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ORIGINAL ARTICLE

Serologic survey in animals of 'Q' fever in Nuevo Leon

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ABSTRACT. The serological prevalence of Q fever in Mexico is unknown. A serological survey for *Coxiella burnetii* was undertaken on a randomly selected population of dairy cattle, beef cattle, goats and sheep flocks. Serological examination of animal sera for antibodies against *Coxiella burnetii* was carried out by the ELISA technique. The 28% of the dairy cattle and 10% of beef cattle examinated were antibody positive. Sera from goats and sheep also had antibodies against this rickettsia, 35% and 40% respectively.

Key words: *Coxiella burnetti*, rickettsia, Q fever.

RESUMEN. La prevalencia serológica de fiebre Q en México es desconocida. Un estudio serológico de *Coxiella burnetii* se realizó en una población de ganado lechero, ganado de carne, ovinos y caprinos seleccionados al azar. Los exámenes serológicos de las muestras de los animales para detectar anticuerpos contra *Coxiella burnetii* fueron mediante la técnica de ELISA. El 28% del ganado lechero y el 10% del ganado de carne muestreado resultó seropositivo. El suero del ganado caprino y ovino presentaron también anticuerpos contra esta rickettsia, observándose un 35% y 40%, respectivamente.

Palabras clave: *Coxiella burnetti*, rickettsia, fiebre Q.

INTRODUCTION

Q fever, caused by the rickettsial organism *Coxiella burnetii*, is a zoonotic infection endemic worldwide in a great variety of vertebrates and invertebrates.[24,27] In cattle, goats and sheep, the organism is found in high concentration in the placenta, amniotic fluid, and other parturient products.[38] In the United States of America human infection has been most often associated with direct contact with aerosols generated during parturition of domestic animals.[21]

In humans most of Q fever cases occur in meat or milk processing plants, slaughterhouses, veterinary schools and research centers, where there is direct contact with animals or their products.[16] Man is the only host of this microorganism known to develop clinical symptoms of infection.[18] The disease is highly infectious with at least two viable microorganisms.[22]

The principal modes of entry of this bacterium into humans are via inhalation of contaminated dust particles and aerosols generated in the milieu of abattoirs as well as dairies and ingestion and handling of infected milk.[3] Intrauterine or neonatal infections may be possible, since *Coxiella burnetii* has been isolated from human placenta and breastmilk.[12] After infection in human a variety of clinical symptoms may be seen. Reports indicate that many cases are mild asymptomatic. Fever for 5 to 57 days is present and symptoms such as severe headache, chills, sweating, cough, painful (and tightness) chest, nausea, abdominal pain, myalgia and bradichardia may be present, and the isolation of the microorganism can be done during the febrile stage from blood or urine of most patients.[10] Others symptoms are shivers and astema o adinamia.[34] *Coxiella burnetii* is an important primary cause of pneumonia and hepatic disease.[19,23,25]

Rickettsial diseases like spotted fever and Q fever may pose a serious public health problem when they are nondiagnosed or misdiagnosed. Although rickettsiae can be isolated from or detected in clinical specimens, a serological test still remain an indispensable tool in the diagnosis of rickettsial diseases.[17] Diagnosis of Q fever can be made by isolation of the organism from clinical specimens by animal inoculation or tissue culture, but this is difficult for most clinical laboratories. The fixation complement (CF) technique has been used to detect antibody response to this disease.[5]

Other methods of serological diagnosis which have been described for *Coxiella burnetii* include agglutination,[15] immunofluorescence test,[2] and ELISA.[28] The diagnosis of this disease requires a high index of suspicion, and in endemic areas serological testing of patients with clinical symptoms suggesting Q fever is recommended. A serological investigations revealed that four patients were suffering from acute Q fever after they had been present at lambing and sheepshearing.[34,35] This disease is a zoonosis related to the existence of the rickettsia in infected animals.[36] In the present paper we study the seroprevalence in domestic ruminants of the disease as a risk factor associated with our veterinary duties in the practice with living animals.

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MATERIAL AND METHODS

Serum. Samples of blood were obtained from the jugular vein of 450 dairy cattle, 190 beef cattle, 90 sheep and 60 goats using Vacutainer® tubes containing separator gel from different herds of Nuevo Leon State in northeastern Mexico with unknown Q fever history. All animals were adults, males and females. Blood were centrifuged at 3000 rpm for 10 min and the serum collected and stored at –20°C until serologic examination for antibodies against *Coxiella burnetii* was performed. Serum was inactivated at 60°C for 30 min.

ELISA procedure. Serum was diluted 1:100 in 0.05M Tris buffer (pH 7.4) containing 0.05% Tween 20 and 0.85% NaCl. The serum, tested for duplicate, was incubated in a microtitration plate coated with antigen[8] (4 mg per well) at room temperature for 90 minutes in a humid camera as described previously to detect antibodies against Q fever.[29] After washing Antiruminant IgG, peroxidase conjugate (1:200) in Tris buffer was added and incubated for 90 minutes. Then after the third wash, 100 µl of chromogen solution was added to each well and the plate was incubated for 20 minutes at room temperature. The developing green-colored reaction was stopped by adding 100 µl of 0.1M hydrofluoric acid. The microplates were read in an automatic ELISA reader (ELX800, Biotech Instruments, Inc.) at 405nm. To calculate the percentage of the ELISA results, which is defined as the binding capacity of the test serum to that of positive control sera on the scale of 0-100%, we used the next equation: (absorbance of the test serum–absorbance of the negative control serum/absorbance of the positive control serum–absorbance of the negative serum) X 100, and the results were analyzed according to a previously described formula to identify the positive and negative samples.[29] Positive and negative control serums were tested simultaneously with all samples.

RESULTS

The results obtained are shown in Table 1. *Coxiella burnetii* seropositive reactions were obtained in 25.5% of the sera tested from all animals (202 of 790).

Antibodies against *Coxiella burnetii* were detectable at titer 1:100 in all animal sera. Multiple reactors were present in all flocks. The highest percentage of positive animals was for sheep (40%), followed closely by goats (35%). It may be noted that these two species had the smaller number of animals tested, 90 and 60, respectively.

Cattle had the lowest prevalence of positive sera. Dairy cattle had a 28%, whereas in beef cattle the percentage of positive animals was 10%. Both dairy and beef cattle had the largest number of animals included in the study, 450 and 190, respectively.

Further increasing the number of sheep and goat tested will give the present evidence stronger support if the prevalence remain as shown.

DISCUSSION

Since its first description in Queensland, Australia, Q fever has been found throughout the world.[13]

In the United States of America, this disease has been reported in 31 States.[9] The present serological examination provides the first report of Q fever in some domestic animals in northeastern Mexico. Results of this small pilot study suggest a high prevalence of antibodies against *Coxiella burnetii*, where 202 of 790 animals reacted positively. The seroprevalence as a percentage of animals tested is 25.5%. Serological results, based on an enzyme-linked immunosorbent assay, indicated a high seroprevalence of 57% for Q fever in a report or this zoonotic disease in the state of Baja California, located in the northwestern region Mexico.[11,30] Serological studies conducted in many countries report seroprevalence in cattle from 6-82% .[1,4,6,7,20,26,32] The distribution of *Boophilus* ticks and a high seroprevalence in animals suggest that these ticks play a role in the transmission and maintenance of *Coxiella burnetii* infection in cattle.[33] The feaces of ticks infected are heavily contaminated with the microorganisms, which remain viable in the feaces for long periods of time and therefore may be a potential source of infection for man and animals.[23] Such infected feaces may become powdered and windborne, thereby infecting the upper respiratory tract of man and animals.[31] *Coxiella burnetii* was detected in 5 of 10 dust samples from a barn housing dairy cattle by the polymerase chain reaction (PCR) method.[37] The zoonosis must be considered as a truly global problem, both in terms of their distribution and the measures required for their control.[14] Some results confirmed that some infections occur frequently in farmers, where the contact with cattle and sheep is associ-

Table 1. Serological status of cattle, sheep and goats against *Coxiella burnetii.*

Animal species	No. of animals	No. positives	% positives
Dairy cattle	450	126	28
Beef cattle	190	19	10
Sheep	90	36	40
Goats	60	21	35
Total	790	202	25.5

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ated with Q fever.[7] Our results showed that exposure to *Coxiella burnetii* is common in animals in the state of Nuevo Leon Mx. The risk of Q fever on people who work with domestic animals is related to contact with the farm environment rather that any specific animal exposure.[35]

Antibodies against *Coxiella burnetii* are highly specific, and no cross-reaction with other microorganisms are known to occur.[8] Because antigen detection methods are currently specialized techniques that may require hazardous and relatively lengthy procedures, serological tests are more important in the diagnosis of Q fever than other diseases. In comparison with the FC test, ELISA and IFI test proved to be more sensitive, rapid, and reliable, and neither test showed any significant cross-reaction with known cases of illness caused by various rickettsiae.[8]

Use of ELISA for the detection of specific antibodies had also been shown to be a sensitive, rapid and reliable mean of screening large number of sera for epidemiological studies or as a reference method for the early and rapid detection of Q fever.[8]

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