Bayesian estimation of the seroprevalence of brucellosis in humans and livestock in Kyrgyzstan

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Summary

Kyrgyzstan reported 77.5 new cases of human brucellosis per 100,000 inhabitants in 2007, which is one of the highest incidences in the world. However, because this number is based on official records, it is very likely that the incidence is underreported. The diagnostic tests most commonly used in Kyrgyzstan are the Rose Bengal test in ruminants and the Huddleson test in humans. The sensitivity and specificity of these tests have never been evaluated under field conditions in Kyrgyzstan, where the strains circulating in livestock and humans are unknown. Therefore, a representative national cross-sectional serological study was undertaken in humans, cattle, sheep and goats to assess the true seroprevalence and to compare different serological tests. In the year of study (2006), few animals were vaccinated against brucellosis in Kyrgyzstan. A total of 5,229 livestock sera and 1,777 human sera from three administrative regions were collected during spring 2006 and submitted to a range of serological tests. The true seroprevalence of brucellosis, estimated using Bayesian methodology, was 7% (95% credibility interval 4%–9%) in humans, 3% (1%–5%) in cattle, 12% (7%–23%) in sheep and 15% (7%–30%) in goats. The Rose Bengal test was confirmed as a useful screening test in livestock and humans, although its sensitivity was lower than that of other tests. The estimates of specificity of all tests were significantly higher than those for sensitivity. The high seroprevalence of brucellosis in humans, cattle and small ruminants in Kyrgyzstan was confirmed. Bayesian statistical approaches were demonstrated to be useful for simultaneously deriving test characteristics and true prevalence estimates in the absence of a gold standard.

Keywords

Animal – Bayesian model – Brucellosis – Human – Kyrgyzstan – Serology – True seroprevalence – Zoonosis.

Introduction

Brucellosis is a bacterial disease of livestock with a high zoonotic impact. Its transmission to humans occurs mainly by the consumption of raw dairy products and by direct contact with the skin or mucosa during parturition and abortion. Cattle (*Bos taurus*) are natural hosts for *Brucella abortus*, and sheep (*Ovis aries*) and goats (*Capra hircus*)

for *B. melitensis* and *B. ovis*. Humans are susceptible to both *B. abortus* and *B. melitensis*, the latter being most frequently reported in humans (1).

Brucellosis occurs worldwide, particularly in developing and transition countries, but it is well controlled in developed countries. Kyrgyzstan has one of the highest incidences of brucellosis in the world, with 36 reported human cases per

100,000 inhabitants in 2002 (2) and 77.5 per 100,000 in 2007 (promedmail.org, Archive Number 20090201.0449, published 1 Feb., 2009). However, it is not known whether the coverage of the Kyrgyz health reporting system is exhaustive and whether all patients have been recorded. It is very likely that the true incidence is underestimated.

Brucellosis can be diagnosed directly by polymerase chain reaction (PCR) or isolation (culture) of the bacteria, or indirectly by antibody detection either in serum or milk (1). The direct *Brucella* culture method proves the existence of the agent in a suspicious sample, but this method of diagnosis is complex and requires a biosafety level 3 laboratory, which is rarely available in the countries where brucellosis is endemic. Hence, serological methods are routinely used for diagnosis in these countries.

Several commercial serological tests are available for humans and animals (1, 3, 4). The Rose Bengal test (RBT) has been recommended as a suitable screening test at the national or local level for diagnosis of brucellosis in animals (1); enzyme-linked immunoassays (ELISA) and the fluorescent polarisation assay (FPA) have recently been added as prescribed tests. They are fairly simple, but robust, tests which can be conducted with a minimum of equipment and are therefore also suitable for smaller laboratories (1, 4). Further serological tests (e.g. the Coombs' test, the serum or plate agglutination test and the immunocapture test) are available, and have specific advantages and disadvantages (1, 3).

For diagnosis of brucellosis in humans, the RBT is recommended as a sensitive screening test, but decisions regarding clinical treatment may require additional confirmatory methods, and specifically strain culture (1). Information on the characteristics (sensitivity and specificity) of the serological tests differs significantly between publications, and this problem is increased if the tests are not applied according to international standards such as the World Organisation for Animal Health (OIE) recommendations (4). In Kyrgyzstan, the tests most commonly used are the so-called Huddleson test for humans and the RBT for animals. The Huddleson test is a plate agglutination test based on the RBT principle but with serial dilution of the sample: a result is defined as positive when agglutination is observed above a defined dilution (5). Although the Huddleson and RBT are both agglutination tests used for screening that do not involve quantitative serum dilution, the latter is performed at acidic pH and is not susceptible to the prozone effect (I. Moriyón, personal communication).

When a diagnostic test's sensitivity or specificity is below 100%, the distortion (bias) has to be accounted for when estimating the true seroprevalence. The Rogan–Gladen estimator is a possible approach that can be used under the condition that both sensitivity and specificity are known (6). This frequentist statistical approach has been applied in several studies (7, 8, 9). However, when the test characteristics are unknown or the expected seroprevalence is low (<5%) this estimator cannot be used. For situations where specific knowledge on test characteristics is lacking but some preliminary information is available, Bayesian methods have been proposed. Several studies have been published describing the use of Bayesian models to estimate prevalence or test characteristics in veterinary medicine (7, 10, 11, 12, 13, 14, 15).

In Kyrgyzstan, exact knowledge of the epidemiology of brucellosis, including its prevalence, is lacking. The characteristics of the Huddleson and Rose Bengal tests, which are currently used in Kyrgyzstan, have never been evaluated under field conditions and are therefore unknown in this setting. The overall objective of this study was to assess the seroprevalence of brucellosis in Kyrgyzstan in a representative sample of livestock and humans and to evaluate the association between apparent seroprevalence values in humans and livestock. The latter was addressed in an accompanying study (16). In the present study the authors used Bayesian models to: a) compare the characteristics of the six applied serological tests without a gold standard, and b) estimate the true seroprevalence of brucellosis within the human, cattle, sheep and goat populations in Kyrgyzstan, taking into account test sensitivity and specificity.

Materials and methods

Dataset (serum samples and population size)

The assessment of human and livestock seroprevalence was planned in an integrated way (17). Serum samples were collected from humans, cattle, sheep and goats during a broad cross-sectional study in spring 2006, which aimed to estimate the seroprevalence of brucellosis in Kyrgyzstan. The sampling procedures are described in detail elsewhere (16). Briefly, a multistage cluster sample, proportional to the size of the small ruminant population, was drawn by levels of oblast (administrative region): Province (*n* = 3), Rayon (district, *n* = 9) and Village (*n* = 90). A total sample size of 1,800 per species was calculated to achieve an absolute precision for the 95% confidence limits of < 3% below and above the estimated seroprevalence. Blood was obtained by venepuncture from humans and livestock (cattle, sheep and goats) into 5 ml and 10 ml Vacutainer® tubes, respectively. The samples were transported to the Rayon health centres (human samples) and veterinary laboratories (livestock samples) and centrifuged. Tubes containing 2 ml of serum were kept at the Rayon veterinary laboratories for testing with the RBT (Kherson Bio-Factory,

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Ukraine; for test description see next section). In addition, portions of all sera were shipped to Bishkek, either to the Centre for Quarantine for human samples or to the Central Veterinary Laboratory for livestock samples, and used in the tests described below.

Data on the population sizes of humans, cattle and small ruminants (sheep and goats) were retrieved from official records for 2006 (www.fao.org/ag/AGP/AGPC/doc/Counprof/kyrgi.htm). In the year of study very few animals were vaccinated against brucellosis in Kyrgyzstan and their influence on the serological results of this study was considered negligible.

Diagnostic tests

The samples were submitted to a range of serological diagnostic tests (Table I). Human sera were subjected to the Huddleson test, the RBT (Bio-Rad Laboratories®), and an immunoglobulin (Ig)G and IgM ELISA (Chekit®, IDEXX Laboratories Inc.) with anti-human goat IgG and IgM conjugates (Sigma-Aldrich Co.). Human sera that were positive in the Huddleson or the RBT test, but negative in the IgG ELISA, were further subjected to the IgM ELISA, and overall classification of the ELISA was positive if the IgG ELISA and/or the IgM ELISA was positive.

Livestock sera were tested using the RBT test from the Kherson Bio-Factory, Ukraine, which is commonly used for livestock samples in Kyrgyzstan. Additionally, livestock sera were tested using an indirect ELISA for ruminants (Chekit®, IDEXX Laboratories Inc.) and the fluorescence polarisation assay (Brucella FPA®, Diachemix, LLC). Serological test results were interpreted according to the manufacturer's recommendations. The cut-off value of the FPA was set at 90 mP (millipolarisation level), and those for the other tests were set using a titration curve, analogous to previous studies (18, 19). Doubtful test results (0% to 5.6% of the test results, depending on the test, and 10.2% in the IgG ELISA for human samples) were handled as positive.

This assumption was based on the consideration that the objective was disease detection (screening).

Bayesian model to estimate test characteristics and true seroprevalence

Given that none of the diagnostic tests utilised can be considered a gold standard with perfect diagnostic sensitivity and specificity, the authors used two Bayesian models to derive estimates for: (a) the true seroprevalence (i.e. the seroprevalence taking into account test characteristics below 100%) in the sample population, and (b) the respective diagnostic test characteristics. The work flow of the data analysis steps and their output is depicted in Figure 1. In the first step, test characteristics of all relevant diagnostic tests were estimated in a 'two dependent tests, two populations' model (2dT2P model). Posterior test characteristics of the 'standard' tests estimated from this model were further incorporated into the 'one test, *n* populations' model (1TnP model) to estimate an overall and Rayon-specific true seroprevalence. In this context, the most common diagnostic test for brucellosis in Kyrgyzstan (Huddleson for humans, RBT for animals) was defined as the 'standard' because it is considered to be the reference approach by the Kyrgyz public health and veterinary authorities. Separate Bayesian analyses were conducted for each of the four species in the study.

The Bayesian models were implemented in the WinBUGS software version 3.0.3 (mathstat.helsinki.fi/openbugs/SoftwareFrames.html) environment, which uses Markov Chain Monte Carlo (MCMC) sampling; the routines were based on published modules (20). In these routines, prior information on test characteristics and disease seroprevalence, mostly described as probability distributions, is combined with the observed data (test results from the respective individuals) to derive posterior density distributions of the parameters of interest. The reported model outcomes were the medians and variation (2.5th and 97.5th percentiles, representing the 95% credibility interval) of the posterior
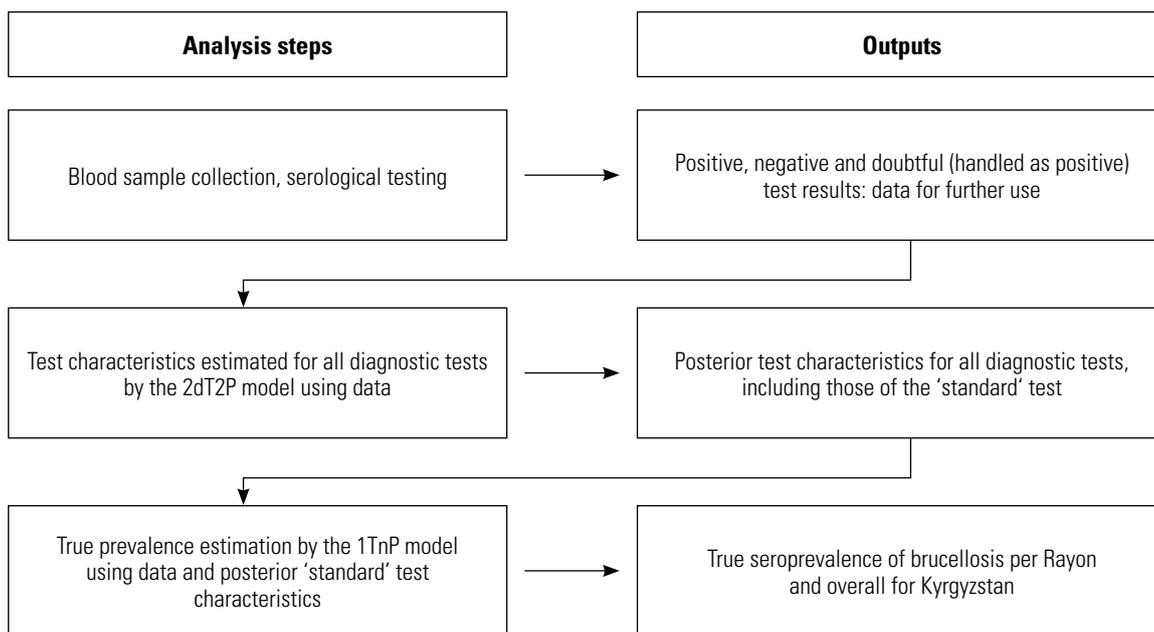
Table I

Total sample size over all Rayons by species and number of samples examined with different diagnostic tests in a seroprevalence survey of brucellosis in Kyrgyzstan in 2006

Species	Total no. of samples	RBT (Bio-Rad)	RBT (Ukraine)	ELISA (ruminant) [a]	ELISA IgG (human) [b]	ELISA IgM (human) [c]	FPA	Huddleson test
Cattle	1,818	737	1,560	1,698	ND	ND	1,691	ND
Sheep	2,101	761	1,853	2,027	ND	ND	2,029	ND
Goats	1,310	764	1,082	1,209	ND	ND	1,176	ND
Humans	1,777	644	ND	ND	1,762	369	253	1,774

FPA: fluorescence polarisation assay
ND: not determined
RBT: Rose Bengal test

(a) Indirect enzyme-linked immunosorbent assay detecting immunoglobulin G in ruminants
(b) Indirect enzyme-linked immunosorbent assay detecting immunoglobulin G in humans
(c) Indirect enzyme-linked immunosorbent assay detecting immunoglobulin M in humans

Analysis steps		Outputs
Blood sample collection, serological testing	→	Positive, negative and doubtful (handled as positive) test results: data for further use
Test characteristics estimated for all diagnostic tests by the 2dT2P model using data	→	Posterior test characteristics for all diagnostic tests, including those of the 'standard' test
True prevalence estimation by the 1TnP model using data and posterior 'standard' test characteristics	→	True seroprevalence of brucellosis per Rayon and overall for Kyrgyzstan

2dT2P: two dependent tests, two populations
1TnP: one test, n populations

Fig. 1
Flow chart of analysis steps and their outputs in a seroprevalence estimation survey of brucellosis in Kyrgyzstan in 2006
As 'standard' tests the Huddleson test was used for humans, the Rose Bengal test (Ukraine) for livestock

parameter distribution. For each estimate, 100,000 iterations were run; the first 10,000 iterations (burn-in phase) were discarded and not used for calculation of the posterior values. The influence on the posterior estimates of both the prior distributions and the starting values (inits) were tested in a sensitivity analysis.

First step: estimation of diagnostic test characteristics by the 'two dependent tests, two populations' model

The sensitivity and specificity of the diagnostic tests were estimated by a 2dT2P model described by Branscum *et al.* and Georgiadis *et al.* (11, 21). The authors employed the model with conditionally dependent tests because all tests were based on serology. The correlation of the two tests is expressed as λ and γ, where λ_{D+} and λ_{D-} reflect the probability of having the same positive and negative results, respectively. The probabilities of contradictory test results are given by γ_{D+} (test 2 positive and test 1 negative) and γ_{D-} (test 2 negative and test 1 positive). Details of the conditional dependence of the two tests are described elsewhere (13, 21). Because no prior information from the literature was available for the two correlation parameters, the authors defined a relatively wide (unspecific) beta-distribution for both λ and γ with a mode of 0.7 and 5th percentile of 0.1, using the freeware tool BetaBuster (22).

The prior distribution mode of one test's sensitivity and specificity was taken from the literature and a relatively wide beta-distribution was determined for variability (Table II). Two subpopulations with distinctly different assumed prevalence were defined by the result of a third diagnostic test, with third-test-positive individuals and third-test-negative individuals belonging to the proposed high and low prevalence populations, respectively. The prior distributions of both the low and the high seroprevalence populations again were defined to be rather wide (the 95th percentile of the high seroprevalence population was set at 0.8 and that of the low seroprevalence population at 0.15), with modes of 0.3 and 0.03 respectively, for all species (Table II).

The different combinations of diagnostic tests which were included in the model, together with the number of positive and negative samples for each test combination, are presented in Table III. This table also lists the third tests, which defines the high and low seroprevalence sub-populations. For the RBT (Ukraine) and the Huddleson tests, where two estimates from different combinations were available, the arithmetic average of the posterior values was used for the second model step.

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Second step: estimation of the true seroprevalence by the Bayesian 'one test, *n* populations' model

To estimate the seroprevalence per Rayon and the overall seroprevalence of brucellosis in Kyrgyzstan by species, specific 1TnP models were employed as described elsewhere (12, 36). Data from the country's standard diagnostic test ('one test'), stratified by the Rayons (*n* = 9) ('*n* populations'), were integrated into the model.

The prior distribution of the test characteristics was transformed from the 2dT2P model's posterior values. The seroprevalence p_i of Rayon *i* was estimated by

$$p_i = \varpi \times \inf_i \qquad (1)$$

where $\inf_i$ denotes a Bernoulli distribution of τ, which reflects the probability of a seroprevalence unequal to 0, i.e. the probability of the presence of brucellosis, and is described as a beta-distribution. The prior value of τ was set very close to 1, because the possibility of freedom from brucellosis in the Rayons can be neglected; ϖ is a beta-distribution of the parameters a and b, which were calculated using

$$a = \mu \times \varphi \qquad (2)$$
$$b = \varphi \times (1 - \mu) \qquad (3).$$

In these equations, μ is beta-distributed and reflects the prior value of the average seroprevalence in the populations (12). For μ, the authors assumed a mode of 0.15 (the mean of the prior values of the low [0.03] and high [0.3] prevalence

Table II
Mode, limit and related beta-distribution values (α, β) of the prior test characteristics for the 'two dependent tests, two populations' (2dT2P) model in a seroprevalence survey of brucellosis in Kyrgyzstan in 2006

Test	Test characteristic	Mode	Source	95% sure that value is	α	β
Cattle						
FPA	Se	0.95	(12, 23, 24)	> 0.8	21.2	2.1
	Sp	0.98		> 0.8	15.8	1.3
ELISA (ruminant)	Se	0.96	(23)	> 0.8	19.1	1.8
	Sp	0.94		> 0.8	23.8	2.5
Sheep						
FPA	Se	0.93	(3, 25)	> 0.5 [a]	5.1	3.0
	Sp	0.98		> 0.5 [a]	4.5	1.1
ELISA (ruminant)	Se	0.95	(3, 26, 27)	> 0.5 [a]	4.8	1.2
	Sp	0.99		> 0.5 [a]	4.4	1.0
Goats						
FPA	Se	0.93	(3, 25)	> 0.8	27.0	3.0
	Sp	0.98		> 0.8	15.8	1.3
ELISA (ruminant)	Se	0.80	(3, 28, 29)	> 0.65	24.1	6.8
	Sp	0.99		> 0.8	14.5	1.1
Humans						
ELISA (human)	Se	0.87	(30, 31, 32, 33)	> 0.7	19.5	3.8
	Sp	0.99		> 0.85	20.6	1.2
RBT (Bio-Rad)	Se	0.95	(1, 30, 32, 34, 35)	> 0.8	21.2	2.1
	Sp	0.95		> 0.8	21.2	2.1
All species						
P low [b]		0.03		< 0.15	1.8	26.7
P high [c]		0.30		< 0.80	1.5	2.3
λD+		0.70		> 0.10	1.376	1.161
λD−		0.70		> 0.10	1.376	1.161
γD+		0.70		> 0.10	1.376	1.161
γD−		0.70		> 0.10	1.376	1.161

ELISA: enzyme-linked immunosorbent assay
FPA: fluorescence polarisation assay
RBT: Rose Bengal test
Se: sensitivity
Sp: specificity

(a) See results of sensitivity analysis for justification of wider prior distribution
(b) Seroprevalence of the population with low seroprevalence
(c) Seroprevalence of the population with high seroprevalence

Table III

Tests of interest for test characteristics and their applied combinations in the 'two dependent tests, two populations' (2dT2P) model in a seroprevalence survey of brucellosis in Kyrgyzstan in 2006

Cattle		Positive		Negative	
Third test for sub-population allocation: **RBT (Ukraine)**		ELISA (ruminant)			
		Positive	Negative	Positive	Negative
FPA	Positive	18	0	11	47
	Negative	9	5	98	1,230
Third test for sub-population allocation: **ELISA (ruminant)**		FPA			
		Positive	Negative	Positive	Negative
RBT (Ukraine)	Positive	18	9	0	5
	Negative	11	98	47	1,230
Third test for sub-population allocation: **FPA**		ELISA (ruminant)			
		Positive	Negative	Positive	Negative
RBT (Ukraine)	Positive	18	0	9	5
	Negative	11	47	98	1,230

Sheep		Positive		Negative	
Third test for sub-population allocation: **RBT (Ukraine)**		ELISA (ruminant)			
		Positive	Negative	Positive	Negative
FPA	Positive	26	18	41	128
	Negative	13	18	83	1,481
Third test for sub-population allocation: **ELISA (ruminant)**		FPA			
		Positive	Negative	Positive	Negative
RBT (Ukraine)	Positive	26	13	18	18
	Negative	41	83	128	1,481
Third test for sub-population allocation: **FPA**		ELISA (ruminant)			
		Positive	Negative	Positive	Negative
RBT (Ukraine)	Positive	26	18	13	18
	Negative	41	128	83	1,481

Goats		Positive		Negative	
Third test for sub-population allocation: **RBT (Ukraine)**		ELISA (ruminant)			
		Positive	Negative	Positive	Negative
FPA	Positive	15	25	18	64
	Negative	10	33	40	715
Third test for sub-population allocation: **ELISA (ruminant)**		FPA			
		Positive	Negative	Positive	Negative
RBT (Ukraine)	Positive	15	10	25	33
	Negative	18	40	64	715
Third test for sub-population allocation: **FPA**		ELISA (ruminant)			
		Positive	Negative	Positive	Negative
RBT (Ukraine)	Positive	15	25	10	33
	Negative	18	64	40	715

Humans		Positive		Negative	
Third test for sub-population allocation: **Huddleson**		ELISA (human)			
		Positive	Negative	Positive	Negative
RBT	Positive	85	10	5	8
(Bio-Rad)	Negative	66	58	94	318
Third test for sub-population allocation: **RBT (Bio-Rad)**		ELISA (human)			
		Positive	Negative	Positive	Negative
Huddleson	Positive	85	10	66	58
	Negative	5	8	94	318
Third test for sub-population allocation: **ELISA (human)**		RBT (Bio-Rad)			
		Positive	Negative	Positive	Negative
Huddleson	Positive	85	66	10	58
	Negative	5	94	8	318

ELISA: enzyme-linked immunosorbent assay   FPA: fluorescent polarisation assay   RBT: Rose Bengal test

populations, respectively) with a 95% certainty that μ was smaller than 0.52. The φ is expressed as a gamma-distribution and is based on the estimated upper percentile of the seroprevalence distribution, as well as on a given certainty that this upper percentile is not exceeded (12, 36). Here, the authors were 99% sure that the seroprevalence was lower than 0.5 for all Rayons and all species. The prior distribution values of the 1TnP model are presented in Table IV.

To estimate the overall true seroprevalence (TP_{ov}) in the sampled regions of Kyrgyzstan, the authors calculated Rayon-specific seroprevalences (p_i) weighted on the Rayon's population size (N_i) from

$$TP_{ov} = \Sigma \, (p_i \times N_i) \, / \, \Sigma \, N_i \qquad (4).$$

TP_{ov} was calculated from equation (4), implemented in WinBUGS; hence, the credibility interval of TP_{ov} was influenced by the credibility intervals of each p_i.

Sensitivity analyses of the Bayesian models

For both applied Bayesian models a sensitivity analysis was conducted by examining the dependence of the posterior values (median and 95% credibility intervals) on both the prior values (mode and variability) and the inits (starting values). To evaluate the dependence on the prior values, the authors ran the model with a varying range of the distributions but a fixed mode of the priors. To estimate the dependence on the inits, three different chains with different sets of starting values were analysed by the Gelman–Rubin convergence statistic, as modified by Brooks and Gelman (37).

Results

True seroprevalence and diagnostic test characteristics

True seroprevalence estimates are presented per Rayon and per species (Table V). The overall seroprevalence in cattle was significantly lower than that in the other livestock species. Posterior values of the estimated sensitivity and specificity of the diagnostic tests, based on the dataset and analysis of the authors, are presented in Table VI. With only the exception of the specificity of the Huddleson test (human 'standard'), no substantial difference was observed when comparing the results for the test characteristics estimated by the two models (2dT2P and 1TnP models). The authors observed a statistically significant higher sensitivity for the ELISA (ruminant) in cattle, for FPA in goats (marginally significant) and for both ELISA (ruminant) and FPA in sheep than for the current 'standard' test (RBT). The RBT (Ukraine) was found to have the highest specificity of all diagnostic tests in livestock. The specificity of the RBT (Bio-Rad) in humans was significantly higher than that of the 'standard' test (Huddleson), but its sensitivity was significantly lower.

Results of the sensitivity analysis

1TnP model

For cattle and goats, the median of the posterior values and variability (credibility interval) increased slightly when using priors with wider distributions. However, none of the differences was significant (no median lay outside

Table IV
Parameters (α, β) of the prior beta-distribution values of the 'one test, *n* population' (1TnP) model in a seroprevalence survey of brucellosis in Kyrgyzstan in 2006

	Species	α	β	Source
Test sensitivity[a],[b]	Cattle	11.0	5.5	Transformation from Table VI by BetaBuster
	Sheep	12.1	14.6	
	Goats	14.9	12.4	
	Humans	45.9	4.4	
Test specificity[a],[b]	Cattle	560.7	6.7	Transformation from Table VI by BetaBuster
	Sheep	464.6	6.6	
	Goats	436.0	23.9	
	Humans	159.5	33.5	
τ [b]	All	15.8	1.3	Assumption
φ [c]	All	4.524	0.387	(11)
μ [b]	All	1.83	5.71	(11)

(a)   Rose Bengal test (Ukraine) for cattle, sheep, goats; Huddleson for humans
(b)   Beta-distribution
(c)   Gamma-distribution

Table V

True seroprevalence (TP) estimates of brucellosis in nine Rayons of Kyrgyzstan for humans, cattle, sheep and goats in 2006

Species	Population size estimate	Sample size for 'standard' test [a]	Positive cases by 'standard' test [a]	TP by Bayesian model; median (95% Cr. Int.)
At-Bashy				
Humans	52,806	206	41	0.13 (0.07–0.21)
Cattle	26,990	200	5	0.02 (0–0.07)
Sheep	123,280	200	21	0.22 (0.12–0.39)
Goats	20,550	200	40	0.31 (0.20–0.51)
Naryn				
Humans	42,427	181	35	0.13 (0.06–0.21)
Cattle	25,490	20	2	0.08 (0–0.28)
Sheep	92,185	181	32	0.36 (0.22–0.60)
Goats	15,370	181	32	0.27 (0.16–0.46)
Kochkor				
Humans	55,978	177	61	0.30 (0.21–0.39)
Cattle	27,004	220	4	0.01 (0–0.05)
Sheep	155,080	ND	ND	ND
Goats	25,850	ND	ND	ND
Jaiyl				
Humans	52,395	197	17	0 (0–0.07)
Cattle	22,747	142	14	0.13 (0.06–0.25)
Sheep	50,430	180	21	0.24 (0.14–0.43)
Goats	8,400	201	22	0.16 (0.07–0.30)
Sokuluk				
Humans	126,718	206	37	0.11 (0.05–0.18)
Cattle	42,936	199	6	0.03 (0–0.09)
Sheep	74,400	211	4	0.03 (0–0.11)
Goats	12,400	190	5	0 (0–0.05)
Yssyk-Ata				
Humans	104,285	203	27	0.06 (0–0.13)
Cattle	40,109	201	3	0.01 (0–0.05)
Sheep	49,650	219	0	0 (0–0.01)
Goats	8,280	85	0	0 (0–0.05)
Karakulga				
Humans	87,077	200	39	0.13 (0.06–0.20)
Cattle	24,504	199	0	0 (0–0.02)
Sheep	86,980	200	0	0 (0–0.02)
Goats	14,500	200	0	0 (0–0.02)
Uzgen				
Humans	166,727	202	0	0 (0–0.01)
Cattle	45,354	179	0	0 (0–0.02)
Sheep	11,080	376	0	0 (0–0.01)
Goats	1,850	25	1	0.04 (0–0.30)
Karasuu				
Humans	299,325	202	16	0 (0–0.06)
Cattle	72,072	200	0	0 (0–0.02)
Sheep	173,055	286	0	0 (0–0.01)
Goats	28,840	ND	ND	ND
Total/overall seroprevalence				
Humans	987,738	1,774	273	0.07 (0.04–0.09)
Cattle	327,206	1,560	34	0.03 (0.01–0.05)
Sheep	971,220	1,853	78	0.12 (0.07–0.23)
Goats	136,040	1,082	100	0.15 (0.07–0.30)

[a] Huddleson for humans, Rose Bengal test (Ukraine) for cattle, sheep and goats; sample size over all Rayons presented in Table I

ND: not determined
TP: true prevalence

Cr. Int.: credibility interval

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the credibility intervals of the other value). For sheep, the median did not change when wider prior distributions were used, but the variability was slightly enhanced. For humans, however, the medians of the seroprevalences were significantly greater when wider prior distributions were implemented in the model. For the overall true seroprevalence, a median of 0.13 (95% credibility interval 0.10–0.17) was calculated when a wider prior distribution was implemented, differing significantly from the median in Table V with the 'original' priors, which was 0.07 (95% credibility interval 0.04–0.09). The posterior values were not dependent on the init values for any Rayon or species.

The 1TnP model was found to be sensitive to the change in the prior modes of the test characteristics: when the prior modes were changed, the median posterior values of the seroprevalence changed in the same direction.

2dT2P model

With regard to the RBT (Ukraine) used in cattle and goats and the Huddleson and ELISA used in humans, the medians of the test characteristics decreased slightly, but not statistically significantly, when using priors with wider distributions. For sheep, however, the model was unstable when the originally planned prior values were used, i.e.

there was no convergence of the three chains with the different inits, but the model converged with wider prior distributions. None of the medians of the test characteristics was dependent on the inits (with the exception of sheep, as mentioned). However, for the sensitivity of the RBT (Ukraine) in sheep and cattle and that of the ELISA (ruminant) in sheep, the convergence of chains was late (at around 50,000 iterations).

In the 2dT2P model, the posterior values of interest (test characteristics of test 2) were quite stable when changing the prior modes of both the test characteristics and the seroprevalence of the two distinct populations. Instability was only visible when sheep data were incorporated into the 2dT2P model, when no convergence of the different inits was possible.

Discussion

The authors used Bayesian models to estimate the true seroprevalence of brucellosis for humans, cattle, sheep and goats in three oblasts of Kyrgyzstan in 2006. To their knowledge this is the first estimation of the true seroprevalence of brucellosis in a Central Asian country published in English.

Table VI
Estimation of sensitivity and specificity of different diagnostic tests in four species

Test	Model used	Sensitivity (95% Cr. Int.) [a]	Specificity (95% Cr. Int.) [a]
Cattle			
RBT (Ukraine)	2dT2P	0.69 (0.47–0.85)	0.99 (0.987–0.998)
	1TnP	0.65 (0.41–0.85)	0.99 (0.986–0.997)
FPA		0.64 (0.46–0.80)	0.96 (0.95–0.97)
ELISA (ruminant)		0.92 (0.79–0.99)	0.93 (0.91–0.94)
Sheep			
RBT (Ukraine)	2dT2P	0.45 (0.30–0.60)	0.99 (0.98–0.996)
	1TnP	0.46 (0.29–0.64)	0.996 (0.991–0.998)
FPA		0.71 (0.57–0.83)	0.92 (0.90–0.94)
ELISA (ruminant)		0.63 (0.49–0.76)	0.94 (0.93–0.96)
Goats			
RBT (Ukraine)	2dT2P	0.55 (0.39–0.71)	0.95 (0.93–0.98)
	1TnP	0.54 (0.37–0.72)	0.97 (0.96–0.98)
FPA		0.70 (0.54–0.84)	0.92 (0.89–0.95)
ELISA (ruminant)		0.43 (0.29–0.58)	0.94 (0.92–0.97)
Humans			
Huddleson	2dT2P	0.93 (0.84–0.98)	0.83 (0.78–0.88)
	1TnP	0.92 (0.82–0.97)	0.91 (0.89–0.94)
ELISA (human)		0.90 (0.81–0.95)	0.77 (0.72–0.81)
RBT (Bio-Rad)		0.60 (0.48–0.70)	0.97 (0.95–0.99)

(a)   Cr. Int.: credibility interval
ELISA: enzyme-linked immunosorbent assay
FPA: fluorescence polarisation assay

RBT: Rose Bengal test
2dT2P: two dependent tests, two populations
1TnP: one test, *n* populations

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Because doubtful test results were handled as positive, the reported seroprevalence may be a slight overestimation of the true seroprevalence. In addition, the intended sample size of 1,800 animals, as calculated elsewhere (16), could not be achieved for goats (Table I) and, therefore, the precision of the seroprevalence estimation for this species may be poorer than 3% (16). The estimated seroprevalence in cattle was lower than that in small ruminants and humans, from which it may be concluded that keeping small ruminants has a higher zoonotic potential than keeping cattle. This is also in line with the higher zoonotic potential of *B. melitensis*, whose natural hosts are small ruminants, than that of *B. abortus*, which is mainly carried by cattle (1). However, Kasymbekov *et al.* isolated only *B. melitensis* from samples in the same dataset as used in this study, including cattle samples (38). The positive correlation of brucellosis seroprevalence in small ruminants and in humans has been documented and presented elsewhere (16).

The authors chose a 2dT2P model to estimate the test characteristics of the standard test because this model was the most stable one; i.e. based on the sensitivity analysis, the posterior values were less influenced by the chosen prior values than for the other model tested. The assignment to low and high prevalence populations by a third test may have been biased, mainly because the results of the third test may be correlated with the results of tests 1 and 2 (all were serological tests). In addition, the authors assumed equal test characteristics for both the high and low prevalence populations, although test characteristics depend on the prevalence in the population to which they are applied (8). However, these two populations were created artificially and did not reflect spatially different populations with diverse prevalence values.

Given the unreliable results in some species–test combinations, not all the laboratory results were considered suitable for further analysis to estimate the true seroprevalence. For humans, the results of the RBT (Bio-Rad), ELISA (human) and the Huddleson test, and for livestock, the RBT (Ukraine), FPA and ELISA (ruminant) were used for the data analysis. Frequentist-based prevalence estimates did not differ substantially from the Bayesian-derived estimates presented here (data not shown). The choice of the 'standard' tests was based only on their common use in Kyrgyzstan. Of all diagnostic tests, they had the greatest influence on the seroprevalence estimates because their data were directly incorporated into the 1TnP model for seroprevalence estimation. However, prior distributions of the 'standard' test characteristics had previously been estimated using the 2dT2P model, using the results of the other diagnostic tests.

The estimates of sensitivity and specificity of the diagnostic tests can be used to compare the different tests. The relatively low sensitivity of the RBT in small ruminants estimated in the present study could have been increased if the test had been adapted for use in small ruminants. High sensitivities can be reached with use of the adapted RBT in sheep and goats (28, 39). In the present study, a 1:1 dilution of serum to antigen was applied for small ruminant samples, according to the manufacturer's recommendation, although the current standard dilution for sheep and goats is 3:1. This may have caused the relatively low sensitivity of the RBT in sheep and goats, and it stresses the importance of performing the tests correctly. The ELISA applied in this study (Chekit®) was based on the use of *B. abortus* antigen as the solid phase. Although the validation of this test showed good results also for *B. melitensis* (Bommeli Diagnostics, Switzerland), the resulting test characteristics may be comparatively low. The low sensitivity of the RBT (Bio-Rad) in humans when compared with the other tests, as estimated from the authors' data, is in contrast with other studies in which high sensitivity of the RBT in humans was described (1). Mert *et al.* revealed 100% sensitivity in detection of antibodies for both the RBT and the Huddleson test (40). However, the estimation of sensitivity in that paper was based only on culture-positive samples; the application of this technique is unrealistic in field study situations.

The RBT currently remains the recommended screening test for brucellosis in humans (1, 41). Although the 'standard' test continues to be used in Kyrgyzstan, another diagnostic test may equal or enhance the precision of diagnosis as a result of better test characteristics. However, besides test characteristics, other issues, including practicability, costs and the specific laboratory settings in Kyrgyzstan, should be taken into account when recommending the 'best' diagnostic tests for brucellosis in Kyrgyzstan. Therefore, information regarding improved diagnostics must be used in cost–benefit calculations related to the choice and correct performance of diagnostic tests. In addition, considerations related to the application of the tests, such as their use as confirmatory versus screening tests, are essential, and guidelines for their application exist (4).

Generally, much smaller credibility intervals were found for specificity than for sensitivity in this study, which may have been due to the clear majority of seronegative samples and the resulting larger sample size for estimation of the specificity when compared with the sensitivity. This observation was also made in a recent study of brucellosis data from Cameroon (42).

In the 1TnP model, no significant influence of either the prior distribution width or inits was observed on the posterior values for cattle, sheep and goats. For humans, however, the median of the overall seroprevalence was significantly higher when a wider prior distribution of the test sensitivity and specificity was incorporated into the model. A possible reason for this could be the extremely narrow prior distribution of the diagnostic test's specificity

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in the first step model (2dT2P model), which resulted in a small range for the prevalence estimation. However, such a narrow prior distribution of the test specificity was given for all species, as a result of the low variability of the posterior values of the specificity estimated by the 2dT2P model.

The posterior values of the 1TnP model seem to be highly sensitive to the prior modes of the test characteristics used. Consequently, reliable prior information is needed for incorporation into the model. Within this study, such information was obtained by applying the 2dT2P model, in which all diagnostic test results were integrated; this increased the reliability of the prior modes. The observed dependence on the incorporated prior values could have been caused by the often conflicting results from the various diagnostic tests. Such considerable dependence of the posterior values on the modes, and especially the width of the prior information, was also found in the study of brucellosis in Cameroon (42). The prior values of the probability that brucellosis is absent in any Rayon and species were set close to zero. This may be questioned, especially with respect to the absence of positive cases in some Rayons and species when the standard test was used (Table V), and may have led to an overestimation of the true seroprevalence. However, it is very unlikely that the true seroprevalence of brucellosis in any species is zero in a Kyrgyz Rayon, when the relatively low sensitivities of the standard diagnostic tests are also considered.

Conclusion

This study confirms the high seroprevalence of brucellosis in Kyrgyzstan. The test characteristics of the different diagnostic tests show that the standard test for brucellosis currently used in Kyrgyzstan for ruminants is less sensitive than other applied tests, but it is more specific. For humans, the standard test was found to be the most sensitive but not the most specific one. Bayesian models enabled estimation of the true seroprevalence without the use of gold-standard diagnostic tests. The results for the sensitivity analyses revealed that reliable prior information on the test characteristics is essential, especially if the various diagnostic tests may produce contradictory results, which is often the case in the diagnosis of brucellosis. The Bayesian models used in the present study can easily be applied, but the choice and influence of (potentially unreliable) priors should always be explored carefully.

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Analyse bayésienne de la prévalence sérologique de la brucellose humaine et animale au Kirghizistan

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Résumé

En 2007, le Kirghizistan a enregistré un taux de 77,5 nouveaux cas de brucellose humaine pour 100 000 habitants, ce qui représente une incidence parmi les plus élevées du monde. Néanmoins, ces chiffres émanent de sources officielles et sont très probablement en-deçà de l'incidence réelle. Les épreuves de diagnostic le plus souvent utilisées au Kirghizistan sont, chez les ruminants, l'épreuve au rose Bengale et, chez l'homme, le test d'Huddleson. La sensibilité et la spécificité de ces tests n'ont jamais fait l'objet d'une évaluation dans des conditions de terrain au Kirghizistan, et l'on ignore quelles sont les souches en circulation dans le bétail et chez l'homme dans ce pays. Pour y remédier, une étude sérologique transversale couvrant le territoire national a été entreprise afin d'évaluer la prévalence sérologique réelle chez l'homme ainsi que chez les bovins, les ovins et les caprins et de comparer les performances de plusieurs tests sérologiques. En 2006, année de l'étude, très peu d'animaux avaient été vaccinés contre la brucellose au Kirghizistan. Pour cette étude, un total de 5 229 sérums issus d'animaux d'élevage et de 1 777 sérums humains collectés au printemps 2006 dans trois régions administratives ont été soumis à une série de tests sérologiques. La prévalence sérologique réelle de la brucellose déterminée au moyen d'une méthode bayésienne était de 7 % chez l'homme (intervalle de confiance [IC] à 95 % compris entre 4 % et 9 %), de 3 % chez les bovins (IC : 1 %-5 %), de 12 % chez les ovins (IC : 7 %-23 %) et de 15 % chez les caprins (IC : 7 %-30 %). L'utilité de l'épreuve au rose Bengale en tant que test de dépistage chez le bétail et l'homme a été confirmée ; ce test est toutefois moins sensible que d'autres. Les mesures de la spécificité de tous les tests évalués ont donné des résultats significativement supérieurs à celles portant sur la sensibilité. Cette étude a confirmé que la prévalence sérologique de la brucellose au Kirghizistan est élevée, aussi bien chez l'homme que chez les bovins et les petits ruminants. Les méthodes statistiques bayésiennes se sont avérées utiles pour établir simultanément les caractéristiques des tests et estimer les prévalences réelles, en l'absence de réactifs et de sérums de référence.

Mots-clés

Animal – Brucellose – Homme – Kirghizistan – Modèle bayésien – Prévalence sérologique réelle – Sérologie – Zoonose.

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Estimación bayesiana de la seroprevalencia de la brucelosis en el ser humano y el ganado en Kirguistán

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Resumen

En 2007 Kirguistán comunicó 77,5 nuevos casos de brucelosis humana por cada 100.000 habitantes, incidencia que se encuentra entre las más altas del mundo. Sin embargo, dado que dicha cifra se basa en los registros oficiales, es muy probable que el número de casos sea en realidad aún mayor. Las pruebas de diagnóstico utilizadas con más frecuencia en Kirguistán son la de rosa de Bengala en los rumiantes y la de Huddleson en las personas. Nunca se han evaluado la sensibilidad y especificidad que ofrecen estas pruebas aplicadas sobre el terreno en Kirguistán, donde se ignora cuáles son las cepas circulantes en el ganado y el ser humano. Por ello se puso en marcha un estudio serológico representativo a escala nacional con personas y con ganado bovino, ovino y caprino, con el objetivo de determinar la seroprevalencia real y comparar entre sí distintas pruebas serológicas. En el año del estudio (2006), pocos animales fueron vacunados contra la brucelosis en Kirguistán. Durante la primavera de 2006 se recogieron en total 5.229 sueros de ganado y 1.777 sueros humanos de tres regiones administrativas, que luego fueron sometidos a una batería de pruebas serológicas. La seroprevalencia real de la brucelosis, estimada con un método bayesiano, resultó del 7% (intervalo de confianza del 95%: 4%–9%) en el ser humano, del 3% (1%–5%) en bovinos, del 12% (7%–23%) en ovinos y del 15% (7%–30%) en caprinos. Se confirmó que la prueba de rosa de Bengala constituía una buena técnica de criba en el ganado y el ser humano, aunque ofrecía menos sensibilidad que las demás pruebas. En todas las pruebas, la estimación de la especificidad arrojó resultados significativamente más elevados que la de sensibilidad. Quedó confirmada la elevada seroprevalencia de la brucelosis en personas, bovinos y pequeños rumiantes en Kirguistán. Se comprobó asimismo que los métodos estadísticos bayesianos resultan útiles para determinar las características de las pruebas y a la vez, a falta de una "regla de oro", estimar la prevalencia real.

Palabras clave

Animal – Brucelosis – Humano – Kirguistán – Modelo bayesiano – Serología – Seroprevalencia real – Zoonosis.

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