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Coxiella burnetii seroprevalence and associated risk factors in dairy and mixed cattle farms from Ecuador

Alfonso Carbonero[a,*], Lucía T. Guzmán[b], Karen Montaño[b], Alicia Torralbo[a], Antonio Arenas-Montes[a], Luis R. Saa[b]

[a] *Department of Animal Health, Veterinary Faculty, Campus de Excelencia Internacional Agroalimentario ceiA3, University of Cordoba, Córdoba 14014, Spain*

[b] *Departamento de Ciencias Agropecuarias y de Alimentos, Laboratorio de Sanidad Animal y Zoonosis, Universidad Técnica Particular de Loja, Loja 110150, Ecuador*

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ABSTRACT

Q fever is a zoonotic disease caused by *Coxiella burnetii*, a bacterial agent for which ruminants are the main reservoir. An extensive cross-sectional study to determine the seroprevalence of and associated risk factors for Q fever was performed in dairy and mixed (dairy–beef) cattle herds in Ecuador. A total of 2668 serum samples from 386 herds were analyzed using an ELISA. In addition, a questionnaire with 57 variables related to management, feeding, facilities, biosecurity and animal health was completed for every cattle farm. A Generalized Estimating Equations model was used to determine the factors associated with *C. burnetii* seropositivity.

The true prevalence of *C. burnetii* seropositivity in dairy and mixed cattle from Ecuador reached 12.6% ($CI_{95\%}$: 11.3–13.9%). The herd prevalence was 46.9% (181/386) ($CI_{95\%}$: 41.9–51.9%), and the within herd prevalence ranged between 8% and 100% (mean: 25.0%; Q1: 12.5%, Q2: 25.0%, Q3: 37.5%). Four factors were included in the GEE model for *C. burnetii* seropositivity: age of the cattle (OR: 1.01; $CI_{95\%}$: 1.006–1.014), feeding of calves with milk replacers (OR: 1.94; $CI_{95\%}$: 1.1–3.3), bovine respiratory syncytial virus seropositivity (OR: 1.54; $CI_{95\%}$: 1.1–2.3), and disinfection of the umbilical cord (OR: 0.60; $CI_{95\%}$: 0.4–0.9).

1. Introduction

Coxiella burnetii, the causative agent of query (Q) fever, is an obligate intracellular bacterium that can infect a wide variety of hosts such as arthropods (particularly ticks), fish, reptiles, birds and several mammals, including humans (Cutler et al., 2007). This bacterial agent, previously included in the family *Rickettsiaceae*, has been recently classified into the Legionellales order and the *Coxiellaceae* family (Bielawska-Drózd et al., 2013). *C. burnetii* can exist in two forms or phases, the smaller of which is a spore-like structure that can persist for long periods in the environment (Marrie, 2010).

Q fever in humans is frequently observed as an acute flu-like illness characterized bya mild form of pneumonia or hepatitis that usually resolves within two weeks (Raya Cruz et al., 2013). However, the infection is sometimes asymptomatic or may cause a more severe, chronic form of endocarditis (Edouard et al., 2014) or arthritis (Angelakis et al., 2014) that could lead to death.

In ruminants, *C. burnetii* mainly causes reproductive disorders (spontaneous abortion, premature delivery,

* Corresponding author. Tel.: +34 957 21 87 25; fax: +34 957 21 87 25.
E-mail address: sa1camaa@uco.es (A. Carbonero).

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stillbirth and weak offspring) in pregnant ewes, goats and cattle (Agerholm, 2013) as well as metritis and infertility in cows (EFSA, 2012). Domestic ruminants are considered to be the most important reservoirs for this agent (Alvarez et al., 2012), as aerosols from ruminants are the main source of infection for humans (Isken et al., 2013). Moreover, this agent can be excreted in milk, urine and faeces and in high numbers in the amniotic fluid, aborted tissues and placenta at birth (EFSA, 2012). A high contamination rate has been reported in dairy products, especially if unpasteurized (Eldin et al., 2013). *C. burnetii* is a highly resistant bacterium, and thus, the environment itself can serve as a reservoir (de Bruin et al., 2013). The importance of ticks in the transmission of *Coxiella* infection remains unclear (Psaroulaki et al., 2006; Pluta et al., 2010; Knobel et al., 2013).

C. burnetii infection in humans has been reported worldwide, with the exception of New Zealand (Cutler et al., 2007). Evidence of *C. burnetii* infection has been reported from all continents: Africa (Dean et al., 2013), Asia (Chang et al., 2010), Europe (Van den Brom et al., 2013), Oceania (Tozer et al., 2011), North America (Anderson et al., 2009) and South America (da Costa et al., 2005). The seroprevalence of *C. burnetii* infection in humans ranges from less than 1% in Canada (Messier et al., 2012) to 52.7% in Cyprus (Psaroulaki et al., 2006). However, the prevalence reaches 65.1% when evaluated in high risk groups (veterinarians) from The Netherlands after the outbreak that occurred between 2007 and 2009 (Van den Brom et al., 2013).

In ruminants, the same geographical distribution of infection has been reported. Studies of *C. burnetii* infection have been published in Africa (Klaasen et al., 2014), Asia (Asadi et al., 2013), Europe (Czaplicki et al., 2012), Oceania (Cooper et al., 2011), North America (Hatchette et al., 2002) and South America (de Ruiz, 1977). The seroprevalence is often higher in ruminants than it is in humans and ranges from 1.1 to 58.4% in sheep (Lange and Klaus, 1992; Dorko et al., 2010), 6.5 to 65.8% in goats (Khalili and Sakhaee, 2009; Pape et al., 2009) and 0.6 to 46.6% in cattle (Htwe et al., 1992; Banazis et al., 2010). The risk factors for *C. burnetii* infection in cattle include female sex, old age, drinking water from a watercourse, and belonging to large herds or dairy herds (McCaughey et al., 2010; Czaplicki et al., 2012; Paul et al., 2014).

Extensive cattle production systems are used in Ecuador. Blood-sucking arthropods such as ticks, which have been described as *C. burnetii* reservoirs (de Bruin et al., 2013), are common in Ecuador, but control measures against tick-borne infection are exceptionally applied. Commercial vaccines for ruminants against *C. burnetii* are not available in Ecuador.

Although the presence of *C. burnetii* in South America has been reported (da Costa et al., 2005), only a single epidemiological study in ruminants was performed 37 years ago. As a consequence, the importance of ruminants as reservoirs and the dissemination of this pathogen are currently unknown. In Ecuador, *C. burnetii* infection in humans has been reported (Manock et al., 2009), but there are no studies in ruminants. The aims of this study were to establish the individual and herd prevalence of *C. burnetii* seropositivity in dairy and mixed cattle farms in Ecuador as well as to determine the distribution of and risk factors associated with this infection.

2. Materials and methods

2.1. Study area

This study was carried out from June 2008 to December 2010 in the main milk-producing provinces of Ecuador (Azuay, Chimborazo, Cotopaxi, Manabí, Pichincha, Santo Domingo, Tungurahua and Zamora-Chinchipe) (Figs. 1 and 2), which produce more than 75.9% of the milk in Ecuador (Instituto Ecuatoriano de Estadísticas y Censos, 2000). Only dairy and mixed (dairy–beef) cattle farms were included.

Ecuador is located in South America and covers an area of 257,217.07 km^2, extending from latitude 01°27′06 N to 05°00′56 S and from longitude 75°11′49 E to 81°00′40 W (WGS84). The continental part of Ecuador is naturally divided into the following three geographical areas: the coast in the west, the Andean mountain system in the central area and the pre-Amazonian forest in the east. As a consequence, Ecuador has many microclimates and the average temperature ranges from 4.4 to 32.1 °C depending on the area. The average annual rainfall varies between 96.7 and 4360 mm depending on the location (Instituto Geográfico Militar, 2013).

2.2. Study design

The following values were used to calculate the required sample size: a 50% expected prevalence (this was selected because there were no previous studies in this area), a 95% confidence interval and an acceptable error of 5%. A minimal sample size of 385 herds was obtained using Win Episcope 2.0.

Due to the lack of a detailed herd and cattle identification system in Ecuador, the following procedure for herd selection was used. During the first stage, we performed a stratified sampling according to the number of herds in each sampled province considering the total number of herds in all provinces included in the study. Thus, we obtained the number of herds to be sampled in every province (Table 1). In the second stage, each province was divided into blocks of 25 km^2. Blocks were randomly selected using random numbers software. Initially, it was unknown how many blocks had to be sampled per province because the number of herds per block was unknown. As a consequence, according to the software, all farms of the first block were visited (cluster sampling), then the second block and so on until the number of herds per province was completed. During the third stage, individual cattle within each herd were randomly selected by means of random number tables. Due to the potential presence of maternal antibodies, only cattle older than 6 months were eligible for selection to avoid false positive test results.

The sample size for detecting *C. burnetii* antibodies within each herd was also calculated with WinEpiscope 2.0, given a within-herd prevalence of 25% (based on preliminary studies), a herd size of 1000 (greater than