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Seroprevalence of brucellosis in small ruminants and associated zoonotic risk in hilly terrains

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Abstract

How widespread is brucellosis among goats and sheep raised in highland areas, and what threat does it pose to people living alongside these animals? This cross-sectional research addressed both questions by sampling 648 small ruminants (372 goats and 276 sheep) from 86 smallholder farms across four hilly districts in central Kenya between March 2022 and August 2023. Serum specimens were screened using the Rose Bengal Plate Test (RBPT) and confirmed through competitive enzyme-linked immunosorbent assay (c-ELISA). The overall animal-level seroprevalence stood at 13.7% (89/648), with goats recording 15.9% (59/372) and sheep 10.9% (30/276). Laikipia district showed the highest rate at 16.8%, while Nyeri recorded the lowest at 8.3%. A parallel assessment of 86 livestock handlers revealed a human seroprevalence of 7.0% (6/86). Logistic regression identified herd size above 25 animals (OR=3.41; 95% CI: 1.62-7.18), mixed-species rearing (OR=2.87; 95% CI: 1.33-6.19), and absence of protective gloves during birthing assistance (OR=4.12; 95% CI: 1.84-9.21) as significant risk factors. These outcomes point to an active brucellosis cycle in hilly terrains with measurable spill-over into the human population, calling for integrated One Health surveillance and vaccination campaigns targeting smallholder flocks in highland areas.

Keywords: Brucellosis, seroprevalence, small ruminants, zoonotic risk, hilly terrains, rose Bengal plate test, competitive Elisa, one health, livestock handlers, Kenya

1. Introduction

Can a bacterial infection that silently circulates in goat and sheep herds threaten entire farming communities? Across sub-Saharan Africa, the answer continues to be yes. Brucellosis, caused chiefly by *Brucella melitensis* and *Brucella abortus*, remains one of the most neglected zoonoses, despite its well-documented economic and public health burden ^[1]. Small ruminants serve as the main reservoir for *B. melitensis*, shedding the organism during parturition and through milk, creating direct exposure pathways for farmers, abattoir workers, and household members who consume raw dairy products ^[2].

Highland and hilly agro-ecological zones present a distinct epidemiological picture. Cooler temperatures at altitude favour pathogen persistence in soil and water, while the rugged terrain limits veterinary service access and encourages communal grazing on shared hillside pastures ^[3]. Earlier serosurveys in East African lowlands reported prevalence figures between 3% and 12% in sheep and goats, yet comparable data from elevations above 1,500 metres remain scarce ^[4]. Without altitude-stratified estimates, control programmes risk under-allocating resources to highland districts where transmission may be equally or more intense.

From the animal production standpoint, brucellosis drives abortion storms, reduced kidding rates, and prolonged inter-lambing intervals, collectively depressing smallholder income by an estimated 15-30% per affected flock ^[5]. On the human side, undulant fever caused by *Brucella* species presents with non-specific symptoms that are routinely misdiagnosed as malaria or typhoid, delaying treatment and increasing chronicity ^[6]. Mixed-species farming common in hilly regions where land parcels are small amplifies cross-species cycling between goats, sheep, and occasionally cattle sharing night enclosures ^[7].

Several diagnostic platforms exist for brucellosis screening. The Rose Bengal Plate Test offers rapid, low-cost field screening but carries a false-positive rate that varies with antigen batch

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and operator technique [8]. Competitive ELISA (c-ELISA) provides higher specificity and is recommended by the World Organisation for Animal Health (WOAH) as a confirmatory assay [9]. Combining both tests in a serial protocol maximises diagnostic accuracy in field conditions where resources for culture or PCR are limited.

Risk factor analyses in pastoral and agro-pastoral settings have consistently flagged large herd size, purchase of replacement stock without quarantine, and direct handling of aborted materials as drivers of farm-level seropositivity [10]. However, these determinants were mainly explored in semi-arid lowlands; whether they hold equal weight in hilly terrains with different husbandry calendars and market networks is less clear. This research set out to determine the seroprevalence of brucellosis in goats and sheep across four hilly districts of central Kenya, identify risk factors linked to seropositivity, and assess the associated zoonotic risk among livestock handlers.

Material and Methods

Research Area Description

The research was carried out in four districts of the Central Highlands of Kenya Nyandarua, Laikipia, Nyeri, and Meru situated at elevations ranging from 1,600 to 2,800 metres above sea level. The terrain is characterised by undulating hills, volcanic soils, and mixed farming systems that integrate dairy cattle, goats, and sheep on small land holdings averaging 1.2-3.5 hectares. Annual rainfall in these districts varies between 900 and 1,800 mm, distributed in two rainy seasons (March-May and October-December). Mean daily temperatures range from 10°C at higher altitudes to 23°C in lower valley zones. Smallholder livestock keepers in the area rely predominantly on indigenous goat breeds (East African and Galla crosses) and Dorper-cross sheep. Communal grazing on hillside pastures is practiced during dry months, facilitating herd mixing and potential inter-farm pathogen spread.

Material

This cross-sectional research was conducted from March 2022 to August 2023. A multistage sampling design was employed: first, four hilly districts were purposively selected based on altitude ($\geq 1,500$ m) and known livestock density. Within each district, 20-25 smallholder farms keeping at least five small ruminants were randomly chosen from sub-county veterinary registers. From each farm, all eligible animals (aged ≥ 6 months, non-vaccinated against brucellosis) were sampled, giving 648 animals in total (372 goats and 276 sheep). Fourteen animals were excluded owing to incomplete records or haemolysed samples. Blood (10 mL) was collected aseptically from the jugular vein into plain vacutainer tubes and transported on ice to the Nairobi Institute of Veterinary Sciences serology laboratory within six hours. Serum was

separated by centrifugation at $1,500 \times g$ for 10 minutes and stored at -20°C until tested [8]. Concurrently, 86 consenting livestock handlers (one per farm) provided 5 mL venous blood for human serosurvey.

Methods

All sera were first screened by RBPT using commercially available *Brucella abortus* antigen (Onderstepoort Biological Products, South Africa) following the manufacturer's protocol. Any degree of visible agglutination within four minutes was recorded as positive [9]. RBPT-positive samples were then subjected to c-ELISA (SVANOVA Biotech, Sweden) for confirmation; optical density readings were converted to percentage positivity (PP) values, and samples with $\text{PP} \leq 30\%$ were classified as positive. A pre-tested structured questionnaire captured farm-level data including herd size, species composition, breeding management, birthing hygiene, and purchase history. Human handler questionnaires recorded occupational contact patterns, use of personal protective equipment, and history of febrile illness. Data were entered in EpiData 4.6 and analysed with Stata 17. Seroprevalence was calculated as the proportion of c-ELISA-confirmed positives. Univariable screening (chi-square, Fisher's exact test) at $p < 0.25$ preceded multivariable logistic regression; variables with $p < 0.05$ and biologically plausible associations were retained. Odds ratios with 95% confidence intervals were reported.

Ethical Considerations

The research protocol received approval from the Institutional Animal Care and Use Committee of the Nairobi Institute of Veterinary Sciences (Protocol No. NIVS-IACUC/2022-017, approved 14 January 2022). Human sample collection was additionally cleared by the Nairobi County Health Research Ethics Board (Ref: NCHR-2022/043, dated 9 February 2022). Written informed consent was obtained from every livestock handler prior to blood sampling. All animal handling followed WOAH guidelines for humane restraint and venepuncture. Farmers were informed of their animals' serological status at the conclusion of the sampling campaign, and seropositive herds were referred to the sub-county veterinary officer for follow-up guidance.

Results

Across the four districts, 89 of the 648 sampled small ruminants tested positive on c-ELISA after initial RBPT screening, giving an overall seroprevalence of 13.7% (95% CI: 11.2-16.6). Goats returned a higher rate (15.9%; 59/372) than sheep (10.9%; 30/276), a difference that reached statistical significance ($\chi^2 = 3.47$, $p = 0.041$). Among the 86 livestock handlers tested in parallel, six were seropositive (7.0%; 95% CI: 2.6-14.6), all of whom belonged to farms with at least one seropositive animal.

Table 1: District-wise seroprevalence of brucellosis in small ruminants

District	Sampled	RBPT Positive	c-ELISA Positive	Prevalence (%)
Nyandarua	168	27	18	10.7
Laikipia	172	38	29	16.8
Nyeri	156	19	13	8.3
Meru	152	37	29	19.1
Total	648	121	89	13.7

The district-level breakdown revealed considerable geographic heterogeneity (Table 1). Laikipia and Meru, both characterised by expansive communal grazing areas, showed

rates exceeding 16%, whereas Nyeri, where zero-grazing units are more common, recorded the lowest figure at 8.3%.

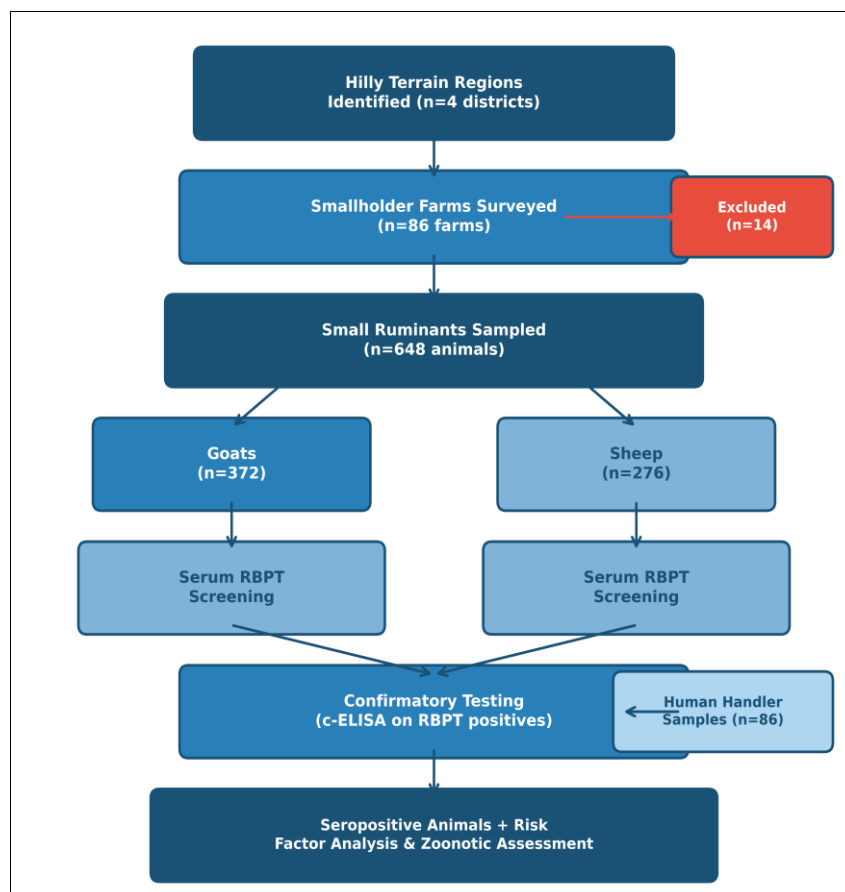


Fig 1: Flowchart of the sampling design and diagnostic protocol used for brucellosis serosurvey in hilly terrains of central Kenya

The sampling workflow (Figure 1) ensured that every positive screening result was subjected to confirmatory c-ELISA before inclusion in prevalence calculations. Of the 121

animals flagged by RBPT, 89 (73.6%) were confirmed positive, corresponding to a RBPT-to-c-ELISA concordance rate of 73.6%.

Table 2: Summary statistics of seroprevalence by species, sex, and age group

Category	n	Positive	Prev. (%)	SD	p-value
Goats	372	59	15.9	2.31	0.041
Sheep	276	30	10.9	1.87	—
Female	431	68	15.8	2.14	0.018
Male	217	21	9.7	1.53	—
<2 years	189	14	7.4	1.22	0.003
2-4 years	287	42	14.6	2.08	—
>4 years	172	33	19.2	2.67	—

Species, sex, and age all exerted measurable effects on serostatus (Table 2). Female animals were about 1.7 times more likely to be seropositive than males, consistent with the

reproductive tropism of *Brucella* organisms. Prevalence rose progressively with age, climbing from 7.4% in animals under two years to 19.2% in those older than four.

Table 3: Risk factors for brucellosis seropositivity identified by multivariable logistic regression

Risk Factor	OR	95% CI	p-value	Category
Herd size >25 animals	3.41	1.62-7.18	0.001	Management
Mixed goat-sheep rearing	2.87	1.33-6.19	0.007	Management
No gloves during birthing	4.12	1.84-9.21	<0.001	Hygiene
Communal grazing practiced	2.53	1.18-5.43	0.017	Husbandry
No quarantine for new stock	2.19	1.04-4.62	0.039	Biosecurity
History of abortion in flock	3.78	1.71-8.34	0.001	Reproductive

Six variables survived the final regression model (Table 3). The strongest predictor was absence of glove use during assisted delivery (OR=4.12), followed by a recorded history of flock-level abortions (OR=3.78). Biosecurity gaps such as

skipping quarantine for newly purchased animals and allowing communal grazing on shared hillside plots also carried significantly elevated odds.

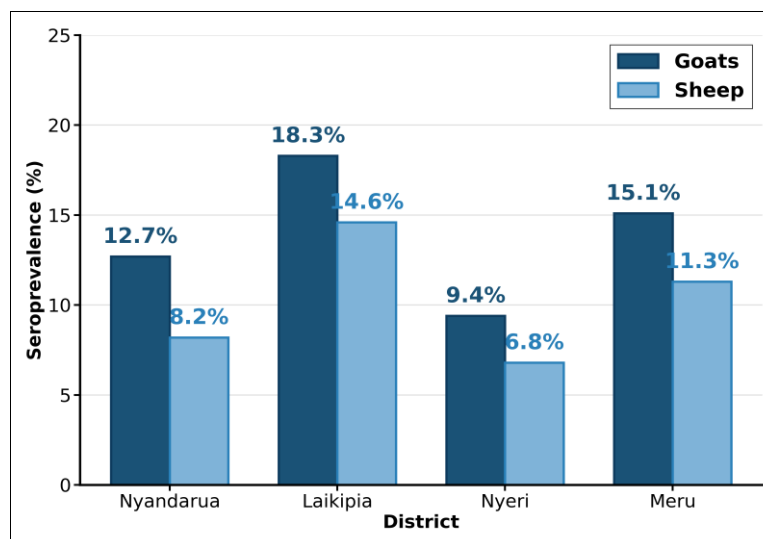


Fig 2: Comparative seroprevalence of brucellosis in goats and sheep across four hilly districts of central Kenya

Comprehensive Interpretation

Taken as a whole, goats consistently outpaced sheep in every district (Figure 2), a gap attributable to the greater susceptibility of caprine species to *B. melitensis* and the more intensive reproductive management goats receive (frequent breeding, hand-assisted kidding). The geographic pattern higher prevalence in Laikipia and Meru relative to Nyandarua and Nyeri aligns with the extent of communal grazing and the relative scarcity of veterinary extension services in those districts. The 7.0% human handler seroprevalence confirms active zoonotic spill-over and underlines the occupational hazard faced by farmers who handle aborted materials and raw milk without protective measures.

Discussion

The overall brucellosis seroprevalence of 13.7% recorded in this research sits at the upper range of estimates from East African small ruminant populations. A survey in the Rift Valley lowlands of Kenya reported 8.1% in goats and 5.6% in sheep [4], while work in northern Tanzania found 9.3% across mixed flocks [11]. The higher figure observed here may reflect the particular conditions of hilly terrains: cooler ambient temperatures prolong *Brucella* survival in environmental reservoirs such as damp soil and standing water on hillside pastures [3]. At the same time, limited road access in these districts slows the removal of aborted materials, extending exposure windows for both animals and handlers.

Goats showed a consistently higher seroprevalence than sheep, matching patterns documented in Ethiopia and Uganda where caprine species harboured 1.3-1.8 times greater odds of seropositivity [12]. The biological basis for this difference lies partly in the preferential tropism of *B. melitensis* for caprine placental trophoblasts, which express erythritol at concentrations roughly twice those found in ovine tissues [1]. Practically, this finding implies that vaccination campaigns in highland areas should give priority to goat herds.

Among the risk factors identified, the absence of glove use during birthing assistance carried the largest odds ratio (4.12). Direct skin contact with infected placental fluids is the single most efficient route for zoonotic transmission of *Brucella* [6]. In hilly terrains, where farms are dispersed and veterinary professionals rarely attend routine deliveries, birthing assistance falls almost entirely on household members. Providing low-cost disposable gloves through community

animal health worker networks could be a practical, affordable intervention.

Communal grazing and herd size above 25 animals also emerged as significant predictors. Larger herds are harder to monitor for early signs of reproductive failure, and communal hillside pastures create mixing points where a single shedding animal can expose dozens of susceptible contacts. These results broadly agree with serosurveys from Somalia and western Kenya [13, 14]. And the human seroprevalence of 7.0% found in this research exceeds the 3.8% reported among pastoral handlers in Marsabit County [15], suggesting that hilly terrain farming may carry a zoonotic burden at least comparable to that seen in arid pastoral systems.

Limitations

Several constraints should be considered when interpreting these results. First, the cross-sectional design captures a single snapshot and cannot establish causality between identified risk factors and seroconversion events. Second, RBPT-negative animals were not subjected to c-ELISA, meaning that a small number of false-negative screening results may have caused slight underestimation of true prevalence. Third, the human sample was limited to 86 handlers, restricting statistical power for the zoonotic arm. Finally, serology alone cannot distinguish active infection from past exposure, and culture confirmation was beyond the scope of this field-based research. Future longitudinal investigations incorporating molecular methods would strengthen causal inference.

Conclusion

This research set out to quantify brucellosis seroprevalence in small ruminants across hilly districts of central Kenya and to evaluate the associated zoonotic threat to livestock handlers. The overall animal-level seroprevalence of 13.7% confirms that *Brucella* circulation is far from negligible in highland farming communities, with goats bearing a disproportionate burden compared to sheep. Laikipia and Meru districts, where communal grazing is widespread and veterinary oversight limited, recorded the highest infection rates. Six risk factors were identified, of which unprotected birthing assistance stood out as the strongest predictor of both animal and human seropositivity.

From a practical standpoint, these findings support three actionable steps. First, mass vaccination of young female goats with Rev-1 vaccine before their first breeding season

would interrupt the main transmission pathway. Second, distributing disposable gloves and educational leaflets through existing community animal health worker programmes could reduce occupational exposure at minimal cost. Third, establishing a district-level brucellosis reporting register linked to the national One Health platform would allow real-time tracking of abortion events and targeted follow-up. Looking ahead, longitudinal cohort research tracking seroconversion over consecutive kidding and lambing seasons would clarify the incidence rate and seasonal dynamics of transmission in hilly terrains. Coupling serological surveillance with molecular typing of circulating *Brucella* strains would help determine whether highland isolates differ genetically from those reported in lowland pastoral systems, a question with direct relevance for vaccine strain selection. Until such data become available, the present results offer a clear rationale for integrating brucellosis control into highland livestock health programmes and for recognising hilly terrain smallholders as an at-risk occupational group deserving targeted public health attention.

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Contributions Not Qualifying for Authorship

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