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ORIGINAL RESEARCH

Brucellosis among smallholder cattle farmers in Zambia

Public health significance

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Abstract A cross-sectional study was performed in Southern and Lusaka provinces of Zambia between March and September 2008 to estimate *Brucella* seroprevalence in cattle kept by smallholder dairy farmers (n=185). Rose Bengal test (RBT) was used as a screening test followed by confirmation with competitive ELISA (c-ELISA). We investigated 1,323 cattle, of which 383 had a history of receiving vaccination against brucellosis and 36 had a history of abortion. Overall seroprevalence was 6.0% with areas where vaccination was practiced having low seroprevalence. Age was associated with *Brucella* seropositivity (P=0.03) unlike cattle breed (P=0.21) and sex (P=0.32). At area level, there was a negative correlation (Corr. coeff=−0.74) between percentage of animals with brucellosis vaccination history (vaccination coverage) and level of brucellosis; percentage of animals with history of abortion (Corr. coeff.=−0.82) and brucellosis vaccination coverage. However, a positive correlation existed between brucellosis infection levels with percentage of animals having a history of abortion (Corr. coeff. = 0.72). History of vaccination against brucellosis was positively associated with a positive *Brucella* result on RBT (P=0.004) whereby animals with history of vaccination against brucellosis were more likely to give a positive RBT test results (OR=1.52). However, the results of c-ELISA were independent of history of *Brucella* vaccination (P=0.149) but was positively associated with history of abortion (OR=4.12). Our results indicate a relatively low *Brucella* seroprevalence in cattle from smallholder dairy farmers and that vaccination was effective in reducing cases of *Brucella* infections and *Brucella*-related abortions. Human exposure to *Brucella* through milk from smallholder farmers could result through milk traded on the informal market since that milk is not processed and there no quality and safety controls.

Keywords Brucellosis · Cattle · Smallholder farmer · Vaccination · Abortion · Zambia

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Introduction

The Zambian cattle industry is broadly divided into two main subsectors: commercial and traditional and has a skewed geographical distribution country wide with livestock concentration present in Western, Southern and Eastern provinces. Commercial dairy production is mainly undertaken along the line of rail by large- and medium-scale farmers. Milk production in the commercial sector is predominantly from Friesian and Holsteins cows, which have an average yield of 25 l/day (Kaluba 1992). Previously, small-scale milk production was mainly done in smallholdings but has now expanded to include traditional farmers who often raise their animals on communal land. Milk in the traditional sector is produced from local cattle, mostly of the Sanga and Zebu types crossed with Tonga, Barotse and Angoni breeds. Mixed dairy crosses between exotic and traditional cattle are also used. Milk yields from traditional cattle are relatively low and range from 2 to 3 l/day (Pandey 2008). Estimates also indicate that

Friesian × indigenous crosses give an average daily yield of 6 l (Kaluba 1992). The traditional sector has the majority of cattle in Zambia and yet contributes a minimal percentage to the national milk market. Milk-production in this sector, therefore, holds a potential to enhance the livelihoods of these communities as an income-generating or employment-creating venture. Traditionally, milk produced in this sector is consumed at home as part of the regular diet but in recent years, some traditional farmers, especially those located along the line of rail, have formed cooperatives through the initiative of Government and non-governmental organisations and are now supplying milk to the processors (Pandey 2008).

Despite all these advances made in the smallholder dairy sector, one area that is requiring attention is the public health aspects of milk production and consumption, especially with respect to transmission of milk-borne zoonoses such as brucellosis and tuberculosis. This is important, especially in situations as existing in Zambia, where a large proportion of milk is sold through informal trade either raw or cultured milk. Further, the recent progress made by some of the smallholder dairy cooperatives, where they have started processing yoghurt and other dairy products, brings to the fore the need to prioritise implementation of sanitary measures in order to protect the public. Among the most important milk borne zoonoses is brucellosis which has been reported in Zambia both in the commercial (Gallagher 1973; Chimana et al. 2010) and traditional sectors, with seroprevalence estimate of 8% (95% CI, 4–11) and 19 (95% CI, 14–28), respectively (Ghirotti et al. 1991; Muma et al. 2006). The prevalence of bovine tuberculosis, which is also an important milk-borne zooneses, is estimated at about 6.8% (95% CI, 4.2–9.5%) among traditional cattle in Southern Province of Zambia (Munyeme et al. 2009) and this further underscores the importance of reducing the public health risk of milk-borne zoonoses from traditional cattle. Prevention of brucellosis in Zambia has traditionally been done through vaccination of cattle with Brucella S19 vaccine, especially among commercial farmers (Gallagher 1973; Schuurman 1983). More recently however, RB51 has also been introduced on the Zambian market. The zoonotic importance of brucellosis has been indicated by the reported human seroprevalence which is estimated to range from about 1–5% in the Southern Province of Zambia where this study was conducted (Muma et al. 2006; Orino et al. 1994). It has been observed that smallholder dairy farmers, especially in the traditional sector, have little knowledge of the zoonotic risk associated with milk consumption (Muma et al. 2008; Munyeme et al. 2010), the trend observed not only in Zambia but also in other countries (Mosalagae et al. 2011). It is generally recognised that, that control of brucellosis in animals results in reduced public risk of exposure (WHO 1981, 2006; Zinsstag et al. 2007). Therefore, disease surveillance, prevention and control need to be sustained to ensure production of safe milk. Serological tests are often used in brucellosis survey, since they are easy to perform (Mohan et al. 1996; Nielsen 2002). In this study, we conducted a serological study of cattle brucellosis among smallholder farmers linked to dairy cooperatives in Southern and Lusaka provinces of Zambia in order to evaluate the risk of brucellosis that could be associated with consumption of raw milk.

Materials and methods

Study area

We conducted a cross-sectional study between March and September 2008 in four districts of Southern province and two of Lusaka province of Zambia. These districts were selected because of the high numbers of smallholder dairy cattle farmers supplying milk to processing companies in Lusaka mainly through cooperatives and also selling milk locally in their communities. The farmers owned different types of animal breeds including traditional, dairy crosses and exotic. Therefore, milk from these animals is of public health significance since a large proportion of it is sold to the general public. A smallholder dairy farmer was defined as any farmer with cattle which was milked for sale to processors, mostly through a local cooperative or to the local communities. The sampling frame was based on a list of farmers obtained from cooperatives. Four districts from Southern province, namely, Mazabuka, Monze, Choma, Kalomo, and two districts from Lusaka province (Lusaka and Kafue) were included in the study as majority of smallholder farmers came from these areas.

Animal sampling

The study design, sampling of herds and individual animals have been described in details elsewhere (Chimana et al. 2010). There were approximately 499 smallholder dairy farmers in the study areas with an average herd size of four animals per herd. We used a convenient sampling, where all farmers who supplied milk to cooperatives were included, as the study was meant to evaluate the zoonotic risk of *Brucella* infections from milk supplied by smallholder farmers. We assumed that brucellosis among smallholder farmers existed at 8% (Chimana et al. 2010). The diagnostic sensitivity and specificity for the Rose Bengal test (RBT) were assumed to be 90% and 75%, and for competitive ELISA (c-ELISA), 98% and 99%, based on previous studies (McGiven et al. 2003; Muma et al. 2007b; Nielsen et al. 1995). Therefore, at individual animal level, the

combined sensitivity and specificity for RBT and c-ELISA in serial (sequential) interpretation were calculated at 88.2% and 99.8%, respectively. Based on the above assumptions, we estimated a sample size of 113 farmers (herds) using the simple random formula (Dohoo et al. 2003), given that our allowable error was set at 5%. We decided to sample at least ten animals from each herd, but for smaller herds (<10 cattle), all animals were sampled while for larger herds (>100 cattle) we sampled at 10% sampling fraction although in some herds more than 10% were sampled. Therefore, our projected sample size was 1,130 animals from 113 farmers assuming that each farmer had at least ten animals.

Only animals aged over ≥2 years were tested since brucellosis is a disease of sexually mature animals. For each animal, information on sex, age, parity and history of abortion in case of cows, was recorded on sample data sheets during blood collection.

Laboratory analysis

Blood samples were shaded for about 10 min to allow for clotting and then maintained at +4°C until processing. In the laboratory, sera were separated by centrifugation at 2,500 rev/min for 15 min (503×*g*) and stored in 2-ml cryovials at −20°C until laboratory tests were performed. Antibodies to *Brucella* spp. were detected by sequential testing of samples using RBT for screening and c-ELISA for confirmation. RBT was done as described by Alton et al. (1975) using standardised *Brucella abortus* antigen obtained from Onderstepoort Veterinary Institute, South Africa. Svanovir™ Brucella-Ab c-ELISA kits (Svanova Biotech, Uppsala, Sweden) were used to determine *Brucella* antibody titers. The threshold for determining seropositivity was according to the manufacturer's recommendations. Antibody titres were recorded as percentage inhibition equivalents of absorbance readings. An animal was considered to be positive if it tested positive on both RBT and c-ELISA.

Data analysis

The database was established in Excel®, and necessary data manipulation done using the same program before transferring to Stata SE 11 for Windows (Stata Corp., College Station, TX). The database included information about each animal. Since age was skewed, we corrected this linearity problem by generating a new variable, "Age-category" by assigning animals to age groups based on quartiles of the number of animals sampled. Overal seroprevalence and seroprevalence according to districts, with 95% confidence intervals, were computed using the survey command estimates in Stata, with adjustments for strata (study areas), primary sampling unit (psu) (herd) Dohoo et al. (2003). To take into account the the effect of imperfect tests true seroprevalance was estimated using the @Risk software with Monte Carlo simulation using 1,000 interations with the following model settings, given the number of positive animals (r) out of the total number of animal tested (n): RiskBeta (α_1,α_2), where $\alpha_1=r+1$ and $\alpha_2=n-r+1$; Test sensitivity, RiskUniform(*min*,*max*), where *min* is the minimum value of sensitivity (Se=88.4) and *max* is the maximum value (100%). The influence of sex and breed on seropositivity was assessed using the Fisher's exact test while the *t*-test was used to compare means between the animals in the *Brucella* positive and negative categories. Relationship between abortion and vaccination were investigated using two-way scatter plots and regression lines (best fit).

Results

We investigated 1323 animals from smallholder dairy farmers (n=185) from Lusaka and Southern provinces, which included traditional (n=620; 46.9%), dairy crosses (n=577; 43.6%) and exotic dairy (n=126; 9.5%). Out of 1,311 animals, 383 (29.2%) had a history of receiving vaccination against brucellosis but information was missing for 12 animals. Similarly, 2.8% (36/1,302) had a history of abortion and information was missing for 21 animals. Overall apparent seroprevalence was estimated at 6.0% and showed some variation across study areas (P=0.058) (Table 1) with areas where vaccination was practiced having low prevalence. When the effect of the imperfect test was taken into account, overall true seroprevalence was estimated at 5.7% (95% CI, 3.4–8.2%) using @Risk which was very similar to the estimated apparent seroprevalence. Age was associated with *Brucella* seropositivity (P=0.03), whereby the mean age among the *Brucella* negatives was 5.5 years (range, 2–13 years) and that of among the *Brucella* positives was 6.0 year (range, 3–13 years). However, *Brucella* seropositivity was not associated with animal breed (P=0.213) and sex (P=0.315).

At the level of study area, there was a negative correlation (Corr. coeff=−0.74) between percentage of animals with history of receiving brucellosis vaccination and percentage of animals with a positive brucellosis test (Fig. 1); and between percentage of animals with history of abortion (Corr. coeff.=−0.82) and percentage of animals with a history of vaccination against brucellosis (Fig. 2). However, a positive correlation existed between the percentage of animals with history of abortion (Corr. coeff.=0.72) and the percentage of animals with a positive brucellosis test (Fig. 3). We also observed a positive association between having a history of vaccination against brucellosis and a positive *Brucella* result on

Table 1 Distribution of proportion of cattle with history of abortion, vaccination against brucellosis and *Brucella* seroprevalence, with 95% confidence intervals, in smallholder dairy cattle (n=1,323) in Southern and Lusaka provinces of Zambia (2008)

Study area	No. of animals sampled (herds)	Proportion with vaccination history	Proportion with abortion history	Proportion seropositive (%)
Batoka	97 (1)	14.3 (9.7–33.4)	3.3 (0.0–6.8)	4.0 (0.0–9.3)
Choma	194 (15)	0.00	5.0 (2.1–7.9)	6.5 (3.0–10.0)
Kalomo	111 (13)	30.8 (5.1–56.5)	1.9 (0.3–3.6)	7.0 (3.3–10.6)
Magoye	245 (45)	100	0.0	0.0
Mapepe	100 (17)	45.4 (13.4–77.3)	3.6 (1.3–5.9)	6.7 (3.4–10.0)
Monze	576 (94)	7.2 (0.0–18.9)	2.7 (0.0–6.4)	5.4 (0.0–11.6)
Overall	1,323 (185)	29.2 (12.6–45.9)	2.8 (1.7–3.9)	6.0 (4.0–8.0)

RBT (P=0.004) whereby animals with history of vaccination against brucellosis were more likely to give a positive *Brucella* antibody test (OR=1.52) compared to those without such a record. However, the results of c-ELISA were independent of history of *Brucella* vaccination (P=0.149) but were positively associated with history of abortion (P=0.039) with animals having a history of abortion being more likely (OR=4.12) to test positive on c-ELISA. There was no positive statistical association between RBT and history of abortion (P=0.11). The performance of RBT and c-ELISA in different categories of animals are presented in Table 2. On average, for every three positive RBT animals, only one tested positive on c-ELISA, except among the abortive animals were the ratio was close to one (Table 2).

Discussion

We investigated brucellosis seroprevalence among smallholder dairy farmers in Southern and Lusaka provinces of Zambia to assess the potential zoonotic risk of *Brucella* transmission to the public since this disease is mainly transmitted from infected animals to human through consumption of contaminated milk (Lulu et al. 1988; Makita et al. 2008; Shaalan et al. 2002). We used a serial interpretation of RBT and c-ELISA which increased the specificity (Sp) of the test regime and reduced sensitivity (Se). This was important considering that we included even animals that were vaccinated against brucellosis using S19 vaccine. The good agreement between our apparent seroprevalence and the true seroprevalence obtained by simulation in @Risk indicated that the test regime of screening animals with RBT and confirming with c-ELISA gives a good estimation of the true seroprevalence. As would be expected, the seroprevalence results based on RBT alone were very high compared to that of c-ELISA and results from this assay were correlated with those of history of vaccination against brucellosis (Gallagher 1973). The RBT is very sensitive and sometimes give a positive result because of S19 vaccination or of false-positive serological reactions with lipopolysacharide (LPS) of *Yersinia enterolitica* O:9 and *Escherichia coli 0157*:OH

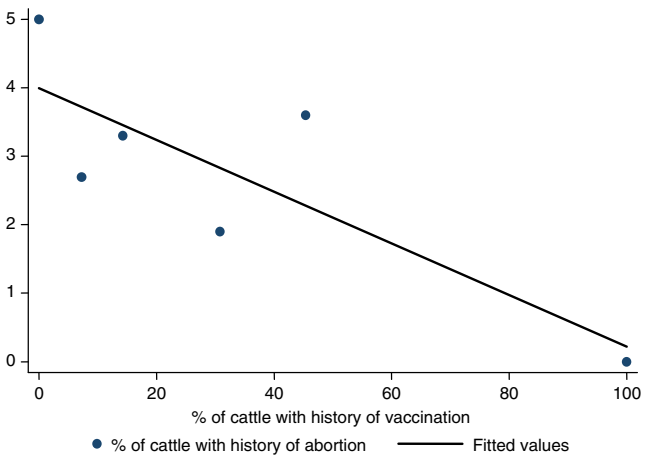

Fig. 1 Negative correlation between percentage of cattle with a history receiving Brucella vaccination and percentage with history of abortion in six study areas where the number of cattle abortions decreased with increasing vaccination coverage

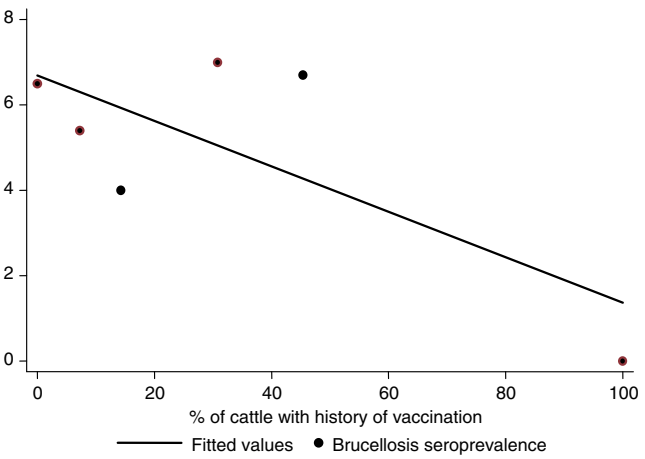

Fig. 2 Negative correlation between percentage of cattle with a history of receiving Brucella vaccination and percentage of cattle testing positive for brucellosis in six study areas where brucellosis seroprevalence decreased with increasing vaccination coverage

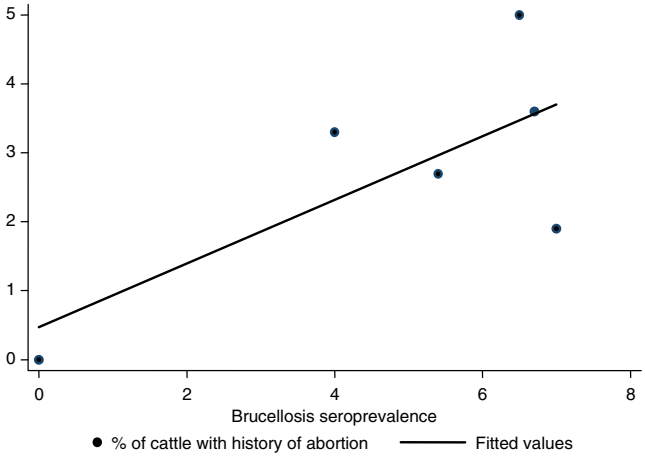

Fig. 3 Positive correlation between percentage (proportion) of cattle with a history of abortion and percentage with a positive brucellosis test in six study areas where the number of animals experiencing abortions increased with increasing brucellosis seroprevalence

(Nielsen et al. 2004, 2006), and is therefore more useful as a herd test (OIE 2010). The fact that all c-ELISA positive sera also tested positive on RBT, demonstrate that it is a useful screening test as earlier observed (Gallagher 1973). On the other hand lack of association between animal vaccination status and c-ELISA test result provides further evidence that the c-ELISA is able to discriminate between antibody production due to vaccination and those from field strains (OIE 2010).

The prevalence observed in this study is similar to that observed among the smallholder farmers in Zimbabwe where a seroprevalence of 5.6% (95% CI, 4.4–6.8%) was observed (Matope et al. 2010) and is also close to the 8% (95% CI, 4–11%) observed among commercial cattle in Lusaka province (Chimana et al. 2010). However, the estimate is significantly lower than that reported in traditional cattle raised in the wildlife/livestock interface of the Kafue flats (Muma et al. 2006). Considering that the animals under investigation are a source of milk supplied to milk cooperatives and also directly to the public, the observed seroprevalence should still be of public health concern. Much of the concern could come from milk traded through the informal market, where there are no safety and quality controls. For milk sold to cooperative, there is a legal requirement in Zambia that milk is sourced from brucellosis and tuberculosis negative animals. This milk entering this chain is normally sold to processing companies and poses little threat to consumers since it undergoes pasteurisation.

We observed a negative correlation between history of vaccination against brucellosis (vaccination coverage) and percentage of animals reacting positive for brucellosis, indicating that vaccination against brucellosis had a protective effect. Vaccination of cattle with S19 is estimated to have a protective efficacy of at least 65% in adult vaccination (Nicoletti 1977) and close to 90% in calf hood vaccination, taking into account potential losses due to cold chain deficiency (Cocks and Davies 1980). Further, we observed negative correlation between frequency of history of vaccination and frequency of history of abortion which indicate that vaccination had a beneficial effect of reducing abortion rates and corroborates what has earlier been observed (Gallagher 1973).

Likewise, we observed positive correlation between percentage of animals with history of abortion and percentage of animals with a positive brucellosis test which provides further evidence that infection due to *Brucella* spp. accounts for a significant proportion of the observed abortions in cattle in Zambia (Muma et al. 2007a). Similar, observations have been made elsewhere in brucellosis endemic countries (Ibrahim et al. 2010; Matope et al. 2011). It is therefore reasonable to hypothesize that brucellosis accounts for a significant proportion of abortions in the study areas and could account for some economical losses through reduced calf-crop and reduced milk production.

Our results indicate relatively low *Brucella* seroprevalence in among smallholder dairy farmers and that vaccination was effective in reducing cases of *Brucella* infections and *Brucella*-related abortions. We therefore recommend that compulsory annual vaccination, including regular testing and elimination of positive cows, be enforced in order to reduce the prevalence of animal brucellosis which will subsequently result in reduced risk of human exposure.

Table 2 Relationship between RBT and c-ELISA test positive proportions with distribution of history of vaccination and abortion among smallholder dairy cattle (n=1,323) in Zambia (2008)

Category	Level	Rose Bengal test (%)	c-ELISA test (%)	RBT/c-ELISA relative ratio
History of vaccination	Not vaccinated	17.1 (13.1–21.0)	5.7 (3.6–7.8)	3.0
	Received vaccination	21.1 (7.0–35.3)	7.8 (1.8–13.9)	2.7
	Unknown status	11.4 (7.2–15.6)	4.2 (1.5–9.9)	2.7
History of abortion	No abortion	16.7 (12.0–21.4)	5.5 (3.4–7.6	3.0
	Abortion present	27.8 (13.3–42.3)	19.4 (4.4–34.5)	1.4

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