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SHORT COMMUNICATIONS

Seroprevalence of bovine brucellosis and associated risk factors in Nakasongola district, Uganda

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Abstract

A cross-sectional study was carried out between November 2015 and January 2016 to determine the seroprevalence of *Brucella* antibodies in cattle raised under communal, fenced farms and tethering systems and the associated factors. Seven hundred twenty-eight bovine sera were collected and tested with rose Bengal test as a screening test and the indirect enzyme-linked immunosorbent assay as a confirmatory test. Animal- and herd-level data were collected and binary logistic regression was used to assess the potential risk factors. True animal- and herd-level prevalence was highest in the fenced farms (4.5% (95%CI, 2.3–6.9) and 19.5% (95%CI, 8.2–32.7) respectively). The risks for natural brucellosis infection were sharing water with wild animals (OR = 0.21, 95%CI, 0.104–0.83), herd size (medium: OR = 0.089, 95%CI 0.017–0.449; large: OR = 0.024, 95%CI 0.003–0.203), fenced farms (OR = 3.7, 95% CI, 1.7–7.9), sex (OR = 0.03, 95%CI, 0.01–0.079), and lactation (OR = 0.013, 95%CI, 0.004–0.049). Changes in rangeland tenure and the shift towards intensive cattle production have influenced brucellosis epidemiology. Future studies should aim at identifying the infecting *Brucellae* and examining the role of wildlife in brucellosis epidemiology.

Keywords Brucellosis · Production systems · Rangeland tenure · Uganda

Abbreviations

iELISA	Indirect enzyme-linked immunosorbent assay
RBT	Rose Bengal test

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Introduction

Brucellosis in cattle is usually caused by biovars of *Brucella abortus* (McDermott et al. 2013) but when cattle are raised together with sheep and goats, *Br. melitensis* may cause the disease (Godfroid 2017). When brucellosis occurs, public health is affected resulting from days lost due to sickness, misery, infertility, and death in rare cases. In cattle, economic losses result from late age at first calving, long calving intervals, low herd fertility, and low milk yields (Ducrotoy and Bardosh 2017). Impact on trade results from restrictions on export and import of livestock (Assenga et al. 2015). Brucellosis is endemic in Uganda with seroprevalence in cattle ranging between 9 and 50% (Makita et al. 2011) constraining the growth of the 14 million cattle subsector.

Nakasongola district is part of Uganda's semi-arid "pastoral ecosystem" where grazing ruminants on previously communal land is the dominant form of land use. However, due to population increase in the late 1990s, government re-allocated this land to pastoralists (Muhereza 2001) with a resultant shift in rangeland tenure from communal to private. This necessitated a change in cattle production systems with the communal system being blended with fenced farms and tethered systems. In this study, we

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Table 1 Animal-level seroprevalence estimates per production system

Test		RBT		iELISA	
Production system	No. sampled	+ [AP]	P value [95%CI]	+ [TP]	P value [95%CI]
Communal	291	8 [2.8]	< 0.001 [1.2–5.3]	7 [2.5]	< 0.001 [0.97–4.9]
Fenced farms	336	36 [10.7]	< 0.001 [7.6–14.5]	14 [4.5]	< 0.001 [2.3–6.9]
Tethered	101	1 [1.0]	< 0.001 [0.03–5.4]	1 [1.04]	< 0.001 [0.03–5.4]
Total	728	45 [6.2]	< 0.001 [4.5–8.2]	22 [3.2]	< 0.001 [1.9–4.5]

+, number of positive animals; *AP*, apparent prevalence; *TP*, true prevalence

examined the sero provenance of *Brucella* antibodies in the three systems above and the associated risk factors.

Materials and methods

Nakasongola district is located on latitudes 055 N 1 40′ N and longitudes 31 55 E and 3250 E, and lies between 3400 and 3800 ft. above sea level. Seven hundred twenty-eight bovine sera were collected from 106 cattle herds selected from six sub counties and one town council by simple random sampling. Cattle 1½ years and above were sampled using systematic random sampling. The sample size was determined using the formula $n = \frac{Z^2 P(1-P)}{d^2}$ (Charan and Biswas 2013) where Z is the z (1.96) statistic for a 95% confidence interval, P the expected prevalence, and d the precision. Using $P = 2.4\%$ (Nizeyimana et al. 2013), $d = 1/2P$ following the guidelines of Naing et al. (2006), and $z = 1.96$, the sample size was 728. The breeds sampled were the Ankole and its crosses with the Holstein, Boran, Sahiwal, and Charolaise.

Five milliliters of venous blood was collected from the jugular vein into clot activation tubes. Serum was extracted into cryo-vials, transported on ice, and refrigerated at − 20 °C. Questionnaires were used to capture animal- and herd-level data (Online Resource 1).

Thirty microliters of RBT and serum were gently mixed on a white tile and rocked for 3 to 4 min at room temperature. Agglutination denoted a positive test and vice versa. To eliminate the risk of false positive serological reactions, RBT-positive samples were subjected to iELISA test (IDEXX brucellosis serum Ab test, IDEXX Montpellier SAS, France) as recommended by the manufacturer. The iELISA offers optimal specificity in the absence of vaccination (Marín et al. 1999) and was suitable for identifying antibodies due to natural infection since all sampled cattle had no vaccination history. Results were obtained by comparing the sample optical density with the positive control mean optical density using the formula $\frac{S}{P} = 100 \times \left(\frac{\text{Sample } A_{450} - \text{NC } A_{450}}{\text{PC}_{\text{mean}} - \text{NC } A_{450}}\right)$, where S/P is the sample to positive percentage, NC the negative control, and PC the positive control. Samples with $S/P \geq 120\%$ were considered positive for the presence of *Brucella* antibodies, those with $S/P < 110\%$ were considered negative, and those with $110\% < S/P < 120\%$ were considered suspect.

Animal- and herd-level true prevalence (TP) was estimated using TP = P + SP − 1 / SE + SP − 1 of Rogan and Gladen (1978) where, P is the prevalence with iELISA, SE the sensitivity, and SP the specificity. Two multivariable logistic regression models based on the expression $Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \ldots + \beta_i X_i + \varepsilon$ which models the probability that the binary response Y is a function of a set of predictor variables X and regression coefficients β were fitted using the backward selection procedure in "R" Version 3.1.2 to assess animal- and herd-level factors. Hosmer and Lemeshow (HL) goodness-of-fit test statistics was used to the best models.

Results

The overall animal-level apparent prevalence (AP) and true prevalence (TP) of *Brucella* antibodies were 6.2% (95%CI,

Table 2 Herd-level seroprevalence estimates per production system

Test		RBT		iELISA	
Production system	No. sampled	+ [AP]	P value [95%CI]	+ [TP]	P value [95%CI]
Communal	44	4 [9.1]	< 0.001 [2.5–21.7]	3 [7.3]	< 0.001 [1.4–18.6]
Fenced farms	44	10 [22.7]	0.0004 [11.5–37.8]	8 [19.5]	< 0.001 [8.2–32.7]
Tethered	18	1 [5.6]	0.0001 [0.14–27.3]	1 [5.9]	0.0001 [0.14–27.3]
Total	106	14 [13.2]	< 0.001 [7.4–21.2]	12 [12.1]	< 0.001 [5.9–18.9]

+, number of positive herds; *AP*, apparent prevalence; *TP*, true prevalence

Table 3 Relationship between exposure variables and herd sero-status

Variable	Categories	P value	OR [95%CI]
Wild animals share water with cattle	No*	–	–
	Yes	0.021	0.21 [0.104–0.83]
Cattle herd size	Small*	0.03	–
	Medium	0.003	0.089 [0.017–0.449]
	Large	0.001	0.024 [0.003–0.203]
Abortion history	No*		–
	Yes	0.122	2.7 [0.765–9.8]

HL statistics; *R*-square = 0.755, X^2 = 4.4, *P* value = 0.729

*Reference category

4.5–8.2) and 3.2% (95%CI, 1.9–4.5) respectively. Animal-level AP and TP were highest in fenced farms at 10.7% (95%CI, 7.6–14.5) and 4.5% (95%CI, 2.3–6.9) respectively (Table 1).

Overall herd-level AP and TP were 13.2% (95%CI, 7.4–21.2) and 12.1% (95%CI, 5.9–18.9) respectively (Table 2).

Herd-level AP and TP were highest in the fenced farms at 22.7% (95%CI, 11.5–37.8) and 19.5% (95%CI, 8.2–32.7) respectively.

The risk of natural brucellosis infection was higher in medium- and large-sized cattle herds (OR = 0.089, 95%CI, 0.017–0.449) and (OR = 0.024, 95%CI, 0.003–0.203) respectively (Table 3).

Herds sharing water with wild animals were more likely to be seropositive (OR = 0.21, 95%CI 0.104–0.83) compared to those that did not.

Cattle in fenced farms were more likely to be seropositive (OR = 3.7, 95%CI, 1.7–7.9) compared to tethered cattle. Female cattle were more likely to be seropositive (OR = 0.03, 95%CI, 0.01–0.079) than male cattle. Lactating cows were more likely to be more seropositive (OR = 0.013, 95%CI, 0.004–0.049) than heifers (Table 4).

Table 4 Relationship between exposure variables and animal sero-status

Variable	Categories	P value	OR [95% CI]
Production system	Tethered*	–	–
	Fenced	0.001	3.721 [1.7–7.9]
	Communal	0.292	0.327 [0.041–2.62]
Sex	Male*	–	–
	Female	0.000	0.010 [0.001–0.079]
Functional status	Heifer*	–	–
	Dry cow	0.000	0.03 [0.01–0.089]
	Lactating cow	0.000	0.013 [0.004–0.049]
History of retained placenta	No*	–	–
	Yes	0.529	1.51 [0.42–5.51]

HL statistics; *R*-square = 0.82, X^2 = 8.7, *P* value = 0.123

*Reference category

Discussion

Whereas bovine brucellosis has been widely studied in Uganda from different perspectives, its relationship to production systems has not been adequately examined. The production system is an important factor in brucellosis epidemiology (Mai et al. 2012). The close contact between cattle in fenced farms could account for the higher seroprevalence of *Brucella* antibodies and higher risk of infection seen in this study. Fencing is necessary for optimal utilization of pasture resources given the small land holdings but offers effective contact between animals which supports transmission of *Brucellae* (Ducrotoy et al. 2014). However, this observation contrasts that of Maurice et al. (2013) who observed lower brucellosis prevalence in smaller landholdings. Since small land holdings cannot support large herds of indigenous cattle, farmers currently keep small- to medium-sized herds of crossbreeds for improved milk yield. These cattle are sourced from herds with unknown *Brucella* status, hence are suspected sources of infection. Similar observations were made by Matope et al. (2011) on smallholder farms that stocked taurine cattle from commercial farms in Zimbabwe for crossbreeding. By contrast, tethered cattle are less likely to mix with other animals, thereby limiting the likelihood of contracting brucellosis.

The higher risk of infection observed in large herds is attributable to easy transmission of *Brucellae* due to close contact between animals (Ducrotoy et al. 2017). Free ranging wildlife is known to sustain sources of *Brucella* infections for livestock (Godfroid 2017). Transmission is possible at watering points when cattle share water with wild animals, which could explain the higher risk of infection in herds that shared water with wild animals.

The higher risk of brucellosis infection in lactating cattle is attributed to the role they play in disseminating *Brucellae* via milk, aborted fetuses, and uterine discharges (Assenga et al. 2015). The higher risk of infection in female cattle is probably due to the longer time they stay in a herd (Kanouté et al. 2017) in addition to the reasons stated above.

The changes in land tenure and the shift towards intensive cattle production have influenced brucellosis epidemiology. Identification of infecting *Brucellae* and a deeper inquiry into the role of wildlife in brucellosis epidemiology should be the focus of future studies.

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Compliance with ethical standards

Statement of animal rights All applicable guidelines for the care and use of animals were followed.

Conflict of interest The authors declare that they have no conflict of interest.

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