Q Fever Epidemic among Employees in a Factory in the Suburb of Zadar, Croatia

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Aim
To examine the role of wind in the spread of Q fever (Coxiella burnetii) from the source of infection (sheep on the pastures) to the factory where there was an outbreak of Q fever among the employees.

Methods
We performed clinical (fever, coughing, myalgias, arthralgias), laboratory (complete blood test, aminotransferases, antibodies to Coxiella burnetii), radiographic (chest X-ray), and epidemiological (questionnaire) analysis on 47 of 110 employees of the plant in a suburb of Zadar. Sera of 182 sheep were tested for antibody to C. burnetii by complement fixation reaction.

Results
During the first half of March 2004, 14 of 110 employees of a factory in a suburb of Zadar were diagnosed with Q fever on the basis of clinical and laboratory findings. In three sections of the plant, directly exposed to the north wind, a diagnosis of Q fever was confirmed in 14 of 110 employees by clinical, laboratory, and X-ray analysis, whereas there were no sick employees in the other four sections. North of the plant there were pastures where many flocks of sheep grazed. Antibodies to C. burnetii were found in 20 out of 182 sheep sera. Employees who were exposed to the north wind, had a significantly higher possibility of acquiring Q fever than did those working in sections protected from the wind.

Conclusion
North wind (bura) containing the aerosolized C. burnetii likely influenced the Q fever outbreak in persons far from the source of the infection.

Q fever is an acute or rarely chronic disease caused by the gram-negative bacterium Coxiella burnetii (1-3). The disease is characterized by high fever, severe headache, muscle aches, often with pneumonia and hepatitis, and rarely with the occurrence of chronic hepatitis, endocarditis, pericarditis, osteomyelitis, meningocerebralitis, hemolytic anemia, chronic fatigue, and exhaustion (4-9). Although it is primarily a zoonosis that occurs in natural foci, the organism has become adapted to domestic animals (sheep, cows, goats), where it survives; domestic animals are the main source of the infection. The organism is found in urine, feces, milk, wool, placenta, amniotic fluid, and amniotic bands of infected animals (1,3). Under unfavorable conditions C. burnetii shows considerable resistance to environmental factors and can survive for months in the form of spores in places where animals had lived. In this way, it presents the source of infection even for those who did not have any direct contact with animals (5).

Infection can be transmitted from an animal reservoir to humans when the human inhales an aerosol contaminated with the organism (4). Because the amount of C. burnetii needed to cause the disease is very small, we presumed that the wind had an important role in carrying an infected aerosol to the people (10-14). Infection
among wild animals is usually spread by ticks, but can also spread by the infected aerosol (1,4).

In the period between March 3 and 14, 2004, there was an outbreak of Q fever among the 110 employees of a factory near Zadar, Croatia. Three to four weeks before the outbreak of the epidemic, a moderate to strong north wind (bura) was blowing in this area. In this study we describe the importance of the wind’s role in spreading *C. burnetii* and causing the epidemic of Q fever among the factory employees.

**Methods**

**Setting**

The factory is surrounded by numerous pastures and situated in a rural agricultural area 1000 m from the urban area of Zadar. The plant is divided into 7 sections (Fig. 1). Section A (7 employees), where the offices are, and section B (43 employees), where the preparation of flexible packaging is done, and section C (6 employees), where the laboratory is, are on the north side of the building. The windows in the sections A and C, which were often open to allow airflow, face the pastures. Sections D (20 employees) and E (12 employees) do not face the pastures. There are no windows on the north side of the section F (20 employees), where cutting of material is performed, is enclosed from the north side, and the ventilation system with air supply duct is in a small room beside the section and is not exposed to the north wind. Section G (2 employees) is a warehouse, completely enclosed from the north.

**Climatic and Meteorological Data**

Climatic and meteorological data were obtained from the National Hydrometeorological Institute (15). Wind speed and direction, average temperature, daily precipitation (rain), air pressure, and relative humidity were analyzed.

**Subjects**

The patient group comprised 14 plant employees, with clinical symptoms, signs, and laboratory diagnosis of acute *C. burnetii* infection.

Controls were 33 randomly chosen plant employees who did not develop the symptoms of Q fever 12 weeks before the occurrence of the first case.

**Diagnostic Criteria**

Clinical symptoms and signs of the disease (fever higher than 38 °C, headache, arthralgias, myalgias, fatigue, coughing, crepitation, and rhonchi), demographic and epidemiological data (age, gender, place of residence, occupation, permanent residence in, or a trip to a rural area, contact with animals, consumption of unboiled milk and fresh cheese, walking through pastures on their way to work, history of tick bite), laboratory findings (ESR, leukocyte count, AST, ALT, creatinine level), chest radiograph, results of serologic tests, and data on treatments and their outcomes were obtained by using a Centers for Diseases Control and Prevention questionnaire (Q Fever Case Report, Centers for Diseases Control and Prevention, Form Approved OMB 0920-0009, Atlanta, GA, USA).

**Serology**

Serum samples to be tested for antibodies to phase II *C. burnetii* antigen were collected in the acute phase of the disease and two weeks later in the convalescence stage. Complement fixation was used for diagnosis, with antigen prepared from yolk sac (strain Nine Mile phase II, Institute of Public Health, Zagreb, Croatia). The initial serum dilution 1:8 a diagnosis of acute Q fever was made on the basis of the following serologic criteria: (a)
seroconversion (antibodies specific to phase II antigen were found in convalescent serum, whereas in acute serum no antibodies were found); (b) fourfold increase in antibody titer in convalescent serum compared to the acute serum (16). Antibody titer ≥32 in both sera was considered as evidence of an earlier or inapparent infection.

Sera of 182 sheep grazing on pastures near the plant when the epidemic occurred, were tested by complement fixation at dilution of 1:10. Antibody titers of 10 to 40 were considered to indicate latent C. burnetii infection. Paired sera were not collected.

Statistical Analysis
Statistical analysis was performed with the EPI-info5 program (Public Domain Software for Epidemiology and Disease Surveillance, Center for Disease Control Epidemiology Program Office, Atlanta, GA, USA; and World Health Organization Global Program on AIDS, Geneva, Switzerland). Data analysis was performed by $\chi^2$ test. $P < 0.05$ was considered significant (17).

Results

Source of Infection
The plant is situated in rural, agricultural surroundings of the town of Zadar. North of the plant there are fields and pastures, where about 700-800 sheep graze every day. Antibodies to C. burnetii were found in the sera of 20 out of 182 (11%) sheep which had been grazing at these pastures when Q fever began occurring in the humans.

Disease and Patients
Between March 3 and 14, 2004 (when the illnesses occurred), laboratory and clinical findings confirmed the diagnosis of Q fever in 14 (12.7%) of 110 employees. Among the 14 patients 8 were men and 6 women (M/F ratio = 1.3:1). Their average age was 32.9 ± 8.1, median 30 (range 21-50 years) (Table 1). In all 14 patients, clinical symptoms and signs of the diseases were found. Of 14 patients, 11 (78.6%) were hospitalized, and 3 (21.4%) were treated in the outpatient clinic. Chest radiograph confirmed the diagnosis of interstitial pneumonia in all 14 patients and in blood tests of 3 (21.4%) patients unsegmented neutrophils were found. All 14 patients had either liver lesions or hepatitis, which was manifested in pathological levels of AST/ALT, 5 times higher than normal (AST = 15-37 U/L; ALT = 10-37 U/L).

Eleven patients showed greater increase in antibody titer (32 to 1024) and seroconversion with significant antibody titer to C. burnetii (second serum titer level was twofold higher than borderline titer 1:32 and fourfold higher than the initial dilution of 1:8 in the first serum) in 3 patients (Table 1).

All patients received doxycycline treatment $2 \times 100$ mg for 14 days, and all became afebrile 48 to 72 hours after the beginning of the treatment.

In section A 3/7 employees (42.8%) became ill, in section B 8/43 (18.6%), in section C 3/6 (50%). In sections D, E, F, and G, none of 54 employees became ill. Control group comprised 33 employees, 18 men and 15 women (F/M ratio 1.2:1); 3 employees from the section A, 11 from section B, 3 from section C, 5 from section D, 4 from section E, 5 from section F, and 2 from section G. Average age in the control group was 33.7 ± 6.3, median 33 (range 22-56 years). In paired sera obtained from 30 controls two weeks apart, no antibodies to C. burnetii were found. In 3 controls titers were lower than the accepted minimum of 32. All subjects, both patients and controls, lived in the town of Zadar. They traveled to work by a main road, which does not lead near the pastures, they did not leave their places of residence in the last 12 weeks, or go to a picnic or to hunt. None had a domestic animal at home or in the neighborhood, or eat unboiled milk or fresh cheese. Nobody had a tick bite, as far as they knew.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Sex</th>
<th>Age</th>
<th>Titer of antibodies</th>
<th>Onset of symptoms</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>M</td>
<td>34</td>
<td>64/152</td>
<td>March 3</td>
<td>cured</td>
</tr>
<tr>
<td>2</td>
<td>M</td>
<td>39</td>
<td>16/256</td>
<td>March 3</td>
<td>cured</td>
</tr>
<tr>
<td>3</td>
<td>M</td>
<td>28</td>
<td>64/128</td>
<td>March 4</td>
<td>cured</td>
</tr>
<tr>
<td>4</td>
<td>F</td>
<td>32</td>
<td>512/1024</td>
<td>March 6</td>
<td>cured</td>
</tr>
<tr>
<td>5</td>
<td>M</td>
<td>30</td>
<td>32/204</td>
<td>March 6</td>
<td>cured</td>
</tr>
<tr>
<td>6</td>
<td>F</td>
<td>30</td>
<td>32/128</td>
<td>March 6</td>
<td>cured</td>
</tr>
<tr>
<td>7</td>
<td>M</td>
<td>29</td>
<td>64/256</td>
<td>March 6</td>
<td>cured</td>
</tr>
<tr>
<td>8</td>
<td>F</td>
<td>25</td>
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<td>9</td>
<td>F</td>
<td>50</td>
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<tr>
<td>10</td>
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<td>30</td>
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</tr>
<tr>
<td>11</td>
<td>M</td>
<td>34</td>
<td>16/256</td>
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<tr>
<td>12</td>
<td>F</td>
<td>21</td>
<td>16/1024</td>
<td>March 12</td>
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<td>M</td>
<td>49</td>
<td>16/64</td>
<td>March 12</td>
<td>cured</td>
</tr>
<tr>
<td>14</td>
<td>F</td>
<td>30</td>
<td>16/64</td>
<td>March 14</td>
<td>cured</td>
</tr>
</tbody>
</table>

* M – man; F – female.
Influence of Wind on Distribution of Q Fever Patients

During the 15-day period between February 1 and 15, 2004, moderate northwest and north winds were blowing with one strong gust of north wind (bura) of 8 m/s and more. Other (average) meteorological parameters were: temperature 7.3 °C, rain 4.8 mm L/m², air pressure 1,024 hPa, relative moisture 72.4%. Statistical analysis was performed to assess the differences between infected and uninfected individuals according to direct exposure to north wind. All 14 subjects from the patient group were directly exposed to north wind whereas there were 33 uninfected individuals exposed to the wind and 16 unexposed controls.

Statistical analysis showed that employees in sections A, B, and C had significantly higher possibility of acquiring this infection than the did employees in sections D, E, F, G, ie employees in sections directly exposed to north wind had a greater possibility of getting Q fever than did employees who were not directly exposed to the north wind (OR = 12.24; 95% confidence interval 1.26-2.59, \( P < 0.05 \)).

Discussion

Fields and pastures of the Zadar region are hosts for sheep flocks throughout the year, with winter months being the season of intensive lambing. Q fever was suspected when employees developed symptoms similar to those of influenza, without there having been epidemiological or clinical data suggesting influenza at that time and in the vicinity of the pastures.

Employees who got the disease worked in sections A, B, and C, which were directly exposed to the north wind blowing through the windows from the pastures. The windows were often left open for ventilation. Air also entered these buildings through the ventilation system with a supply duct on the north side of the building. In sections A and B which were most directly exposed to the wind, half of the employees became ill, whereas in section C 18.6% of employees became ill. In the sections of the plant that were completely protected from the wind, no one acquired Q fever. Antibodies to \( C.\ burnetii \) found in the sera of sheep that grazed daily near the plant, pointed to the occurrence of infection in the flock. However, as the second serum was not analyzed, due to lack of financial resources, it was not clear whether the sheep had latent infection or these were residual antibodies. Climate and meteorological parameters in the Mediterranean area such as dry and cold wind, low precipitation, relatively low temperatures, and daily relative humidity, although unfavorable, obviously were not sufficiently unfavorable to destroy these resilient organisms. Moreover, the cold and dry north wind which blows usually in this area in February and the first half of March, was also blowing during incubation period and appears to have been effective in carrying aerosols towards the plant, so that employees were inhaling air containing \( C.\ burnetii \) organisms.

Statistical analysis of the relation between direct exposure to a north wind and acquisition of Q fever showed significant correlation. From this result we conclude that the north wind had a crucial role in the pattern of distribution of employees with symptoms and signs of Q fever.

It is well known that people far from the source of infection can acquire Q fever if they inhale an aerosol containing \( C.\ burnetii \) (10-12,18). During lambing, sheep with latent \( C.\ burnetii \) infection secrete huge amounts of the organism to the environment, especially from amniotic bands and placenta to pastures and fields. The organism, even in unfavorable weather conditions, can survive for months in the form of spores, thus presenting a source of infection even for those who are not in direct contact with sheep (5,10-12,18). The hypothesis that wind plays a role in spreading \( C.\ burnetii \) was based on studies showing the small amount of infected aerosol needed for the outbreak of infection, seroprevalence of antibodies to \( C.\ burnetii \) in the areas where the disease has been recorded and different wind directions (13,14).

Q fever, because of its clinical significance and epidemiological prevalence worldwide, presents an important public health problem. Sheep have chronic infection, but have no symptoms of the disease, and secrete \( C.\ burnetii \), thus presenting a permanent source of infection (5). Today various measures (health control, vaccination of animals, adequate disinfection, and disposal of animal products containing \( C.\ burnetii \)) to prevent the occurrence and spread of the disease are implemented (18). Sheep infected with \( C.\ burnetii \) and a Q fever epidemic in employees of a plant in rural Zadar region point to the necessity
for protective measures at this site such as enclosed pastures far from human activities, building protective baffles on the north side of the plant, installation of ventilations systems with special filters which would prevent \( C.\ burnetii \) from entering the plant and installing air supply ducts on sides of the plant that do not face the pastures. Aerogenic transmission of the causative agent, the likelihood of transmission by arthropods, low infectious doses and very high resistance to environmental stresses make \( C.\ burnetii \) a potential agent of Q fever in epidemic proportions not only in rural but also in urban areas (5,18). This epidemic and the growing urbanization of rural areas suggest that future outbreaks of Q fever may occur in the Zadar region. Therefore, we must heighten awareness of epidemiologists, veterinarians, and medical staff in primary and hospital care settings if we are to prevent, recognize, and efficiently treat Q fever in this area.

References


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