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CASE REPORT

Epidemiological Investigation of Bovine Brucellosis in Indigenous Cattle Herds in Kasulu District of Tanzania

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Background: Livestock-wildlife interfaces create unique hotspots of many infectious diseases including brucellosis. **Methods**: A combination of epidemiological field studies utilizing Participatory Epidemiology tools and cross-sectional studies investigating Abortion Associated Syndrome (AAS) was conducted in livestock wildlife interface areas in Kasulu district, Tanzania from 23 to 28 July 2019. A total of 285 cattle from 27 herds were examined and sampled. Individual animal and herd-level data were collected using a structured questionnaire. Serum samples were screened for anti-*Brucella* antibodies using the Rose Bengal Plate Test (RBPT). **Results**: Ranking and proportional piling showed cattle to be the most important animal species kept but also significantly contribute to the livelihood of the livestock keepers. Matrix scoring results showed weak to moderate agreement between informant groups perception on the AAS and risk factors. The overall seroprevalence of anti-*Brucella* antibodies in individual animal was 30.8% (95% Confidence intervals (CI) = 25.5 – 36.2) and the corresponding herd prevalence was 77.7% (95% CI = 59.2 – 89.4). Fifty-one (37.0%) out of the 138 cows that had history of abortion over the previous 2 years (2018-2019) prior to the study was seropositive on RBPT. Univariate logistic regression analysis showed sex and age of the animals as a potential predictor for individual animal seroprevalence. **Conclusions**: The results showed that farmer knowledge and perception about diseases including AAS to be moderate and further confirm brucellosis to be prevalent and widely distributed locally. Heightened routine surveillance, further studies, and institution of preventive and control measures particularly among young female stock should be implemented. Creating disease awareness especially amongst livestock keepers and general public who are at high risk of contracting brucellosis is desirable.

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Abbreviations: AAS, Abortion associated syndrome; PE, Participatory Epidemiology; FMD, Foot and Mouth Disease; ECF, East coast fever; CBPP, Contagious Bovine Pleuropneumonia; RBPT, Rose Bengal Plate Test; TSHZ, Tanzania Short Horn Zebu; TVLA, Tanzania Veterinary Laboratory Agency

Keywords: *Brucella*, Cattle, Participatory technique, Seroprevalence, Kasulu, Tanzania

Author Contributions: AJM: Conception; Design; Data collection and analysis; Writing and Editing. ESS: Conception; Design; Data analysis; Writing and Editing. BGL: Data collection; Writing and Editing. SLC: Conception; Design; Data collection and analysis; Writing and Editing. All authors reviewed the manuscript.

INTRODUCTION

Brucellosis is ranked by the Food and Agriculture Organization (FAO), the World Health Organization (WHO), and the World Organization for Animal Health (formerly Office International des Epizooties) (OIE) as one of the most widespread zoonoses in the world [1]. The disease is endemic in many regions of sub-Saharan Africa (SSA) [2,3], including Tanzania, particularly within pastoral systems where people live in close contact with livestock, increasing the risk of human infection [4-8]. In cattle, the disease is usually caused by *Brucella abortus* (a gram-negative, facultative intracellular coccobacilli bacterium), however, other species, namely *B. melitensis*, *B. ovis*, and *B. suis* have been found to be circulating in Tanzania affecting goats, sheep, and pigs [9,10]. Brucellosis is characterized by late term abortion; infertility and reduced milk production as a result of retained placenta, endometritis, and a varying degree of sterility in the males and cows [11].

Collaboration between Tanzanian governmental sectors responsible for animal, human, and environmental health has identified brucellosis as one of six priority zoonotic diseases of great public health concern. Understanding the disease behavior along livestock wildlife interface and the risk factors of infection data can be used to guide control and clinical decision making, contribute towards the prevention and control strategy for brucellosis in Tanzania [12], and improve understanding of brucellosis epidemiology in SSA.

In Kigoma region, the disease has been insufficiently investigated and information relating to livestock keepers perception, its magnitude, distribution, and risk factors is scant. Such information is important when designing appropriate strategies that would help reduce its prevalence and effects. We used a combination of participatory epidemiology tools and cross-sectional studies in order to gather additional information likely to contribute to data that may be used to devise appropriate national strategies for the control of the disease.

Therefore, the objectives of the study were: i) to assess perception and knowledge of brucellosis in affected herds, ii) to characterize the disease with respect to perceived risk factors, incidence and impact iii) to estimate, identify and quantify risk factors associated with brucellosis in the study herds.

MATERIAL AND METHODS

Area and Study Population

This study was carried out in Kasulu district, one of the six districts of Kigoma region. The district is located in the north-western part of the region. Geographically the district is located between latitudes 4 25’ and 6 24’ S and longitudes 30 20’ and 32 26’ E. The district livestock population is estimated to be 285,830 cattle, 129,048 goats, 51,230 sheep, 17,161 pigs, 3,189 dogs, and 489 cats (Kasulu district monthly reports, 2016). The area is characterized by a long wet rainy season beginning from late October to May with a short dry spell of 2-3 weeks in January or February followed by a prolonged dry season. The mean annual rainfall varies from 600 to 1500 mm/year. The relative humidity ranges from 60% to 90% for most of the year. Mean daily ambient temperatures range between 25°C December to January and 28°C in September. The vegetation consists of closed and open woodland interspersed by bushy grassland and swamps. The main study sites and participating village was Katoto (Figure 1a, b). Administratively, Katoto village belongs to Kagera, the Nkanda ward and Makere Division, a livestock/wildlife interface area and previously reported to have history of cattle abortion. Sub-villages sampled included Mkumboni, Igalula, Mulutwana, Muyonga, Malenge, and Mfunda. This livestock-human-wildlife interface area hosts a population of 1,020 people and approximately 15,000 cattle. Field study was conducted from 23-28 July 2019.

Cattle Husbandry System

The main economic activities in Katoto village are livestock keeping and paddy rice production (small scale rice cultivation/production in flooded or wet fields). The study animals were indigenous breeds of cattle, mainly Ankole and Tanzania short horn zebu (TSHZ) breeds reared under extensive husbandry systems of which the latter allows free grazing, usually mixed with livestock from other herds and villages. The breeding system used is natural mating, with bulls running freely with females all year round.

DATA COLLECTION

Participatory Epidemiological Studies

The participatory epidemiological (PE) studies consisted of focal group discussions with livestock keepers, of which sub-villages were the unit of analysis, and key informant interviews. The focal group discussions took place in three sub-villages, comprising of an average of four to eight informant groups. In each sub-village, focal groups were convened which consisted of between seven and 20 people, most of them were men aged between 16 and 70 years and majority of them were ethnically Tusi and Sukuma. During the focal group discussions, a variety of PE tools, previously described in the literature [13-15] were utilized to encourage participation of each member in the groups. Due to time constraints, however, not all sub-villages were covered, also not all tools

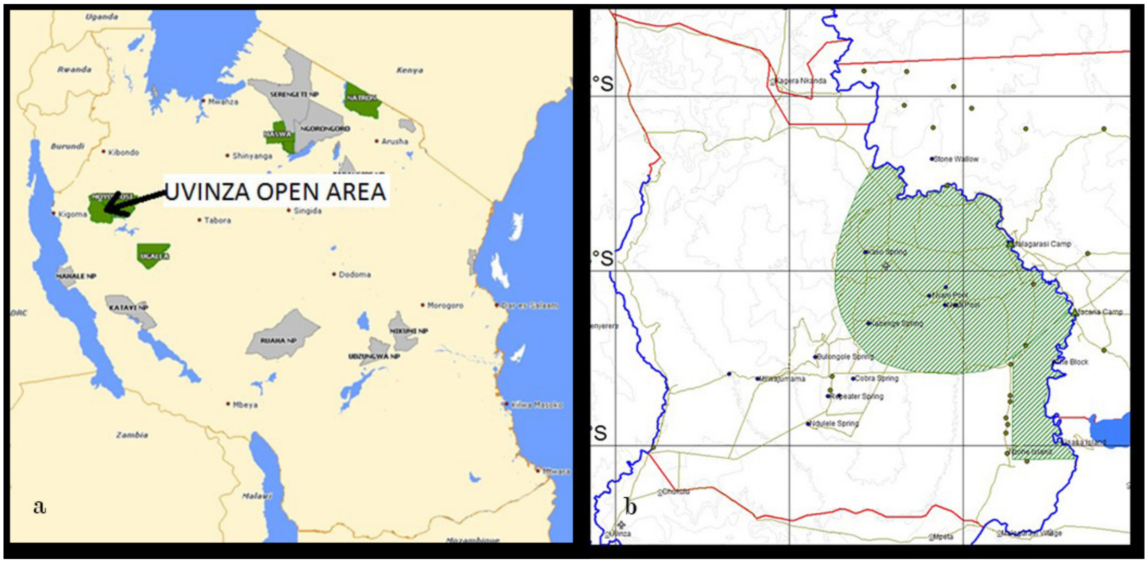

Figure 1. **a**. Map of Tanzania showing Uvinza open area. **b**. A zoomed map of Uvinza open area in Tanzania.

were applied in every sub-village. These PE tools were complimented by direct observation of the herd and their environment.

Semi-structured Interviews

These were used to collect general information about knowledge and experience with respect to brucellosis focusing on history and the occurrence of abortion over the past 2 years prior to the study. The interviews were guided by a pre-tested and adjusted checklist of open-ended questions prior to the initiation of the study. Interviews were conducted using the national Swahili language.

Proportional Piling

This tool was used to rank livestock species by numbers and relative contribution to livelihoods, using the method described in the literature [15]. For this, participants first listed the animal species kept. A circle was drawn on the ground representing each species. Participants allocated 100 counters (bean seeds) to the circles according to the relative numbers of each species. The exercise was then repeated, except this time participants were asked to allocate the counters in proportion to the relative contribution each species made to their livelihoods. Follow-up questions explored the range of benefits that each livestock species provided.

Perceived Association between Diseases and Risk Factors

Informal interviews with livestock keepers were used to identify five most important bovine diseases to be used in the matrix scoring along with Abortion Associated Syndrome (AAS) using the proportional piling technique [14,15]. These five diseases and their local names were East Coast fever (ECF) or locally branded as ('*Madundo*'), Foot and Mouth disease (FMD) or ('*Suna*'), Lumpy Skin Disease (LSD) or ('*Mapele*'), Diarrhea in calves ('*Ichuto*'), Fascioliosis ('*Sundo*'), AAS (locally called '*Kusunta' or Mguu/Muganyazi'*), Contagious bovine Pleuropneumonia (CBPP) or ('*Homa ya Mapafu'*), and Heart water/Ormillo ('*Kizungu zungu'*). Presented clinical signs identified to be associated with cattle diseases were excessive salivation, lameness, swollen joints, diarrhea, superficial lymph node (Parotid) enlargement, rough hair coat, and loss of body weight. Simple matrices were constructed on the ground or flip chart paper. Various disease impacts formed the y-axis and five diseases, one of which was AAS, formed the x-axis. Likewise, risk factors and clinical signs formed the y-axis and diseases formed the x-axis. Twenty counters were allocated for each disease and the participants were asked to divide the counter in relation to the relative importance of each symptoms/clinical signs, disease impact, or risk factors played in the disease.

Estimating Relative Incidence and Case Fatality Rates Due to AAS and Other Related Cattle Diseases

Relative incidence and mortality due to AAS was estimated using a proportional piling exercise. A total of 100 seeds representing the total population of a cattle species were used. The first step involved the distribution of 100 seeds into two piles; one representing the proportion of animals that got sick over the last 1 year prior to the study and the other the proportion that remained healthy.

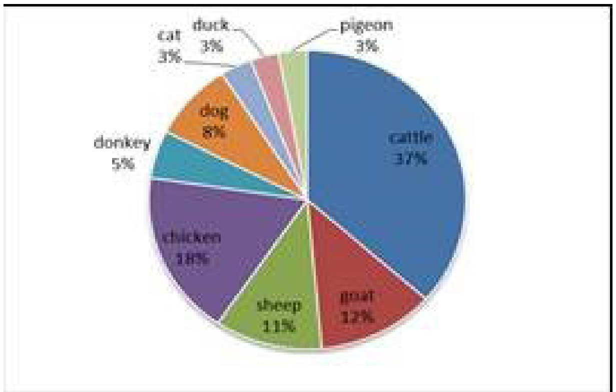

Figure 2. Proportion of the main animal species kept at Katoto village of Tanzania, July 2019.

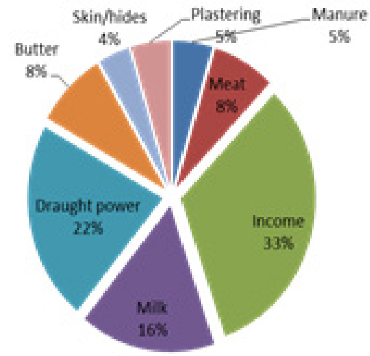

Figure 3. Benefits derived from livestock in Katoto village of Tanzania, July 2019.

Before proceeding to the next step, the participants were asked to give reasons that guided the piling of seeds in this step. In the second step the participants were asked to distribute the seeds from the sick pile category. The score represented the relative incidence of that disease. The third step involved the distribution of seeds allocated into two other piles for those that died and those that recovered from the disease. The scores in the pile that represent animals that died of AAS and other diseases are considered the case fatality rate due to the disease. The score from the exercise were then recorded.

Key-informant Interviews

These were conducted with various officials who had experiences on cases related AAS, such as livestock field extension officers, village elders, and village leaders.

CROSS SECTIONAL STUDIES

A purposively cross-sectional study was undertaken during the same period, July 2019. Owing to the practical consideration of logistics and funds, the sampling frame was limited to herds within Katoto village and her sub-villages. The data bases of livestock keepers under the Katoto village and her sub-villages were estimated to be 1,020 distributed evenly across the entire sub-villages. Information about each herd and the animals kept was collected by means of a structured questionnaire, complemented by focus group discussion (FDG) and key informant interviews (KII). Questionnaire administration was completed at all the selected herds on a single visit. The questionnaire was designed to comprise mostly close ended (categorical) questions to ease data processing, minimize variation, and improve precision of responses [16]. The questionnaire was pretested in the field and adjusted as required. A questionnaire was administered to 27 livestock keepers in 27 households where blood samples from animals were collected. Interviews were conducted with a member of the household who was more conversant about the herd using Swahili dialect by a trained investigator team member, mainly the veterinary officer. Data on risk factors for *Brucella* seropositivity, livestock owner's knowledge, attitudes and practices regarding brucellosis were also collected. Information on herd level and individual animal variables (age, sex, previous history of abortion) was recorded separately on sample data sheets.

Blood Sampling

To establish seroprevalence, about 5ml of blood was collected from the jugular vein of each animal using a plain vacutainer tube and needle (Zhejing Kangshi Medical Device Co., Ltd, China). Each sample was labelled by using codes describing the specific animal and herd. The samples were left to clot at room temperature overnight and subsequently centrifuged at 3000 rpm for 10 minutes to obtain clear serum. About 2ml of serum was collected into cryovials and stored in a cool box loaded with an ice pack. Four days later, samples were shifted to zonal Tanzania Veterinary Laboratory Agency (TVLA), Tabora for storage and analysis.

Laboratory Analysis of Samples

All collected sera were screened using Rose Bengal Plate Test (RBPT) for anti-*Brucella* antibodies according to the test procedure recommended by the OIE [17]. RBPT antigen, positive and negative freeze-dried serum (for internal quality control) were cordially provided by the Veterinary Laboratory Agency, Weybridge, UK. Briefly, 30µl of RBPT antigen and 30µl of the test serum were placed alongside on the glass plate and mixed thoroughly. After 4 minutes of rocking, any visible agglutination was considered a positive result. A herd or animal was considered positive for the disease when at least a

Table 1. Mean relative livestock production challenges as scored by livestock keepers.

Production challenges	%
Diseases (*n* = 3)	35
Pastures (*n* = 3)	25
Dip tank (*n* = 3)	16
Vet service (*n* = 3)	14
Market (*n* = 3)	10

n – The number of groups involved in the proportional piling exercises.

single animal tested positive for RBPT.

DATA MANAGEMENT AND ANALYSIS

Two sets (semi-quantitative data obtained from scoring and ranking and Cross-sectional) of databases were created and constructed in Microsoft Excel 2007 (Microsoft Corporation). Stored data (after cleaning) were later exported to EPI info™ Version 7.1.1.14 (Center for Disease Control, Georgia, Atlanta, USA 2007) and MedCalc™ version 13.0.2 (MedCalc software, Acacialaan 22, B-8400, Ostend, Belgium) for analysis using non-parametric and descriptive statistical methods. Analysis involved computing percentages, medians, and ranges of the score and generating graphs. For cross-sectional data, descriptive and inferential statistics of the outcome variable (*Brucella* sero-status: negative or positive) were calculated as proportions. The associations between individual animal and herd level factors and brucellosis seroprevalence was explored in Mantel–Haenszel $\chi 2$ tests and by univariate logistic regression. A p-value of <0.05 was considered indicative of a statistically significant difference.

RESEARCH CLEARANCE AND ETHICS

Approval to conduct the study was granted by the Department of Veterinary Services Tanzania and the District Veterinary Office, Kasulu, Tanzania. The research was performed in accordance with the guidelines and regulations prescribed by the above animal health authorities. Written informed consent for study participation was obtained from each participant using forms translated into Swahili, the local area language.

RESULTS

Relative Livestock Population, Herd Structure, and Importance

Cattle (37.0%) constitute the largest proportion of animal species kept in the six sub-villages visited. Chicken (18.0%) population comes second to cattle while goat (12.0%) and sheep (11.0%) are the third and fourth in population size. Other animals kept, however, in small numbers, were dogs, donkeys, cats, ducks, and pigeons (Figure 2).

Table 2. The relative importance of AAS as scored by livestock keepers interviewed (*n* = 3).

Rank[a]	Disease	Mean scores (%),± SE
1	CBPP	19.4 ± 6.5
2	East Coast fever	16.4 ± 10.4
3	FMD	14.3 ± 6.8
4	Calf diarrhea	13.3 ± 6.29
5	Hygroma (*Mugayanzi*)	8.7 ± 1.47
6	AAS	8.3 ± 3.4

[a]The perceived scale of importance of a disease from high (1) to low 6); *n* – the number of groups involved in the proportional piling exercises; SE – standard error of mean.

In all surveyed sub-villages, livelihoods depended largely on livestock and paddy rice production. Cattle were the animal species that made by far the greatest contribution to livelihoods. For all livestock species, the main benefit derived from livestock was reported to be income (from sales of surplus or unwanted animals due to ill-health), draught power (for transportation of agricultural produce and water), food, which included milk, meat, and fat. Other minor benefits were manure for paddy rice production, house plastering, as well as skin and hides (Figure 3).

Livestock Production Challenges

The respondents ranked diseases as the most important livestock production challenges in terms of causing losses in production and deaths (Table 1). Inadequacies' of pasture both in sufficient amount and quality due to seasonal variation of annual rainfall were ranked second. Other challenges included lack of cattle dip for tick and other pest control, lack of field staff to support animal husbandry and extension, and the lack of live animal markets. Sampling dates coincided with the drought weather at Katoto and outbreak of FMD was common amongst investigated herds. Other minor observed ill-health conditions included photosensitization and hygroma. Tick control infrastructure like dipping or spray races were not seen during the transect walk and > 90.0% of the animals investigated were found carrying ticks of different species, mainly *Rhipicephalus appendiculatus*, *Boophilus*, and *Amblyomma* spp.

Upon probing, participants mention lack of boreholes and water troughs for both human and animal use.

Table 3. Mean scores (out of a total of 20 counter per risk factor) derived from simple matrices constructed with livestock keepers showing five cattle related diseases with respect to their perceived strength of association with predetermined risk factors (*n* = 3).

Risk factor	Assigned score, Mean±StDev and (Median) (Range)					
	ECF[a]	CBPP	FMD	Calf D¥	AAS[b]	Mugayanzi/Hygroma[a]
Animal interaction	0	14±6.0 (14), 8-20	20±0 (20), 20-20	5.3±9.25 (0), 1-16	8.3± 38.3 (10), 0-15	6.0±11.5[a] (0), 0-20
Vectors (tick/tsetse)	6.6±11.5 (0), 0-20	2±3.4 (0), 0-6	0	1.3±2.3 (0), 0-4	5.0±5.5 (5), 0-10	0
Lack of vaccine	0	2.3±4.0 (0), 0-7	0	0	0	0
Climate[¥]	0	0.67±1.15 (0), 0-2	0	6.7±11.5 (0)0-20	0	0

n – the number of groups involved in the matrix scoring exercises; [¥]climate - related to drought, lack of water and fodders; ¥Calf diarrhea, [a]Only one groups reported vector to be associated with ECF and Mugayanzi/Hygroma. [b]Only two groups reported AAS being associated with interaction.

Table 4a. The mean relative incidence due to AAS and others as scored by livestock keepers involved in the study (*n* = 3).

Interviewed groups	Assigned morbidity (incidence) score[a], Mean ± SDev and Median (Range)			
	Disease			
	AAS	CBPP	FMD	Calf diarrhea
Group 1 (*n* = 15)	34	97	100	99
Group 2 (*n* = 8)	66	90	100	98
Group 3 (*n* = 18)	0	57	100	0
Overall av (*n* = 5)	**33.3 ± 33.0, 34 (0-66)**	**81.3 ± 21.3, 90 (57-97)**	**98.6 ± 2.3, 100 (96-100)**	**65.5 ± 56.6, 98 (0-99)**

n – the number of participants involved in the proportional piling exercises; * - Case morbidity represents the animals scored as having getting sick from the pile out of 100, Calf D – calf diarrhea often associated linked to enterotoxaemia; SDev- Standard deviation; [a]: The higher the score, the more strongly livestock keepers perceived the disease to be of importance.

Table 4b. The mean relative case mortality rates due to AAS and others as scored by livestock keepers involved in the study (*n* = 3).

Interviewed group	Assigned mortality score[a], Mean ± SDev and Median (Range)			
	Disease			
	AAS	CBPP	FMD	Calf diarrhea
Group 1 (*n* = 15)	5	68	28	97
Group 2 (*n* = 8)	3	81	24	75
Group 3 (*n* = 18)	0	59	3	0
Overall av (*n* = 15)	**2.6 ± 2.5, 3 (0-5.5)**	**69.3 ± 11.6, 68 (59-81)**	**18.3 ± 13.4, 24 (3-28)**	**57.3 ± 50.6, 75 (0-97)**

n – the number of participants involved in the proportional piling exercises; * - Case mortality represents the animals scored as having died from the pile of those that got 'sick, Calf D – calf diarrhea often associated linked to enterotoxaemia; SDev- Standard deviation; [a]: The higher the score, the more strongly livestock keepers perceived the disease to be of importance.

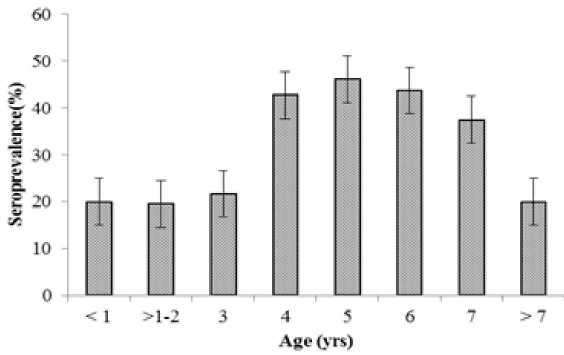

Figure 4. Age sero-prevalence profile (± 95% CI) of *Brucella* in the sampled cattle at Katoto village, Kasulu, Kigoma region of Tanzania, July 2019.

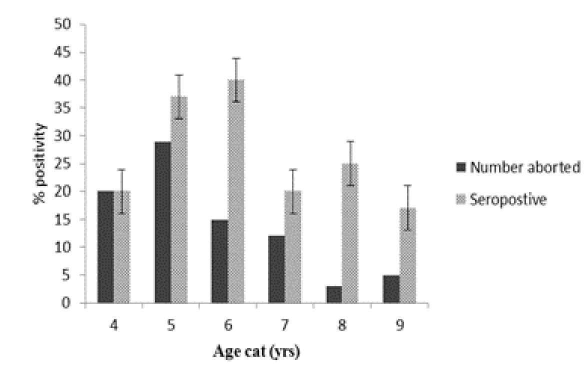

Figure 5. Age sero-prevalence profile (± 95% CI) of *Brucella* in the aborted cattle at Katoto village, Kasulu, Kigoma region, Tanzania (July 2019) (black bar: Number aborted; stippled bar: % seropositive).

Impact of AAS on Livestock Keeper Livelihoods

The impact of AAS and other diseases on livelihoods derived from cattle was assessed using disease impact matrix scoring, and the mean scores for every disease were determined to provide total impact of the disease on cattle-derived livelihoods (Table 2). Pre-determined impact factors were reduced income, milk, public health threats, treatment costs rated to be high, and reduced value of the animal (loss of draught power). Out of the six diseases used in the disease impact matrix scoring, AAS and Hygroma ('*Muganyazi'*) were ranked number five and six, respectively. CBPP, Vector borne diseases namely ECF and LSD were ranked high.

Disease Association with Risk Factors

A matrix scoring exercise was carried out to establish the respondents' perception in associating risk factors to AAS and other cattle diseases. The results from this exercise show that AAS and Hygroma ('*Muganyazi'*) were weakly associated with climate and lack of vaccine, but strongly linked to contact with infected animals during grazing or at watering points or with newly introduced infected animals from other herds (Table 3). On probing it was established that the participants had high knowledge of risk factors associated with FMD or '*Suna*' and ECF.

Estimating Disease Incidence

The relative incidence of the four cattle diseases including AAS and case mortality was explored through proportional piling to ascertain the perceived morbidity and mortality associated with the disease (Table 4a, b). The result shows the average relative incidence and case mortality rate due to AAS to be 33.3% and 2.6%, respectively.

SEROLOGICAL RESPONSES TO *BRUCELLA* INFECTION

All 27 of the selected herds were visited and the farmers were interviewed, resulting in a 100% response rate for participation in the study. The average herd size of participating farms was 124 (range 34-599). The mean number of sampled animals per herd was 11±StDev 3.2, range (6-25). The overall seroprevalence of brucellosis at the animal level was 30.8% (95% CI: 25.5 – 36.2). The corresponding overall herd seroprevalence (at least one animal or herd seropositive) for *Brucella* antibodies was 77.7% (95% CI: 59.2 – 89.4). Seroprevalence of brucellosis were higher in mature cows mainly dry (46.0%) and lactating cows (42.0%) and less in breeding bulls (31.2%) and yearlings (16.6%) as indicated in Table 5. The relationship between age and seroprevalence is shown in Figure 4. The prevalence of cattle seropositive for anti-*Brucella* antibodies increased from 42.0% in animals 4 years old to 43.0% in cattle 6 years of age, and showed a declining trend for animals beyond 7 years old. From the 21 seropositive herds, the mean intra-herd sero-prevalence was 37.9 % (95% CI: 27.5 – 48.3) and the seroprevalence between herds ranges from 10.0% to 80.0%. Grazing (100.0%) was the predominant system employed and no history of *Brucella abortus S19* vaccination was recorded during sampling.

History of Abortion and Exposure to Brucella *Infection*

Abortion history was recorded in 48.5% (*n*=138) and 51.8% (*n*=14) of the individual animals and herds, respectively. Fifty-one (37.0%) of one hundred and thirty-eight (138) mature cows that recorded evidence of abortion at some stage in their life span was seropositive based on RBPT. Cases of abortion were prominent for cows aged 5 and 6 than 7 years old (Figure 5).

Table 5. The proportions of animal in each category of each variable investigated during the study (n = 285).

		No. of animals		
Variable	**Categories**	**Proportion (n, %)**	**Seropositivity (n, %)**	**Univariable regression OR (95% CI)**
Sex	Male	63 (22.0)	14 (22.2)	ref
	Female	222 (78.0)	74 (33.3)	0.57 (0.28-1.08)*
Source of animals	Brought	24 (8.5)	2 (9.0)	ref
	Home bred	261 (91.5)	86 (33.0)	0.18 (0.03-0.69) *
Source brought in (*n* = 24)	Gift	5 (21.0)	2 (9.0)	ref
	Purchase	19 (79.0)	3 (15.8)	0.30 (0.029-3.43) **
Breed	Ankole	218 (76.5)	67 (30.7)	ref
	TSHZ	76 (23.5)	21 (31.3)	1.16 (0.65-2.10) **
Herd status	Breeding bull	16 (5.6)	5 (31.25)	
	Dry cows	13 (5.2)	6 (46.1)	
	Heifers	39 (13.7)	9 (23)	
	Lactating cows	123 (43.1)	52 (42.3)	
	Suckling	28 (9.8)	5 (17.8)	
	Weaners	18 (6.3)	3 (16.6)	
	Yearling	48 (16.8)	8 (16.6)	
Lactation status (*n* = 175)	Lactating	123 (70.3)	71 (57.7)	ref
	Non-lactating	52 (29.7)	37 (71.1)	1.80 (0.90-3.7) **
Pregnancy status (*n* = 175)	N/pregnant	162 (92.7)	61 (37.6)	ref
	Pregnant	13 (7.4)	6 (46.1)	1.41 (0.42-4.5) **
Age category (yrs)	< 2	122 (42.8)	19 (15.5)	
	> 2 to 4	51 (17.9)	22 (43.1)	
	> 4 to 6	86 (30.0)	39 (45.3)	
	> 6 to 8	20 (7.0)	7 (35.0)	
	> 8	6 (2.1)	1 (16.6)	
Interaction wildlife	No	123 (43.2)	35 (28.0)	ref
	Yes	162 (56.8)	53 (32.7)	1.22 (0.73-2.04) **
Abortion history (*n* = 138)	No	98 (71.0)	34 (37.7)	ref
	Yes	38 (27.5)	17 (44.7)	1.51 (0.70-3.28) **

*Significant at P< 0.05; **Not significant P>0.05; OR = Odds ratio; 95% CI; Confidence Interval; ref: reference variable.

Factors Influencing Seroprevalence of Bovine Brucellosis Univariate Analysis

The association of the animal and herd-level categorical explanatory variables and brucellosis is shown in Table 5. At an animal-level, older animals (≥ 4 years, 45.3%) were more likely to be seropositive compared to animals ≤4 years old ($P = 0.02$). The animals introduced into the herd (from within and outside study areas) had low risk of being seropositive (90%) compared with homebred counterparts (33.0 %) ($P = 0.0126$). There was no statistically significant association between animals being Ankole or TSHZ, contact with wildlife, and *Brucella* infection seropositivity outcome ($P > 0.05$). Results of Fisher's Exact Test showed that history of previous abortion ($P > 0.05$) in the individual animals were not significantly associated with *Brucella* seropositivity. None of the other investigated variables were associated with differences in prevalence values.

DISCUSSION

Participatory Epidemiology

PE tools have been widely used by researchers to investigate animal health related topics in resource poor areas [15,18]. In this study, our results showed that livestock production, mainly cattle, to be the major animal species kept and contributes significantly to the livelihoods of the livestock keepers in Katoto village, consistent with other findings [19,20]. The main livestock derived benefits identified by participants are aligned with findings reported in previous studies [21]. CBPP, ECF, FMD, and Calf diarrhea were prioritized as the most frequently occurring but economically important diseases in cattle herds in the past year. Of the sampled herds, at least 40.0% had animals showing the signs of FMD. The consistent and high allocation of the scores to the risk factors perceived to be associated with FMD and ECF confirms that the community had great knowledge of the disease risk factors. However, it should be noted that the same communities clearly and appropriately associated known risk factors with CBPP and Calf diarrhea. These results affirm previous findings in the region [5-7]. Important zoonotic diseases such as brucellosis locally branded as '*Kutupa Mimba*' or '*Mugayanzi/Hygroma*' collectively termed in this study as AAS were ranked fifth and sixth, respectively, despite recent reports of high seroprevalence in cattle within the high interface zone [8]. The fact that this disease was not ranked highly by participants could be explained by the lack of awareness or lack of distinct clinical manifestations that allow differential diagnosis with other diseases. However, the epidemiological perceptions and frequencies of abortion cases (40.0%) amongst investigated herds over the past two years (2018-2019) prior to the study, were very well supported by the serological survey findings. The frequent occurrence of prioritized diseases in the study sub-villages is not surprising given the absence of comprehensive vaccination strategies, limited veterinary services, high costs of vaccines and drugs and limited surveillance systems in the area that was very well identified during the transect walk, ranking and scoring exercises. Based on matrix scoring, FMD, CBPP, ECF, and Calf diarrhea had the greatest impact on cattle benefits, mostly on milk production, draught power, animal losses, and income from livestock sales. These impacts could have caused huge burden to the households dependent on livestock for draught power, milk production, and income generation on daily basis.

The overall animal and herd seroprevalence of brucellosis in this study was 30.8% and 77.7%, respectively, suggesting that the disease is endemic and widespread locally. The sex of the animal was statistically significantly associated with seropositivity in this study, and this is in disagreement with the report of Kubuafor et al. [22]. However, a small proportion of males compared with females were sampled, which is likely to explain the observed sex-risk difference. In the present study, a history of newly introduced animals from other areas or herds was associated with a decreased risk for *Brucella* infection seropositivity. We postulate that introduced animals were more likely to have encountered and recovered from *Brucella* infection. However, in our study, we are unable to unveil the possibility of naïve susceptible animals to have been exposed after they were introduced into the herd sampled. The relevant data from East and Central Africa and from other environments similar to those found in Tanzania, and wildlife interfaces also indicate that *Brucella* antibodies can be quite widespread among the cattle production systems. For example, in the pastoral animal and herds RBPT-based investigations, seroprevalences of 0.77% and 46.1%, 9.3% and 31%, 14.1% and 46.2% have been reported in Ethiopia, Ghana, and Zambia, respectively [23-25]. The apparent geographical variation in the seroprevalence may reflect differences in the levels of natural immunity, management, and husbandry practices employed, and sensitivities and specificities of the diagnostic methods used among researchers as well as genetic variation in disease resistance among the breeds maintained in the systems [24,26].

The seroprevalence for *Brucella* antibodies increased with age, consistent with previous reports [27,28]; younger animals are more resistant to primary infection and frequently clear infections, although latent infections occur [29]. The increased likelihood of *Brucella* infection in older animals (≥4 years) could be explained by increased susceptibility to infection due to nutritional stress combined with the lowered immunity that developed following acute infection [11,30]. Our study dates coincided with the dry period; fodder and water were scarce and animal body condition scores were poor. These may have contributed to the lowered immunity of animals and hence easily succumbed to disease conditions, including AAS. In our study, we sampled a wide age range of animals; therefore, the seroprevalence reported here is likely to be rather cumulative as a result of continuous exposure to *Brucella* infection over time.

The difference in prevalence between lactating, non-lactating, pregnant, and non-pregnant was not significant, consistent with reports by Mekonnen et al. [31], but inconsistent with findings by Ibrahim et al. [32]. This is of public health importance particularly in those groups who drink milk directly from the udder or who do not have traditions to pretreat milk before consumption. Studies have shown *Brucella abortus* being isolated from milk in Fulani cattle in Nigeria [33] and antibodies being detected in bulk collected milk in Tanzania [34].

A history of previous abortions or stillbirths was not significantly associated with seropositivity, a finding which is in contrast with reports from other studies [23,35]. Forty-four percent of the RBPT positive animals reported a history of abortion, compared to 37.7% of the

RBPT negative animals. This could be explained by the fact that causes of abortion such as vector and non-vector borne diseases which are prevalent in the study area were important factors rather than brucellosis [36]. Similar observations were made by other investigators [37].

Our study was limited by its cross-sectional design; therefore, we are unable to conclude on the causal relationship between the identified risk factors and *Brucella* infection serological status outcomes. However, from participatory epidemiology results/finding, there was indication from the livestock keepers that *Brucella* is one of the major disease conditions in the study area. Perceived abortion syndrome which had no seasonal pattern and the link between abortion and '*Hygroma'* or '*Mugayanzi'* further support our hypothesis that brucellosis is likely to be animportant animal and public health problem in such settings. Description and characterization of the disease with perceived risk factors showed some level of agreements as indicated in the grey literature. Animal and herd-level data were collected in a binary fashion and age was based on farmer recall or being estimated from the number of calving periods, so no information about magnitude or duration of contact with wildlife is known, which may have an effect on prevalence estimations. Moreover, we could not perform criterion standard *Brucella* confirmatory testing in our study due to logistical reasons. Although, the test employed was RBPT, the recommended screening test for *Brucella* infection [17], the test has some challenges in discriminating natural infection against vaccine induced immunity and also the specific prevailing *Brucella* spp. pathogen. However, our questionnaire results/finding showed no evidence of *B. abortus* S19 vaccination history and most likely the exposure evidence we recorded in this work is likely to be derived from natural infection.

We cannot conclude on the transferability of the findings to other zones of the country, although, extensive studies have been conducted in pastoral and livestock wildlife interface areas indicating brucellosis to be one of the important disease conditions [5,6]. Therefore, the results of this work should be interpreted with caution and serve as baseline information on *Brucella* spp. exposure status in the Katoto village, western Tanzania. The findings of this study should be validated formally by prospective studies and a broader range of diagnostic tests applied to elicit better epidemiological information.

CONCLUSION

In conclusion, application of PE tools/techniques showed cattle to be an important animal species kept and the diseases including brucellosis (AAS) as an important challenge. Farmers were able to associate perceived impacts, clinical signs and risk factors consistent to the information described in official veterinary literature. The overall animal and herd seroprevalence was high, suggesting that Brucellosis is endemic, widespread locally, and animals and humans are at high risk. Females were more likely to be positive reactors than males; possibly because they stay longer on farms and consequently, they are more likely to be exposed to *Brucella* pathogens. Consistently, seroprevalence increased with age most likely due to enhanced susceptibility to infection which may be associated with nutritional stress combined with the lowered immunity that developed following acute infection. Brucellosis is an important disease in cattle-keeping communities in Katoto village and integrating vaccination against brucellosis into the annual vaccination program of livestock is highly recommended. Additionally, further structured studies employing criterion standard confirmatory tests should be performed to understand the epidemiology of the disease in interface areas and its association with human brucellosis.

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