



Assessment of Haematological Profile Clustering Differences in Brucella-Infected Goats across Urban, Peri-Urban, and Rural Areas of Central Uganda

Hellen Ndagire^{1*}, Kizito M Muwonge², Julius Mulindwa³, Charles Kakuhikire Twesigye¹

Abstract

Goats provide income to small and medium-scale farmers in developing countries through sale of meat and skins. Brucella is a zoonotic intracellular pathogen with poorly established impact on haematological profiles among goats. The haematological profiles serve as prior indicators of preclinical and para-clinical assessments. Disease and livestock management systems affect their haematological profiles. This study aimed to assess seroprevalence of asymptomatic brucellosis in apparently healthy goats in central Uganda and establish their associated haematological alterations. Haematological profiles of 396 apparently healthy goats from Kassanda [rural, n = 155, 39%], Wakiso [peri-urban, n = 150, 38%] and Kampala [urban, n = 91, 23%] Districts were determined. Body condition scores and farming systems were recorded. Venous blood samples were screened for brucellosis with Rose Bengal plate test. A complete blood count was determined by microscopy. PCV and haemoglobin were determined by haematocrit and colorimetry, respectively. Brucellosis seroprevalence was 3.33% [n = 13/396] among goats, whereas it was 4.5% [n = 7/155], 3.3% [n = 5/150] and 1.09% [n = 1/91] among animals from rural, peri-urban and urban districts, respectively. Principal component analysis based on haematological profiles generated three clusters from brucellosis-positive samples by site of origin. Haematological profiles varied among urban, peri-urban and rural Brucella seropositive apparently healthy goats owing to grazing systems at different sites. These conditions possibly impacted the stress levels and body condition scores, which allowed the goats to respond differently to the infection.

Keywords: Brucellosis; Goats; Haematological profiles; Uganda

Introduction

Goats are a source of income, meat and skins and provide spiritual satisfaction requirements to small and medium-scale farmers in low-and middle-income countries [1]. Brucella affects goats and other livestock, reducing their welfare and economic gains [2]. Brucella impacts the welfare and economic benefits of infected wildlife, livestock and human beings [3,4]. Humans are infected through the consumption of contaminated animal products such as meat and milk, direct contact with fluids from infected animals or inhalation of aerosols from animals [5]. Brucella causing bacteria spread by sexual contact among mature animals, contact to aborted placenta, foetus, foetal fluids, or vaginal discharge [6]. Brucella presents with prolonged latent periods, abortion and infertility among animals [4]. Brucellosis impacts the blood elements of infected animals [7]. Haematological and biochemical variations in blood parameters are indicators of the health, nutritional and physiological status of ruminants. Physical or environmental stressors may

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also affect the haematological and biochemical profiles [8]. The variations also occur in Brucella seropositive animals, with varying body condition scores indicative of stored fat and muscle protein [9,10]. Fat and protein are required for production, reproduction and maintenance. The reserves indicate previous nutritional status and are used in stressful conditions and periods when the animal requires high energy consumption [11]. Farming systems affect the body condition scores of goats. Extensive farming system provides superior welfare compared to intensive or semi-intensive practices [12]. Semi-intensive farming systems generate higher BCSs than does intensive goat management [13]. The Lake Victoria crescent in Uganda is predominantly covered by extensive, semi-intensive and intensive farming systems in rural, peri-urban and urban settings, respectively.

There are no widespread Brucella vaccination or sensitization programmes for goats in the Lake Victoria crescent in Uganda. Brucellosis vaccines for goats are costly and not readily available to small and medium - scale farmers in Uganda. [14,15]. Lack of brucellosis vaccination programmes among goats at community and farm levels facilitates the spread of disease within and between herds and other livestock [16]. Animal herds on extensive and semi-intensive farming systems within the study setting often share grazing lands, shelter and water sources and hence facilitate brucellosis transmission [17]. Small- and medium -scale farmers share bucks in the study setting [18]. Previous studies have reported brucellosis among herds irrespective of symptomatic or asymptomatic presentation in Uganda [19–23]. Smallholder farmers acquire asymptomatic goats from each other or from local animal markets on the basis of their physical appearance without laboratory investigations. Lack of proper diagnostic processes, prior to the acquisition of the animals, may lead to spread of infections between farms [24,25]. The aim of this study was to determine the seroprevalence of asymptomatic brucellosis in apparently healthy goats in central Uganda and to assess the association of the disease with haematological changes and body condition scores across urban, peri-urban, and rural areas, taking into account the role of different farming systems and management conditions in shaping the occurrence and severity of the disease's effects.

Materials and Methods

Study design and sampling sites

A cross-sectional study was conducted from June to November 2023 by randomly selecting 396 apparently healthy adult goats, without any prior abortion from consenting households from three district sampling sites: Kassanda [rural], Wakiso [peri-urban] and Kampala [urban] in Uganda, East Africa (Figure 1). Body condition scores [BCSs] were recorded and natural non improved pasture grazing with or

without supplementation was noted. Goat rearing practices were categorised as extensive, intensive or semi-intensive. Breed descriptors, including coat colour and appearance, presence or absence of horns, size and shape of breed's ears, presence of a mane, and body size were recorded. Vaccination of the selected goats against brucellosis was noted. Drinking water sources were also recorded.

Sample size

The sample size of the goats was determined according to Daniel [1999]. Three hundred ninety-six goats [396] were sampled from three sites by proportions according to the number of goats as reported by Uganda Bureau of Statistics [26]. One hundred and fifty goats were collected from the peri-urban district sampling sites, one hundred and fifty-five goats from the rural district sampling sites and ninety-one goats from the urban district sampling sites. A random starting point was established at the village level. Every fifteenth household in rural and peri-urban areas was selected. Every fifth household in the urban area was selected. No more than 10% of the flock in the household was sampled. If the targeted homestead did not rare goats or did not consent, then the adjacent household was considered.

Sample collection and analysis

Venous blood was drawn aseptically into EDTA tubes and another without an anticoagulant from each animal and transported to the laboratory at 4°C. Rose Bengal plate test was performed to detect Brucella antibodies. 16S rRNA sequencing confirmed selected Brucella seropositive samples. Haematological parameters were analysed within four to six hours of collection. Microscopy was used to establish Total Red blood cell counts [TRBCs] and leucocyte counts of TWBCs, neutrophils, lymphocytes, eosinophils, monocytes and basophils. Packed cell volume [PCV] was determined by haematocrit [MRC-HCEN]. Haemoglobin levels were determined by colorimetry [MRC-CEN-202]. Mean corpuscular volume [MCV], mean corpuscular haemoglobin [MCH] and mean corpuscular haemoglobin concentration [MCHC] were calculated from pre-attained values. Red cell indices and leucocyte counts were referenced with MSD manual of Veterinary Medicine [27].

Statistical analysis

Principal component analysis [PCA] was performed on the haematological scores of the samples. Dendrograms were established to illustrate the clustering and interpretation of relationships between variables among the samples. Analysis of variance was used to compare means of haematological profiles with their sites of origin, followed by Least Significant Difference [LSD] - post hoc test using R programming software. Statistical significance was considered at 95% confidence level [$p < 0.05$].

Study location

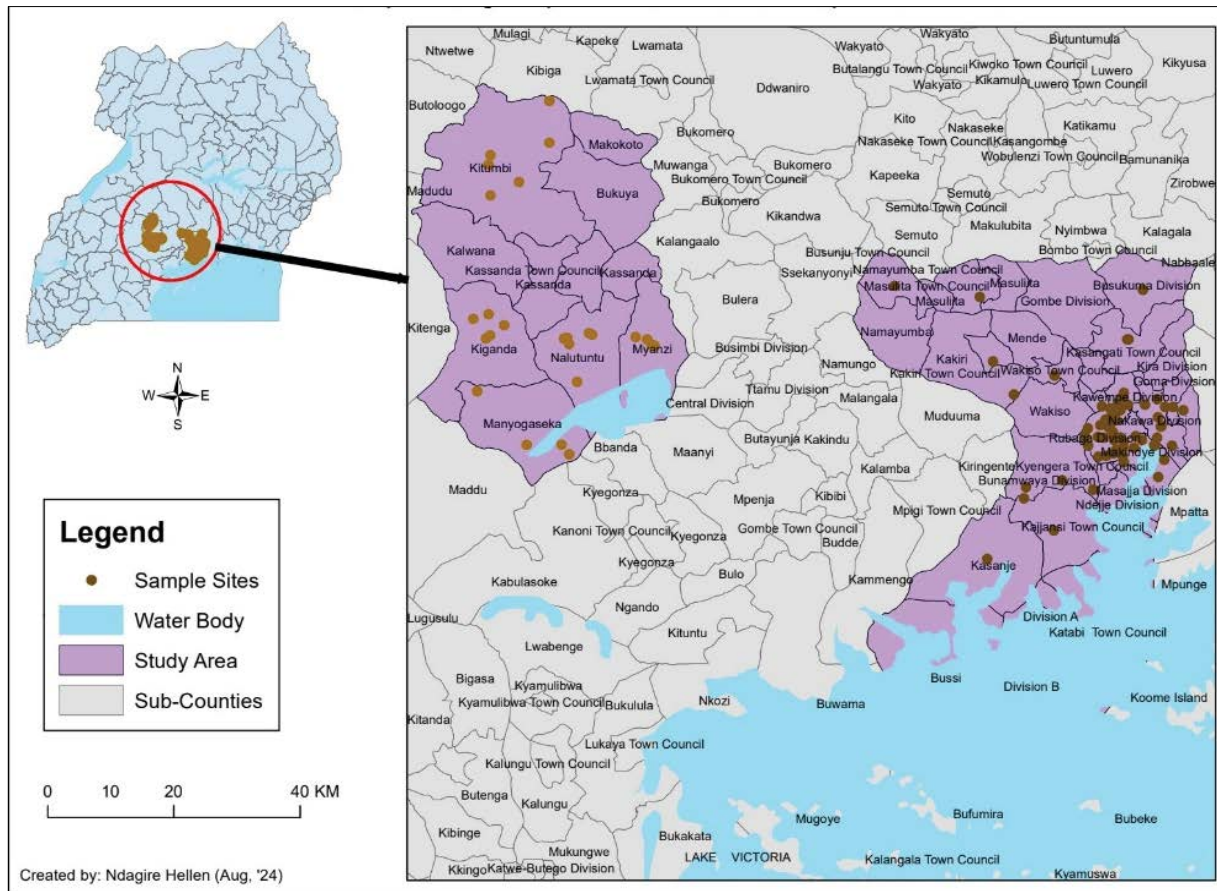


Figure 1: Map showing the sampling sites in Wakiso, Kampala and Kassanda Districts in Central Uganda.

Results

Goat breed distribution

Goats from the rural district were pasture grazing without supplementation under extensive farming. The peri-urban goats were managed mostly through extensive and semi-intensive rearing with limited intensive practices. The urban goats were from farmers who mostly practiced intensive rearing with the following breed ratios sampled: Boer, 1.8% [n = 7/396]; KigeziXSMEA, 1.5% [n = 2/396]; Mubende, 12% [n = 48/396]; MubendeXBoer, 5.3% [n = 21/396]; MubendeXKigezi, 0.3% [n = 1/396]; Savanna, 2% [n = 8/396]; SavannaXBoer, 0.3% [n = 1/396]; SMEA, 33.1% [n = 131/396]; SMEA XBoer, 36.9% [n = 146/396]; SMEA XKigezi, 0.3% [n = 1/396]; and SMEA X Mubende, 7.6% [n = 30/396].

Farming practices at the district sampling sites

The goats in this study were not vaccinated against brucellosis. Drinking water for animals at the sites in Kassanda District was obtained from shared springs or

shallow wells/boreholes, streams, swamps and shorelines of Lake Wamala. Drinking water for animals at the sites in Wakiso and Kampala Districts was obtained from springs or shallow wells/boreholes, streams, swamps and shorelines of Lake Victoria. A few farmers at the Wakiso and Kampala study sites provided chlorinated drinking water to their animals. Chlorination minimises the exposure of animals to disease-causing microorganisms. Most of the goats were fed nonimproved natural pastures [83%], and the rest were on both natural pastures and supplementation with grown pasture like legumes and mineral blocks [17%] [Figure 3]. Most of the feed supplementation among goats occurred in peri-urban settings, whereas the lowest amount occurred in urban areas (Figure 4).

Brucella seropositivity prevalence among the goats

Rose Bengal plate test detected *Brucella* antibodies in 3.3% [n = 13/396] of the goats. *Brucella* seropositivity was approximately 4.5% [n = 7/155], 3.3% [n = 5/150] and 1.09% [n = 1/91] at the rural, peri-urban and urban study sites, respectively.

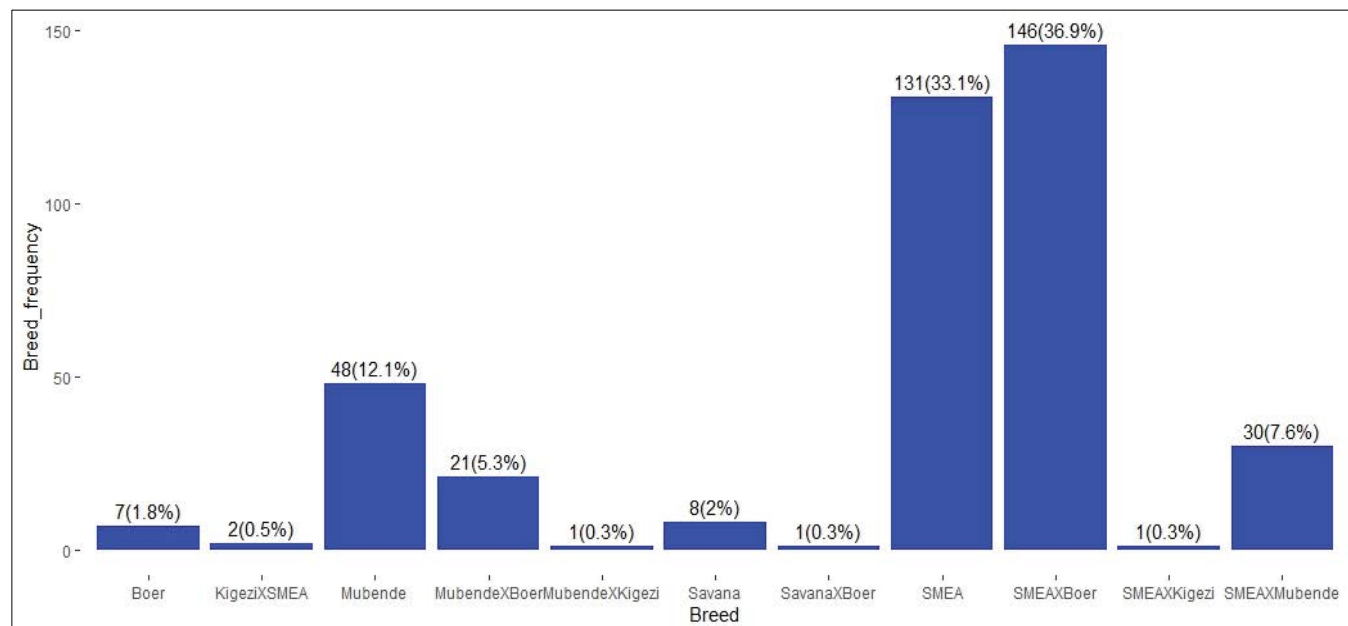


Figure 2: Frequency of sampled goat breeds at sampling sites in Kassanda, Wakiso and Kampala Districts

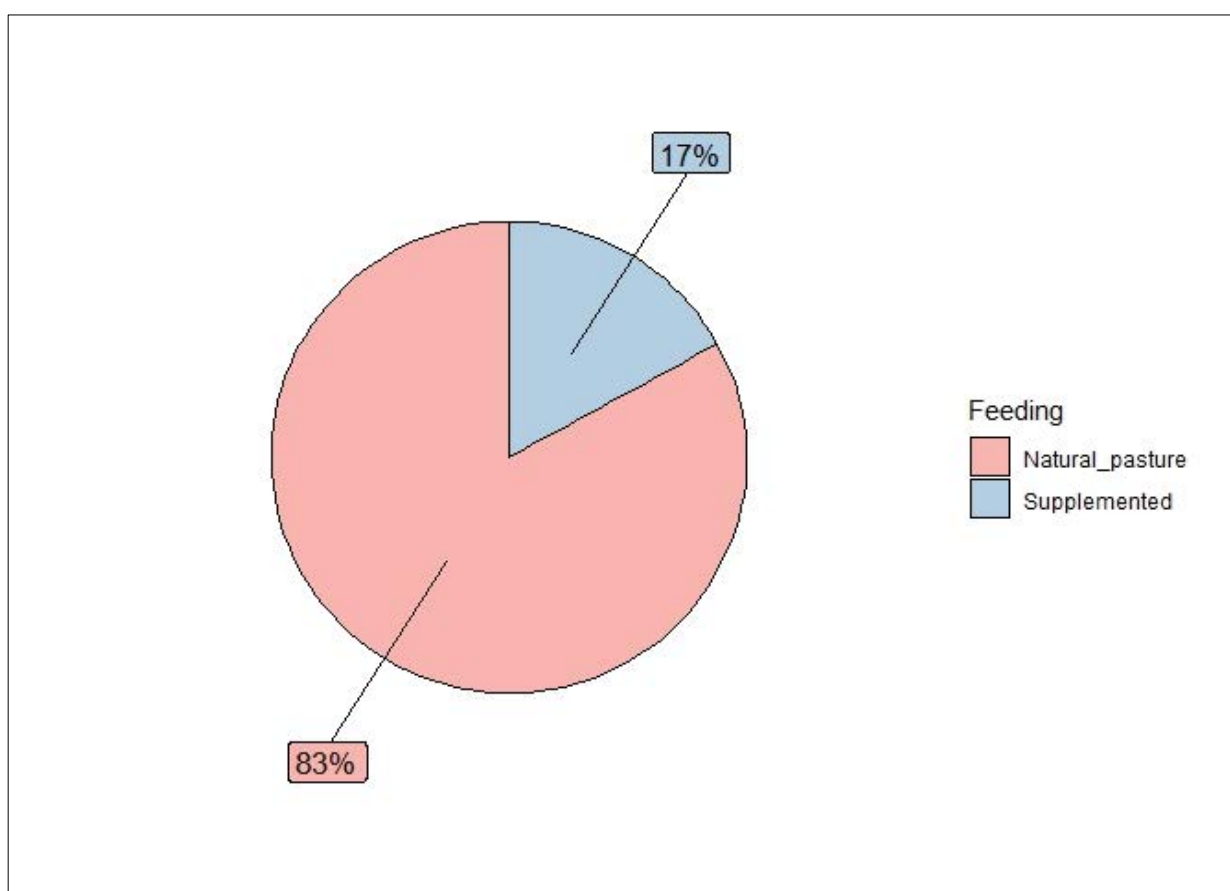


Figure 3: Ratios of feeding methods among the sampled goats.

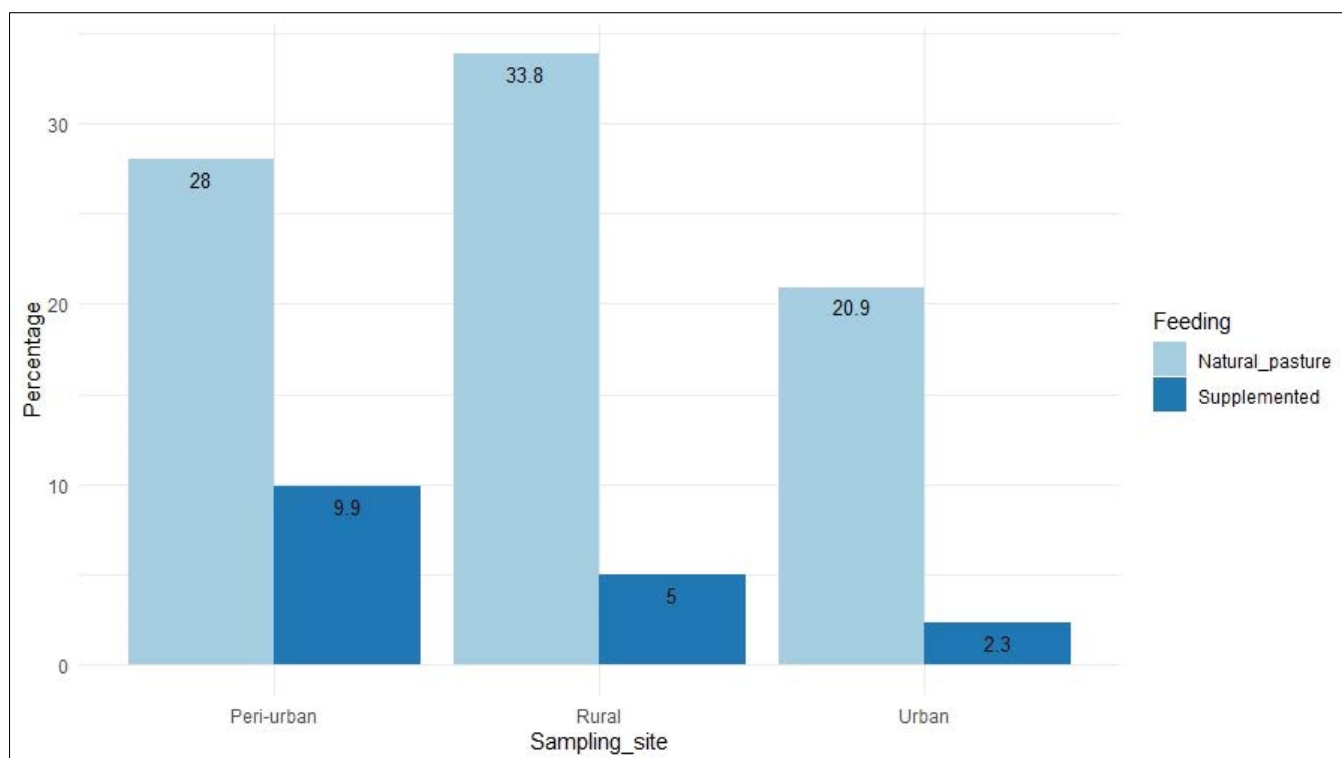


Figure 4: Percentages of different feeding methods used at the different sampling sites.

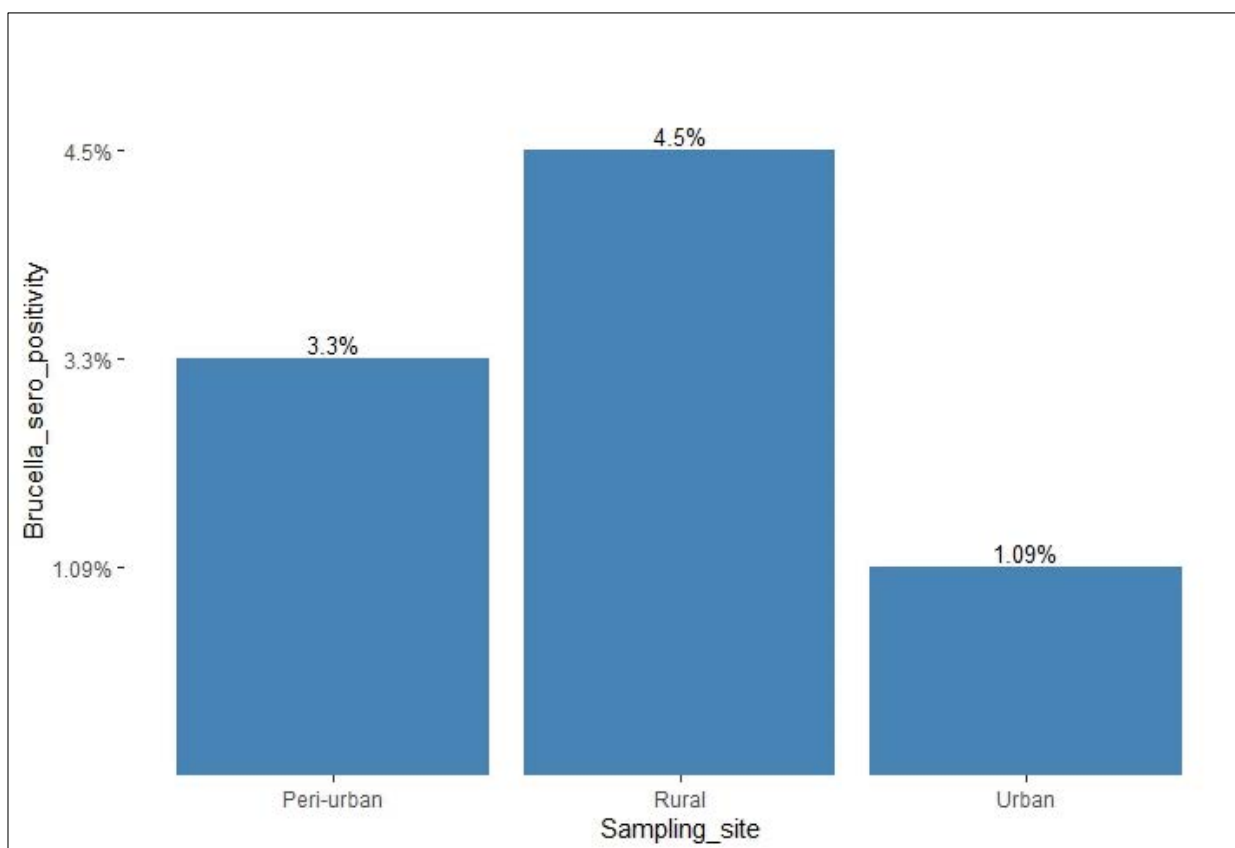


Figure 5: Brucella seroprevalence at the sampled sites.

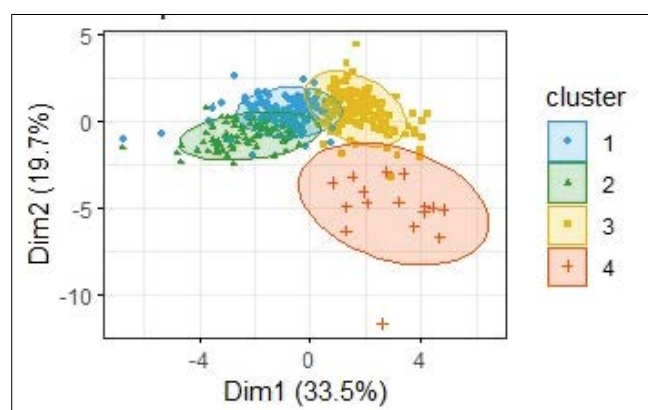


Figure 6: Clusters of haematological profile variations among the goats.

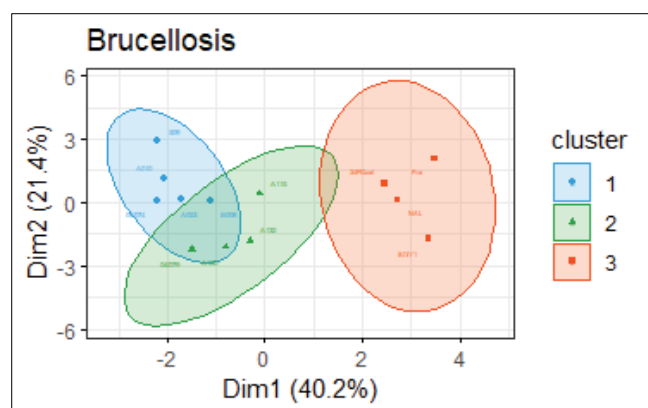


Figure 7: Clusters of haematological profile variations in Brucella-positive goats.

Haematological profiles of the goats

Clustering of the hematological profiles of the goats was performed using principal component analysis. Relationships among variables exhibited distinct patterns of the haematological profiles. The first principal components [Dim1 and Dim2] explained 53.2% of the total variance.

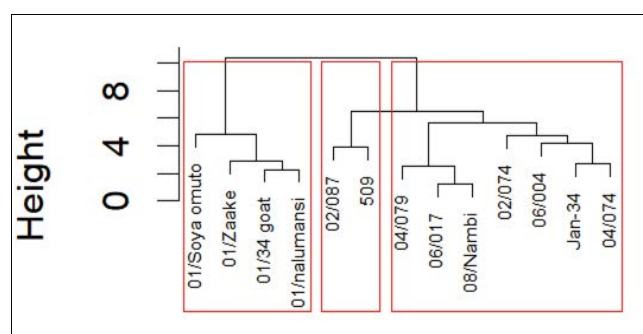


Figure 8: Hierarchical clustering of Brucella-positive samples.

The data points displayed as dots [Figure 6] represent the distribution of points that tightly clustered near the origin. Therefore, the majority of the goats did not vary drastically across components. The outliers presented distinct blood profiles. The goats formed four clusters near the origin, indicative of minimal variation in haematological profiles, except for outliers.

Haematological profiles of Brucella-positive goats

Principal components [Dim1 and Dim2] explained 40.2% and 21.4% of the data, respectively [Figure 7]. Blood pictures were linked to brucellosis among the goats. Haematological profiles reflected the immunological response. Three clusters were formed in reference to haematological profile variations. The clusters indicated the contribution of the haematological parameters to variations in haematological profiles among Brucella-positive goats.

Brucella-positive goats were singled out in a dendrogram [Figure 8]. Clusters and clades formed in response to variations in haematological profiles. Goats in cluster I had higher body condition scores than their counterparts in clusters II and III did [Table 1]. Goats in cluster I were under extensive farming and were either SMEAXMubende or Mubende by breed. Distribution of goats in clusters and clades is indicated in Table 1.

Table 1: Details of the samples in the different clusters.

Cluster	Member	BCS	Sampling site	Breed	Feeding
I	01/soya omuto	High	Rural	SMEAXMubende	Pasture
	01/Zaake	High	Rural	SMEAXMubende	Pasture
	01/34goat	High	Rural	Mubende	Pasture
	01/Nalumansi	High	Rural	SMEAXMubende	Pasture
II	02/087	Moderate	Peri-urban	SMEAXBoer	Supplemented
	509	Modrate	Rural	MubendeXBoer	Pasture
III	04/079	Moderate	Rural	Mubende	Pasture
	06/017	Moderate	Peri-Urban	Mubende	Pasture
	08/Nambi	Moderate	Peri-Urban	SMEAXBoer	Pasture
	02/074	Low	Peri-Urban	SMEAXBoer	Supplemented
	06/004	High	Peri-Urban	Mubende	Pasture
	Jan-34	Low	Urban	SMEA	Pasture
	04/074	Moderate	Rural	SMEAXBoer	Pasture

Table 2: Average means of haematological profiles for Brucella seropositive samples.

Parameter	Cluster I: Mean± SD	Cluster II: Mean± SD	Cluster III: Mean± SD
TWBC X10 ³ /μl	12.68±2.02 ^a	11.15±2.00 ^a	10.90±2.70 ^a
Lymphocytes X 10 ³ /μl	6.24±1.37 ^a	7.11±1.63 ^a	6.13±1.58 ^a
Neutrophils X 10 ³ /ul	5.46±2.51 ^a	3.20±0.35 ^a	3.95±1.12 ^a
Eosinophils X 10 ³ /ul	0.47±0.37 ^a	0.38±0.03 ^a	0.40±0.08 ^a
Monocytes X 10 ³ /ul	0.36±0.14 ^a	0.33±0.08 ^a	0.22±0.08 ^a
Basophils X 10 ³ /ul	0.14±0.08 ^a	0.13±0.06 ^a	0.21±0.08 ^a
TRBC X 10 ⁶ /ul	13.22±0.55 ^a	11.20±0.62 ^b	7.83±0.10 ^c
PCV%	29.42±1.91 ^a	22.67±1.15 ^b	20.78±1.21 ^b
HB, g/dl	10.06±0.72 ^a	7.72±0.26 ^b	7.50±0.54 ^b
MCV, fL	22.24±1.02 ^b	20.25±0.52 ^b	26.54±1.25 ^a
MCH, pg	7.61±0.4 ^b	6.90±0.28 ^b	9.58±0.59 ^a
MCHC%	34.21±0.95 ^b	34.06±0.72 ^b	36.09±1.09 ^a

Key: ^{a, b & c} are hypothetical letters implying significant difference. [LSD, p = .05]

Significant differences were observed across the three clusters when haematological parameters of Brucella seropositive samples were analysed [Table 2]. The TWBC, lymphocyte, neutrophil, eosinophil, monocyte and basophil counts were not significantly different across the clusters. Therefore, the goats exhibited similar leucocyte response in brucellosis. However, lymphocyte counts were highest in Cluster II [7.11 ± 1.63]. In contrast, neutrophil counts were lowest in Cluster II [3.20 ± 0.35]. Basophil levels slightly increased in Cluster III [0.21 ± 0.08].

We found significant variation in total red blood cell [TRBC] counts, where Cluster I presented with the highest mean value, whereas Cluster III presented the lowest [Table 2]. Similarly, packed cell volume [PCV] was highest in Cluster I [29.42 ± 1.91] and lowest in Cluster III [20.78 ± 1.21], suggesting a reduction in red blood cell volume, especially in Cluster III. Clusters II and III were not significantly different. Haemoglobin levels were significantly lower in Cluster II [7.72 ± 0.26] and Cluster III [7.50 ± 0.54], compared to Cluster I [10.06 ± 0.72], suggesting brucellosis-induced anaemia. Mean corpuscular volume [MCV] and mean corpuscular haemoglobin [MCH] were highest in Cluster III, indicating macrocytic changes in red blood cells and further supporting severe anaemia in Cluster III. Mean corpuscular haemoglobin concentration [MCHC] was significantly greater in Cluster III [36.09 ± 1.09] compared to Clusters I and II, suggesting a higher haemoglobin concentration in red blood cells in Cluster III. Overall, the haematological profiles of Cluster I goats were least altered among those with brucellosis. Cluster III indicated the most pronounced haematological changes, particularly in the erythrocyte indices. This highlights the varying severity of brucellosis among goats.

Discussion

Asymptomatic latent brucellosis is prevalent among livestock and humans, with limited data concerning their corresponding haematological profiles. There is paucity of data concerning asymptomatic brucellosis among goats in Uganda. Previous studies have reported combined rates of brucellosis among asymptomatic and symptomatic goats in Uganda [19,23]. In contrast, our study investigated the prevalence of asymptomatic brucellosis in apparently healthy goats in central Uganda. The overall rate of asymptomatic latent brucellosis was 3.33% in the three study districts, which was consistent with the 3.92% reported among goats in Karenga District, Uganda [23]. However, our findings of the prevalence of brucellosis among goats in Kassanda, Wakiso and Kampala study sites were inconsistent with the findings of Miller et al. [20], who reported seropositivity of 16% among serum samples from goats in southwestern Uganda. Higher rate of Brucella seropositivity was reported by Mugabi et al. [19] among goats in Kiruhura District, Uganda. Brucella seropositivity in the studies by Miller et al. [20] and Mugabi et al. [19] was irrespective of pre-clinical or clinical manifestations of the disease. In this study, the rate of asymptomatic brucellosis was highest at the rural site [Kassanda, 4.52%], which may be attributable to more frequent sharing and exchange of bucks among rural farmers in Uganda, as reported by Kaumbata et al. [18].

Breed distribution and feeding systems at the study sites

The major indigenous goat breeds in this study were SMEA [33.1%], Mubende [12%] and Kigezi [1.5%], in agreement with previous reports [28,29]. The goat population in Uganda is composed of more than 95% indigenous goat

breeds [30]. There is indiscriminate crossbreeding among goats in Uganda [31]. SMEAXBoer was the major cross breed in this study, similar to previous reports [28,31,32].

Majority of the goats [83%] in this study were on nonimproved natural pastures, which is in agreement with Nantongo et al. [28]. Extensive management systems are the main livestock production method in Uganda, where goats are grazed in open noncultivable areas or fenced farms. Free-range animals have the ability to select their fodder, increasing the quantity and quality of their diet [28,33].

Hierarchical clustering of goats by haematological profiling

In the present study, clustering occurred near the origin, indicative of minimal variation in haematological profiles, similar to the insignificant differences in blood parameters reported by Shamsa et al. [34]. However, the study by Shamsa et al. [34] reported significant differences in lymphocytes. The current study revealed differences in haematological profiles of *Brucella* seropositive goats, similar to the findings of Mohammed et al. [35], who reported significant differences in haematological parameters among different goat breeds under warm and humid weather conditions.

Hierarchical clustering of samples positive for brucellosis using haematological profiles

Brucella seropositive goat samples clustered into three groups [I, II and III] by haematological profiling. Four indigenous goats constituted cluster I, with three MubendeXSMEA cross breeds and a Mubende goat breed. The goats in cluster I were all from rural sampling sites [Kassanda district] and fed on nonimproved natural pastures under an extensive care system without supplementation. Cluster I was characterised by elevated TWBCs, neutrophils, TRBCs and PCV. Similarly, a previous study by Allam [9] reported increased TWBCs and PCV among *Brucella* seropositive goats. Elevated TWBCs in *Brucella* seropositivity may be attributable to recognition of the infection, and the immune system builds up to manage the infection [36].

One of the clades in cluster I consisted of a single leaf [01/soya omuto] with both brucellosis and anaplasmosis. The difference in clade distance from the rest of the candidates in cluster I may be attributed to coinfection. Cluster I was characterised by elevated TWBCs, neutrophils, TRBCs and PCV with high BCSs. Similarly, Allam [9] reported moderately high values of PCV and TWBCs among goats and sheep in Egypt and reported that the body condition score [BCS] was related to the haematological characteristics of *Brucella* seropositive ruminants.

Cluster II consisted of two goats, Mubende X Boer and SMEA X Boer cross breeds from the rural and peri-urban [Wakiso] sampling sites, respectively. Lymphocytes and

monocytes were slightly increased among goats in cluster II while the other haematological parameters did not differ from those in clusters I and III. Elevated lymphocytes and monocytes in brucellosis are attributed to the presence of *Brucella* organisms, regardless of disease symptoms or asymptomatic state [9].

Cluster III was characterised by increased basophils and lower TWBCs, TRBCs and PCVs. Allam [9] reported low HB, reduced RBC and lower PCV values among animals with low BCSs, which is in agreement with the findings of this study. According to Allam's report, [9] increased monocytes and basophils were proportional to high BCS among *Brucella* seropositive animals. This study did not reveal any significant differences in lymphocyte counts among all the clusters, which differed from the findings of Allam et al. [9], who reported the presence of lymphocytosis in clinical or subclinical cases irrespective of the BCS. This study also revealed that brucellosis affected the haematological profiles of goats, which contrasts with the findings of Skider [37], who reported that *Brucella* infection cannot be attributed to significant changes of haematological profiles of animals.

In this study, the body condition scores were high for the goats in the extensive care farming systems, which were practiced predominantly in the rural sampling site. Body condition scores are linked to health status, production and welfare of goats [38]. An animal's body condition indicates the amount of lipid [fat] and protein [muscle] reserves that are available for maintenance, reproduction and production [10]. Fat reserves are utilised during high energy demand periods, such as stress, or under conditions of under nutrition [10]. Intensive and semi-intensive farming practices require herding with frequent human handling. Low BCSs have been previously reported in intensive care systems among goats with frequent human handling, which harms the appetite center of the hypothalamus in the brain. Disruption of the hypothalamus causes reduced feed intake and thus decreased weight [39]. However, Kharrat [40] reported a decreasing BCS with low nutritive value pastures in Lebanon. Similarly, Bushara et al. [41] reported reduced BCS among goats during drought conditions associated with poor natural pastures in extensive farming systems in Sudan. In the present study, the low BCS may be attributable to frequent human handling in the intensive care system [39].

Variation in haematological profiles of goat samples

Cluster I presented insignificant differences in haematological profiles of *Brucella* seropositive goats. However, cluster I exhibited elevated TWBCs, similar to the findings of a previous report by Muayard [42] and their study reported elevated TWBCs among extensively managed goats in Malaysia. Extensive system in goat farming subjects the animals to numerous microbial challenges [43], which is in agreement with our findings of elevated neutrophils,

eosinophils, and monocytes. The elevated values in cluster I, although not significant, simulated the findings of Olayemi et al. [44], reported significantly higher values of TWBCs, neutrophils, and eosinophils among extensively reared West African dwarf goats in Nigeria.

Goats in cluster I on an extensive management system had significantly higher TRBC, PCV and HB in contrast with reduced TRBC, PCV and HB values, as previously reported by Olayemi et al. [44]. However, our findings were obtained during a rainy season, whereas Olayemi et al. [44] reported contrasting results for a study in a dry season with poor pastures. Our findings of elevated TRBC, PCV and HB in goats with brucellosis are in agreement with those of a previous study by Elsa and Onyeyili [45] during the rainy season in southern Nigeria.

Conclusion

The effects of brucellosis on haematological profiles varied among urban, peri-urban and rural apparently healthy goats owing to the different grazing systems in the different areas, which resulted in different stress levels for the animals and differences in body condition, which allowed the goats to respond differently to the impact of the infection. We recommend a larger sample size of apparently healthy indigenous, crossbred and exotic goats to affirm the impact of brucellosis on animals in the different ecological zones and grazing systems in Uganda.

Conflict of interest

The authors declare that they have no conflicts of interest.

Ethical approval

Ethical approval was obtained from the Institutional Animal Care and Use Committee of the School of Veterinary Medicine and Animal Resources, Makerere University [Reference number: SVAR-IACUC/97/2021].

Informed consent

All the farmers provided written consent to participate in this study.

Consent for publication

Not applicable

Source of funding

No external funding was received.

Author contributions

HN conceptualised the research design, performed the laboratory activities and analysis, and drafted the initial manuscript. KMM conducted the laboratory analysis. JM and CTK supervised the research activities. KMM, JM and CTK revised the manuscript. All the authors read and approved the final manuscript.

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