Cross-sectional study of brucellosis in Jordan: prevalence, risk factors and spatial distribution in small ruminants and cattle

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Abstract

Brucellosis is considered endemic in many Middle Eastern countries including Jordan. To determine the frequency, risk factors and spatial distribution of ruminant brucellosis in Jordan, a nationwide cross-sectional study was conducted. Small ruminant flocks (n=333) and cattle herds (n=204) were randomly selected, and their disease status was ascertained by testing individual serum samples using the Rose Bengal Test and a competitive ELISA (sheep and goats) and milk samples using an indirect ELISA (cattle). Information on putative risk factors was collected using standardised questionnaires. A logistic model with a binomial outcome was built to identify risk factors for being seropositive. The estimated true seroprevalence values were 18.1% (95% CI: 11-25.3) (cattle-only herds), 22.2% (95% CI: 16.5-28.8) (sheep flocks), 45.4% (95% CI: 30.3-61.6) (goat herds), 70.4% (95% CI: 55.5-84.9) (mixed sheep-goat flocks), 34.3% (95% CI: 28.4, 40.4) (all small ruminant flocks) and 38.5% (95% CI: 24.3-51.8) (mixed herds of cattle and small ruminants). Only 1.5% of small ruminant flocks were vaccinated. The seroprevalence was higher in northern areas, where livestock density is also higher. The logistic model fitted the data well and had a very high predictive ability. In the small ruminant model, five variables were significantly associated with a higher odds of seropositivity: lending/borrowing rams (OR=8.9, 95% CI: 3.0-26.1), feeding aborted material to dogs (OR=8.0, 95% CI: 3.5-18.1) the presence of goats (OR=6.9, 95% CI: 3.1-15.4), introducing new animals to the flock (OR=5.8, 95% CI: 2.5-13.6), and a large flock size (OR=2.2, 95% CI: 1.0-4.6). Conversely, separating newly-introduced animals (OR=0.16, 95% CI: 0.05-0.47), separating animals that had aborted (OR=0.19, 95% CI: 0.08-0.46) and using disinfectants to clean pens (OR=0.37, 95% CI: 0.16-0.83) were significantly associated with a lower odds of being seropositive. The main risk factor for cattle herds being seropositive was the introduction of new animals (OR=11.7, 95% CI: 2.8-49.4); while separation of newly-introduced animals (OR=0.09, 95% CI: 0.03-0.29), herd disinfection (OR=0.04, 95% CI: 0.01-0.15) and having calving pens (OR=0.14, 95% CI: 0.05-0.43) significantly reduced the odds of infection. Brucellosis is endemic at high levels across Jordan, and the current vaccination program, which is limited to small ruminants, has very low coverage. A revised brucellosis control program is required in Jordan. Given the high baseline prevalence, it should be based on vaccination accompanied by measures to promote

hygiene and husbandry practices that minimize the risk of introduction and maintenance of *Brucella* spp., and thereby the risk of human infection.

Keywords: brucellosis, Jordan, prevalence, ruminants, risk factors, cross-sectional, *Brucella*

1. Introduction

Brucellosis is a highly contagious zoonotic disease affecting humans and a wide range of animals, including all domestic ruminants (Radostits *et al*., 2000). There are ten known *Brucella* species and five of them have been isolated from human cases (Sohn *et al*., 2003). The World Health Organisation (WHO) estimates that more than 500,000 new human cases of brucellosis occur worldwide annually (Corbel, 1997); however the number is probably underestimated as a result of underreporting and misdiagnosis (Jennings *et al*, 2007). The vast majority of human cases are acquired through consumption of contaminated dairy products or contact with infected animals, in particular ruminants, thus the control of ruminant brucellosis is key to the prevention of human infection (Corbel, 2006). In addition to its impact on human health, ruminant brucellosis is responsible for considerable economic losses due to abortion in pregnant animals, loss in milk production and infertility in adult males (OIE, 2009). *B. abortus*, which is the species sustained in cattle populations, has been eradicated from many developed countries through a combination of vaccination and test-and-slaughter of positive animals (CFSPH, 2009). However, the control of *B. melitensis*, which mainly infects small ruminants, is proving challenging in most endemic areas (Blasco, 2010). Possible reasons why progress in control of *B. melitensis* is slow with the infection persisting at high levels in regions such as the Middle East and Central Asia include i) a higher infectivity and transmissibility of this species compared to *B. abortus* (Cloeckaert, 2002), ii) the pastoralist and transhumant small ruminant production systems favouring transmission and iii) control programs that are often intermittent or inadequate when they do exist (Blasco, 2010). Successful control of *B. melitensis* has been achieved in some endemic areas such as in Tajikistan, where the implementation of a vaccination program over five years in eight of the Tajik districts preceded a relative drop in seroprevalence of 80% (Ward *et al*, 2011).

The decision on how to better allocate resources to brucellosis control within a certain country or region should be based on, among other considerations, the existing frequency of infection. However, reliable frequency estimates of ruminant brucellosis are notably lacking in endemic areas such as most Middle Eastern countries including Jordan.

In Jordan, some conclusions may be drawn from previous surveys -which have mostly been geographically-circumscribed and non-probabilistic- as follows. Firstly, brucellosis is likely to be

endemic both in small and large ruminants; and secondly, *B. melitensis* biovar 3 is present in Jordan and has caused infections in sheep (Al-Talafhah *et al*., 2003), goats (Al-Majali, 2005), camels (Hawari, 2008) and humans (Shehabi *et al*., 1990). Furthermore, the number of human cases of brucellosis is thought to be increasing (Abo-Shehada &Abu-Halaweh, 2013), and some evidence suggests a heterogeneous distribution of infection with a higher prevalence in the northern parts of the country, which have a higher density of livestock (Al-Talafhah *et al*., 2003, Samadi *et al*, 2010). International organisations such as the Food and Agriculture Organisation (FAO) and the World Organisation for Animal Health (OIE) have produced guidelines for the control of brucellosis in endemic areas (FAO, 2009) depending on the level of seroprevalence. At high levels of prevalence, vaccination is preferred; while at low prevalence levels, test-and-slaughter may allow eradication of the disease without vaccination. Consideration of the baseline level of infection is therefore essential for the formulation of appropriate control strategies (Hegazy *et al*. 2009). Moreover, the identification of risk factors for infection and spatial heterogeneities in the disease distribution could allow control efforts to be targeted at selected subpopulations of herds/flocks or parallel control measures to be tailored to vaccination and/or test-and-slaughter programmes. The overall aim of this study is to generate baseline information on the frequency and distribution of ruminant brucellosis in Jordan to inform the national control programme. Our specific objectives are i) to estimate the true seroprevalence of *Brucella* spp. infection in small ruminants and cattle ii) to identify risk factors associated with *Brucella* spp. seropositivity at herd/flock level and iii) to describe the spatial distribution of ruminant brucellosis in Jordan.

2. Materials and methods

2.1. Study design and study population

A nationwide cross-sectional study was conducted in Jordan from May to October 2013. The study unit was the herd or flock, defined as animals (cows, sheep or goats) owned by the same person/household and usually kept in the same location. The target population included all small ruminant flocks and cattle herds in the country. Ethical approval for this study was granted by the Ethics and Welfare Committee of the Royal Veterinary College. Informed consent for questionnaire administration and collection of biological samples was sought verbally from individual farmers.

The climate in Jordan is semi-arid in summer with average temperatures around 35 °C, and around 13°C in winter, when practically all of the precipitation occurs. The main livestock species in Jordan are sheep, goats, cattle and camels, with sheep and goats accounting for more than 97% of the total ruminant population and cattle comprising 2% (MOA, 2012). Sheep and goats are mainly kept in flocks that are very variable in size or as part of small household flocks, sometimes with other species. Awassi sheep and Baladi goats are the predominant breeds. The lambing season starts in November and lasts until May the following year. During the dry period some sheep flocks move from the East to communal grazing areas in the West of the country. The most predominant cattle breed in Jordan is the Holstein Friesian, which is kept for milk production and is mainly reared using intensive or semi-intensive systems, with a smaller number kept in small herds or as household animals. The density of both small ruminants and cattle is higher in the northern governorates.

2.2. Sampling strategy and sample size

2.2.1 Sampling of herds/flocks

Administrative divisions in Jordan include governorates (n= 12), departments (also known as districts) and villages. A list of all herds and flocks present in each governorate was provided by the Ministry of Agriculture (MOA). This list included: unique identification of herds/flocks, district where they are registered, the number of animals of different species within each herd or flock and complete vaccination history (MOA, annual report, 2012). These lists are updated annually, and in the opinion of the senior author -supported by personal experience- they are reasonably accurate. Selection of herds/flocks within each governorate was carried out by simple random sampling from the list provided by the MOA. The owner of each selected herd/flock was contacted by the local veterinarian and visited to explain the purpose of the study. If the owner refused to participate or if the herd/flock had been vaccinated against brucellosis during the previous year, the next herd/flock in the list was contacted. The total number of cattle herds and small ruminant flocks to be sampled in order to generate herd/flock-level prevalence estimates with a predefined precision was obtained as:

$$N = \left(\frac{1.96}{d}\right)^2 * \frac{(\{(HSe * P) + (1 - HSp) * (1 - P)\} * \{(1 - HSe * P) - (1 - HSp) * (1 - P)\}}{(HSe + HSp - 1)^2}$$

Where N is the sample size (number of herds or flocks to be sampled), 1.96 is the Z-value corresponding to a 95% confidence interval of the standard normal distribution, d is the expected absolute error (6%), P is the expected prevalence at cattle herd or small ruminant flock level (15% and 35%, respectively, based on the most recent estimates available) and HSe and HSp are the herd or flock-level sensitivity and specificity of the serological tests used. Based on estimates in published literature, Se and Sp values for the Rose Bengal Test (RBT) are: $0.89 \leq Se \leq 1$; $0.924 \leq Sp \leq 1$ and for cELISA: $0.95 \leq Se \leq 1$; $0.96 \leq Sp \leq 1$ (Ramirez-Pfiffer *et al*, 2007). For iELISA, Se and Sp values were obtained directly from the manufacturer and are as follows: $0.98 \leq Se \leq 1$ and $0.99 \leq Sp \leq 1$. For the purpose of sample size calculations, fixed values of Se and Sp were used. For the series combination of RBT and cELISA, CSe = 0.89 and CSp = 0.97, and for iELISA, Se = 0.98 and Sp =0.99.

The publicly-available application HerdAcc (Jordan, 1995) (http://epitools.ausvet.com.au/content.php?page=HerdSens4) was used to estimate the herd-level sensitivities (HSe) and specificities (HSp) achieved by testing different numbers of individual animals (see details in section 2.2.2) with plausible values of individual-animal level sensitivity and specificity. After exploring a range of scenarios, likely (conservative) herd-level sensitivity and specificity values were obtained for cattle herds ($HSe = 0.95$, $HSp = 0.95$) and for small ruminant flocks ($HSe = 0.92$, $HSp = 0.96$).

The total number of cattle herds and small ruminant flocks to be sampled was distributed across governorates in proportion to their weight in the total population. These numbers of herds and flocks would allow us to detect an odds ratio (OR) ≥2 for risk factors present in 20% of cattle herds and small ruminant flocks with a study power of 94% and 95% for cattle herds and small ruminant flocks, respectively.

2.2.2 Within herd/flock sampling

The number of animals that had to be tested within a herd or flock in order to reach a certain confidence of detecting at least one positive animal was calculated as:

$$k = \left[1 - \left(1 - p^{1/d}\right)\right] * \left[N - \frac{d}{2}\right] + 1$$

Where k is the number of animals to be sampled from each herd or flock; p is the probability of detecting at least one positive animal; d is the expected number of infected animals in an infected herd or flock and N is the average herd or flock size. For the calculations, a 25% seroprevalence of brucellosis within cattle herds and 35% within small ruminant flocks was assumed. The probability of detecting at least one animal if the herd is infected (p) was set as 90% and herd and flock sizes of 30 and 100, respectively, were used. The results from these calculations suggested that 9 cows and 8 sheep/goats would be sufficient to reach the desired probability of detection under the assumptions above.

2.3 Ascertainment of disease status

Each selected herd/flock was visited between May and October 2013. Individual animals were selected as they passed through the door of the pen, using a flock-size specific sampling interval. When this was not possible, the shepherd or herder was asked to point to individual animals without a specific rule. In cattle herds, 50 ml milk samples were collected from 9 cows- when the number of lactating cows at the time of the visit was more than 9 - or, from all lactating cows if there were 9 or fewer in total. Samples were obtained from each individual cow in a sterile screw cap polyethylene tube; 5 ml of 5% formalin was added immediately after collection. Samples were kept refrigerated until tested. In each of the selected sheep flocks and goat herds, eight blood samples were obtained aseptically from the jugular vein in a sterile centrifuge tube without anticoagulant and transported directly to the laboratory. Both ewes and rams were sampled. Serum was separated by centrifugation and divided into aliquots of 0.5 ml using cryogenic vials and stored at -20°C until being tested.

Milk samples were tested by an indirect ELISA (Brucelisa 160M). A cut-off optical density (OD) was calculated as 10% of the mean of the 8 positive control wells. Any test sample giving an OD equal to or above this value was considered positive (OIE, 2009A).

Serum samples from sheep and goats where first tested by RBT. Any observed agglutination by the naked eye was considered to be a positive reaction; positive samples by RBT were confirmed by a competitive ELISA (COMPELISA). A cut-off OD was calculated as 60% of the mean of the 4 conjugate control wells. Any test sample with an OD equal to or below this value was considered positive. Animals which gave positive results in both RBT and cELISA tests were considered

seropositive, while negative animals were those which gave negative results to either RBPT or cELISA (OIE, 2009B).

Diagnostic procedures for the two ELISA tests were carried out according to the manufacturer instructions (OIE brucellosis reference centre - Animal Health Veterinary Laboratories Agency (AHVLA)) at the laboratory facilities of the Jordan Food and Drug Administration and optical densities were measured using an ELISA reader (BioTek, ELX800, USA).

2.4 Data collection

A structured questionnaire (with sections for small ruminant flocks and for cattle herds) including closed-ended questions was designed to capture information concerning i) the identification and location of the herd/flock; ii) its structure and composition; and iii) husbandry and health management practices. Variables of interest for cattle herds and small ruminant flocks are shown in Table 1 and Table 2 respectively. The questionnaire was piloted in ten herds/flocks. Questionnaires were administered to the owner or person in charge of the herd/flock either by the primary author or by a veterinarian from the local veterinary services at the same time of the visit to collect biological samples. The geographical location of each sampled herd/flock was recorded as the latitude and longitude at the point where animals were kept overnight, by means of a Global Positioning System (GPS) device (Garmin, eTrex20).

2.5 Data analysis

2.5.1 *Seroprevalence Estimation*

A herd/flock was considered to be positive if at least one animal tested positive. The apparent seroprevalence of brucellosis among cattle herds (AP_{CH}) and small ruminant flocks (AP_{SRF}) was calculated as the total number of seropositive herds or flocks divided by the total number of herds or flocks sampled. Herd/flock sensitivity (HSe) and specificity (HSp) were calculated as follows:

$$HSe = 1-(1-AP_{POS})^{n}$$

$$AP_{POS} = P*Se + (1-P)\,(1-Sp)$$

$$HSp = Sp^{n}$$

Where P is the within herd/flock prevalence, Se is the individual level sensitivity, Sp is the individual level specificity and n is the number of tested animals in the herd/flock.

225 The true seroprevalence at herd (TP_H) and flock (TP_F) levels were calculated after adjusting for *HSe* and
226 *HSp* as $TP_H = (AP_H + HSp - 1) / (HSe + HSp - 1)$. Monte-Carlo simulation implemented in @Risk 6.2
227 for Excel (Palisade Corporation Inc., Newfield, NY, USA) was used to account for variability and
228 uncertainty in the performance of the diagnostic tests at individual-animal level and the effect of the
229 within-herd/flock sampling fraction. The 95% confidence intervals for the estimated seroprevalence at
230 herd/flock levels were obtained from the outputs of a simulation of 10,000 iterations.
231 2.5.2 *Univariate associations between herd/flock- level serological status and potential risk factors*
232 A conceptual framework was developed to represent causal pathways and assist with selection of
233 candidate variables identified as putative risk factors. Candidate variables were selected based on the
234 biological plausibility of their contribution to the risk of introduction of *Brucella* spp. in the herd/flock
235 or to the maintenance of the infection in the herd/flock following its introduction (Tables 1 and 2).
236 Univariate analysis of associations was carried out considering the serological status of the herd/flock as
237 a binary outcome (positive or negative). The variable representing herd/flock size was categorised into
238 three levels based on percentiles. With regard to small ruminants, flock species was categorized as:
239 flocks with goats (goats only or goats and sheep) vs. flocks without goats (sheep only). Following
240 exploration of the data, this was considered an appropriate categorisation given the small number of
241 goat (only) flocks and the fact that goats are more susceptible to brucellosis than sheep, therefore
242 "presence of goats" regardless of the presence of sheep was considered to be a plausible risk factor.
243 Each risk factor was tested for significant association with the serological status using the Chi-squared
244 test of association. Prevalence ratios and their 95% confidence intervals for herd and flock-level risk
245 factors were obtained, as shown in Table 1 and Table 2 respectively.
246 *2.5.3 Multivariable analysis*
247 Significant variables in the univariate analysis ($p \leq 0.05$) were assessed for collinearity by means of
248 Cramer's phi-prime (φ) statistic; variables were considered collinear if (φ) > 0.7). When a pair of
249 variables was found to be collinear, only the more biologically plausible variable was kept for further
250 analysis by means of logistic regression. Analysis was carried out considering the serological status of
251 the herd/flock as a binary outcome. The least significant variables were removed using a backwards
252 stepwise procedure when $p \geq 0.05$ and if removal of the variable did not alter the odds ratio of other

253 variables by more than 20%. The analysis was then repeated using forward selection starting with
254 variables with lowest *p* value in the univariate analysis to ensure that the same results were obtained.
255 Only variables with $p < 0.05$ were retained in the final model. Hosmer-Lemeshow χ^2 was used as a
256 goodness of fit test and the area under the Receiver Operating Characteristic (ROC) curve (AUC) was
257 obtained as a measure of the predictive ability of the model. Univariate and multivariable data analyses
258 were carried out using R (3.0.2) and STATA v.12.1 (STATA Corporation, Texas, USA).
259 *2.5.4 Spatial distribution of infection*
260 In order to visualize the geographic distribution of ruminant brucellosis in Jordan, two choropleth maps
261 representing the estimated true prevalence of seropositive small ruminant flocks and cattle herds per
262 governorate were created using ArcGIS 10 (ESRI, 2010).
263 **3. Results**
264 **3.1 Seroprevalence estimation**
265 A total of 2,664 blood samples from 333 small ruminant flocks were collected: 229 sheep flocks, 52
266 goat herds and 52 mixed (sheep and goat) flocks. On ten occasions owners refused to participate
267 because they did not want their animals to be sampled, and on five occasions flocks were not sampled
268 because they had previously been vaccinated against brucellosis.
269 With regard to cattle herds, a total of 1,810 milk samples were collected from 204 herds. Nine samples
270 per herd were obtained except on 12 occasions when the number of lactating cows in the herd was fewer
271 than 9. The *HSe* and *HSp* were estimated at 0.85 and 0.96 (sheep flocks), 0.85 and 0.96 (goat herds),
272 0.89 and 0.96 (mixed sheep-goat flocks), 0.84 and 0.94 (all small ruminant flocks), 0.85 and 0.96 (cattle
273 herds) and 0.92 and 0.97 (mixed herds of cattle and small ruminants). The true seroprevalence values of
274 herds/flocks with at least one seropositive animal were in ascending order: 18.1% (95% CI: 11, 25.3) in
275 cattle herds, 22.2% (95% CI: 16.5, 28.8) in sheep flocks, 38.5% (95% CI: 24.3-51.8) for mixed herds of
276 cattle and small ruminants, 45.4% (95% CI: 30.3, 61.6) in goat herds and 70.4% (95% CI: 55.5, 84.9) in
277 mixed sheep-goat flocks. The true prevalence across all small ruminant flocks was estimated as 34.3%
278 (95% CI: 28.4, 40.4) and. Figure 1 summarizes the seroprevalence estimates for the different types of
279 flocks and herds.
280 **3.2 Spatial distribution of infection**

Figure 2A shows the study area in relationship to other countries of the region and the distribution of seropositive cattle herds and small ruminant flocks in each of the 12 Jordanian governorates (Figures 2B and 2C respectively). The choropleth maps show that there was marked spatial variation in seroprevalence, which was higher for both small ruminant flocks and cattle herds in the northern governorates.

3.3 Risk Factor Analysis

Small ruminant flocks

Out of 14 studied variables, ten were significantly associated ($p<0.05$) with seropositive status in the univariate analysis, as shown in Table 1. The final logistic regression model included eight variables of which large flock size, presence of goats in the flock, lending/borrowing a ram for reproduction, feeding aborted material to dogs and introducing new animals into the flock in the previous year were associated with higher odds of seropositive status. Separating newly-introduced animals, disinfecting pens and isolating aborted animals were negatively associated with the odds of infection (Table 3). The Hosmer – Lemeshow test of goodness of fit was not significant ($p = 0.79$) and the AUC was 0.94, indicating that the model fit the data well and had a high predictive ability.

Cattle Herds

Six out of 12 studied variables were significantly associated ($p< 0.05$) with the serological status of cattle herds against *Brucella* spp. in the univariate analysis (Table 2**)**. The final logistic regression model included four variables: adding new animals during the last year was associated with higher odds of being positive and separating newly–introduced animals, having calving pens and herd disinfection were negatively associated with the odds of infection (Table 4**)**. As in the small ruminant model, the Hosmer-Lemeshow test was not significant ($p = 0.86$) and the high AUC (0.93) suggested that the model was good at discriminating between seropositive and seronegative herds.

4. Discussion

Brucellosis remains a major public health concern in the Middle East, where similar livestock systems, environmental conditions and cultural aspects are shared across countries (Refai, 2002; Gwida, 2010). Ruminants are assumed to be the main source of human infection and a number of control options exist, with vaccination and/or test-and-slaughter of positive animals being the cornerstones of most control

programs. The suitability of different control strategies largely depends on the baseline frequency and distribution of infection across ruminant subpopulations (FAO, 2009). It is therefore surprising that, to our knowledge, this is the first nationwide survey of ruminant brucellosis in the region formally designed to generate unbiased prevalence estimates.

The results of this study confirm that ruminant brucellosis is widespread in Jordan with approximately one in five cattle herds and one in three small ruminant flocks estimated to be seropositive in the absence of vaccination, and therefore presumed to be infected. Although comparisons are difficult due to different methodologies, our results are similar to those obtained in a smaller scale survey conducted in Jordan more than 10 years ago (Al-talafaha *et al*, 2003) and to estimates from neighbouring countries such as Egypt, where in 2008, Hegazy *et al*. (2011) estimated that 41% of sheep flocks and 32% of goat flocks in a Governorate of the Nile Delta were seropositive. Compared to other endemic countries in the Mediterranean such as Morocco, Greece and Turkey (Benkirane, 2006; Iyisan *et al*, 2000), the prevalence in Jordan appears to be higher which could partly be explained by less intensive control efforts to date. *Rev 1* vaccine is used for the vaccination of small ruminants, but vaccination coverage is minimal and no vaccine is used for cattle in Jordan. Based on our findings - during the process of identifying eligible flocks and herds for this study- only 1.5% of small ruminant flocks were vaccinated in 2013.

Brucella spp. is transmitted either in-utero or by direct contact between infected and susceptible animals (Radostits *et al*., 2000). The high seroprevalence estimates found in this study are indicative of long-term maintenance of infection within herds/flocks and/or high frequency of contact between flocks/herds as is likely to be the case in Jordan, where a considerable number of small ruminant flocks move freely across the country. This uncontrolled movement of flocks compromises the usefulness of vaccination and must be taken into consideration should the existing vaccination programme be revised. The higher seroprevalence found in small ruminant flocks compared to cattle herds indicates that introduction and/or within-herd/flock circulation of *Brucella* spp. following introduction is more intense in small ruminant flocks than cattle herds. A higher risk of introduction in small ruminant flocks could possibly be explained by the itinerant or semi-nomadic management of many of them. Furthermore, previous studies isolating *B. melitensis* suggest that this species, which is more adapted to small

ruminants (and in particular goats) than cattle, is the predominant strain in Jordan (Crespo, 1994; Elzer *et al*., 2002; Al-Talafhah *et al*., 2003; Al-Majali, 2005; Hawari, 2008; Samadiet *et al*., 2010). Accordingly, in our study small ruminant flocks with goats had much higher odds of positive status than flocks with sheep only (adjusted OR: 6.9; 95% CI: 3.1, 15.4).

The risk of seropositivity is heterogeneous across the country, with northern governorates having a higher seroprevalence, in both small ruminants and cattle. This is probably associated with higher livestock density in these governorates. Other factors such as different environmental conditions influencing the persistence of *Brucella* spp. in the environment and differences in husbandry systems may also contribute to the spatial variability in seroprevalence. Regional differences are of interest as they may offer opportunities for targeted control effort in those areas with higher prevalence, perhaps in combination with zoning/compartmentalization within the country as proposed by FAO and WHO (FAO, 2009).

As expected, a number of flock/herd characteristics were associated with the likelihood of a herd/flock being seropositive. The different statistical modelling approaches (binary vs. multinomial outcome, backwards vs. forward variable selection) yielded very similar results and the high AUC obtained for the binary model confirms that the model is a very good classifier of flocks or herds as either seropositive or seronegative.

The small ruminant binary model included five variables significantly associated with higher odds of a flock being seropositive and three variables significantly associated with lower odds of seropositivity (Table 3). It is reasonable that a larger flock size increases the risk of infection by increasing the contact rate between susceptible and infected animals (Coelho *et al*, 2008). The remaining factors highlight the importance of management and hygiene practices to mitigate the risk of introduction and/or persistence of *Brucella* spp. in a flock. Specifically, the implementation of biosecurity and quarantine measures and appropriate management of cases of abortion including proper disposal of aborted materials could make a significant contribution to any control strategy for ruminant brucellosis in Jordan. Our results strongly suggest that the facilitation and promotion of such practices should be part of a control programme in addition to the use of vaccines. The significant role of exchanging rams for service highlights the importance of including these animals in vaccination or other control programs as it has been shown that

rams can be infected with *Brucella* spp. in the absence of clinical signs such as orchitis and epididymitis (CFSPH, 2009).

The main risk factor for seropositivity in cattle herds was the introduction of new animals. Separation of newly-introduced animals, using disinfectant to clean pens and having calving pens significantly reduced the odds of infection. The interpretation of these findings is similar to those of small ruminant herds, highlighting the potential contribution of quarantine and restricted movement of animals from infected herds and hygienic management within the herd as important components of a control program. The use of milk rather than serum to detect the presence of antibodies in cattle was a limitation of the study as this meant that non-lactating animals including bulls and cows that had aborted were not sampled, which would have had an impact on herd-level sensitivity. The reasons for including only lactating cows were logistics, costs and acceptability by livestock keepers. However, our sampling and testing strategy is likely to have achieved a high sensitivity of 95% and we therefore considered the exclusion of non-lactating animals to be a reasonable approach.

Ruminant brucellosis is endemic at high levels in Jordan and although not quantified in this study, it is reasonable to assume that it poses a high public health burden on the Jordanian population and considerable financial losses for livestock keepers. The existing control programme relies on vaccination of small ruminants with *B. melitensis* Rev. 1 vaccine and achieves very low coverage. At the moment there is no vaccine used for cattle in Jordan. A revised control programme is needed and, given the high baseline prevalence, it is recommended that it is based on vaccination. Consideration should be given to concentrating control efforts in areas of higher prevalence or that are central in the network of uncontrolled animal movement. It seems reasonable that the control strategy focuses, at least initially, on small ruminants given that all *Brucella* spp. isolates from all host species (humans, sheep, goats, cattle and camel) so far have been *B. melitensis*. We have shown that the risk of flock/herd infection is highly dependent on biosecurity and hygiene practices. Based on our models, a small number of flock/herd attributes determine to a large extent whether the flock is infected or not. Accordingly, while vaccination should be the cornerstone of the control effort, it should be accompanied also by measures to facilitate and promote the adoption of hygiene and husbandry practices that minimise the risks of introduction and maintenance of *Brucella* spp. as well as the risk of human

infection. Some questions that could provide a stronger rationale for the formulation of an improved brucellosis control programme remain unanswered, in particular the incidence of human infection, the likely public health impact of reducing prevalence in ruminants, and the role of cattle in sustaining infection. Restricting vaccination to small ruminants could be justified if cattle simply act as spill-over hosts.

6 Conclusions

Brucellosis is endemic at high levels in domestic ruminant species in Jordan. The infection is heterogeneously distributed, with some farms at high risk as a result of practices such as exchanging rams for service and introducing new animals without quarantine. Mass vaccination of small ruminants, in addition to the adoption of hygiene and biosecurity practices, is recommended as a control strategy in Jordan.

Acknowledgments

The authors are grateful to Yousef Jamel scholarship for sponsoring this study as part of the PhD studentship of IM. However, this sponsor played no part in the study design, in the collection, analysis or interpretation of data, in the writing of the manuscript or in the decision to submit this manuscript for publication.

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478

Table 1 Descriptive statistics and univariate associations between potential flock-level risk factors and *Brucella* spp. serological status in small ruminants flocks in Jordan. Results from 333 small ruminant flocks included in a nationwide cross-sectional study carried out between May and October 2013.

Variable	Role[1]	Categories	No +ve/total (%)	*p*
Flock size	I&M	Small: ≤127 animals	21/111 (19)	0.01
		Medium: 127-200 animals	28/111 (25)	
		Large: >200 animals	47/111 (42)	
Flock species	I&M	Sheep only	43/229 (19)	< 0.01
		Goats (only or with sheep)	53/104 (51)	
Presence of a dog (if the flock owner keeps a dog with the flock)	I&M	No	13/57 (23)	0.27
		Yes	83/276 (30)	
Presence of equines (horses or donkeys)	I&M	No	30/90 (33)	0.21
		Yes	66/243 (27)	
Mix with other flocks for water or grazing, regularly	I	No	69/284 (24)	0.01
		Yes	27/49 (55)	
Lending /borrowing ram for service	I	No	72/286 (25)	< 0.01
		Yes	24/47 (51)	
Flock type	I	Non- nomadic	83/318 (26)	0.01
		Nomadic	13/15 (87)	
Introducing new animals to the flock in the previous year	I	No	19/153 (12)	0.01
		Yes	77/180 (43)	
Newly-introduced animals are kept in separate pen or house for a certain period	I	No	89/239 (37)	0.01
		Yes	7/94 (7)	
Pen disinfection (whether pens are regularly cleaned with disinfectant)	M	No	75/181 (41)	0.01
		Yes	21/152 (14)	
Using sponges for oestrus synchronization	M	No	30/103 (29)	0.93
		Yes	66/230 (29)	
Disinfect synchronization gun between animals	M	No	33/72 (31)	0.36
		Yes	33/92 (26)	
Isolation of aborted animals (kept separate for some time after abortion)	M	No	83/192 (43)	0.01
		Yes	13/141 (9)	
Feeding aborted material to dog	M	No	25/225 (11)	0.01
		Yes	71/108 (66)	

[1] Plausible role as risk factor for seropositive status: facilitates introduction of *Brucella* into the flock (I), facilitates maintenance of *Brucella* following introduction (M), facilitates both, introduction and maintenance (I&M)

485 **Table 2** Descriptive statistics and univariable associations between potential herd-level risk factors and

486 *Brucella* spp. serological status in cattle in Jordan. Results from 204 cattle herds included in a nationwide

487 cross-sectional study carried out between May and October 2013

Variable	Role[1]	Categories (code)	No. +ve/ total (%)	*p*
Herd size	I&M	Small: <27 animals	9/ 68 (14)	
		Medium 27–44 animals	17/ 68 (25)	0.09
		Large >44 animals	12/68 (18)	0.49
Presence of small ruminants (if the herd owner keeps sheep or goats within the herd)	M	No	24/ 171 (15)	0.01
		Yes	14/33 (43)	
Method of service (What is the method of service that herd owners use regularly)	I&M	Natural	30/179 (17)	0.09
		AI	8/25 (32)	
Borrowing /lending bulls for service	I	No	32/181 (18)	0.34
		Yes	6/23 (27)	
Introducing new animals to the herd in the previous year	I	No	3/86 (4)	0.01
		Yes	35/118 (30)	
Separate new animals (when new animals are introduced from other herds they are always or almost always kept in a separate pen or herd for a certain period of time)	I	No	30/62 (49)	0.01
		Yes	8/142 (6)	
Having calving pens	M	No	31/97 (32)	0.01
		Yes	7/107 (7)	
Herd disinfection (whether the herd owner applies disinfectant to clean herd pens routinely)	M	No	32/75 (43)	0.01
		Yes	6/129 (5)	
Having visitors on the farm, regularly	I	No	19/104 (19)	0.89
		Yes	19/100 (19)	
Isolating aborted animals (keep aborted animals separate in a place for a period of time)	M	No	24/97 (25)	0.04
		Yes	14/107 (14)	
Feeding aborted material to dog	M	No	29/168 (18)	0.29
		Yes	9/36 (25)	
Throwing aborted material in fields	M	No	24/150 (16)	0.12
		Yes	14/54 (26)	

488 [1] Plausible role as risk factor for seropositive status: facilitates introduction of Brucella spp. into the herd (I),

489 facilitates maintenance of Brucella spp. following introduction (M), facilitates both, introduction and

490 maintenance (I&M)

491 **Table 3** Results of a multivariable logistic regression on serological status of small ruminant flocks against

492 *Brucella* spp. (333 small ruminant flocks included in a nationwide cross-sectional study carried out between

493 May and October 2013 in Jordan)

Variable (category)	**Odds Ratio**	**95% CI**	***p***
Lending/Borrowing ram (yes)	8.9	3.0, 26.1	< 0.01
Feeding aborted material to dog (yes)	8.0	3.5, 18.1	< 0.01
Flock species (flocks with goats)	6.9	3.1, 15.4	< 0.01
Introducing new animals to the flock in the last year (yes)	5.8	2.5, 13.6	< 0.01
Flock size (linear trend)	2.2	1.0, 4.6	0.04
Separate newly-introduced animals (yes)	0.16	0.05, 0.47	< 0.01
Isolating aborted animals (yes)	0.19	0.08, 0.46	< 0.01
Pen disinfection (yes)	0.37	0.16, 0.83	0.02

494 [1] Hosmer – Lemeshow $\chi^2 = 4.67$; $p = 0.79$ at 8 d.f.; AUC = 0.94

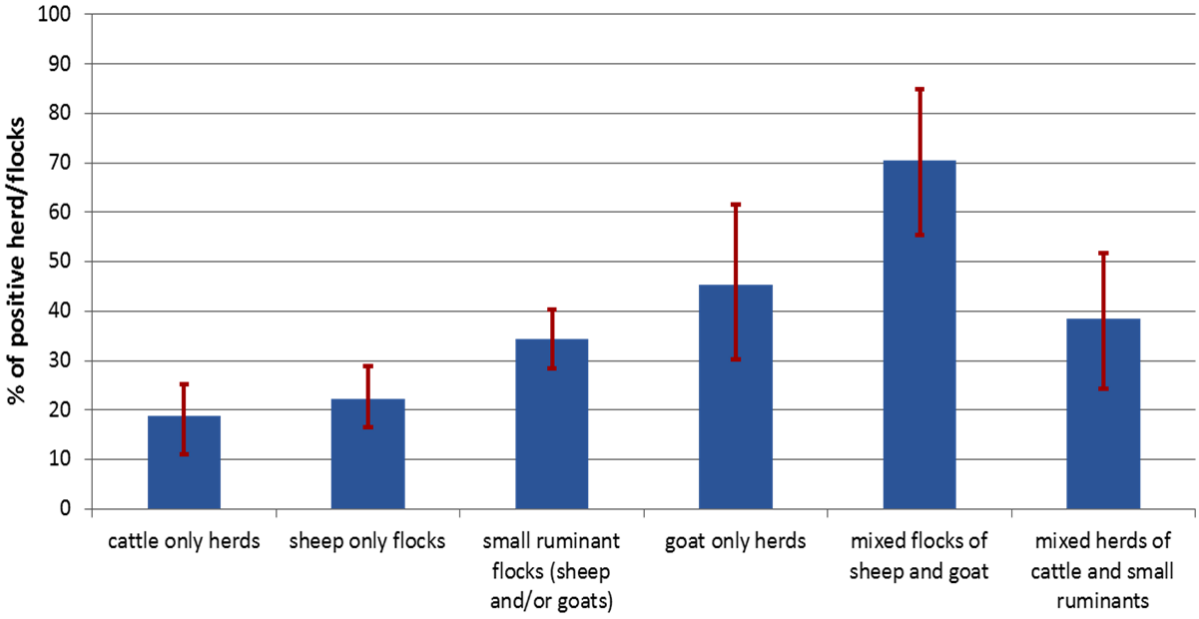
495

Table 4 Results of a multivariable logistic regression on serological status of cattle herds against *Brucella* spp. (204 cattle herds included in a nationwide cross-sectional study carried out between May and October 2013 in Jordan.)

Variable (category)	**Odds Ratio**	**95% C.I.**	***p***
Adding new animals to the herd in the last year (yes)	11.7	2.8, 49.4	< 0.01
Herd disinfection (yes)	0.04	0.01, 0.15	< 0.01
Separate newly-introduced animals (yes)	0.09	0.03, 0.29	< 0.01
Having calving pens (yes)	0.14	0.05, 0.43	< 0.01

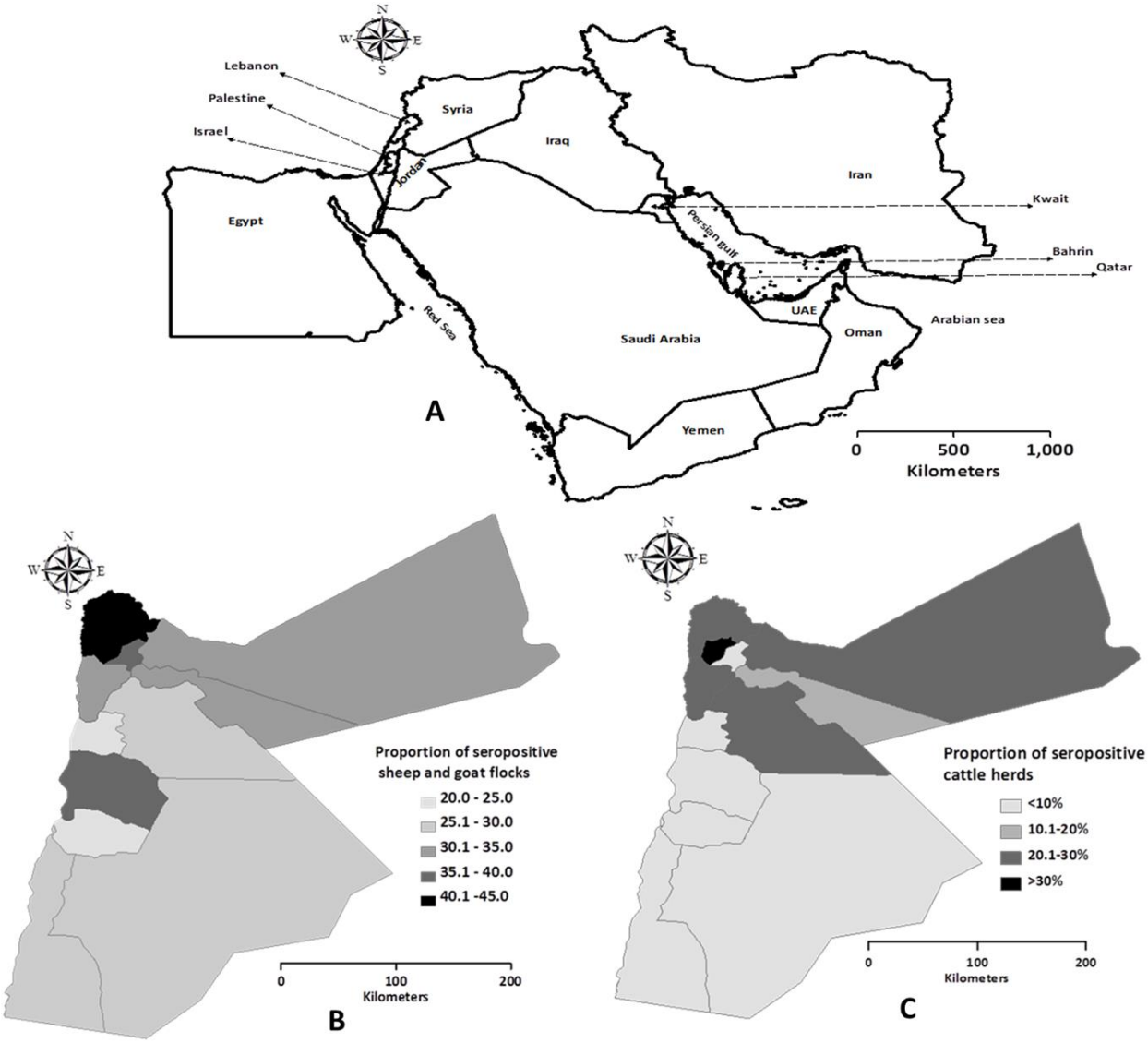
[1]Hosmer – Lemeshow χ^2= 3.24, p= 0.86, at 7d.f.; AUC = 0.93

Figure 1 Estimated true seroprevalence of herd/flocks by species of animals and 95% Confidence Intervals of the estimates.



505 **Figure 2** Choropleth maps representing the study area (A) and the estimated true prevalence of seropositive

506 small ruminant flocks (B) and cattle herds (C) in the Jordanian Governorates (May – October, 2013)**.**



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