Seroprevalence of *Chlamydia Abortus* in captive ruminants of Mexico

Seroprevalencia de *Chlamydia Abortus* en rumiantes en cautiverio de México

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ABSTRACT
Background. *Chlamydia abortus* is the etiological agent of ovine enzootic abortion and is the most common cause of reproductive failure in ruminants. In Mexico, the prevalence of anti-*Chlamydia abortus* IgG antibody was determined by ELISA assay and the molecular identification by PCR in sheep with a clinical history of abortion. However, information regarding the prevalence of *C. abortus* infections in wild mammals is unknown. Objective. The study aimed to determine the seroprevalence of *C. abortus* using a specific recombinant ELISA followed by the identification of risk factors associated with captive ruminants in the Zacango Zoo and Ocotal Park, Mexico. Methodology. A total of 103 specimens corresponding to 15 different species of captive ruminants at the Zacango Zoo and Parque Ocotal, Mexico, were tested to determine the presence in sera of antibodies against *C. abortus* using a specific recombinant ELISA kit. In addition, the influence of risk factors such as age, sex, and the site, were evaluated on seropositivity. Results. Seropositive species included bighorn sheep (*Ovis canadensis*) (1/2), barbary sheep (*Ammotragus lervia*) (4/23), red deer (*Cervus elaphus*) (1/2), and a domestic goat (*Capra hircus*) (1/3). A total frequency of 7/103 (6.8%) was obtained. The analysis of antibody values about risk factors showed that the older animals (3.1 years) were seropositive with a frequency of 7/80 (8.7%) and three males (3/47) and four females (4/56) also were seropositive; however, no statistical significance was observed. Seropositive specimens from Ocotal Park (5/28) showed a higher risk compared to Zacango Zoo. Conclusion. The prevalence of *C. abortus* in captive ruminants at both sites was low; however, the health impacts to these species are unknown. Further research is warranted on the transmission of *C. abortus* within captive ruminants, and diagnostic of domestic livestock, and humans.

Keywords: *Chlamydia abortus*, captive ruminants, serologic survey.

RESUMEN
Antecedentes. *Chlamydia abortus* es el agente etiológico del aborto enzoótico ovino y es la causa más común de falla reproductiva en rumiantes. En México, la prevalencia de anticuerpos frente a *Chlamydia abortus* se han determinado mediante ELISA y se ha realizado la identificación molecular de la bacteria a través de PCR en ovinos y caprinos con historia clínica de aborto. Sin embargo, la información sobre *Chlamydia abortus* en animals de fauna silvestre, sigue siendo desconocida. Objetivo. El objetivo del estudio fue determinar la seroprevalencia de *C. abortus* mediante un ELISA recombinante específico seguido de la identificación de factores de riesgo asociados con rumiantes en cautiverio en el Zoológico de Zacango y Parque Ocotal, México. Metodología. Se analizaron un total de 103 animales correspondientes a 15 especies diferentes de rumiantes en cautiverio en el Zoológico de Zacango y el Parque Ocotal, México, para determinar la presencia de anticuerpos contra *C. abortus* utilizando un kit ELISA recombinante específico. La influencia de factores de riesgo como la edad, el sexo y el sitio, fue evaluada sobre la seropositividad. Resultados. Las especies seropositivas incluyeron el borrego cimarrón (*Ovis canadensis*) (1/2), el borrego de Berbería (*Ammotragus lervia*) (4/23), el ciervo rojo (*Cervus*

elaphus) (1/2) y una cabra doméstica (*Capra hircus*) (1/3). La frecuencia total fue de 7/103 con un 6,8%. El análisis de los valores de anticuerpos en relación con los factores de riesgo mostró que los animales de mayor edad (3,1 años) fueron seropositivos con una frecuencia de 7/80 (8,7%) y tres machos (3/47) y cuatro hembras (4/56) también lo fueron; sin embargo, los resultados no fueron estadísticamente significativos. Los especímenes seropositivos del Parque Ocotal (5/28) mostraron un mayor riesgo en comparación con los del Zoológico de Zacango. Conclusión. La prevalencia de *C. abortus* en rumiantes cautivos en ambos sitios fue baja; sin embargo, se desconocen los impactos en la salud de estas especies. La investigación sobre la transmisión de *C. abortus* en rumiantes cautivos debe continuar incluyendo diagnóstico del ganado doméstico y humanos.

Palabras clave: *Chlamydia abortus*, rumiantes en cautiverio, estudio serológico.

1 INTRODUCTION

Chlamydia abortus (*C. abortus*) is a Gram-negative, obligate intracellular bacteria and the etiologic agent of ovine enzootic abortion (OEA) reported in many countries. It affects sheep and goats causing abortion during the last third of gestation or delivery of weak neonates and, it is considered a zoonotic agent associated with occupational exposure (Sachse *et al*., 2015). *C. abortus* presents a biphasic replicative cycle, consisting of an extracellular infectious form called the elementary body (EC) and an intracellular form, identified as the reticular body (CR). ECs are spherical structures about 0.2–0.3 µm in diameter, antigenic, non-proliferative, responsible for carrying out intracellular adhesion, fusion, and infection (Beeckman *et al*., 2014). The RCs are the metabolically active intracellular form of the bacterium, they are structures that measure approximately 0.5 – 2 mm in diameter. RCs are not antigenic and have fewer disulfide bonds between their membrane proteins, so are labile and osmotically more permeable, which allows them to obtain nutrients from the cell's cytoplasm (Omsland *et al*., 2014).

Pathologies associated with infection with *C. abortus* refer to damage to the placenta of pregnant females, with abortion in the last third of gestation and for males, cause transitory infertility (Borel *et al.,* 2018).

Molecular tests such as PCR and serological tests such as complement fixation, cell cultures with immunofluorescence labeling, and ELISA-type tests are used for diagnosis. PCR tests have allowed the differentiation between Chlamydias species with greater specificity compared to the rest of the previously mentioned diagnostic techniques (Sachse *et al*., 2009). Serologic diagnosis may be affected due to cross-reactivity tests that may arise from other Chlamydial species and Gram-negative bacteria such as Acinetobacter spp. (Livingstone *et al*., 2005), therefore, specific diagnostic tests are required to properly understand the epidemiology of the disease and in the implementation of control strategies.

Several ELISA tests have been developed to improve the serological diagnosis of *C. abortus*. A specific recombinant ELISA based in a fragment of 80-90 kDa has proven to be an effective method (Vretou *et al*., 2007).

The first isolates of *Chlamydia* (*Chlamydia psittaci*) in Mexico were obtained in 1996 from sheep (Escalante-Ochoa *et al*., 1996), when isolation in cell cultures was successful in 92.88% of the trials, with a high incidence that led to serious consideration of the pathogenic role of *Chlamydia* in Mexico. The prevalence of anti-*Chlamydia abortus* IgG antibody was determined by ELISA assay and the molecular identification by PCR in sheep with a clinical history of abortion of samples carried out in 35 flocks of sheep from Xalatlaco, Mexico. The seropositive rate was 31.1% (14/45) for healthy ewes and 21.3% (65/304) for ewes with a clinical history of abortion (Jimenez-Estrada *et al*., 2008). However, information regarding the prevalence of *C. abortus* infections in wild mammals is unknown. Sheep and goats are considered natural hosts, however, isolates from cattle, swine, and other animals have been reported (Sachse *et al*., 2015). *C. abortus* has been linked to reproductive disorders in other ruminants, including yak (*Bos grunniens*) in Tibet, China (Liang *et al*., 2021) in which *C. abortus* was isolated suggesting that played a substantial role in abortions. The goal of the study was to determine the seroprevalence of *C. abortus* using a specific recombinant ELISA and the identification of risk factors associated with captive ruminants in the Zacango Zoo and Ocotal Park, Mexico.

2 MATERIALS AND METHODS

Study Area

The study was performed at the Zacango Zoo, Metepec-Santa Maria Nativitas (99°37'02"W; 19°10'25"N) and the Ocotal Park, Atlacomulco-San Bartolo (99º47'24"W; 20º03'01"N), during March and October 2016.

Serum Samples

A total of 103 sera from 15 species of ruminants were tested: Alpaca (*Lama pacos)*, Bactrian camel (*Camelus bactrianus)*, Barbary sheep *(Ammotragus lervia)*, Bighorn sheep (*Ovis canadensis)*, Black buck (*Antilope cervicapra)*, Call (*Lama glama)*, Dromedary (*Camelus dromedarius)*, Elk (*Cervus canadensis)*, Fallow deer (*Dama dama)*, Goat (*Capra hircus)*, Guanaco (*Lama guanicoe)*, Kirk's dik-dik (*Madoqua kirkii)*, Red deer *(Cervus elaphus)*, Sheep *(Ovis aries)*, White-tailed deer *(Odocoileus virginianus)*. Samples originated from Zacango Zoo were 75 and 28 from Ocotal Park, providing 44/103 (45%) males and 59/103 (55%) females. Blood samples were obtained from captive ruminants by jugular venipuncture using tubes without anticoagulant (Vacutainer system ©

Becton Dickinson, USA). Tubes were centrifuged at 1318g for 5 minutes and sera stored in aliquots at -20 °C until tested.

Serological diagnostic

In the laboratory, sera were tested for anti-C. abortus antibodies using a recombinant ELISA kit (*C. abortus* serum verification, version p00700/05-25/05/04. Pourquier Institute, Montpellier, France) following the manufacturer's protocol and previously validation (Vretou *et al*., 2007). Briefly, 96-well microtitre plates coated with the recombinant antigen POMP 80-90KDa, were blocked with 5% non-fat dried milk PBS for 60 min at 37^0C. The plates were washed and then sheep sera (30µl) were added to appropriate wells and incubated for 1h at 37^0C. After further washing, horseradish peroxidase-conjugated donkey anti-sheep IgG was added and incubated for 60 min at 37^0C. Optical densities were read in a microtitre plate reader (CERES 900 Hdi. Bio-TEK instrument. Winooski, VT, USA) at a wavelength of 405 nm. Each test was performed with positive, negative, and blank controls. Serum Positive Percentage (S/P) was calculated, concerning the average of the positive control sera, using the following formula: S/P = (OD of sample x 100): Average OD of positive control. Final values were expressed as Sample/Positive control % (S/P %), serum samples that yielded less than 50% were considered negative, samples with S/P values between 50-60% were scored as doubtful, and sera with S/P values greater than 60% were considered positive.

Risk factors

Information about age, sex and management units of the animals were collected. Two regions (Zacango Zoo and Ocotal Park, Mexico) and sex (Male and Female) were analyzed in this study. Animals' ages were divided into categories: young ≤ 0-3 years and adults > 3.1 years.

Statistical analyses

Descriptive statistics included the calculation of percentages of seropositive animals for species, sex, and location. An odds ratio (OR) and confidence intervals were determined for age, sex, and location, for a P-value < 0.05 to be statistically significant between factors and prevalence. The OR > 1 indicated that the presence of this factor is related to the appearance of seropositive animals. All statistical analyses were performed using the SAS Program Statistics version 9.0.

3 RESULT

The frequency of antibodies against *C. abortus* was 7/103 (6.7%), being barbary sheep, bighorn sheep, goat, and red deer the species that demonstrated at least one positive individual (Table 1).

Table 1. The frequency of antibodies against *C. abortus* in wild ruminants in units of management, conservation, and research of the State of Mexico determine by a recombinant ELISA kit.

Species	Positive animal/Animal tested (% positive) at Zacango	Positive animal/Animal tested (% positive) at Ocotal
Alpaca (*Lama pacos)*	0/1 (0)	0/0 (0)
Bactrian camel (*Camelus bactrianus)*	0/1 (0)	0/0 (0)
Barbary sheep*(Ammotragus lervia)*	0/0 (0)	4/23 (17.4)
Bighorn sheep (*Ovis canadensis)*	0/0 (0)	1/2 (50)
Black buck (*Antilope cervicapra)*	0/12 (0)	0/0 (0)
Call (*Lama glama)*	0/23 (0)	0/0 (0)
Dromedary (*Camelus dromedarius)*	0/1 (0)	0/0 (0)
Elk (*Cervus canadensis)*	0/2 (0)	0/0 (0)
Fallow deer (*Dama dama)*	0/16 (0)	0/0 (0)
Goat (*Capra hircus)*	1/2 (50)	0/1 (0)
Guanaco (*Lama guanicoe)*	0/4 (0)	0/2 (0)
Kirk's dik-dik (*Madoqua kirkii)*	0/1 (0)	0/0 (0)
Red deer (*Cervus elaphus)*	1/2 (50)	0/0 (0)
Sheep (*Ovis aries)*	0/9 (0)	0/0 (0)
White-tailed deer (*Odocoileus virginianus)*	0/1 (0)	0/0 (0)

The samples obtained from Ocotal Park had a significantly higher risk factor for *C. abortus* antibodies -OR 4.56, P = 0.052 than results obtained for Zacango Zoo samples (Table 2).

Table 2. Characteristics and risk analysis for age, management units, and sex factors as described by odds ratio (OR), 95% confidence interval (CI), and *P*-value.

Risk Factors	Number of positive/Number of animals tested (%)	OR (95% CI)	*P*-value
Age			
0-3	0/23 (0)	0.21 (0.01, 3.79)	0.289
>3.1	7/80 (8.7)	4.80 (0.26,87.18)	0.289
Conservation and management units			
Zacango Zoo	2/75 (2.6)	0.13 (0.02, 0.69)	0.017
El Ocotal	5/28 (17.9)	7.93 (1.44, 43.67)	0.017
Sex			
Female	4/56 (7.1)	1.13 (0.24, 5.31)	0.879
Male	3/47 (6.3)	0.88 (0.18, 4.17)	0.879

Seropositive species included bighorn sheep (*Ovis canadensis*) (1/2), barbary sheep (*Ammotragus lervia*) (4/23), red deer (*Cervus elaphus*) (1/2), and a domestic goat (*Capra hircus*) (1/3). The analysis of antibody values about risk factors showed that the older animals (3.1 years) were seropositive with a frequency of 7/80 (8.7%) and three males (3/47) and four females (4/56) also were seropositive; however, no statistical significance was observed. Seropositive specimens from Ocotal Park (5/28) showed a higher risk compared to Zacango Zoo.

4 DISCUSSION

The study is the first report of *C. abortus* seroprevalence in captive ruminants in Mexico. *C. abortus* antibodies have been detected using a similar ELISA in sheep from the State of Mexico obtaining prevalences ranging from 21.3% to 31.1% (Jiménez-Estrada *et al*., 2008). Additionally, the seroprevalence in goats ranged from 3.44% and 13.51% (Campos-Hernández *et al.*, 2014). *C. abortus* antibodies have been detected in horses (1.32%) and cattle (48%) in the State of Mexico suggesting that other domestic animals may be involved in the epidemiology of the disease (Rubio-Navarrete *et al*., 2017).

Recently, the use of a commercial ELISA allowed determining for a total of 5, 231 serum samples, 581 (10.92 %) as positive. Positive samples were identified in the State of Tlaxcala for a 13.08% (73/558); Sonora 12.45% (102/819); Chihuahua 11.56% (107/925); Hidalgo 11.34% (97/855); Chiapas 10.15% (60/591); Querétaro 9.69% (79/815) and the State of Mexico 7.09% (63/758). The frequency of seropositive herds was 43.34% (140/323) (Palomares-Rezendi *et al*., 2020).

A high prevalence (41.7%) of Chlamydiaceae antibodies has been documented in free-ranging ungulates in Spain specifically, *C. abortus* antibodies ranged from 7-40% (Salinas *et al*.,

2009). Other studies in wild ruminants using complementation fixation test (CFT) showed the prevalence of 45.1% in European bison (Bison bonasus) (Salwa *et al*., 2007). In addition, the prevalence also has been determined with values of 24% to 37% in fallow deer (Dama dama), mouflon (Ovis mussimon), red deer, and Spanish ibex (Capra pyrenaica) (Cubero-Pablo *et al*., 2000). *C. pecorum* and *C. psittaci* antibodies have been reported in red deer suggesting that wild ruminants may be a potential reservoir for Chlamydiae (Di Francesco *et al*., 2012).

Serological diagnostic by ELISA, using recombinant polymorphic outer membrane proteins (POMPs) fraction as antigen, is specific for *C. abortus* and distinguishes between *C. pecorum* and *C. abortus*. The assay is based on the detection of IgG antibodies specific to a recombinant fraction of the major outer membrane protein of *C. abortus* (POMP 80-90 kDa), showing no cross-reactivity against other bacteria of the same genus or Enterobacteria (Vretou *et al*., 2007).

It is possible that previous studies using less-sensitive assays could have been overestimating *C. abortus* seroprevalence in wild populations, the use of a specific assay for specific species to avoid false positives or false negatives is necessary.

Future studies with sensitive techniques and larger numbers of animals need to be expanded to understand the epidemiology of the disease. During this study, the site represented a significant risk factor potentially due to the confinement and management conditions. For example, a goat was introduced to Ocotal Park and then found positive to *C. abortus*. Previous studies have shown that domestic animals which had contact with free-ranging wildlife are the source of infection (Joseph *et al*., 2015). Once exposed, there is the potential of wild animals to serve as reservoirs of the disease, but further research is needed.

Another study of the relationship of risk factors with the prevalence of the disease was indicative at the most important risk factor that favors the spread of the disease in herds is the introduction of animals that have not been previously certified as negative for the disease tests. Authors inferred that animals from production units in other states or even from other countries, upon arrival at the new center, can spread the infection more easily (Palomares-Rezendi *et al*., 2020).

In the present study, no significant differences between sex and age were obtained, however, differences in exposure related to these risk factors have been documented, for example, bison from Poland resulted in seropositive with 18/48 (37.5%) males and 37/74 (50%) females (Salwa *et al*., 2007). Another relationship between *C. abortus* seropositivity and age has been previously reported (Yin *et al*., 2014). In addition, in Mexico, was observed that as age increases, the probabilities of exposure to the disease increase proportionally, therefore, the number of seropositive sheep increases (Palomares-Rezendi *et al*., 2020).

The study of risk factors associated with seroprevalence to *C. abortus* in Tibetan sheep in Gansu province, Northwest China, revealed that gender was not statistically significantly a difference of the seasons, ages, and different geographic distribution, that represents a higher risk (Si-Yuan *et al*., 2014).

The seroprevalence of *C. abortus* among sheep and goat flocks from the Eastern Province of Saudi Arabia and the identity of flock management and animal risk factors were associated with *C. abortus* seropositivity. Multivariable logistic regression revealed that the introduction of goats to the flocks (OR: 1.9; 95% CI: 1.2–3.0) was identified as a risk factor, whereas good farm hygiene (OR: 0.3; 95% CI: 0.2–0.7) was indicative of a protective factor. In addition, the introduction of new sheep to the flocks (OR = 2.6; 95% CI: 1.5–4.4), type of breeding system (OR = 1.8; 95% CI: 1.0–3.4), flocks allowing females in (OR = 1.9; 95% CI: 1.1–3.3) or females out (OR = 2.2; 95% CI: 1.1–4.3), and sheep age 1.4–2.8 years (OR = 1.9; 95% CI: 1.3–2.9) were risk factors for *C. abortus* seropositivity (Fayez *et al*., 2021).

The identification of chlamydial infections in captive and wild ruminants is important because of their impact on animal health and the relationship to those strains also found in humans and domestic animals. The presence of traditional pathogens of the family Chlamydiaceae such as *C. abortus* in wild animals is a risk factor for infections in domestic animals and/or humans (Burnard and Polkinghorne, 2016).

5 CONCLUSION

C. abortus prevalence in captive ruminants from Mexico was low compared to other studies. Further research aimed at determining the impact of *C. abortus* in reproductive disorders and the potential of interspecific transmission is necessary.

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CONFLICT OF INTERESTS

The authors declare they have no conflict of financial or non-financial interests.

COMPLIANCE WITH ETHICAL STANDARDS

The authors declare that all the procedures that contributed to the realization of this work comply with the ethical standards of the UAEM, and with the authorization by the bioethical committee of the Faculty of Veterinary Medicine and Zootechnics, UAEM, 4359/2017.

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