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Original Research

Prevalence of *Chlamydia abortus* Antibodies in Horses From the Northern State of Mexico and Its Relationship With Domestic Animals

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ABSTRACT

Chlamydia abortus is obligate intracellular bacteria that cause a wide range of reproductive and respiratory diseases in animals and humans. Serologic and molecular evidences of *C. abortus* in Mexico have been reported in sheep and goats. The presence of *C. abortus* antibodies in horses (n = 301) was detected by a commercial indirect enzyme-linked immunosorbent assay test. Additionally, *C. abortus* antibodies in bovines (n = 25), goats (n = 8), and sheep (n = 94) reared with horses were detected to identify probable animal species that could infect horses. The prevalence obtained was 1.32% for horses, 48% for bovines, 12.5% for goats, and 29.7% for sheep. The 75% of horses seropositive to *C. abortus* were obtained from farms with the presence of sheep seropositive to *C. abortus*. These results suggest that the presence of *C. abortus* in horses from Mexico exist and can be related with the presence of sheep positive to *C. abortus*.

1. Introduction

Chlamydia abortus (*C. abortus*) is a Gram-negative, obligate intracellular bacteria that are found in a wide range of hosts and causes reproductive and respiratory diseases in animals and humans in many countries around the world [1].

In horses, abortion induced by *Chlamydia* is generally considered rare; however, in some studies, evidence was presented that *Chlamydia* played a major role in equine abortion [2], for example, the abortions and conjunctivitis reported in horses in South Africa [2]. Furthermore, Henning et al [3] isolated *Chlamydia* from an equine fetus aborted at ninth month of gestation from a mare with respiratory disease and fever. In contrast, the detection of *Chlamydia* in 27.1% and 55% of aborted foals has been described. However, no detection of chlamydial antigens in placental tissues, liver, or lung from aborted foals has been reported. Forster et al [4] concluded that the chlamydial infections could be implicated in respiratory diseases of horses but are not related with abortions. Chlamydial infections have also been reported in yak, buffalo, and horses, where as the chlamydiosis in horses was related with pneumonia [5].

Animal care statement: None.

Ethical approval statement: The handling of animals was done according to international bioethical standards and NOM-062 - ZOO-1999.

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Reports of *Chlamydia psittaci* in horses have been documented. *Chlamydia psittaci* serotype 1 was later named *Chlamydophila abortus* [6] and recently named *C. abortus* [7]. It is therefore probably that some isolates identified as *C. psittaci* could now be identified as *C. abortus*. McChesney et al [8] isolated *C. psittaci* from lung tissues of a horse with a fatal respiratory disease. Subsequently, the infected ponies developed subclinical disease and the microscopic studies reveled a generalized chlamydial infection, and the authors concluded that *C. psittaci* could cause pneumonia and become invasive [8]. Additionally, Moorthy and Spradbrow [9] reported the isolation of *C. psittaci* from the nasal tract of horses with acute respiratory disease; however, chlamydia was not isolated from tissues of 14 aborted foals. Szeredi et al [10] identified a high prevalence of *C. psittaci* infection in fetal membranes of aborted equine fetuses from Hungary. Tests by immunohistochemistry, polymerase chain reaction (PCR), and modified Ziehl-Neelsen staining suggested that the chlamydial infection of the genital tract was a possible factor in equine reproductive disorders. Recently, Jelocnik et al [11] reported the identification of *C. psittaci* strain involved in equine placentitis, which was associated with subsequent human psittacosis, highlighting the potential role of *C. psittaci* in equine abortions and the zoonotic potential of the abortion caused for *Chlamydia*. Additionally, *Chlamydia pnueumoniae* has been identified from clinically healthy horses in 26.5% of samples tested [12].

Chlamydia abortus has also been reported in some studies in horses, for example, the *C. abortus* isolated from cases of abortion in horses, rabbits, guinea pigs, and mice [6]. In Germany, *C. abortus* has been identified in clinically healthy and horses with recurrent airway obstruction [13]. Ninety-one percent of positive equine placenta tissue sampled for *Chlamydia* spp. were identified as *C. abortus* by real-time PCR, which confirms the importance of *C. abortus* as a pathogen in reproductive disorders for horses [14].

In Mexico, there is evidence of the presence of *C. abortus* in sheep and goats. Escalante-Ochoa et al [15] were the first to report in Mexico, the isolation of *C. psittaci* serotype 1, now known as *C. abortus*, in a herd of goats causing 28.6% of abortions.

Arteaga-Troncoso et al [16] identified 80% of a herd of sheep positive for *C. abortus* antibodies with an average seropositive of 21.5%. In 2008, the seroprevalence in sheep of the State of Mexico was 21.3%, and the detection of *C. abortus* by specific PCR was 0.65% [17]. Barbosa-Mireles et al [18] detected the presence of antibodies for *C. abortus* in veterinarians and flock workers in a region of the State of Mexico. This was with the presence of sheep positive to *C. abortus*, and they identified a prevalence of 9.6%.

The presence of *C. abortus* in goats of Mexico has been reported, in commercial dairy goat farms of the Guanajuato state with a seroprevalence ranging between 3.44% and 13.51% [19]. In 2015, the seroprevalence of *C. abortus* reported was of 9.6% in the same state, with 26.98% of the isolates in goats [20] which confirms the presence of *C. abortus* in Mexico. However, the presence and impact of *C. abortus* in horses in Mexico is unknown. In Mexico, the breeding of different animal species is common. Evidence exists of these bacteria infecting a broader range of animal hosts. Consequently, the presence and impact *C. abortus* on domesticated animals and the relationships of those with other animal species is important. The objective of the present study was to evaluate the presence of *C. abortus* antibodies in horses and the possible species involved.

2. Material and Methods

2.1. Samples

For the determination of sample size, the binomial sampling formula for infinite populations was used (expected prevalence of 25%, confidence level 95%, and error of 5%) [21]. A total of 301 samples of serum were obtained from horses in Acambay (n = 125), Jilotepec (n = 29), Temascalcingo (n = 30), Toluca (n = 14), and Villa del Carbón (n = 63) municipalities of the Northern State of Mexico, Mexico, and the delegation Miguel Hidalgo (n = 40) of Mexico City, Mexico. The samples were obtained from male (n = 143) and female (n = 158) horses and were collected in the months of May to November 2012. Additionally, random samples of goats, sheep, and cattle (in coexistence with equines) sera were collected from animals in the same corral and which were grazing with the horses. Blood samples were obtained from the jugular vein of each animal included in the study. The samples were analyzed at the Centro de Investigación y Estudios Avanzados en Salud Animal, Facultad de Medicina Veterinaria y Zootecnia, Universidad Autónoma del Estado de México, Mexico.

2.2. Enzyme-linked Immunosorbent Assay

All serum samples were tested for *C. abortus* antibodies using an indirect multispecies enzyme-linked immunosorbent assay (ID Screen *Chlamydophila abortus* indirect multispecies, ID Vet, Grabels, France). The assay uses a recombinant fragment 80–90 kDa (POMP) protein specific of *C. abortus* and was performed according to the instructions of the manufacturer. Briefly, 90 μL of buffer dilution and 10 μL of each of the samples obtained and the positive and negative control (provided by the manufacturer) were added to each well of the plate included. After incubation for 45 minutes, the plates were washed three times with the wash solution included. Later, 100 μL of the conjugate solution was incubation for 15 minutes followed by addition of 100 μL of the substrate solution and incubated for 30 minutes. Consequently, 100 μL of the stop solution was added and the absorbance determined by a colorimetric reader (CERES 900 Hdi. Bio-TEK instrument, Winooski, TV) at a wavelength of 450 nm. The absorbance was expressed as percentages with values higher than 60% considered as positive for horse serum and values higher than 40% considered as positive for serum obtained from goats, sheep, and cattle as per the manufacturer's instructions.

2.3. Statistical Analysis

The risk ratio (RR) was calculated to estimate if the presence of species in coexistence with the horses was related to the presence of *C. abortus* seropositive horses [21].