Short Report: Brucellosis in Dairy Cattle and Goats in Northern Ecuador

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Abstract. The purpose of this study was to conduct a convenience study for brucellosis prevalence in dairy-producing animals in northern Ecuador. In total, 2,561 cows and 301 goats were tested. Cattle sera were tested using the Rose Bengal card antigen test (RBCT), yielding an overall apparent prevalence of 5.5% (95% confidence interval [95% CI] = 4.7–6.5%) and true prevalence of 7.2% (95% CI = 6.0–8.5%). Prevalence varied by herd size and was highest in larger commercial herds. Polymerase chain reaction was used to test goat milk and lymph nodes, resulting in 9% and 8% positivity, respectively. The RBCTs from goat sera yielded an adjusted true prevalence of 17.8% (95% CI = 6.2–44.2%). Our findings are similar to other overall prevalence estimates for dairy herds but show higher prevalence in commercial herds compared with small groups (less than five animals). We also identify urban milking goats living in metropolitan Quito as a potential source of zoonosis.

Brucellosis is a chronic infection caused by an intracellular bacterium, which affects almost every organ system of the body.¹ *Brucella abortus* and *B. melitensis* are the two most important species of *Brucella* that infect food-producing animals and humans. Most commonly, infection in humans occurs through consumption of unpasteurized dairy products, causing chronic bone, joint, and reproductive diseases, spontaneous abortion, and chronic undulant fever that typify brucellosis. Infection with *B. melitensis* can also cause fatal cardiac disease.¹ The global data on human cases are believed to be a massive underestimation of the true prevalence of brucellosis.¹ Extensive eradication programs in food animals have been successful in some countries, leading to the elimination of human brucellosis. However, brucellosis remains endemic in many parts of the world because of insufficient capabilities for diagnosis and reporting; thus, it continues to limit rural economic development and human wellbeing.^{2,3}

South American countries are considered to have high brucellosis prevalence in humans, despite low numbers of human cases reported by the World Organization for Animal Health.^{1,4} Official government reports in Ecuador from 1990 to 2008 estimated human prevalence of 0.21 cases per 100,000 persons.⁵ However, recently, in the first and only published human case report for *B. abortus* infection, the International Center for Zoonosis suggested human prevalence in northern Ecuador to be 2% or 2,000 cases per 100,000 persons.⁶

There are no peer-reviewed published reports of *Brucella* epidemiology in Ecuador, and reported data on the subject are variable. As part of a national brucellosis disease control program, a division of the Ecuadorian Ministry of Agriculture, Agrocalidad, estimated prevalence of brucellosis in food animals from 1979 to 2008 to range from 1.92% to 10.62% in cattle in highland provinces and from 4.12% to 10.62% in coastal provinces.⁷ Data published in different graduate theses from Ecuadorian universities show significant variability

in cattle disease prevalence, ranging from 1–9.73% to 24–48%.^{8,9} The aim of this study was to estimate brucellosis prevalence (from a convenience sample) in food animals that the authors work with on a regular basis, including commercial dairy herds, cattle in rural communities, and urban milking goats in the northern Ecuadorian highland provinces of Imbabura and Pichincha and the city of Quito, respectively. The dairy herds tested were selected by convenience and are clients of one of the authors (L.L.), who is a bovine practitioner based in Cayambe. We have long-standing working relationships with the animal-owning families in the rural communities that we tested as part of a field school with the University of Wisconsin–Madison Global Health Institute. Dairy goats raised in urban areas, such as the nation's capital city of Quito (population of 2.4 million people in 2010 from the Instituto Nacional de Estadísticas y Censos), are used to provide meat and fresh milk from street vendors. Cattle are slightly different, because there are two distinct populations of cattle in northern Ecuador: cattle owned by individual families in rural communities that are managed as individuals or small groups (less than five animals) and cattle that are managed in commercial herds. The different management methods are important, because the two populations of animals provide food in different ways. Individually managed animals in rural villages are more likely to provide milk (unprocessed, unpasteurized, or raw) to a family or small group of people. Cattle managed in herds, defined in this study by five or more animals, tend to supply milk to creameries for later commercial retail sale after pasteurization and packaging.

In total, 101 dairy herds and three rural communities were included in this study, covering a total population of 2,561 cattle from 2011 to 2013. The largest commercial dairy herd was 600 animals, and herd size was determined by total number of cattle on the farm. To the best of our knowledge of animal demographics on each farm (complete and accurate herd records are not readily available on many farms in the area), we tested all female animals over 6 months of age. One exception was the largest herd of 600 cattle, of which only 130 animals were available to be tested during the time that our team was on the farm. In rural communities for

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TABLE 1

Seroprevalence of *Brucella* sp. (*B. abortus* or *B. melitensis*) in dairy cattle in northern Ecuador by herd size

Herd size	No. herds tested	Cattle tested	No. positive	Apparent prevalence % (95% CI)	True prevalence % (95% CI)
< 5	86	108	0	0.0 (0.0–3.4)	0.0 (0.0–4.0)
5–24	69	635	20	3.1 (2.0–4.8)	3.8 (2.2–6.2)
25–49	13	413	19	4.6 (3.0–7.1)	5.9 (3.3–9.3)
50–74	7	282	1	0.4 (0.1–2.0)	0 (0.0–2.0)
75–99	3	171	16	9.4 (5.8–14.7)	12.5 (7.3–19.9)
≥ 100	9	952	86	9.0 (7.4–11.0)	12.0 (9.6–14.8)
Total	101	2,561	142	5.5 (4.7–6.5)	7.2 (6.0–8.5)

Data from animals managed in commercial dairy herds and small groups (less than five animals) by individual families in rural communities. The number of individual animal owners (families who do not sell milk commercially) represents the number of herds tested for herd size less than five animals.

individual families, we offered testing for all animals over 6 months of age as part of voluntary animal health clinics held in the central communal areas of the communities. It is unknown what proportion of the total animals in those communities was tested, also because of the lack of animal ownership records. Blood samples from cattle were collected by veterinarians or veterinary students under direct supervision of veterinarians (K.P.P. and/or L.L.); 18 of 101 commercial herds (727 animals tested) reported concurrent or historical use of vaccines for cattle brucellosis. Both Strain 19 and RB51 modified live vaccines are used and available for purchase without a prescription from a veterinarian in Ecuador. Because of the lack of complete herd records, we did not separate vaccinated cattle by vaccine type received. Cattle sera were tested with the Rose Bengal card antigen tests, and all reagents were purchased from the National Veterinary Laboratory located in Ames, IA and performed as prescribed by the World Organization for Animal Health Brucellosis Manual.¹⁰ The card test was performed by veterinarians or veterinary students under direct supervision of veterinarians (K.P.P. and/or L.L.). The card antigen test is a widely accepted method for herd diagnostic testing, and it is 99.6% specific, with 72.2% sensitivity in cattle for both *B. abortus* and *B. melitensis*.¹¹ The 95% confidence intervals (95% CIs) for apparent prevalence were calculated using the Wilson binomial approximation method.¹² The prevalence per dairy herd was estimated as the apparent prevalence followed by correction into the true prevalence using the Rogan–Gladen correction with the sensitivity and specificity for individual animals tests.¹³ The sensitivity of the Rose Bengal card test used was 72.2%, and the specificity was 99.6%.¹¹ Blaker's exact 95% CIs for true prevalence estimates were calculated as described by Reiczigel and others.¹⁴ The apparent prevalence of brucellosis was 5.5% (95% CI = 4.7–6.5) (Table 1). No positive animals were found in the three communities tested where animals were managed individually or in groups of less than five animals (Table 1).

Polymerase chain reaction (PCR), which has a detection limit of 1 pg for *B. abortus* and *B. suis* DNA and 10 ng for *B. melitensis* DNA, was used to detect *B. melitensis* in goat milk as previously described.¹⁵ Sensitivity and specificity data for this method have yet to be made available, and therefore, positive PCR reactions were confirmed by DNA sequencing of the amplicons obtained from samples looking at homology to the *bcspl31* gene (laboratory of G.T.). Milk from 100 urban goats in Quito, representing a convenience sample of goats living in the city, resulted in nine samples that were PCR-positive for *Brucella* spp. Serum (101 samples) and inguinal lymph node (100 samples) collected by trained personnel (laboratory of G.T.) at the main slaughterhouse for goats in Quito resulted in 18 and 8 positive results from Rose Bengal card test and PCR assay, respectively (Table 2). No two samples were taken from the same goat. The card test is reported to be 80.2% sensitive and 99.6% specific for goats, and prevalence calculations for goat samples were adjusted accordingly with the Rogan–Gladen correction method, resulting in 17.8% (95% CI = 6.2–44.2%) seropositivity.^{13,16}

True prevalence calculation is important for future data collection and continued disease monitoring, because it allows for informed sample sizes to detect seropositive animals that includes the concept of uncertainty around the point estimates of the prevalences.¹⁷ True prevalence, based on herd size, ranged from 0% to 12.5%. Overall true prevalence was 7.2%, which allows for significantly smaller sample sizes within a herd to detect positive animals compared with sampling the entire herd for future testing.

Overall, vaccination status-specific and herd size-specific apparent prevalences were calculated by dividing the number of positive outcomes by the corresponding number tested (i.e., overall, specific vaccination status, and herd size) (Table 3). Vaccinated herds had higher apparent and true prevalence than unvaccinated herds, which is counterintuitive. There are several potential explanations for vaccine status not being identified as a significant predictor, but the most important explanations are likely the lack of permanent herd records and the lack of standardized methods for *Brucella* vaccination. Lack of permanent record and identification of vaccinated animals makes vaccine status of any herd a matter of speculation. This finding is especially true, because there is no regulation of animal sale or movement within Ecuador. The herds with indicated vaccinated cattle were only vaccinated sporadically when a *Brucella* vaccine was available, but there is not a consistent supply of vaccine in the region. Also, vaccines are never administered by trained personnel, and therefore, quality control from appropriate handling of the vaccine that is a biological product subject to spoilage cannot be guaranteed. We commonly identified poor vaccine handling as a potential source for vaccine failure during consultation with owners about herd testing results. Many owners who invested

TABLE 2
Brucellosis in urban goats in Quito, Ecuador

Animals sampled	Sample	Method of testing	N	No. positive (% positive)
Goats at slaughterhouse	Inguinal lymph node	PCR	100	8 (8)
Goats at slaughterhouse	Serum	Rose Bengal card test	101	18 (17.8; 95% CI = 6.2–44.2%)
Urban milking goats	Milk	PCR	100	9 (9)

No two samples were taken from the same goat (total N = 301).

TABLE 3

Seroprevalence of *Brucella* sp. (*B. abortus* or *B. Melitensis*) in dairy cattle in northern Ecuador by vaccination status

Vaccine status	Cattle tested	No. positive	Apparent prevalence % (95% CI)	True prevalence % (95% CI)
Vaccinated	727	46	6.3 (4.8–8.3)	8.3 (6.0–11.1)
Unvaccinated	1,383	31	2.2 (1.6–3.2)	2.6 (1.6–3.8)
Unknown	451	65	14.4 (11.5–18.0)	19.5 (15.2–24.4)
Total	2,561	142	5.5 (4.7–6.5)	7.2 (6.0–8.5)

time and financial resources into *Brucella* vaccines were obviously disappointed to find that vaccinated herds had a higher prevalence of seropositive animals compared with unvaccinated herds. Our data show that current vaccine programs are inadequate, and we suggest that there is a significant opportunity for improvement of disease control with little additional costs. Client education and working with a veterinarian can improve vaccine efficacy with better vaccine handling, identification of vaccinates, and strategic selection of animals to receive vaccine. Client education should also include the indication for use of vaccine in the herd. Many animal owners start vaccination without consultation with a veterinarian for several reasons, such as perceived need or peer use. However, because of the low availability of diagnostic testing, lack of trust by animal owners and veterinarians in diagnostic laboratories, and sparse availability of veterinary expertise in rural areas, it is difficult for animal owners to make informed decisions to invest in brucellosis vaccination.

Overall, our brucellosis prevalence findings for cattle are similar to those findings reported by the Ecuadorian National Veterinary Service and Agrocalidad.⁷ However, some herds had extremely high disease prevalence, which was consistent with data published in a graduate thesis project.⁹ The finding of very little or no prevalence in rural communities (where animals are managed individually) or small groups (less than five animals) is consistent with the pathophysiology of the bacteria, which requires direct contact (more common in herds) for transmission. There are limitations on the small sample size of cattle managed individually or in small groups, and therefore, we cannot rule out seropositivity in rural communities. However, this finding is novel for brucellosis disease ecology in Ecuador, because to the authors' knowledge, all available testing data are solely from dairy herds.

Larger and pilot-informed sample sizes may yield more informative and representative future results concerning predictors of prevalence, including location and seasonality, and it is clear that improvements in methods for brucellosis vaccination and data recording are needed. With ongoing prevalence monitoring, multiple previously described brucellosis vaccine strategies, using either Strain 19 or RB51 modified live vaccines, could be implemented and tested for effectiveness in this particular region, because a single approach will likely fail in a country as ecologically diverse as Ecuador.^{18,19} In conclusion, our findings can aid in continued efforts for brucellosis control in the northern highlands of Ecuador by identification of goats living in urban areas as potential sources of zoonosis for brucellosis. Limited resources for disease control may be targeted for higher prevalence in larger commercial dairy herds with appropriate vaccine protocol training as prescribed by the World Organization for Animal Health and the Ecuadorian Ministry of Agriculture.

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