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Serological Evidence of Antibodies Against *Coxiella burnetii* in Sheep Flocks of Mexico

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Abstract: The study aimed to determine the frequency of *Coxiella burnetii* in sheep flocks located in some states of Mexico. The analysis of 5.552 serological samples was carried out in 317 flocks in 61 municipalities of seven states of the Mexican Republic. Serology was performed with a commercial ELISA-kit and the frequency of *C. burnetii* seropositive animals was used to calculate the real prevalence per animal and flock level by state. The apparent prevalence of *C. burnetii* by flock in each state was: Sonora 50%; Chiapas 35.1%; Queretaro 28.1%; Hidalgo 28.3%; Tlaxcala 27.8%; Chihuahua 27% and the State of Mexico 10.9%. The real prevalence by state was: Tlaxcala 13.2% (IC95 10.4-16.0%); Querétaro 12.6% (CI95 10.4-14.9%); State of Mexico 12.4% (CI95: 10.0-14.8%); Chihuahua 11.8% (IC95: 9.7-13.9%); Hidalgo 11.0% (IC95: 8.9-13.1%); Chiapas 7.9% (IC95: 5.8-10.1%) and Sonora 2.5% (IC95: 1.5-3.4%). Although, Q-fever is considered an exotic disease in Mexico, this study evidenced the presence of antibodies against *Coxiella burnetii* in sheep flocks located in seven states of Mexico.

INTRODUCTION

Coxiella burnetii is an obligate intracellular bacterium and is the causal agent of Q-fever in humans and ruminants. This bacterium is worldwide distributed and it has been found in ticks, birds and other mammalian species[1-2]. Coxiellosis in cattle is predominantly asymptomatic, although, as a reproductive syndrome, causes spontaneous abortion, delivery of weak offspring or infertility[3]. Infected animals often excrete *C. burnetii* in milk, urine, feces and by placentas the fluids of abortions or delivery. Although, ewes are generally asymptomatic carriers, they are reported to be capable of shedding high amounts of bacteria during parturition and intermittently in various secretions[4-5]. The life cycle of *C. burnetii* includes a sporiform phase in which it has the ability to resist desiccation, allows the bacteria to be carried by the wind and is capable of produce outbreaks in far places from the initial infection focus[4].

The first serological evidence of C. burnetii in Mexico was reported by Silva[6] with 2 and 1.8% of seropositive cases at the Comarca Lagunera and Mexico City, respectively. The first pilot study in Mexico was carried out in the state of Hidalgo by

Araujo-Melendez *et al*.[7] after receiving three cases diagnosed with Q-fever, the research group decided to study the presence of antibodies against *C. burnetii* in patients with or without symptoms, finding 10.69% of persons with antibodies; the seroprevalence in patients with and without clinical signs related with risk factors to infection was 47.83 and 4.41%, respectively.

In Mexico, *Coxiella burnetii* is considered an exotic disease and is included in the Group 1 of the official notification diseases[8]. Precisely because it is considered exotic in our country, there are no diagnostic tests available for the opportune detection of the agent or the presence of antibodies in farm animals. However, in the state of Nuevo Leon, Mexico, a study reported the prevalence of antibodies against *C. burnetii* in domestic ruminants which suggests the presence of the microorganism in the national territory for several years[9].

For years, the introduction to Mexico of sheep and rams from countries (where the coxiellosis is endemic) has been happening mainly for genetic purposes but without a diagnostic test. The ease of infection and spreading of *C. burnetii*, the suggestive clinical cases observed by veterinarians and producers as well as the previous reports of the presence of the bacteria in humans, suggest the presence of *C. burnetii* in different sheep flocks in different states of Mexico. Due to its rapid spread and economic impact on animals and public health, justifies the serological study in the Mexican Republic to measure the prevalence of the disease and to generate more effective diagnostic tests and control strategies. Therefore, the objective of this study was to determine by serology the frequency of *Coxiella burnetii* in sheep flocks in seven states of Mexico.

MATERIALS AND METHODS

Study design and animal sampling: In a cross-sectional study, 5.552 sera from female sheep were collected from 317 flocks located in 61 municipalities of seven states of Mexico: Hidalgo, Tlaxcala, Querétaro, Chihuahua, Sonora, Chiapas and Mexico. These states were selected for their representativeness in the ovine activity and background of active animal mobilization for genetic improvement purposes.

The proportion of animals sampled was calculated according to the known population of each state (Table 1) using the formula:

$$n = \frac{(N \times Z_{\alpha}^{2}) \times (p \times q)}{e^{2}(N-1) + Z_{\alpha}^{2} \times (p \times q)}$$

Where:

N = Known value of the population of each state of Mexico

$Z\alpha^2$ = The value of the desired normal distribution

p = The unknown prevalence value (p = 0.50) for *C. burnetii* (q = 1-p)

e^2 = Desired precision

Most of the females were of Mexican origin and the imported came from Australia, New Zealand and the United States of America. The initial flock selection was carried out with the approval of specialized associations of sheep producers and the support of local technicians. The flocks were randomly selected. To preserve the proportion and representativeness of the population of each flock, 20% of the females with >7 months into the flock or with abortions background. In the case of small flocks (<30 animals), 10 animals were sampled.

Samples collection and serological diagnosis: With puncture of the jugular vein (Vaccutainer® Extraction Equipment), approximately 10 mL of blood was taken in vacuum tubes and were transported (4°C) to the Small Ruminant Diseases laboratory of the Centro Nacional de Investigacion Disciplinaria en Salud Animal of the Instituto Nacional de Investigaciones Agricolas, Forestales y Pecuarias (INIFAP), in Mexico City. The blood samples were centrifuged 3000×g for 10 min and the serums were extracted and transferred to microtubes (1.5 mL) and stored at -20°C. The serological diagnosis was determined by indirect-ELISA using a commercial kit (Q-Fever AB Test *Coxiella burnetii* Idexx Pourquier, Montpellier, France IDEXX®) to identify IgG antibodies against *Coxiella burnetii* from sera samples. This test has a sensitivity and specificity between 89-95% and 100%, respectively. The plates were read (BioRad® plate reader) according to the manufacturer's

Table 1: Animal population, the number of flocks sampled and the seroprevalence of *Coxiella burnetii* by state

	Flocks				Animals				
State	*N	n	(+)	$_{flo}$p (%)	n	(+)	$_{app}$p (%)	$_{real}$p (%)	CI95 (%)
Queretaro	152,720	64	18	28.1	815	105	12.9	12.6	10.4, 14.9
State of Mexico	1,379,974	55	6	10.9	758	96	12.7	12.4	10.0, 14.8
Hidalgo	1,131,718	46	13	28.3	855	97	11.3	11.0	8.9, 13.1
Tlaxcala	284,037	36	10	27.8	558	75	13.4	13.2	10.4, 16.0
Chihuahua	306,348	37	10	27.0	925	112	12.1	11.8	9.7, 13.9
Sonora	68,626	42	21	50.0	1.050	35	3.30	2.50	1.5, 3.4
Chiapas	306,348	37	13	35.1	591	50	8.50	7.90	5.8, 10.1
Total		317	100	28.7	5,552	570	10.3		

*Servicio de Informacion Agroalimentaria y Pesquera (SIAP); $_{flo}$p = apparent prevalence of *C. burnetii* at flock level; $_{app}$p = apparent prevalence of *C. burnetii* in animals; $_{real}$p = real prevalence of *C. burnetii* in animals

specifications and the results in the perceptual absorption were interpreted as <30% negative ≥30% a <40% doubtful and ≥40% positive.

Statistical analysis: The apparent prevalence ($_{app}p$) was calculated using the frequency of seropositive animals *Coxiella burnetii* (COX^+) in the ELISA test in relation to n ($_{app}p = COX^+/n$) of each state. Aflockwas considered positive if had at least one COX^+. The prevalence of seropositive flocks ($_{flo}p$) was in relation to the number of sampled flocks by state. The real prevalence ($_{real}p$) of *C. burnetii* and the confidence interval at 95% (CI95) were calculated on the WinEpi platform (http://www.winepi.net/), considering the N of each state, the sensitivity (95%) and specificity (100%) of the ELISA test.

RESULTS AND DISCUSSION

From 5.552 serum samples collected, 570 were positive in the serological ELISA test to detect antibodies against *Coxiella burnetii* (Table 1). The detected prevalence in flocks and animals were $_{flo}p = 28.7\%$ and an $_{app}p = 10.3\%$, respectively. The State of Mexico had the lowest proportion of affected flocks ($_{flo}p = 10.9\%$) while Sonora was the state with the highest number of affected flocks ($_{flo}p = 50.0\%$). Even when Sonora had the highest distribution of *Coxiella burnetii* in flocks was the state with the lowest number of COX^+ animals ($_{real}p = 2.5\%$ (CI95: 1.5, 3.4%).

A retrospective review of the frequency of Q-fever of 34 studies carried out (from 1970-2010) in countries of five continents, presents 23 studies of individual prevalence and 11 studies of sheep flocks, reporting a crude average of 15%. However, at the flock level, there were dissimilar frequencies ranging from 0-83% and Oceania was the unique continent without positives sheep[10].

The Q-fever received international attention after an outbreak in the Netherlands and other European countries that involved humans and domestic animals[11]. In Pakistan, a prevalence of 17.9% was reported in sheep using the serological ELISA test[12]. In another study, two commercial ELISA (IDDEX and Pourquier) tests and the complement fixation test were compared for the diagnosis of *C. burnetii* in domestic ruminants, both commercials ELISA tests were able to detect the majority of infected animals[13].

According to the agreement settled down in Mexico, among the exotic and endemic diseases and pests for official notification in terrestrial and aquatic animals, *C. burnetii* is classified in Group 1. In this grouping, there are exotic diseases and pests absent in the Mexican territory or have been eradicated from the country. Due to their ease and rapid dissemination can affect the terrestrial and aquaculture animal population and in some cases such as *C. burnetii* can be a risk to public health. These diseases are considered as official immediate notification to the National System of Epidemiological Surveillance of Mexico[8]. However, in Mexico there is a background of the presence of *C. burnetii* in both animals and humans.

In the state of Nuevo León, 450 serums were collected from dairy cattle, 190 from beef cattle, 90 from sheep and 60 from goats, from different herds of that state, finding a seropositive frequency of 28, 10, 40 and 35%, respectively[9]. This suggests that the disease can spread to other species besides sheep including humans. In Mexico there are three reports in humans; one of them dates back in 1998 of a reported case in a 10-year-old girl with vegetative endocarditis in the aortic valve, fever, hepatomegaly, splenomegaly and the bacteriological isolation from blood was negative but the serology for *C. burnetii* was positive[14]; the second case was in the state of Hidalgo of a 24-year-old man (a hardware store employee) who had close contact with animals (dogs, cattle, horses and sheep), presented intense headache, fever (39º-40ºC) every day predominantly during the evening, moderate diaphoresis, accompanied with myalgias, arthralgias, hypoxia, general malaise and weight loss (not quantified), *C. burnetii* was diagnosed by the compatible clinical signs and suggestive serology by indirect immunofluorescence[15]. The third case of Q-fever was reported in 2009, the patient presented a hepatic granuloma with no epidemiological history of the disease and no typical clinical signs of Q-fever[16].

CONCLUSION

The present study confirmed the presence of specific antibodies against *C. burnetii* in sheep flocks of the Mexican territory, despite Q-fever is considered an exotic disease in Mexico.

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Bioethical standards: The procedures in the sampled animals of this study were done before the consent of each flock owner and performed by veterinarians in agreement with the ethical standards of the Declaration of Helsinki written in 1964 (including the later amendments in 2008) and were approved by a group of researchers of the INIFAP and ITSON.

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