High seroprevalence of *Coxiella burnetii* infection in Eastern Cantabria (Spain)

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**Background** *Coxiella burnetii* is the causative agent of Q fever, a worldwide zoonosis which shows a wide diversity of clinical manifestations. The objective of this seroepidemiological study was to estimate the prevalence of antibodies to *C. burnetii* in a representative population of the Eastern area of the Cantabrian Community (Northern Spain).

**Methods** A cross-sectional study was conducted in 595 subjects from Eastern Cantabria. Four different zones (one urban and three rural zones) were defined according to the geographical characteristics of the area and the economic activities of the population. The population sample for this study was collected between December 1994 and March 1995, and was stratified by age, sex and municipality of residence. IgG and IgM antibodies against *C. burnetii* were assayed by an indirect immunofluorescence technique.

**Results** The prevalence of anti-phase II *Coxiella* IgG (titre $\geq 1:16$) was 48.6% (95% confidence interval: 44.6–52.6%), with 81.3% having a titre of $\geq 1:64$. The prevalence of IgG increased with age, ranging from 12.0% in people <15 years to 70.3% in those $\geq 65$ years ($P < 0.001$). The antibody detection rate was higher in males (53.6%) than in females (43.3%) ($P < 0.01$). The prevalence of anti-phase II *Coxiella* IgG was less among subjects living in the urban zone (32.8%) than in those living in the other three rural zones: the two situated on the coast (54.0% and 54.9% respectively) and the inner mountainous zone (82.3%) ($P < 0.001$).

**Conclusions** The prevalence of *C. burnetii* infection observed in this study is one of the highest reported in Europe. The eastern area of Cantabria is a hyperendemic area for Q fever.

**Keywords** Q fever, *Coxiella burnetii*, antibodies, prevalence, seroepidemiological study

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Q fever is a zoonosis distributed throughout the world and caused by *Coxiella burnetii*. Ungulate mammals (sheep, goats, and cattle) are the main reservoirs and the most important source of infection for humans. Q fever has a broad spectrum of clinical manifestations ranging from asymptomatic infections to a febrile syndrome with or without signs of pneumonia, hepatitis or other symptomatology. The infection may become chronic, producing endocarditis, osteoarthritis, etc. Q fever is an endemic disease in Spain and its incidence seems to be particularly high in the north of the country, especially in the Basque Country. However, there is little information on the prevalence of past infections for large areas of Spain. In this paper we report the findings of a seroepidemiological study of Q fever carried out in Eastern Cantabria, in the north of Spain.

**Methods**

**Geographical description**

The Cantabrian Community resides in a mountainous area fronted by the Cantabrian Sea (Atlantic Ocean). Its eastern area (the study area) is 1032 km² and has a population of 74 936 inhabitants (Figure 1). The eastern area has mild climate with high and regular rainfall; the vegetation is composed of eucalyptus plantations, brushwood, and extensive grasslands and pastures. Cattle are the predominant livestock (around 80 000 head). Based on the geographical characteristics of the study area and the economic activities of the population, four zones can be defined: (1) Urban zone: consisting of centres of population located on the coast (Laredo, Santona and Colindres)
whose economy depends on fishing and service sector activities; and (2) and (3) western and eastern coastal zones, situated on either side of the urban zone. These zones are formed by foot-hills of varying heights up to 500 m which become larger in the eastern zone. They have small towns (Meruelo, Gama, Guriezo, Limpias and Ampuero) or larger concentrations of population (Castro-Urdiales) involved in rural and farming activities. The inner mountainous zone (4) has deep valleys and mountains as high as 1000 m. The population here works in farming activities (Alto Asón and Voto).

Population study
The population sample for our seroprevalence study consisted of 595 subjects (302 males and 293 females) enrolled between December 1994 and March 1995. The number of subjects included in this group was determined on the basis of the size of population in Eastern Cantabria (1993 Census population6), and the results of a previous study which showed a seroprevalence of 48% (worst acceptable result of 44%, 95% confidence level). The group was stratified according to the age, sex and municipality of residence. A predetermined quota was chosen from each stratum of consecutive subjects attending for outpatient care in the Public Health Centres and for whom their practitioners requested a routine (checkup or diagnostic) laboratory test. Patients who were suspected of having acute infectious disease or were immunosuppressed were excluded. A serum sample from each subject was stored at —40°C until processing.

Laboratory methods
The presence of IgG against C. burnetii was measured by an indirect immunofluorescence technique (IFI) using commercial slides containing C. burnetii antigen in phase II and anti-human IgG goat immunoglobulins marked with fluorescein isothiocyanate (BioMérieux, Marcy l’Etoile, France). The sera were initially tested against C. burnetii IgG at 1:16 dilution (phosphate buffered saline pH 7.6) and further studied at higher dilutions that produced reactivity. An antibody titre of $\geq 1:16$ was considered indicative of previous infection. The positive sera for IgG were tested in order to detect specific IgM against C. burnetii antigen in phase II (BioMérieux) at 1:64 dilution, after absorption with anti-IgG (RF Absorbens, Boehringerwerke, Munich, Germany). For those sera with IgG against C. burnetii (phase II) $\geq 1:1024$, the presence of IgG against phase I C. burnetii was also investigated by IFI. (Antigen in phase I was kindly provided by Dr Kazar, Virological Institute of Bratislava, Slovak Republic.)

Data analysis
Standard statistical methods were used for data analysis. The $\chi^2$ and $\chi^2$ for trend (Mantel-Haenszel) tests were used to compare prevalence. The level of significance chosen was $\alpha = 0.05$.

Results
Of the 595 subjects studied, 289 had anti-phase II Coxiella IgG (48.6%, 95% CI: 44.6–52.6%). A progressive increase in the seroprevalence of this antibody according to age was observed, so that 12.0% of the subjects <15 years (10/83) showed anti-phase II Coxiella IgG, increasing up to 70.3% in the subjects >65 years (78/111) ($\chi^2$ for trend = 95.4; $P < 0.001$) (Figure 2). The mean age ±SD of the group was 44.8 ± 21.8 years (range 1–91: <14 years old [83], 15–30 years [86], 31–45 years [114], 46–64 years [201], >65 years [111]). Of these subjects, 262 lived in the urban area, 91 lived in the western coastal zone, 163 in the eastern coastal zone and 79 in the mountainous area. Anti-phase II Coxiella IgG was observed in 86.4% (19/22) of the subjects >80 years. The antibody detection rates in males and females were 53.6% (162/302) and 43.3% (127/293) respectively ($\chi^2 = 6.3; P = 0.01$; odds ratio [OR] = 1.51, 95% CI: 1.08–2.12). The prevalence of anti-phase II Coxiella IgG was lower among the population who lived in the urban zone (32.8%; 86/262) than those observed among people living in the western coastal zone (54.9%; 50/91), the eastern coastal zone (54.0%; 88/163) and in the inner mountainous zone (82.3%; 65/79) ($\chi^2$, 3 d.f. = 65.3, $P < 0.001$) (Table 1). Among the antibody-positive subjects, 54.7% (158/289) had low titres (38/158 had 1:16; 16/158 had 1:32; and 104/158 had 1:64), 41.2% (119/289) intermediate titres (1:128–1:512) and 4.2% (12/289) high titres (>) 1:1024. In the latter, the presence of anti-phase I Coxiella IgG was investigated. In eight subjects this antibody was below the detection limit (<1:64), and it was positive in four subjects at low titre: 1:64 (2); 1:128 (1); and 1:256 (1).

Thirty-five subjects (5.8%) showed anti-Coxiella IgM and five of them (0.8% of the total) had titres of $>1:256$. In five instances the presence of specific IgM $>1:1024$ and IgM $>1:64$ (one of them with IgM $>1:256$) was detected at the same time. Anti-Coxiella IgM was detected in 1.2% of the sera from subjects <15 years; prevalence increased slowly up to the 9.4% observed among the 46–64 year old group ($\chi^2$ for trend = 8.1; $P = 0.004$) but it decreased in the >65 years group (3.6%) (Figure 2).

Discussion
The data obtained in this study showed that C. burnetii infection is common in Cantabria. Among those studied, 48.6% had anti-phase II Coxiella IgG. In seroepidemiological studies conducted in Spain, only in Salamanca province (west of Spain) has a similar prevalence rate been found: Of the 400 subjects from
Coxiella burnetii.

Studies of

0.001.

\( \text{area} \) lead to greater exposure to the microorganism. Similar obser-

vations have been made in other provinces of Spain in which samples of rural and urban populations have been studied.8,9,11

In Cantabria, due to the number of cattle, it is presumed that cattle constitute the prime reservoir of C. burnetii. Studies of seroprevalence of C. burnetii infection among animals are scarce in Spain and there are no such studies in Cantabria. In Cata-

luña9 sera from cattle in 19 towns showed only five towns free of infection; the remaining 14 were distributed in two groups: one with a high proportion of animals infected (16.7–60.9%) and the other with a low proportion (1.7–7.1%). In another study from the Basque Country and Navarra, in 1981–1984, the seroprevalence of C. burnetii infection was: 1.8% among cattle, 2.4% among sheep, and 4.8% among goats.20

The increase in the prevalence of C. burnetii infection during life is expected because of cumulative exposure, and since IgG antibodies persist. In this study, almost all the subjects >80 years had been in contact with C. burnetii. Similar prevalence has only been noted in Spain in a group at high risk of contracting Q fever such as slaughterhouse workers,21 indicating that exposure to C. burnetii among the population of eastern Cantabria is very high. As reported in other studies, the prevalence of C. burnetii infection was higher in males than in females, probably because Q fever is an occupational disease.

The prevalence of anti-Coxiella IgM increases up to the sixth decade of life (Figure 2), and in contrast to IgG prevalence, decreases subsequently, indicating that the incidence of new infections at this time is lower since more subjects have already been infected. The age at which the highest IgM prevalence was observed among the subjects studied (adults) correlates well with the age at which the highest Q fever incidence is reported in other studies.1 Almost 6% of the investigated population had anti-Coxiella IgM. We do not believe that all these subjects had acute Coxiella infection. Probably, such a high rate is related to the persistence of this antibody.22 This makes an isolated determination of limited use in the diagnosis of the acute Q fever in our environment, especially if the titre is low. Therefore, in endemic areas, our data reinforce the use of restrictive serological criteria in order to confirm the diagnosis of an acute Q fever infection, e.g. IgM titre \( \geq 1:256 \) by indirect immunofluorescence, as recommended in the Basque Country.23

In the light of the data obtained in this study, the combined values proposed by Tissot et al. (IgM \( \geq 1:64 \) and IgG \( \geq 1:1024 \)) could also be accepted.24

In conclusion, the eastern region of Cantabria is a hyper-

demic area for Q fever. Taking into account the potentially serious consequences of this disease (pneumonia, hepatitis, end-}

docardiitis, etc.) it can be considered to constitute an important public health problem in our geographical area. It is essential to identify the reservoirs of this zoonosis in Cantabria in order to initiate any preventive and control measures which may be considered appropriate.

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References


