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Short communication

Seroprevalence of brucellosis in livestock within three endemic regions of the country of Georgia

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ABSTRACT

Brucellosis is the one of most common livestock zoonoses in Georgia, resulting in significant economic losses. Livestock were sampled in three regions of Georgia (Kakheti, Kvemo Kartli, Imereti). Districts that historically reported high numbers of brucellosis related morbidity were selected for serological, bacteriological and molecular surveys. Surveying efforts yielded samples from 10,819 large and small ruminants. In total, 735 serological tests were positive on Rose Bengal and 33 bacterial isolates were recovered and identified as *Brucella melitensis* or *Brucella abortus* by microbiology and AMOS-PCR. A Bayesian framework was implemented to estimate the true prevalence of the disease given an imperfect diagnostic test. Regional posterior median true prevalence estimates ranged from 2.7% (95% CI: 1.4, 7.2) in Kvemo Kartli, 0.8% (95% CI: 0.0, 3.6) in Kakheti, to an estimate of 0.6% (95% CI: 0.0, 2.9) in Imereti. Accurate and efficient surveillance of brucellosis is not only of economic value, but also informs efforts to reduce the disease impact on the human population.
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1. Introduction

Brucellosis is a serious infection in livestock globally despite efforts to mitigate its presence (Seleem et al., 2010). Independent nations of the former Soviet Union have been disproportionately burdened by some of the highest global rates of the disease in both livestock and humans (Pappas et al., 2006). Decreased funding for veterinary health surveillance brought on by the collapse of the Soviet Union have contributed to an already problematic situation in endemic areas and have likely contributed to a largely unknown disease status among livestock in several nations of Central Asia and the Caucasus region.

In Georgia, brucellosis is one of the most common bacterial zoonoses of livestock, causing significant economic losses. Multiple species of the genus *Brucella* contribute to chronic and acute health complications in both animal and human populations across the country. In livestock, complications from brucellosis range from infertility and low milk output to increased calf mortality (Renukaradhya et al., 2002). Recent studies have utilized Bayesian

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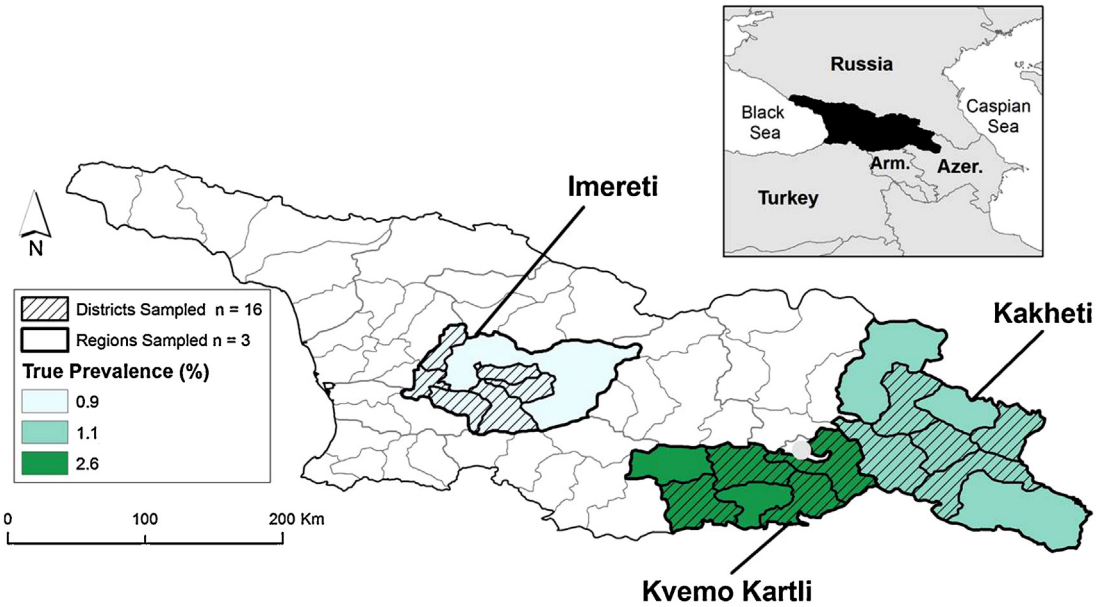

Fig. 1. Study area for the survey in ruminants depicting areas in Georgia that were sampled districts ($n = 16$) by cross-hatching, and the regions that were sampled ($n = 3$) outlined in black. Colored regions show the posterior mean estimates of the true prevalence of ruminant brucellosis as a percent for each of the regions sampled. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

methods to help elucidate the infection status of livestock in the face of an imperfect diagnostic test (Branscum et al., 2004). Bayesian analyses can be used to estimate the true prevalence (*tp*) of disease, which may differ from the ratio of test positives to test negatives or apparent prevalence (*ap*) (Pozzato et al., 2011), by incorporating prior information on the sensitivity (*se*) and specificity (*sp*) of the diagnostic test.

The primary goals of this study were to: (1) enhance the capacity and knowledge of veterinary and public health management by sampling, testing, and identifying areas of high disease potential; and (2) estimate the true prevalence of livestock brucellosis in three endemic regions of Georgia using Bayesian techniques.

2. Methods

2.1. Study population

A team from the Laboratory of the Ministry of Agriculture (LMA) collected samples during each of two seasons (spring, fall) from 2008 to 2011 using a random sampling approach within districts and regions to establish estimates of livestock brucellosis. Three regions historically designated as areas of concern for ruminant brucellosis, Kakheti, Kvemo Kartli and Imereti were selected (Fig. 1). Locations were randomly sampled within districts, however in some instances access to animals and farms was difficult or restricted resulting in small sample sizes in some areas. In each of the regions blood and milk samples were taken from large ruminants (cattle/bovines) and small ruminants (sheep/ovines and goats/caprines). Additional information regarding the gender and age of the animals was also recorded for a subset of the sampled animals.

2.2. Sample analysis

The Rose Bengal Test (RBT) was used to detect antibodies against *Brucella* spp. (OIE, 2004) in the collected sera. Seropositive blood and available milk samples were cultured in vitro using *Brucella* selective medium (Oxoid), and the pathogen isolated. A basic bacteriological algorithm was used to identify the genus and determine species (Alton et al., 1988). Genus identity was confirmed by extracting DNA from pure isolate cultures and testing with the Idaho Technology Inc. real-time PCR *Brucella* species assay (Brucellosis Target 1) using a LightCycler instrument (Roche). The 5-primer AMOS PCR assay (Bricker and Halling, 1994), which differentiates species-specific IS711 insertions, was used to confirm the species of the isolates as determined by the microbiological tests.

2.3. Statistical analysis

Prevalence estimates for *ap* and *tp* based on blood samples were calculated using a Bayesian framework to account for the se and sp of the RBT. Models were run using the WinBugs software version 3.1 with 100,000 iterations after a burn-in of 20,000 iterations were discarded. Formulation of the model for *tp* estimates given *ap* was based on binomial sampling of 1 test, 1 population (Branscum et al., 2004) with code obtained from the website (http://www.epi.ucdavis.edu/diagnostictests/module03.html). Prior beta estimates for *se* and *sp* were derived using the BetaBuster software (http://www.epi.ucdavis.edu/diagnostictests/betabuster.html) with a mode for $se = 0.75$ (95% confidence interval: >0.60) and $sp = 0.85$ (95% confidence interval: >0.75). Posterior median

Table 1
Brucella abortus and *Brucella melitensis* recovery rates from cattle, sheep, and goat blood and milk. CI = 95% binomial exact confidence intervals.

Livestock group	*Brucella* spp.	Bacteria recovery rate (recovery/sampled)	
		Blood[a]	Milk
Cattle	*Brucella abortus*	0.828 (4/483; CI: 0.23–2.11)	5.99 (13/217; CI: 3.23–10.03)
Sheep		0.476 (1/210; CI: 0.01–2.62)	5.405 (2/37; CI: 0.66–18.19)
Goat		0 (0/42; CI: 0–8.4)	0 (0/17; CI: 0–19.5)
Cattle	*Brucella melitensis*	0 (0/483; CI: 0–0.76)	2.304 (5/217; CI: 0.75–5.29)
Sheep		0.476 (1/210; CI: 0.012–2.62)	13.514 (5/37; CI: 4.54–28.77)
Goat		2.38 (1/42; CI: 0.060–12.57)	0 (0/17; CI: 0–19.51)

[a] Samples sizes in denominator reflect seropositive samples across all regions by livestock type presented in Table 2.

estimates of *ap* and *tp* were reported with 95% credible intervals (CI). Bacteriology recovery rates were calculated by dividing the number of isolates recovered by livestock group and bacterial species for both blood and milk samples. Exact binomial confidence intervals were calculated using the epitools package in R (http://medepi.com/). The Mann–Whitney *U*-test was used to evaluate whether or not there was a statistically significant difference between the age of test positives and test negatives using R.

3. Results

A total of 33 bacterial isolates were recovered from seropositive blood ($n = 735$) or milk ($n = 271$) and identified as *Brucella melitensis* ($n = 12$) or *Brucella abortus* ($n = 21$) using microbiology and AMOS PCR. Bacterial recovery rates and sources are summarized in Table 1. All bacteria were recovered from blood and milk in cattle, sheep and goats with the exception of a single *B. abortus* isolate recovered from fetal tissue from an aborted bovine calf. AMOS PCR was successful for *B. melitensis* isolates, but not for *B. abortus* isolates recovered.

Total seropositive blood samples are presented by region in Table 2. Regional *ap* estimates ranged from 5.1% to 10.4%. Bayesian *tp* estimates showed little variation between the three sampled regions (Table 2). The *tp* estimate was highest in Kvemo Kartli 2.7% (95% CI: 1.4, 7.2) and lowest in Imereti 0.6% (95% CI: 0.0, 2.9). Overlapping credible intervals of *tp* estimates indicated no significant difference in seroprevalence between the three regions.

3.1. Age and gender of ruminant seropositives

Sampled cattle on average were older than sheep and goats with females representing a greater number of surveyed livestock. Results from the Mann–Whitney *U*-test ($W = 14438$, $p < 0.001$) indicated that the mean age of test positive animals 4.0 years (95% CI: 3.8, 4.2) was significantly greater than that of test negative animals 3.0 years (95% CI: 2.9, 3.1).

4. Discussion

This study analyzed brucellosis seroprevalence in small and large ruminants from three regions in the country of Georgia using a Bayesian framework to estimate the true prevalence of disease given an imperfect diagnostic test. Additionally, we employed an algorithm to recover bacteria from blood and milk samples collected during animal sampling. The overall presence of brucellosis in these areas of the country have most likely been impacted by the dramatic governmental upheaval and transition since the disintegration of the Soviet Union, which has included cuts in funding for public and veterinary health control programs, as well as a shift towards the privatization of animal ownership (Pappas et al., 2006). *B. abortus* represented 63.6% of the bacteria recovered from livestock, while *B. melitensis* represented 36.4% of recoveries. Recovery rates were higher from milk samples than blood samples for both cattle and sheep (Table 1). AMOS PCR was useful for the *B. melitensis* isolates but not for the *B. abortus* strains recovered in this study. Bricker and Halling (1994) stated that several *Brucella* biovars cannot be detected using the AMOS assay and that from *B. abortus* only biovars 1, 2 and 4 can be identified. It thus appears that the veterinary isolates recovered identified as *B. abortus* by microbiological tests

Table 2
Regional numbers of cattle, sheep, and goats tested for brucellosis with Rose Bengal tests in the country of Georgia. Total livestock population estimates are provided for each region by livestock group for comparison. Apparent and true prevalence estimates were derived from a WinBugs simulation.

Region	Cattle (−/+)[a]		Sheep (−/+)		Goat (−/+)		Total Sampled	Total Positive	Population (thousands) [C, S, G][e]	AP[b] (%)	TP[c] (%) (95% CI)[d]
Kvemo Kartli	1517	137	1192	161	124	23	3154	321	153, 202, 18	10.4	2.7 (1.4, 7.2)
Kakheti	2019	207	1914	49	418	14	4621	270	119, 250, 14	6.7	0.8 (0.0, 3.6)
Imereti	1654	139	507	0	739	5	3044	144	268, 26, 14	5.1	0.6 (0.0, 2.9)

[a] Total number of negative (−) and positive (+) test results.
[b] Posterior median of the apparent prevalence derived from a WinBugs.
[c] Posterior median of true the prevalence derived from a WinBugs.
[d] Credible intervals derived from Winbugs.
[e] Total population of livestock: C = cattle, S = sheep, and G = goats.

in this study do not fall within the biovars of *B. abortus* that can be detected and typed using this assay.

Seroprevalence estimations in these regions may be partly attributed to uncontrolled livestock movements both to and from seasonal pastures as well as trans-boundary movements between neighboring endemic countries (Taleski et al., 2002). Intermixing of animals and sharing of pasture lands may be a contributing factor to the disease status. In the Kakheti region of Georgia it was recently suggested that that the intermixing of livestock was a common practice (Havas et al., 2012). The three regions surveyed in this study represent a large proportion (~45%) of the bovine, caprine and ovine milk production as well as representing ~45% the total population of livestock in Georgia (GeoStat, 2011). The large scale agriculture practices in these regions taken in conjunction with the new seroprevalence estimates obtained in this study suggest that these areas may also represent a focus of human brucellosis. Akhvlediani et al. (2010) identified regions that reported a high prevalence of human disease that correspond in part to the regions of livestock disease identified in this study. The age and gender distribution of seropositive animals was in line with expectations related to the sampling design of the study and the natural history of the livestock types. Females were shown to comprise a larger portion of the livestock surveyed, which was a design of the study since *Brucella* spp. can be shed in the milk. Additionally, the age distribution of livestock sampled may have been skewed since bovids live longer and sexually mature later than other species sampled. Differences in the proportion of animals sampled was also a byproduct of the availability and access to animals at a given location.

The *tp* estimates presented here may represent actual districts with an increased presence of the disease or they could be an artifact of the sampling effort. Every effort was made to sample adequately among locations; however, in some areas access to animals was an issue, which prohibited the estimation of individual and herd level prevalence estimates. Future studies may incorporate risk factors that are associated with the presence of brucellosis in livestock in order to better assess spatial differences in the level of possible exposures.

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