ORIGINAL RESEARCH ARTICLE

Presence of *Chlamydia suis* and *Chlamydia abortus* in Pigs from Mexico; first report

Presencia de Chlamydia suis y Chlamydia abortus en cerdos de México, primer reporte

Chlamydia suis e Chlamydia abortus em suínos do México: primeiro relato

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Abstract

Background: The genus *Chlamydia* comprises obligate intracellular Gram-negative bacteria responsible for various diseases in animals and humans. In pigs, the species *C. suis* is transmitted via aerogenic, genital, and oral routes, with its pathogenicity associated with the conjunctival tissue, respiratory system, gastrointestinal tract, and reproductive system. In addition to *C. suis*, other *Chlamydia* species have been identified in pigs. In Mexico, chlamydiosis was considered an exotic disease until 2016, the year it was recognized as endemic in birds and ruminants; however, there are no reports of this disease in pigs. **Objective:** This study aimed to detect the presence of *Chlamydia suis* and *Chlamydia abortus* in sows that had experienced abortion and conjunctivitis. **Methods**: This study examined the presence of *Chlamydia suis* and *Chlamydia abortus* in pigs from four farms in central Mexico between 2016 and 2022, focusing on animals exhibiting reproductive issues, conjunctivitis, or diarrhoea. A total of 141 samples, including vaginal exudates (122), conjunctival exudates (17), and faeces (2), were analysed using real-time PCR (qPCR) to detect *Chlamydiaceae* and specific *Chlamydia* species. **Results:** The results showed that 57 samples were positive for the family *Chlamydiaceae* (40.42%). Within this group, 26 animals (45.61%) were identified with a specific *Chlamydia* species, while in 31 animals (54.39%) the infecting species could not be determined, highlighting the necessity for further genomic analysis. Among the identified species, 12 animals were positive exclusively for *C. abortus*, one animal was positive exclusively for *C. suis*, and 13 animals presented coinfection with *C. abortus* and *C. suis*. **Conclusions:** These results align with global reports on *Chlamydia* prevalence in pigs and underscore the significance of co-infections, which were observed in vaginal exudates, feces, and conjunctival samples. This study constituted the first report of porcine chlamydiosis in Mexico, raising concerns about potential health risks for pig farms and zoonotic implications for humans. The study emphasized the need for improved surveillance of *Chlamydia* species across production stages and advocates further research into the genetic diversity and prevalence of *Chlamydia* in Mexico.

Keywords: *Chlamydia abortus; Chlamydia suis; Coinfection; Pigs.*

Resumen

Antecedentes: *Chlamydia* es un género de bacterias Gram negativas, intracelulares obligadas, que causan diversas enfermedades en animales y humanos. En cerdos, *C. suis* se transmite por vías aerógena, genital y oral, afectando el tejido conjuntival, el sistema respiratorio, el tracto gastrointestinal y el sistema reproductivo. Además de *C. suis*, se han identificado otras especies de *Chlamydia* en cerdos. En México, la clamidiosis fue considerada exótica hasta 2016, cuando se reconoció como endémica en aves y rumiantes; sin embargo, no hay reportes en cerdos. **Objetivo:** Detectar la presencia de *Chlamydia suis* y *Chlamydia abortus* en cerdas con antecedentes de aborto y conjuntivitis. **Métodos:** Se investigó la presencia de *C. suis* y *C. abortus* en cerdos de cuatro granjas del centro de México entre 2016 y 2022, priorizando animales con problemas reproductivos, conjuntivitis o diarrea. Se analizaron 141 muestras: exudados vaginales (122), conjuntivales (17) y heces (2), mediante PCR en tiempo real (qPCR) para detectar *Chlamydiaceae* y especies específicas de *Chlamydia*. **Resultados:** Los resultados mostraron que 57 muestras fueron positivas para la familia *Chlamydiaceae* (40.42%). Dentro de este grupo, 26 animales (45.61%) fueron identificados con una especie específica de *Chlamydia*, mientras que en 31 animales (54.39%) no fue posible determinar la especie infectante, lo que resalta la necesidad de análisis genómicos adicionales. Entre las especies identificadas, 12 animales fueron positivos exclusivamente a *C. abortus*, un animal fue positivo exclusivamente a *C. suis* y 13 animales presentaron coinfección con *C. abortus* y *C. suis*. **Conclusiones:** Estos hallazgos coinciden con informes globales sobre la prevalencia de *Chlamydia* en cerdos y resaltan la importancia de las coinfecciones. Este estudio representa el primer informe de clamidiosis porcina en México, lo que plantea preocupaciones sobre su impacto en granjas y su potencial zoonótico. Se destaca la necesidad de mejorar la vigilancia de *Chlamydia* en la producción porcina y de ampliar la investigación sobre su diversidad genética y prevalencia en México.

Palabras clave: *Cerdos; Chlamydia abortus*; *Chlamydia suis*; *Coinfección.*

Resumo

Contexto: O gênero *Chlamydia* compreende bactérias Gram-negativas intracelulares obrigatórias, responsáveis por diversas doenças em animais e humanos. Em suínos, a espécie *C. suis* é transmitida por vias aerogênica, genital e oral, estando sua patogenicidade associada aos tecidos

conjuntivais, ao sistema respiratório, ao trato gastrointestinal e ao sistema reprodutivo. Além de *C. suis*, outras espécies de *Chlamydia* foram identificadas em suínos. No México, a clamidiose foi considerada uma doença exótica até 2016, ano em que foi reconhecida como endêmica em aves e ruminantes; no entanto, não há registros dessa doença em suínos. **Objetivo:** Este estudo teve como objetivo detectar a presença de *Chlamydia suis* e *Chlamydia abortus* em porcas que apresentaram aborto e conjuntivite. **Métodos:** Investigou-se a presença de *Chlamydia suis* e *Chlamydia abortus* em suínos de quatro granjas no centro do México entre 2016 e 2022, com foco em animais que apresentavam problemas reprodutivos, conjuntivite ou diarreia. Um total de 141 amostras, incluindo exsudatos vaginais (122), exsudatos conjuntivais (17) e fezes (2), foram analisadas por PCR em tempo real (qPCR) para detectar *Chlamydiaceae* e espécies específicas de *Chlamydia*. **Resultados:** Os resultados mostraram que 57 amostras foram positivas para a família *Chlamydiaceae* (40.42%). Dentro desse grupo, 26 animais (45.61%) foram identificados com uma espécie específica de *Chlamydia*, enquanto em 31 animais (54.39%) a espécie infectante não pôde ser determinada, destacando a necessidade de análises genômicas adicionais. Entre as espécies identificadas, 12 animais foram positivos exclusivamente para *C. abortus*, um animal foi positivo exclusivamente para *C. suis* e 13 animais apresentaram coinfecção com *C. abortus* e *C. suis*. **Conclusões:** Esses resultados estão em conformidade com relatos globais sobre a prevalência de *Chlamydia* em suínos e ressaltam a importância das coinfecções, observadas em exsudatos vaginais, fezes e amostras conjuntivais. Este estudo constitui o primeiro relato de clamidiose suína no México, levantando preocupações sobre potenciais riscos sanitários para granjas de suínos e implicações zoonóticas para humanos. O estudo enfatiza a necessidade de melhorar a vigilância das espécies de *Chlamydia* em todas as fases da produção e defende mais pesquisas sobre a diversidade genética e a prevalência de *Chlamydia* no México.

Palavras-chave: *Chlamydia abortus*; *Chlamydia suis*; *Coinfecção; Suínos.*

Introduction

The genus *Chlamydia* comprises obligate intracellular Gram-negative bacteria responsible for various diseases in animals and humans. Currently, 14 pathogenic species are recognised, affecting mammals, birds, and reptiles (Staub *et al.*, 2018; Taylor-Brown and Polkinghorne, 2017; Vorimore *et al.*, 2013; Zareba-Marchewka *et al.*, 2020).

In pigs, the species *C. suis* is transmitted via aerogenic, genital, and oral routes, with its pathogenicity associated with the conjunctival tissue, respiratory system, gastrointestinal tract, and reproductive system. In addition to *C. suis*, other *Chlamydia* species have been identified in pigs. *C. abortus* has been linked to reproductive disorders, *C. pecorum* has been associated with keratoconjunctivitis, polyarthritis, and encephalomyelitis, and *C. psittaci* has been detected in the lungs and genital tract (Kieckens *et al.*, 2018; Schautteet and Vanrompay, 2011).

Co-infections involving *C. suis* with either *C. abortus* or *C. pecorum* have also been reported, resulting in clinical presentations such as those mentioned above (Dean *et al.*, 2013). Despite its growing recognition as an emerging pathogen in the swine population worldwide, *Chlamydia suis* remains significantly understudied in Mexico and across Latin America.

In Mexico, chlamydiosis was considered an exotic disease until 2016. It is now endemic in birds and ruminants (Limon-Gonzalez *et al.*, 2022; Ornelas-Eusebio *et al.*, 2020). Although the presence of *C. suis* has been demonstrated in pig farms in the United States (Hoque *et al.,* 2020), the lack of epidemiological data in Mexican pig farms may be attributed to diagnostic limitations, underreporting due to nonspecific clinical signs, and low prioritization in national surveillance programs. Given the economic importance of the swine industry in Mexico and the increasing global concern over antimicrobial resistance, investigating the prevalence, pathogenicity, and transmission dynamics of *C. suis* is essential. It is hypothesized that *C. suis* may already be present in Mexican pig farms, but remains undetected due to current diagnostic and surveillance limitations, as well as the presence of other viruses that produce similar clinical signs, such as Porcine Reproductive and Respiratory Syndrome virus, porcine parvovirus, or bacteria such as *Leptospira* spp., or *Mycoplasma* spp., among others. Such research would not only fill a critical gap in veterinary public health but also contribute to the development of targeted biosecurity and diagnostic strategies within the region.

This study aimed to detect the presence of *Chlamydia suis* and *Chlamydia abortus* in sows that had experienced abortion and conjunctivitis.

Material and methods

Ethical considerations

The procedures were performed according to institutional guidelines and with approval from the Institutional Subcommittee for the Care and Use of Experimental Animals of the "Facultad de Medicina Veterinaria y Zootecnia, Universidad Nacional Autónoma de Mexico", protocol DC-2023/3-3.

Animals and samples

The study was observational and descriptive, based on case reports and case series, with the objective of identifying clinical presentations through non-probabilistic sampling of available subjects (Manterola and Otzen, 2014; Tyrer and Heyman, 2016). It was conducted on four pig farms located in central Mexico, each with distinct production objectives. Farms were selected based on the presence of reproductive, conjunctival, or diarrheal disorders reported between 2016 and 2022. Farm 1 (Tenancingo, State of Mexico; 18°57'46.6"N 99°35'37.2"W) was a semi-technified farrow-to-wean unit with 315 sows. Farm 2 (Jocotitlán, State of Mexico; 19°42'27.7"N 99°47'19.2"W) was a semi-technified farrow-to-finish unit with 215 sows. Farm 3 (Jilotepec, State of Mexico; 19°57'24.9"N 99°30'43.8"W) was a farrow-to-finish unit with 130 sows. Farm 4 (Tláhuac, Mexico City; 19°16'17.2"N 99°00'31.4"W) was a backyard unit with 63 sows, some of which exhibited reproductive disorders. A total of 141 samples were obtained using sterile swabs: 122 vaginal exudate samples, 17 conjunctival exudate samples, and two faecal samples collected from the rectum of diarrhoeic animals.

The samples were placed in a Sucrose/Phosphate/Glutamine (SPG) transport medium and maintained at 4°C during transportation to the CENID-SAI laboratory, where they were stored at -80°C until processing.

DNA analysis and Chlamydia diagnostics

Total DNA was extracted from vaginal, conjunctival, and rectal swab samples using the DNeasy Blood and Tissue Kit (Qiagen, Hilden, Germany), according to the manufacturer's instructions. Subsequently, DNA concentration was assessed using a UV micro-spectrophotometer (NanoDrop 1000; Thermo Fisher Scientific, MA, USA) at an absorbance of 260 nm. DNA purity

was determined by calculating the 260/280 nm absorbance ratio. Sample concentrations ranged from a minimum of 5 ng/µL to a maximum of 150 ng/µL.

Amplification and detection of a 111 bp fragment of the 23S rRNA gene—present in all members of the *Chlamydiaceae* family—were performed by real-time PCR (qPCR) on a LightCycler 480 II system (Roche, Rotkreuz, Switzerland), using primers and probes described by Ehricht *et al*. (2006) with the modifications detailed below. A TaqMan® hydrolysis probe (Biosearch Technologies, Hoddesdon, UK) was used.

Reactions were performed in 96-well plates in 20 µl volumes containing: 10 µl of QuantiNova Probe PCR Kit (Qiagen, Hilden, Germany; final concentration 1×), 0.4 µM of each primer, 0.1 µM of hydrolysis probe, and 2 µl of template DNA. Cycling conditions were: initial denaturation at 95 °C for 2 min, followed by 40 cycles of denaturation at 95 °C for 5 s and annealing/extension at 65 °C for 2 s.

Positive samples were subsequently tested for species identification. A 23S rRNA gene fragment specific to *C. suis* and an *ompA* gene fragment specific to *C. abortus*, *C. psittaci*, and *C. pecorum* were amplified, using primers and probes reported by Pantchev *et al.* (2010), with the following modifications: reactions (20 µl) contained 10 µl of QuantiNova Probe PCR Kit (Qiagen; final concentration 1×), 0.4 µM of each primer, 0.1 µM of hydrolysis probe for *C. abortus* and *C. suis*, 0.2 µM of hydrolysis probe for *C. psittaci* and *C. pecorum*, and 2 µl of DNA. All hydrolysis probes were obtained from Biosearch Technologies. The cycling conditions were: initial denaturation at 95 °C for 2 min, followed by 40 cycles of denaturation at 95 °C for 5 s and annealing at 60 °C for 3 s.

The primers and probes used are listed in Table 1. Detection thresholds (Ct values) were established for each qPCR assay using standard curves (Supplementary Figure 1). Thresholds were as follows: *Chlamydiaceae* 23S rRNA and *C. pecorum*, Ct ≤ 36; *C. suis*, Ct ≤ 37; and *C. abortus* and *C. psittaci*, Ct ≤ 35.

Table 1. Primers and probes used for the identification of different species of *Chlamydia*

qPCR	Target gene	Primers and probes (5′- 3′)	Amplicon size	Reference
Chlamydiaceae	23S rRNA	Ch23S-Fw: CTGAAACCAGTAGCTTATAAGCGGT Ch23S-Rv: ACCTCGCCGTTTAACTTAACTCC Ch23S-P: FAM-CTCATCATGCAAAAGGCACGCCG-TAMRA	111bp	Ehricht et al., 2006
C. abortus	*omp*A	CpaOMP1-Fw: GCAACTGACACTAAGTCGGCTACA CpaOMP1-Rv: ACAAGCATGTTCAATCGATAAGAGA CpaOMP1-P: FAM-TAAATACCACGAATGGCAAGTTGGTTTAGCG-TAMRA	82 bp	Pantchev, *et al*., 2010
C. suis	23S rRNA	C suis23S-Fw: CCTGCCGAACTGAAACATCTTA Csuis23S-Rv: CCCTACAACCCCTCGCTTCT Csuis23S-P: FAM-CGAGCGAAAGGGGAAGAGCCTAAACC-TAMRA	118 bp	Pantchev, *et al*., 2010
C. psittaci	*omp*A	CppsOMP1-Fw: CACTATGTGGGAAGGTGCTTCA CppsOMP1-Rv: CTGCGCGGATGCTAATGG CppsOMP1-P: FAM-CGCTACTTGGTGTGAC-TAMRA	76bp	Pantchev, *et al*., 2010
C. pecorum	*omp*A	CppecOMP1-F: CCATGTGATCCTTGCGCTACT CppecOMP1-R: TGTCGAAAACATAATCTCCGTAAAAT CppecOMP1-P: FAM-TGCGACGCGATTAGCTTACGCGTAG-TAMRA	76 bp	Pantchev, *et al*., 2010

Results

Of the 141 samples collected, 57 tested positive for the *Chlamydiaceae* family. Of these 57 positive samples, 51 were vaginal exudates, four were conjunctival exudates, and two were faecal samples from diarrheic animals.

Among the 57 *Chlamydiaceae*-positive samples, 26 animals were identified with a specific *Chlamydia* species; notably, 31 samples positive for the *Chlamydiaceae* family, did not test positive for any of the four *Chlamydia* species examined using these primers. Among the identified species, 12 animals were positive exclusively for *C. abortus*, one animal was positive exclusively for *C. suis*, and 13 animals presented coinfection with *C. abortus* and *C. suis*. All samples were negative for *C. pecorum* and *C. psittaci*.

Of the 25 *C. abortus*-positive samples, 19 were vaginal exudates, of which seven also tested positive for *C. suis*, indicating co-infection in these animals. One vaginal exudate sample was positive only for *C. suis*. The four conjunctival exudates and two positive faecal samples for *C. abortus* also showed co-infection with *C. suis*. In total, 13 samples demonstrated co-infection with both species (*C. abortus* and *C. suis*), as shown in Table 2.

Table 2. Results of samples tested for the *Chlamydiaceae* family and *Chlamydia* species. Of the samples that tested positive for the *Chlamydiaceae* family, 31 did not yield positive results for any of the four *Chlamydia* species assessed.

Type of sample	Number of samples	Positive to *Chlamydiaceae*	Positive to *C. abortus*	Positive to *C. suis*	Coinfection *C. abortus* & *C. suis*
Vaginal swabs	n=122	51	12	1	7
Conjunctival swabs	n=17	4	0	0	4
Rectal swabs	n= 2	2	0	0	2
Total	141	57	12	1	13

Discussion

Most studies on chlamydiosis in pigs have been conducted on commercial farms in European countries, involving both fattening and breeding pigs in intensive production systems and small herds. However, the global prevalence of the disease remains unknown (Englund *et al.*, 2012; Hoffmann *et al.*, 2015; Hoque *et al.*, 2020; Li *et al.*, 2017; Rypula *et al.*, 2016; Vanrompay *et al.*, 2004). In Mexico, there are no reports of chlamydiosis in pigs despite its endemic presence in birds and small ruminants (DOF, 2016; Limon-Gonzalez *et al.*, 2022; Ornelas-Eusebio *et al.*, 2020).

This study demonstrated the presence of *C. suis* in eight sows with reproductive problems and four pigs (two males and two females) in the fattening stage. However, *C. abortus* was the most frequently identified species.

A meta-analysis conducted in China reported a 21.86% prevalence of *Chlamydia* in pigs with reproductive disorders and 21.43% in fattening pigs. In this analysis, *C. abortus* showed a prevalence of 41.35%, while *C. psittaci* was detected at 18.41%. The authors suggested strengthening *Chlamydia* detection in healthy and symptomatic pigs to control bacterial spread, particularly in intensive farming. Although *C. suis* was not evaluated in that study, our findings underscore the importance of analysing multiple *Chlamydia* species, as several may co-circulate within a single farm (Sheng *et al.*, 2021).

A noteworthy result in this study was the co-infection of *C. suis* and *C. abortus*, identified in vaginal exudates from sows with reproductive problems. Co-infection was also detected in conjunctival exudates and fecal samples from pigs with conjunctivitis and diarrhea, respectively. However, in the same farm, no pathogen was detected in vaginal exudates from pigs that also presented reproductive problems, suggesting potential variability in species distribution or infection dynamics.

Several studies have reported *Chlamydia* co-infections. Pantchev *et al*. (2010) detected co-infections in 21.1% of DNA samples from several hosts, including pigs, aligning with our findings. These authors highlighted the pig's susceptibility to co-infections. In Switzerland, Hoffmann *et al*. (2015) found *C. suis* as the predominant species in fattening pigs, with a correlation between *Chlamydia* presence and diarrhoea but not conjunctivitis. Mixed infections of *C. suis*, *C. pecorum*, and *C. abortus* were linked to proximity to ovine flocks, a factor absent in our study.

Rypula *et al.* (2018) evaluated the impact of *C. suis* infection on reproduction in 64 herds, finding reproductive issues in 77.3% of sampled sows. In our study, we prioritised samples from sows with abortion issues, resulting in most samples being vaginal exudates (122/141), followed by conjunctival exudates and faecal samples in fewer cases.

Schautteet *et al.* (2013) studied herds in Belgium, Israel, and Cyprus with reproductive problems, detecting *C. suis* in 58.97% of animals sampled, with some cases showing positive results for only specific sample types. Similarly, Schautteet and Vanrompay (2011) highlighted the importance of collecting multiple sample types to improve detection success, as *Chlamydia* may circulate in different production groups and excrete via diverse routes. Our study confirmed vaginal exudates, faeces, and conjunctival exudates as suitable samples for detecting the pathogen.

In 31 samples (54.4%), we could not identify the specific *Chlamydia* species, leaving them as positive for the *Chlamydiaceae* family. Camenisch *et al*. (2004) reported similar findings, with 25% of "*Chlamydia*-like" samples from sows with reproductive issues remaining unidentified. In such cases, sequencing could be helpful, though our study faced limitations due to insufficient *Chlamydiaceae* DNA quantities for genomic analysis. Similar constraints have been noted in avian studies (Aaziz *et al.*, 2015).

Our study highlights that vaginal, conjunctival, and rectal samples are effective for detecting *Chlamydia* species in pigs, consistent with the pathogen's pathogenicity in this species (De Puysseleyr *et al.*, 2017; Rypula *et al.*, 2018; Unterweger *et al.*, 2021).

Finally, this study identified *C. abortus* as the predominant species, followed by *C. suis*, in pigs presenting reproductive issues, conjunctivitis, and/or diarrhoea. Notably, co-infections involving both species were detected, marking the first report of porcine chlamydiosis in Mexico.

Understanding the presence of this disease in Mexico provides producers with the necessary knowledge to implement appropriate sanitary measures, thereby preventing its spread within pig herds and increasing awareness of its potential zoonotic implications.

Further research covering all stages of pig production is essential to determine the future prevalence of this disease in Mexico.

Declarations

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Conflicts of interest

The authors declare no conflicts of interest.

Author contributions

Paola-Alexis Hernández-Ramírez: Sampling and collecting the data from farms, performing experiments, analyzing the data, and writing the manuscript. María-Magdalena Limón-González performed experiments, analyzed data, and wrote the manuscript. Nelson Pérez-Romero: Sampling and collecting the data from farms, analysis of data, and a critical review of the manuscript. Gabriela Palomares-Resendiz: Performed experiments, analyzed data, wrote and reviewed the manuscript. Rigoberto Hernández-Castro: Analysis of data, wrote and reviewed the manuscript. Efrén Díaz-Aparicio: Conception of the study, wrote and reviewed the manuscript. Beatriz Arellano-Reynoso: Design of the study; administered the project, wrote and reviewed the manuscript.

Use of artificial intelligence (AI)

During the preparation of this manuscript, the authors used ChatGPT in order to verify correct English writing. After using this tool, the authors reviewed and edited the content as needed, taking full responsibility for the publication's content.

Data Availability

The information is available upon request from the reader

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