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Original Contribution

Representative Seroprevalences of Brucellosis in Humans and Livestock in Kyrgyzstan

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Abstract: Kyrgyzstan reported 77.5 new human brucellosis cases per 100,000 people in 2007, which is one of the highest incidences worldwide. In Kyrgyzstan, the currently used diagnostic tests in humans and animals are the Rose Bengal Test and the Huddleson test. A national representative cross-sectional study using cluster sampling proportional to size in humans, cattle, sheep, and goats was undertaken to assess the apparent seroprevalence in humans and animals. A total of 4,936 livestock sera and 1,774 human sera were tested in Naryn, Chuy, and Osh Oblasts. The overall apparent seroprevalences of brucellosis were 8.8% in humans (95% CI 4.5–16.5), 2.8% (95% CI 1.6–4.9%) in cattle, 3.3% (95% CI 1.5–6.9%) in sheep, and 2.5% (95% CI 1.4–4.5%) in goats. Naryn Oblast had the highest seroprevalences in humans and sheep. More men than women were seropositive (OR = 1.96; $P < 0.001$). Human seroprevalence was significantly associated with small ruminant seroprevalence but not with cattle seroprevalence. Annual incidence of human brucellosis exposure, measured by serological tests, was more than ten times higher than the annual incidence of reported clinical brucellosis cases. This indicates an under-reporting of human brucellosis cases, even if only a fraction of seropositive people have clinical symptoms. In conclusion, this study confirms the high seroprevalence of brucellosis in Kyrgyzstan and warrants rapid effective intervention, among others, by mass vaccination of sheep and goats but also of cattle.

Keywords: apparent prevalence, incidence, brucellosis, human, livestock, serology, Kyrgyzstan

INTRODUCTION

Brucellosis is a bacterial disease of livestock with a high zoonotic potential. Its transmission from livestock to humans occurs mainly by consumption of raw dairy products and by direct contact during delivery and abortion. *Brucella*

abortus is mainly found in cattle and *B. melitensis* and *B. ovis* in goats and sheep. Humans are susceptible to both *B. abortus* and *B. melitensis*, the latter being most frequently reported in humans (Corbel 2006).

Brucellosis occurs worldwide, particularly in developing and transition countries, but it is well controlled in industrialised countries. Kyrgyzstan has one of the highest brucellosis incidences worldwide with 36 reported annual

human cases per 100,000 people in 2002 (Pappas et al. 2006), and 77.5 per 100,000 people in 2007 (promedmail.org, Archive Number 20090201.0449, published Feb 1, 2009).

However, it is not known if the Kyrgyz health system coverage is exhaustive and if all patients have access to care. It is likely that the true incidence is underestimated. An earlier study by Kozukeev et al. (2006) indicated the importance of cattle for the transmission of brucellosis in Kyrgyzstan, but the role of small ruminants for the transmission of brucellosis in humans is not clear. Brucellosis can be diagnosed by isolation (culture) of the bacteria, direct PCR of *Brucella* spp. genome in contaminated specimens or indirectly by antibody detection either in serum or milk (Corbel 2006). The culturing of *Brucella* spp. from animal samples is complex and dangerous and requires biosafety level 3, which is not currently available in Kyrgyzstan. Hence, we rely on available and more recent serological methods for diagnosis. The objective of this study was to assess representative brucellosis seroprevalences of livestock and humans for Kyrgyzstan and the association between human and livestock brucellosis seroprevalence. Representative estimates should be related to official reports and inform Kyrgyz public health and veterinary policy. The test characteristics of the Huddleson test in humans and Rose Bengal Test in livestock, which are currently used in Kyrgyzstan, are not known and there is no gold standard.

MATERIALS AND METHODS

Partnership Between Public Health and the Veterinary Sector

In the Fall of 2006, an integrated assessment of human and livestock brucellosis seroprevalence was undertaken jointly by the Republican State Center for Veterinary Diagnostics, the Republican Centre for Quarantine and especially dangerous diseases, the State Sanitary Epidemiological Department of the Kyrgyz Republic and the Swiss Tropical and Public Health Institute. Complementary Support of Community Action for Health facilitated this partnership (Zinsstag et al. 2009).

Study Design

For this survey, the national census data on sheep and goat populations was used. A multistage cluster sampling proportional to size was determined by levels of Oblast (province), Rayon (district), and village (Bennett et al. 1991;

Schelling et al. 2003). Three out of seven Oblasts were sampled. Naryn Oblast was selected by convenience (availability of previous serological studies), and the two others were sampled randomly in proportion to their size. In every Oblast, three Rayons, and in every Rayon, ten villages, were selected randomly in proportion to their size as shown in Fig. 1 (Bennett et al. 1991). In this way, a total of 90 villages were selected randomly and used as cluster units. We assumed an intraclass correlation roh of 0.2 between clusters and a design effect of 4.8. Sampling of 20 humans or livestock in every cluster provided a total sample size of 1,800 per species and 95% confidence limits of the estimate of $<3\%$ below and above the estimated seroprevalence, which is representative for the whole country.

This study was approved by the Ethical committee of the Cantons of Basel and the Kyrgyz Health Authorities. Informed written consent was provided by all persons participating in the study or of young children's mothers, after they had received detailed patient information. Overall, the proportion of sheep to goats was estimated at 6:1. Due to the lack of more detailed information, we assumed that this proportion is true for all Rayons. In the year of study (2006) very few animals were vaccinated against brucellosis in Kyrgyzstan and their influence on the serological results of this study were considered negligible.

Human and Livestock Sample Collection

The study was conducted by three field teams composed of one veterinarian and one physician in the spring of 2006. A total of 103 villages in the nine selected Rayons were visited. Venous blood was taken with 5 ml Vacutainer® tubes and the age, sex, and names were recorded for all participants. Blood of livestock (cattle, sheep, and goats) was obtained by venipuncture with 10 ml Vacutainer® tubes. Livestock and human samples were not necessarily collected from the same households as the participation was voluntary. Human blood was transported to the Rayon Health Center and animal blood was transported to the Veterinary laboratory and centrifuged. All sera were shipped to Bishkek either to the Centre for Quarantine for testing of human sera with the Huddleson agglutination test or to the Central Veterinary Laboratory in Bishkek for other tests described below.

Diagnostic Tests

In Kyrgyzstan, the most common test is the Huddleson agglutination test for humans (official test) and the Rose
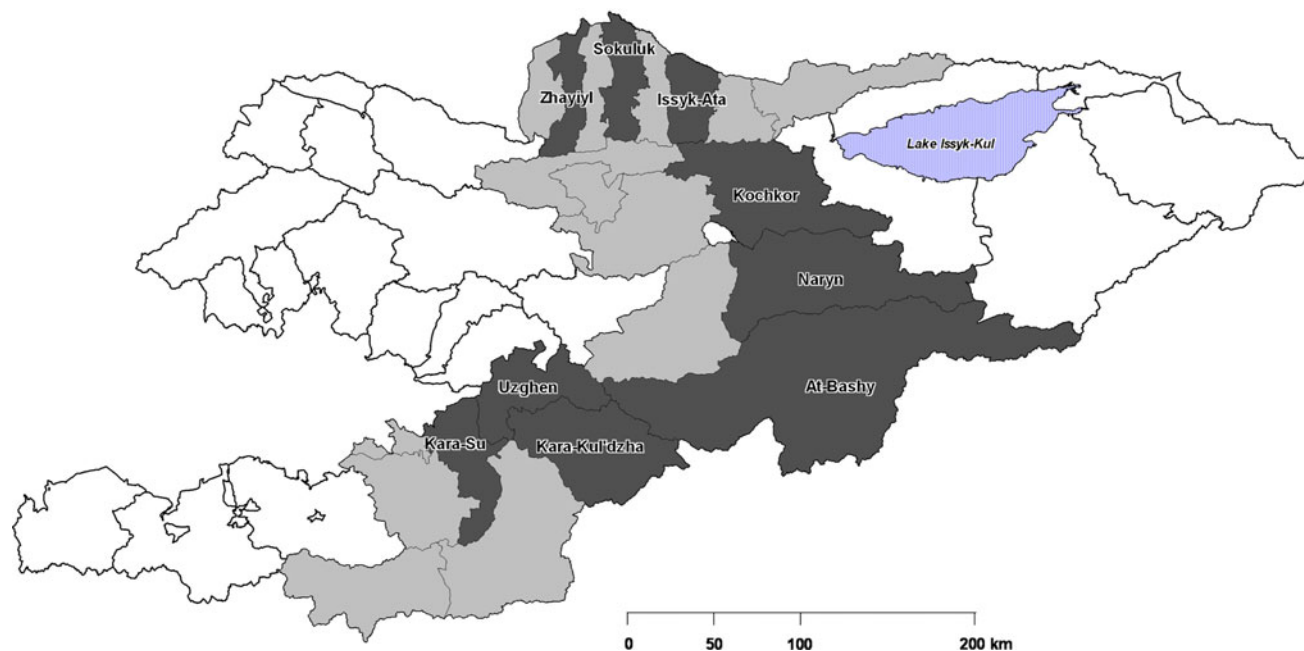
Figure 1. Map of Kyrgyzstan with selected Oblasts and Rayons. *Light gray* Rayons belong to selected Oblasts but not selected Rayons, *dark gray* rayon are the nine in this study selected Rayons (*oblast* = region and *rayon* = district).

Bengal Test (RBT) for animals. Human sera were subjected to the Huddleson test, the RBT (Bio-Rad Laboratories®), and an IgG and IgM ELISA (Chekit® IDEXX Laboratories Inc.) with anti-human-goat IgG and anti-human-goat IgM conjugates (Sigma-Aldrich Co®). The latter IgM test was only done for sera that were Huddleson test or RBT positive and IgG ELISA negative. Classification of the ELISA was then positive if either the IgG ELISA and/or the IgM ELISA were positive. Livestock sera was tested with a RBT from the Kherson Bio-Factory, Ukraine, an indirect ELISA for ruminants (Checkit® IDEXX Laboratories Inc.) and the Fluorescence polarization assay (FPA) (Brucella FPA®, Diachemix, LLC). Tubes of 2 ml of sera were kept for further testing and parallel tested in the Rayon veterinary laboratory with the RBT.

Serological test results were interpreted according to the manufacturers' recommendations. Cut-off values were determined by a titration curve analogous to Bonfoh and Steinmann (Bonfoh et al. 2002; Steinmann et al. 2005). With the exception of the FPA, all values were recorded as negative, doubtful, and positive. The cut-off value of the FPA was at 90 mP. Since, agreements of pair wise comparison of tests (Kappa statistics) within species were generally better when all doubtful results were classified negative than as positives, for binary classification of results, doubtful sera were classified as negative for all tests, although the best agreement between two serological tests

was only a moderate agreement (Kappa < 0.6). For livestock sera, agreements between tests were better for cattle than for sheep and goats. For further statistical analyses, we used the Huddleson agglutination test for human sera and the ELISA results for livestock sera.

Data Analysis

Serological results were converted into dichotomous outcomes (1 = seropositive, 0 = seronegative), depending on the cut-off value of each test. Logistic regression, modeling for the outcome of seropositive humans and livestock (SP) included random effects (re) at various levels as follows: (1) at the level of rayon for the national representative estimate and for Oblast level, SP $\sim$ re(Rayon) (Table 2). (2) For the analysis of human sera the level of village was used as random effect. Univariable models related SP individually with sex, age, and Oblast (Table 3). (3) Assessing a possible relationship between human and livestock seropositivity we have regressed SP $\sim$ proportions of human and livestock seropositivity at rayon level (Table 4).

Analyses of Human Sera

Participants with the same family name within a village were regrouped in a unique family code. Age in years was categorized in steps of 10 years (0–10 years up to

80–90 years) and in three categories that represent the life stage: 0–18 years; 19–45 years; and ≥46 years. A unique code was created for all villages. We have tested if age, sex, and Oblast were associated with seropositivity in humans using a logistic regression model (with a random effect at the village level) based on the likelihood-ratio test (LRT) (xtlogit procedure in STATA 10). The variances of the Huddleson test (humans) and ELISA (livestock) were highest at the Rayon level (district) when compared to the levels of family, village, and Oblast (province). For the logistic regression of human sera, we considered village as a random effect. Age, sex, and Oblast were tested as univariable variables. The LRT test was used to test the significance of a variable (Table 3). Apparent seroprevalences are presented, because the test characteristics were not known for the different species.

Correlation Between Human and Livestock Seropositivity

Regressions have been done with SP ~ proportions of human and livestock seropositivity (all livestock, small ruminants [sheep and goats together] and the single species cattle, sheep, and goats) at rayon level. Not more than one livestock category was tested in a model due to strong correlation between sheep and goat seropositivity on Rayon level. The confidence intervals were constructed using a bootstrap re-sampling technique and the information on total number of individuals tested per rayon.

Estimation of Incidence of Apparent Brucellosis Seropositivity in Humans

The age-specific apparent seroprevalence of the Huddleson tests was used to estimate the incidence of brucellosis

seropositivity of a catalytic two way model under equilibrium conditions (Muench 1959). We used data between the 2nd and 8th decade of life because data on younger or older patients were too sparse.

$$dS/dt = -aS + bI \tag{1}$$

$$dI/dt = aS - bI, \tag{2}$$

where S is the susceptible population and I is the seropositive population. The parameter a is the incidence of seroconversion and b the rate of loss of sero-positivity. Under equilibrium conditions the apparent seroprevalence P is related to a and b (Eq. 3).

$$P = a/(a + b). \tag{3}$$

We estimated the seroconversion rate a and the loss rate b simultaneously from the data using Vensim (Ventana Inc.) software, using a Powell algorithm analogous to (Zinsstag et al. 2005).

Results

A total of 4,936 different livestock sera (1659, 1642, 1635 from Naryn, Chuy, and Osh Oblast, respectively) and a total of 1,774 human sera (564, 606 and 604 from Naryn, Chuy, and Osh Oblast, respectively) were tested with at least one serological test (Table 1).

Representative Apparent Seroprevalences and Human Incidences

In Table 2, human and livestock seroprevalences are presented for every Oblast. The highest human brucellosis seroprevalence is found in Naryn Oblast and the lowest in

Table 1. Total sample size by species and number of samples examined with different diagnostic tests

Species	Total	RBT (Biorad)[a]	RBT (Ukraine)[b]	ELISA (ruminant)[c]	ELISA IgG (human)[d]	ELISA IgM (human)[d]	FPA[e]	Huddleson[f]
Cattle	1,813	737	1,560	1,698	0	0	1,691	0
Sheep	2,076	761	1,855	2,029	0	0	2,029	0
Goats	1,286	764	1,082	1,209	0	0	1,176	0
Humans	1,775	644	0	0	1,762	369	0	1,774

[a]Rose–Bengal Biorad.

[b]Rose–Bengal Ukraine.

[c]Indirect enzyme-linked immunosorbent assay detecting IgG in ruminants.

[d]Indirect enzyme-linked immunosorbent assay detecting IgG and IgM, respectively, in humans.

[e]Fluorescence polarization assay.

[f]Huddleson test.

Table 2. Apparent seroprevalence estimates of brucellosis in Kyrgyzstan for humans, cattle, sheep, and goats per oblast, 2006

Oblast species	n	Seroprevalence[a]	95% CI
Naryn Oblast			
Humans	564	18.3	14.0–23.7
Cattle	536	2.2	0.8–6.0
Sheep	562	8.9	5.8–13.5
Goats	561	2.5	0.9–7.0
Chuy Oblast			
Humans	606	8.9	6.9–11.5
Cattle	598	5.7	4.1–7.9
Sheep	610	3.0	0.0–9.7
Goats	434	2.7	1.3–5.7
Osh Oblast			
Humans	604	2.2	0.1–27.6
Cattle	564	1.6	0.5–4.5
Sheep	857	1.3	0.4–3.9
Goats	214	2.8	1.3–6.1

[a]Seroprevalences (and 95% CI) calculated with a logistic regression model specifying Rayon as a random effect.

Osh Oblast. Using Rayon as a random effect, the overall representative apparent seroprevalences of brucellosis for Kyrgyzstan were 8.8% in humans (95% CI 4.5–16.5), 2.8% (95% CI 1.6–4.9%) in cattle, 3.3% (95% CI 1.5–6.9%) in sheep, and 2.5% (95% CI 1.4–4.5%) in goats. The average duration of brucellosis seropositivity ($1/b$) was estimated at 10.9 years. Keeping this constant, human incidence of apparent sero-conversion is estimated at 0.88% (95% CI 0.43–1.77%) per year for the Huddleson test. This means that on average 880 (95% CI 400–1,770) persons per 100,000 are exposed to brucellosis per year. Extrapolated to the total Kyrgyz population of 5.2 millions on average 45,882 persons per year get exposed to brucellosis.

Analysis of Human Sera for Risk Factors of Brucellosis Seropositivity

Additional data on age, sex, and village was available for 1,761 people. The proportion of sampled female participants varied between 0.42 and 0.78 in the 9 Rayons and was 0.57 over the whole sample. The median age was 39 years (5–95% percentiles 17–66). More male participants were seropositive compared to females (in all three age classes) (OR = 1.96; p(LRT) < 0.001) (Table 3).

Table 3. Risk factors of human seropositivity determined with the Huddleson test as outcome variable

Variable	Huddleson test		Univariable logistic regression model	
	n	Pos%	OR	p(LRT)
Sex				
Female	1,011	8.4	1	<0.001
Male	750	14.9	1.96 (1.4–2.7)	
Age category				
0–18	128	14.1	1	0.60
19–45	1,049	11.7	1.2 (0.6–2.3)	
>45	584	9.6	1.03 (0.5–2)	
Oblast				
Naryn	560	18.4	1	<0.001
Chuy	600	8.3	0.4 (0.2–0.7)	
Osh	601	7.3	0.2 (0.1–0.5)	

The uni- and multivariable models used a random effect on village level. P values of the log LRT are presented.

Table 4. Regression coefficients for human seropositivity and livestock seropositivity

	Intercept (95% CI)	Slope (95% CI)
All livestock species	0.09 (0.05–0.12)	0.81 (−0.1 to 1.7)
Small ruminants	0.08 (0.05–0.11)	0.97 (0.3 to 1.7)
Cattle	0.15 (0.11–0.18)	−0.88 (−1.8 to 0.2)
Sheep	0.07 (0.05–0.10)	0.83 (0.3 to 1.4)
Goats	0.09 (0.07–0.12)	0.99 (−0.06 to 2.1)

Correlation Between Human and Livestock Seropositivity

On the Rayon level, both pooled small ruminant and sheep seroprevalences were correlated positively with human seroprevalences: an increase of 1% of small ruminant and sheep seroprevalences, increased human seroprevalence by 0.97 and 0.83%, respectively (Table 4).

DISCUSSION

This study was designed as a representative cross-sectional assessment of brucellosis prevalence in humans and livestock in Kyrgyzstan in 2006. Because of the highly endemic epidemiological situation, the true incidence of clinical brucellosis cannot be stated. The estimated incidence of

apparent seropositivity using the Huddleson test of 880 (95% CI 400–1,770) per 100,000 is 11 times higher than the officially reported number of brucellosis cases. There is no data available in the literature indicating the proportion of seroconversion that leads to clinically manifested brucellosis in endemic areas. If 10% of the people that seroconverted showed clinical symptoms, the incidence of clinical brucellosis would be in agreement with the reported data. However, if 50% of the people that seroconverted showed clinical symptoms, the level of under-reporting would be 5.6 (95% CI 2.5–11.4). Studies in Saudi Arabia indicate a high proportion of clinical illness among seropositive family members of acute brucellosis cases (Almuneef et al. 2004; Alsubaie et al. 2005). Further studies are needed to assess the true incidence of human cases. The estimated duration of seropositivity of 10.9 years is in agreement with Beklemishev in Kazakhstan (cited by v.Oldershausen 1968), but we were not able to estimate a confidence limit, without time series data.

In our study, the apparent seroprevalences between the Oblasts ranged from 2.2 to 18.3% in humans and between 1.3 and 8.9% in livestock species with the highest variance for both on the Rayon level. Although, the Oblast seroprevalences of the three livestock species were comparable, we have seen a significant correlation between human and small ruminant seropositivity on the Rayon level, thus suggesting Rayon specific clustering. This likely reflects the relatively localized contact networks and marketing system of Kyrgyz livestock. A sheep–cattle relationship has been confirmed by the detection of a *B. melitensis* strains in sheep and in two cattle (data not shown). In humans, male participants were more frequently seropositive than female participants but no difference between age classes was detected. More data is needed to understand the frequency of brucellosis in children, where transmission may be due to both direct exposure to contaminated livestock material and consumption of raw livestock products. The higher risk of male seropositivity in human adults indicates that exposure in these rural villages may more likely be due to direct (professional) close contact with infected livestock, than by exposure through contaminated livestock products, but this hypothesis requires further investigation. In a study about risk factors of human brucellosis in Kyrgyzstan, Kozukeev et al. (2006) reported a higher, however both non significant, odds ratio (OR) for keeping cattle (adj. OR = 4.5) followed by goats (adj. OR = 1.6). However, we find no correlation between cattle and human seropositivity (indeed, we found a negative correlation) and we only

find a significant correlation with sheep, but not for goats. Such contradictory results, also due to different study designs, reflect the difficulty in determining an association of infection between humans and animals for zoonotic diseases. Confirmation of transmission chains by molecular analysis of strains isolated from humans and different livestock species is warranted.

This study was intended to inform Kyrgyz policy on brucellosis epidemiology by providing representative data using existing and new diagnostic tests. As it is important to adapt assessments to local health policy decision pathways (Habicht et al. 1999), our analysis of human apparent seroprevalence is based on the Huddleson agglutination test, which is the officially recognized diagnostic test in Kyrgyzstan.

In a complementary analysis, we assess the test characteristics of all used tests using a Bayesian model for the estimation of true seroprevalence in the absence of a gold standard, which are published separately. The results will be further used to estimate the cost of brucellosis to the Kyrgyz economy analogous to Roth et al. (2003).

The presented study is an example of an integrated human and animal study design under a "One Health" paradigm, facilitating identification of the source of a zoonosis in the animal reservoir in a single step because of a connected study design and the ability to assess the impact of the disease in multiple sectors, notably the health and livestock production sectors. It is thus an example of the added value of a closer cooperation between human and animal health practitioners (Zinsstag et al. 2009).

Conclusion

Our study confirms the high seroprevalence of brucellosis in Kyrgyzstan and suggests that the annual incidence of human brucellosis exposure, measured by serological tests, is more than ten times higher than the annual incidence of reported clinical brucellosis cases. This indicates an underreporting of human brucellosis cases even if only a fraction of the seropositive persons have clinical symptoms. Human brucellosis seroprevalence was most closely associated with brucellosis seroprevalence in sheep. Effective mass vaccination of sheep, goats, and cattle, following the guidelines by the World Animal Health Organization (OIE) are warranted to control human brucellosis at its source. Further research is needed to further confirm the human–livestock linkages by molecular typing of brucella strains

from humans and livestock, to relate human Brucellosis seropositivity and clinical symptoms in a highly endemic area like Kyrgyzstan and to monitor control policies for their effectiveness.

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