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Coxiella burnetii in bulk tank milk and antibodies in farm workers at Montería, Colombia¤

Coxiella burnetii en leche de tanque y anticuerpos en trabajadores rurales en Montería, Colombia

Coxiella burnetii em leite de tanque bovina e anticorpos em trabalhadores rurais de Montería, Colômbia

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Summary

Background: Q fever is a zoonotic disease caused by *Coxiella burnetii*. In Colombia it is not a notifiable disease in humans and is most likely under diagnosed. There are no studies about its prevalence in important reservoir species, such as cattle. **Objective:** the aim of this study was to investigate the frequency of *C. burnetii* infection in cattle farms and determine the frequency of antibodies in farm workers at risk in rural areas of Montería, Córdoba (Colombia). **Methods:** eleven cattle farms were randomly chosen to investigate the infection by *C. burnetii.* Bulk tank milk samples of each farm were analyzed by conventional PCR for DNA detection of transposase gene IS*1111* of *C. burnetii*. Serum samples from 61 apparently healthy people living in eight farms were analyzed by indirect inmunofluorescence against phase II IgG antibodies to *C. burnetii.* **Results:** we report the presence of *C. burnetii* DNA in 45% of bulk tank milk samples from cattle farms and a 61% frequency of antibodies (IgG phase II ≥1/64) in farm workers at risk. **Conclusion:** our results demonstrate the circulation of this bacterium in the studied farms in Montería, Colombia, showing that at-risk farm workers have a high antibody frequency.

Keywords: *cattle, prevalence of diseases*, *Q fever, serology, zoonoses.*

Resumen

Antecedentes: la fiebre Q es una zoonosis causada por *Coxiella burnetii*. En Colombia no es una enfermedad notificable en humanos y probablemente es subdiagnosticada. De otro lado, no se han realizado estudios acerca de su prevalencia en importantes reservorios como los bovinos. **Objetivos:** el objetivo de este

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estudio fue determinar la frecuencia de infección por *C. burnetii* en fincas de ganado bovino y determinar la frecuencia de la presencia de anticuerpos en trabajadores de fincas en riesgo en áreas rurales del municipio de Montería, Córdoba (Colombia). **Métodos:** once fincas de ganado bovino fueron aleatoriamente seleccionadas para investigar la frecuencia de infección por *C. burnetii.* Muestras de leche de tanque de cada finca fueron analizadas mediante PCR convencional para detección del gen transposasa IS*1111* de *C. burnetii*. Asimismo, se colectaron muestras de suero sanguíneo de 61 personas aparentemente saludables que vivían en ocho de las fincas estudiadas, las cuales fueron analizadas mediante el ensayo de inmunofluorescencia indirecta para detección de anticuerpos IgG contra fase II de *C. burnetii.* **Resultados:** en este estudio se reporta la presencia de ADN de *C. burnetii* en 45% de las muestras de leche de tanque de las fincas ganaderas estudiadas y una frecuencia de anticuerpos contra *C. burnetii* (IgG Fase II ≥1/64) del 61% en trabajadores de fincas en riesgo. **Conclusiones:** los resultados de este estudio demuestran la circulación de *C. burnetii* en las fincas estudiadas de un área de Montería, Colombia. También, los trabajadores de fincas en situación de riesgo presentan una alta frecuencia de anticuerpos contra este patógeno.

Palabras clave: *bovinos, fiebre Q, prevalencia de enfermedades, serología, zoonosis.*

Resumo

Introdução: a febre Q é uma zoonose causada por *Coxiella burnetii*. Na Colômbia não é uma doença de notificação obrigatória em seres humanos e é provavelmente subdiagnosticada. Além disto, não há estudos sobre sua prevalência nas principais espécies de reservatórios, como os bovinos. **Objetivos:** determinar a frequência de infecção por *C. burnetii* em fazendas de gado de leite e determinar a frequência de anticorpos em trabalhadores rurais em risco do município de Montería, Córdoba (Colômbia). **Métodos:** 11 fazendas de gado leiteiro foram selecionadas aleatoriamente para investigar a frequência de infecção por *C. burnetii*. Amostras de leite do tanque de cada fazenda foram analisadas por PCR convencional para a detecção do gene IS*1111* transposase de *C. burnetii*. Além disso, amostras de soro de 61 pessoas aparentemente saudáveis que vivem em oito das propriedades estudadas foram analisadas por imunofluorescência indireta para a detecção de anticorpos IgG contra *C. burnetii* fase II. **Resultados:** neste estudo, o DNA de *C. burnetii* foi encontrado em 45% das amostras de leite do tanque, e uma frequência de anticorpos contra *C. burnetii* (fase II IgG ≥ 1/64) de 61% em trabalhadores rurais em risco. **Conclusões:** os resultados deste estudo demonstram a circulação de *C. burnetii* em algumas fazendas de gado em uma área de Montería, Colômbia. Além disso, os trabalhadores rurais em situação de risco têm uma alta frequência de anticorpos contra este patógeno.

Palavras chave: *bovinos, febre Q, prevalência de doenças, sorologia, zoonose.*

Introduction

Q fever is a zoonotic disease caused by *Coxiella burnetii* that concerns public health throughout the world. Domestic ruminants such as cattle, goats and sheep are known to be the principal source of human infection (Maurin and Raoult, 1999). Bacterial shedding occurs in mammals trough placenta, birth fluids, vaginal mucus, feces, and milk (Berri *et al*., 2001; Arricau-Bouvery *et al.,* 2003; Guatteo *et al.,* 2007a; Rodolakis *et al.,* 2007). *C. burnetii* is a frequent cause of reproductive disorders in these hosts (Berri *et al.,* 2002; Guatteo *et al.,* 2007b). In goats, *C. burnetii* has been reported as a cause of abortions and stillbirths in 19% of 211 cases reported in California, USA (Moeller, 2001). It was identified in Switzerland as a cause of abortions in 10% of 144 cases (Chanton-Greutmann *et al*., 2002). These reports are more abundant than those found for cattle and sheep (approximately 1% or less; Kirkbride, 1993; Muskens, *et al*., 2012). Metritis and infertility are clinical signs of infection in cattle; however the infection is frequently subclinical (Rodolakis, 2009) despite the occurrence of *C. burnetii* shedding (Rodolakis *et al.,* 2007).

Inhalation of dust contaminated with the bacteria from feces, urine or birth products from infected animals is the main source of infection in humans, resulting in several clinical manifestations; however, 60% of infections could lead to asymptomatic seroconversion (Maurin and Raoult, 1999). Most human patients experience a nonspecific febrile illness during acute infection. Atypical pneumonia or febrile hepatitis may also occur. The disease can become chronic, manifested as endocarditis, chronic hepatitis,

and osteomyelitis, or as a chronic vascular infection (Anderson *et al.,* 2013).

Only few cases of Q fever have been reported in Colombia, though it could be more prevalent. A human case of endocarditis caused by *C. burnetii* was reported in Medellín, Colombia in 2012 (Betancur and Múnera, 2012) and at the end of that year a case of pneumonia caused by *Coxiella* was reported in Cali, Colombia (Cardona, 2012). There are no previous studies conducted in domestic ruminants. It is not known if people in contact with cattle are at risk of infection. The aim of this study was to investigate the frequency of *C. burnetii* infection in cattle farms and to determine the frequency of antibodies in farm workers from rural areas of Montería, Colombia.

Materials and methods

Ethical statement

The research ethics committee of the Institute of Tropical Biological Research at Universidad de Córdoba approved the study through Act 026-2011. Samples were taken after participants signed an informed consent.

Sampling

Eleven cattle farms in rural areas of Montería, Colombia (8º 48´4694" N; 75º 54´5415" W) were randomly chosen for *C. burnetii* infection research during July and August, 2012. These farms were chosen from a dairy area, which receives milk from 60% of the 3341 registered farms in Montería. The farms studied had a dual-purpose production system (milk and meat). All farms had between 150 and 600 animals and initial *C. burnetii* infection status was unknown. Cases of sporadic abortions and mastitis were common in all farms tested. 50 ml samples of bulk tank milk (BTM) per farm were collected into sterile plastic tubes and screened using PCR methods. Serum samples from 61 apparently healthy people who lived in eight farms were analyzed by indirect inmunofluorescence assay (IFA). Epidemiological data was gathered from all participants who were asked about the possibility of Q fever occurrence in the past. InfoStat software (Version 2013; Group InfoStat, FCA, Argentina) was used for statistical analysis (Di Rienzo *et al.*, 2013). A Chi square was conducted to establish dependence between variables obtained from humans and frequency of antibodies against *C. burnetii*.

DNA extraction and molecular detection of Coxiella burnetii

Milk samples and a negative control (sterile water) were subjected to DNA extraction following the manufacturer's instructions (Purelink, DNA mini kit, Invitrogen, CA, USA). DNA extraction was performed directly from 400 µl of homogenized whole milk and eluted in a final volume of 100 µl. To ensure there was no contamination, a negative control (sterile water) was included for DNA extraction. Purified DNA was stored at -20 ºC until use as a template for polymerase chain reaction (PCR). A conventional PCR was conducted with primers CoxP4 (5´-GGCTGCGTGGTGATGG; Genbank accession number: M80806) and CoxM9 (GTCCCGGTTCAACAATTCG), which amplify a 435 bp product of the transposase gene of *C. burnetii* (IS*1111*; Panning *et al*., 2008). Positive control was not used to avoid contaminations. Amplification was carried out in 50 µl total reaction volume with 1 PCR buffer 1X, 4 µl MgCl2 (25mM), 2 µl DNTPs (10 mM), 25 µl Taq Polymerase (made at home), 1.5 µl of each primer (5 µM), and 2 µl total DNA. The products were separated by electrophoresis on agarose gel (1.5%), stained with ethidium bromide and examined using an ultraviolet (UV) transilluminator.

Indirect inmunofluorescence assay (IFA)

Blood serum samples were used to detect IgG antibodies against phase II of *C. burnetii* using an indirect inmunofluorescence kit according to manufacturer's instructions (Ref. PCOBUI+II, Vircell, Granada, Spain). Sensitivity and specificity of this particular IgG test are 97 and 99%, respectively (Vircell, Granada, Spain). A negative and positive control was included in each assay, and the positive samples were confirmed. A title ≥ 1:64 was taken as suggestive of past or present infection. Positive sera with IgG titers ≥ 1:64 were diluted at 1:128, 1:256, 1:512, and 1:1024.

Results

Coxiella burnetii DNA was detected in 5 out of 11 BTM samples (45%). After sequencing positive samples, all of them were 100% identical to the sequence of *C. burnetii* strain CbuK 154Q (Genbank accession number CP001020). Location of farms with positive and negative bulk milk samples is indicated in Figure 1.

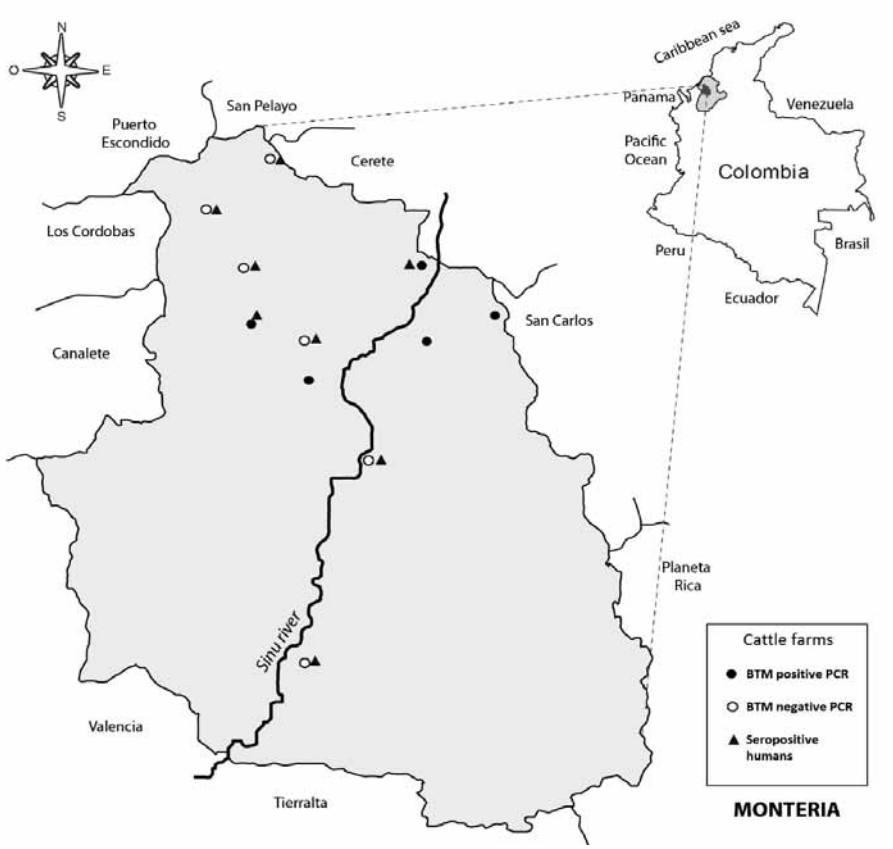

Figure 1. Detection of *Coxiella burnetii* in bulk tank milk samples from cattle farms, and antibodies in people at risk in Montería, Colombia. Black and white circles correspond to farms with positive and negative BTM by PCR, respectively. Triangles correspond to farms with seropositivity in humans.

Of the 61 people involved in the serological study, 52 were male and 9 female; 84% of men were farm workers and their mean age was 38.26 years (SD 13, 63). 61% (37/61) had IgG antibodies against *C. burnetii* phase II. End titers were determined for all seropositive participants: IgG II ≥1:64 (n = 24); ≥1:128 (n = 3), ≥1:256 (n = 7), and ≥1:1024 (n = 3). The epidemiological features of the studied population are summarized in Table 1. No differences were found between antibody frequency according to gender, age and time living in the area.

Discussion

We report the presence of *C. burnetii* DNA in BTM of cattle from Montería, Northern Colombia, and have not found similar reports in this country. These results demonstrate the circulation of *C. burnetii* in some cattle herds of an area of Montería. PCR analysis of BTM is a useful tool that could be used to detect, at large scale, infection by *C. burnetii* in cattle considering the low cost of the technique and the easiness of sample collection (Guatteo, 2007). In this study, the use of CoxP4 and CoxM9 primers (Panning *et al.,* 2008) for amplification of a fragment (435 pb) from IS*1111*gene allowed to increase assay sensitivity, because this gene is present at multiple copy number (7 to 110 copies), depending on *C. burnetii* strains (Klee *et al.,* 2006).

According to our findings, although the number of samples analyzed was low, BTM infection

Table 1. Epidemiological features of the population studied.

Variable	People tested (n = 61)	Frequency of IgG II antibodies against *Coxiella burnetii* (%)	p-value*
Sex			0.737
Male	52	60	
Female	9	67	
Occupation			0.205
Farm workers	46	65	
Farm owners	3	0	
Housewives	9	67	
Students	3	33	
Time living in the area (years)			0.99
≤5	18	56	
>5 to 15	16	56	
>15	19	63	
Age range (years)			0.258
≤15	2	50	
>15 to 30	20	45	
>30 to 50	25	76	
>50	14	57	
Direct contact with bovines			0.646
Yes	43	63	
No	18	56	
Previous work in another farm			0.655
Yes	36	58	
No	25	64	

*p ≤0.05

frequency was high (45%). These results are similar to a report from the United States, where 9 of 21 raw milk samples (42%) from seven states contained *C. burnetii* DNA (Loftis *et al.,* 2010). Other studies based on BTM testing showed that more than 90% of US dairy herds were infected with *C. burnetii* (Kim *et al.,* 2005). PCR detection of *C. burnetii* in bovine milk has not been totally standardized because bacterium excretion patterns by cattle can be intermittent or continuous (Rodolakis, 2007). We cannot conclude that the farms with negative results were totally free of *C. burnetii* infection (Guatteo *et al.,* 2007). Although small ruminants (sheep and goats) are also an important source of *C. burnetii* (Rodolakis, 2009), presence of these animals in the farms was not investigated in this study. Further epidemiological studies are necessary to establish the frequency of *C. burnetii* shedding in cattle and small ruminants.

People sampled worked for eight of the studied farms. Antibody presence was detected in people belonging to all BTM-negative farms and two farms

with positive BTM (Figure 1). We were not able to obtain human blood samples from the remaining three BTM-positive farms. A high frequency (61%) of IgG antibodies against *C. burnetii* phase II was found, but we cannot conclude that the infection of these individuals was acquired in the studied farms because 59% of them had previously worked in other farms. However, no differences were found in antibody frequencies in people with or without history of working in other premises (Table 1). All individuals tested in our study were clinically healthy and did not have a previous Q fever diagnosis, although febrile episodes were frequently recorded. According to these data, it is very feasible that human cases of Q fever are underdiagnosed in the studied area. Accordingly, physicians should be informed and look for this condition.

These results are higher than those informed in a previous study where 24% overall seroprevalence was found in rural workers from five villages of Córdoba and Sucre, Colombia (Máttar, 2006). Population-proportion differences of both studies were highly significant ($p<0.001$). However, seroprevalence among the five villages in the previous study varied from 0% to 61.5%, which is similar to our study. On the other hand, our results are higher than reports from other countries where Q fever is prevalent. A 3% seroprevalence was reported in Dane farmers in close contact with cattle (Bosnjak, *et al.,* 2012), and 19.5% seroprevalence was reported in healthy Turkish farm workers (Seyıtoğlu, *et al.,* 2006). The results of the present study are similar to a report from the Netherlands where overall seroprevalence in cattle farm residents was 72%. Of these, 87%, 54%, and 44% of farm workers, housewives and children had antibody titers, respectively. Antibody frequency in housewives and farm workers was similar in our study (Table 1).

In conclusion, our study suggests that *C. burnetii* is frequent in some Montería cattle farms. At-risk farm workers had high antibody frequency; all of them were clinically healthy and were not previously diagnosed with Q fever.

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Conflicts of interest

The authors declare they have no conflicts of interest with regard to the work presented in this report.

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