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Presence of *Chlamydia abortus* in goats with a history of abortions in Mexico

Presencia de *Chlamydia abortus* en cabras con historial de abortos en México

Sánchez-Rocha Liliana[1ID], Arellano-Reynoso Beatriz[1*ID], Hernández-Castro Rigoberto[2 ID], Palomares-Resendiz Gabriela[3 ID], Barradas-Piña Francisco[4 ID], Díaz-Aparicio Efrén[3 ID]

[1]Departamento de Microbiología, Facultad de Medicina Veterinaria y Zootecnia, Universidad Nacional Autónoma de México, Circuito Exterior de Ciudad Universitaria, Coyoacán, Ciudad de México, 04510, México. [2]Departamento de Ecología de Agentes Patógenos, Hospital General "Dr. Manuel Gea González", Tlalpan, Ciudad de México, 14080, México. [3]CENID Salud Animal e Inocuidad, Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias, Carretera Federal México-Toluca Km. 15.5, Cuajimalpa, Ciudad de México, 05110, México.[4]CE La Posta, Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias, Km. 22.5 Carretera Federal Veracruz-Córdoba Paso del Toro, C.P. 94277, Municipio de Medellín de Bravo, Veracruz, México. *Corresponding author and correspondent author: Beatriz Arellano Reynoso. E-mail: liliana_srocha@hotmail.com, arerey@yahoo.com, rigo31@yahoo.com, gabio_1704@hotmail.com, fcobarradast@gmail.com, efredia@yahoo.com

ABSTRACT

Chlamydia abortus causes a series of reproductive disorders in ruminants, including abortions, premature births and stillbirths. Additionally, as a zoonosis, it can cause miscarriages or pneumonia in people who come in contact with sick animals or their secretions. The objective of this study was to isolate *C. abortus* from Mexican goats with a history of abortion and from recently parturient goats with a history of abortion; 186 samples were collected from 49 herds in the states of Coahuila, Jalisco, Puebla, Veracruz and Querétaro. Bacterial isolation of the clinical samples was performed using the mouse fibroblast cell line L929 and molecular identification was achieved by amplification of a 342 bp fragment corresponding to the 16S-23S ribosomal intergenic spacer RNA region. The amplification products were sequenced and compared with the GenBank database. Isolation identified 23.1% of the samples and PCR identified 9.6% as positive. Homology search revealed 100% identity with *Chlamydia abortus* EF486854, U76710, U68444, among others. The presence of *C. abortus* was confirmed in goats with a history of abortion in Mexico by bacterial isolation, PCR and sequencing. These findings suggest that *C. abortus* played a substantial role in goats with a history of abortion in Mexico.

Keywords: *Chlamydia abortus,* goats, abortions, Mexico, chlamydiosis.

RESUMEN

Chlamydia abortus causa abortos o nacimientos prematuros en rumiantes; adicionalmente, es una zoonosis que puede causar abortos o neumonías en personas que tienen contacto con animales enfermos o sus secreciones. El objetivo de este estudio fue aislar *C. abortus* de cabras mexicanas con historial de aborto y de cabras recién paridas con antecedentes de abortos; se recolectaron 186 muestras de 49 rebaños, en los estados de Coahuila, Jalisco, Puebla, Veracruz y Querétaro. El aislamiento bacteriano de las muestras clínicas fue realizado utilizando la línea celular de fibroblastos de ratón L929 y la identificación molecular se logró mediante la amplificación de un fragmento de 342 pb correspondiente a la región 16S-23S espaciador intergénico ribosomal RNA. Los productos de amplificación se secuenciaron y se compararon con la base de datos GenBank. El aislamiento identificó el 23.1% de las muestras y la PCR identificó el

9.6% como positivas. La búsqueda de homologías reveló una identidad del 100% con *Chlamydia abortus* EF486854, U76710, U68444, entre otras. La presencia de *C. abortus* se confirmó en cabras con antecedentes de aborto en México mediante aislamiento bacteriano, PCR y secuenciación. Estos hallazgos sugieren que *C. abortus* jugó un papel sustancial en cabras con antecedentes de aborto en México.
Palabras clave: *Chlamydia abortus*, caprinos, abortos, México.

INTRODUCTION

Chlamydiosis is an infectious disease that occurs in sheep, goats, and cows globally, causing reproductive disorders including abortions, stillbirths, and weak or premature offspring that die shortly after birth. The etiologic agent, *Chlamydia abortus*, is the main cause of reproductive losses in goats and sheep in northern European countries, and causes approximately 44% of all diagnosed infectious abortions in the United Kingdom (Stuen and Longbottom, 2011). As a zoonosis, *C. abortus* presents the greatest risk to pregnant women, because it is able to colonize the human placenta leading to abortion. Atypical respiratory pneumonia has also been described in workers who came into contact with ruminants (Longbottom and Coulter, 2003; Ortega *et al*., 2015; Pichon *et al*., 2020). *Chlamydia* is a Gram-negative bacterium and an obligate intracellular parasite. It presents an asynchronous multimorphic development cycle with two stages, the elementary and reticular bodies, both of which possess lipopolysaccharides. Unlike the typical bacterial cell wall, *Chlamydia* cell walls do not present muramic acid (Rodolakis and Laroucau, 2015).

In Mexico, chlamydiosis in goats and sheep, as well as the role of *Chlamydia* in ewe abortions were first reported in the 1990s (Escalante *et al*., 1996). The first isolation of *C. abortus* (then *C. psittaci* serotype 1) in goats was published in 1997 (Escalante *et al*., 1997). In 2001, *Chlamydia* was shown to be present in a zoonotic process that originated in an infected goat herd (Escalante *et al*., 2001). *C. abortus* (then *Chlamydophila abortus*) was demonstrated in goats using serology and isolation in 2004, and again in 2005, 2006 and 2008 (Lazcano, 2006; Soriano *et al*., 2011). In 2011, a study found *C. abortus* in 26.9% of sampled goats in the state of Guanajuato, Mexico, reaching 9.60% seropositivity (Mora *et al*., 2015). Palomares *et al*. (2020) evaluated individual and herd serological frequency, as well as risk factors for *C. abortus* in seven sheep-producing states in Mexico, and identified enzootic abortion in ewes. Given this background, the goal of this study was to determine whether *C. abortus* is present in cases of goat abortion across different regions of Mexico.

MATERIAL AND METHODS

Samples

This study was carried out between August 2016 and February 2018. We obtained a total of 186 vaginal exudates from 49 herds with reported abortions across five states in

Mexico. The state of Coahuila provided 82 samples (44.10%) from 24 herds, followed by Veracruz with 67 samples (36.02%) from 18 herds, Jalisco with 25 samples (13.44%) from three herds, Querétaro with seven samples (3.76%) from one herd, and Puebla with five samples (2.68%) from three herds.

All sampled does were mixed-breed, and husbandry conditions varied by state. Samples from Jalisco and Queretaro came from stabled herds kept as breeding stock under intensive management. These animals were fed balanced diets with mineral supplementation and did not coexist with other animal species. In contrast, goats from Puebla, Coahuila and Veracruz were kept under extensive management conditions for meat production, either commercially or for non-market consumption. These herds spent mornings grazing on unmanaged vegetation and along roadsides and were penned overnight without additional food supplementation. These goats coexisted with other animal species in the corrals, mainly with sheep and chickens, as well as horses, dogs and cats.

We sampled goats that had recently aborted or does that had recently given birth with a history of abortions (no more than 30 days in both cases). Vaginal samples were taken using sterile swabs and transported in tubes with 2 ml of sucrose-phosphate/glutamate (SPG) medium (217 mM sucrose, 4 mM KH2PO4, 7 mM K2HPO4, and 1% L-glutamine), supplemented with 10% fetal bovine serum (FBS; GIBCO, USA) and antibiotics (100 µg/ml streptomycin, 50 µg/ml gentamicin; Invitrogen, USA) (Sachse *et al*., 2009). In the laboratory, samples were kept at -20°C until processing. Experiments involving samples and live *Chlamydia* were performed in a type III biosafety laboratory. For the immunofluorescent technique and PCR reactions, we used a C. *abortus* A.22 strain kindly donated by Petter C. Griffiths from The Central Veterinary Laboratory, United Kingdom, in 1993. This sample was imported under zoosanitary certificate No. 27491.

Bacterial isolation

Bacterial isolation was performed in mouse fibroblasts from cell line L929. Cells were cultured in Eagle's essential minimum medium (EEMM; Invitrogen, USA), supplemented with 10% fetal bovine serum (FBS), 1% non-essential amino acids, 1% L-glutamine at 37°C, and 5% CO_2 (Escalante *et al*., 1996). To identify chlamydial bodies, we used 0.9×10^5 cells per well, in 24-well polystyrene culture plates with 12-mm diameter sterile glass coverslips (Mora *et al*., 2015). Identification of intracytoplasmic inclusions produced by *C. abortus* was carried out via direct immunofluorescent technique, using commercial IMGEN Chlamydia tests (OXOID, UK) according to manufacturer's instructions. Intracytoplasmic inclusions were visualized in an UV microscope (Leica DM1000) with

40X and 100X objectives. A sample was considered to be negative after two blind passages without intracytoplasmic inclusion detection.

DNA extraction

Vaginal exudate samples were homogenized and then 500 µl from each one were transferred to a sterile micro tube that was inactivated at 80°C for 20 min. Then, 100 µl of NET Buffer (50 mM NaCl, 125 mM EDTA, 50 mM Tris HCl, pH 7.6), and 50 µl of 24% SDS (3.4% final concentration) were added and incubated at 80°C for 10 min. Afterwards, we added RNAse at 75 µg/ml final concentration, and incubated for 2 h at 50°C, followed by proteinase K (USB, Ohio, USA) at a final concentration of 325 µg/ml and incubated at 50°C for 90 min more. Then, we added a 25:24:1 volume of phenol, chloroform and isoamilic alcohol (Sigma-Aldrich, USA), mixed for 15 min, and centrifuged at 16,060 *g* for 5 min at room temperature.

Finally, 0.6 volumes of isopropanol were added to the supernatant and centrifuged at 16,060 *g* for 15 min. The resulting DNA was washed with 70% cold ethanol and centrifuged at 16,060 *g* for 5 min. DNA was resuspended with 25 µl of DNAase-free water.

Molecular identification

Molecular identification was performed via PCR using primers that amplify a 342 bp of 16S-23S ribosomal RNA intergenic spacer. Primers were designed with the IDT SciTools Primer QuestSM program, using the *C. abortus* A.22 ribosomal operon sequence deposited in GenBank with accession number U68444.1 (Everett *et al*., 1997). PCR reactions were performed in a final volume of 50 µl, including 1X PCR buffer, 3 mM MgCl2, 400 µM dNTP´s, 25 pmol of each primer, 1 U DreamTaq Polymerase (Thermo, USA), and 50 ng DNA. *C. abortus* A.22 strain was used as positive control.

The amplification protocol consisted of an initial denaturing cycle of 5 min at 95°C, 40 cycles at 95°C for 1 min, 63°C for 30 s, 72°C for 1 min, and a final extension at 72°C for 10 min. Amplification products were observed in a 1% agarose gel (Thermo, USA) stained with ethidium bromide (0.5 µg/ml).

Samples found to be positive for *C. abortus* through PCR were purified using the commercial QIAquick gel extraction system, following manufacturer's instructions. Product sequencing was performed in both directions by means of the Taq FS Dye Terminator Cycle Sequencing Fluorescence-Based Sequencing method. The consensus sequence and sequence alignment were achieved with the Vector NTI program.

The sequences of 16S-23S ribosomal RNA intergenic spacer obtained in this study were subjected to BLAST searches in GenBank database. Multiple alignments were

established using sequences of the genus *Chlamydia* available in the GenBank with Clustal W and Muscle algorithms included in MEGA software version 7.0.26 (Kumar *et al*., 2016). Phylogenetic reconstruction was conducted using a Bayesian approach with MrBayes version 3.2 (Ronquist *et al*., 2012). The analysis was performed for 3,000,000 generations with sampling trees every 100 generations. Trees with scores lower than those at the stationary phase ('burn-in') were discarded, and trees that reached the stationary phase were collected and used to build majority consensus trees.

RESULTS

L929 cells were infected with clinical samples and immunofluorescence was used to visualize chlamydial inclusions. In total, inclusions were found in 43 out of 186 assessed samples (23.11%), or 18 out of 82 samples from Coahuila, 13 out of 67 from Veracruz, seven out of 25 from Jalisco, and five out of seven from Queretaro (Table 1).

Table 1. Overview of the obtained results from the bacteriological and PCR techniques employed on the vaginal exudates samples

Origin – States	Samples	Positive to bacterial isolation	Positive to PCR
Veracruz	67	13	2
Jalisco	25	7	5
Coahuila	82	18	5
Queretaro	7	5	6
Puebla	5	0	0
Total	186	43	18
Total percentages	100 %	23.19 %	9.68 %

PCR analysis revealed 18 of the 186 vaginal exudate DNA samples were positive for *C. abortus*. Across sampled regions, Querétaro had six positive samples, Coahuila and Jalisco each had five positive samples, Veracruz had two, and samples from Puebla had no positive results.

We identified the amplification zone included 70 bp of the 16S ribosomal subunit, a 105 bp intergenic region, the 115 bp 5S ribosomal subunit, another 3 bp intergenic region, and the 49 bp 23S ribosomal subunit. The sequences were edited with Vector NTI program and homology search was performed in the GenBank database (BLASTn), finding a 100% identity with *Chlamydia abortus* EF486854, U76710, U68444, among others. The *Chlamydia abortus* FMVZ455365 was the only strain that presented different homology to the other strains, this due to a simple change in a C/T base at position 236, however this strain showed 100 identities with *C. abortus* CP031646, LS450958 and KX870501, among others. The complete sequences obtained for *C. abortus* were deposited in the GenBank under accession numbers: FMVZ0Y55 (MZ093042), FMVZ455376 (MZ099638),

FMVZ0Y54 (MZ099636), FMVZ455365 (MZ093041), FMVZ45535 (MZ093043), FMVZ9505 (MZ099635), and FMVZ9595 (MZ099634) (Figure 1).

DISCUSSION

Results revealed the presence of *C. abortus* in goats from four of the five sampled Mexican states. In a previous study, *C. abortus* was isolated from goats of the state of Guanajuato that had aborted (Mora *et al*., 2015).

There are different causes of infectious or non-infectious abortion in ruminants, and malnutrition during gestation appears to be the most common in extensive goat production systems. Lack of food, long distances between grazing and shelter areas, exposure to high temperatures and a shortage of drinking water are frequent grazing conditions during in the dry season that can cause stress that leads to abortion. However, given that *C. abortus* is always a pathogen and not part of the bacterial flora, isolating this microorganism in herds where several animals present reproductive failure can be taken as a definitive and accurate diagnosis (Mellado *et al*., 2004; Urrutia *et al*., 2015; OIE, 2018).

Isolation is the gold standard test to demonstrate the presence of *C. abortus*, but inconsistencies in cell culture, including death during transport, inadequate sample preservation, or contamination can lead to low sensitivity (Thejls *et al.*, 1994; Sachse *et al*., 2009). By complementing isolation with PCR, we found 18/186 (9.23%) positive samples. Our results are lower than those described by Mora *et al*. (2015), who reported 30/125 (24%) PCR-positive vaginal exudate samples and were able to confirm 88.23% of the positive samples obtained from isolation. In the present study, PCR only confirmed 42.8% of the positive results observed through isolation. We may have observed false negatives due to low sample bacterial load, sample cross-contamination or because other elements present in the vaginal exudate samples may have inhibited the PCR (OIE, 2018). Some elements normally present in clinical samples are known to sometimes affect assay sensitivity, or even prevent DNA amplification (Schrader *et al*., 2012). These elements include polysaccharides, calcium, collagen, hemoglobin, sucrose and proteinases that either originate in the animal itself or its bacterial flora.

In contrast, while lower than Mora *et al*. (2015), our PCR positivity confirmation rate was higher than that reported by Campos-Hernández *et al*. (2014). In that study, which assessed 246 samples via PCR, the presence of *C. abortus* could only be confirmed in the spleen of an aborted fetus. Likewise, our PCR positivity results are higher than those

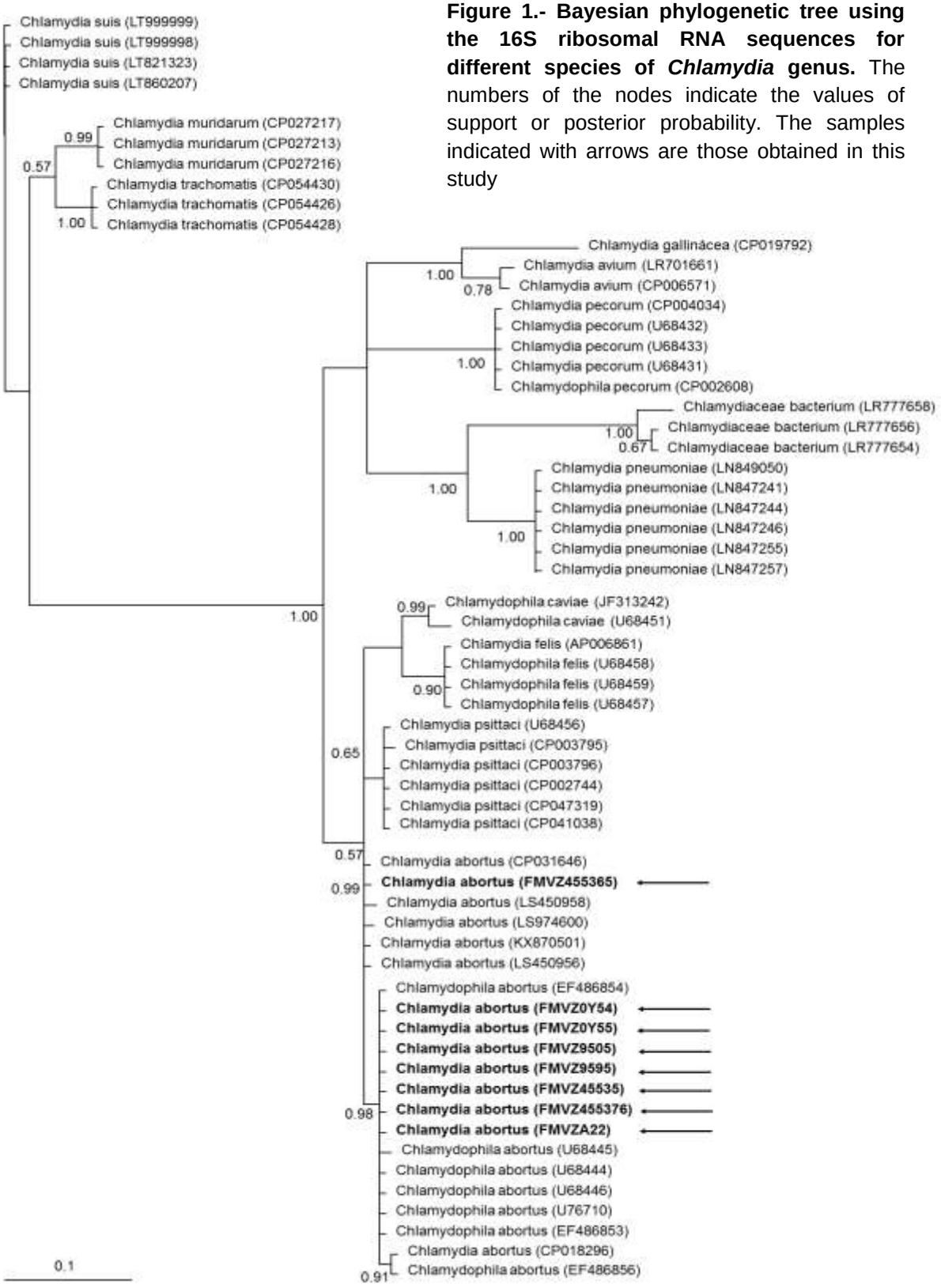

Figure 1.- Bayesian phylogenetic tree using the 16S ribosomal RNA sequences for different species of *Chlamydia* genus. The numbers of the nodes indicate the values of support or posterior probability. The samples indicated with arrows are those obtained in this study

from some European studies. A report from Italy (Chisu *et al.,* 2013) used PCR to detect 3/40 (7.5%) positive samples in sheep herds with high incidence of abortions. In contrast to our study, their evaluation had fewer samples, but these came from placentas. On the other hand, a study in Germany (Lenzko *et al.*, 2011) sampled 32 goat herds with abortion rates below 1%. They analyzed 352 vaginal exudates using PCR, and their results revealed that 28 animals (7.95%) were positive to *C. abortus*. These data indicate that *Chlamydia* infections occur frequently in this region even in the absence of high abortion rates, this could be due to the endemicity of the disease in the region (Lenzko *et al.*, 2011).

While placentas and aborted fetuses are the tissues with the highest bacterial load, and thus, the best source for bacterial isolation (Sachse *et al*., 2009; Rodolakis and Laroucau, 2015), the sampled herd husbandry conditions made it unfeasible to collect these tissues. Most of the herds in our study are kept under extensive management systems, where unsupervised goats roam many kilometers to reach communal pastures. As these field conditions make the recovery and collection of fetuses and placentas unfeasible, we sampled vaginal exudates instead. Studies from both Mexico and elsewhere have shown that vaginal exudates are adequate samples and can be widely used for diagnosis. (Papp *et al.*, 1994; Jiménez-Estrada *et al.*, 2008; Gutierrez *et al.*, 2011; Campos-Henández *et al.*, 2014; Mora *et al*., 2015; Laroucau *et al.*, 2018; O' Neill *et al.*, 2019).

Albeit it is accepted that working with tissues that have a high bacterial load favors the PCR sensitivity level (Livingstone *et al*. 2009), other studies in Mexico have successfully used PCR to detect *C. abortus* in vaginal exudates. In 2008, a study analyzed 304 vaginal exudates from sheep with a history of abortions coming from the state of Mexico (Estado de México). This study found 0.65% positivity (Jiménez-Estrada *et al.*, 2008) which is a lower positivity rate than that of our results. It is possible that husbandry practices that allow interactions between sheep and goat predispose the dissemination of *Chlamydia* between herds.

As part of the identification of *Chlamydia* species involved in our study, and specifically to discard the possibility of another bacterium being responsible for abortions in goats (namely *C. pecorum*), it was necessary to complement the PCR with amplified fragment sequencing.
The sequences showed high identity with most of the *C. abortus* sequences, available in the databases. Similar result was obtained when phylogenetic analyzes were carried out, observing little variation in the clade of *C. abortus* FMVZ455365, which an apparently separate of *C. abortus* Mexican strains with posterior probability of 98 and 99%, respectively.

As of 2016, chlamydiasis has been considered endemic in Mexico (DOF, 2016). Before this, it had been considered exotic, which prevented the implementation of diagnostic techniques and control measures, which in turn favored the spread of the disease. It is possible that the consequences of *Chlamydia abortus* presence in Mexico are similar to those reported worldwide, and that the lack of necessary detection tools have fostered its presence in Mexico without it becoming evident (Sachse *et al*., 2009). Furthermore, analysis of both sheep and goat studies may show that the bacterium has become disseminated throughout the country, but not at the same proportion in different regions. Indeed, positive serology results from areas in Mexico with important goat populations (distributed through the North, Center, West, and East of the country) seem to show that the lack of routine diagnosis before animal transporting has fostered disease spread, and concomitant sanitary and socioeconomic risks (Mora *et al*., 2015; Campos-Henández *et al.*, 2014; Jiménez-Estrada *et al.*, 2008).

The goal of our study was to determine *Chlamydia* spp. presence in some important goat producing regions in Mexico. While the type of sampling we used did not allow us to measure disease prevalence, our results showed that *Chlamydia abortus* was isolated in 23% of the goats that had endured abortions. This is evidence that *C. abortus* is an important pathogen that requires attention as well as the implementation of control measures, both in goats and other productive species in which we have evidence of the disease. Recent serological studies in Mexican dairy cows with abortions and reproductive disorders showed that out of a total 833 analyzed samples, 90 (10.8%) tested positive for *C. abortus.* In the state of Guanajuato, 6% (15/237) of animals tested seropositive and 18.5% (15/81) of the sampled herds have at least one seropositive animal (Limón *et al.*, 2011). A serological study performed in sheep herds between 2011 and 2013, analyzed 5,321 ewe blood samples from 209 production units in 61 municipalities across seven states in Mexico, and found positivity rates between 24% and 67% (Palomares *et al.*, 2020). It is necessary to carry out further research on both prevalence and distribution of *C. abortus* to better understand its impact on goats and production in Mexico. Pertinent measures to control and prevent *C. abortus* transmission between animals also need to be implemented in order to avert human infection. Furthermore, special attention needs to be paid in regard to education and disease risk awareness for people working directly with animals as well as in microbiology laboratories, given that human cases of *C. abortus* infections stem from exposure to infected sheep, goats, aerosols, and contaminated materials (Longbottom and Coulter, 2003; Ortega *et al*., 2015; Pichon *et al*., 2020).

CONCLUSION

The present study confirms that *Chlamydia abortus* is found in Mexican goats who have had abortions, in the states of Coahuila, Jalisco, Queretaro, and Veracruz. Furthermore, that *Chlamydia abortus* is the etiologic agent of chlamydiosis, which supports and confirms the presence of chlamydiosis in Mexico. It is of the utmost importance to study the distribution of this bacterium across Mexico, in order to implement prevention and control measures to avoid spread of disease, as well as to diminish the risk of human infection.

Conflict of interest statement
The authors have nothing to disclose.

REFERENCES

CAMPOS-HERNÁNDEZ E, Vázquez-Chagoyán JC, Salem AZ, Saltijeral-Oaxaca JA, Escalante-Ochoa C, López-Heydeck SM, de Oca-Jiménez RM. 2014. Prevalence and molecular identification of *Chlamydia abortus* in commercial dairy goat farms in a hot region in Mexico. *Tropical Animal Health and Production*. 46(6):919-924. https://doi.org/10.1007/s11250-014-0585-6

CHISU V, Porcu R, Tanda A, Masala G. 2013. First isolation and characterization of *Chlamydophila abortus* from abortion tissues of sheep in Sardinia, Italy. *Veterinaria Italiana*. 49(4):331-334. https://doi.org/10.12834/VetIt.1303.10

DOF (Diario oficial de la Federación). 2016. Acuerdo mediante el cual se dan a conocer en los Estados Unidos Mexicanos las enfermedades y plagas exóticas y endémicas de notificación obligatoria de los animales terrestres y acuáticos. 4 de mayo de 2016. http://www.dof.gob.mx/nota_detalle.php?codigo=5436016&fecha=04/05/2016

ESCALANTE OC, Díaz A, Segundo Z, Suárez G. 1997. Isolation of *Chlamydia psittaci* involved in abortion of goats in Mexico: First Report. *Revista Latinoamericana de Microbiología*. 39(3-4):117–121. PMID: 10932720. https://pubmed.ncbi.nlm.nih.gov/10932720/

ESCALANTE OC, Lazcano A, Soberón M. 2001. *Chlamydophila* spp. Como agente zoónotico en México: Reporte de un caso. Abstracts of XXXVII Reunión Nacional de Investigación Pecuaria. Tuxtla Gutierréz, Chiapas, México. Pp. 41. ISSN: 24485284.

ESCALANTE OC, Rivera F, Trigo T, Romero M. 1996. Detection of *Chlamydia psittaci* in enteric subclinical infections in adult sheep through cell culture isolation. *Revista Latinoamericana de Microbiología*. 38(1):17–23. PMID: 8783901. https://pubmed.ncbi.nlm.nih.gov/8783901/

EVERETT KD, Andersen AA. 1999. Identification of nine species of the *Chlamydiaceae* using PCR-RFLP. *International Journal of Systematic Bacteriology*. 49(2): 803–813. https://doi.org/10.1099/00207713-49-2-803

EVERETT KD, Andersen AA. 1997. The ribosomal intergenic spacer and domain I of the 23S rRNA gene are phylogenetic markers for *Chlamydia* spp. *International Journal of Systematic Bacteriology*. 47(2): 461-473. https://doi.org/10.1099/00207713-47-2-461

GUTIERREZ J, Williams EJ, O'Donovan J, Brady C, Proctor AF, Marques PX, Worrall S, Nally JE, McElroy M, Bassett HF, Sammin DJ, Markeya BK. 2011. Monitoring clinical outcomes, pathological changes and shedding of *Chlamydophila abortus* following experimental challenge of periparturient ewes utilizing the natural route of infection. *Veterinary Microbiology*. 147(1-2):119-126. https://doi.org/10.1016/j.vetmic.2010.06.015

JIMÉNEZ-ESTRADA JM, Escobedo-Guerra MR, Arteaga-troncoso G, López-hurtado M, De Haro-Cruz MDJ, Montes de Oca JR, Guerra-Infante FM. 2008. Detection of *Chlamydophila abortus* in Sheep (*Ovis aries*) in Mexico. *American Journal of Animal and Veterinary Sciences*. 3(4):91-95. https://thescipub.com/abstract/ajavsp.2008.91.95

KUMAR S, Stecher G, Tamura K. 2016. MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets. *Molecular Biology and Evolution*. 33(7):1870-1874. https://doi.org/10.1093/molbev/msw054

LAROUCAU K, Aaziz R, Vorimore F, Menard MF, Longbottom D, Denis G. 2018. Abortion storm induced by the live *C. abortus* vaccine 1B strain in a vaccinated sheep flock, mimicking a natural wild-type infection. *Veterinary Microbiolology*. 225:31-33. https://doi.org/10.1016/j.vetmic.2018.09.012

LAZCANO AC. 2006. Detección de *Chlamydophila* spp. en rebaños caprinos del Estado de Michoacán mediante técnica inmunodiagnóstica ELISA y aislamiento bacteriológico. Tesis de Licenciatura. Universidad Nacional Autónoma de México, CDMX, México. https://ru.dgb.unam.mx/handle/DGB_UNAM/5/browse?type=author&order=ASC&rpp=20&value=Lazcano+Arrevillaga%2C+Cnidia

LENZKO H, Moog U, Henning K, Lederbach R, Diller R, Menge C, Sachse K, Sprague LD. 2011. High frequency of chlamydial co-infections in clinically healthy sheep flocks. *BMC Veterinary Research*. 7(29). https://doi.org/10.1186/1746-6148-7-29

LIMÓN GMM, Favila HLC, Herrera LE, Lozano DRR, Meléndez SRM, Morales AJF, Díaz AE, Vitela MI, Córdova LD. 2011. Clamidofilosis en vacas lecheras con problemas reproductivos en Guanajuato y Aguascalientes: Resultados preliminares. Abstracts of XLVII Reunión Nacional de Investigación Pecuaria, Mérida, Yucatán, México. ISBN: 978-60-425-582-9.

LIVINGSTONE M, Wheelhouse N, Maley SW, Longbottom D. 2009. Molecular detection of *Chlamydophila abortus* in post-abortion sheep at oestrus and subsequent lambing. *Veterinary Microbiology*. 135(1-2):134-141. https://doi.org/10.1016/j.vetmic.2008.09.033

LONGBOTTOM D, Coulter LJ. 2003. Animal chlamydioses and zoonotic implications. *Journal of Comparative Pathology*. 128(4):217-244. https://doi.org/10.1053/jcpa.2002.0629

MELLADO M, Valdez R, Lara LM, García JE. 2004. Risk factors involved in conception, abortion, and kidding rates of goats under extensive conditions. *Small Ruminant Research*. 55 (1-3):191-198. https://doi.org/10.1016/j.smallrumres.2003.10.016

MORA DJC, Díaz AE, Herrera LE, Suárez GF, Escalante OC, Jaimes VS, and Arellano-Reynoso B. 2015. Isolation of *Chlamydia abortus* in dairy goat herds and its relation to abortion in Guanajuato, Mexico. *Veterinaria Mexico OA*. 2(1). https://doi.org/10.21753/vmoa.2.1.339

OIE (Organización mundial de sanidad animal). 2018. Manual terrestre de la OIE. Capítulo 3.7.5. Aborto enzoótico de las ovejas (Clamidiosis ovina) (Infección por *Chlamydia abortus*. https://www.oie.int/fileadmin/Home/esp/Health_standards/tahm/3.07.05_Aborto_enz_ovejas.pdf

O'NEILL LM, Keane OM, Ross PJ, Nally JE, Seshu J, Markey B. 2019. Evaluation of protective and immune responses following vaccination with recombinant MIP and CPAF from *Chlamydia abortus* as novel vaccines for enzootic abortion of ewes. *Vaccine*. 37(36): 5428–5438. https://doi.org/10.1016/j.vaccine.2019.06.088

ORTEGA N, Caro MR, Gallego MC, Murcia-Belmonte A, Alvarez D, Del Rio L, Cuello F, Buendia AJ, Salinas J. 2015. Isolation of *Chlamydia abortus* from a laboratory worker diagnosed with atypical pneumonia. *Irish Veterinary Journal*. 69, 8. https://doi.org/10.1186/s13620-016-0067-4

PALOMARES REG, Mejía SP, Aguilar RF, De la Cruz CL, Jiménez SH, Leyva CJC, Morales PMI, Díaz AE. 2020. Frecuencia y factores de riesgo asociados a la presencia de C*hlamydia abortus,* en rebaños ovinos en México. *Revista mexicana de ciencias pecuarias*.11(3):783-794. ISSN 2428-6698. https://doi.org/10.22319/rmcp.v11i3.5269

PAPP JR, Shewen PE, Gartley CJ.1994. Abortion and subsequent excretion of *Chlamydiae* from the reproductive tract of sheep during estrus. *Infection and Immunity*. 62:3786-3792. PMID: 8063395. https://iai.asm.org/content/62/9/3786.short

PICHON N, Guindre L, Laroucau K, Cantaloube M, Nallatamby A, Parreau S. 2020. *Chlamydia abortus* in Pregnant Woman with Acute Respiratory Distress Syndrome. *Emerging Infectious Diseases.* 26(3): 628-629. https://doi.org/10.3201/eid2603.191417

RONQUIST F, Teslenko M, van der Mark P, Ayres DL, Darling A, Höhna S, Larget N, Liu L, Suchard MA, Huelsenbeck JP. 2012. MrBayes 3.2: Efficient Bayesian phylogenetic inference and model choice across a large model space. *Systematic Biology*. 61(3): 539-542. https://doi.org/10.1093/sysbio/sys029

SACHSE K, Vretou E, Livingstone M, Borel N, Pospischil A, Longbottom D. 2009. Recent developments in the laboratory diagnosis of chlamydial infections. *Veterinary Microbiology*. 135(1-2):2-21. https://doi.org/10.1016/j.vetmic.2008.09.040

SCHRADER C, Schielke A, Ellerbroek L, Johne R. 2012. PCR inhibitors - occurrence, properties and removal. *Journal of Applied Microbiology*. 113(5): 1014-1026. https://doi.org/10.1111/j.1365-2672.2012.05384.x

RODOLAKIS A, Laroucau K. 2015. *Chlamydiaceae* and chlamydial infections in sheep or goats. *Veterinary Microbiology*. 181(1-2):107-118. https://doi.org/10.1016/j.vetmic.2015.07.010

SORIANO VE, Jiménez EJM, Salgado MC, López HM, Escobedo GMR, Guerra IFM. 2011. Identificación de C*hlamydophila abortus* en un aborto ovino en Almoloya de Juárez, México. *Revista electrónica de veterinaria*. 12(11): 1-6. http://www.redalyc.org/articulo.oa?id=63622049007

STUEN S, Longbottom D. 2011. Treatment and control of chlamydial and rickettsial infections in sheep and goats. *Veterinary Clinics of North America: Food Animal Practice*. 27(1):213-233. https://doi.org/10.1016/j.cvfa.2010.10.017

THEJLS H, Gnarpe J, Gnarpe H, Larsson PG, Platz-Christensen JJ, Ostergaard L, Victor A. 1994. Expanded gold standard in the diagnosis of *Chlamydia trachomatis* in a low prevalence population: diagnostic efficacy of tissue culture, direct immunofluorescence, enzyme immunoassay, PCR and serology. *Genitourinary Medicine*. 70 (5):300-303. https://sti.bmj.com/content/70/5/300

URRUTIA MJ, Vera AHR, Villagómez-Amezcua ME. 2015. “Aborto no infeccioso”. In: Díaz-Aparicio E, Tórtora-PÉREZ J, Palomares-Resendiz E, Gutiérrez-Hernández J. Enfermedades de las cabras. Mexico: INIFAP, SAGARPA. ISBN: 978-607-37-0411-3.