Seroprevalence and Risk Factors for Brucellosis in Free-Range Goats

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ABSTRACT

The prevalence of *Brucella* antibodies in serum of unvaccinated grazing goats as well as risk factors for brucellosis in northeastern Mexico were evaluated using data from a cross-sectional study between 2002 and 2008. Data were from 99 goat flocks. Serum samples from 12,127 goats were analyzed with the Rose-Bengal plate-agglutination test (RBPT). 9.3% of goats had antibodies against *Brucella,* and the disease affected 64% of goat flocks. A multiple logistic regression model identified altitude of grazing site (OR = 1.40), average annual precipitation (OR = 1.68), flock size (OR = 1.21) and breed of goats (indigenous goats more resistant than imported ones) as risk factors for seropositivity for *Brucella*. It was concluded that brucellosis is quite widespread in flocks in the region studied. Also, flocks with <50 goats, low geographical elevation of flock location, increased shrub cover and lower annual rainfall were associated with increased odds of testing positive for *Brucella*.

Keywords: Rose Bengal Plate Test; *Brucella*; Criollo goats; Nubian goats; Rangeland.

INTRODUCTION

Brucellosis is a nationwide zoonotic bacterial disease in goats in Mexico (1, 2). The animal-level seroprevalence of this disease in different parts of the country is around 10% (2-4). The widespread presence of this disease in goats in Mexico is due to the extensive nature of goat operations, which facilitates multiple contagious routes, such as sharing the same grazing land by multiple goat flocks, convergence of flocks of goats in a common water point, sharing bucks and does among producers and the use of highly crowded pens (<5 square meters per goat). Additionally, vaccination programs against *Brucella* in goats are limited, or vaccination campaigns are conducted on a small-scale. Also, these immunization campaigns frequently are inappropriately executed: goats are sometimes repeatedly vaccinated with the Rev 1 *Brucella melitensis* vaccine or they are wrongly vaccinated with the RB51 vaccine, which provokes that goats go unnoticed using the routine diagnosis tests for brucellosis and some pregnancies results in abortions and stillbirths (5-6), spreading *B. melitensis* through vaginal discharges (6).

Furthermore, attempts to manage this disease in Mexico by test-and-slaughter have failed because of limited allocation of needed resources to control and eradicate brucellosis, or the unwillingness of goat producers to get rid of their seropositive goats, because of the generalized believe that once the infected goats recover from the insidious onset of this infection, they become resistant to this disease and their productivity is similar to that of healthy animals.

In the arid and semi-arid zones of Mexico, a great deal of peasants depend on goats for living, mostly in pastoral and mixed agricultural/pastoral systems (7). Given that most of these goats production systems are not subjected to health programs, people who hinge on this kind of livestock are at high risk of brucellosis infection (1).

Brucellosis is transmitted to humans by direct or indirect routes such as consumption of unpasteurized dairy products

contaminated with *B. melitensis* (8). The milk of infected goats may contain large numbers of viable organisms which become concentrated in cheeses. In fact, unpasteurized cheese has been recognized as a major vehicle of infection in many countries (9-11). Limited information is available on the prevalence of brucellosis in pastoral communities on rangeland, due to the difficulty in gathering information in extended areas. Because these communities of peasants are economically and culturally dependent on goats, it is important to identify some risk factors associated with brucellosis in grazing goats.

Many of the studies conducted on brucellosis in Mexico have been from the humid zones (3,6) or subtropical regions (2). To the best of knowledge of the authors, no large surveys have been carried out on the prevalence of brucellosis in goats operations on rangeland. The primary aim of the current study was to investigate the individual and herd-level seroprevalence of *Brucella* infections in grazing goats in a semi-arid environment. The secondary aim was to identify some risk factors for testing positive to brucellosis in goats from medium to large-scale flocks under rangeland conditions.

MATERIAL AND METHODS

Site description

The study was carried out in Nuevo Leon State, Mexico (23 to 27° N and 98 to 101° W). The climate is semi-arid, and the annual precipitation ranges from 300 to 600 mm, 70% of which occurs between June and October. Most precipitation events are brief, intense convecting storms. The average annual temperature for the study area is 23°C. The terrain consists of valleys, rolling hills and mountains with elevations ranging from 500 to 3700 m. Vegetation is characterized as Chihuahuan desert rangeland. For decades, pastures have been heavily grazed by bovines, equines, sheep and goats.

Goat management

Animal care and experimental procedures were conducted in accordance with international guiding principles for biomedical research involving animals and institutional policies for animal health and well-being and approved by the Autonomous Agrarian University Antonio Narro Animal Care and Use Committee.

Goat flocks ranged in size from 14 to 419 adult goats, with a median of 195 animals. All goats were female as male goat kids in this zone are slaughtered around 45 days of age. Goats were Nubian, Criollo (descendent from animals introduced by the Spaniards in the XVI century) and crosses between Criollo and European dairy breeds and Criollo and Nubian. All goats were from commercial flocks and foraged exclusively on native vegetation, most of them without feed or salt supplementation throughout the year. Goats shared the rangeland with bovines, equines and sheep.

Water sources in pastures were surface reservoirs, where goats were taken once daily. Animals were penned near the household of peasants at night without access to feed and water. Pens were built with local materials, mainly, tree and shrub branches, with a very limited space per goats (3-4 square meters per animal). All flocks were protected by several guardian mixed-breed dogs, which were deeply bonded to goats.

Goats grazed daily for approximately 7 h (from 1100 to 1800 h) on open range, herded by a goat keeper. Goats were taken to different grazing sites every day, and animals walked approximately 5 km daily from the pen. Goats were not vaccinated against endemic diseases (including brucellosis) and were not treated against internal and external parasites. In most flocks, group mating took place during 4 weeks at different periods of the year. Goats were milked for approximately 6 months (40 to 60 liters/lactation).

Group mating took place during all months of the year, except April, during a 4-week period. Bucks ranged from 1.5 to 5-year of age and the doe buck ratio did not exceed 40:1. Kiddings occurred in all season. When parturition was imminent, most does were not taken out for grazing, thus, most kiddings occurred in confinement. No bedding was used in pens, therefore all newborn kids laid on the accumulated manure. Because guardian dogs were underprovided with food year-round, these animals readily ingested placentas of all goats that gave birth. Female kids remained with their dams throughout the lactation period. Stocking rate were approximately 7 ha per goat, which was above the carrying capacity of these rangelands.

Study population and study design

A total of 99 goat flocks from all agro-ecological regions (desert scrub, coastal plain scrub, piedmont scrub, montane low forest, montane mesic forest, montane chaparral, subalpine humid forest, alpine meadow) of the state of Nuevo Leon, Mexico which were representative of the entire goat

population of the study area were randomly selected. In order to simplify the statistical analysis and to make the association between vegetation and prevalence of brucellosis more comprehensible, only shrub coverage was considered instead of different ecological regions. Target sample size for simple random sampling was calculated with the following formula: $(1.96)^2[p^*q]/d^2$, where p is the prevalence of brucellosis in the zone, q=(1-p), and d is the precision of the estimate, for a 10% prevalence of caprine brucellosis in Mexico (2-4), 95% confidence limits and 5% relative precision. However, to ensure adequate power for the objective of this study, a much larger sample size (12,127 goats) was used. Of these animals 5,714 (47%) were Criollo, 5,019 (41%) were mixed-breed goats (Criollo crossbred with dairy breeds or Criollo crossbred with Nubian) and 973 (8%) were Nubian. The study was a cross-sectional type with data collected between 2002 and 2008. We acknowledge that data are not recent, but due to the scarce strategies to effectively control this disease in northern Mexico, it is presumed that the results obtained in this study reflect the current situation of prevalence of antibodies to *Brucella* spp. in goat herds in the studied area. In fact, brucellosis in traditional pasture-based systems around the world tends to remain relatively stable (12).

Climatological data included in the study were obtained from weather stations in each of the regions where goats grazed. Average altitude of terrain and type of vegetation where goats were turned out for grazing/browsing was recorded by a technician trained in range management.

Blood samples and testing for brucellosis

Only pluriparous goats were included in this investigation. Blood samples (10 ml) were collected aseptically from the jugular vein throughout the year, using disposable needles and vacutainer tubes. Blood samples were clotted at room temperature and the sera were separated by centrifugation and stored at -20˚C until testing. All collected sera were screened for the presence of antibodies against *Brucella* using the Rose Bengal plate test (RBPT) (13). The antigen used was the 1119-3 *B. abortus* whole cells at 8% concentration and pH of 3.65 in a lactate buffer solution. Agglutination observed within 4 min after mixing was deemed a positive result.

Briefly, the procedure consisted of placing 30 µl of the sera onto the plate and 30 µl of RBPT antigen was dropped alongside the sera. The plate was rocked for 4 min and the test was read by comparing it with the positive and negative control sera by looking for agglutination. Micro-agglutination was observed with a magnifying glass. Samples with no agglutination were deemed negative, while those with agglutination were considered positive. A herd was considered positive for *Brucella* infection if at least one goat on the flock tested positive with the RBPT.

It should be noted that testing positive for *Brucella* is a result of exposure to the bacteria, the presence of cross-reacting organisms, or true infection with field strains of this microorganism. Antibody development is not considered to be due to environmental of geographical conditions.

Data analyses

Descriptive statistics were used to determine the percentage of flocks with seropositive goats. All serum samples were used to estimate the overall seroprevalence. 95% confidence intervals for discrete variables were described with their frequencies (SAS Proc Freq/binomial; SAS Inst. Inc., Cary, NC, USA). To analyze factors contributing to the probability of positive reaction to brucellosis (binary outcome; individual goats as units of analysis), a multiple logistic regression model of SAS was used. The model included the following potentially explanatory variables of interest: the average annual temperature of sites where goats were raised, altitude of grazing terrain, shrub cover of rangeland where goats were kept, mean annual precipitation, flock size and breed of goats (Criollo, Nubian and mixed-breed). Year was included in the model as covariate.

The average annual temperature was classified as being ≤22 or >22˚C. Altitude of grazing terrain was categorized as lower or greater than 500 m. Shrub cover was coded as less or higher than 50%. Annual precipitation was classed as less or greater than 550 mm. Flock size was organized into fewer than 220 goats or greater than 220 animals. The criteria for variable separation were a value around their mean. A segmented regression was used (CurvExpert professional 2.0 software) to described the association between flock size and seroprevalence for brucellosis.

RESULTS

Sixty-four percentage of the flocks (67/104) had at least 1 seropositive animal (Fig. 1). Of the 12,127 goat sera tested in all the regions under investigation, antibodies to *Brucella* spp. were detected in 1,131 animals, thus the prevalence of *Brucella* was 9.3% in this population (95% CI, 8.8-9.9). The

Figure 1: Frequency of goat flocks under pastoral production systems on rangelands (n = 99) with *Brucella* seropositive goats in the state of Nuevo Leon; Mexico.

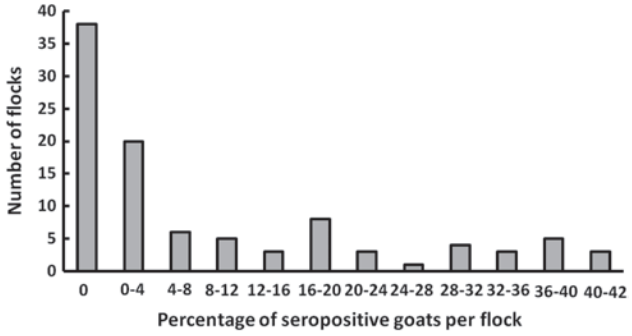
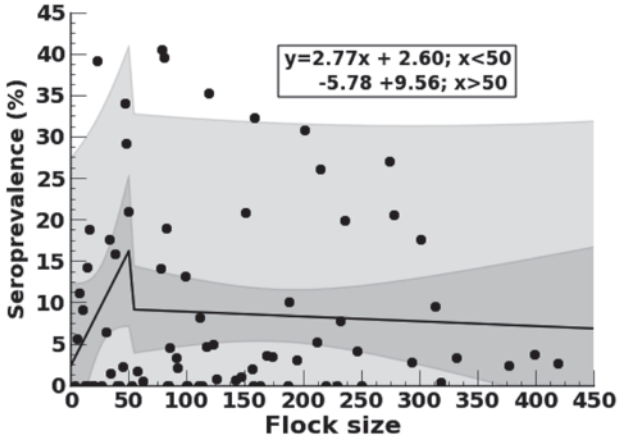


Table 1: Odds ratios (OR) for the likelihood of brucellosis in range goats as a function of mean annual ambient temperature; altitude; shrub coverage; mean annual precipitation and flock size.

Variable	Brucellosis prevalence	Odds ratio (OR)[1]	95% CI OR
$\bar{x}$ annual ambient temperature (°C)			
≥ 22	9.2 (794/8663)		
< 22	9.7 (373/3464)	1.03	0.84 – 1.27
$\bar{x}$ elevation (m)			
≥ 500	8.7 (612/6999)		
< 500	10.1 (519/5128)	1.40*	1.12 – 1.76
Shrub coverage (%)			
≥ 50	10.2 (540/5305)		
< 50	8.7 (591/6822)	0.83*	0.74 – 0.95
$\bar{x}$ annual precipitation (mm)			
≥ 550	6.9 (351/5089)		
< 550	11.1 (780/7038)	1.68*	1.48 – 1.92
Flock size			
≥ 220	8.4 (445/5288)		
< 220	10.0 (686/6839)	1.21*	1.07 – 1.38
Breed of goats			
Criollo	7.8 (444/5714)		
Mixed breed (Criollo x dairy breeds)	11.8 (594/5019)	1.6*	1.4 – 1.8
Nubian	8.4 (82/973)		
Criollo	7.8 (444/5714)	0.92	0.72 – 1.17
Nubian	8.4 (82/973)		
Mixed breed (Criollo x dairy breeds)	11.8 (594/5019)	1.2*	1.07 – 1.36

[1] Odds ratios measure how much more or less likely the outcome is among variables with a given risk factor; compared with those without it; or reference category (odds ratio of 1.0).
* P < 0.01.

Figure 2: Association between *Brucella* seroprevalence in individual goats and flock size in commercial goats operations on rangeland in the state of Nuevo Leon; Mexico. Bands represent 95% confidence intervals for predicted (darker) and real (lighter) data.



range for within-herd animal-level seroprevalence was 0 to 42.0% with a median of 2.63%. Mean within-herd seroprevalence in positive flocks was 12.6% (95% CI, 11.9-13.3%).

The results of the serological examination associated to the mean annual temperature, number of goats in flocks, vegetation characteristics, altitude of grazing terrain and breed of goat are presented in Table 1. Seropositivity to *Brucella* was not affected by mean annual ambient temperature. Grazing at an altitude lower than 500 meters was positively associated with seropositivity to *Brucella*; goats raised in lowlands were 40% more likely to test positive for *Brucella* antibodies than goats kept in higher zones (Table 1). Goats kept in terrains with less than 50% shrub cover were less likely to test seropositive for *Brucella* than goats in flocks grazing in denser vegetation. Compared to goats grazing in zones with rainfall >550 mm, goats grazing in drier areas were 1.7 times more likely to have positive titres to *Brucella*.

The odds of goats being positive for *Brucella* infection were 1.2 times greater for goats in flocks <220 animals compared to the odds of being in larger flocks. However, seroprevalence of caprine brucellosis was affected by flock size in a different manner; in flocks <50 goats *Brucella* reactors were more prevalent as the size of flock increased, whereas seropositivity to *Brucella* antibodies decreased as flock size increased in flocks >50 animals, as indicated by a segmented regression (r=0.32; Fig. 2).

Regarding goat genotype, mixed-breed goats were 1.6 times more likely to test serologically positive to *Brucella* than Criollo goats. The odds of mixed-breed goats being positive

for *Brucella* infection were 1.2 times higher than Nubian goats. On the other hand, odds of *Brucella* seropositivity were similar for Nubian and Criollo goats.

DISCUSSION

The present study represents the largest prevalence of *Brucella* antibodies report for extensive pastoral system of goats in the semi-arid zones in north eastern Mexico to date. Point prevalence estimates for serological positivity to *Brucella* in this study is consistent with data of other researchers (3) in unvaccinated grazing goats in a subtropical zone of Mexico, but much lower than the prevalence of 38% found in other studies (14) in unvaccinated goat flocks in pastoral extensive systems in southwestern Mexico. The percentage of seropositive flocks found in the present study is higher than the 51.6% infected flocks (unvaccinated) found in a subtropical zone of Mexico (2).

However, direct comparisons among regions are problematic as the seroprevalence for *Brucella* may vary due to screening examination methods, breed of goats, feeding and health management, agro-climatic conditions and production systems. The high number of flocks (64%) with at least one *Brucella* seropositive goat in the present study indicates that this disease is widespread in the region studied. These data validate the notion that goats represent an important threat to humans from this zoonotic pathogen, mainly via the consumption of raw milk and unpasteurized goat milk cheeses. In fact, 93% of human brucellosis cases in Mexico are infected with *B. melitensis* of caprine origin (1).

Grazing in terrains of low altitude favored higher seroprevalence for *Brucella*. This effect was independent of factors related to climate, because mean annual temperature did not modify seroprevalence of this infectious disease. It is unclear how lower terrains could influence seropositivity for brucellosis. It could be that close encounters with other farm animals in terrains of lower altitude, which are fundamentally important to disease dynamics, were more frequent in lowlands, because bovine, sheep and equines in this zone are concentrated in lower terrains, due to their inability to graze steep and rugged landscapes as goat do. Common grazing pastures allow intermingling of infected flocks with brucellosis free flocks. Considering the contagious nature of *Brucella* spp., sharing grazing land and drinking water among farm animals is likely to make easy transmission of this disease (15). Under these circumstances the existence of cross-infections with *B. melitensis* may be more frequent, being the most common cause of infection when farm animal species are reared together (15). Other authors have documented the association of the contact with other farm animals with *Brucella* seropositivity in goats (15-18).

Shrub cover was an important factor for seropositive antibodies to *Brucella*. The lower the shrub cover in the grazing site the lower goats tested positive for Brucellosis. Shrub cover possibly affected abiotic conditions that might influence the reservoirs for *Brucella*. The mechanisms by which *Brucella* is disseminated in these pastoral extensive systems is exposure to the disease from multiple sources such as vaginal discharges of aborted goats, fetal membranes, fetuses, direct contact with infected animals and possibly from airborne particles. A great deal of parturitions in these systems occur in the pen, but goats kidding on rangeland with high shrub cover possibly increased the contact of healthy goats to become in contact with uterine material following abortion. This could be so due to denser vegetation leading to a more cohesive group of goats while grazing. Additionally, placental membranes are more difficult to find by guardian goats that readily ingest them. This data highlight the importance of vegetation structure and composition in brucellosis dynamics in goat flocks on rangeland.

Precipitation was the single most important risk factor for *Brucella* seropositivity in goats. *Brucella* reactors were more prevalent in zones with rainfall <550 mm. Many infectious agents and its rate of pathogen replication are sensitive to climatic conditions (19, 20). In the present study it is believed that lower rainfall prompted a greater airborne spread of brucellosis, because placental tissue and vaginal discharges of infected animals dried more rapidly, liberating *Brucella* to the environment, which may be inhaled as aerosols by healthy goats (21-23). Thus, we postulate that *Brucella* may persist in the environment in a viable state during times of low rainfall and this may lead towards a higher infection rates via inhalation of *Brucella* as the mode of transmission in drier landscapes.

Prevalence of antibodies to *Brucella spp*. was affected by flock size. In flocks >50 goats *Brucella* reactors were more prevalent as size of the flock increased, which was in line with previous studies with small flocks (17, 24-26). This association between seropositivity to *Brucella* antigens with larger flocks has been ascribed to crowding in the pen and an increase in stocking density creating a higher bacterial load in the envi-

ronment and increasing the odds of disease transmission. On the other hand, in flocks with <50 goats this study links the seropositivity for *Brucella* antigens with smaller flocks. This response could be due to the fact that the greater the flock the more ample are the goat pens, so that dissemination of this zoonotic microorganism could be limited, because many goats do not get close to the site of parturition (presence of tissue and fluids overload with *Brucella*) of infected goats. Additionally, despite the cohesiveness of goat flocks while grazing on rangeland, goat dispersion in patchy, roughed and low-producing landscapes is greater in large flocks than in small flocks, which reduces the likelihood of contact between infected and non-infected goats. It is generally accepted that an increase in herd size is accompanied by an increase in stocking density, but this is not the case in the present study, where goats had ample grazing land, which did not allow crowding on open range, due to the wide spatial grazing patterns of goats. These data highlights the importance of defining what a large herd is because contrasting results can be obtained with different sizes of flocks with unrestricted grazing distribution in extensive pastures.

Higher prevalence of antibodies to *Brucella* was revealed in mixed-breed goats compared to Criollo goats. Likewise seropositive reactors in Nubian were lower than in mixed-breed goats. These results indicate that no apparent advantage for heterozygosity to brucellosis resistance exists in crossbred goats. We examined goats under the same production system, housing and fairly the same type of vegetation, therefore management factors did not seem to be involved here. Therefore, this data support the notion that non-adapted breed of goats to hot-arid environments are more susceptible to be seropositive for *Brucella*, compared with goats which evolved surviving entirely on arid rangeland (27). Thus, these data highlight the advantage of using indigenous disease-resistant goats instead of local adapted crossbred goats.

Natural resistance against brucellosis has been demonstrated in bovines, particularly in cattle adapted to harsh environments, and is linked with the ability of macrophages to prevent intracellular replication of *Brucella abortus* (28) and the Nramp1 gene, which enhances innate and adaptative immunity favoring bacterial killing by macrophages (29). No studies comparing the prevalence of brucellosis between dairy goats of European origin and native breeds (Spanish origin) on rangeland are available. These results support the hypothesis that the hardy well-adapted Criollo breed has greater resistance factors for brucellosis than goats bearing dairy breed genes. Thus, farmers in extensive pastoral systems should be aware of introducing "genetically better" stock developed in high input production systems, because lack of fitness of these animals makes them more susceptible to test positive to antibodies against *Brucella*.

Finally, several limitations need to be taken into account. Although the study sample is representative of the total goat population of the Nuevo Leon State in Mexico, the prolonged sampling period may not adequately reflect the prevalence and accurate risk factors for brucellosis in goats in the areas studied.

In conclusion, results indicate that seroprevalence for brucellosis among goats in extensive pastoral systems in the region studied in northeastern Mexico is not greater than seroprevalence among goats in other parts of the country. A number of easily identifiable risk factors associated with seropositive antibodies against *Brucella* were detected. Large flock size (flocks with <50 goats), low elevation of grazing terrain, increased shrub cover and lower annual rainfall were associated with increased odds of testing positive to *Brucella*. Goat producers in extensive pastoral systems also are provided with evidence that strong variation in breed susceptibility to this infectious disease exists, with solid evidence that the well adapted sturdy Criollo goats are less susceptible to the presence of serum antibodies for brucellosis than less adapted goats (local x dairy animals).

DECLARATION OF CONFLICT OF INTEREST

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

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