Empirical Bayes estimation of farm prevalence adjusting for multistage sampling and uncertainty in test performance: a Brucella cross-sectional serostudy in southern Kazakhstan

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SUMMARY

Estimation of farm prevalence is common in veterinary research. Typically, not all animals within the farm are sampled, and imperfect tests are used. Often, assumptions about herd sizes and sampling proportions are made, which may be invalid in smallholder settings. We propose an alternative method for estimating farm prevalence in the context of Brucella seroprevalence estimation in an endemic region of Kazakhstan. We collected 210 milk samples from Otar district, with a population of about 1000 cattle and 16 000 small ruminants, and tested them using an indirect ELISA. Individual-level prevalence and 95% confidence intervals were estimated using Taylor series linearization. A model was developed to estimate the smallholding prevalence, taking into account variable sampling proportions and uncertainty in the test accuracy. We estimate that 73% of households that we sampled had at least one Brucella-seropositive animal (95% credible interval 68–82). We estimate that 58% (95% confidence interval 40–76) of lactating small ruminants and 14% (95% confidence interval 1–28) of lactating cows were seropositive. Our results suggest that brucellosis is highly endemic in the area and conflict with those of the official brucellosis-testing programme, which found that in 2013 0% of cows and 1.7% of small ruminants were seropositive.

Key words: Brucellosis, diagnostics, empirical Bayes, livestock, prevalence, surveillance.

INTRODUCTION

Brucellosis is a bacterial zoonosis that reduces reproductive performance and milk production in cattle (predominantly Brucella abortus), sheep and goats (predominantly B. melitensis) and other livestock species [1]. Both B. abortus and B. melitensis infections cause a range of syndromes in humans that include fever and joint pain and range from mild to debilitating. Brucella sp. can be transmitted to humans via contact with aborted fetuses, parturition fluids or via consumption of unpasteurized milk and dairy products. [2]

Similarly to other Central Asian countries, Kazakhstan has a high human incidence of brucellosis. According to reports from the Ministry of Health, nine cases were reported per 100 000 humans in 2012. The reported incidence is higher in the south of the country, for example in Almaty Oblast (region) where incidence increased between 2007 and 2010, from 19.5 to 30 cases/100 000 [3, 4]. Despite widespread testing of livestock, the percentage of positive tests has been consistently
very low in all oblasts according to data provided by the Kazakh Ministry of Agriculture in Astana (generally <1% of cattle and small ruminants). Considering the high human incidence, and the likelihood of under-reporting of human cases, this suggests there may be either a systematic bias in selection of animals for testing, an inaccurate testing regimen or inaccurate reporting. There are very few alternative reliable data sources on the current prevalence and distribution of the disease in livestock or humans in Kazakhstan [5–8].

Control of livestock diseases, particularly zoonoses, is important in Kazakhstan because a large proportion of the population relies on small-scale agriculture for subsistence, particularly in rural areas [9]. Brucellosis has been identified as a priority by the Kazakh Ministry of Agriculture and the current control strategy is in the process of being revised.

Accurate estimation of herd-level prevalence (or seroprevalence) is essential to the planning and implementation of cost-effective disease control programmes. Theoretical aspects of defining ‘herd status’ based on testing of individual animals were reviewed by Christensen & Gardner [10].

In prevalence surveys of livestock diseases, often not all animals in each household are sampled, the number and proportion of animals sampled varies per household, the test(s) used is (are) not perfect and the sensitivity and specificity of the test(s) are uncertain. It is not uncommon to ignore these potential biases when calculating herd-level prevalence, and if the sampling fraction within each herd is high, and the test has a high sensitivity and specificity, this may be justified [11, 12]. However, if this is not the case, it is good practice to adjust ‘apparent’ prevalence to generate ‘true prevalence’ estimates that account for potential misclassification. One approach for herd-prevalence estimation has been to estimate the herd-level sensitivity and specificity from individual-level sensitivity and specificity values, and then to calculate a so-called ‘true prevalence’ taking into account the likely numbers of false-positive and false-negative herds based on the values of herd-level sensitivity and specificity [13–15]. However, in order to calculate a single herd-level sensitivity and specificity it is necessary to make the implicit assumption that the herd-level sensitivity and specificity are the same for each and every herd/household, which is unlikely to be the case when there is variability in herd/household size and the number of animals sampled in each herd/household. More critically, a somewhat arbitrary cut-off has to be chosen for ‘design prevalence’, a threshold below which it is assumed the disease cannot be present. The trade-offs between sample size, ‘design prevalence’ and herd-level sensitivity and specificity have been explored in detail elsewhere [16]. Simulation has been used to account for uncertainty in the true herd-level sensitivity and specificity, but it has still been assumed that there are a single set of true values that are applied to every herd [13, 14].

These problems are exacerbated by very small herd sizes, which often occur in smallholder settings where brucellosis and other livestock diseases are often most prevalent. For example, when testing one cow out of three in a household, if the cow tests negative, it is nonsensical to state that the household is ‘negative’, or that the prevalence is ‘0·05 after adjusting for herd-level sensitivity and specificity’. However, it does make sense to say that the house has a given probability of being ‘negative’ (meaning that all three cows are negative). If this approach was applied to each household/farm in the study one-by-one, household-level prevalence could then be estimated. This general approach has been used widely in risk assessment. An extension of this probabilistic approach is Bayesian estimation of prevalence, which has been reviewed by Branscum et al. [17], who also proposed a method for estimation of herd-level prevalence implemented in WinBUGS, which was adopted by Verdugo et al. [18], Pruvot et al. [19] and others; however, the method relies on the Binomial approximation for sampling of animals, which is not suitable for small herds. Suess et al. [20] simulated the true status of each animal within each herd, removing the need to assume a certain herd-level sensitivity and specificity; however, in their model they assumed that the same number of animals was sampled within each herd. In both cases, prior distributions for prevalence and uncertainty distributions for sensitivity and specificity were generated using the opinions of experts.

Here, we present a method of calculating exact probabilities of positive household status for each household, one-by-one, based on a discrete (rather than continuous) probability distribution of the number of true positives in the household, that is generated separately for each household. Then in a second step we estimate household-level prevalence. We make no assumptions about the sizes of the herds or the numbers of animals sampled per herd. The prior distributions of within-household prevalence for each herd is a discrete distribution that gives the probability of each possible number of positives in that herd, given the number of animals in the herd. This prior distribution
is generated from the within-household prevalence distribution of the other herds in the survey, and sensitivity and specificity uncertainty distributions are generated from the data used by the manufacturer to validate the test. The self-contained program runs in R [21] and the only input required by the user is the input of the survey data and the sensitivity and specificity validation data (or point values of sensitivity and specificity, if preferred).

The aims of this study were: (1) to estimate the prevalence of brucellosis antibodies in milk, from cattle and small ruminants in a typical rural village in one region in the south of Kazakhstan; and (2) to develop a method for estimating true herd-level prevalence taking account of a range of sampling fractions used on each smallholding and uncertainty in the sensitivity and specificity of the test used.

METHODS
Study area
We selected Otar Selskiy Okrug (district) for our study site as it is a typical rural Kazakh district, is conveniently located near to the laboratory and the necessary permissions from the local veterinarians, regional veterinary office and Ministry of Agriculture were granted. Otar is also in southern Kazakhstan where the human incidence of brucellosis is high. It has a population of 10 759 humans, 1054 cattle and 16 050 small ruminants (sheep and goats), many of which are kept by smallholders. These animals are kept at the household during the night and share grazing around the villages during the day. In Otar Selskiy Okrug there are 1525 households with livestock and 1300 of these are in the main village, Otar. There is only one other large village, Matybulak (with 110 households with livestock), which is located ~3 km from Otar village (in Almaty Oblast) and animals from both villages share grazing areas. Throughout Kazakhstan, brucellosis vaccination is prohibited, and a national test-and-slaughter programme is being carried out, that involves twice-yearly testing of all sheep, goats and cattle.

Original study design
We planned to conduct systematic random sampling of households. We estimated that we needed to sample 250 cows and 360 small ruminants in order to estimate individual-level prevalence within 1.7% absolute error with 95% confidence, with a hypothesized prevalence of 2%, a design effect of 1.4 and a finite population size of 500 lactating cows and 8000 lactating small ruminants. We planned to select the required number of animals needed to detect disease (assuming a minimum of 10% seroprevalence) with 95% confidence in each household according to the number of livestock present.

Amendments to study design
Due to practical limitations, we had to resort to convenience sampling of households. The households were selected either by the local veterinarians because they were already planning to visit them for routine brucellosis blood testing, or they were relatives or friends of the research team, or they were neighbours or friends of these people. In addition we sampled four large farms in the area, one of which was the research farm belonging to the laboratory and the remaining three were contacts of the research team. These animals grazed on the steppe during the day and were brought into an enclosure at night.

Due to limitations in accessing households, we sampled as many as possible of the cattle, sheep and goats on each household or farm. We tried to avoid any obvious bias in selection of animals for sampling, but random sampling was not possible.

Data collection
The identification number or description, species, breed and age of each animal was recorded when available. We completed an interview (in Russian or Kazakh) with each owner, using a pre-designed form including questions on the number of animals of each type on the household and the gender and age of members of the household who regularly milked the livestock or assisted with parturition.

Sample collection, processing and testing
Ten millilitres of milk were collected from each quarter into a single plain polyethylene tube, after cleaning and drying the teats. The samples were placed immediately into a cool box and placed in a refrigerator within a few hours. The samples were left to stand to allow the lactoserum to separate from the fat layer, or they were centrifuged, and the lactoserum was pipetted into Eppendorf tubes. The samples were then frozen for up to 5 months before de-frosting at room temperature.

The milk samples were tested using an indirect ELISA for brucellosis antibodies (ID Screen®
Individual-level seroprevalence

The individual-level percentages of test-positive milk samples were estimated using the ‘survey’ package [22] in R [21], which produces both a point estimate that is weighted according to the sampling fraction, and a confidence interval that is adjusted for clustering (in this case within a smallholding or farm) by adjusting the standard error using Taylor series linearization [23]. The sampling fraction was calculated as the number sampled/the number of lactating animals in the household at the time of the visit. There were seven households for which the number of lactating small ruminants in the household was not recorded, and five for which the number of lactating cows was not recorded. The mean of the available sampling fractions was used for these households. Uncertainty distributions for the sensitivity and specificity of the iELISA were generated based on data used for validation by the manufacturer (see Table 1).

The point estimates and confidence intervals were adjusted for the sensitivity and specificity using the uncertainty distribution, in R, using the following formula:

\[
TP = \frac{AP + Sp - 1}{(Se + Sp - 1)},
\]

where \( TP = \) true prevalence, \( AP = \) apparent prevalence, \( Se = \) sensitivity and \( Sp = \) specificity. [24].

The median values of the resulting uncertainty distributions for point estimate, lower and upper confidence intervals are presented.

Household-level prevalence

The household-level prevalence of brucellosis (the proportion of households with at least one animal with antibodies against Brucella spp. in the milk) was estimated taking into account the imperfect sensitivity and specificity from (1) estimation of the true sensitivity and specificity from a sample of ‘truly infected’ and ‘truly non-infected’ samples, and (2) uncertainty arising from the imperfect sensitivity and specificity of the test.

### Table 1. Sensitivity and specificity values assumed in this analysis

<table>
<thead>
<tr>
<th>Test used</th>
<th>Species</th>
<th>Sensitivity/%</th>
<th>Specificity/%</th>
<th>Supporting data</th>
</tr>
</thead>
<tbody>
<tr>
<td>Indirect ELISA</td>
<td>Small ruminants</td>
<td>Beta distribution with parameters ((a,b)), where ( a = s + 1 ), ( b = n - s + y ), ( s ) (the number of successes in a binomial process) = 18 and ( n ) (the number of trials in the binomial process) = 18</td>
<td>Beta distribution with parameters ((a,b)), where ( a = s + 1 ), ( b = n - s + y ), ( s = 650 ) and ( n = 650 )</td>
<td>Data provided by ID.vet: 650/650 milkings from Brucella-free cattle in France tested negative 18/18 milkings from RBT-positive cattle from Italy and Albania tested positive</td>
</tr>
<tr>
<td>Indirect ELISA</td>
<td>Cattle</td>
<td>As above</td>
<td>As above</td>
<td>As above</td>
</tr>
<tr>
<td>PAT</td>
<td>Small ruminants</td>
<td>0.771</td>
<td>0.999</td>
<td>Mean of values cited in [30]</td>
</tr>
<tr>
<td>PAT</td>
<td>Cattle</td>
<td>0.771</td>
<td>0.999</td>
<td>Mean of values cited in [30]</td>
</tr>
<tr>
<td>CFT</td>
<td>Small ruminants</td>
<td>0.926</td>
<td>0.999</td>
<td>Meta-analysis [25]</td>
</tr>
<tr>
<td>RBT</td>
<td>Small ruminants</td>
<td>0.925</td>
<td>0.999</td>
<td>Meta-analysis [25]</td>
</tr>
<tr>
<td>RBT</td>
<td>Cattle</td>
<td>0.981</td>
<td>0.998</td>
<td>Meta-analysis [25]</td>
</tr>
<tr>
<td>RBT, CFT and RBT in series</td>
<td>Small ruminants</td>
<td>0.660</td>
<td>1.000</td>
<td>Combined sensitivity: ((Sn) = Sn(PAT) \times Sn(CFT) \times Sn(RBT)) Combined specificity: ((Sp) = 1 - (1 - Sp(PAT)) \times (1 - Sp(CFT)) \times (1 - Sp(RBT)))</td>
</tr>
<tr>
<td>PAT, CFT and RBT in series</td>
<td>Cattle</td>
<td>0.726</td>
<td>1.000</td>
<td>As above</td>
</tr>
</tbody>
</table>

CFT, Complement fixation test; RBT, Rose Bengal test; PAT, plate agglutination test.
infected’ animals and (2) sampling of only a proportion of each household (the proportion being different in each household). A model was constructed in R (R Core Team, 2015) as follows. (The complete model is available as Supplementary material.)

**Step 1.** The probability that each given household was negative ($P_n$) (i.e. that there were no lactating animals with antibodies in the milk) was calculated for each household individually as follows:

$$P_n = O_n/(1 + O_n),$$

where $O_n$ is the (posterior) odds that the household was negative, and was calculated according to Bayes theorem as follows:

$$O_n = \frac{(\text{Prior}_n \times \text{Likelihood}_n)}{\sum (\text{Prior}^i \times \text{Likelihood}^{ijk})},$$

where Prior$_n$ is the prior probability of that there were zero positives on the farm (this was a discrete probability for each iteration of the model); Likelihood$_n$ is the likelihood of obtaining the laboratory results, given that there were zero positives on the farm; Prior$^i$ is the prior probability that there were $i$ positives on the farm (where $i$ is a vector from 1 to the total number of lactating animals on the farm) (these were discrete probability values for each iteration of the model); and Likelihood$^{ijk}$ = the likelihood of obtaining the laboratory results, given that there were $i$ disease-positives in the household, $j$ disease-positives among the tested animals and $k$ false positives. For each household, each possible permutation of the number of true positive lactating animals in the household† ($i$) and the sample ($j$), and the number of false positives ($k$) that could result in the laboratory results was generated programmatically and the likelihood was calculated for each permutation, as shown in the worked example in Figure 1.

The prior distribution of the number of true positives on the farm was also calculated for each household separately as follows. First, the frequency distribution of within-household prevalence values from all households in this study was multiplied by the number of lactating animals in the particular household, and second, the resulting numbers of positives were rounded to whole numbers, to create a discrete probability distribution of each possible number of true positives on the farm.

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† For households where the number of lactating animals in the household was not available, the number of female animals (or total number of animals) was used as a conservative approximation.

**Step 2.** For each run of the model, each household was simulated to be positive or negative by drawing one random sample from a binomial distribution with probability of success of $(1 - P_n)$.

**Step 3.** Steps 1–2 were repeated 1000 times to create an uncertainty distribution, where the 2.5th and 97.5th percentiles give a 95% credible interval and the 50th percentile gives the most likely household-level prevalence.

**Comparison of field-study results with official seroprevalence data**

In order to compare the field-study results with the official data, we conducted the following analysis. The number of official tests conducted in 2013 and the number of seropositives were obtained from the local veterinary office. It was assumed that the results were obtained by combining the plate agglutination test (PAT), complement fixation test (CFT) and Rose Bengal test (RBT) in series. Sensitivity and specificity estimates for each test were obtained based on a published meta-analysis [25] where available, or literature review (Table 1). Combined sensitivity and specificity were estimated as shown in Table 1 (which assumes the tests are independent from one another).

Based on the combined sensitivity and specificity, adjusted seroprevalence estimates were obtained using the method by Reiczigel et al. [26] implemented online in ‘Estimated true prevalence and predictive values from survey testing’ (http://epitools.ausvet.com.au/content.php?page=TruePrevalence). This assumes that the results were obtained by simple random sampling.

The difference between the adjusted seroprevalence values obtained based on the field-study and official results, and an exact 95% confidence interval (CI) was estimated using the following formula:

$$95\% \text{ CI} = \text{RD} - \sqrt{[(p_1 - l_1)^2 + (u_2 - p_2)^2]} \quad \text{to} \quad \text{RD} + \sqrt{[(p_2 - l_2)^2 + (u_1 - p_1)^2]},$$

where $\text{RD}$ = the risk difference, $l_1$ to $u_1$ is the 95% CI of the first proportion, $p_1$ and $l_2$ to $u_2$ is the 95% CI of the second proportion, $p_2$ [27].

**Ethical standards**

Ethical approval for the study was granted by the Royal Veterinary College ethics committee (URN 2011 1097). Written consent was obtained from one member of each household.
RESULTS

Descriptive analysis

Farms and households

In total, three farms and 31 smallholdings in Otar Selskiy Okrug were visited. Interviews were conducted on all three farms, 22 of the smallholdings from which milk samples were taken and a further three smallholdings which had no lactating animals at the time of the visit.

The farms were all mixed species and had between one and three lactating cows (median 2) and 150–318 small ruminants (median 284). Of the 25 smallholdings
for which data were available, seven kept only small
ruminants (range 1–40, median 30), three kept only
cattle (range 1–3, median 2) and 15 kept both small
ruminants (range 7–85, median 20, data missing for
two farms) and cattle (range 1–16, median 2).

A range of 1–5 individuals on each smallholding had
contact with the livestock via milking or delivering new-
born animals (median 2, missing data for 7/31 small-
holdings). Of these individuals (n = 48), 21 were female
and 27 were male, and their ages ranged from 13 to
75 years (median 47, missing data for 5/48 individuals).

Milk samples

We collected 210 milk samples from all three farms and
28 of the smallholdings. The milk samples came from
43 cows and 167 small ruminants (129 sheep, 23
goats, 15 not specified). The cows ranged in age be-
tween 3 and 15 years (median 5, data missing for 15/
43 cows); the small ruminants ranged in age between
1 and 7 years (median 4, data missing for 86/167
small ruminants). The breeds of the livestock were fre-
cently unknown or not recorded, but cattle breeds
included mixed, Kazakh, Zerno-pestreesa and Alatau;
sheep breeds included mixed, Kazakh, Merino and
Yedilbai; and goat breeds included mixed and Angora.

After accounting for the multistage sampling that
resulted in variable sampling fractions with households,
the percentage of households with at least one seros-
itive to Brucella (Table 2). The percentage of official
serological tests reported positive in Otar Selskiy Okrug was 53% lower (95% CI 38–73)
for small ruminants, suggesting the results were incom-
patible with one another. For cattle our results were
13–2% lower than the official serological results but
the 95% CI included zero, suggesting that the results
were not incompatible at the 95% level, although our
sample size was possibly not large enough to detect a
statistically significant difference.

The apparent percentage of households with at least
one seropositive lactating animal was 64% (14/22
households for which sufficient data were available)
and the adjusted percentage was 73 (95% credible
interval 68–82). (Note, this is a sample estimate not
a population estimate.)

DISCUSSION

The results strongly suggest that brucellosis is endemic
in small ruminants is much higher than suggested by
official results. (A larger sample of cows would be
needed to estimate the seroprevalence in cows more
precisely.) It is unlikely that this difference is due to
systematic bias in selection of animals for official test-
ing, due to the large numbers officially tested, and the
size of the difference between our results and official
results. It seems unlikely that selection of animals in
our study was heavily biased towards animals with
brucellosis, as owners would be less likely to allow
testing if disease was suspected, and many of the
households were known to have been included in the
official testing programme. It also seems unlikely
that such a large difference would be observed due
to testing milk as opposed to blood samples.

Finally, we used an indirect ELISA, whereas the
official testing involves the PAT, RBT and CFT. We
used a commercial indirect ELISA to detect Brucella
antibodies because there were good laboratory facil-
ities available to perform this test, and the laboratory
staff had experience with ELISAs. The agglutination
tests (RBT and PAT) and CFT also measure anti-
bodies; however, sensitivity and specificity values
vary between the different tests, and there can be
slight differences in the sensitivities to different classes
of antibody (IgM, IgG or IgA). There is no gold
standard test for detecting antibodies to Brucella
[28]; however, all of the tests have been validated by
previous authors using samples from known infected
and non-infected animals [25]. We adjusted the official
prevalence values according to published sensitivities
and specificities, and the ELISA results were adjusted
according to sensitivity and specificity data provided
by the manufacturer of the ELISA. These values
were based on cows and B. abortus only, and the
cows were from different contexts to Kazakhstan.
Sensitivity and specificity values can vary in different
populations. Data specific to Kazakhstan or to small
ruminants generally are lacking, and it is possible
that there is a higher incidence of cross-reactions in
our study; however, it seems unlikely that this would
explain the high proportion of positives that we found.

About 33 cases of (culture-positive) brucellosis were
reported per 100 000 people in 2009 in Kordai rayon
(a larger administrative division than Selskiy Okrug – data not available at Selskiy Okrug level),
which would suggest that a higher seroprevalence in
livestock would be expected than is of
officially reported, based on data from other endemic
countries.

Possible explanations for the difference in results
include poor sensitivity of tests used in official
laboratories, or false reporting. The stringent requirement for all three official tests to be positive for a sample to be classified as positive means that a deficient sensitivity in any one of the tests could result in a very low combined sensitivity. The consequences of culling a positive animal may be very severe for households in Otar Selskiy Okrug, despite the compensation that is given, as the owners are heavily reliant on a few animals, and this may help to explain a bias towards classifying animals as negative in doubtful situations.

There were several additional limitations to this study. Despite planning to do random sampling, we had to resort to convenience sampling. However, obvious biases were avoided where possible, and we sampled typical households in a village setting, as far as we could tell. In addition, we were sampling within a small geographical area with a population of ∼16 000 small ruminants, most of which share grazing pastures. Although there could be some bias in the sampling, it seems reasonable to conclude that brucellosis is highly endemic in the area, given the high proportion of positives that we obtained.

We conclude that brucellosis is highly endemic in Otar Selskiy Okrug and due to the structure of the livestock system, a large proportion of people in the area are likely to be at risk of exposure to *Brucella*. The official census data shows that there are >1500 households with livestock in Otar Selskiy Okrug, an area with a human population of ∼11 000. Our interview data suggested that in most households more than one person had regular contact with the household livestock, and the ages of those in contact ranged from 13 to 75 years.

The methods we developed for estimating the herd-level prevalence could be applied to any multi-stage prevalence study in order to account for variable sampling proportions and imperfect tests, simultaneously. The method also incorporates uncertainty arising from estimation of sensitivity and specificity, based on a small validation study. Furthermore, this approach could readily be tailored to various study designs, could be extended to use in analytical studies aimed at identifying risk factors, or could also be used as a ‘module’ in a quantitative risk assessment involving heterogeneous farms, for example.

Brucellosis control has historically been extremely challenging in Central Asia, and there is very little precedent for the success of test-and-slaughter in endemic areas [5, 29]. A wide-scale review of the national test-and-slaughter scheme in Kazakhstan is needed, bearing in mind that Kazakhstan is a large country and our study was only conducted in a small geographical region.

**SUPPLEMENTARY MATERIAL**

For supplementary material accompanying this paper visit http://dx.doi.org/10.1017/S0950268816001825.

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DECLARATION OF INTEREST

None.

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