>
<td>31</td>
<td>20</td>
<td>64.5</td>
</tr>
<tr>
<td>Siracusa</td>
<td>985 (8)</td>
<td>17</td>
<td>18</td>
<td>7</td>
<td>39</td>
</tr>
<tr>
<td>Trapani</td>
<td>378 (4)</td>
<td>8</td>
<td>6</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>10572</td>
<td>190</td>
<td>198</td>
<td>77</td>
<td>-</td>
</tr>
</tbody>
</table>
was composed of 375,840 cattle belonging to 10,572 farms.

**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.

**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.

**Serological tests**

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

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

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

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

<table>
<thead>
<tr>
<th>Farm size</th>
<th>N. of animals to test</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤ 30</td>
<td>≤ 28</td>
</tr>
<tr>
<td>≤ 40</td>
<td>≤ 37</td>
</tr>
<tr>
<td>≤ 50</td>
<td>≤ 45</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Province</th>
<th>Minimum</th>
<th>1st quartile</th>
<th>Median</th>
<th>3rd quartile</th>
<th>Maximum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Agrigento</td>
<td>1</td>
<td>3</td>
<td>10</td>
<td>23,25</td>
<td>266</td>
</tr>
<tr>
<td>Caltanissetta</td>
<td>1</td>
<td>13</td>
<td>33</td>
<td>41,2</td>
<td>250</td>
</tr>
<tr>
<td>Catania</td>
<td>1</td>
<td>8</td>
<td>23</td>
<td>61</td>
<td>231</td>
</tr>
<tr>
<td>Enna</td>
<td>1</td>
<td>9</td>
<td>25</td>
<td>47,7</td>
<td>264</td>
</tr>
<tr>
<td>Messina</td>
<td>1</td>
<td>8</td>
<td>20</td>
<td>39</td>
<td>425</td>
</tr>
<tr>
<td>Palermo</td>
<td>1</td>
<td>5</td>
<td>14</td>
<td>31</td>
<td>664</td>
</tr>
<tr>
<td>Ragusa</td>
<td>1</td>
<td>11</td>
<td>24,5</td>
<td>53</td>
<td>619</td>
</tr>
<tr>
<td>Siracusa</td>
<td>1</td>
<td>12</td>
<td>31</td>
<td>66</td>
<td>781</td>
</tr>
<tr>
<td>Trapani</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>5</td>
<td>14</td>
</tr>
</tbody>
</table>
Blood samples were taken from the coccygeal vein into a 10 ml vacuum tube, stored in a refrigerated bag and confedered 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.

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, Mellilli 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 previously 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. 2010) with 59% positive herds, Portugal (Agger et al. 2010) with 59% positive herds, Portugal with 61.1% positive herds (Pimenta et al. 2015).

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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**References**


