SHORT REPORT: SEROPREVALENCE OF HUMAN INFECTION BY COXIELLA BURNETII IN BARCELONA (NORTHEAST OF SPAIN)

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Abstract. Coxiella burnetii is the causal agent of Q fever, a worldwide-distributed zoonosis, which is endemic in Spain. C. burnetii has an extensive reservoir, including farm animals and pets. The aim of this study was to determine the seroprevalence of C. burnetii in humans in Vallés Occidental (Barcelona, northeast of Spain) and its possible related risk factors. The prevalence of phase II antibodies from 216 subjects was determined by indirect immunofluorescence assay (IFA). Age, sex, living place, occupation, and contact with animals were surveyed. A 15.3% seroprevalence was found (≥ 1/40), and 8.8% of samples had titers ≥ 1/80. Seropositive cases were significantly higher in patients > 44 years of age. No statistically significant correlation was found between seropositivity and the remaining variables studied. Therefore, infection by C. burnetii seems to be endemic in our region, with a prevalence ranging from 9% to 15%, depending on the titers that are to be considered significant.

Coxiella burnetii is an obligate intracellular, small, gram-negative bacterium. This microorganism is the causal agent of Q fever, a worldwide distributed zoonosis considered a public health problem in many countries. C. burnetii has an extensive reservoir, including many wild and domestic mammals, birds, and arthropods such as ticks. The most frequent sources of human infection are farm animals, especially cattle, goats, and sheep. It has been shown that infected cats, rabbits, and dogs can also transmit C. burnetii to humans. Animals are often chronically infected, shedding bacteria in feces, milk, urine, and especially, birth products of mammals.

The aerosol route is the primary mode of human infection. Contamination by aerosols may occur mainly from parturient fluids. The organism may be spread by the wind; therefore, patients without any contact with animals could be infected. Ingestion (raw eggs and mainly, drinking raw milk) and person-to-person transmission (e.g., contact with parturient women, blood transfusion, autopsies, sexual transmission) are minor routes. C. burnetii is resistant in adverse conditions and can survive for months to years in a sporelike state, contaminating water or soil.

In humans, Q fever presents as a clinical polymorphism and usually is asymptomatic (~60% of the infections are asymptomatic seroconversions) or mild, as a flu-like disease with spontaneous recovery. The infection has two forms in humans: acute and chronic. In the acute form, clinical manifestations such as pneumonia, prolonged fever, and granulomatous hepatitis occur. The most frequent clinical manifestation of the chronic form is endocarditis. The acute form is often underdiagnosed because of a non-specific clinical picture, and thus serology is extremely important in the diagnosis of the disease.

Seroprevalence studies have a 2-fold interest: epidemiologic and diagnostic. The first shows the prevalence in a given geographical area. The second allows for adjusting the cut-off point of the serologic titers for diagnostic purposes in the study region.

The aim of this was to determine the seroprevalence of C. burnetii infection in a representative sample of 391,546 inhabitants from a region where our hospital is a reference facility.

The study was undertaken in Vallés Occidental (Barcelona), a predominantly urban county near the coast in the northeast of Spain. A total of 11 municipalities (391,546 inhabitants) participated in the study.

Two hundred sixteen serum samples from patients who had attended at Sabadell Hospital were collected during a 5-month period from September to January. The sample included adults undergoing minor surgery and children cared for at the Pediatrics Emergency Service for non-infectious diseases. Informed consent was obtained from all adult participants and from parents or legal guardians of minors.

Taking into account the actual population of Vallés Occidental, the study population was stratified by age (0–14, 15–29, 30–44, 45–64, and > 64 years) and by living place (rural: < 5,000 inhabitants, semi-urban: 5,000–50,000 inhabitants, urban: > 50,000 inhabitants). For each study subject, the following variables were surveyed: age, sex, place of residence, contact with wild animals, contact with farm animals, contact with pets, and occupation. Those inhabitants unable to answer the epidemiologic survey were excluded.

Sera were studied by indirect immunofluorescence, using a commercially available antigen (Coxiella burnetii spot IF; Bio-Mérieux, Marcy L’Étoile, France) obtained from Vero cell cultures infected with C. burnetii (phase II antigen). Antibody determination was made by double serial dilutions, beginning with a 1/20 sampling dilution, and using a fluorescein-labeled IgG immunoglobulin. The technique was conducted according to the manufacturer’s recommendations. Titers ≥ 1/40 were considered positive.

Data were analyzed with the SPSS package using Student t test to compare quantitative variables. Univariate group comparisons were performed using χ² and Fisher exact test. A P < 0.05 was considered significant.

Of the 216 subjects, 117 (54.16%) were men and 99 (45.84%) women. The age ranged from 0 to 91 years. Subjects were reported by 11 towns, and 149 (68.98%), 51 (23.61%), and 16 (7.41%) subjects lived in urban, semi-rural, and rural areas, respectively. In the group of 161 adults (> 18 years), there were 11 (6.8%) students, 27 (16.8%) retired, 37 (23%)...
TABLE 1
Titers obtained when matching the study sera with the phase II antigen Coxiella burnetii

<table>
<thead>
<tr>
<th>Living place</th>
<th>No. of subjects</th>
<th>No. of positives</th>
<th>&lt;1/40</th>
<th>1/40</th>
<th>1/80</th>
<th>1/160</th>
<th>1/320</th>
<th>1/640</th>
<th>1/1,280</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urban</td>
<td>149 (68.98%)</td>
<td>24 (72.72%)</td>
<td>125</td>
<td>12</td>
<td>6</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Semi-urban</td>
<td>51 (23.61%)</td>
<td>6 (18.18%)</td>
<td>45</td>
<td>1</td>
<td>3</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Rural</td>
<td>16 (7.41%)</td>
<td>3 (9.09%)</td>
<td>13</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>216</td>
<td>14</td>
<td>10</td>
<td>3</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
</tbody>
</table>

Distribution of results by living place.

TABLE 2
Titers obtained when matching the study sera with the phase II antigen Coxiella burnetii

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>No. of subjects</th>
<th>No. of positives</th>
<th>&lt;1/40</th>
<th>1/40</th>
<th>1/80</th>
<th>1/160</th>
<th>1/320</th>
<th>1/640</th>
<th>1/1,280</th>
</tr>
</thead>
<tbody>
<tr>
<td>0–14</td>
<td>50 (23.15%)</td>
<td>0 (0%)</td>
<td>50</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>15–29</td>
<td>50 (23.15%)</td>
<td>3 (9.10%)</td>
<td>47</td>
<td>2</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>30–44</td>
<td>43 (19.91%)</td>
<td>9 (27.27%)</td>
<td>34</td>
<td>6</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>45–64</td>
<td>46 (21.29%)</td>
<td>14 (42.42%)</td>
<td>32</td>
<td>4</td>
<td>5</td>
<td>3</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>≥ 65</td>
<td>27 (12.5%)</td>
<td>7 (21.21%)</td>
<td>20</td>
<td>2</td>
<td>3</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Distribution of results by age groups.

housewives, 53 (32.9%) workers, and 26 (16.1%) unem-
ployed. In seven people, the occupation was unknown.

Thirty-three (15.3%) of the sera samples analyzed showed antibodies against Coxiella burnetii (≥ 1/40). Positive sera titers ranged from 1/40 to 1/1,280, and 19 (8.8%) had titers ≥ 1/80. Fourteen (6.48%) of the positive samples had an IgG titer of 1/40, 10 (4.63%) had a titer of 1/80, 3 (1.39%) had a titer of 1/160, 2 (0.93%) had a titer of 1/320, 2 (0.93%) had a titer of 1/640, and 2 (0.93%) had a titer of 1/1,280.

From 33 seropositive subjects, 19 (57.6%) were men and 14 (42.4%) were women (non-significant). Coxiella burnetii seroprevalence was 16.10% in urban areas, 11.76% in semi-urban areas, and 18.75% in rural areas (Table 1), which was non-significant. There were no significant differences in the rates of antibodies to Coxiella burnetii related to either contact with any kind of animals or occupation.

The prevalence of infection varied among the different age groups (Table 2). Seropositive cases were significantly higher in 45- to 64-year-old patients (P < 0.05; OR: 3.91). Of the 73 subjects > 44 years of age, 21 (28.8%) had titers ≥ 1/40, which was similar to those with ages between 30 and 44 years who had 9/43 seropositive titers (27.27%). In contrast, only 3 of 100 (3%) patients 0–29 years of age had positive titers against Coxiella burnetii.

Coxiella burnetii infection is a public health problem in many countries, such as European countries, Canada, Japan, Turkey, Israel, and Australia.\(^1\,3\,8\,–\,11\) In Spain, Q fever is endemic,\(^6\,7\,12\,\) although its declaration is not mandatory. Therefore, epidemiologic and clinical features are known from research studies. Seropositive rates differ from one region to another, probably because of different epidemiologic and climatic environments. In effect, the highest seroprevalence data are found in northern Spain (Cantabria [48.6%],\(^13\) Basque country [38.5%]\(^14\)), Salamanca (50.2%),\(^15\) and Leon (40.6%),\(^16\) probably because of more cattle-raising activities. In contrast, Q fever seems to be less prevalent in central Spain (Madrid [12.7%]\(^17\)) and southern Spain (Huelva [4–6%]\(^18\)).

In this study, the seroprevalence of Coxiella burnetii was 15.3%, showing the endemic infection by Coxiella burnetii in our predominantly urban area. This result agrees with those obtained in a previously carried out in Catalonia (northeast Spain) whose seropositive rates ranged from 16% to 39%.\(^19\)

No statistically significant correlation was found between seropositivity and living place. Although seroprevalence has been considered higher in rural areas because farm animals are the main reservoirs,\(^8\,9\,13\,14\,17\,19\) some studies have shown a rise in cases in people living in urban areas.\(^20\) Increased exposure to farm animals caused by travels to rural areas and outside activities, contact with pets, urbanization of rural areas, and windborne spread could contribute to the rising probability of being infected among urban residents.\(^1\,4\,6\,20\,21\) However, the small number of patients in our rural area requires conservative conclusions.

Q fever is more frequently in men in areas where the most common risk factor is exposure to infected cattle, including most European countries, California, and Australia.\(^13\,14\,16\,22\)

In contrast, our data showed a sex ratio similar to 1:1, proving that humans from our region could also be infected by other sources. For instance, exposure to parturient cats is the primary mode of Coxiella burnetii infection in Nova Scotia, where the sex ratio is 1:1.\(^23\)

Although Q fever remains an occupational hazard among people in contact with farm animals,\(^1\,3\,8\,10\,21\) no significant difference was found between seropositive rates and either contact with animals or occupation. However, the small number of people who reported contact with animals may be a limitation.

Moreover, spread by the wind\(^1\,3\,4\) contact with pets,\(^2\,3\,23\) minor routes such as ingestion or person-to-person transmission,\(^3\,5\) arthropod-borne transmission,\(^24\) and the resistance of Coxiella burnetii in adverse conditions\(^9\) may contribute to humans being exposed to unknown sources. Therefore, further studies are necessary to elucidate risk factors in our region (predominantly urban).

Like other studies,\(^13\,–\,16\,23\) seroprevalence to Coxiella burnetii tends to increase with age. Significant prevalences of past infections in older ages could also be explained because of the longer exposure time experienced by older people.

Seroprevalence studies are essential to mark a cut-off point with diagnosis purposes. This is especially important in a dis-
ease like the Q fever, which has a rather unspecific clinical course, and where serologic results are determining. In our study, even considering a $\geq 1/80$ titer, 8.8% of the population was positive, which forces us to use titers $\geq 1/160$ for diagnosis purposes.

In conclusion, 15.3% of a sample of people representative of the Vallés Occidental County had antibodies against C. burnetii, which confirms the endemic nature of this infection.

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