

Seroepidemiology and risk factors associated with *Leptospira* and *Chlamydia abortus* in goat herds in Guanajuato, Mexico

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Abstract

A cross-sectional epidemiological study was conducted from March 2022 to April 2023 to determine seropositivity for chlamydiosis and leptospirosis, as well as to identify risk factors associated with their transmission in goat herds in the municipality of Juventino Rosas, Guanajuato. A total of 741 samples were collected from 32 herds. For serological diagnosis, the microscopic agglutination test was employed for *Leptospira*, and an indirect ELISA was used for *Chlamydia abortus*. Logistic regression analysis was applied to identify risk factors. The study found that 14.1 % and 52.5 % of samples were seropositive for chlamydiosis and leptospirosis, respectively. The primary serovar detected for leptospirosis was the national strain H-89 (Hardjo) at 40.8 %. A significant causal association for leptospirosis was observed ($P < 0.0211$) in herds with a history of abortions ($OR = 8.88$), in goats older than 6 years ($OR = 6.91$), with the loaning of bucks ($OR = 5.6$), in the buying and selling of animals ($OR = 3.54$), in herds with more than 60 animals ($OR = 3.44$), in the presence of rodents ($OR = 2.86$), and with humidity ($OR = 1.79$). For chlamydiosis, a significant causal association ($P < 0.0456$) was observed when pregnant females were not separated ($OR = 4.28$), when grazing areas were shared ($OR = 3.4$), and in herds with more than 60 animals ($OR = 2.1$). Given these findings, it is essential to enhance technical assistance to implement biosafety measures aimed at reducing the occurrence of these diseases.

Keywords: Risk factor; Goats; Serovar; Chlamydiosis; Leptospirosis.

Submitted: 2023-09-13

Accepted: 2024-08-07

Published: 2024-12-03

Additional information and declarations can be found on page 11

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Cite this as:

Tufiño-Loza C, Gutiérrez-Hernández JL, Palomares-Resendiz EG, Musito-Moreno AS, Guzmán-Ojeda M, Martínez-Pérez A, Díaz-Aparicio E. Seroepidemiology and risk factors associated with *Leptospira* and *Chlamydia abortus* in goat herds in Guanajuato, Mexico. Veterinaria México OA. 2024;11. doi: 10.22201/fmvz.24486760e.2024.1266.

Study contribution

Leptospirosis and chlamydiosis cause reproductive disorders that significantly impact goat production. Therefore, this study aimed to determine seropositivity for these diseases and identify risk factors in goat herds in the municipality of Juventino Rosas, Guanajuato. The study found a 14.1% seropositivity rate for chlamydiosis, with a causal association observed in shared grazing areas, when pregnant females were not separated, and in herds with more than 60 animals. For leptospirosis, a seropositivity rate of 52.5% was observed, with a causal association identified in herds with a history of abortions, in goats over six years old, with the loan of bucks, with the purchase and sale of animals, in herds with more than 60 animals, in presence of rodents, and humid conditions. Based on these findings, it is crucial to enhance technical assistance to implement biosafety measures that reduce the incidence of these diseases.

Introduction

Goat farming continues to be a traditional family-based activity, that provides both income and a vital food source for many families. This sector typically relies on family labor and operates extensively with low technological input. Despite these limitations, goat farming has proven its adaptability and profitability through the production and marketing of milk and meat. However, many goat farmers lack the technical assistance necessary to maximize the profitability of their herds.⁽¹⁾ Poor management of animal health further exacerbates this issue, significantly hindering their development. This is evident in the low reproductive efficiency characterized by an increased number of abortions, prolonged calving intervals, low prolificacy, higher mortality rates at birth, fewer kids per goat per year, and low milk production during lactation.^(2, 3)

Leptospirosis and chlamydiosis cause reproductive disorders that impair the efficiency of goat production and are also zoonotic diseases. Leptospirosis caused by the genus *Leptospira*, is distributed worldwide and affects most domestic species. Free-living animals, such as rats, armadillos, coyotes, skunks, and reptiles, serve as reservoirs, acting as carriers and shedding the bacteria, thereby contaminating the environment.^(3, 4) Chlamydiosis, primarily caused by *Chlamydia abortus*, leads to reproductive disorders including abortions during the last third of gestation and the birth of weak kids with high mortality in the first days of life.^(5–7) Both diseases are endemic in Mexico, with serological evidence of their spread found in regions with significant goat populations.^(8–20)

The municipality of Santa Cruz de Juventino Rosas is the largest contributor to goat milk production in Guanajuato, Mexico, which ranks second nationally with a 25.2% share.⁽²¹⁾ Consequently, leptospirosis and chlamydiosis can have significant socioeconomic impacts on both animal and public health, as these diseases not only compromise productivity but also pose risks to the personnel involved in goat farming. Therefore, the objective of this study was to determine the seropositivity for leptospirosis and chlamydiosis and to identify the risk factors contributing to their transmission and dissemination in goat herds in Santa Cruz de Juventino Rosas, Guanajuato, Mexico. This will enable the establishment of specific strategies for controlling these diseases.

Materials and methods

Ethical statement

This research did not require the approval of an institutional committee for the care and use of animals, as blood sampling was only taken from the animals once throughout the research, however consent letters were sent to the farmers who agreed to participate voluntarily, indicating the handling that would be carried out on the animals.

Study area

Santa Cruz de Juventino Rosas is situated in the north-central of Guanajuato, Mexico (20° 39' 00" N 101° 00' 00" W), covering 1.4% of the state's total area. It borders the municipalities of Salamanca, Allende, and Comonfort to the north; Comonfort and Celaya to the east; Celaya, Villagrán, and Salamanca to the south; and Salamanca to the west. Ecologically, the southern of the municipality is classified as a temperate zone, while the central-northern region is arid. The climate is characterized as warm, semidry, and temperate subhumid, with an average annual temperature of 18.8 °C and average annual rainfall from 600 to 800 mm.

According to the National Population Council, this municipality is classified as having a low marginalization index.⁽²²⁾ The primary economic activities are agriculture and livestock. Land use is predominantly agricultural, with 80% of the area utilized for irrigated or seasonal crops in plains, while the remaining 20% consists of scrubland, mountain ranges, and highlands. In the region, goat farming is mostly semi-intensive (85%), with a smaller proportion (15%) being intensive, primarily focused on milk production.

Study design and sample collection

A cross-sectional epidemiological study was conducted from March 2022 to April 2023, with the voluntary participation of 32 cooperating farmers from the communities of San Juan de la Cruz, Emiliano Zapata, Cerrito de Gasca, Naranjillo, La Purísima, San José de los Manantiales, and the periphery of the municipal seat. These areas are considered to have a low marginality index within Santa Cruz de Juventino Rosas. The goat production units (GPUs) were selected based on a maximum of 70 livestock and were characterized by relying on backyard agriculture and livestock farming as their primary source of income. Preference was given to units utilizing regional resources, such as rangeland or ejidal lands for grazing. Additionally, the selected units were those with minimal or no technical assistance.

Risk factors

Each farmer was visited between March and June 2022 to conduct a survey, which provided insights into their production characteristics, facilities, and sanitary and reproductive management practices. The evaluated risk factors included: age (animals under one year, one to two years, three to five years, and six to eight years), sex (female or male), history of abortion, production system (stabled, semi-stabled, extensive), the presence of other types of livestock (cattle, sheep, pigs, backyard birds), sharing of grazing areas with other herds, communal use of vans for trans-

porting animals, disinfection of navel after birth, purchase and sale of animals, whether pregnant females give birth alongside the rest of the herd, disposal of birth or abortion waste (e.g., feeding it to dogs), loan of bucks between GPUs for free mating, presence of humidity in pens, and the presence of harmful fauna (rodents, birds, and wildlife) in the GPU.

Inclusion criteria

For sampling, all animals from the GPUs older than three months of age that were considered replacements for breeding stock were included.

Blood sampling

A clinical inspection was conducted on all animals from each GPU, and blood samples were collected via jugular vein puncture using vacuum tubes without anticoagulant (Vacutainer®). For each animal, individual records were kept, including details on age, breed, sex, body condition, productive status, and any specific characteristics observed. The samples were transported under cold chain conditions to the Small Ruminant Diseases Laboratory at National Center for Disciplinary Research in Animal Health and Safety (CENID-SAI, for its acronym in Spanish) of the National Institute of Forestry, Agriculture, and Livestock Research (INIFAP, for its acronym in Spanish) for processing. Blood samples were centrifuged at $3\ 000 \times g \times 10$ minutes to separate the serum, which was then transferred into microtubes and stored at $-20\ ^\circ\text{C}$ until analysis.

Serological examination for leptospirosis

Serological diagnosis for leptospirosis was performed using the microscopic agglutination test (MAT), following the reagents and methodology described by INIFAP, Mexico.⁽²³⁾ This involved a panel of eight *Leptospira* serovars, including four reference strains (Icterohaemorrhagiae, Bratislava, Hardjo, Tarassovi, and Wolffi) and three national isolates (Palo Alto: Icterohaemorrhagiae, H-89: Hardjo, and Portland-Vere: Canicola). Serum samples were diluted 1:10 and mixed with an equal volume of each *Leptospira* serovar. The final serum dilution (including the added antigen) of 1:20 was used for the preliminary examination.

For samples that tested positive in the preliminary examination and reacted with one or more serovars, a series of two-fold dilutions was prepared to determine the endpoint titer with $\geq 50\%$ agglutination. MAT was conducted using five titers: 1:40, 1:80, 1:160, 1:320, and 1:640. Results were evaluated using a dark field microscope with $\times 100$ magnification. The antibody titer was defined as the highest dilution containing $\geq 50\%$ agglutinated leptospire. Samples with a titer of $\geq 1:40$ were considered positive. Samples showing no detectable agglutination were deemed negative. GPUs were classified as *Leptospira*-positive if at least one individual exhibited a titer of 1:40 or higher for any serovar.

Serological examination for chlamydiosis

Antibodies against *C. abortus* infection were detected using a commercial indirect-ELISA kit, following the manufacturer's instructions. Serum samples and controls were diluted to 1:10. The microplate was read at a wavelength of 450 nm to obtain optical densities (OD). The ratio of the OD of the sample to the OD of the positive control (S/P %, Sample / Positive) was calculated for each sample using the following equation:

$$S/P \text{ (\%)} = \frac{\text{OD sample}}{\text{OD positive control}} \times 100$$

Samples with an S/P % of 50 % or less were considered negative. Samples with an S/P % greater than 50 % but less than 60 % were classified as suspicious, while samples with an S/P % greater than 60 % were deemed positive.

Statistical analysis

The data collected from each production unit were compiled into a database for analysis to determine the frequencies and risk factors associated with each disease. Risk factor association analysis was conducted in two phases. Initially, χ^2 tests for proportion homogeneity were performed, with factors showing a Pvalue < 0.1 considered statistically significant. These factors were then included in a multivariate logistic regression analysis using SPSS software (Version 25, 2017), where factors with a Pvalue < 0.05 were deemed statistically significant.

Results

Serological frequencies

Out of a total of 741 goat serum samples analyzed, 389 (52.5 %, 95 % confidence interval [CI]: 48.9, 56.07) were positive for antibodies against leptospirosis, while 105 (14.17 %, 95 % CI: 11.84, 16.87) were positive for antibodies against chlamydiosis. Clinical inspection of the sampled animals revealed that all were healthy. However, antibodies against both diseases were detected in 86 sera (11.61 %, 95 % CI: 9.5, 14.11), originating from 13 GPUs across six of the seven communities included in the study. The communities of San José de los Manantiales and Emiliano Zapata exhibited significantly higher seropositivity for both diseases ($P < 0.0001$) (Figure 1).

Based on the frequency of animals seropositive for the different serovars of *Leptospira* assessed in this study, the serovar with the highest detection rate was the national strain H-89 (Hardjo) at 40.89% (95 % CI: 37.41, 44.47). This was followed by the serovar Portland-Vere (Canicola) at 16.6% (95 % CI: 14.09, 19.45), Bratislava at 11.61 % (95 % CI: 9.5, 14.11), the national strain Palo Alto (Icterohaemorrhagiae) at 10.12 % (95 % CI: 8.15, 12.5), Icterohaemorrhagiae at 9.79% (95 % CI: 7.77, 12.27), Wolffi at 9.18% (95 % CI: 7.3, 11.47), Hardjo at 3.37 % (95 % CI: 2.3, 4.93), and Tarassovi at 2.7 % (95 % CI: 1.75, 4.13) (Table 1 for their frequency by community).

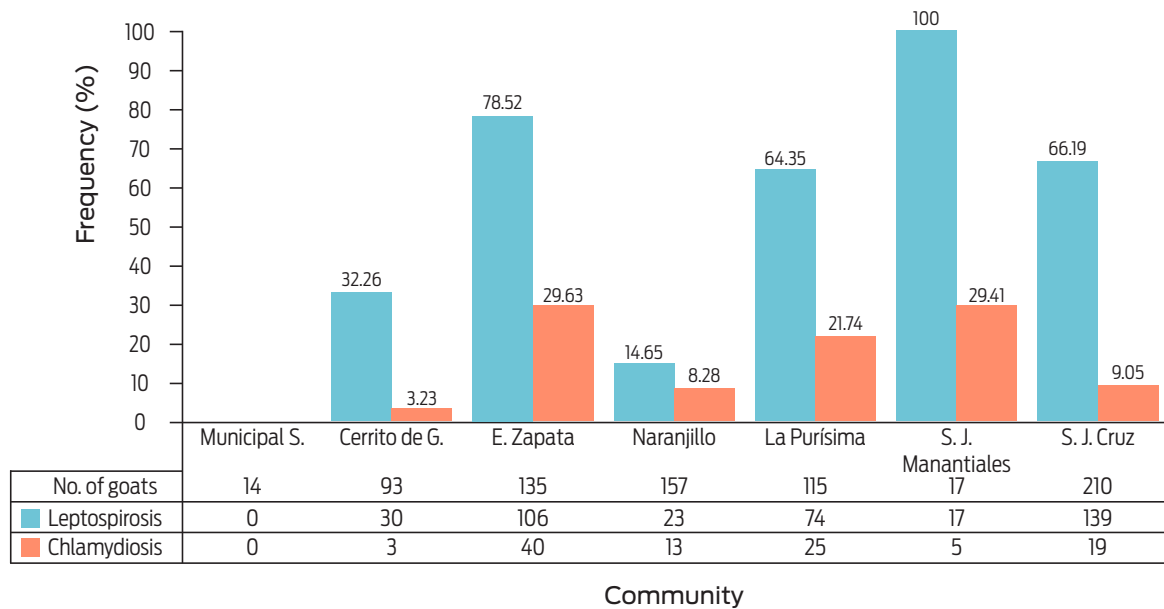


Figure 1. Percentage of goats seropositive for leptospirosis and chlamydia by community in Santa Cruz de Juventino Rosas, Mexico.

Abbreviations: Municipal S.: Municipal Seat, Cerrito de G.: Cerrito de Gasca, E. Zapata: Emiliano Zapata, S. J. Manantiales: San José de los Manantiales and S. J. Cruz: San Juan de la Cruz.

Table 1. Frequency by the *Leptospira* serovar per community in Santa Cruz de Juventino Rosas, Mexico

Community	Serovar (%)							
	Wolffi	Hardjo	Tarassovi	H-89	Palo Alto	Icterohaemorrhagiae	Portland-Vere	Bratislava
Municipal Seat	0	0	0	0	0	0	0	0
Cerrito de Gasca	2.15	2.15	0	17.2	5.38	16.28	5.38	6.45
E. Zapata	11.85	4.44	0.74	71.85	8.15	8.89	11.85	3.7
Naranjillo	0	0	4.46	4.46	2.55	6.37	4.46	6.37
La Purísima	9.57	5.22	0.87	57.39	6.96	13.89	6.96	14.78
S. J. Manantiales	23.53	11.76	0	100	17.65	0	23.53	2.33
S. J. Cruz	16.67	4.29	5.24	47.62	20.95	9.52	39.52	21.9

Abbreviations: E. Zapata: Emiliano Zapata, S. J. Manantiales: San José de los Manantiales and S. J. Cruz: San Juan de la Cruz.

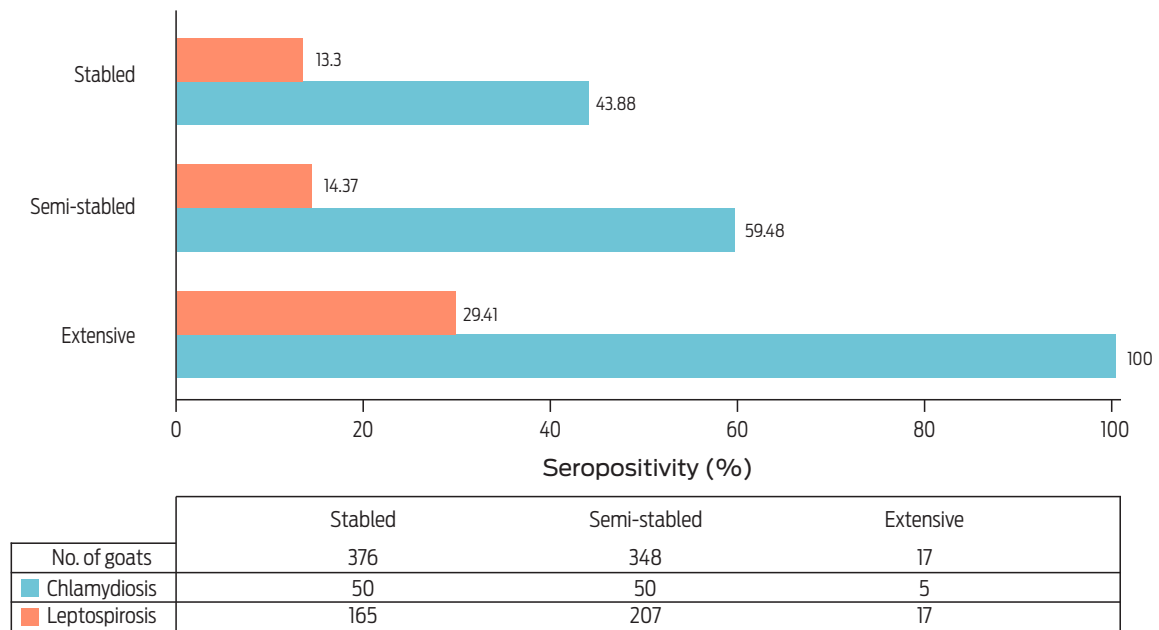


Figure 2. Percentage of goats seropositive for chlamydiosis and leptospirosis by production system in Santa Cruz de Juventino Rosas, Mexico.

The GPUs have a higher proportion of adult goats aged between three and eight years, where increased seropositivity was observed for both *C. abortus* and at least one serovar of *Leptospira* ($P = 0.0231$). Additionally, GPUs were managed under stabled, semi-stabled, and extensive production systems, with a higher frequency of leptospirosis detection in semi-stabled and extensive systems ($P < 0.0001$). In contrast, no significant difference was observed for chlamydiosis across these management systems (Figure 2).

Risk factors

For leptospirosis, a significant causal association is observed for the history of abortions in the GPUs (OR = 8.88), for goats older than 6 years (OR = 6.9), for the loan of bucks between GPUs for free mating (OR = 5.6), for the buying and selling of animals (OR = 3.5), for herds larger than 60 animals (OR = 3.4), for the presence of rodents as harmful fauna in the GPU (OR = 2.9), and the presence humidity in pens (OR = 1.8) (Table 2).

For chlamydiosis, a significant causal association was observed when pregnant females were not separated from the rest of the herd (OR = 4.3), when grazing areas were shared with animals from other herds (OR = 3.4), and for herds with more than 60 animals (OR = 2.1) (Table 3).

Discussion

The primary animal health issue reported by farmers is the occurrence of abortions at a rate of 36.4%, which hinders the proper replacement of goats within the GPU and impacts the demand for milk and meat. Consequently, serological detection of chlamydiosis and leptospirosis was performed.

Table 2. Logistic regression analysis of flock and animal risk factors associated with leptospirosis seropositivity in goats from herds in Santa Cruz de Juventino Rosas, Guanajuato

Risk factor	Category	OR	DF	95 % CI	P-value*
Abortions	Yes	8.88	1	5.02–15.72	0.0001*
	No				
Age	6 and 8 years	6.91	1	1.61–29.64	0.0092*
	< a 6 years				
Buying- Selling	Yes	3.54	1	1.89–6.64	0.0001*
	No				
Humidity	Yes	1.79	1	1.09–2.94	0.0211*
	No				
Rodents	Yes	2.86	1	1.94–4.22	0.0001*
	No				
Loan of bucks	Yes	5.62	1	2.42–13.04	0.0001*
	No				
Flock size	> 60 goats	3.44	1	1.63–7.25	0.0011*
	< 60 goats				

DF: Degrees of freedom; OR: Odds ratio; CI: Confidence interval; *: P < 0.05 significant causal association.

Table 3. Logistic regression analysis of flock and animal risk factors associated with chlamydiosis seropositivity in goats from herds in Santa Cruz de Juventino Rosas, Guanajuato

Risk factor	Category	OR	DF	95 % CI	P-value*
Age	6 to 8 years	1.15	1	0.79–1.67	0.4785
	< 6 years				
Flock size	> 60 goats	2.14	1	1.32–3.46	0.002*
	< 60 goats				
Grazing areas shared	Yes	3.39	1	1.02–11.29	0.0463*
	No				
Absence of goat subdivision	Yes	0.98	1	0.49–1.94	0.9612
	No				
Abortions	Yes	2.07	1	0.67–6.41	0.2077
	No				
Pregnant females give birth with the rest of the animals	Yes	4.29	1	1.02–17.85	0.0456*
	No				
Waste birth or abortions for dogs	Yes	1.64	1	0.43–6.29	0.4677
	No				
Rodents	Yes	1.09	1	0.29–3.89	0.9082
	No				
Birds	Yes	1.47	1	0.58–3.68	0.4162
	No				
Wildlife	Yes	2.37	1	0.59–9.41	0.2219
	No				

DF: Degrees of freedom; OR: Odds ratio; CI: Confidence interval; *: P < 0.05 significant causal association.

In this study, 52.5% of the samples showed antibody titers against at least one *Leptospira* serovar. Research conducted in Guanajuato, Mexico, which features dry and semidry, warm, and temperate subhumid climates, has demonstrated an increasing trend in the serological frequency of leptospirosis, rising from 37.9% in 2016 to 45.5% in 2019.^(15, 20) Reports from other regions of Mexico are as follows: in a warm subhumid climate like Guerrero, the serological frequency was 64.26%; in a humid temperate climate like the central zone of Veracruz, 25.5%; in a dry climate like the Comarca Lagunera region, 60.1%; and in a very warm semidry climate like Nuevo León, 14.7%.^(9-11, 16)

The main serovar identified in our study was the national strain H-89 (Hardjo), with a prevalence of 40.89%. This differs from previous studies where the *Icterohaemorrhagiae* serovar was predominant.^(15, 20) The Hardjo serovar, part of the Sejroe serogroup, is known to be adapted to small ruminants and is a major cause of reproductive issues in clinically healthy animals,⁽³⁾ as observed in the goats in our study. These adapted strains are less influenced by regional factors or environmental conditions such as topography or seasonal rainfall, leading to subclinical infections that are significant for disease transmission to humans and other animals.⁽²⁴⁾ It's also noteworthy that while the Hardjo serovar primarily affects cattle,^(25, 26) its presence has been observed in sheep flocks with no contact with cattle, highlighting its adaptability to other ruminants.⁽²⁷⁾

The history of abortions in GPUs is a risk factor that impacts the occurrence of leptospirosis in animals. Our study suggests this is likely due to subclinical infections, as the animals appear clinically healthy during sampling but show high seropositivity. Subclinical infections are primarily associated with reproductive issues, including infertility, an increased number of services per conception, extended calving intervals, abortions, stillborn animals, and the birth of weak kids.⁽³⁾ Another identified factor was goats older than six years. This can be explained by the increased number of opportunities these animals have to come into contact with even older goats, some up to eight years old, as observed in this study. Dos Santos et al.⁽²⁸⁾ also found that adult goats had up to three times the risk of contracting the disease compared to younger animals.

The loan of bucks between GPUs and the buying and selling of animals are practices closely linked to the introduction of new animals into the GPUs, which poses a risk of introducing diseases not originally present in the region or even the country. Herds with more than 60 animals were also identified as a risk factor, as the likelihood of animal contact increases with herd size.⁽¹⁸⁾ Additionally, rodents, being the primary reservoir of leptospirosis, significantly contribute to the risk; their presence in GPUs can triple the probability of finding seropositive animals.⁽²⁹⁾ Finally, humidity in pens creates an ideal environment for the maintenance and spread of leptospirosis, as this humidity primarily consists of urine from animals that are reservoirs of the bacteria. For instance, the serovar *Icterohaemorrhagiae*, which causes acute clinical symptoms,⁽³⁰⁻³³⁾ was not observed in this study.

Various research groups have found serological evidence of chlamydiosis in areas with significant goat populations.^(8, 13, 19, 34, 35) In this study, the global seropositivity for chlamydiosis was 14.17%, with local variations ranging from 0% to 29.6%, and over 50% of the GPUs had at least one positive animal. These data are higher than those reported in previous studies in Guanajuato, Mexico by Campos-Hernández et al.⁽¹²⁾ (4.87%), and Díaz et al.⁽¹⁴⁾ (9.6%). More recent

investigations have reported higher frequencies, with García-López,⁽¹⁷⁾ 31.5%, and Rueda,⁽¹⁸⁾ 41.5% in municipalities near Juventino Rosas. These findings, along with those from this study, suggest that the disease is present and continues to spread, influenced by certain management practices and conditions inherent to the production systems used by farmers in the region.

According to the logistic regression results, there is a positive causal association with the practice of not separating pregnant females. When these animals are not isolated, other animals come into contact with secretions and birth products, such as placentas and fetuses, which contaminate the environment (including food, water, and soil). A significant number of bacteria are shed in vaginal discharges, the placenta, and on the skin of aborted kids, and to a lesser extent, through urine, milk, and feces for several days after delivery or abortion. Ewes' secretions can contain chlamydiae from about one day before an abortion until 2 to 3 weeks after, while goats' secretions can contain chlamydiae from as early as 9 days before to 2 weeks after an abortion.⁽³⁶⁾ This situation poses a risk of disease transmission both orally and through aerosols to other animals, as well as to personnel working in the GPUs.⁽³³⁾

Sharing grazing areas with goats from other herds was also identified as a risk factor for chlamydiosis. Bacterial shedding can continue for several weeks after an abortion and may be detected intermittently and at low concentrations. Despite this, prolonged exposure can significantly impact transmission.⁽³⁷⁾ Continuous bacterial excretions contaminate grazing areas that are commonly used by multiple herds, creating a risk of exposure to *C. abortus* through ingestion.^(38,39) Gebren-tensay et al.⁽⁴⁰⁾ observed in Ethiopia that sheep flocks with access to communal pastures had a higher prevalence and risk of abortion compared to those without such access. This increased exposure to secretions and abortion products from *Chlamydia* spp., *Coxiella burnetti*, or *Toxoplasma gondii* can facilitate the spread of these pathogens between animals in communal grazing areas.

Herd size was another identified risk factor, with herds of more than 60 animals showing higher seropositivity to chlamydiosis. In the GPUs studied, animals are fed primarily through grazing and are then housed together in pens at night, increasing their contact and facilitating the transmission and spread of *C. abortus*.^(12, 34) Tejedor-Junco et al.⁽⁴¹⁾ also found an association between chlamydiosis and herds of 50–100 goats under stabled and semi-stabled production systems.

Conclusions

The detection of antibodies against at least one serovar of *Leptospira* and *C. abortus* in goats Juventino Rosas, Guanajuato, Mexico, indicates the presence and ongoing spread of both diseases. The national strain H-89 (Hardjo) was the most frequently detected serovar. To address the identified risk factors for these diseases, it is recommended to enhance technical assistance for implementing and adapting biosafety measures. These measures are currently lacking in the analyzed GPUs but could help reduce disease prevalence, thereby increasing productivity and promoting the health of the animals.

Data availability

All relevant data is found within the manuscript and its supporting information files are fully available in SciELO Dataverse, doi: 10.48331/scielodata.T9P8FH.

Acknowledgments

Thanks to the DVM. Salvador Arellano González, President of the Local Livestock Association, for the technical support and connection with the farmers of the municipality of Santa Cruz de Juventino Rosas, Guanajuato, Mexico for the sampling carried out on the goats and application of the epidemiological survey.

Funding statement

This research was partially financed by the project CONAHCYT “Programa Sanitario en Rebaños Caprinos para Aumentar la Eficiencia Productiva y la Seguridad Alimentaria, en Zonas Marginales del Municipio de Juventino Rosas, Guanajuato, México.” and by the fiscal Project INIFAP: “Seguimiento epidemiológico de un programa sanitario en rebaños caprinos, ubicados en zonas marginales del municipio de Juventino Rosas, Guanajuato, México”. Catalina Tufiño Loza is grateful for the postdoctoral fellowship from CONAHCYT.

Conflicts of interest

The authors have no conflicts of interest to declare regarding this publication.

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