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Research Article

Presumptive diagnosis of brucellosis and determination of risk factors for seropositivity among members of cattle keeping households in a high cattle traffic area in the South Western region of Uganda

Abstract

Brucellosis is worldwide and affects human, cattle health and international trade. Human *Brucella* seroprevalence in Ugandan communities is not well known since most of the health centers are not able to screen for brucellosis. The study presumptively determined: seroprevalence, identified risk factors associated with *Brucella* infection in cattle keeping household members and within their herds in Kyangyenyi sub county, Sheema district, South Western region of Uganda. A one-month cross-sectional study that used a simple two stage cluster sampling method was conducted where 216 households were randomly selected from 18 rural villages. Questionnaires were administered to household participants. Humans and cattle from same households were screened for *Brucella* antibodies using the Rose Bengal Test.

Overall, from 1820 cattle, 839 cattle were sampled from 216 households and about 4 cattle were sampled per household. One third (33%; 95% confidence interval (CI), 27%, 39%) of 216 household members had probable seropositivity. Of the 216 herds sampled, (71%; 95% CI, 64%, 77%) had at least one presumptively seropositive animal and 34.7% (291/839) of the total number of cattle sampled were presumptively seropositive. Having a presumptively seropositive herd increased the odds of having a probable seropositive household member (2.03; 95% CI, 1.01, 4.07), p=0.045. Households with presumptive seropositive herds were more likely to have probable seropositive members. Health promotion and awareness in these communities to avert increasing *Brucella* infection is important.

Abbreviations

°C: Degrees Centigrade; FREC: Faculty Research Committee; MUIRC: Mbarara University Institutional Review Committee; ML: Milliliters; No: Number; RBT: Rose Bengal Test; REC: Research Ethics Committee; Spp: Species; S-LPS: Smooth Lipopolysaccharide; UK: United Kingdom; IgA: Immunoglobulin A; UNCST: Uganda National Council for Science and Technology; Vol: Volume.

Introduction

Brucellosis is a highly contagious zoonosis of major public health and veterinary concern globally [1]. *Brucella* causes disease primarily in domestic and in some wild animals. Most *Brucella* spp. are pathogenic in humans [2-4]. Brucellosis is caused by gram-negative facultative intracellular coccobacilli of *Brucella* genus [2,5]. Cattle are majorly infected with *Brucella abortus* but cases of infection with *Brucella meltensis* are known to happen [6,7]. Humans get infected by direct or indirect contact with infected cattle or their products and also from contaminated environments. Humans also get infected from occupational exposure due to direct contact with infected cattle and from food borne transmission [4,8,9]. Person-to-person transmission is rare but has been observed when infective biological products such as: infective blood used for transfusion, infective tissue or bone marrow used in transplants and also sexually from an infected person [4]. The clinical presentation of brucellosis in humans appears as a nonspecific flu-like syndrome (relapsing fever, headaches, general body malaise),

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while brucellosis in livestock causes reproductive losses and is also chronic, causing economic losses [4,10-13]. The disease is common in Sub Saharan Africa, South America, Mediterranean region and Asia where effective diagnosis or control in humans and animals is often not available [4,14]. Herd management practices are known as important risk factors: keeping large herd sizes [10,13,15], communal grazing where commingling of flocks and herds from different owners happens; purchasing animals from unscreened sources [4,8] and sharing male breeding stock [12]. *Brucella* can be shed in the milk of infected animals for a variable length of time, but often throughout their life time [4,16]. *Brucella* can be transmitted to calves vertically and also through drinking contaminated milk [4,16,17]. Although sexual transmission usually plays little role in the epidemiology of bovine brucellosis, artificial insemination can transmit the disease with semen from an infected animal [17]. In unvaccinated cattle herds, infection can spread rapidly and many abortions may occur [12,13,18].

Uganda a Low and Medium Income Country (LMIC) has limited resources devoted to the control of brucellosis therefore cost friendly, amenable, adaptable, effective and efficient means of screening are needed such as RBT. Previous studies in Uganda have reported a relatively high seroprevalence in humans (11% and 14.9% in south western Uganda; 17% in central Uganda) as well as among cattle (14%), bovine milk (29%) and goats (17%) [19-21]. Majority of screening in humans done in rural areas is presumptive and World Health Organization (WHO) classifies presumptive laboratory diagnosis or probable diagnosis as use of Rose Bengal Test (RBT) (http://data.unaids.org/publications/irc-pub04/surveillancestandards_en.pdf). According to World Organization for Animal Health (OIE), in cattle, RBT is very sensitive and false-negative reactions occur rarely and the test is adequate as a screening test for detecting infected herds or to guarantee the absence of infection in brucellosis-free herds or flocks especially in Brucella endemic country like Uganda [22]. According to the Food and Agriculture Organisation (FAO), the buffered *Brucella* antigen tests are suitable for screening herds and individual animals and in situations where no control programs are going on, on-farm active seroprevalence testing can be done with RBT (http://www.fao.org/3/y4723e/y4723e08.htm). In endemic areas with high seroprevalence, RBT is sufficient to give a true seroprevalence of brucellosis in animals according to the Kappa results obtained from the study by Madut et al., [23]. There is an intense livestock trade in western and south-western districts of Uganda as reported by the district veterinary officers of the western region; there are weekly and monthly livestock markets, where cattle from different areas comingle. Cattle from western Uganda are known to move to all regions of the country and to neighboring countries as well [15]. Animal health service providers in southwestern Uganda performing limited and non-routine cattle screening for cautious cattle breeders and traders have constantly reported an increasing incidence of *Brucella* infection in cattle due to abortions and results from field tests. Previous studies have reported that unscreened animals, especially cattle moved from western Uganda to other regions of Uganda potentially expose animals of those other regions to *Brucella* infection [15]. On the basis of previous studies, and information collected from the animal health service providers on the potentially increasing incidence of brucellosis in western Uganda, the present study used a presumptive laboratory diagnosis as recommended by WHO and OIE by using RBT for screening so as to determine the probable human and cattle seroprevalence and also identify associated risk factors for *Brucella* infection among the cattle keeping household members and their herds in a high cattle traffic area in the western region of Uganda. This study will provide data needed to justify the need for continuous brucellosis surveillance, control and prevention in highly endemic areas.

Materials and Methods

We conducted a one month cross-sectional study using a two stage cluster sampling method [24,25] in Kyangyenyi Sub County, Sheema district, Western region of Uganda (Figure 1). This district is an important catchment area for traders and farmers from Uganda, Rwanda and Burundi in need of breeding animals especially Friesian cross breeds. Kyangyenyi Sub County has 31,263 inhabitants [26].

Expected household member seroprevalence of brucellosis was 7% [27], with a 5% precision at a 95% confidence interval. From six study parishes of Kyangyenyi Sub County, three villages were randomly selected and 12 households were sampled per cluster/village. Study villages had an average of 115 households with 4.66 persons per household [26]. The study was conducted for one month and 216 households were visited. Each Household had a herd or eligible animal.

Household eligibility was based on having at least one female bovine (i.e. associated risk with milk and pregnancy as potential sources for *Brucella* infection) [22,28]. Household members were eligible for the study if they satisfied at least one of the following criteria: lived together under same roof for more than one week; shared meals from a common cooking pot, took care of the cattle; carried out milking and preparing animal products for consumption. The village health team members listed all the households that fit the inclusion criteria; households were then randomly chosen from these lists. Eligible members from each randomly chosen household were enumerated; one member was randomly chosen, and if the person rescinded, another raffle without replacement was done to select another person from the household sampling frame.

The cattle were selected from households participating in the study. In-calf heifers, milking cows, cows with history of parturition and cows older than two years were eligible for sampling. In case of herds with less than ten animals, all animals in the herd were sampled if the animals fit the inclusion criteria. Herds with more than ten, only ten animals, due to resource constraints were randomly sampled. Numbers were first assigned to the eligible animals in the herd; numbers were then drawn randomly without replacement to choose animals to be sampled.

Human blood samples were collected in the households of the study participants according to the Clinical and

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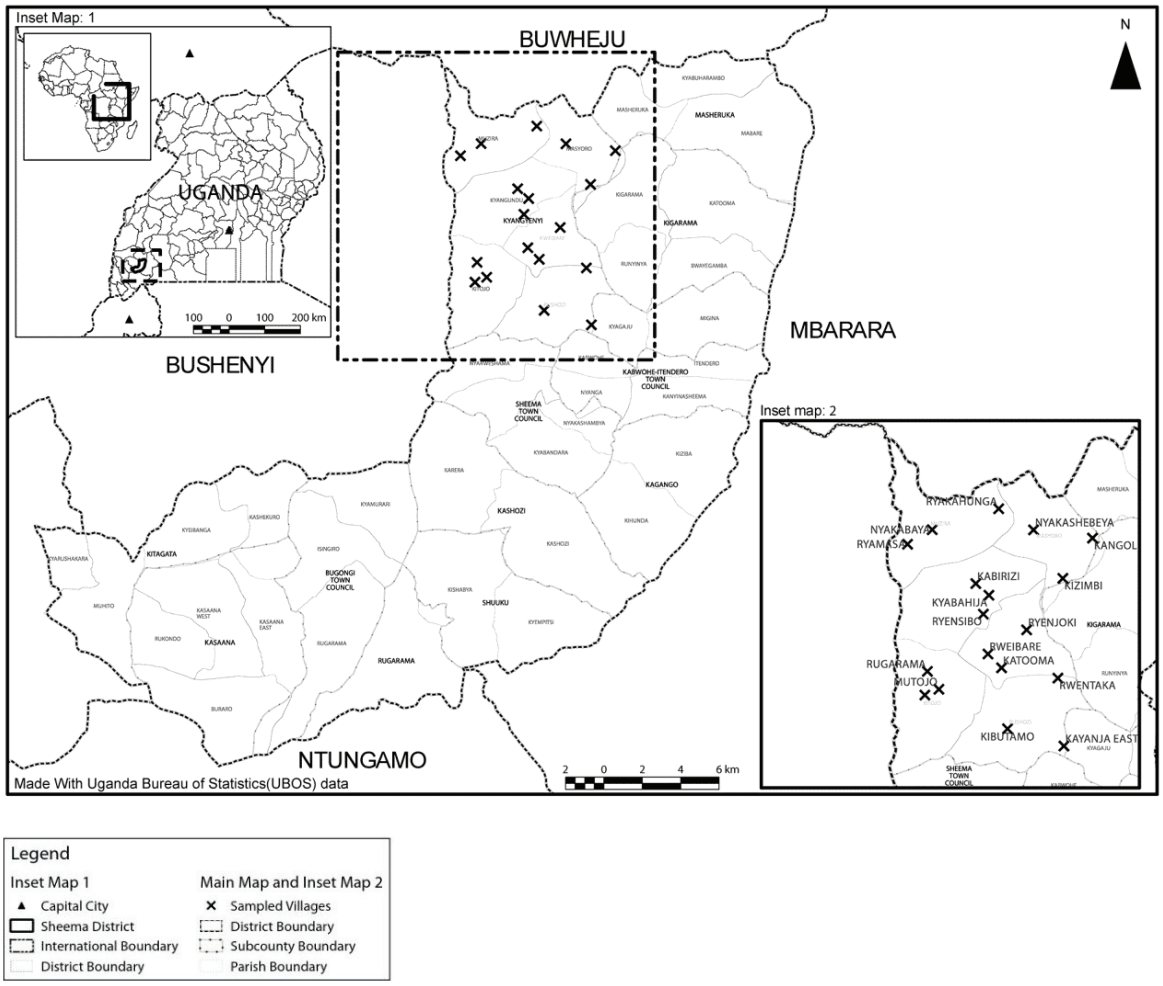

Figure 1: Map showing parishes within Sheema district and its neighbouring districts in South Western Uganda with inset map 1 showing Uganda within Africa and inset map 2 showing Kyangyenyi Sub County and villages where study was carried out.

Laboratory Standards Institute (i.e. National Committee for Clinical Laboratory Standards; Procedures for the Collection of Diagnostic Blood Specimens by venipuncture. Approved Standard - Fifth Edition H3-A5, Volume.23 Number.32.). Blood samples were collected into sterile dry vacutainer tubes (Becton Dickinson®, Plymouth, United Kingdom.), labeled and allowed to clot for 30 minutes in the field; serum was then harvested into 2 milliliter (mL) cryogenic vials and kept at -4°C for less than 24 hours. Serum samples were then transported to Mbarara western regional veterinary laboratory to be stored in liquid nitrogen. The serum was processed within three days.

Blood from cattle was collected from the mid coccygeal or jugular vein and put into sterile dry vacutainers. Collected blood samples were labeled and allowed to clot. Serum was harvested into 2 mL cryogenic vials and kept at -4°C. The serum was then transported to the Mbarara western regional veterinary laboratory within 24 hours where it was stored at -80°C and processed within three days.

Serum samples were screened for anti-*Brucella spp.* antibodies using the classical rapid slide-type agglutination assay called Rose Bengal test (RBT). Like in previous studies and as recommended by OIE, FAO and WHO, Rose Bengal reagent, a pinkly stained *B. abortus* suspension at pH 3.6–3.7 was used on both human and animal serum [4,22,29,30]. *Brucella abortus* antigen, positive and negative control sera (Cypress Diagnostics, Belgium), were used to detect *Brucella* antibodies following the manufacturer's instructions. Reacting samples (i.e. agglutinating) were considered positive.

For human serum, RBT protocol for incubation time was adjusted from four to eight minutes considering that sera with blocking immunoglobulin A (IgA) or with high titers of non-agglutinating antibodies will need up to eight minutes to develop the bacterial clumps or the characteristic rim of positive RBT [31].

Demographic data was collected from the randomly selected household members before blood was collected from them. Data on associated risk factors for *Brucella* infection in both humans and cattle was also collected using structured interviews guided by a questionnaire. Data on herd management practices was collected: Location, herd size, calf feeding method, history of vaccination and method of disposal of afterbirth and other reproductive refuse. Animal data collected included: age, history of vaccination and breeding method (natural or artificial), pregnancy and lactation status. Data on history of

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abortion, retained placenta or other reproductive disorders was also collected. Herd personnel knowledge on awareness of brucellosis and its modes of transmission was queried. History of raw milk consumption was also collected. No *Brucella* vaccination has ever been carried out in the study area, according to official reports from the district veterinary office. Data was filled into the Epi-info® software, transferred to Stata 11.0 (Stata Corporation, College Station, Texas, USA) and analyzed using Generalized Estimating Equations models using the logit link function and exchangeable correlation within the villages was assumed, using village as a random effect.

For this study, RBT was used for presumptive or probable diagnosis in humans and cattle. All positive cases were termed as presumptive or probable cases. The primary outcome of the study was probable *Brucella* seropositivity among members from cattle keeping households, which was defined as the proportion of household members testing seropositive among the total number of household members sampled (i.e. only one member from each household was randomly screened). The secondary outcome for the study was presumptive *Brucella* seroprevalence in cattle which was defined as the proportion of seropositive cattle over the total number of cattle sampled. All households sampled had a herd or an animal; and any herd that had at least one animal reacting positive was considered as a potentially exposed herd [16,22,32,33]. Secondary objectives of the study were: the identification of risk factors that were associated with probable *Brucella* seropositivity in household members and identification of risk factors associated with presumptive *Brucella* seropositivity within the herds belonging to households whose members were enrolled in the study.

The study which involved collection of human and cattle blood samples was approved by the Faculty of Medicine Research Committee (FREC) at Mbarara University of Science and Technology and also by the Research Ethics Committee (REC) of Mbarara University of Science and Technology (Study number 160121) and Uganda National Council of Science and Technology (approval number HS 165). Written consent was obtained from the respondents and identification numbers were assigned to all participants for confidentiality. We maintained confidentiality and privacy during the data collection. Willing study participants provided their personal telephone numbers on a voluntary basis. The results of the laboratory diagnosis for both human and cattle blood were timely shared with the participants. Also, our study team invited participants that were feeling ill to attend the Government Health Center where they were offered adequate treatment.

Results

The descriptive demographic characteristics of the 216 participants (Table 1) showed: A predominantly male 61% (131) population with median age of 48 years. 6% admitted drinking raw milk and 94% of the responders were aware of a disease called brucellosis which is locally known as *Obutorogye* or *Okutoroga* in animals. From 1820 cattle from 216 households, 839 cattle were sampled and on average, 3.9 cattle were sampled per household. 34.7% (291/839) cattle reacted seropositive to RBT. A third (33%; 95% Cl, 27%, 39%) of the household members tested seropositive and close to three quarters (71%; 95% Cl, 65%, 77%) of the household herds screened seropositive with RBT meaning 153 herds had at least one animal that screened seropositive. Of the 153 herds, 72 herds (47%) had more than one presumptively seropositive animal while 81 herds (53%) had only one animal that was presumptively seropositive. 27.9 % (19/68) of the small herds, 54.5 % (36/66) of the medium sized herds and 89.5% (17/19) of the large herds had more than one animal that reacted positive with RBT.

For associated risk factors for probable *Brucella* seropositivity in household members (Table 2), with level

Table 1: Demographic and baseline characteristics; cattle keepers and their cattle enrolled in the Brucellosis study in Kyangyenyi, Sheema district, rural south-western Uganda, 2018.

Cattle Keepers' Characteristics	Variable	Number (%) N=216
Age (Years)	Median age	48 [34.5, 63.5]
	<35	69 (32.0)
	35-50	54 (25.0)
	>50	93 (43.0)
Sex	Male	131 (60.6)
	Female	85 (39.4)
Level of education	>primary	49 (22.7)
	Primary	25 (11.6)
	Uneducated	142 (65.7)
Occupation	Farmer	171 (79.2)
	Attendant	24 (11.1)
	Other	21 (9.7)
Consume raw milk		12 (5.6)
Assisted in Calving		152 (70.4)
Knowledge of brucellosis	in animals	204 (94.4)
	as a zoonotic	151 (69.9)
Knowledge of zoonotic transmission modes	knows two	48 (22.2)
	Know >2	7 (3.2)
	knows one	91 (42.1)
	do not know	70 (32.4)
FARM CHARACTERISTICS		
Median herd size [IQR]		6 [3.0-10.0]
Herd size categories	Small (n≤ 6)	119 (55.1)
	Moderate (n=7-15)	77 (35.6)
	Large (n≥16)	20 (9.3)
Calf feeding pattern	Residual	73 (33.8)
	Bucket	126 (58.3)
	Both	17 (7.9)
Received Vet service in last 2 years		211 (97.7)
Abortion history on farm in the last 3years		106 (49.1)
Placenta retention record on farm		82 (38.0)
Disposal of placenta, aborted, still birth material	Bury	14 (6.5)
	Given to dogs	46 (21.3)
	Open disposal	156 (72.2)

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Table 2: Univariate analysis of factors associated with probable *Brucella* seropositivity in cattle keeping households in Kyangyenyi sub-county Sheema district, rural south-western Uganda, 2018.

Variable	Negative (%)	Positive (%)	Crude OR [95%CI]	P value
Cattle Keepers' characteristics				
Age in years				
• >50	59 (63.4)	34 (36.6)	1.00	
• 35-50	43 (62.3)	26 (37.7)	1.04 [0.55-1.96]	0.908
• <35	43 (79.6)	11 (20.4)	0.48 [0.22-1.01]	0.055
Sex				
• Male	92 (70.2)	39 (29.8)	1.00	
• Female	53 (62.3)	32 (37.7)	1.41 [0.80-2.50]	0.237
Level of education				
• Beyond primary	35 (71.4)	14 (28.6)	1.00	
• Primary	18 (72.0)	7 (28.0)	1.03 [0.37-2.88]	0.951
• No education	92 (64.8)	50 (35.2)	1.35 [0.67-2.68]	0.400
Occupation				
• Other	16 (76.2)	5 (23.8)	1.00	
• Farmer	110 (64.3)	61 (35.7)	1.54 [0.57-4.16]	0.397
• Attendant	19 (79.2)	5 (20.8)	0.74 [0.19-2.86]	0.658
Consume raw milk				
• No	137 (67.2)	67 (32.8)	1.00	
• Yes	8 (66.7)	4 (33.3)	0.98 [0.30-3.21]	0.975
Assisted in calving				
• No	44 (68.8)	20 (31.2)	1.00	
• Yes	101 (66.4)	51 (33.6)	1.09 [0.59-2.03]	0.784
Received Vet service in last 2 years				
• Yes	142 (67.3)	69 (32.7)	1.00	
• No	3 (60.0)	2 (40.0)	1.19 [0.2-7.2]	0.847
Disposal of placenta, aborted and still birth material				
• Bury	13 (92.9)	1(7.1)	1.00	
• Given to dogs	29 (63.0)	17 (37.0)	6.97 [1.00-48.36]	0.049
• Open	103 (66.0)	53 (34.0)	5.81 [0.89-38.15]	0.067
Parish				
• Kitojo	24 (66.7)	12 (33.3)	1.00	
• Kyangundu	26 (72.2)	10 (27.8)	0.77 [0.40-1.48]	0.433
• Masyoro	21 (58.3)	15 (41.7)	1.43 [0.76-2.67]	0.263
• Muzira	24 (66.7)	12 (33.3)	1.00 [0.53-1.89]	1.000
• Rushozi	26 (72.2)	10 (27.8)	0.77 [0.40-1.48]	0.433
• Rweibare	24 (66.7)	12 (33.3)	1.00 [0.53-1.89]	1.000
Having a presumptively *Brucella* seropositive herd				
• No	48 (76.2)	15 (23.8)	1.00	
• Yes	97 (63.4)	56 (36.6)	1.86 [0.98-3.56]	0.06
Knowledge of brucellosis in animals				
• Yes	135 (66.2)	69 (33.8)	1.00	
• No	10 (83.3)	2 (16.7)	0.39 [0.08-1.81]	0.229
Knowledge of brucellosis as a zoonotic				
• Yes	100 (66.2)	51 (33.8)	1.00	
• No	45 (69.2)	20 (30.8)	1.01 [0.57-1.79]	0.978
Knowledge of zoonotic transmission modes				
• Knows two	31 (64.6)	17 (35.4)	1.00	
• Knows > 2	5 (71.4)	2 (28.6)	0.66 [0.11-3.87]	0.642
• Knows one	60 (66.0)	31 (34.0)	1.03 [0.51-2.10]	0.925
• Do not know	49(70.0)	21 (30.0)	0.99 [0.47-2.09]	0.977
Herd size				
• Small(n≤ 6)	81 (68.1)	38 (31.9)	1.00	
• Moderate(n=7-15)	50 (64.9)	27 (35.1)	1.10 [0.63-1.89]	0.742
• Big(n≥16)	14 (70.0)	6 (30.0)	0.95 [0.36-2.50]	0.919
Calf feeding pattern				
• Residual suckling	53 (72.6)	20 (27.4)	1.00	
• Bucket	79 (62.7)	47 (37.3)	1.47 [0.82-2.62]	0.198
• Both	13 (76.5)	4 (23.5)	0.89 [0.24-2.73]	0.840
Abortion history on farm in the last 3 years				
• No	72 (65.5)	38 (34.5)	1.00	
• Yes	73 (68.9)	33 (31.1)	0.81 [0.49-1.37]	0.436
Placenta retention record on farm				
• No	91 (67.9)	43 (32.1)	1.00	
• Yes	54 (65.9)	28 (34.1)	1.09 [0.62-1.92]	0.762

of significance at $p<0.05$, a liberal p value of 0.2 was set as a cut off to allow inclusion of 4 variables for multivariable regression. The adjusted Odds ratios for associated risk factors for probable *Brucella* seropositivity among household members (Table 3) were: the adjusted odds ratio for probable *Brucella* seropositivity in household members with age category <35 years was (0.41; 95% CI, 0.18, 0.94). Secondly; having a presumptively *Brucella* seropositive herd increased the odds for probable *Brucella* seropositivity in household members (2.03; 95% CI, 1.01, 4.07). Thirdly, the adjusted odds ratio for probable *Brucella* seropositivity in household members when reproductive refuse was openly disposed was (7.43; 95% CI, 0.93, 59.49); and when fed to dogs, the adjusted odds for probable *Brucella* seropositivity in household members was (6.87; 95% CI, 0.91, 51.41). Fourthly: when calves were bucket fed, the adjusted odds ratio for probable *Brucella* seropositivity in household members was (1.69; 95% CI, 0.91, 3.13).

For risk factors associated with *Brucella* seropositivity in herds (Table 4): The location of the farm i.e parish was important risk factor associated with *Brucella* seropositivity in the herds.

Compared to other parishes, Kitojo and Kyangundu had significantly higher odds ratios of seropositive herds that had animals that reacted positive with the RBT.

Discussion

The scope of the study was to measure the level of human

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Table 3: Multivariable analysis of factors associated with probable *Brucella* seropositivity at household level, Kyangyenyi sub-county in Sheema district, rural south-western Uganda, 2018.

Variable	OR*	P
Having a presumptively *Brucella* seropositive herd		
No	1.00	
Yes	2.03 [1.01-4.07]	0.045
Disposal of reproductive refuse		
Bury	1.00	
Open disposal	7.43 [0.93-59.49]	0.059
Fed to dogs	6.87 [0.91-51.41]	0.061
Age category		
>50	1.00	
35-50	1.18 [0.61-2.28]	0.625
<35	0.48 [0.22-1.06]	0.071
Calf feeding pattern		
Residual suckling	1.00	
Bucket	1.69 [0.91-3.13]	0.098
Both	0.69 [0.20-2.38]	0.559

Caption: * = Odd Ratio Adjusted at 95% Confidence Interval.

Table 4: Univariate analysis of factors associated with presumptive *Brucella* seropositivity in farms/herds in Kyangyenyi sub county Sheema district, rural south-western Uganda, 2018.

Farm characteristics Negative (%)	Positive (%)	Crude OR [95% CI]	P Value
Calf feeding pattern			
Residual suckle	22 (30.1)	51 (69.9)	1.00	
Bucket	37 (29.4)	89 (70.6)	1.01 [0.53-1.92]	0.968
Both	4 (23.5)	13 (76.5)	1.24 [0.37-4.20]	0.725
Received Vet service in last 2 years			
Yes	62 (29.4)	149 (70.6)	1.00	
No	1 (20.0)	4 (80.0)	1.92 [0.19-19.31]	0.578
Abortion history on farm in the last 3 years			
No	34 (30.9)	76 (69.1)	1.00	
Yes	29 (27.4)	77 (72.6)	1.19 [0.65-2.16]	0.573
Placenta retention record on farm			
No	42 (31.3)	92 (68.7)	1.00	
Yes	21 (25.6)	61 (74.4)	1.41 [0.76-2.61]	0.278
Disposal of placenta, aborted and still birth material			
Buried	3 (21.4)	11 (78.6)	1.00	
Fed to dogs	7 (15.2)	39 (84.8)	1.70 [0.38-7.63]	0.490
Open disposal	53 (34.0)	103 (66.0)	0.52 [0.14-1.92]	0.328
Parish			
Kitojo	13 (36.1)	23 (63.9)	1.00	
Kyangundu	4 (11.1)	32 (88.9)	4.52 [2.17-9.43]	<0.001
Masyoro	5 (13.9)	31 (86.1)	3.50 [1.76-6.98]	<0.001
Muzira	11 (30.6)	25 (69.4)	1.28 [0.72-2.30]	0.398
Rushozi	14 (38.9)	22 (61.1)	0.89 [0.50-1.56]	0.681
Rweibare	16 (44.4)	20 (55.6)	0.71 [0.40-1.24]	0.224

and cattle exposure to *Brucella* in a high risk rural setting using the most readily available RBT screening tool. RBT was found to be optimal for use in this resource limited study area just like in other studies done before [13,29,31,32]. According to the WHO, case classification of a positive case when RBT is used is regarded as a probable case and according to OIE a positive case in animals when RBT is used is regarded as a presumptive case. For the control of brucellosis in herds at the national or local level, RBT is considered as a suitable screening test and rarely gives false negatives [33]. From this study, more than a third (34.7%) of the animals sampled were presumptively seropositive, this is in line with field reports from animal service providers, who non-routinely carry out *Brucella* screening in livestock in this western region and had persistently reported an increase in seroprevalence. This seroprevalence is within the range of the one in a study that was carried out in South Sudan (31%) where RBT was used [23]. The RBT was used qualitatively and disease confirmation was not done because titres were not taken, never the less, presumptive herd seroprevalence of 71% was considered high and as a matter of serious public and veterinary health concern since animals solely indirectly or directly infect humans and serious economic losses occur when animals have brucellosis [34]. Literature also guides us that in an endemic area with high seroprevalence in animals, the RBT is sufficient test without need for a confirmatory test to give a population picture of the disease status [23]. The presence of anti-*Brucella* antibodies suggests exposure to *Brucella* spp., seropositivity does not most times indicate that the animals have current or active infection at the time of sampling. Studies have shown that animal species susceptible to *Brucella* infection can lose their antibody titers. This means that the actual prevalence of brucellosis may be higher than that indicated by antibody screening [35]. This high seroprevalence seen in the current study could be as a result of high demand for breeding heifers from this region by governmental, non-governmental organizations, traders from other regions in Uganda and neighboring countries (Burundi, Rwanda). This reasoning is in accordance with available official disease surveillance reports from District Veterinary Offices of western Uganda that indicated that the many traders and organizations that source animals from farms within the Uganda Western region, select and purchase in calf-heifers and cows that screen RBT negative. This inadvertently increases *Brucella* seroprevalence and exposure in the region since those that screen positive with RBT are rejected and left behind. The government purchase orders issued to livestock suppliers explicitly indicate that the animals to be purchased and distributed in government programs should be free of Brucellosis. Based on the high seroprevalence at animal and herd level noted in this study, it is not surprising that the probable human seropositivity in this study was 33%. This is almost similar to the seroprevalence got in South Sudan of 33.3% where also no control program for the disease is taking place [23]. In a study done at a community hospital in south western Uganda, probable seroprevalence of febrile patients who were screened for brucellosis was 14.9% [21]. Compared to the facility based surveillance, the current study gives a good indication of what might be happening in the communities away from the health centers where probable seropositivity

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(33%) captured all those with and without clinical signs, those with actual disease, self-limiting infection and those that recovered from illness hence illuminating the level of exposure and infection within this cattle keeping populations where close interactions with infected animals and consumption of their infective products put household members at risk of infection. Furthermore when RBT is used qualitatively to depict magnitude of infection in an endemic area, seroprevalence is expected to be higher than disease prevalence because some infections may be self-limiting or some of the infected population could have recovered after treatment and remained with antibodies hence false positive disease cases are expected but rarely does it exclude true *Brucella* infection. In the study by Migisha et al, all the confirmatory brucellosis cases had all initially reacted with the RBT [21,36,37].

Belonging to households that owned a presumptively *Brucella* seropositive herd, significantly increased the odds for probable *Brucella* seropositivity in household members by (2.03; 95% CI, 1.01, 4.07) and this is true since from literature it is known that animals are solely responsible for infecting human beings directly or indirectly [1,4], furthermore, studies have shown that interaction with infected animals causes infections in household members that rear livestock [8,38]. When animal reproductive materials are improperly disposed off in the open or given to dogs, this most likely exposes the dogs to infection [39]. *Brucella* in the environment persists long enough to effectively infect susceptible hosts [17,18,40]. Infected dogs and infective biological products act as quasi-constant sources of exposure for households and livestock [12,18,41]. The associated risk factors in the model: Age category, belonging to households that owned a suspected *Brucella* seropositive herd, mode of disposal of reproductive refuse and calf feeding pattern are important for estimating *Brucella* seropositivity in household members.

From univariate analysis, location of the herds within the parishes of Kyangundu and Kitojo increased the odds for having herds that were presumptively *Brucella* seropositive, it was also noted from the official communication from the office of the district veterinary officer that farmers in those parishes are seriously engaged in cattle trade with constant selling and restocking of farms with unscreened cattle, and this most likely contributes to higher odds of *Brucella* seroprevalence within the herds in those parishes compared to other parishes. Most of these farmers within the study area source cheaply from within the study area and many times also purchase animals with reproductive problems such as history of abortions, still births and repeat breeders who fail to conceive and these animals are pone off to naïve farmers. There is unregulated movement of animals between farms in the study area which is very important in spread of infection within the farms.

The strength of this study was its ability to use RBT as a low-cost screening test to depict increasing *Brucella* infection in cattle and humans. Another important strength of the study was the assessment of important risk factors associated with *Brucella* infection in cattle keeping communities and involvement of the department of Health, Village Health Team members, Veterinary personnel, security and political structures within the study area which exemplified a One Health approach that is being advocated for in the country. The weakness of the study was that confirmatory diagnosis was not done and the results were probable and presumptive.

Conclusions

The study highlights the intricate nature of *Brucella* infection in cattle keeping communities especially within households, between animals and from animals to humans. *Brucella* seropositive herds are a likely source of zoonotic infection to household members. Future studies that would assess the ease of use of rapid tests in brucellosis surveillance and diagnosis especially in rural cattle keeping areas in Uganda would be important.

Cattle vaccination against brucellosis needs to be encouraged in cattle keeping communities and continuous surveillance encouraged for both animals and humans. Efforts should be made to create awareness about the zoonotic potential of infected livestock in the rural cattle keeping communities so that they can be able to improve their knowledge and actively participate in community based surveillance where they can detect early and quickly report signs and symptoms for early response.

Declarations

This study which involved collection of human and animal blood samples was approved by both the Uganda National Council of Science and Technology (UNCST). The approval number is HS 165 and the Research Ethics Committee (REC) of Mbarara University of Science and Technology known as Mbarara University Institutional Research Committee: (MUIRC 1/7; June 6, 2016. Study number 160121). Written consent was sought from the respondents during study and identification numbers were accorded to all participants and cattle for confidentiality. Participants' defined privacy was accorded during collection of data and samples.

Human blood samples were collected in the households of the study participants according to the Clinical and Laboratory Standards Institute (i.e. National Committee for Clinical Laboratory Standards; Procedures for the Collection of Diagnostic Blood Specimens by venipuncture. Approved Standard - Fifth Edition H3-A5, Vol.23 No.32.). Bearing in mind the fundamental principles of the Basel Declaration, written consent from the cattle owners was also sought before any blood samples were collected from the cattle. Cattle blood samples were collected humanely from the prescribed sites (Australian Code of Practice for the Care and Use of Animals for Scientific Purposes) and the study in animals was also approved by Uganda National Council of Science and Technology (UNCST). The approval number is HS 165.

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Author's contributions

AE and FB designed the study, supervised the data collection, analysis, interpretation. AE wrote the first draft. JPG, TTS and TG participated in the design of the study, interpretation of results and assisted in manuscript write-up. All authors read and approved the final draft

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