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Sero-prevalence and Risk Factors Associated with Bovine Brucellosis in Central Equatoria State, South Sudan

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Abstract: Brucellosis is one of the most important zoonotic diseases of livestock and human in South Sudan. A cross-sectional study was conducted in peri-urban Juba town and rural Terekeka County to estimate sero-prevalence and determine risk factors of bovine brucellosis. Ninety (90) respondents were randomly interviewed on demographic information and potential risk factors to brucellosis. Out of the 90 respondents interviewed, 44 were from 44 cattle herds in peri-urban Juba and 46 from 46 cattle herds in rural Terekeka County. Sera of 502 cattle were screened for *Brucella* antibodies using Rose Bengal Plate test. The positive sera on RBPT were then confirmed by competitive Enzyme-linked Immunosorbent Assay. SPSS version-18 and frequencies were used for sero-prevalence. Chi square and binomial logistic regressions were used for analysis of risk factors. RBPT and c-ELISA tests showed positive reactors and an overall individual animal sero-prevalence of 23.2%, 95% confidence interval (CI): (18.4 - 28.8) and 19.2%, 95% CI: (2.5 – 14.0), respectively in peri-urban Juba town. However, in rural Terekeka County the respective results showed sero-prevalence of 40.5%, 95% CI: (34.5 – 46.4) and 39.3%, 95% CI: (33.3– 45.2). The overall herd level sero-prevalence on c-ELISA revealed 61.4% and 90.0% for peri-urban Juba town and rural Terekeka County cattle herds, respectively. The individual animal level risk factors in the study area revealed abortion history (OR= 4.941 and (CI): 2.077-11.753) and (OR= 6.251 and (CI): 2.920-13.379) significantly associated with brucella sero-positivity, respectively. No risk factor determined at herd level in peri-urban Juba town. However, in rural Terekeka County herds, number of cattle above 300 in a herd (p= 0.005, OR= 44.934) was significantly associated with brucellosis sero-positivity. Further epidemiological studies are needed for developing appropriate control strategies against bovine brucellosis in South Sudan.
Keywords: Brucellosis, Cattle, Sero-prevalence, Risk factors, South Sudan

INTRODUCTION

Brucellosis poses a major threat to human, wildlife and livestock health [1]. This zoonotic disease is considered as one of the most-widespread zoonoses in the world [2]. The economic loss due to brucellosis in livestock production is enormous particularly in low-income countries. This is attributed to high rates of abortion, stillbirth, infertility and calf mortality as well as reduced growth and longer calving intervals of infected animals [3]. Brucellosis has been eradicated in many developed countries in Europe, Australia, Canada, Israel, Japan and New Zealand, but it remains endemic and an uncontrolled episode in vast regions of Africa, including Mediterranean, some parts of Middle East, Asia and Latin America [4]. Incidence of brucellosis is reported to range from 0.85-23.3% in several endemic countries [5]. Some of the risk factors that have been determined to be playing a major role in the infection and spread of bovine brucellosis include herd size, age, and interaction with wildlife, communal grazing [6] as well as introduction of asymptomatic infected animals into a herd [7].

Brucellosis and other zoonoses pose a great threat in post-independent South Sudan. This is attributed to the collapse of veterinary services during decades of civil war which devastated animal disease control systems. Although there is paucity of data on livestock-human-disease situation in South Sudan, bovine brucellosis was identified as one of the most predominant livestock diseases [8]. South Sudan is fraught with several potential risk factors that could be fueling the brucellosis spread among livestock and their human owners. Key to these factors is the traditional pastoralist's practice of conglomerating several herds into cattle camps with close livestock-human interactions. The traditional husbandry practices of mixing herds in the cattle camps, mixing different species as well as poor awareness are risk pointers to brucellosis occurrence and perpetuation in the livestock

and disease exposure to humans. Additionally, other brucellosis risk indicators include the rampant animal herder's practice of vulval blowing to facilitate milk letdown during cow milking and the practice of direct udder-to-mouth consumption of raw milk.

The aim of the present study was to estimate the prevalence and determine the risk factors of bovine brucellosis in peri-urban cattle camps of Juba town and rural Terekeka County, Central Equatoria State (CES), South Sudan.

MATERIALS AND METHODS

Study area, population and design

This was a cross-sectional study conducted targeting all cattle breeds including Nilotic zebu, Lugbara and cross-bred of both sexes and older than 6 months in different cattle camps in peri-urban Juba Town and rural Terekeka County, CES, South Sudan (Figure 1). Juba town is geographically located between 4°51'00''N and 31°36'00''E.

While Terekeka County is located between 5°26'56.14''N and 31°45'8.63''E and lies on the west bank of the White Nile river distant an 85Km north of Juba town. Cattle population in CES is estimated at 2,260,333 heads, of which Terekeka County is endowed with 55.3%. Nilotic Zebu cattle are the predominant breeds and mostly kept on communal husbandry system for earning livelihoods of the agro-pastoralist and pastoralist communities. Cattle are intermixed with other animal species including dogs, sheep and goats.

Field visits were made to the study area which was divided into two strata, the peri-urban (Juba town) and the rural (Terekeka County). With the help of veterinary officers, paraveterinarians and veterinary extension workers, a list of cattle camps around Juba town and in the rural Payams of Terekeka County was prepared. Study cattle camps were randomly selected from the total list obtained. In each cattle camp interviews using structured questionnaires were administered to selected respondents of each constituent cattle herd by a trained research assistant knowledgeable in English, Arabic and respective local language in each selected cattle camp. The questionnaires were administered to each selected owner or the head of each cattle herd within a cattle camp. The questionnaires elicited information on the demographics of the cattle keepers, cattle management and husbandry practices in the region, biosecurity measures and brucellosis awareness among communities, farmer's education levels and other possible factors that predispose to brucellosis. In particular information on cattle camp density, grazing system used, herd management, cattle interaction with humans, wildlife as well as other animals were recorded. Following questionnaires administration, target cattle were randomly selected from each camp for blood sampling.

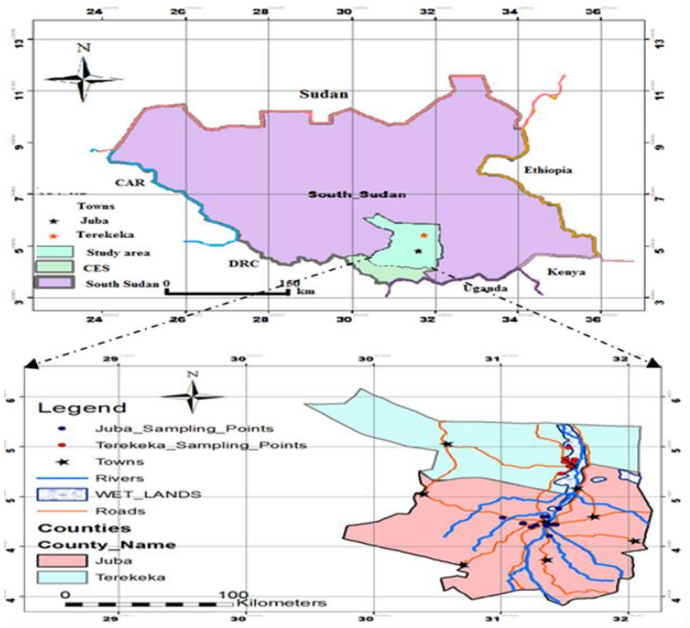

Fig-1: Map showing the locations of study areas, Central Equatoria State, South Sudan

Ethical Approval

This study involves an administration of questionnaires to the farmers as well as blood sampling from cattle. Therefore, the study protocol was assessed and approved by the Ethical Review Committee of the College of Veterinary Medicine, Animal Resources and Biosecurity (COVAB), Makerere University, Uganda vide the reference number of SBLS/REC/15/120. Herdsmen's consents were obtained prior to the start of data collection. Moreover, import and export permits of the biological samples were obtained from Ministry of Agriculture, Animal Industry and Fisheries (MAAIF),

Uganda and Ministry of Livestock and Fisheries (MLF), South Sudan, respectively, prior to shipment from and to designate country.

Sample size

The sample size was estimated and calculated using the formula for a cross-sectional study design [9]. The total number of cattle sampled in this study was calculated using an expected individual level prevalence (P) of 25.3% [8] for the rural cattle camps of Terekeka County and a prevalence of 7.5%, from neighboring country, Uganda, [10] for the peri-urban cattle camps of Juba town.

$$n = \frac{\left[Z\alpha\sqrt{2PQ} + Z\beta\sqrt{(PeQe) + (PcQc)}\right]^2}{(Pe - Pc)^2}$$

The sample size that achieves 5% desired absolute precision at 95% confidence interval and 0.84 at 20% level for type II error was calculated as described by [9]. Based on the above formula, a total of 85samples were supposed to be collected from each stratum. However, 250 samples were collected from peri-urban Juba and 252 from rural Terekeka, CES. This was intended to increase the study precision.

Collection of blood samples

Blood samples were collected from the jugular veins of the selected cattle from each camp using plain vaccutainers. Detailed information on the date, geographical coordinates, Payam, cattle camp, animal ID, breed, sex, age, abortion history and reproductive status of each animal bled was labeled for each blood sample. Sera were harvested by centrifugation at 1000xg for 10 –15 min at the Central Veterinary Research Laboratory (CVRL) in Rejaf West Juba County, South Sudan. The harvested sera were collected in Eppendorf tubes and kept at -20°C pending analysis.

Serological detection of Brucella antibodies by Rose Bengal plate test

All serum samples collected were screened for Brucella antibodies using the Rose Bengal Plate Test (RBPT) at the CVRL, South Sudan. The RBPT antigens were obtained from the Veterinary Research Institute (VRI) in Soba, Khartoum Sudan and kept at 5°C. Testing was done according to the procedures stipulated by [11]. Briefly, the serum samples and antigens were brought to room temperature (22 ± 4°C). Only sufficient antigen for the day's tests was removed from the refrigerator. Then 30 µl of each serum sample was placed on a clean white tile and mixed with an equal volume of antigen. Subsequently, an equal volume of antigen was placed near each serum spot. The serum and antigen were mixed thoroughly using a clean tooth pick to produce a circle approximately 2 cm in diameter and the mixture was agitated gently for 4 min. at ambient temperature and the result was noted based on the presence or the absence of agglutination.

Serological confirmation using c-ELISA

The positive sera on RBPT were confirmed by c-ELISA using a commercial kit (Svanovir ® Biotech AB, Uppsala, Sweden). Analysis of the sera for detecting brucella antibodies using c-ELISA was conducted at the COVAB, Makerere University, Uganda, following the manufacturer's instructions. The kit comprised positive, weak positive and negative control sera which were run in duplicates. While the samples were run singly. Briefly, 45 µl of Sample Dilution Buffer was added into each well that was used for serum samples, serum controls and conjugate. Then 5 µl of serum controls, conjugate and test samples were added to each of the appropriate wells. This was followed by addition of 50 µl monoclonal antibody (mAb) solution into all wells used for controls and samples. The time difference between control/sample and mAb solution addition was not exceeding 10 min. The plate was sealed and the reagents were mixed thoroughly for 5 min by a plate shaker and followed by an incubation period of 30 min at 22 ± 4°C. The plate was rinsed 4 times with phosphate buffer saline (PBS)-Tween Buffer. Then100 µl of conjugate solution (goat anti-mouse IgG antibody conjugate with horseradish peroxidase) was added to bind any mAb to the smooth-lipopoly saccharides (S-LPS) on the plate. The plate was again sealed and incubated at 22 ± 4°C for 30 min. This was then followed by rinsing of the plate 4 times with PBS-Tween Buffer. Unbound materials were removed by rinsing with PBS- Tween Buffer. Then addition of 100 µL substrate solution to each well was made, followed by incubation for 10 min at 22 ± 4°C. The begin timing was after the first well was filled. Finally, the reaction was stopped by addition of 50 µl of sulphuric acid to each well in the same order as the substrate solution was added. Color development was observed due to the conversion of the substrate by the conjugate. The optical density was measured by spectrophotometer at 450 nm. The interpretation of the status of a test sample was determined by Percent Inhibition (PI), < 30% was negative and ≥ 30% was considered positive.

Data analysis

The data from the questionnaires and serological analyses were coded and entered into SPSS version 18 (SPSS Inc., Chicago, USA). The data were checked thoroughly for any errors by cross-checking against the original questionnaires and laboratory result sheets. At the individual animal level the dependent variable was brucella sero-positivity of each animal tested and the independent variables were sex, age, breed, abortion, retained placenta and parity number. At the herd level brucella sero-positivity was the dependent variable and the presence of one sero-positive animal qualified the herd to be positive. Cross tabulation of the independent variables with the dependent variables were computed separately for both the individual and herd level risk factors in both the peri-urban Juba town

and rural Terekeka County herds at 95% level of confidence. Initially, univariable analyses of all the variables were performed at both individual and herd levels separately. Variables with a p-value <0.25 on likelihood ratio Chi-square test were subjected to multivariable analysis. In the multivariable logistic regression analysis, the model was fitted with the variables that were significant at the univariable analysis. Variables were then removed one at a time and their interactions between significant variables were tested using Enter selection method until variables in the model showed a P-value of < 0.05. Hosmer-Lemeshow test was used to ascertain the goodness of fit of the models.

RESULTS

Descriptive statistics of study animals

A total of 502 cattle sera were collected, of these 250 were from 44 cattle herds of 9 randomly selected cattle camps in peri-urban areas of Juba town and 252 were from 46 cattle herds of 8 randomly selected cattle camps in rural Terekeka County. Female cattle constituted the majority (82.2% and 86.5%) and also the Nilotic zebu breed predominated (78.8% and 98.4%), in peri-urban Juba town and rural Terekeka County, respectively. No cross-bred cattle were found in the rural Terekeka County cattle camps and most cattle sampled were adults over 6 years of age (Table 1).

Table 1: The descriptive characteristics of sampled cattle from peri-urban Juba town and rural Terekeka County, Central Equatoria State, South Sudan

Variable	Category	Peri-urban Juba		Rural Terekeka	
		Frequency (%)	95% CI	Frequency (%)	95% CI
Sex	Male	43(17.8)	12.4-22.4	34(13.5)	9.5-17.9
	Female	207(82.2)	77.6-87.6	218(86.5)	82.1-90.5
Age (years)	0.6-2	67(26.8)	21.6-32.8	33(13.1)	8.7-17.5
	>3-4	34(13.6)	9.6-18.4	22(8.7)	5.2-12.3
	>5-6	64(25.6)	20.4-30.8	126(50.0)	44.0-56.0
	>6	85(34.0)	28.4-40.0	71(28.2)	23.0-33.3
Cattle breed	Nilotic zebu	197(78.8)	73.6-83.2	248(98.4)	96.8-99.6
	Lugbara	48(19.2)	14.8-24.4	4(1.6)	0.4-3.2
	Cross-bred	5(2.0)	0.4-4.0	-	-

Demographic statistic of respondents interviewed

A total of 90 respondents were interviewed, 44 from peri-urban Juba town and 46 from rural Terekeka County cattle herds. Male respondents predominated in both peri-urban Juba and rural Terekeka herds, 27(61.4%) and 38(82.6%), respectively. The majority of the respondents were aged between 36 – 45 years (27.3%) in peri-urban Juba town compared to age group 26 – 35 years (37.0%) in rural Terekeka County cattle herds. Most of the respondents (95.7%, n=44) in rural Terekeka County did not attend formal education compared to 77.3% (n=34) of respondents in peri-urban Juba town cattle herds. Most of the respondents in both study sites were married (Table 2).

Table 2: Demographic descriptive characteristics of the respondents in peri-urban Juba town and rural Terekeka County, Central Equatoria State, South Sudan

Variable	Category	Peri-urban Juba		Rural Terekeka	
		Frequency (%)	95% CI	Frequency (%)	95% CI
Sex	Male	27(61.4)	45.5 – 77.3	38(82.6)	71.7 – 93.5
	Female	17(38.6)	22.7 – 54.5	8(17.4)	6.5 – 28.3
Age (years)	18-25	11(25.0)	13.6 – 38.6	8(17.4)	6.5 – 30.4
	26-35	11(25.0)	13.6 – 38.6	17(37.0)	23.9 – 50.0
	36-45	12(27.3)	13.6 – 40.9	15(32.6)	19.6 – 45.7
	46-55	7(15.9)	6.8 – 27.3	4(8.7)	2.2 – 17.4
	>56	3(6.8)	0.0 – 13.6	2(4.3)	0.0 – 10.9
Marital status	Married	23(52.3)	38.6 – 65.9	32(69.6)	56.5 – 82.6
	Single	15(34.1)	22.7 – 47.7	10(21.7)	10.9 – 34.8
	Separated	6(13.6)	4.5 – 22.7	4(8.7)	2.2 – 17.4
Education level	Not attended	34(77.3)	63.6 – 88.6	44(95.7)	89.1 – 100.0
	Basic	9(20.5)	9.1 – 34.1	2(4.3)	0.0 – 10.9
	Secondary	0(0.0)	-	-	-
	Diploma/Degree	1(2.3)	0.0 – 6.8	-	-

Sero-prevalence of bovine brucellosis

Bovine brucellosis sero-reactors were detected in cattle camps in both study sites. The individual animal RBPT and c-ELISA based sero-prevalence of

bovine brucellosis in peri-urban Juba town were 23.2% (n=58) and 19.2 %(n=48), respectively. While in the rural Terekeka County an individual animal sero-prevalence rate of 40.5% (n=102) on RBPT and 39.3% on c-ELISA was observed. The overall individual animal sero-prevalence of bovine brucellosis in the studied cattle was 31.9% (n=160) and 29.3% (n=147), by RBPT and c-ELISA, respectively. On the other hand, the herd level sero-prevalence of bovine brucellosis in peri-urban Juba cattle herds was 65.9% (n=29) and 61.4% (n=27) by RBPT and c-ELISA, respectively. In rural Terekeka County cattle herds, the RBPT and c-ELISA tests revealed a herd level sero-prevalence of 91.3% (n=42) and 89.1% (n=41), respectively. The overall herd level sero-prevalence of bovine brucellosis in the studied cattle herds was 78.9% (n=71) and 75.6% (n=68), by RBPT and c-ELISA, respectively.

The risk factors for bovine brucellosis at individual animal level

Univariable logistic regression analyses of individual level risk factors in peri-urban Juba revealed five significant variables namely, sex (P=0.025), age (p= 0.001), abortion history (p= 0.001) and parity (p < 0.001) to be associated significantly with brucella sero-positivity in the cattle herds (Table 3). Comparatively, the results of the same analyses in rural Terekeka County cattle herds revealed that all the variables tested (sex, breed, age, retained placenta, abortion history and parity) were found to be significantly associated with brucella sero-positivity (Table 4).

When variables with p-value less than 0.25 in the univariable analyses of individual animal level risk factors for brucellosis sero-positivity in peri-urban Juba town cattle herds were subjected to multivariable analysis, only previous history of abortion (P < 0.001, OR= 4.9) was significantly associated with brucella sero-positivity. The Hosmer-Lemeshow test at the individual animal level in peri-urban Juba town cattle herds showed that the model had fitted the data at ($\chi 2$ = 1.421, df= 6, p= 0.965).

Similarly, of all the variables identified in rural Terekeka County, only previous history of abortion (p < 0.001, OR= 6.251) was determined as a risk factor associated with brucella sero-positivity when individual animal level risk factors were subjected to multivariate analysis. The Hosmer-Lemeshow test at the individual animal level in rural Terekeka cattle herds showed that the model had fitted the data at ($\chi 2$ = 3.523, df= 8, p= 0.897).

Table 3: Descriptive statistics and univariable logistic regression analyses of individual animal risk factors for sero-positivity to Brucella in peri-urban Juba town cattle herds, Central Equatoria State, South Sudan

Variable	Category	Frequency (%)	c-ELISA positive	P-value	OR*	95% CI
Sex	Male (ref)	43(17.2)	3	-	-	-
	Female	207(82.8)	45	0.025	3.704	1.095–12.531
Breed				0.463		
	Cross-bred (ref)	5(2.0)	0	-	-	-
	Nilotic zebu cattle	197(78.8)	40	< 0.001	0.200	0.094 - 427
	Lugbara	48(19.2)	8	< 0.999	0.000	-
Age (years)				0.001		
	0.6 – 2 (ref)	67(26.8)	4	-	-	-
	3 – 4	34(13.6)	3	< 0.001	0.097	0.030 – 0.317
	5 – 6	64(25.6)	16	< 0.001	0.333	0.189 – 0.587
	>6	85(34.0)	25	< 0.001	0.417	0.261 – 0.664
Retained placenta	No (ref)	120(82.2)	29	-	-	-
	Yes	26(17.8)	11	0.060	2.301	0.951 – 5.565
Abortion history	Absent(ref)	94(64.4)	15	-	-	-
	Present	52(35.6)	25	< 0.001	4.877	2.247– 10.585
Parity number				0.009		
	Heifer(ref)	61(29.6)	5	-	-	-
	Produced once	37(18.6)	7	0.001	0.233	0.102 – 0.531
	Produced twice	40(19.4)	12	0.014	0.429	0.218 – 0.843
	Produced more than twice	68(33.0)	21	0.002	0.447	0.267 – 0.47

*OR=Odd Ratio

Table 4: Frequency and Univariable logistic regression analyses of individual animal level risk factors for bovine brucellosis in rural Terekeka County cattle herds, Central Equatoria, State South Sudan

Variable	Category	Frequency (%)	c-ELISA positive	P-value	OR	95% CI
Sex	Male (ref)	34(13.5)	4	-	-	-
	Female	218(86.5)	95	<0.001	5.79	1.97 – 17. 01
Breed	Lugbara (ref)	4(1.6)	0	-	-	-
	Nilotic zebu cattle	248(98.4)	99	0.157*	1.664	1.504 – 1.842
Age(years)				< 0.001		
	0.6 – 2(ref)	33(13.1)	2	-	-	-
	3 – 4	22(8.7)	5	0.016	0.294	0.109 – 0.797
	5 – 6	126(50.0)	59	0.476	0.881	0.621 – 1.250
	>6	71(28)	33	0.553	0.868	0.545 – 1.384
Retained placenta	No (ref)	136(73.1)	53	-	-	-
	Yes	50(26.9)	37	< 0.001	4.457	2.170 – 9.155
Abortion history	Absent(ref)	81(43.8)	18	-	-	-
	Present	104(56.2)	71	< 0.001	7.530	3.865–14.672
Parity number				0.011		
	Heifer(ref)	33(15.1)	6	-	-	-
	Produced once	45(20.6)	20	0.457	0.800	0.444 – 1.440
	Produced twice	56(25.7)	30	0.593	1.154	0.682 – 1.951
	Produced more than twice	84(38.5)	39	0.513	0.867	0.564 – 1.331

* = Fisher's exact test

Bovine brucellosis herd level risk factors

Two herd level risk factors, keeping a number of cattle above 300 (p=0.043, OR= 3.91) in a cattle camp and handling of aborted fetus (p=0.017, OR= 49), were found to be statistically associated with brucella sero-positivity in peri-urban Juba town cattle herds at the univariable analysis(Table5). Similarly univariable analysis identified two herd level risk factors to be significantly associated with Brucella sero-positivity in rural Terekeka County, i.e. keeping a number of cattle above 300 per a cattle herd (p <0.001, OR= 39.000) and the presence of wildlife (p= 0.015, OR= 8.00) (Table 6)

On multivariable analysis, no significant risk factor was found to be associated with Brucella sero-positivity in the peri-urban Juba town cattle herds. However, multivariable analysis identified keeping a number of cattle above > 300 (p= 0.005, OR= 44.934) in a camp as the sole risk factor associated with Brucella seropositivity in rural Terekeka County cattle herds. The Hosmer-Lemeshow test showed that the model had fitted the data at the herd level ($\chi 2$= 0.759, df= 4, p=0.944).

Table 5: Result of the herd level univariable analysis of brucella risk factors in peri-urban Juba town cattle camps, Central Equatoria State, South Sudan

Variable	Category	P-value	OR	95% C.I
Number of cattle in a camp	50 - 99 (ref)	-	-	-
	>300	0.043	3.91	1.004–15.240
Mixed herd	No(ref)	-	-	-
	Yes	0.242	2.11	0.598 – 7.448
Breeding System	Bull on own herd (ref)	-	-	-
	Communal bull	0.583	1.41	0.416 – 4.753
Presence of wildlife	No (ref)	0.507	0.64	0.172 – 2.391
	Yes	-	-	-
Other herds share water source	Yes	-	-	-
	No (ref)	0.510	0.66	0.193 – 2.268
Recently introduced cow	Yes	-	-	-
	No (ref)	0.800	1.19	0.308 – 4.604
Handling of aborted fetus	Throw out (ref)	-	-	-
	Feeds to dogs	0.017	4.95	1.270–19.288

Table 6: Herd level risk factors at Univariable analysis in rural Terekeka County cattle camps, Central Equatoria State, South Sudan

Variable	Category	P-value	OR	95% C.I
Number of cattle in a camp	50 - 99 (ref) >300	- < 0.001	- 39.000	- 3.046-499.323
Mixed herd	No (ref) Yes	- 0.432	- 0.415	- 0.044 – 3.928
Breeding method	No (ref) Yes	- 0.141	- 1.379	- 1.140 – 1.669
Presence of wildlife	No (ref) Yes	- 0.015	- 8.000	- 1.238– 51.690
Other herds share water source	No (ref) Yes	- 0.195	- 1.290	- 1.092 – 1.525
Recently introduced cow	No (ref) Yes	- 0.362	- 2.357	- 0.359– 15.496
Handling of aborted fetus	Throwing out (ref) Feeds to dogs	- 0.058	- 6.167	- 0.782– 48.640

Prevalence of practices that could predispose to human infection by Brucella bacteria

The most common practices noted in both peri-urban Juba town and rural Terekeka County that could predispose to Brucella infections of people were; eating aborted fetuses, blowing through vulva, drinking un boiled milk and aiding cow during parturition. All these risky practices were more prominent in rural Terekeka County compared to peri-urban Juba town (Table 7).

Table 7: Common practices that could predispose to Brucella infection among pastoralists

Type of practice	Category	Peri-urban Juba town	Rural Terekeka County
		Frequency (%)	Frequency (%)
Eating aborted fetuses	No (ref) Yes	29(65.9) 15(34.1)	9(19.6) 37(80.40
Blowing through vulva	No(ref) Yes	28 (63.3) 16(36.4)	9(19.6) 37(80.4)
Drinking unboiled milk	No(ref) Yes	4(9.1) 40(90.9)	- 46(100.0)
Aiding cow during delivery	No(ref) Yes	2(4.5) 42(95.5)	- 46(100.0)

DISCUSSION

This present study has revealed the occurrence of bovine brucellosis in the study area. The disease was more prevalent in rural Terekeka County cattle herds compared to Juba town herds. The herd level sero-prevalence of 89.1% based on c-ELISA is similar to 90% reported in Khartoum [12]. However, the individual sero-prevalence rate of 40.5% on RBPT and 39.3% on c-ELISA in Terekeka was higher than the 24.9% individual animal prevalence rate reported in Kuku dairy Scheme Khartoum North, Sudan. Comparatively, the ostentation of herd level sero-prevalence in peri-urban Juba town was 61.4% on c-ELISA, however the overall individual animal RBPT and c-ELISA based sero-prevalence was 23.2% and 19.2 %,respectively, indicating a lower prevalence of brucellosis in Juba compared to Terekeka or to Khartoum [12]. This study showed higher prevalence compared to several studies conducted on sero-prevalence of bovine brucellosis across the region. However, our results concur with those in Eastern Africa including Uganda which experiences a high prevalence of bovine brucellosis in the rural areas compared to urban and peri-urban areas [10]. Similarly, a study conducted in Niger had found higher prevalence of brucellosis of 4.6% (95% CI: 3.3-6.2) in rural areas opposed to 2.0% and 1.8% in urban and peri-urban areas, respectively [13]. It seems that the high sero-prevalence rate in rural Terekeka County could be attributed to the mixing of a large number of livestock and herds within the cattle camps, this practice increases the chances of contacts and contaminations. Moreover it could be explained by the presence of communal grazing areas and interactions of cattle from different cattle camps especially during the grazing and in water points in the dry season. Most cattle camps in South Sudan during the dry season periods migrate far distances to swampy areas (toichs) in search of pasture

and water and this can provide high spots for the transmission of brucella . This study has shown the communities in the study area predominantly keep female cattle since these constituted the majority of the animals randomly samples. This could be attributed to the social prestige and cultural norms of our pastoralist communities in keeping livestock for quantity as cattle serve as a store of wealth [14]. Nilotic zebu cattle were predominant which could be related to the norms, customary myths and beliefs of pastoralists in that those exotic breeds of cattle are easily vulnerable to diseases and deaths. Indeed no cross-bred cattle were found in the rural Terekeka County cattle camps rural settings. Most herders in the urban settings in and around Juba were adults over 36 years of age whereas more active youth 26 – 35 engaged in cattle herding's activities in the pastoralist rural Terekeka County. This may be explained by the very active age of pastoralists to cope with the dynamics and threats in the face of cattle raiding or rustling that may arise in the grazing areas. As such males were the most involved in cattle herding activities in the camps as opposed to the female counterparts. This dominance of the male gender is plausible under the traditional pastoralist system in South Sudan. Pointedly, the hard living condition in the cattle camps, the remoteness and insecurity accruing from cattle raiding, are all situations not favorable to females. Moreover, the dominance of male in cattle rearing may predispose them to brucella infection. A study conducted in Terekeka County health facility found more cases of brucella infection in male compared to female and high prevalent of human brucellosis among age group 20 – 30 years old [15]. The low education levels in rural Terekeka County could be attributed to the heavy involvement of children and youth in cattle herding activities as opposed to the case in urban Juba town.

Risk factors associated with bovine brucellosis are imperative for elucidating epidemiological studies of such disease of public health implications. In peri-urban Juba town and rural Terekeka County cattle herds, this study showed that animals with prior history of abortion ($p < 0.001$, OR= 4.941) and ($p < 0.001$, OR= 6.251) were associated with brucella sero-positivity. This is in line with the findings of [16] who reported a higher sero-positivity of bovine brucellosis in animal with a history of abortion ($\chi 2 = 24.50$, $p < 0.001$). Similarly, a significant association ($p = 0.042$) of brucella sero-positivity in animals with history of abortion was reported by [17].

In peri-urban Juba town, no significant risk factor was determined at the herd level as opposed to rural Terekeka County, where a number of cattle above 300 ($p= 0.005$, OR= 44.934) in a cattle camp was found to be associated with brucella sero-positivity. This could be explained by the fact that cattle camps in rural Terekeka County are very big-sized and can accommodate 4,000 to 6,000 heads of cattle. This big number might lead to very fast exposures especially when a disease is contagious by nature. [18] found that large herd size (OR= 1.3 (95% CI: 1.1 – 1.5), $p < 0.001$) was associated with brucella sero-positivity. Similarly, [6] found herd size ($p=0.009$) to be significantly associated with bovine brucellosis.

CONCLUSION

The current study has revealed the occurrence of bovine brucellosis in peri-urban Juba and rural Terekeka cattle herds. The sero-prevalence was higher in rural Terekeka County compared to peri-urban Juba town cattle herds. Abortion history was the sole individual animal risk factor determined as significantly associated with brucella sero-positivity in both the study sites. Large herd size was identified as the risk factor for brucella sero-positivity in rural Terekeka County cattle herds. Further studies are needed to understand the disease's epidemiology and hence develop appropriate control measures against bovine brucellosis in South Sudan.

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