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Title: Prevalence of and factors associated with *Brucella* sero-positivity in cattle in urban and peri-urban Gulu and Soroti towns of Uganda.

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ABSTRACT

Brucellosis is a key zoonosis of major public health, animal welfare, and economic significance, and is endemic in livestock in Uganda. A cross-sectional epidemiological study was carried out to estimate the sero-prevalence of brucellosis and identify factors associated with sero-positivity in cattle in urban and peri-urban **Gulu** and Soroti towns of Northern and Eastern Uganda, respectively. A total of 1007 sera and data on biologically plausible risk factors from 166 herds and their spatial locations, were collected from cattle reared in urban and peri-urban Gulu and Soroti towns of Uganda. The sera were analyzed using indirect ELISA and sero-positive reactors confirmed by competitive ELISA. Multivariable models were used to investigate for risk factors. The overall animal-level and herd-level sero-prevalence was 7.5% (76/1007, 95% Confidence Interval (CI): 6.15 – 9.4%) and 27.1% (45/166, 95% CI: 20.9% - 34.3%), respectively. Herd-level sero-prevalence was significantly ($p<0.001$) higher in Soroti than Gulu. In Gulu town, sero-positivity increased with an increase in herd size ($p=0.03$) and age ($p=0.002$), and was higher in cattle brought in from western Uganda ($p<0.0001$). In Soroti town, introduction of new cattle into a herd was significantly ($p=0.027$) associated with herd sero-positivity. There was a geographically differential risk (clustering) of *Brucella* sero-positivity in herds in Soroti, while sero-positivity was homogeneously distributed in Gulu. The data highlights brucellosis occurrence and major risk factors for its transmission in cattle in urban and peri-urban areas.

Keywords: brucellosis, risk factors, sero-positivity, Uganda, urban and peri-urban cattle.

INTRODUCTION

Livestock farming in urban and peri-urban areas is increasing globally in parallel to the rapid urbanization in low-income countries and is the major source of unprocessed foodstuffs to the increasing urban populations [5, 16]. However urban and peri-urban farming is faced by a number of challenges among which is a high disease burden due to changes in the environment- host-pathogen interface [4]. Some of the diseases affecting urban and peri-urban farming also may pose a threat to human health [18, 32]. Among such diseases is brucellosis.

Brucellosis in cattle is almost exclusively caused by various biovars of *Brucella abortus*, but biovars of *Brucella melitensis* and *Brucella suis* have also been implicated [2]. It is a key zoonosis of major public health, animal welfare, and economic significance [25, 31]. In humans, brucellosis is debilitating, while in domestic animals the main problem is reproductive failure [31]. In particular the economic significance of this disease in animals stems from abortion, mastitis, reduced milk production, retained placenta, metritis, impaired fertility and arthritis/bursitis.

Currently in most high-income countries brucellosis has been eradicated or severely curtailed by a combination of strict veterinary hygiene measures, control programs, and improved food safety measures [7, 31]. However, in Uganda, a low income country, the disease remains endemic in cattle with reported sero-prevalence of 10.2% to 15.8% in pastoralist areas, 5% in a major urban setting and National average of 10% [6, 19, 23]. Brucellosis sero-prevalence in other Sub-Saharan countries follows a similar trend as that in Uganda, with higher prevalence of up to 26.3% reported in pastoral systems

[12], and lower prevalence of 5.5% reported in small holder semi-intensive systems [15]. Epidemiological studies done in western and central Uganda, other East African countries and Africa at large have identified some of the potential risk factors for the spread of brucellosis in cattle, among which is large herd size, sharing of grazing grounds, mixing and trading of animals and extensive production system [9, 12, 13, 15, 30]. Studies on the epidemiology of brucellosis in urban and peri-urban cattle in Eastern Africa have mainly concentrated on major towns [9, 13, 30]. The sero-prevalence and risk factors for this disease in small towns with a high rate of rural to urban human migration is largely unknown. This study sought to estimate the sero-prevalence and risk factors for *Brucella* sero-positivity in urban and peri-urban cattle in Gulu and Soroti, which are the two rapidly growing towns in Northern and Eastern Uganda, respectively.

MATERIALS AND METHODS

Study area and population

The study was carried out in the urban and peri-urban areas of Gulu and Soroti towns in Northern and Eastern Uganda, respectively (Fig. 1). Gulu town had a population of 119,430 people in 21,622 households while Soroti town had 41,711 people living in 9,768 households according to the 2002 household survey [29].

The cattle population in Gulu and Soroti Districts, where Gulu and Soroti towns are located, was estimated to be 40,130 and 271,634, respectively [28]. We assumed that only 2% of the urban households in Gulu (432) and Soroti (195) kept cattle as was the case in the capital city Kampala since no accurate data was available for these two towns [28]. The cattle were kept in herds as a mixture of different breeds, indigenous breeds

(Ankole or Zebu), exotic breed (Friesian), or crosses of indigenous and exotic breeds.

Study design

A cross-sectional epidemiological study was carried out to investigate the sero-prevalence of *Brucella* and factors associated with *Brucella* sero-positivity in cattle in urban and peri-urban areas of Gulu and Soroti towns.

Each town was divided into urban and peri-urban areas using the population dynamics rapid rural mapping method according to Makita *et al.* [14]. This characterizes urban areas as those with a dense human population with back yard farming on small plots, while peri-urban areas are transition areas from rural to urban, with a high rate of population increase, new human residential units, and larger plots for crop cultivation. There were four cattle grazing systems in the study area; communal grazing, tethering, paddocking and zero-grazing with the characteristics according to Table 1 [22]. A list of herds in the study areas was generated with the help of extension workers and farmers. The herds were assigned numbers and the selection of herds for inclusion in the study was done by simple random sampling. Based on assumption that only 2% of urban and peri-urban households kept cattle [28], and desirous of sampling at least 25% of cattle keeping households in respective study areas as done by Matope *et al.* [15], a total of 116 herds were selected in Gulu and 50 in Soroti. The total targeted number of cattle to be sampled per District was 385 basing on the expected *Brucella* sero-positivity in cattle of 50% (since no studies had been done), 95% confidence interval and a desired precision of 5% as described by Thrusfield [27]. However, more animals than these were sampled in order to increase on the study precision. In each randomly selected herd with less than 10 adult cattle (> 6 months old), all animals were sampled, whereas in herds with more than 10 cattle, 10 cattle

were randomly sampled plus an additional 20% of the remaining cattle.

Ethical statement

This study entailed collection of animal samples and interviewing of farmers. Ethical clearance was obtained from the Ethical Review Committee of the College of Veterinary Medicine, Animal Resources and Biosecurity, Makerere University. The farmers were informed about the study and their verbal consent was sought prior to commencement of data collection.

Collection of data and blood samples

Blood samples, individual cattle and herd data were collected in the dry season from the study herds from May to late July 2011 for Gulu and January to early March 2012 for Soroti. In each selected herd, an interview was carried out using a structured open and closed ended questionnaire administered by trained local research assistants conversant in English and respective local languages. The information elicited included farmer's awareness of brucellosis risk factors in cattle, the grazing system used, cattle numbers, breeding method, species kept on the farm, and the intra-household gender involvement in the keeping of cattle. These interviews and the data are thus based on self-reporting from farmers. The questionnaire template used can be obtained from the first author on request.

Blood was collected from the mid coccygeal or the jugular vein into sterile vacutainer tubes without anticoagulant (BD, Plymouth UK). Variables were collected for all the samples taken including; town, date, herd, urban or peri-urban, geographic location (latitude and longitude coordinates collected by a Global Positioning System (GPS)

receiver), identity of animal sampled, breed, sex and brucellosis vaccination status. Blood samples collected and properly identified were allowed to clot and serum was then harvested immediately in the field in 2 ml cryogenic vials (Sarstedt, Germany). The sera was transported at 4° C to the College of Veterinary Medicine, Makerere University, Kampala, and stored at -20° C in sterile cryogenic vials until tested.

Detection of antibodies to *Brucella*

Antibodies to *Brucella* in serum were detected using *Brucella* indirect ELISA kit (Svanova™ *Brucella*-Ab i-ELISA). The positive reactors were confirmed using *Brucella* competitive ELISA kit (Svanova™ *Brucella*-Ab c-ELISA). Both kits were procured from Svanova Biotech AB Uppsala, Sweden, and the analyses on the sera were performed following the manufacturer's recommendations. The Svanova™ *Brucella*-Ab i-ELISA has sensitivity (Se) of (95.1%) and specificity (Sp) of (97.6%), and if this is coupled with the higher sensitive (99.5%) and specific (99.6%) Svanova™ *Brucella*-Ab c-ELISA, false positives due to vaccination six months after vaccination may be ruled out [20, 21]. However, there is no National vaccination program against brucellosis in Uganda. The i-ELISA assay was *B. abortus* and bovine specific and the c-ELISA assay was a multispecies assay capable of detecting both *B. abortus* and *B. melitensis* antibodies, and both have higher sensitivity than standard tests like complement fixation test (CFT, 91.8% Se), but with comparable specificity to CFT (99.9%) [21].

Each serum sample was run in duplicates on *Brucella*-Ab i-ELISA kit and samples with a percent positivity (PP) ≥ 40 were considered positive. The PP was calculated as $(\text{Mean optical density (OD)}_{\text{sample}} / \text{Mean OD}_{\text{positive control}}) \times 100$ where OD is the Optical Density. The *Brucella*-Ab i-ELISA positive samples were then run in duplicates on

Brucella-Ab c-ELISA kit and samples with a percent inhibition (PI) $\geq 30\%$ were considered as positive. The PI was calculated as $100 - [(Mean OD_{samples} \times 100) / (Mean OD_{Conjugate\ control})]$. Positive and negative control sera were included in each test for test validity according to instructions from the manufacturer.

Statistical analysis

The data obtained was captured in the MS Excel, edited for accuracy and then exported to the statistical package for social scientists (SPSS) version 17.0 program to establish associations between sero-prevalence of brucellosis and the risk factors at 95% level of confidence. Continuous variables e.g. age, were categorized to ease the analysis. Summary statistics, univariable and multivariable analyses were carried out to identify associations between selected risk factors and *Brucella* sero-positivity at individual animal and herd levels. The confidence intervals of point estimates of proportions were calculated using vassarstats software (www.vassarstats.net). The animal level sero-prevalence was the proportion of positive animals out of the total number of animals sampled and analyzed, while the herd level sero-prevalence was the proportion of herds with at least one positive animal out of the total number of herds sampled.

Following the summary statistics that showed a considerable difference in sero-prevalence between the two towns, the analysis for factors associated with *Brucella* sero-positivity was made separately for the two towns.

Initially, univariable analyses were performed on all herd and animal level variables for each of the two regions. Variables with a p value of <0.25 on likelihood ratio chi-square test were included in multivariable models, after checking for collinearity. Variables were

deemed collinear if they had a variance inflation factor (VIF) of ≥ 10 and in such a case only one of the collinear variables deemed biologically more plausible was included in the multivariable model. Abortion history and retained placenta at individual animal level were considered in a model involving only females above two years of age. Two multivariable logistic regression models were built to investigate risk factors associated with *Brucella* sero-positivity on herd and animal level, respectively. The Wald's backward selection and iterative maximum likelihood tests were used until variables in the models showed a p value of ≤ 0.05 . Interaction between significant variables was tested. Hosmer-Lemeshow test and diagnostic plots were used to ascertain the goodness of fit of the models. Adjustment for intra-herd clustering for individual animal risk factors was done by fitting a Generalized Linear Mixed Model (GLMM) including the fixed effects for the regressors and the random effect of the herd.

Spatial analyses of sero-positive and sero-negative herds were done using QGIS (Quantum GIS 1.7.3 Wroclaw). Spatial epidemiological analyses for herd level *Brucella* seropositivity clustering effects were analyzed using the Bernoulli model in spatial scan statistics, SaTScan version 9.1.1. Significant clusters based on the distribution of likelihood ratio and its corresponding p -value were identified after running the analysis for 999 permutations (type I error set at 0.001) by Monte-Carlo simulation. We used a standard purely spatial scan statistic at 10% that imposed a circular scanning window.

RESULTS

Descriptive statistics:

A total of 500 sera were collected in 116 cattle herds in Gulu and 507 sera collected from

50 herds in Soroti town. The individual animal-level and herd-level sero-prevalence were 7.5% (76/1007, 95% CI: 6.15 – 9.4%) and 27.1% (45/166, 95% CI: 20.9% - 34.3%) respectively. Individual animal level sero-prevalence tended ($p=0.08$), to be higher in Soroti than in Gulu and the herd-level sero-prevalence was significantly higher ($p<0.001$) in Soroti (Table 2).

In both Gulu and Soroti, males headed the majority of households keeping cattle (67% and 90%, respectively), and owned the majority of the herds (57% and 92%, respectively). In Soroti, 98% of the herds shared a grazing ground compared to 43% in Gulu. Soroti reported 24% herds with abortion and 13% Gulu. Out of 300 female cattle of reproductive age (above 2 years old), in Gulu 5% had aborted and 3% had retained placenta. None of the animals that had aborted or had retained placenta were sero-positive for *Brucella* in Gulu. Out of 310 female cattle of reproductive age in Soroti, 4% had aborted and 3% had retained placenta. Sero-prevalence of brucellosis in cattle with a history of abortion and retained placenta in Soroti was 8% and 11%, respectively. There was no significant association between history of abortion and sero-positivity, or retained placenta and sero-positivity. The gender of household head, the ownership of the cattle, sharing grazing ground, abortion history at herd and individual animal level, an individual animal's history of retained placenta and keeping goats in addition to cattle showed p -values >0.25 in both districts and are not presented in detail. None of the cattle sampled had a history of brucellosis vaccination. The characteristics of the farms and plausible risk factors ($p<0.25$) for sero-positivity in the two towns are shown in Table 3.

Univariable analyses of herd level risk factors in Gulu identified farm location, cattle attendant, herd size, herd breed, grazing system, watering system, introduction of new

cattle and keeping sheep and dogs as plausible risk factors with $p < 0.25$. In Soroti town the herd size, breeding system, grazing system, management of aborted material and after birth, introduction of new cattle in the last two years and keeping dogs were identified (Table 3). Univariable analyses of individual animal level risk factors in Gulu identified animal breed, age and region of origin as plausible risk factors with $p < 0.25$ while in Soroti town, animal age was the only plausible risk factor (Table 4).

Risk factors for *Brucella abortus* sero-positivity at individual and herd level in Gulu

Herd size, the breed and age of the animal and its region of origin (place of purchase) were significantly ($p < 0.05$) associated with *Brucella* sero-positivity in Gulu in the univariable analysis (Table 3 and 4). In the multivariable model adjusting for intra-herd clustering of individual animal risk factors in Gulu town, the age of the animal ($p = 0.007$) and its region of origin ($p < 0.0001$) (place of purchase) were associated with sero-positivity (Table 5). Large herd size was significantly associated with sero-positivity ($p = 0.003$), as indicated in table 6 where herds with > 20 cattle were 7.8 times more likely to test sero-positive compared to those of 1-5 cattle. Ankole cattle kept in herds with more than 20 cattle were 3.3 times more likely to test sero-positive compared to other breeds or their counterparts in smaller herds ($p = 0.016$; Odds Ratio (OR) = 3.3; 95% C.I for OR: 1.2-8.9). The Hosmer-Lemeshow test showed that the models fitted the data at both the herd and individual level ($\chi^2 = 0.234$, df = 4, $p = 0.994$; $\chi^2 = 2.510$, df = 4, $p = 0.643$).

Risk factor for *Brucella abortus* sero-positivity at individual and herd level in Soroti

Introduction of new cattle was significantly associated with *Brucella* sero-positivity both at univariable ($p = 0.04$) and multivariable ($p = 0.027$) analysis (table 3 and 6). The Hosmer-Lemeshow test showed that the model fitted the data at herd level ($\chi^2 = 4.591$, df = 7, $p =$

0.71). No risk factors were significantly ($p < 0.05$) associated with sero-positivity at individual animal level in Soroti and thus the intra-herd clustering effect was not computed.

Spatial distribution of sero-positive herds and their characteristics

The spatial distribution of sero-positive and sero-negative herds in the two study areas are shown in Fig. 2 and 3. In Soroti two patterns of *Brucella* herd seropositivity were observed, one in which disease was homogenously distributed (39 herds) and the other in which it was clustered (11 herds). This significant ($p = 0.033$) cluster of sero-positive herds was centred at 33.613270 N, 1.642567 E in Obila village, Otatai parish, Asuret sub-county, Soroti and had 11 herds of which 9 were observed to be sero-positive and in a radius of 5 Km². The number of expected cases of sero-positive herds was 3, assuming that the cases were randomly distributed in space. The log likelihood ratio for this cluster was 8, and the relative risk within this cluster was 3.5 at 95% level of confidence. In Gulu, there was no evidence for a geographically differential risk of *Brucella* sero-positivity in cattle at herd level.

DISCUSSION

This was the first study to document the sero-positivity to *Brucella* antibodies and factors associated with sero-positivity in cattle in two towns of Northern and Eastern Uganda at different stages of urbanization. We found significant differences in the herd sero-prevalence between the towns and common as well as different risk factors for sero-positivity in the two towns.

The overall herd and individual animal-level sero-prevalence in this study were lower (7.5%) than what was found in studies in rural areas of Uganda (15.8%, 10.2% and 10%) [6, 19, 23] but higher than that found in the urban peri-urban area of Kampala (5%), the capital city of Uganda [13]. This observation suggests that there may be variations in risk factors for *Brucella* transmission in cattle related to the geographical location of the animal i.e. rural or urban. This finding is consistent with previous reports on the disease, where the sero-prevalence was highest in rural areas, followed by peri-urban areas and least in urban areas [9].

Soroti town in Eastern Uganda had higher herd *Brucella* sero-prevalence than Gulu town in Northern Uganda. Our study finding of higher herd-level *Brucella* sero-prevalence in Soroti than Gulu may be difficult to explain, but the observed predominant communal grazing and watering systems that allow more mixing of herds in the former as opposed to zero grazing and individual herd watering in the latter, may be implicated. These findings concur with previous studies that reported high *Brucella* sero-prevalence in herds that practiced free-grazing as compared to restricted grazing [13, 17]. It could also be related

with the introduction of more new cattle in Soroti (66%) than Gulu (28%), since herds that introduced new cattle were more likely (OR=4.4) to test positive in Soroti. This is supported by the evidence in previous studies where management practices, the scale of the herd and the production system influenced sero-positivity and culling [11, 17, 20, 30].

In Gulu town, the herd size was significantly associated with herd sero-positivity, and the animal's age and its region of origin were significantly associated with individual animal sero-positivity. Large herds were more likely to test sero-positive than smaller herds. This is most likely due to increased chances of animal to animal contact in larger herds than smaller ones [17]. The high individual animal sero-prevalence observed in cattle from western Uganda concurs with previous studies that reported sero-prevalence of 21.5% and 10.2% respectively [10, 19]. The reported high risk in older cattle compared to young cattle agrees with the literature about the disease, which is due to increase in the chances of getting exposed to *Brucella* over time [2]. Sero-prevalence being higher in the 1-2 years age category than the 2-5 years age category could be due to presence of maternal antibodies in animals of 1-2 years [3]. The observed high sero-prevalence in the Ankole cattle compared to other breeds suggests that there is breed variation in susceptibility agreeing with findings of Tatiane *et al.* [26] and Mekonnen *et al.* [17].

Considering not doing a complete coverage of all herds in the study area for spatial analyses, the interpretation of the results from this analysis needs to be interpreted carefully. However, the observed clustering of *Brucella* in Soroti herds could be due to the herd structure and management practices within the herds in the *Brucella* sero-positive cluster that facilitated mixing of the herds. Likely, the small herd size observed in the cluster

facilitated their easy movement and eventual congregation in communal grazing areas where they were tethered or grazed freely increasing the animal density, thus facilitating transmission of *Brucella*. This finding is consistent with that of Hegazy *et al.* [8] in Upper Egypt.

The study finding of insignificant association between history of abortion and sero-positivity, or retained placenta and sero-positivity points to possible occurrence of other abortifacients and causes of retained placenta in cattle in the study areas. Probable causes could be tick borne diseases which are known to be highly prevalent in the study areas as established by Ocaido *et al.* [24], or infectious causes of abortion like leptospirosis that has been reported in Uganda [1] or dietary deficiencies as most of these cattle were not supplemented. This calls for further research to determine the exact cause of these abortions.

In conclusion, the present study reports high herd-level prevalence of *Brucella antibodies in cattle* in two towns in northern and eastern Uganda as well as a set of risk factors for sero-positivity. In Gulu town, older cattle, region of origin of the cattle and large herd size were identified as risk factors for *Brucella* sero-positivity, whereas in Soroti town introduction of new cattle was identified. This shows that risk factors for *Brucella* sero-positivity in urban and peri-urban cattle in the two studied areas differed. This in turn calls for a tailored approach when designing control measures for prevention of transmission of bovine brucellosis in different areas.

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Tables

Table 1: Cattle production systems in Urban and peri-urban Gulu and Soroti

Grazing system	Characteristics
Communal grazing	Indigenous breeds kept on a communal grazing land, often with water and pasture scarcity and low level of commercial in-puts.
Tethering	Semi-intensive system with indigenous and cross-bred cattle restrained by ropes in intensively cropped areas, or urban centres and normally with herd sizes of 1-5 cattle and limited level of commercial in puts.
Paddockling	Intensive or semi-intensive system with exotic or cross- bred cattle confined in fenced units, often with improved pastures and fodder. Herd sizes vary with acreage.
Zero-grazing	Intensive system with exotic or cross-bred cattle kept and fed on fodder and crop residues in stalls. Herd size is usually small (1-3 or up to 10 cattle for large scale farmers).

Source: Mwebaze, 2006.

Table 2: Number of included herds and animals and *Brucella* sero-prevalence

District	Gulu	Fraction	95% CI:		Soroti	Fraction	95% CI:	
			Lower	Upper			Lower	Upper
Urban herds	28	-	-	-	9	-	-	-
Herd sero-prevalence	28.6%	8/28	14%	48.9%	44.4%	4/9	15.3%	77.3%
Serum samples	81	-	-	-	108	-	-	-
Animal sero-prevalence	12.3%	10/81	6.4%	22%	5.6%	6/108	2.3%	12.2%
Peri-urban herds	88	-	-	-	41	-	-	-
Herd sero-prevalence	15.9%	14/88	9.3%	25.6%	46.3%	19/41	31%	62.4%
Serum samples	419	-	-	-	399	-	-	-
Animal sero-prevalence	4.8%	20/419	3%	7.4%	10%	40/399	7.3%	13.5%
Total herds sampled	116	-	-	-	50	-	-	-
Total serum samples	500	-	-	-	507	-	-	-
Overall herd sero-prevalence	19%	22/116	12.90%	27.10%	46%	23/50	33%	59.60%
Overall Animal sero-prevalence	6%	30/500	4.20%	8.40%	9.10%	46/507	6.90%	11.90%

Table 3: Descriptive statistics and univariable analyses of plausible risk factors for seropositivity to *Brucella* at herd level in Gulu and Soroti (Note: only factors associated with seropositivity at $p < 0.25$ in at least one of the towns are shown).

Variable	Category	Gulu			Soroti		
		N (%)	% sero pos	p-value	N (%)	% sero pos	p-value
Farm location	Peri-urban	88 (76)	16	0.17	41 (82)	46	1.00
	Urban	28 (24)	29		9 (18)	44	
Cattle attendant	Husband	27 (23)	11	0.12	12 (24)	50	0.64
	Wife	46 (40)	15		5 (10)	20	
	Children	22 (19)	18		13 (26)	46	
	Hired worker	21 (18)	38		20 (40)	50	
Herd size	1-5 cattle	75 (65)	16	0.001	4 (8)	0	0.03
	6-10 cattle	17 (15)	0		20 (40)	50	
	11-20 cattle	11 (10)	27		11 (22)	27	
	> 20 cattle	13 (11)	54		15 (30)	67	
Herd breed	Exotic breed	53 (46)	15	0.11	1 (2)	0	0.523
	Mixed breeds	31 (27)	32		10 (20)	50	
	Indigenous breed	24 (21)	8		39 (78)	46	
	Cross breed	8 (7)	25		0 (0)	0	
Breeding system	Artificial insemination	57 (49)	18	0.65	0 (0)	0	0.21
	Communal bull	45 (39)	18		48 (96)	44	
	Non communal bull	14 (12)	29		2 (4)	100	
Grazing system	Zero-grazing	54 (47)	17	0.13	0 (0)	0	0.18
	Communal grazing	19 (16)	32		38 (76)	40	
	Tethering	36 (31)	11		12 (24)	67	
	Paddocking	7 (6)	43		0 (0)	0	
Watering system	Individual water	70 (60)	14	0.15	2 (4)	50	1.000
	Communal water	46 (40)	26		48 (96)	46	
Management of aborted material and afterbirth	Bury them	51 (44)	16	0.48	17 (34)	59	0.24
	Do nothing	65 (56)	22		33 (66)	39	
Introduction of new cattle in last 2 years	No	84 (72)	16	0.18	17 (34)	23	0.04
	Yes	32 (28)	28		33 (66)	58	
Keeping sheep	Yes	20 (17)	17	0.06	27 (54)	41	0.57
	No	96 (83)	83		23 (46)	52	
Keeping dogs	Yes	63 (54)	24	0.16	38 (76)	53	0.11
	No	53 (46)	13		12 (24)	25	

Table 4: Descriptive statistics and univariable analyses at individual animal level in Gulu and Soroti (Note: only factors associated with sero-positivity at $p < 0.25$ in at least one of the towns are shown).

Variable	Category	N (%)	Gulu		N (%)	Soroti	
			% sero pos	p-value		% sero pos	p-value
Animal breed	Cross-bred	22 (4.4)	5	0.01	20 (3.9)	5	0.812
	Friesian	96 (19)	9		1 (0.2)	0	
	Zebu	317 (63)	4		469 (93)	9	
	Ankole	65 (13)	14		17(3)	6	
Age	1- 2 years	91 (19)	6	0.01	100 (20)	5	0.18
	>2 - 5 years	288 (58)	4		161 (32)	8	
	>5 - 7 years	77 (15)	10		135 (27)	10	
	> 7 years	44 (8.8)	16		111 (22)	14	
Region of origin	Western Uganda	51 (10)	16	0.001	0 (0)	E*	*
	Central Uganda	42 (8.4)	14		0 (0)	E*	
	Eastern Uganda	0 (0)	0		507 (100)	9	
	Northern Uganda	407 (81)	4		0 (0)	E*	

No statistic is computed because region of origin is constant, E result undefined

Table 5: GLMM analyses adjusting for intra-herd clustering of animal level risk factors in Gulu.

Variable	Category	p-value	OR	95% C.I for OR	
				Lower	Upper
Age		0.007			
	1 - 2 years (ref)	-	1.0	-	-
	>2 - 5 years	0.144	0.4	0.11	1.38
	>5 - 7 years	0.186	2.5	0.64	9.87
	> 7 years	0.096	3.5	0.80	14.84
Region of origin of the cow		< 0.0001			
	Western Uganda (ref)	-	1.0	-	-
	Central Uganda	0.067	0.14	0.02	1.15
	Northern Uganda	0.009	0.09	0.02	0.57

Table 6: Multivariable analyses of herd risk factors for *Brucella* seropositivity in cattle in Soroti and Gulu

	Category	p-value	OR	95% C.I for OR	
				Lower	Upper
Variable in Soroti					
Introduction of new cattle in last 2 years	No (ref)	-	1.0	-	-
	Yes	0.027	4.4	1.2	16.4
Variable in Gulu					
Herd size		0.03			
	1 - 5 cattle (ref)	-	1.0	-	-
	6 - 10 cattle	0.998	-	-	-
	11 - 20 cattle	0.22	2.6	0.6	12
	> 20 cattle	0.002	7.8	2	29

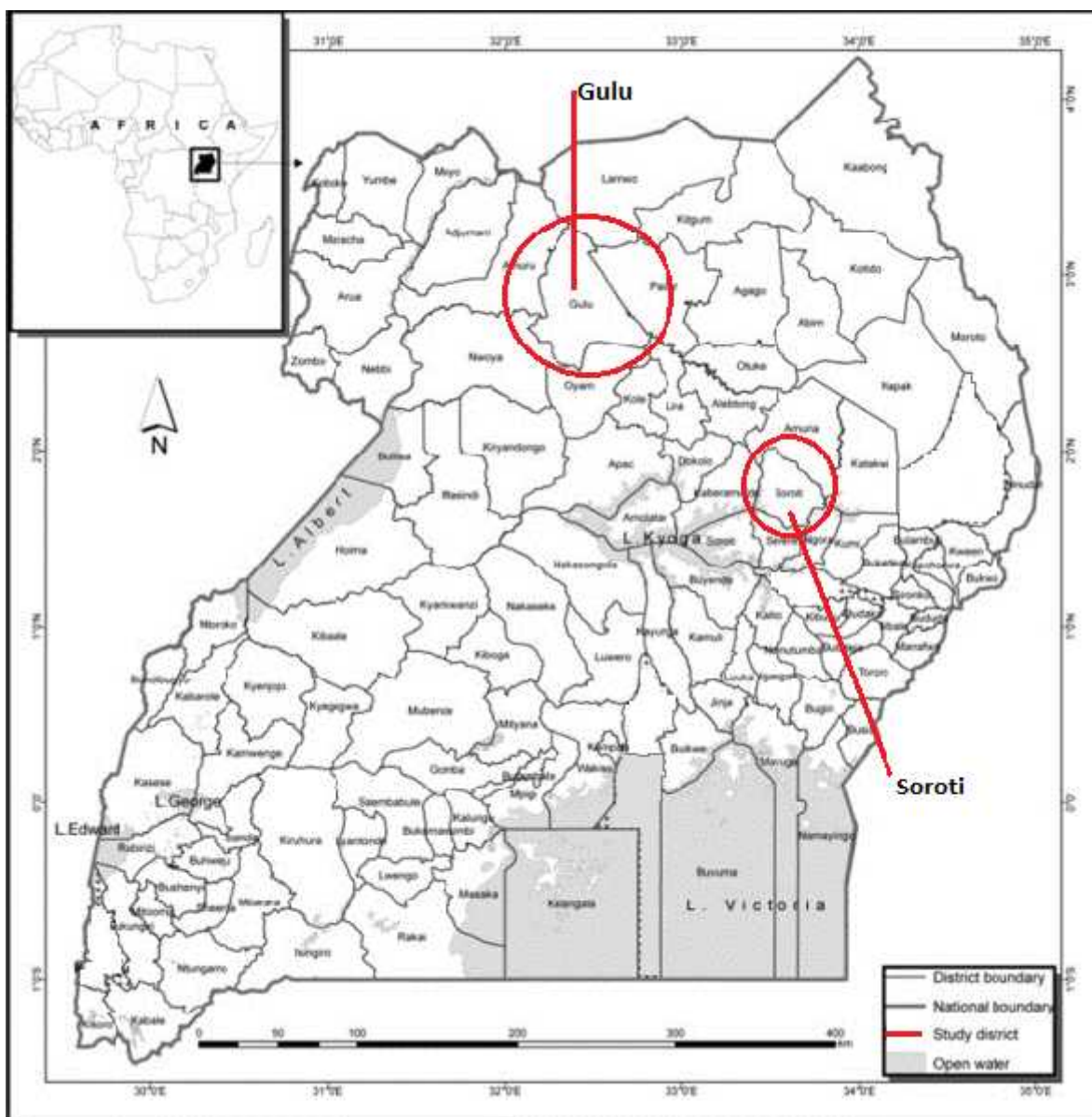


Figure 1: Map of Uganda showing the location of Gulu and Soroti Districts.

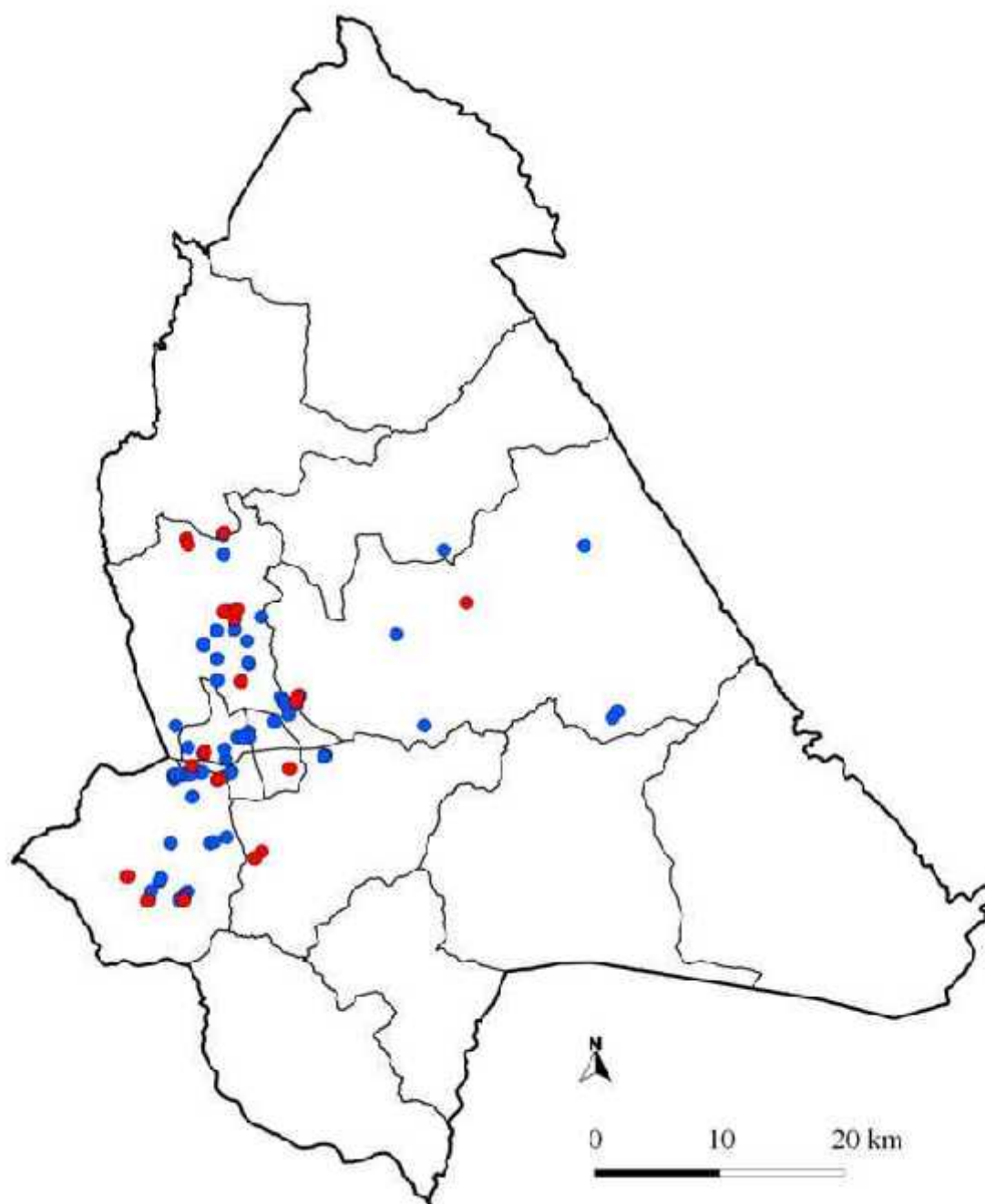


Figure 2: Map of Gulu District showing the location of cattle herds included in this study. Please note that the locations of some herds may overlap.

Key:

Red dots represent *Brucella* sero-positive herds

Blue dots represent *Brucella* sero-negative herds

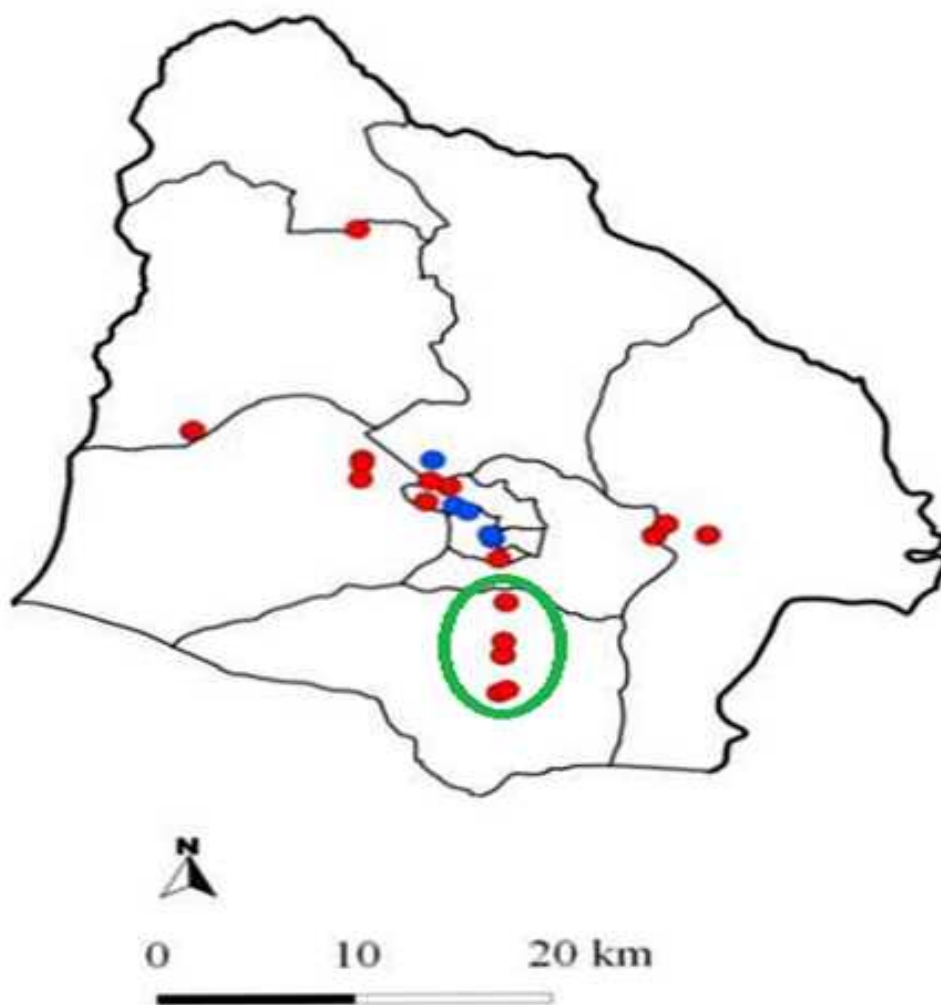


Figure 3: Map of Soroti District showing the location of cattle herds included in this study. Please note that the locations of some herds may overlap.

Key:

Red dots represent *Brucella* sero-positive herds

Blue dots represent *Brucella* sero-negative herds

Green circle represents the significant cluster