

Detection of *Chlamydophila abortus* in Sheep (*Ovis aries*) in Mexico

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Abstract: *Chlamydophila abortus* is one of the pathogens which induce abortion in small ruminants; this pathogen has a tropism for ruminant placenta and causes the disease commonly referred to as Ovine Enzootic Abortion (OEA). In Europe are estimated economic losses of around 20 million pounds a year by OEA. In the American Continent the disease has been reported only in Canada, the United States, Colombia and Chile while in Mexico it is unknown whether OEA is common and it is causing abortions in flocks of sheep from "Estado de Mexico". The objective of this study was investigating the prevalence of anti-*Chlamydophila abortus* IgG antibodies and detection of *C. abortus* DNA in sheep with clinical abort history by mean of ELISA assay (*C. abortus* ELISA, Institute Pourquier, Montpellier, France) and molecular identification of the principal outer membrane protein (POMP 90-91B) gene by PCR, respectively. A cross-sectional study was carried out to enroll and random sample of ewes from november 2003 until march 2005. A total of 349 sera and vaginal swabs samples were collected from 35 flocks of sheep from Xalatlaco. The results showed that the seropositive rate was 31.1% (14/45) for healthy and 21.3% (65/304) for sheep with history clinical of abort. In vaginal swabs, the PCR showed 0% (0/45) for healthy animals and 0.65% (2/304) for aborted sheep. Samples from the lungs and liver of the fetus of one of these animals were also positive for *C. abortus*. In conclusion, these results confirmed that infection with *C. abortus* is common and is affecting sheep flocks in the Mexican highlands. Therefore, is necessary that the authorities responsible for animal welfare in Mexico (SAGARPA) to set up appropriate epidemiological surveillance and control programs to eradicate this disease.

Key words: *Chlamydiaceae*, *Chlamydophila abortus*, ovine enzootic abortion, ovines, POMP 90-91B

INTRODUCTION

Chlamydophila abortus is the most common infectious bacteria causing abortion in small ruminants in Europe, the disease is called Ovine Enzootic Abortion (OEA). Clinically is characterized by abortion in last month of pregnancy, stillbirths or premature births of weak kids with low birth weight and by

occurrence by polyarthritis in lambs. Occasionally, some affected goats develop a persistent cough, polyarthritis or keratocojunctivitis. In males, *C. abortus* can cause vesiculitis, epididymitis and orchitis^[1,2].

In both Northern Europe and the United Kingdom^[1], the OEA causes estimated economic losses of around 20 million pounds a year^[3]. The estimated prevalence of infection measured by the presence of

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anti-*C. abortus* antibodies is of 8.6% in the United Kingdom^[4], 21.8% in Spain^[5], 19% in Switzerland^[6] and 50.5% in Jordan^[7].

In the American Continent, the presence of *C. abortus* in sheep flocks has been reported to International Office of Epizootics (OIE) by Canada, the United States, Colombia and Chile (<http://www.oie>). In Mexico, animal welfare authorities consider that infection with this bacterium is exotic. In the municipality of Xalatlaco in 'Estado de México' high prevalence (86%) of abortions in ewes has been reported^[8], here, we suspected that *C. abortus* could participate as sheep abortigenic agents. The aim of this study was to investigate the prevalence of anti-*C. abortus* antibodies and the detection of *C. abortus* by mean PCR in sheep from Xalatlaco in 'Estado de México'.

MATERIALS AND METHODS

A total of 349 sera and vaginal swabs samples were collected from 35 flocks of sheep with reproductive failure from Xalatlaco, Estado de México (located between 19°10'39.17"/99°29'24.05"). Of these sheep, 304 had history of abortion and 45 without history of abortion. The sera were tested for presence of IgG antibodies against *C. abortus* using *C. abortus* ELISA (version P00700/04-18/02/05, Institut Pourquier, Montpellier, France), the assay was performed according to the instructions of the manufacturer. This ELISA assay uses recombinant fragment 80-90 kDa (POMP) protein of *C. abortus*. The final values were expressed as sample/positive control % (S/P %). Sera with S/P% equal to or lower than 50% were considered negative, sera with an S/P% between 50 and 60% were doubtful and sera with an S/P% >60% were positive for OEA-infection.

The vaginal swabs were used to detection of *C. abortus* DNA by mean PCR. The vaginal swabs were put into tubes which had previously contained a sucrose-phosphate-glutamate transport medium and kept at 4°C until their arrival at the laboratory. Two hundred microliters of samples were deposited in Eppendorf tubes and centrifuged at 3500X g for 30 min. Two hundred µL of lysis solution [tris-hydroxymethyl-aminomethane (trizma base) pH 7.6 1 M, ethylenediaminetetraacetic acid (EDTA) pH 8.0 1 M, NaCl 1 M, sodium dodecyl sulfate (SDS) 10%, Proteinase K at 0.2 mg mL⁻¹] were added to the cell button. The tubes were shaken by vortex for 1 min and incubated at 55°C for 2 h. Then 200 µL of phenol and 200 µL of chloroform were added to each tube. The tubes were shaken and centrifuged at 750 X g, for 10 min and then the organic phase was recuperated in another sterile Eppendorf tube. Two washes with 200 µL of chloroform by centrifugation were performed. Then, 0.1 volume of 1M sodium chloride and 2 volumes (v/v) of absolute ethanol were added and

mixed by inversion. The tubes were centrifuged at 10,000 X g at 4°C. for 5 min, they were subsequently incubated overnight at -20°C. Then, they were centrifuged at 10,000 X g for 10 min. The precipitate was washed twice by centrifugation with 2 mL of 70% ethanol and mixed by inversion, then the alcohol was evaporated. Finally, 50 µL of Tris-EDTA buffer (Trizma base, 1mM - EDTA, 1 mM) were added. The presence of DNA was evaluated by 0.8% agarose gel electrophoresis. The detection of *C. abortus* was performed by the identification and amplification of the polymorphic outer membrane protein (POMP 90-91B) gene by Polymerase Chain Reaction (PCR) with primers CpaX-1 [5'ACGGTCACTTGGAAACAAGG3'] and CpaX-2 [3'AGCAGAGGTTGGGCTCACTA5'] (GenBank access number: EF372585), that hybridize in the region of 1541 bp to 2452 bp of POMP 90-91-B gen (GenBank access number: U65942.1) and which amplify a product of 912 bp. PCR was performed on a final volume of 25 µL containing 5X PCR buffer, 1.5 mM MgCl₂, 400 µM of deoxynucleotide triphosphate, 25 pmol of each primer, 1 U *Taq Polymerase* (GoTaq Flexi DNA Polymerase Promega USA) and 100 ng of DNA samples. The amplification began with 5 min of denaturation at 95°C, followed by 30 cycles of amplification. Each cycle consisted of denaturation at 95°C for 1min, annealing at 60°C for 30 sec, extension at 72°C. for 1 min and a final extension at 72°C for 10 min. As a positive control, the *C. abortus* AB7 strain was used (donated by Dr. L. Jesús Salinas Lorente, Director of the Department of Animal Welfare at Universidad de Murcia, Spain) and as a negative control ovine DNA of uninfected animals was used. The PCR products were examined by 2.5% agarose gel electrophoresis and sequenced. Nucleotide sequencing was performed by using reagents provide in ABI PRISM BigDye Terminator Cycle Sequencing Ready Reaction Kit by using automated ABI PRISM 377-96 DNA sequencer (Perkin-Elmer, CA, USA).

RESULTS

The Table 1 shows the results of serology, PCR detection of *C. abortus* agent from vaginal swabs and aborted fetuses. The seropositive rate was 31.1% (14/45) for healthy and 21.3% (65/304) for sheep with history of abortion. Vaginal swabs were sampled from all aborted sheep and healthy animals and screened with PCR for presence of *C. abortus* MOMP gene (Fig. 1). The PCR detection rate was 0% (0/45) for healthy animals and 0.65% (2/304) for aborted sheep. The PCR detection rate aborted fetuses was 1/1 for sheep (Fig. 1). The MOMP gene sequences of positive vaginal swabs and aborted fetus were 98.9 -100% homologues among themselves and with the POMP 90-91B gene (Access number: U65492.1) (Fig. 2).

Table 1: No. of animals with anti-Chlamydial IgG antibodies o DNA *Chlamydomphila abortus*

Test	Healthy	Aborted
Serum IgG	14/45 [†]	65/304 [†]
Vaginal swab (PCR)	0/45	2/304
Aborted fetus (PCR)	NA*	1/1

[†]: No. positives/Total No. of samples tested, *: Not available

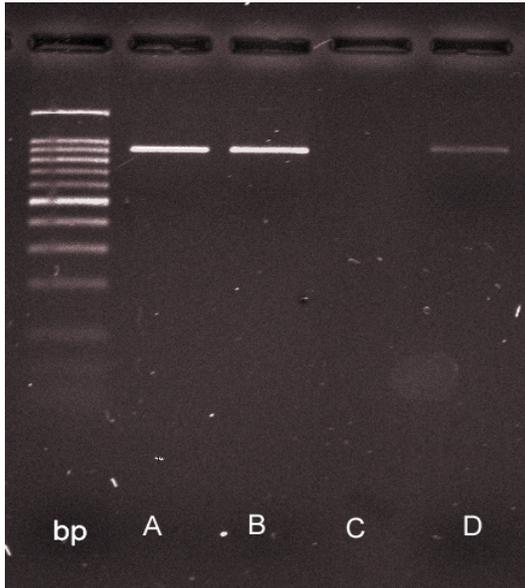


Fig. 1: Electropherogram of amplicon of 912 bp of POMP 90-91B gene of *Chlamydomphila abortus*, (bp) Molecular size markers of 100 bp, (A) vaginal swap of PB1 sheep, (B) lung of aborted fetus from PB2 sheep, (C) DNA of seronegative sheep, (D) *Cp. abortus* AB7 strain (Strain donated by the Department of Animal Welfare, Faculty of Veterinary Medicine, Universidad de Murcia, Spain)

DISCUSSION

This study confirmed the relative high seroprevalence of *C. abortus* in health sheep and sheep with history of abortion. Some anti-Chlamydia IgG antibodies may be induced by natural infection with *C. abortus* since a vaccine has not been used in this country. Nevertheless, due to known cross-reactivity among Chlamydomphila genus, some anti-Chlamydia IgG antibodies detected may have been induced by *C. pecorum*, this could explain for that a high percentage of healthy animals are positive to *C. abortus*. In spite of the above-mentioned, the Pourquier-ELISA assay used in this study has been reported as a test highly specific since it did not react with any of the sera from the SPF-lambs experimentally



Fig. 2: Alignment of the sequences of POMP 90-91B gene of *Chlamydomphila abortus* (accession number U65942) and Pb2 sample. Pb2 is a positive sample to amplicon of 912 bp of POMP 90-91B gene of *Chlamydomphila abortus* by PCR. Consensus sequences are shown at the bottom (*)

infected with various subtypes of *C. pecorum*^[9], therefore, we could think that healthy sheep that are positive to Pouquier-ELISA is due to a recent infection with *C. abortus* without history of abortion, thus the seroprevalence rate reported in this study should have been overestimated.

In Mexico, so far there has been not investigation on the situations surrounding the incidence of several infectious diseases that cause abortion in sheep and goats such as: *C. abortus*^[10], *Salmonella abortus ovis*^[11], *Champylobacter foetus*^[12], *Champylobacter jejuni*, *Toxoplasma gondii*^[13], *Leptospira pomona*^[14] and *Listeria monocytogenes*^[15]. However, goat brucellosis is endemic in most areas in Mexico. For

example, in some areas like the Bajío region of Michoacan the prevalence of brucella is 9.8% in goats^[16], while northeast of Mexico is reported of 9% in goats^[17], in Xalatlaco, Estado de Mexico is unknown. Due to the above-mentioned, is difficult to estimate how much of contributed *C. abortus* for provoke abort in these animals. If we considered that 21.3% of aborted sheep tested positive by serum IgG and that only two were PCR positive to *C. abortus*, we could be speculated that 80% of animals with history of abortion could be due to several pathogens to *C. abortus*.

During this study, one of the sheep, which had tested positive for *C. abortus* by PCR, aborted. Samples from the lungs and liver of the fetus were analyzed by PCR and they tested positive for the amplicon of 912 bp, sequencing of this fragment confirmed 100% homology.

CONCLUSION

In conclusion, the seroprevalence of *C. abortus* in Mexican's sheep is high, therefore, is necessary that the authorities responsible for animal welfare in Mexico (SAGARPA) to set up appropriate epidemiological surveillance and control programs to eradicate the disease caused by this pathogen.

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