

A comparative study of the seroprevalence of brucellosis in commercial and small-scale mixed dairy-beef cattle enterprises of Lusaka province and Chibombo district, Zambia

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Abstract

A cross-sectional study was conducted between January 2007 and February 2008 to estimate seroprevalence of brucellosis and identify risk factors associated with *Brucella* infections in commercial cattle in 3 districts of Lusaka province (Chongwe, Luangwa, Kafue; $n=849$) and in one rural district from the Central province ($n=48$). A total of 897 serum samples were randomly collected from 55 farms along with animal level data such as sex, age and parity. Sera were screened for presence of anti-*Brucella* antibodies using Rose Bengal Test (RBT) and positive samples were confirmed using competitive enzyme linked immunosorbent assay (c-ELISA). At animal level, seroprevalence was estimated at 7.9 % (95% CI: 4.4-11.4%) in the Lusaka province and 18.7 % (95% CI: 7.5-29.9%) for Chibombo district. Brucellosis seroprevalence varied according to district, with Chongwe district recording the highest compared to other districts. Seroprevalence also varied according to sex with bulls ($n =96$) having higher seroprevalence (12.5%; 95% CI: 3.8-21.1 %) compared to females (8.1%; 95% CI: 4.6-11.6). Similarly, seroprevalence varied according to age groups, with the age category 1-4 years recording the highest (10.7%). The study recorded relatively low *Brucella* seroprevalence in commercial farms in Lusaka, compared to the traditional small-scale farms. We suggest that testing and stamping out of infected animals is likely to improve the situation and significantly reduce the public health risk associated with *Brucella* infections in animals.

Key words: brucellosis, commercial cattle, Lusaka province

Abbreviations

c-ELISA	Competitive enzyme linked immunosorbent assay
RBT	Rose Bengal test
Se	Sensitivity
Sp	Specificity
ZNFU	Zambia national farmers union

Introduction

Cattle brucellosis is usually caused by *Brucella abortus* biovars and occasionally by *B. melitensis* (OIE, 2008). The disease is mainly characterised by abortion, stillbirths or weak calves and lactating cows may show decrease in milk yield (Matope et al., 2010b; Muma et al., 2007a). In bulls brucellosis may manifest as unilateral or bilateral orchitis and sterility, while in all age groups, hygromata involving one or more leg joints may be observed (OIE, 2008). Brucellosis is transmitted among animals mainly through ingestion of contaminated feed and water, and occasionally by inhalation of aerosols or by direct contact with infected materials (Maurin, 2005; McDermott and Arimi, 2002). (Muma et al., 2007a) observed that brucellosis contributed to low livestock productivity in traditional cattle and further, reported, a seroprevalence of 5% in humans, signifying the zoonotic importance of brucellosis in Zambia (Muma et al., 2008). Since consumption of raw milk is a tradition practiced by people in cattle producing areas of Zambia, it is important to understand animal brucellosis in order to have insight in the associated public health risks. Despite the slaughterhouse survey by Ahmadu et al., (1999), information on brucellosis in the commercial sector is lacking. The aim of this study was to estimate and compare the seroprevalence of brucellosis in commercial cattle farms in Lusaka province and a rural district of Chibombo in Central province.

Materials and methods

Study sites and design

A cross sectional study was conducted between January 2007 and February 2008 in 3 districts of Lusaka province located in central and southern parts of Zambia. These districts were selected because of the high number of commercial cattle farms supplying milk to Lusaka city. A commercial farm was defined as a farm where cattle were reared not only for subsistence, but also for sale of milk or animals. The sampling frame was based on a list of farmers obtained from Zambia National Farmers Union (ZNFU). Three districts namely, Lusaka (6 farms), Kafue (25 farms) and Chongwe (19 farms) were included in the study. Chibombo district with small-scale farms (5 farms) in the Central province was included for comparison purposes because it supplies milk to Lusaka city. There were approximately 95 commercial farms in the study areas with an average herd size of 350 animals. Using the procedure of (Dohoo et al., 2003), a sample size of 76 herds was calculated from the study areas by assuming that brucellosis existed at 17% (Muma et al., 2006). The sample sizes of individual animals per farm were estimated using Herdacc® (Jordan, 1995), by assuming that the sensitivity (Se) and specificity (Sp) for RBT were 90% and 75% (Muma et al., 2007c), and the c-ELISA, Se and Sp 98% and 99%, respectively (Nielsen et al., 1996). Based on the above assumptions, herd sensitivity (Hse) and herd specificity (Hsp) were estimated at 98.0% and 100%, respectively, when a 10% sampling fraction was used and farms classified as positive when at least a single brucellosis positive animal was detected.

Sampling procedure and laboratory analysis

Cattle were selected by systematic random sampling and only animals ≥ 2 years, with no history of vaccination against brucellosis were included. In herds with ≤ 10 animals all were sampled and

blood samples were taken. During blood sampling, information on sex, age, parity, breed and identity was obtained.

The separated sera were stored in 2.5ml cryovial tubes at – 20 °C until analysis. The RBT conducted as previously described (OIE, 2008) was used to screen sera for anti-*Brucella* antibodies. The antigen was obtained from Onderstepoort Veterinary Institute, South Africa. The Svanova c-ELISA (Svanova® Biotech AB, Uppsala, Sweden) kits were used for confirmation of RBT positive cases. The c-ELISA was done according to the manufacturer’s instructions as described by Muma et al., (2006).

Data analysis

Data was analysed using STATA® version SE 10.0 (StataCorp., Texas, USA). Only animals positive on both RBT and c-ELISA were classified *Brucella* seropositive. The proportion seropositive was estimated using the survey command in STATA®. The association between *Brucella* seropositivity and the various animal level risk factors were investigated using the Fisher’s exact test in univariable analyses.

Results

A total of 897 cattle from 55 farms were investigated during the study period (Table 1). The estimated overall seroprevalence in Lusaka province was 7.9 % (95% CI: 4.4-11.4) while that for Chibombo district was 18.7% (95% CI: 7.5-30.0) (Table 2). Seroprevalence in the Chibombo district, which is more rural, was even higher (Table 2). At animal level, seroprevalence varied according to sex with bulls 116 (n=96) having slightly higher odds of being seropositive (OR=1.7) compared to females; according to age groups with the age category 1-4 years recording the highest seroprevalence (10.7%; 95% CI: 4.9-16.9%) (Table 3), and according to district (p<0.001) with Chongwe district recording the highest in Lusaka province.

Table 1 Serum samples collected from commercial cattle (n =897) in the province of Lusaka between January 2007 and February 2008

Study area (District)	No. of farms targeted	of Targeted number of cattle	No. of farms sampled	Actual number of cattle sampled	Median herd size
Kafue	19	190	25	427	66
Chongwe	19	190	19	359	58
Lusaka	19	190	6	63	25
Chibombo	10	100	5	48	15
Total	67	670	55	897	41

Table 2 Brucellosis seroprevalence estimates in commercial cattle in the Lusaka province ($n = 849$) and Chibombo district ($n = 48$), January 2007-February 2008

Study area (District)	Number of serum samples tested on RBT	% Positive on RBT (95% CI)	% Positive on c-ELISA (95% CI)	% Individual seroprevalence (95%CI)*	% Herd brucellosis seroprevalence (95% CI)
Kafue	427	7.5 (0.4, 14.6)	4.9 (0.0, 10.0)	4.0 (0.0, 9.3)	20.0 (3.0, 36.1)
Chongwe	359	22.3 (13.9, 30.7)	12.8 (7.9, 17.7)	12.8 (8.2, 91.7)	70.0 (49.2, 90.7)
Lusaka	63	6.3 (0.0, 13.0)	3.2 (0.0, 6.5)	3.1 (0.0, 6.5)	40.0 (4.3, 84.3)
Overall (Lusaka province)	849	13.7 (7.8, 19.5)	8.1 (4.5, 11.8)	7.9 (4.4, 11.4)	100% (0.0, 0.0)
Chibombo (Central province)	48	25.0 (10.7, 39.3)	18.8 (7.5, 30.0)	18.7 (7.5, 29.9)	46.2 (32.5, 60.0)

*Serial interpretation of RBT and c-ELISA results

Table 3 Brucellosis seroprevalence in cattle at animal level for the province of Lusaka according to age group during the period January 2007 and February 2008 (Serial interpretation of Rose Bengal test and competitive ELISA)

Variable	Level	Seroprevalence (95% CI)
Sex	Oxen ($n = 10$)	0 (0.0, 0.0)
	Cows ($n = 791$)	8.1% (4.6, 11.6)
	Bulls ($n = 96$)	12.5% (3.8, 21.1)
Age category	1-4 years	10.7% (4.9, 16.9)
	4.5-5 years	4.2% (0.0, 7.8)
	5.5-7 years	6.0% (2.0, 9.9)
	>7 years	9.9% (2.3, 17.0)

Discussion

In this study, brucellosis seroprevalence in Zambia was investigated in commercial cattle in Lusaka province in comparison to the rural district of Chibombo. Although we did not sample all the 67 targeted herds, the 55 herds sampled were considered sufficient to ensure minimal effect on the validity of the results. Serial interpretation of RBT and c-ELISA results were used because it gives a better estimation of the seroprevalence (higher specificity) compared to the parallel interpretation (higher sensitivity). The latter tends to overestimate sensitivity, because of the diagnostic confusion resulting from the RBT assay, especially in *B. abortus* S19 vaccinated herds and cross reactions with antibodies against the smooth lipopolysaccharides of other Gram-negative bacteria such as *Yersinia enterocolitica* O:9 (Mainar-Jaime et al., 2005). The observed seroprevalence in the Chibombo district corroborates previous findings while that in Lusaka district was lower (Muma et al., 2006). The lower seroprevalence in commercial cattle could be attributable to differences in animal husbandry practices where the commercial enterprises tend to control brucellosis or take some precautions when purchasing their replacement stock. In contrast, traditional cattle farmers share communal areas for grazing and the resultant mixing of cattle is an important risk factor for exposure to *Brucella* spp. (Matope et al., 2010b; McDermott and Arimi, 2002; Muma et al., 2007b). Additionally, small-scale cattle farmers regularly purchase cattle from other farms where the screening of these cattle for brucellosis is not carried out due to limited availability of veterinary services and this further increases chances of contact with infected herds (Matope et al., 2010a; Muma et al., 2007b; Omer et al., 2000). Within the Lusaka province, herd level *Brucella* seroprevalence was relatively higher in Chongwe district compared to other study areas. Again, Chongwe was more rural compared to Lusaka and Kafue. At individual animal level, more seropositive cases were recorded in bulls compared to females and oxen. Our results were higher than what has been recorded for bulls in traditional cattle (Muma et al. 2006). The difference could not be explained with the observed data, but suggested that bulls could be exposed via the venereal route (Silva et al., 2000). However, no equivocal statement can be made since our samples size was limited. Our seroprevalence data for oxen was lower than what has been reported previously for the traditional sector (Muma et al., 2006). In traditional cattle, oxen tend to be kept for longer periods than in the commercial farms and this may account for the observed high number of seropositive oxen in the former compared to those in the latter since age is a known risk factor to *Brucella* infection (Muma et al., 2007b). The observed effect of age on *Brucella* infection is related to sexual maturity of animals and this corroborates findings of other studies (Gul and Khan, 2007; Muma et al., 2006; Omer et al., 2000). In conclusion, the study has demonstrated that commercial cattle in the province of Lusaka and the Chibombo district are exposed to *Brucella* spp., and that seroprevalence differed according to age and sex of animals and the district of origin.

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