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IDENTIFICATION OF *Chlamydia abortus* AND *Chlamydia pecorum* IN WATER BUFFALOES (*Bubalus bubalis*) AND COWS COHABITATING THE SAME HERD

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ABSTRACT

The aim of this study was to determine the presence of *Chlamydia* spp. in a dual-purpose zebu and water buffalo-mixed herd cohabitating in a ranch located in southern Mexico. The zootechnical purpose of this herd is milk and cheese production. A total of 52 vaginal exudate samples were obtained from clinically healthy water buffaloes, less than one month after parturition, and two vaginal exudate samples were taken from cows that had recently aborted. L929 cells were used for bacterial isolation. Two of these cultures were infected, confirmed by direct immunofluorescence. Total DNA was extracted for analysis with two types of real-time PCR, a *Chlamydiaceae*-specific real-time PCR and another species-specific real-time PCR for *C. abortus, C. psittaci* and *C. pecorum*. Two *Chlamydia* isolates were obtained, one from a water buffalo positive to *C. abortus* and *C. pecorum*, the other from a cow positive to *C. abortus.* This is the first report of the presence of *C. abortus* and *C. pecorum* in water buffaloes in Mexico. *C. abortus* was also detected in one cow living in the same herd as the water buffaloes.

Keywords: *Bubalis bubalis*, water buffaloes, cattle, *Chlamydia abortus*, *Chlamydia pecorum*, Mexico

INTRODUCTION

The water buffalo (*Bubalus bubalis*) or domestic water buffalo is a large bovid that originated in India, Southeastern Asia, and China. The world population of water buffalo is approximately 230 million head: more than 95% are in Asia; 2% in Africa, particularly in Egypt; 2% in America and less than 1% in Australia and Europe. The largest number of dairy water buffalo are in India, Pakistan, China, Egypt, and Nepal (Vale, 2017).

Water buffalo was introduced to Mexico in 1992. Nowadays, their production spreads out to the states of Puebla, Oaxaca, Chiapas,

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Nayarit, Sinaloa, Campeche, Tabasco, Jalisco, and Guanajuato, and it involves an activity of 120 thousand head (Aguilar-Domínguez *et al*., 2018). The health conditions of water buffalo herds and the impact they could generate on both, public health, and the animal population they share paddocks with, are unknown. Chlamydiosis can cause the birth of weak calves, stillbirths, and abortion within the last third of gestation in ruminants. *Chlamydia abortus (C. abortus)* is the specific principal agent. However, *Chlamydia pecorum (C. pecorum)* and C*hlamydia psittaci (C. psittaci)* may also be involved (Longbottom and Coulter, 2003; Mohamad and Rodalakis, 2010).

Moreover, the presence of *C. abortus* has been reported as the cause of abortion and fertility reduction in cows (Godin *et al*., 2008; Reinhold *et al*., 2011). Although previous reports have mentioned the presence of *C. psittaci* and *C. abortus* in water buffalo in Europe (Greco *et al*., 2007; Roncoroni *et al*., 2007; Greco *et al*., 2008) and Africa (Osman *et al*., 2012) no species of *Chlamydia* have been notified in water buffalo within the American continent.

This study started with etiological research on abortions in brucellosis-free cattle heard that shared facilities with water buffaloes. Both cows and water buffalo were used for milk production and had no history of reproductive disorders or abortions.

At sampling time, however, two cows had aborted within the last third of gestation. Due to their close coexistence with water buffaloes, the samples were collected from both cows and water buffaloes with less than one month of parturition.

The objective of this study was to determine the presence of *Chlamydia* species in a mixed cattle and water buffalo herd located in southern Mexico.

MATERIALS AND METHODS

Collection of animal samples

Samples of vaginal exudates were collected from a mixed herd in the state of Chiapas, Mexico. The herd contained one hundred dual-purpose bovids and one hundred and one water buffaloes (*Bubalus bubalis*), sixty-one females and forty replacements, including studs. The zootechnical purpose of the herd is milk and cheese production. The herd was brucellosis-free. A total of fifty-two samples were obtained from water buffaloes with less than one month of parturition and without previous abortion. Two samples were collected from cows that had aborted within their last month of pregnancy. These samples were collected 10 days after abortion. The sterile swabs used for the collection of vaginal exudates were transported in a sucrose phosphate glutamate (SPG) medium at 4ºC, then maintained at -80°C until arrival at the INIFAP laboratory in Mexico City. The samples were stored at -80°C until usage.

Cell culture isolation and direct immunofluorescence

The vaginal swab samples were inoculated into L929 mouse fibroblasts cell-line for bacterial isolation (Escalante *et al*., 1997). Inclusion bodies in infected cultures were identified by direct immunofluorescence using the IMAGEN *Chlamydia* test commercial kit from Oxoid.

Determination of *Chlamydiaceae* family and *Chlamydia* spp.

A genomic DNA extraction was performed using the commercial kit (Qiagen, Germany) following the manufacturer's instructions; DNA was then stored at -20ºC. The identification of the *Chlamydiaceae* family was performed using

TaqMan real-time PCR, targeting the 23S rRNA gene, in accordance with Ehricht *et al*. (2006). *Chlamydia* species were identified by TaqMan real-time PCR assays that detect an *ompA* gene fragment, specific to *C. abortus, C. psittaci* and *C. pecorum* as previously described by Pantchev *et al*. (2010).

RESULTS

Chlamydia spp. was isolated and identified in cell culture and by direct cell immunofluorescence from vaginal exudates of 1/52 (1.9%) water buffaloes and one out of two cows (50%). The two positive isolates of *Chlamydia* spp. were also positive to the *Chlamydiaceae* family by real-time PCR. *Chlamydia* species determination by real-time PCR revealed that the water buffalo isolate was positive to both, *C. abortus* and *C. pecorum*, while the recently aborted cow isolate was positive only to *C. abortus*. The real-time PCR test for *C. psittaci* was negative for both samples. All animals were negative for *C. psitacci.*

DISCUSSIONS

Previous reports on bovine chlamydiosis in Mexico are few. One serologic study involving two states of the country reported frequency in individual animals was 10.97% (Limón *et al*., 2011). In another study in seven states, 36 out of 157 (22.9%) cows with abortions were positive to *Chlamydia* spp. according to the isolation and identification results (Limón *et al*., 2019).

To date, water buffalo breeding in Mexico is incipient and limited; the Department of Agriculture and Animal Husbandry recognized water buffalo as a domestic species for dietary goods production until 2018. Thus, it has not been included in any health campaign yet. Most water buffalo herds share paddocks and facilities with other cattle, making them vulnerable to infections coming from other bovids and *vice versa*. These infections also represent a potential danger for public health (Aguilar-Domínguez *et al*., 2018).

The present report is the first in our country to point out the presence of chlamydiosis in water buffaloes; the spp. of the bacterial genuses that infect them had not been previously determined.

Other studies have reported reproductive disorders in water buffalo infected with *C. abortus* and *C. pecorum*. The present study, however, found that while cows cohabitating with the water buffalo in the same ranch presented abortions due to the presence of *C. abortus*, infected water buffalo did not show this reproductive disorder.

Given that there are diseases like chlamydiosis which may be ignored in veterinary practice, our present findings on Chlamydia infection in water buffaloes without signs of reproductive disorders are relevant to preserve the health status of the herd. In previous reports from other countries, *C. psittaci* and *C. abortus* infections have been found to cause abortions in water buffalo (Greco *et al*., 2007; Roncoroni *et al*., 2007; Osman *et al*., 2012). *C. abortus* and *C. pecorum* co-infections have been detected by nested PCR in vaginal swab samples taken from buffaloes presenting abortions, as well as in aborted fetuses, which were in a buffalo herd with an abortion rate of 36.8% (Greco *et al*., 2008). This differs from our present results in which the water buffaloes detected with a *C. abortus* and *C. pecorum* co-infection did not present reproductive disorders nor abortions In agreement with our study, De Graves *et al*. (2003) reported cows from which *C. pecorum* was isolated that did not present

reproductive disorders and were clinically healthy. This latter study demonstrated that the presence of *C. psittaci* and *C. pecorum* caused genital tract infections in healthy heifers.

In a comparative study considering water buffaloes and cows with and without reproductive disorders, including abortion, *C. psittaci and C. abortus* were isolated and identified by RFLP-PCR in approximately 49% in both water buffaloes without reproductive disorders and buffaloes presenting no clinical reproductive disorders (Osman *et al*., 2012).

Mixed infections of *Chlamydia* spp. have been reported in other ruminant spp. For example, in a study of sheep herds in Germany, simultaneous infections of *C. abortus, C. pecorum* and *C. psittaci* were detected in herds with less than 1% of abortions (Lenzko *et al*., 2011). This is similar to our findings, since the water buffaloes in our study did not have a history of abortion.

Mixed infections have also been reported in females with recent abortions in Algerian sheep and goat farms, where simultaneous infection of *C. abortus* and *C. pecorum* was found in the samples from vaginal exudates of three animals (Merdia *et al*., 2015). Similarly, *C. psittaci* and *C. abortus* have been simultaneously isolated in sheep and goat vaginal exudates (Berri *et al*., 2009). Finally, *C. abortus* and *C. suis*, *C. abortus* and *C. psittaci*, as well as *C. suis* and *C. pecorum* have also been found in pigs (Pantchev *et al*., 2010).

This study is the first in Mexico to report the confirmed presence of *C. abortus* and *C. pecorum* in clinically healthy water buffalo without reproductive disorders.

The presence of *Chlamydia* spp. in cattle represents a risk to the human population due to the zoonotic potential of the bacteria. Moreover, it strongly underlines the necessity of preventive sanitary measures in order to reduce the risk of infection. The presence of *Chlamydia* in cows and water buffaloes living in the same herd calls for additional studies to clarify the circulation of this bacterium among animal species. Furthermore, since mixed infections are not uncommon, it is important that they are diagnosed. This would represent important information for epidemiological studies; it would also stress the need to control the disease.

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