An outbreak of Q fever in Bulgaria

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Summary. Q fever is an acute febrile illness due to Coxiella burnetii. In the Balkans, Q fever in humans has been reported since World War II, and in countries such as Bulgaria the number of cases has increased since the early 1990s. We report an investigation of an outbreak in the town of Botevgrad, Western Bulgaria. Overall, 220 cases were identified between May 1 and June 9, 2004. Of the cases, 168 were from Botevgrad; the others were from neighbouring towns. This has been the largest outbreak in Bulgaria in the last 20 years. Q fever outbreaks in urban areas are not common. Flocks of sheep and goats were the most likely source of infection, as suggested by the observation that flocks grazed in, or had travelled on, the roads and the gardens of the town, and for the prevalence of anti-C. burnetii antibodies among animals in the area. This large outbreak highlights how zoonoses such as Q fever may represent a public-health threat also for urban populations.

Key words: Q fever, outbreak, Bulgaria.

INTRODUCTION

Q fever is an acute febrile illness due to Coxiella burnetii, an intracellular gram-negative bacterium. In more than half of the cases, primary infection is symptomless [1]. Although the reservoir of C. burnetii consists of various species, including mammals, birds, and ticks, the most common sources of human infection are farm animals, such as cattle, goats, and sheep [2]. C. burnetii is typically transmitted by the aerosol route and is endemic in virtually all countries, except New Zealand [2].

Several outbreaks have been recently reported in different European countries, such as Germany, The Netherlands, Scotland, and Slovenia [3-6]. In Balkans countries, such as Bulgaria, Q fever in humans has been reported since World War II, and the number of cases tended to increased since the early 1990s [7]. During the last decade, the number of cases reported in Bulgaria varied from year to year, with annual incidence rates ranging from less than 1 per 100 000 to more than 3 per 100 000 during years with reported outbreaks (i.e., such outbreaks occurred in 1993 in the town of Panagiurishte, in 1996 in Karlovo, and in 2002 Etropole) [7].

Hereby we report the results of an investigation of an outbreak in the Municipality of Botevgrad, Western Bulgaria.
METHODS

The Municipality of Botevgrad (36 846 inhabitants) includes the homonym town and several small villages. The town of Botevgrad (20 504 inhabitants) is approximately 45 km North-East of Sofia and is located in a rural area, in a valley close to the Stara Planina Mountains. Small farms with 5-10 animals are present in the main part of the town, and sheep and goats roam freely around the town. Each day, these animals are gathered from around the town by shepherds and brought to graze in area outside of the town.

A case was defined as a person who presented with fever and who tested positive for *C. burnetii*. Case finding was carried out by contacting hospitals and healthcare providers in the area. Laboratory diagnosis was made according to the criteria established by the Bulgarian Ministry of Health [8], which include clinical signs and symptoms and laboratory confirmation.

Serology was performed on all suspected cases for Q-fever using an immunofluorescence assay (IFA) with phase II antigen (i.e., for IgM and IgG combined). A clinical case was confirmed as affected by Q-fever if i) a high titre (i.e., =>164) of specific antibodies was detected in a single convalescent-phase serum sample, or ii) seroconversion, as defined by the positivity for specific antibodies in the convalescent phase in an individual who was negative during the acute phase of the disease, was demonstrated.

Throat swabs from patients with clinical manifestations suggestive of Q-fever were analysed by PCR. Throat swabs were collected within the first two weeks after the onset of clinical symptoms. Target sequence for PCR was the multicopy insertion element IS1111, known to be species-specific for *Coxiella burnetii* [9]. Less than 10% percent of the serologically confirmed cases were positive by PCR. Swab samples were collected from healthy volunteers and children with *Bordetella pertussis* infection, that were used as a control group, in order to exclude unspecific PCR results, false positives, or cross contamination. Other infectious agents (i.e., *Legionella pneumophila*, *Mycoplasma pneumoniae*, *Chlamydia pneumoniae*, influenza virus) were excluded through molecular methods [10].

To evaluate the extent of the spread of the infection, a sero-epidemiological survey of 104 pregnant women living in the town were also conducted. Moreover, 270 animals, including sheep (including flocks that had passed through the town), goats, and cattle were tested serologically, using the same laboratory methods described above.

RESULTS

Overall, 220 cases were identified between May 1 and June 9, 2004, with an attack rate of 5.96 per 1000 inhabitants. The epidemic curve is shown in (Figure 1). Of the cases, 168 were from the town of Botevgrad and the remaining 52 were from neighbouring villages of the same Municipality. Attack rate were higher in the town of Botevgrad (i.e., 8.19 per 1000) than in the other small villages of the Municipality (i.e., 3.18 per 100 000). The median age of the cases was 37.5 years; 22 patients were less than 5 years old, whereas 2 were older than 80 years. Regarding gender, 131 were men and 89 women (male-to-female ratio of 1.47:1).

![Fig. 1](image-url) Shows the epidemic curve observed during the outbreak. On the ordinate are reported the number of cases observed in each month reported on the abscissa.
Throat swab samples were available from 54 of the 220 serologically confirmed cases. Of them, 3 (5.5%) were positive for *C. Burnetii*. All the samples were negative for the other investigated agents. All the control group swabs were negative for *C. Burnetii*.

A serosurvey was conducted in order to evaluate the spread of the infection among humans. Of the 104 pregnant women, 8 (7.7%) were positive for anti-*C. burnetii* antibodies, confirming high levels of exposure to the agent in the local population.

Serological surveys were also conducted on different animal species with the aim of identifying possible sources of infection. Overall, 74 of the 270 (27.4%) tested animals were positive: *i.e.*, 21 of 100 sheep (21%), 20 of 50 (40%) goats, and 25 of 120 (20.8%) cattle.

**DISCUSSION**

This is the largest outbreak in Bulgaria in the last 20 years. Flocks of sheep and goats were the most likely source of infection, as suggested by the observation that, at the time of the outbreak, flocks grazed in, or had travelled on, the roads and the gardens of the town, and that the prevalence of anti-*C. burnetii* antibodies was high among animals in the area. However, since sheep and goats were present within the town well before the outbreak, other unidentified cofactors might have favoured the spread of the infection. Among these, lambing (*i.e.*, because of the high bacterial load of the placenta) and dry weather (*i.e.*, because of dust contamination and dispersion) merit special consideration. Actually, lambing did not appear to play a role: in fact, the epidemic occurred in the late spring, about one month after the birthing season; no newborn lambs were present in the flocks during the outbreak. Thus, we investigated whether the outbreak was due to dust dispersion favoured by a dry climate. May is usually a rainy month, but dust storms at the beginning of May were reported by people resident in the town of Botevgrad [11]. In particular, thunder storm with no rain precipitations were reported on the 2nd of May, whereas abundant precipitations (14 l/m²) on the 3rd of May; then, no more precipitations were reported (apart from 2 l/m² on the 7th) until the 10th of May. During the first half of May, wind speed more than 5 m/sec was reported for three days, from the 4th to the 6th of May.

Q fever outbreaks in urban areas are not common. A cluster in South Wales was probably due to indirect contact with animal secretions/excretions through contaminated straw [12], whereas distant exposure to sheep flocks was a determinant of an outbreak in a semi-urban area in Northern Italy [13]. Finally, a large Q fever outbreak due to sheep farming near residential areas has recently been reported in Germany; short distance from the grazing area, male gender, and age (25 to 62 years) were associated to higher risk of infection [14].

The outbreak was investigated as an urgent public health threat and several draconian measures were taken for a two-month period. All patients were hospitalised, and schools were closed for one month during the epidemic peak. All animals were kept outside the town in a controlled yard, and those passing through the town had to follow certain roads, which were disinfected every day. Sheep and goats were treated by washing, whereas cows were disinfected by spraying acaricides. Protective facial masks were used by people involved in the above mentioned activities. Moreover, severe fines were charged to farmers who did not remove animal excrement and disinfect places contaminated by animal faeces. Unemployed citizens were involved in cutting, transporting outside the town and then disinfecting or burning potentially contaminated material. Milk collection and drinking were forbidden.

In conclusion, this large outbreak highlights how zoonoses such as Q fever may represent a public-health threat also for urban populations and emphasizes the need for strengthening control measures against this emerging problem in the Balkans.

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