

## Low seroprevalence of *Chlamydia abortus* in dairy cows of hot environment in southern of Mexico

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**Abstract:** *Chlamydia abortus* (*C. abortus*) are Gram-negative obligate, intracellular bacteria responsible of major economic losses due to infection and subsequent induction of abortion in several animal species, including humans. To test the prevalence of *C. abortus* in dairy cows in Mexico, 271 sera were tested for the presence of anti- *C. abortus* antibodies. Detection of anti- *C. abortus* antibodies was carried out with a commercial indirect ELISA kit. Optical density readings specific to *C. abortus* were low ( $14.53 \pm 9.55$ ) ranging from 2.81% - 90.21%. Only two sera from State of Mexico exhibited positive readings, showing a prevalence of 0.73%. It is concluded that the seroprevalence of *C. abortus* in dairy cattle in Mexico is low.

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### 1. Introductions

All members of the family *Chlamydiaceae*, are spherical, obligate intracellular, Gram negative bacteria which are pathogenic to different animals, including birds and human beings (Everett, 2000; Everett et al., 1999). *Chlamydia abortus* (*C. abortus*) causes different diseases in bovines. In bulls, it causes epididymitis, orchitis and vesiculitis (Gomes et al., 2001; Storz et al., 1968). It is also the etiological agent of Epizootic Bovine Abortion (EBA) in milking cows (Rekiki et al., 2002; Rodolakis et al., 1998; Szeredi and Bacsadi, 2002). It also causes mastitis, reproductive issues such as endometritis, changes in length of open period and vaginitis, pneumonia, conjunctivitis, enteritis, polyarthrititis and encephalitis (Corner et al., 1968; Twomey et al., 2003). Infected animals also give birth to weak/premature calves (Idtse, 1984; Pérez-Martínez and Storz, 1985).

Animals can become infected at any time of the year, especially via ingestion of elemental bodies (EB) from aborted foetus, placentas, and uterine discharge from infected individuals that contaminate drinking water or food (DeGraves et al., 2004; Longbottom and Coulter, 2003). Infected heifers are latent carriers until the next gestation period where risks of abortion are high. These animals remain as carriers for the rest of their productive life (Koehler et al., 1997).

Several studies had confirmed the presence of *C. abortus* in bovines. In India, 80.7% of cows were positive to *C. abortus* using complement fixation (CF)

tests in placenta tissue samples (Nanda, 1992). Works carried out in Sao Paulo (Brazil) showed a prevalence of 12.5% in female bovines with reproductive issues using CF tests (Igayara-Souza et al., 2004). Studies carried out using a recombinant Enzyme-linked immunosorbent assay (rELISA) suggested a seroprevalence of 35% in India (Bandyopadhyay et al., 2009). In Taiwan, vaginal sponges and sera from cows that experienced abortion were analysed using PCR. Prevalences were 34.9% y 71.4%, respectively. These data suggested a high prevalence of *C. abortus* (Wang et al., 2001). In Switzerland, PCR-based studies showed a prevalence of 2.55% from bovine placentas and of 0.4% using rELISA (Borel et al., 2006; Godin et al., 2008). Studies from Turkey also using rELISA showed seroprevalence of 8.33% in dairy cows (Igayara-Souza et al., 2004). To date, there are no published works on bovine chlamydiosis in Mexico. However, studies carried out in ovine species have shown seropositivity, and have also reported the isolation and molecular characterization of *C. abortus* (Jiménez-Estrada et al., 2008). The present study establishes for the first time the seroprevalence of *C. abortus* in dairy cows in Mexico using the Enzyme-linked immunosorbent assay (ELISA).

### 2. Materials and Methods

#### 2.1. Animals and samples

A specific survey targeting antibodies against *C. abortus* was carried out in dairy cows. The sample

included primi and multiparous Holstein and Jersey breed dairy cows. Animals were selected regardless of productive stage. A total of 217 sera were obtained from four Mexican states: 50 from Puebla, 65 from Hidalgo, 102 from State of Mexico and 45 from Tabasco. Blood (5-7 ml) was aseptically obtained through vacutainer venipuncture of caudal vein. Blood samples were centrifuged 7 minutes at  $1.7 \times 10^3 \times g$ . Serum was then removed and stored at  $-20^\circ C$  until assayed.

#### 2.2. Enzyme-linked immunosorbent assay (ELISA)

Antibodies anti- *C. abortus* were detected using a commercial ELISA kit specific for *C. abortus* (ID Screen<sup>®</sup> *Chlamydophila abortus* Indirect MS Multi Species, Montpellier, France) following the manufacturer's protocol. The kit uses a synthetic peptide antigen from a major outer membrane protein (MOMP) specific to *C. abortus* and allows discrimination of infections by *C. pecorum*. Seroprevalence was estimated by calculating the percentage of seropositive results to the total number of cattle.

### 3. Results

Optical density (OD) values specific to *C. abortus* in 271 sera from dairy cows from four states of Mexico were generally low ( $14.53 \pm 9.55$ ; 2.81% - 90.21%). Only two sera from State of Mexico (SoM) showed values above the cut-off value (90.21 and 63.21, respectively) and were considered positive to *C. abortus*. Total seroprevalence was 0.73%. State-specific seroprevalence was 1.9 % for SoM and 0% for Puebla, Hidalgo and Tabasco.

### 4. Discussion

Data obtained from the present study exhibited a low seroprevalence of antibodies against *C. abortus*. This suggests that this bacterial species is rarely present in dairy cows. However, occurrence of seropositive animals suggests previous contact with *C. abortus*, especially in herds from SoM.

When *C. abortus* interacts with its mammalian host, it elicits a strong immune response, which may protect animals from subsequent abortions. However, bacteria can be intermittently excreted from the reproductive tract, as previously shown in ovine species (Papp et al., 1994).

Since only two individuals were positive, further screening using alternative techniques should be performed. These could include cell culture and PCR, which have shown the highest sensitivity and specificity compared to ELISA and have are currently regarded as standards (Da Silva et al., 2006). The ELISA carried out in the present study uses a synthetic peptide antigen from the VS2 fraction of the major outer membrane protein (MOMP) (Livingstone et al.,

2005). This peptide is specific for *C. abortus* and allows discriminating between infections caused by *C. abortus* and *C. pecorum*. It does not cross-react with Gram-negative bacteria such as *Acinetobacter* spp. as previously described (Pérez-Martínez and Storz, 1985). This assay has high sensibility (95.7%) and specificity (100%) and is a powerful tool to test both suspected *C. abortus* -infected and healthy herds.

A similar work performed in Sweden by Godin *et al.* (2008) showed a prevalence of 0.4% using rELISA. In that study, low prevalence was associated with small mean herd size as well as reduced contact with different herds due to confinement periods of over 9 months. Similarly to the present study, in the Swedish research sample size of herds was small due to the small-scale nature of production units. However, in Mexican herds from SoM, two sera were positive for *C. abortus*. Interestingly those two individuals interacted with herds from different farms. In sheep from SoM, prevalence was found to be higher in individuals that were in contact with animals from different herds. Prevalence was also associated with large mean herd size (Jiménez-Estrada et al., 2008).

In the present study, a questionnaire was designed to record the relationship between *C. abortus* infection and reproductive issues in cattle. However, it was not possible to fully determine reproductive failure due to lack of registers in all sampled productive units. Previous studies have shown that *C. abortus* is responsible for several clinical and pathological issues in bulls and dairy cows (Gomes et al., 2001, Szeredi and Bacsadi, 2002), and that prevalence is higher in animals that show reproductive failure (Godin et al., 2008; Wehrend et al., 2005; Wittenbrink et al., 1993).

Although *C. abortus* can produce zoonotic infections and may have negative economic impact in dairy farms, this pathogen has not been included in the cattle diseases list of the World Organisation for Animal Health (OIE, 2011). Previous works carried out in Mexico using different diagnostic techniques have shown that *C. abortus* is present in ovine and wild ruminants (Jiménez-Estrada et al., 2008; Manjarrez et al., 2010). This suggests that dairy cows that interact with ovine herds might show a higher prevalence. This will be further researched in future work. Seroprevalence of *C. abortus* in dairy cows from four Mexican states was low. Samples were assayed using ELISA. Positive individuals were only found in production units from State of Mexico. This suggests that positive animals were exposed to *C. abortus*.

#### Competing Interests

The authors declare that they have no competing interests.

#### Authors Contribution

ARPA drafted the manuscript, carried out sampling of animals and serology analysis. AZMS

participated in writing and revision of manuscript. VCG participated in implementation of ELISA and interpreted the results. PFR participated in writing and revision of manuscript. RMJ participated in project integration, implementation of ELISA and writing and revision of manuscript.

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