Molecular detection of *Chlamydia* sp. in small ruminants from the central region of the state of Veracruz, Mexico

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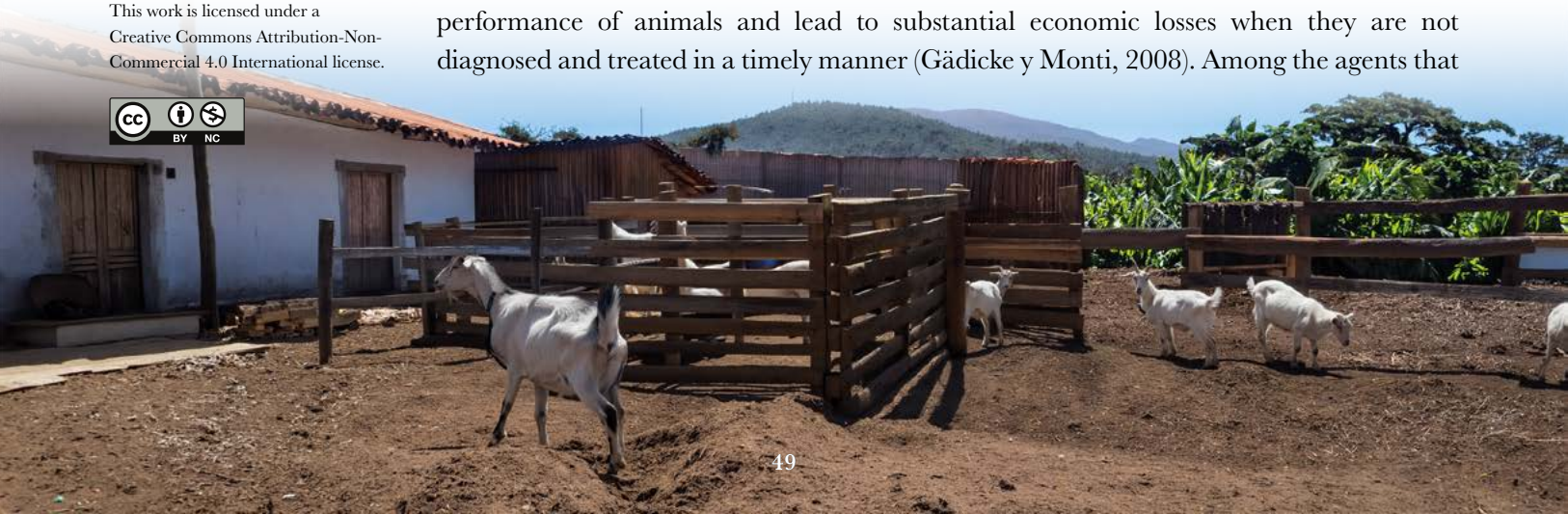
ABSTRACT

Objective: To identify the presence of *Chlamydia* sp. in the municipality of Coatepec, Veracruz, Mexico.
Design/methodology/approach: A total of 60 animals from five dairy goat farms were included, comprising 57 females and three males of varying ages and breed phenotypes. Samples were obtained via vaginal and preputial swabs and placed in sterile conical tubes. Endpoint PCR was performed for the detection of the *ompA VD2* gene with an amplification of 576 bp. Statistical analysis was conducted using the chi-square test for prevalence and a 2×2 contingency table for the analysis of risk factors.
Results: An overall prevalence of 5.0% was obtained from the samples collected, with three females (5.26%) aged 48 months-old (6.1%), and 36 months (12.5%). A 6.8% of positive samples belonged to the Alpine and Saanen breed phenotypes; however, no risk factor attributable to the variables found was identified ($p>0.05$).
Limitations on study/implications: The study has a small sample size and and farms number, which may limit the representativeness of the results, which may underestimate the true prevalence of the agent.
Findings/conclusions: The presence of *Chlamydia* sp. in goats in the central region of the state of Veracruz has been confirmed, highlighting the usefulness of PCR as a diagnostic tool. These results underscore the need to expand health surveillance and strengthen prevention measures in goat production systems.

Keywords: Goats, molecular diagnostics, prevalence, zoonoses.

INTRODUCTION

Abortions caused by bacterial diseases represent a highly significant problem in goat production and reproduction, as they have a considerable impact on the reproductive performance of animals and lead to substantial economic losses when they are not diagnosed and treated in a timely manner (Gädicke y Monti, 2008). Among the agents that



cause this type of problem is *Chlamydia* sp., recognized for its ability to cause reproductive disorders that can be confusing or even go unnoticed; furthermore, most research related to chlamydial infections has focused on sheep, which limits specific knowledge on goats. (Mora-Díaz *et al.*, 2015).

Chlamydiosis, also known as abortion in small ruminants is a highly contagious disease and is also considered a zoonosis, which poses a risk to people who are in contact with sick goats, particularly immunocompromised individuals or pregnant women (Castro-Flores *et al.*, 2021).

This disease can cause abortions, vesiculitis, epididymitis, and orchitis; however, abortions caused by chlamydiosis are more common in sheep than in goats, which makes it difficult to determine the true status of the disease (WOAH, 2018). The diagnosis of chlamydiosis is based on bacteriological isolation or the identification of nucleic acids in aborted fetuses or vaginal secretions, although its identification is often complicated due to the similarity it has with *Brucella* sp. or *Coxiella burnetii*; there is a possibility of a misdiagnosis (Esmaeili *et al.*, 2024).

In Mexico, chlamydiosis was included in group 3 of endemic diseases and exotic pests, those that must be reported monthly to veterinary authorities (DOF, 2016). In 1996, the first case of chlamydiosis in small ruminants affected by *Chlamydia psittaci* serotype 1, which is currently known as *C. abortus*, was reported in Mexico. Escalante-Ochoa *et al.* (1996) mentioned that the high incidence found led to considering *C. abortus* as playing an important role in reproductive losses in goat herds. Subsequently, microscopic lesions related to the agent were identified in fetuses aborted by goats (Escalante *et al.*, 1997).

Currently, the epidemiological situation of *Chlamydia* sp. is still limited. Studies have been conducted identifying the presence of the pathogen and finding prevalences ranging from 9.6% to 55.6% (Castro-Flores *et al.*, 2021; Sánchez-Rocha *et al.*, 2021; García-López *et al.*, 2019; Campos-Hernández *et al.*, 2014). Callejas-García (2017) found a *Chlamydia* sp. seroprevalence of 5.5%, and confirmed the presence of *C. abortus* (21.05%) in the central area of the state of Veracruz through PCR testing.

The importance of addressing chlamydiosis in the central region of Veracruz becomes particularly significant, considering that this area is a central hub for goat production. By virtue of this, it is imperative to establish constant monitoring of this pathology as a preventive measure against potential disease outbreaks. For this reason, the objective of the present research was to evaluate the presence of *Chlamydia* sp. in the municipality of Coatepec, Veracruz, Mexico using molecular techniques.

MATERIAL AND METHODS

Study Area

A cross-sectional epidemiological study was carried out, considering five goat farms located in the municipality of Coatepec, in the central area of the state of Veracruz, Mexico (19° 21' and 19° 32' north latitude, 96° 47' and 97° 06' west longitude, 500-2,900 meters above sea level) (INEGI, 2010). The goat farms specialized in milk production, with Alpine, Saanen, and crossbred phenotypes, in a semi-intensive system.

Study samples

The sample size was estimated for an expected prevalence of 50%, based on the population of the farms. The collected samples were vaginal and preputial swabs, which were transferred to sterile conical tubes, kept refrigerated, and later frozen at −20 °C until processing. A total of 60 samples were collected, consisting of 57 females and three males, during April 2023.

Identification of *Chlamydia* sp. by PCR

The laboratory diagnosis was carried out using the endpoint PCR method for the identification of *Chlamydia* sp. DNA extraction was carried out using Chelex 100®, and a 576 bp fragment of the ompA VD2 gene was amplified using the following primers: CMGP-1F: 5′-CCTTGTGATCCTTGCGCTACTTG-3′/CMGP-1R: 5′-GTGAGCAGCTCTTCGTTGAT-3′, and CMGP-2F: 5′-GCCTTAAACATCTGGGATCG-3′/CMGP-2R: 5′-GCACAACCACATTCCCAT-AAAG-3′ (Chahota *et al.*, 2006). The amplifications were visualized on a 2% agarose gel in 1X TAE at 85 volts for 45 min.

Statistical analysis

Statistical analysis was carried out using the Chi-square test in the mode of estimating proportions with the online program Vassarstats®, and for the identification of risk factors with the online software Win Episcope®.

RESULTS AND DISCUSSION

The presence of *Chlamydia* sp. was detected by PCR, for the amplification of the fragment at 576 bp (Figure 1).

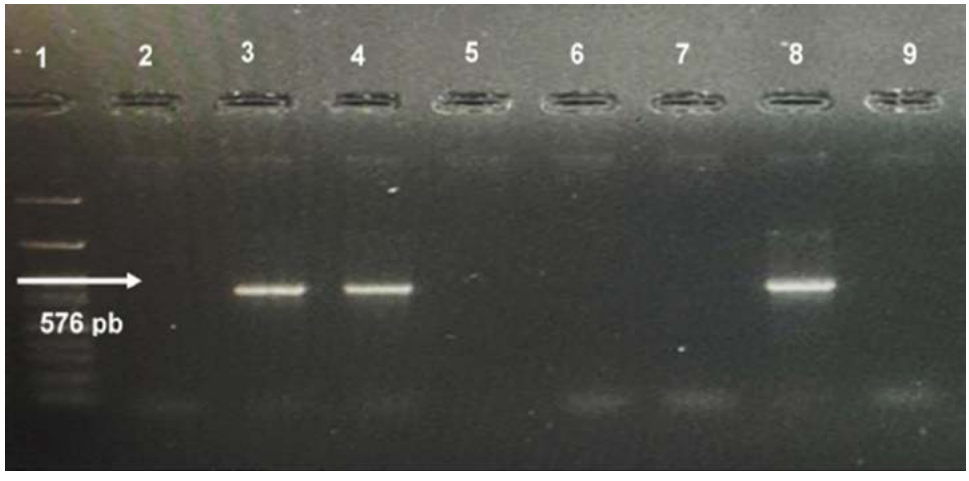


Figure 1. Electrophoretic profile of the amplification of *Chlamydia* sp. (576 bp). Lane 1: molecular weight marker (100 bp); Lane 2: Negative sample; Lane 3: Positive sample; Lane 4: Positive sample; Lane 5: Negative sample; Lane 6: Negative sample; Lane 7: Negative control, nuclease-free H_2O; Lane 8: Positive control, *Chlamydia* sp.; Lane 9: Empty lane.

The results obtained show an overall prevalence of 5.0% (95% CI: 1.7-17.7; 3/60). Out of a total of 57 females, three tested positive (5.26%; 95% CI: 1.8-14.37). Of the positive samples, in relation to age, two samples of 48 months-old (6.1%; 95% CI: 1.6-19.61) and one of 36 months-old animal (12.5%; 95% CI: 2.2-47.1) were identified. Regarding the racial type, 6.8% (95% CI: 2.3-18.2) of the positive animals belonged to Saanen and Alpine crossbreeds. No pregnant or lactating females were identified among the positive animals.

The statistical analysis did not show a significant association between the variables evaluated, so no risk factors were identified ($p>0.05$) (Table 1). These results identify the presence of *Chlamydia* sp. in the municipality of Coatepec, Veracruz; however, this area is where nearly 90% of the goat inventory in Veracruz is concentrated (Román-Ramírez *et al*., 2017). Previous studies conducted in Mexico reported prevalences of 9.6% to 55.6% in areas such as Guanajuato, Morelos, Coahuila, Querétaro, Puebla, and Veracruz (Castro-Flores *et al*., 2021; Sánchez-Rocha *et al*., 2021; García López *et al*., 2019). However, Callejas-García (2017), in his research in the central area of the state of Veracruz, reported a seroprevalence of 5.9%, finding that the municipalities of Perote and Villa Aldama were risk factors ($P<0.05$) and that no positive cases were identified in the municipality of Coatepec. However, in the present study, positive animals were indeed found.

The environmental conditions in this mountainous region are usually favorable for *Chlamydia* sp., as temperatures range from 12 to 25 °C. This results in the pathogen being released into the environment through fluids or excretions, allowing the microorganism to survive for several weeks in cold and humid conditions. On top of that, the lack of strategies on farms to separate females nearing parturition and newly kidding goats promotes easier infection, since during birth and abortions a large load of *Chlamydia* bacteria is released, contaminating the feed, water, and bedding, and favoring the transmission and persistence of the agent in affected herds (Rodolakis y Laroucau, 2015; Talafha *et al*., 2012).

Table 1. Prevalence and risk factors associated with the presence of *Chlamydia* sp. in the central area of the state of Veracruz, Mexico.

Variable	n	+	Prevalence (%)	$_{95\%}$ CI	OR*	$_{95\%}$ CI	P
Sex							
Female	57	3	5.3	1.8-14.4	-	-	-
Male	3	0	-	-	-	-	-
Age, months*old							
60	2	0	-	-	-	-	-
48	33	2	6.1	1.6-19.6	1.0	-	-
36*	8	1	12.5	2.2-47.1	2.2	0.17-27.98	0.49
24	3	0	-	-	-	-	-
12	14	0	-	-	-	-	-
Breed phenotype							
Alpine	11	0	-	-	-	-	-
Saanen	5	0	-	-	-	-	-
Crosses	44	3	6.8	2.3-18.2	-	-	-

* OR (Odds Ratio) should be interpreted with caution due to the small sample size.

Certainly, the presence of *Chlamydia* sp. in flocks of small ruminants depends on various factors such as geographic location, the breed, age, sex, and number of parturitions, as well as the type of management, hygiene and preventive medicine practices, and the movement of animals (Afrisham *et al*., 2023). In the present study, the animals that tested positive were females aged 36 to 48 months-old, but abortions were rare, according to the information provided by farmers.

Castro-Flores *et al*. (2021) mentioned that females are usually affected by chlamydiosis at a young age and with a lower number of parturitions; these authors also pointed out that the interval between abortion, kids birth, and sample collection is of utmost importance to ensure a timely diagnosis; in addition, Palomares-Reséndiz et al. (2020) reported that goats between 37 and 48 months-old show a higher frequency of positive cases of chlamydiosis. In this regard, it can be considered that the timing of sampling and the low frequency of abortions may influence the detection of the agent, independently of the reproductive signs of disease on the evaluated farms. Although the bacteriological isolation of *Chlamydia* sp. is still considered the reference method for identification (WOAH, 2018), molecular PCR techniques are considered the ideal tests to confirm the agent in clinical cases suspected of chlamydiosis, as well as for epidemiological surveillance. Official organizations consider molecular tests as complementary diagnostic tests, which must be corroborated by pathological analysis to confirm the diagnosis; however, microbiological diagnosis can fail due to the loss of bacterial viability, so techniques like PCR can detect the agent even when the reference method is not viable (Esmaeili *et al*., 2024; Castro-Flores *et al*., 2021).

In Mexico, Sánchez-Rocha *et al*. (2021) identified the presence of *C. abortus* in goats that had experienced abortions; these authors reported 9.6% positives with samples of vaginal exudates in the states of Coahuila, Jalisco, Querétaro, and Veracruz; in this last state, they only found two positive samples, which is similar to what was identified in the present study.

Rodolakis and Laroucau (2015) mentioned that the ideal samples for PCR analysis are aborted fetuses and placentas, due to their high bacterial load, although it is also possible to demonstrate their presence in vaginal exudates, which can be effectively used for the diagnosis of chlamydiosis in small ruminants (Mora-Díaz *et al*., 2015; Campos-Hernández *et al*., 2014).

Likewise, the introduction of animals without a prior diagnosis represents a significant risk factor for the spread of the agent, particularly due to the absence of a standardized reference test, which hinders the timely detection of the agent, since it is considered a rare or exotic disease (Palomares-Reséndiz *et al*., 2020).

The results of the present study confirm the presence of *Chlamydia* sp. in the central area of the state of Veracruz, although there is a previous study in 14 municipalities of the central area of Veracruz, where the presence of Chlamydia sp. was reported by PCR, but the municipality of Coatepec was found to be negative (Callejas-García, 2017), which suggests that the agent is circulating in the area and highlights the need to strengthen epidemiological surveillance in the contiguous urban areas.

CONCLUSIONS

The present study confirms the presence of *Chlamydia* sp. in the municipality of Coatepec, in the central region of the state of Veracruz in female goats. Although it was not possible to identify the species circulating in the region, the results found, coupled with literature available in the central area of Veracruz, confirm and provide evidence of the circulation of the agent in mature animals. Likewise, the need for monitoring the different reproductive stages of females is highlighted. These results underscore the need to implement preventive measures and epidemiological surveillance to mitigate the impact of *Chlamydia* sp. in the region.

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