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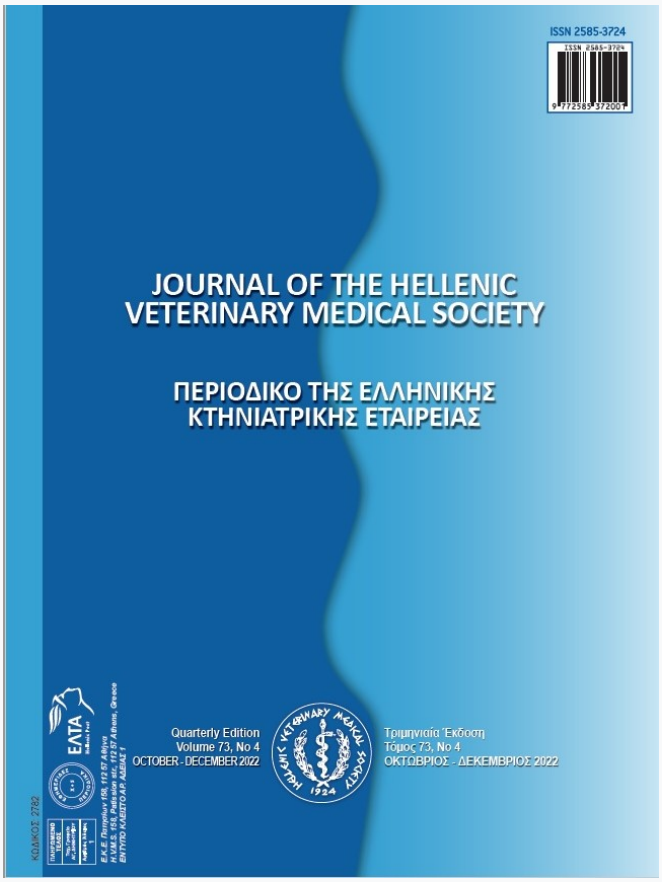

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Research article
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Sero-epidemiology of brucellosis in goats and sheep on rangeland in northern Mexico

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ABSTRACT: The seroprevalence of *Brucella* antibodies in the serum of goats and sheep reared on semi-arid pastoral systems of northeastern Mexico was assessed. Additionally, species, gender, and predominant breed were evaluated as risk factors for seropositivity to brucellosis. Data were from 375 herds of goats or co-mingled goats and sheep. Serum samples from 11001 goats and 4741 sheep collected between 2016 and 2019 were analyzed with the brucellosis card test. The disease affected 12% of herds. The overall prevalence of brucellosis seropositivity in goats and sheep were estimated at 2.1% (95% CI = 1.78-2.31) and 0.8% (95% CI = 0.58-1.10), respectively. Brucellosis seropositivity was two times more likely ($p < 0.01$) in goats than sheep. The risk of seropositivity to brucellosis for goats and sheep was 2.3 and 3.2 times higher ($p < 0.01$) in females than in males. Toggenburg goats were more likely ($p < 0.01$) to be seropositive to brucellosis than all other breeds of goats. In contrast, criollo sheep were more likely to be seropositive to brucellosis than hair sheep composite breeds. The current study revealed that brucellosis is not widely distributed in the study area, despite the absence of *Brucella* vaccination programs in recent years. Also, of all animals screened, seropositivity to *Brucella* infection was highest in Toggenburg compared to other dairy and meat breeds; Criollo sheep also presented the highest seropositivity to brucellosis compared to hair sheep composite breeds. Finally, females compared to males had increased odds of testing positive for brucellosis.

Keywords: breed, brucellosis, card test, goat, sheep.

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INTRODUCTION

Sheep and goat farming has been practiced in northern Mexico for centuries. Most goats and sheep in Mexico are raised under an extensive pastoral production system where health programs are uncommon. Under these conditions, caprine brucellosis remains endemic in most areas of the country (Acosta-Gonzalez et al., 2009; Marin et al., 2016) and constitutes an important threat to humans (Luna-Martinez and Mejia-Teran, 2002) mainly due to the ingestion of unpasteurized goat milk and consumption of soft cheeses elaborated with *Brucella*-contaminated goat milk (Guzmán-Hernández et al., 2016).

The risk of contracting this disease among small ruminants kept on communal rangeland is very high due to the close contact between goat herds in overgrazed shrublands of the arid and semi-arid zones of northern Mexico (Marin et al., 2016). Additionally, most sheep in the arid zones of northern Mexico are raised mixed with goats. Therefore, these animals have a great risk of brucellosis due to the high pathogenicity of *B. melitensis* (Christopher et al., 2010).

B. melitensis infection in goats and sheep reared on rangeland has been usually ignored because sheep and goat production in arid zones is practiced in marginal rural areas by communal low-income farmers. Due to these extensive farming systems, controlling and eradicating this zoonotic disease is extremely difficult. The control and eradication of brucellosis in small ruminant herds have been used in many countries with success using surveillance programs and culling of seropositive animals (Blasco and Molina-Flores, 2011); however, the low-quality local veterinary services, the fluctuating availability of human resources to carry out the serosurvey program for this disease and the erratic coordination and administrative organizations for control programs make extremely difficult to implement an integrated control strategy to control this disease.

Hence, serologic studies focusing on risk factors for brucellosis in goats and sheep on rangeland are useful to know the magnitude of this disease in goat and sheep farms and to identify the factors that impact the occurrence of brucellosis in sheep and goat herds in arid ecosystems.In addition, a sporadic detailed survey has been conducted to know the exact incidence of the disease in arid zones of northern Mexico (Marin et al., 216). Thus, considering the paucity of epidemiological reports on brucellosis in northeastern Mexico, information on the seroprevalence of brucellosis in small ruminants is necessary to define control measures for this zoonotic disease in the area.

This study aimed to determine the individual seroprevalence of brucellosis and the risk factors involved in the occurrence of this disease in mixed herds of sheep and goats in a semi-arid ecosystem.

MATERIAL AND METHODS

The experimental procedures and animal management were carried out following international (FASS, 2010) and national (NAM, 2002) guidelines for animal welfare. This study was approved by the Committee for Animal Ethics on Animal Experimentation of the Research Department of the Autonomous Agrarian University Antonio Narro (Protocol number 3811142503001-2418).

Study site

The study was conducted in the southeastern district of Coahuila State in northeastern Mexico (24° 51’ 19’’ to 25° 34’ 41’’ N, and 100° 49’ 37’’ to 102° 33’ 14’’ W) in an area of approximately 25,100 km^2. The climate is semi-arid, with annual precipitation ranging from 300 to 450 mm. The dry season extends from November to the end of May, while the wet season comprises June to October. The mean annual temperature is 18 to 20 °C, and the grazing area consists of semi-arid shrublands. The terrain consists of rolling hills, valleys, and mountains with elevations ranging from 550 to 2800 m. The plant communities are dominated by creosote bush (*Larrea tridentata* (DC.) Coy.). Other important browse species are lechuguilla (*Agave lechuguilla* Torr.) and resinush (*Viguiera greggii* (Gray) Blake). Common grasses are blue grama (*Boutelouagracilis* H.B.K.) and buffalo grass (*Buchloe dactyloides* (Nutt) Engelm). Major forbs are rosval (*Croton dioicus* Cav.), globemallow (*Sphaeralcea angustifolia* (Cav.) D. Don.), and silver leaf nightshade (*Solanum elaeagnifolium* Cav.). These rangelands have been heavily grazed by bovines, equines, sheep, and goats for decades. The study area was chosen because brucellosis in small ruminants and the human population of this zone has been present for many decades. Brucellosis control programs for small ruminants in this zone, including vaccination and test-and-slaughter strategy,have been implementedfor many years, but these efforts have been inconsistent and intermittent.The human population in this district is about 1 million, whereas goats and sheep are approximately 250,000 and 50,000.

Goat and sheep management

Herds of goats or sheep and goat co-mingled herds ranged in size from 65 to 319 adult animals. For both goats and sheep, most animals were females as young male kids, and lambs are sold at a young age. Most goats and sheep were crossbred animals (milk or beef-type for goats and beef-type for sheep) of different ages and parity with 30 to 45 kg adult live weight. The breed of animals was classified according to the predominant phenotype observed. Sheep breeds included Criollo, Dorper, Pelibuey, Katahdin, Texel, and Merino. Because of the limited numbers of some breeds of sheep, Texel and Merino were excluded from the data set when the breed of sheep was analyzed.

Breed of goats included the traditional dairy breeds, Boer and Criollo. All animals were reared in the same areas where they were tested, had no health intervention, except for infrequent and inconsistent brucellosis tests, and did not receive feed or mineral supplements throughout the year. Goats and sheep are reared under a traditional extensive village system on communal rangelands throughout the year. The grazing period is approximately 7 hours daily (from 1100 to 1800 hours), and herdsmen lead animals. Goats and sheep are penned near the household at night without access to feed and water. Brucellosis test in all herds studied had not been made in the last five years.

Kiddings occurred throughout the year, but two peaks were manifest in June and November. When parturition was imminent, does were left in pen; therefore, most kiddings occurred in the pens, and newborn kids laid on the accumulated manure. Guardian dogs readily ingested placentas and aborted fetuses immediately after birth. Female kids remained with their dams throughout the lactation period. The grazed areas have been continuously grazed at the rate of 1.5 to 15 ha per goat/sheep for several decades. Besides goats and sheep, most rangelands were grazed by cattle and equines.

Animal sampling, sample collection, and handling

According to this district's agriculture office, a cross-sectional approach involving goats and sheep was conducted, using commercial herds randomly selected based on the area's total herds.The total number of herds to be included in this study was calculated using an expected herd-level seroprevalence "*p*" of 9.3% for caprine brucellosis (Marín et al., 2016) and 7.3% (Marín et al., 2015) for sheep in this zone, a confidence level of 95%, desired absolute precision (*d*) of 0.05 and using the following formula $p= (1.96)^2 [p*q]/d^2$, where p is the prevalence of brucellosis in the zone, q=(1-p), and d is the precision of the estimate. Accordingly, the sample size was 100 goat/sheep herds. However, to ensure adequate power for the objective of this study, a much larger sample size (375 herds) was used, screening 100% of animals in the herds selected.

A total of 11001goats and 4741 sheep were sampled. Five mL of blood were collected aseptically via jugular venipuncture from goats and sheep using 10 mL blood collection tubes (BD Vacutainer, Franklin Lakes, NJ, USA) and disposable 1½ inch, 18-20gauge needles (Greiner Bio-One, Kremsmünster, Austria). Blood was allowed to clot, placed in ice, and transported to an accredited laboratory. Sera were separated from blood without centrifugation, decanted into labeled Eppendorf® microcentrifuge tubes, and stored at -20 °C until serologic testing. The brucellosis card test examined the serum samples. The test was performed using Rose Bengal antigens, prepared by the National Producer of Veterinary Biologicals (CDMX, Mexico). The antigen had a pH of 3.6 and was prepared with lactate buffer using the strain 1119-3 of *B. abortus* at 3% cell concentration.

Animals' variables such as animal species, predominant breed, and gender were recorded. Given that this study aimed to assess the seroprevalence of brucellosis in a large population of sheep and goats on rangeland, only a screening approach was made without a confirmatory diagnosis. We acknowledge that the validation of such diagnostic tests is an issue; therefore, the true prevalence of this disease in small ruminants was calculated according to the following formula:

True Prevalence = P^T + Specificity - 1/ Sensitivity + Specificity - 1, where P^T = Prevalence observed by the test. Values for sensitivity (100%) and sensibility (98%) were taken from previous studies in goats (Díaz-Aparicio et al., 1999).

Statistical analyses

Descriptive statistics were used to calculate the percentage of herds with seropositive animals and the percentage of seropositive animals within herds (Proc Freq/binomial of SAS; SAS Inst. Inc., Cary, NC, USA). 95% confidence intervals were calculated using Proc Freq of SAS for seroprevalence.

A univariate logistic regression model of SAS was

used to analyze factors contributing to the probability of positive reaction to brucellosis (binary outcome; individual goats as units of analysis). Possible risk factors evaluated for animals included species, predominant breed of goats or sheep, and gender; the year was included in the model as a covariate. Values of $p < 0.05$ were considered statistically significant

RESULTS AND DISCUSSION

There were 45 seropositive goats or co-mingled goat and sheep herds among the 375 herds (12%) under investigation. Of the 11001goat sera tested, antibodies to *Brucella* were detected in 225animals (2.05%); in the case of sheep, 40 animals resulted positive out of 4741 tested animals (0.84%). The sampling herds employed in this investigation were generally dissimilar to those in other published work in México because herds in the present study had been tested with the removal of serologically positive animals several years ago. However, no serious and consistent effort exists to achieve a disease-free status eventually. Different from our findings, earlier studies in Mexico have reported herd-level incidences of seropositive animals between 6.8 - 9.3% (Acosta-Gonzalez et al., 2009; Marin et al., 2016). Therefore, these results emphasize the importance of continuous and consistent control efforts against caprine/ovine brucellosis.

Although sheep have been found previously to be infected with brucellosis in northern Mexico (Nuñes-Torres et al., 1999; Marin et al., 2015), reports from Mexico are few, based entirely on occurrence in animal samples from just a single site and often just one survey over a limited time. In the present study, we detected anti-*Brucella* antibodies in just 0.84% of sheep; this indicates that *Brucella spp.* infections are relatively rare in grazing sheep populations in the study site. This is comparable to the 1.2% incidence found in eastern Sudan (Gumaa et al., 2014). In the present study, *Brucella* species was not isolated, but brucellosis due to *B. melitensis* has been described in sheep in the region where this study took place (Marin et al., 2015). Thus, probably *B. melitensis* spills over to sheep from the goat reservoir. Lambing or kidding in crowded pens, as was the case in the present study, favors the spread of the organism. At the same time, open-air parturition in a dry environment results in decreased transmission (Yilma et al., 2016).

Goats in these extensive production systems were two times more likely to develop brucellosis than sheep. Other studies in nomadic and sedentary herds in arid zones have also found that a higher proportion of goats tested seropositive to brucellosis than sheep (Brisibe et al., 1996). However, in the center of Spain, brucellosis-seropositivity was higher for sheep (0.7%) than goats (0.1%) in an area where some herds of these species shared a communal grazing area (Reviriego et al., 2000). Given the high diffusion of *B. melitensis* in a highly susceptible host (sheep) and the lack of a consistent brucellosis control program in Mexico, the low seroprevalence of this disease in sheep in the present study is intriguing. Mainly because lambing or kidding in crowded pens, as was the case in the present study, favors the spread of the organism (Yilma et al., 2016). Additionally, dogs are never removed from these herds, and they can be infected, as these animals readily eat placentas or aborted fetuses of infected sheep and goats.

For goats, females had higher odds of seropositivity to brucellosis than males. Likewise, ewes were 2.3 times more likely to be positives to antibodies to *Brucella spp*. than males (Table 2). These results are in line with findings of studies with sheep and goats (Brisibe et al., 1996; Mahboub et al., 2013), only sheep (Kotadiya et al., 2015), and only goats (Priya et al., 2010) in arid environments, where males were at decreased risk to seropositivity to brucellosis than females. On the other hand, no differences in the prevalence of *Brucella* antibodies between males and females were found in indigenous breeds of goats of Nigeria (Olufemi et al., 2018). Results of the present study could be due to the much higher number of females in the herds because most bucks and rams in the herds studied are joined with females just for breeding purposes; hence goat farmers rear fewer males. During the kidding season, they have lower chances of being exposed to bacteria shed in the birth fluids or fetus, placenta, and abortion secretions of infected females, as bucks and rams are typically isolated to have controlled breeding seasons.

Compared with Saanen, Toggenburg genotypes had 6.1 higher odds ($p < 0.01$) of being seropositive to brucellosis. Besides, Toggenburg goats were 2.2 times more likely to be diagnosed with brucellosis than Criollo goats ($p < 0.01$). Thus, these findings support the hypothesis that some breeds of dairy goats are more resistant to brucellosis infection. Differences in goat breeds regarding brucellosis susceptibility have been described in different countries (Solorio-Rivera et al., 2007; Ali et al., 2015; Aworh et al., 2017), although

Table 1: Seroprevalence to brucellosis based on the card test in grazing goats and sheep in semi-arid rangelands of northern Mexico.

Species	Total No. tested	Number positive	Seroprevalence (serop; 95% CI)	True serop	Odds ratio[1*] (95% CI)
Goat	11001	225	2.05 (1.78-2.31)	2.11	2.5 (1.8-3.4)
Sheep	4741	40	0.84 (0.58-1.10)	0.88	1.0
Total	15742	265	1.68 (1.48-1.88)	1.73	-

[1]Odds ratios indicate how much more or less likely the outcome is among variables with a given risk factor; compared with those without it. The reference odds ratio is 1.0.
*p < 0.01.

Table 2: Seroprevalence of brucellosis based on the card test in male and female grazing sheep and goats in semi-arid rangelands of northern Mexico.

Gender	Total No. tested	Number positive	Seroprevalence (serop; 95% CI)	True serop	Odds ratio (OR)[1*]
Goats					
Female	10620	225	2.07 (1.80-2.34)	2.13	2.3 (1.7-3.3)
Male	381	5	1.31 (0.17-2.46)	1.36	1.0
Sheep					
Female	4254	38	0.89 (0.61-1.18)	0.93	3.2 (0.6-16.7)
Male	487	2	0.41 (0.00-0.98)	0.44	1.0
Total					
Female	14874	258	1.73 (1.52-1.94)	1.79	2.2 (1.0-4.6)
Male	868	7	0.81 (0.21-1.40)	0.85	1.0

[1]Odds ratios indicate how much more or less likely the outcome is among variables with a given risk factor; compared with those without it. The reference odds ratio is 1.0.
*p < 0.01.

Table 3: Seroprevalence of brucellosis based on the card test in different breeds of grazing sheep and goats on semi-arid rangelands of northern Mexico.

Species and breed	Total number tested	Number positive	Seroprevalence (sero; 95% CI)	True sero	Odds ratio (OR)[1*]
Goats					
Alpine (A)	997	18	1.81 (1.1-2.8)	1.87	A vs. T; 1.8 (1.0-3.2)
Boer (B)	1076	17	1.58 (0.9-2.5)	1.63	B vs. T; 2.1 (1.2-3.8)
Criollo (C)	2039	31	1.52 (1.0-2.1)	1.57	C vs. T; 2.2 (1.3-3.5)
Nubian (N)	4696	117	2.49 (2.0-3.0)	2.56	N vs. T; 1.3 (0.9-1.9)
Saanen (S)	1086	6	0.55 (0.2-1.2)	0.58	S vs. T; 6.1 (2.5-14.4)
Toggenburg (T)	1107	36	3.25 (2.2-4.4)	3.34	
Sheep					
Criollo (C)	669	14	2.09 (1.1-3.4)	2.15	C vs. P; 0.6 (0.1-2.8)
Dorper (D)	487	2	0.64 (0.4-0.9)	0.67	D vs. P; 2.1 (0.5-8.9)
Katahdin (K)	283	1	0.35 (0.1-0.9)	0.38	K vs. P; 3.8 (0.3-42)
Pelibuey (P)	151	2	1.32 (0.2-4.7)	1.37	

[1]Odds ratios indicate how much more or less likely the outcome is among variables with a given risk factor; compared with those without it. The reference odds ratio is 1.0.
*p < 0.01.

no differences in susceptibility to seropositivity to brucellosis have been found in indigenous breeds of Nigeria (Ogugua et al., 2014; Olufemi et al., 2018).

In the present study, Saanen goats demonstrated the ability to maintain a lower seropositivity response to brucellosis than other dairy and meat breeds of goats. Lower-yielding and autochthonous dairy cattle breeds are known to possess higher resistance to diseases when compared to high-yielding cows (Gandini et al., 2007; Curone et al., 2018). This partially applied to the present study as Saanen goats had a low prevalence of antibodies against brucellosis, but Toggenburg goats presented the highest seropositivity to this disease. It could be that this breed of goats had a higher mobilization of body reserves from adipose and muscle tissue, and negative energy status in the days immediately after kidding may lead to further health problems (Gandini et al., 2007). It is worth mentioning that many goats used in this study were not purebred; therefore, further studies are necessary to conclude a causal association between breed and seropositivity to brucellosis on rangeland.

Compared with Katahdin, Pelibuey sheep had 3.8 higher odds of being seropositive to brucellosis, and 2.1 higher odds than Dorper ($p < 0.01$, Table 3). Surprisingly, Criollo sheep presented higher seropositivity to brucellosis than all other breeds of sheep. This response is intriguing because Criollo is a rustic breed well adapted to harsh environmental conditions. Other authors have found breed differences in the prevalence of *Brucella sp.* antibodies in indigenous sheep (Mahboub et al., 2013; Patel et al., 2017; Shuaiband Mansour, 2018). These discrepancies in seroprevalence between breeds could be related to genetic variation involved in host resistance (Bishop, 2010). These findings suggest that hair sheep composite breeds maintain high heterosis for brucellosis resistance; however, further investigations are required to reiterate this view.

CONCLUSIONS

The study demonstrated that brucellosis persists at low endemic levels in sheep and goats pastoralist systems on the rangeland of the region studied. Goats showed two-fold higher seropositivity to brucellosis than sheep, implying that these animals can serve as a potential threat to sheep and cattle in the area where this study took place. Additionally, this field study under extensive conditions has provided epidemiological evidence that crossbred goats with a predominance of Toggenburg were more susceptible to seropositivity to brucellosis than other meat and dairy breeds; criollo sheep, on the other hand, presented higher seropositivity to brucellosis compared to hair sheep composite breeds, which implies promise for increased resistance to this disease through the use of sheep composite breeds.

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CONFLICT OF INTEREST

The authors declare that no conflict of interest could be perceived as prejudicing the impartiality of the research reported.

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