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Seroprevalence, molecular detection, risk factor analysis and public health perceptions of brucellosis in donkeys across different production systems in Kenya

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Introduction: Donkeys are vital to livelihoods in Kenya, yet their exclusion from national disease surveillance leaves potential health risks underexplored. Brucellosis, a significant zoonosis, remains poorly characterized in donkeys despite frequent close contact with humans. This study aimed to determine the seroprevalence, molecular detection, and risk factors for *Brucella* spp. infection in donkeys, and to assess owner knowledge, attitudes, and practices (KAP) across seven Kenyan counties representing diverse production systems.

Methods: Between October 2024 and February 2025, a cross-sectional survey sampled 392 donkeys. Serum was tested using the Rose Bengal Plate Test (RBPT) and indirect ELISA (iELISA). Donkeys testing seropositive on either test (n = 42) had their corresponding whole blood samples subjected to DNA extraction for PCR analysis, targeting *Brucella abortus* and *Brucella melitensis*. Structured interviews with owners were conducted to assess knowledge, attitudes, and practices (KAP). Mixed-effects logistic regression in R was used to identify risk factors.

Results: Overall seroprevalence was 10.7% by RBPT, 2.0% by iELISA, and 0.0% by PCR. All iELISA-positive cases (n = 8) were from Turkana (4), Narok (3), and Nairobi (1). Young donkeys (<3 years) had significantly higher odds of being seropositive (aOR = 11.8; 95% CI: 1.70–81.99; p = 0.013). Owner knowledge was low—only 25.3% had heard of brucellosis and risky practices were common, with 91.1% assisting foaling without protective equipment and 19.4% consuming donkey products, often raw.

Conclusion: Donkeys in Kenya may contribute to *Brucella* transmission within mixed livestock systems and to humans. Inclusion of donkeys in brucellosis surveillance, targeted community education, and improved diagnostics are recommended. These findings provide the first field-based evidence of donkey brucellosis in Kenya and underscore the importance of integrating donkeys into One Health strategies to reduce zoonotic risk.

KEYWORDS

brucellosis, donkeys, Kenya, One Health, zoonosis

1 Introduction

Donkeys are vital working animals in many developing countries, providing affordable transport, supporting agriculture, and generating income for millions of households (1). The global donkey population is estimated at about 50 million, with roughly half in Asia and more than a quarter in Africa. Ethiopia has the largest national herd, while smaller populations are distributed across West, Southern, and East Africa, including Kenya (2, 3). In Kenya, the most recent census recorded 1,176,293 donkeys (4), concentrated in diverse production systems such as arid, semi-arid, urban, and high-potential (5). Their adaptability to harsh environments (6) and cost-effectiveness compared to mechanized transport make them indispensable, particularly for women whose livelihoods depend on them (1).

Brucellosis, caused mainly by *Brucella abortus* and *Brucella melitensis*, is a zoonotic disease of economic and public health importance. In donkeys, infection can result in abortion, infertility, orchitis, and epididymitis, leading to productivity losses (7, 8). The disease is endemic in East Africa (9) and transmitted through direct animal contact, ingestion of contaminated materials, or inhalation of aerosols (10).

In Kenya, brucellosis contributes to major livestock losses from infertility, abortion, and extended calving intervals (11). Human brucellosis is also significant, with seroprevalence estimates ranging from 10.8% in Marsabit (12) to 16.5% in Kajiado (13), and incidence rates exceeding 40% among febrile patients (14). Risk factors include raw milk consumption, handling of birth materials, and frequent livestock contact (15). In rural and peri-urban areas where donkeys play central roles, the potential for zoonotic transmission is particularly high (16).

Globally, donkey brucellosis prevalence averages 10.2% but varies widely (0–63.7%) depending on geography, management, and diagnostic methods (17). Pastoral systems generally report higher prevalence due to herd mixing, communal grazing, and wildlife contact (18, 19). Despite this, donkeys remain largely excluded from surveillance and control programs, which may underestimate their reservoir role (17).

Control of brucellosis relies on One Health strategies such as vaccination, surveillance, biosecurity, test-and-slaughter, and public education. While near-eradication has been achieved in some high-income countries (20), progressing Africa is constrained by limited resources, weak veterinary infrastructure, and low community awareness (21). In Kenya, most brucellosis research has focused on cattle, camels, goats, and sheep, with little epidemiological data available for donkeys (17, 22).

To address this gap, we applied a One Health approach to investigate brucellosis in donkeys across seven Kenyan counties representing different production systems. Specifically, we aimed to: (i) estimate seroprevalence, (ii) detect *Brucella* species using PCR, (iii) identify associated risk factors, and (iv) assess community perceptions of the disease.

2 Materials and methods

2.1 Study design and data collection

This study employed a cross-sectional design conducted between October 2024 and February 2025 across seven counties in Kenya: Turkana, Kitui, Narok, Nakuru, Nairobi, Kiambu, and Bungoma (Figure 1). These counties were purposively selected to represent diverse donkey production systems, including arid, semi-arid, urban, and high-potential agro-ecological zones. The selected communities are characterized by informal animal health systems, close interspecies contact, and reliance on donkeys for daily livelihoods.

The study population comprised donkeys aged six months and above, managed under traditional husbandry systems. Donkeys younger than six months or belonging to owners who declined consent were excluded. A sample size of 384 was calculated using the (23) formula, assuming a 50% expected prevalence, 5% margin of error, and 95% confidence level. To improve representativeness, samples were proportionally distributed across counties based on donkey population size. At the end of the exercise, 392 donkeys were sampled. Within each county, one or more sub-counties were purposively selected based on security, logistical access, and presence of active donkey-owning communities. Donkey welfare groups were identified with support from local non-governmental organisations, and within each group, households were randomly selected. Where an owner had more than one donkey, one animal was randomly chosen for sampling.

Data collection involved a combination of structured questionnaires and blood sampling. The questionnaire was developed using previously published KAP tools on zoonoses, adapted to donkeys, and pre-tested in one county to check clarity. It was then digitized, and administered via Google Forms on mobile devices by trained enumerators. Responses were scored and grouped into knowledge, attitudes, and practices domains. The tool captured information on owner demographics, knowledge, attitudes, and practices related to brucellosis, as well as donkey characteristics, reproductive history, and husbandry practices. Interviews were conducted in English or local languages, depending on participant preference.

Following the interview, approximately 10 ml of blood was aseptically collected from the donkey's jugular vein using sterile vacutainer tubes. Samples were stored in cool boxes (4–8 °C) and processed at county laboratories, where serum was separated for serological testing and EDTA blood retained for molecular analysis. All samples were tested in parallel using the Rose Bengal Plate Test (Pourquier® Rose Bengal antigen - Innovative Diagnostics [IDVET], France), valued for its sensitivity, and indirect ELISA (ID Screen® Brucellosis Serum Indirect Multi-species kit (IDVET, France), known for specificity. Samples positive on either test were further analyzed by conventional PCR (QIAamp® DNA Blood Mini Kit (Qiagen, cat. no. 51106, Hilden, Germany) to detect and

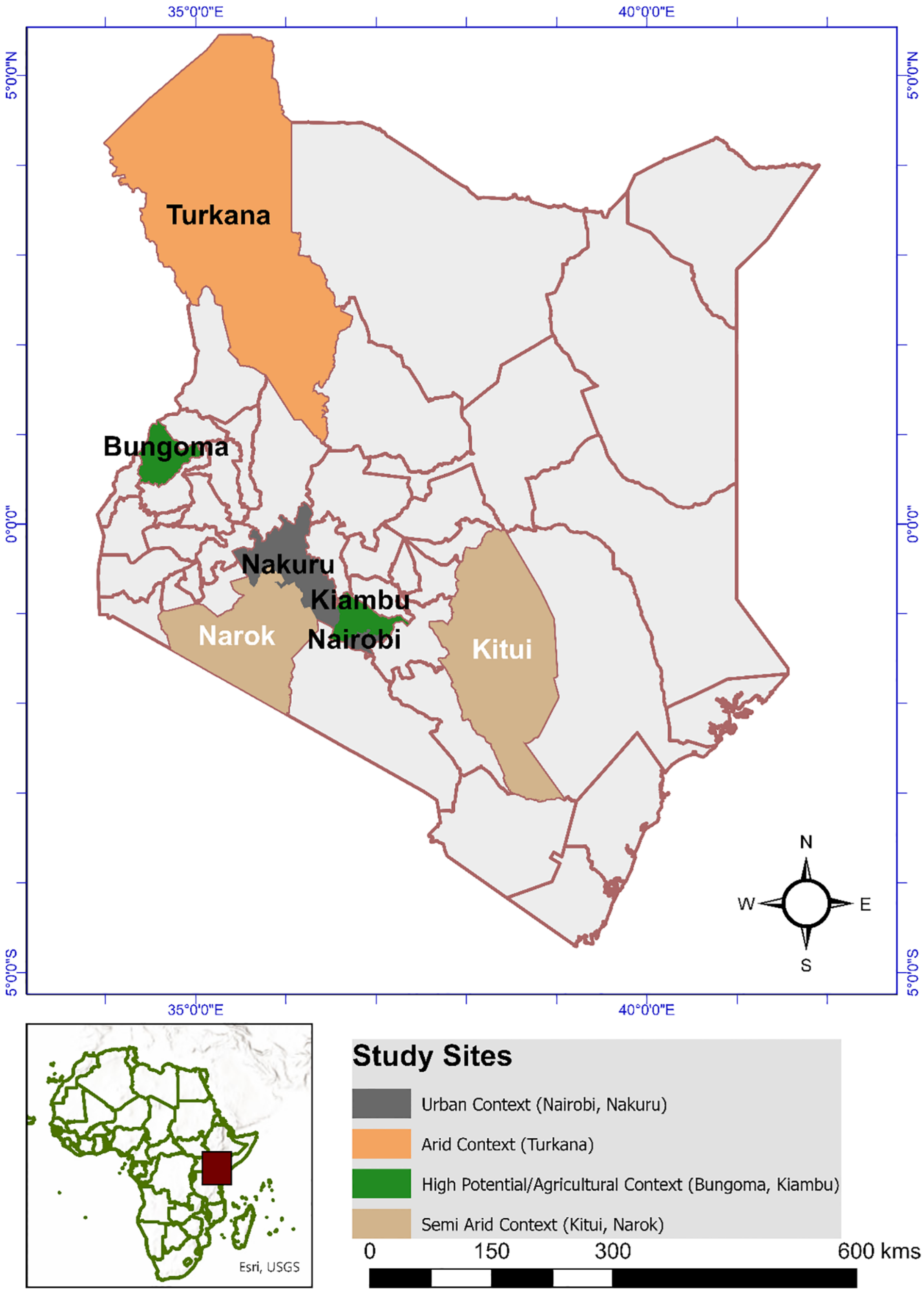

FIGURE 1
A map of Kenya showing study counties and categorized into production systems (Urban, Semi-arid, high potential and Arid. [Source: The map was generated using ArcMap version 10.8.2., ESRI, California, USA]).

differentiate *Brucella abortus* and *Brucella melitensis*. Laboratory protocols followed international standards, with appropriate positive and negative controls.

2.2 Ethics

Ethical approval for the study was obtained from the University of Nairobi Faculty of Veterinary Medicine Biosafety, Animal Use and Ethics Committee (Ref: FVM BAUEC/2024/117). Research authorization and permits were also granted by the National Commission for Science, Technology and Innovation (NACOSTI), Permit No. NACOSTI/P/24/38872.

All animal handling and blood collection were performed humanely and non-invasively, using minimal restraint to avoid distress, and in accordance with approved animal welfare and biosafety protocols. Participation in the accompanying questionnaire survey was voluntary, and written informed consent was obtained from all donkey owners prior to interviews after explaining the purpose and confidentiality of the study.

2.3 Data management and analysis

Data from questionnaires and laboratory results were merged into a single dataset, cleaned for consistency, and analysed using R version 4.1.2 (R Foundation for Statistical Computing, Vienna, Austria). Descriptive statistics were used to estimate seroprevalence with 95% confidence intervals. Associations between potential risk factors and *Brucella* seropositivity were initially assessed using Chi-square tests. Mixed-effects multilevel logistic regression models were then used to identify independent predictors, accounting for clustering by county. Variables with p-values <0.20 in univariable analysis were considered for multivariable models.

3 Results

3.1 Seroprevalence of donkey brucellosis and by county

A total of 392 donkey serum samples were collected across seven Kenyan counties; Bungoma, Kiambu, Kitui, Nairobi, Nakuru, Narok, and Turkana to assess the seroprevalence of *Brucella* spp. infection. Using RBPT, 42 donkeys (10.7%) tested positive, while iELISA confirmed only 8 donkeys (2.0%). All iELISA-positive samples were also RBPT-positive, indicating concordance in true positives but highlighting the lower specificity of RBPT. The iELISA-derived prevalence (2.0%) is considered the more accurate estimate of *Brucella* exposure in donkeys, while RBPT results are best regarded as preliminary screening. Substantial variation in seroprevalence was observed across counties. Nairobi (24.1%) and Bungoma (23.3%) reported the highest RBPT prevalence, followed by Turkana (10.0%) and Kitui (9.0%). Lower RBPT seroprevalence was observed in Narok (8.1%), Kiambu (6.7%), and Nakuru (5.0%). iELISA detected positives in Nairobi (3.4%), Narok (4.8%), and Turkana (4.0%) only. The proportion of dual-positive samples mirrored the iELISA results, reflecting the concordance between assays.

A Chi-square test indicated a statistically significant association between county and RBPT seroprevalence ($\chi^2 = 13.12$, df = 6, $p = 0.041$). Cohen's Kappa statistic revealed a fair agreement between RBPT and iELISA ($\kappa = 0.30$), suggesting some degree of diagnostic discordance, likely due to differences in test sensitivity and specificity as detailed in Table 1 below. However, none of the 42 seropositive samples yielded *Brucella* DNA by PCR, indicating absence of detectable infection in blood at the time of sampling.

3.2 Seroprevalence by sex, age, and body condition score

Among the 392 donkeys sampled, 188 were male and 204 female. Seroprevalence based on the RBPT was slightly higher in males (11.7%) compared to females (9.8%). Both sexes recorded 4 positive cases by iELISA, yielding nearly identical prevalence rates (2.1% in males *vs*. 2.0% in females). Logistic regression showed no significant difference by sex for either RBPT (OR = 1.22, p = 0.544) or iELISA (OR = 1.09, p = 0.914).

Age-based analysis revealed that young donkeys (<3 years) had the highest RBPT (13.3%) and iELISA (6.7%) positivity, followed by mature (10.7%, 2.0%) and old donkeys (9.7%, 0.0%). The low number of positive iELISA results limited statistical inference, and no associations were statistically significant.

Body condition also showed modest variation. RBPT seroprevalence was highest in very fat donkeys (25.0%), followed by thin (14.3%), fat (12.2%), and moderate (10.1%) animals. For iELISA, only moderate (1.9%) and fat (4.1%) donkeys recorded positive results. Odds ratios comparing fat to moderate donkeys were elevated (OR = 2.21, p = 0.3394), though not statistically significant (Table 2).

3.3 Molecular detection of *Brucella* species by PCR

A total of 42 seropositive samples, identified by RBPT and/or iELISA, were subjected to conventional polymerase chain reaction (PCR) to confirm the presence of Brucella DNA and determine species identity. The analysis was performed at the Department of Public Health, Pharmacology and Toxicology, University of Nairobi, using species-specific primers targeting Brucella abortus and Brucella melitensis (Table 3).

DNA was successfully extracted from all samples, and PCR amplification was conducted in triplicate to enhance detection reliability. Despite this, none of the samples yielded amplification products for either B. abortus or B. melitensis, indicating no detectable Brucella DNA in the tested sera.

3.4 Analysis of risk factors for donkey brucellosis

A total of 392 donkeys were tested using iELISA, yielding an overall seroprevalence of 2.04%. Univariable logistic regression was used to explore associations between *Brucella* seropositivity and key variables: sex, age, county, body condition score (BCS), and recent movement history.

In univariable analysis, none of the variables reached statistical significance. However, younger donkeys (below 3 years) showed higher odds of testing positive (OR = 3.5; p = 0.136), suggesting possible recent exposure or age-related susceptibility. Seropositivity clustered in Turkana, Narok, and Nairobi counties, but the odds ratios were unstable due to zero cases in other counties. Movement history and BCS showed no significant association.

Multivariable logistic regression was conducted including all the variables. Only age group remained statistically significant: young donkeys had an adjusted odds ratio (aOR) of 11.8 (95% CI: 1.70–81.99; p = 0.013), confirming that younger age was independently associated with higher risk. Body condition (moderate *vs*. fat) also showed significance (aOR = 0.07; p = 0.011), though caution is needed due to small sample sizes in some BCS categories.

TABLE 1 Seroprevalence of brucellosis in donkeys by county (n = 392).

County	No. tested	RBPT positive (%)	iELISA positive (%)	Dual positive (%)	RBPT OR	RBPT p-value	iELISA OR	iELISA p-value
Bungoma	30	23.3 (7/30)	0.0 (0/30)	0	0.3	0.005	7.88×10^{-12}	0.99
Kiambu	30	6.7 (2/30)	0.0 (0/30)	0	0.23	0.088	1	1
Kitui	100	9.0 (9/100)	0.0 (0/100)	0	0.32	0.043	1	1
Nairobi	29	24.1 (7/29)	3.4 (1/29)	3.4	1.05	0.942	4.53×10^{9}	0.99
Nakuru	40	5.0 (2/40)	0.0 (0/40)	0	0.17	0.038	1	1
Narok	62	8.1 (5/62)	4.8 (3/62)	4.8	0.29	0.05	6.45×10^{9}	0.99
Turkana	101	10.0 (10/101)	4.0 (4/101)	4	0.36	0.065	5.23×10^{9}	0.99
Total	392	10.7 (42/392)	2.0 (8/392)	2	—	—	—	—

All other variables (sex, county, movement) were not significant in the multivariable model. The findings highlight young age as the strongest and most consistent predictor of *Brucella* exposure in donkeys (Table 4).

3.5 Knowledge, attitude, and practices of donkey owners on brucellosis

A cross-sectional survey involving 392 donkey owners across seven counties in Kenya was conducted to evaluate their knowledge, attitudes, and practices (KAP) related to brucellosis a neglected but important zoonotic disease. The demographic profile of the respondents showed an even gender distribution (50% male and 50% female), with the majority aged between 19 and 49 years. Educational attainment was generally low: 69% had only completed primary school, 28.1% had secondary education, and less than 4% had attained post-secondary qualifications. This background provides important context for understanding the observed knowledge and behavioural gaps.

The level of awareness about brucellosis among donkey owners was strikingly low. Only 25.3% had ever heard of the disease, and fewer (20.7%) recognized its potential to transmit to humans. Just over half (54.6%) could identify at least one clinical sign suggestive of the disease in donkeys, such as abortion or infertility. When knowledge was measured using a structured scoring scale, the majority (62.2%) fell into the “very low” category, and only 2.0% attained a “very high” score. This lack of knowledge was significantly associated with several factors. Higher education levels were positively linked to better understanding (χ^2 = 17.57, p = 0.007), as were county of residence (χ^2 = 52.78, p < 0.001), awareness of donkey vaccination history (χ^2 = 11.54, p = 0.003) and movement history (χ^2 = 10.35, p = 0.006). Variables such as respondent age and gender showed no statistically significant associations with knowledge levels.

Attitudes toward brucellosis risk were similarly concerning. A significant proportion of owners underestimated the potential impact of the disease, with 63.5% rating it as a “low” or “very low” risk. Only 24% of respondents perceived brucellosis as a “high” or “very high” threat. Individuals who had previously observed

TABLE 2 Donkey brucellosis seroprevalence by sex, age, and body condition score.

Category	No. tested	RBPT positive (n, %)	iELISA positive (n, %)	RBPT OR	RBPT p-value	iELISA OR	iELISA p-value
Sex							
Male	188	22 (11.7%)	4 (2.1%)	1.22	0.544	1.09	0.914
Female (Ref)	204	20 (9.8%)	4 (2.0%)	1	Ref	1	Ref
Age group							
Young (<3 yrs)	30	4 (13.3%)	2 (6.7%)	—	—	—	—
Mature (Ref)	300	32 (10.7%)	6 (2.0%)	Ref	—	Ref	—
Body condition							
Thin	21	3 (14.3%)	0 (0.0%)	1.49	0.54	—	—
Moderate (Ref)	318	32 (10.1%)	6 (1.9%)	Ref	Ref	Ref	Ref
Fat	49	6 (12.2%)	2 (4.1%)	1.25	0.641	2.21	0.339

TABLE 3 The primers used in this study were based on previously validated protocols.

Target species	Primer name	Sequence (5′–3′)	Expected product size (bp)	Reference
Brucella spp.	*Brucella* spp-F/R	F: GCT CGG TTG CCA ATA TCA ATG R: *GGG TAA AGC GTC GCC AGA AG*	223	(24)
Brucella abortus	Bab-F/Bab-R	F: *GCG GCT TTT CTA CGG TAT TC* R: *CAT GCG CTA TGA TCT GGT TAC G*	800	(25)
Brucella abortus	B4/B5	F: *TGG CTC GGT TGC CAA TAT CAA* R: *CGC GCT TGC CTT TCA GGT CTG*	223	(26)
Brucella melitensis	BM-F/BM-R	F: *AAC AAG CGG CAC CCC TAA AA* R: *CAT GCG CTA TGA TCT GGT TAC G*	279	(27)

signs suggestive of brucellosis in their donkeys (such as abortion or joint swelling) were more likely to report a willingness to seek veterinary care (61.8%) compared to those without such experiences (28.9%). This suggests that firsthand experience, rather than formal knowledge, plays a major role in shaping risk perception and response behaviour.

Risky practices were widespread across the study population. An overwhelming 91.1% of owners reported assisting donkeys during foaling without any personal protective equipment, and none reported routine use of disinfectants during animal handling or within donkey shelters. These practices greatly increase the risk of zoonotic transmission, especially in the absence of formal training or veterinary oversight. About 19.9% of owners claimed their donkeys had been vaccinated against brucellosis, although no such vaccine is currently licensed or administered for donkeys in Kenya. This likely reflects a misunderstanding or confusion with other livestock vaccination programs.

Consumption of donkey products also emerged as a potential route of human infection. While not widespread, a notable proportion of respondents reported consuming donkey meat (11.2%), donkey blood (5.1%), and raw milk (2.3%). Others had consumed combinations of these products, further compounding risk. Such practices, when combined with poor hygiene and low awareness, pose a significant public health threat.

Environmental factors further exacerbated these risks. Donkeys were commonly reported to share grazing areas (84.2%), water sources (79.5%), and shelters (62.3%) with other livestock, particularly cattle, goats, and sheep. These close interspecies interactions, in the absence of routine health checks and preventive measures, create an ideal environment for the transmission of brucellosis within and between herds (Table 5).

4 Discussion

This study provides the first comprehensive One Health oriented investigation of *Brucella* spp. seroprevalence in donkeys across seven diverse Kenyan counties namely Turkana, Kitui, Narok, Nakuru, Bungoma, Nairobi, and Kiambu, integrating serological data with donkey owner Knowledge–Attitudes–

TABLE 4 Analysis of risk factors for donkey brucellosis.

Variable	Reference category	Comparison group	Univariable OR (p-value)	Multivariable OR (95% CI)	Multivariable p-value	Significant
Sex	Female	Male	1.09 (0.91)	1.30 (0.25–6.74)	0.754	No
Age group	Mature	Young (<3 yrs)	3.50 (0.136)	11.80 (1.70–81.99)	0.013	Yes
	Mature	Old (>10 yrs)	– (0.999)	–	1	No
County	Kiambu	Kitui	1.00 (1.000)	1.12 (0.00–∞)	1	No
	Kiambu	Nairobi	– (1.000)	–	1	No
	Kiambu	Nakuru	1.00 (1.000)	1.07 (0.00–∞)	1	No
	Kiambu	Narok	– (1.000)	–	1	No
	Kiambu	Turkana	– (1.000)	–	1	No
BCS	Fat	Moderate	0.45 (0.339)	0.07 (0.01–0.54)	0.011	Yes
	Fat	Thin	– (0.999)	–	1	No
	Fat	Very fat	– (1.000)	–	1	No
Movement	No	Yes	0.73 (0.672)	0.93 (0.19–4.67)	0.934	No

TABLE 5 KAP summary table for donkey brucellosis.

KAP component	Variable assessed	Key response (Yes/No/Category)	Proportion (%)	Statistical association	p-value
Knowledge	Brucellosis awareness	Yes	25.3	–	–
	Aware it is zoonotic	Yes	20.7	–	–
	Knowledge score	Very low	62.2	χ^2 = 784.0, df=8	<0.0001
	Can mention ≥1 clinical sign	Yes	54.6	–	–
	Risk perception (High/Very High)	Yes	24	χ^2 = 26.64, df=8	0.001
Attitudes	Would seek vet if signs present	Yes	61.8	χ^2 = 1.34, df=2	0.513
Practices	Assists foaling without PPE	Yes	91.1	χ^2 = 1.34, df=2	0.513
	Claims donkey vaccinated	Yes	19.9	χ^2 = 11.54, df=2	0.003
Risk behaviours	Consumed donkey meat	Yes	11.2	χ^2 = 93.11, df=14	<0.0001
	Consumed donkey milk	Yes	2.3	χ^2 = 93.11, df=14	<0.0001
	Consumed meat and milk	Yes	0.8	χ^2 = 93.11, df=14	<0.0001
	Consumed meat and blood	Yes	5.1	χ^2 = 93.11, df=14	<0.0001

Practices (KAP). Donkeys, while integral to livelihoods in pastoral, peri-urban, and rural settings, remain largely excluded from brucellosis surveillance and control efforts (28, 29).

The marked disparity in seroprevalence, 2.0% by indirect iELISA compared with 10.7% by RBPT, with almost no agreement (κ ≈ 0), highlights the well-recognised limitations of RBPT, particularly its lower specificity and potential cross-reactivity with non-*Brucella* bacteria. In contrast, iELISA has been validated as a more specific assay in equids and other livestock and is therefore recommended for epidemiological studies (28, 30). In this study, iELISA-derived seroprevalence is considered the more reliable estimate of *Brucella* seroprevalence in donkeys, while RBPT findings are best interpreted as preliminary screening. Similar test discrepancies have been reported in livestock across East Africa, further underscoring the importance of confirmatory testing. For example, in Amibara, Ethiopia, camel brucellosis prevalence was 7.6% by RBPT but only 3.2% when confirmed by the complement fixation test (31). Likewise, in Kajiado County, Kenya, iELISA outperformed RBPT in detecting bovine seropositive animals (32). These findings strengthen the case for prioritizing iELISA or other confirmatory assays when assessing brucellosis in donkeys.

Despite identifying 42 seropositive donkeys, none yielded positive PCR results for *Brucella abortus* or *B. melitensis*. Similar discrepancies between serological and molecular findings have been reported elsewhere, including studies in Ethiopia where livestock that tested seropositive by RBPT or ELISA were PCR-negative when only blood samples were analyzed, even though *Brucella* organisms were later isolated from reproductive tissues and lymph nodes of the same animals (33). Such variation between serology and PCR outcomes can be attributed to both biological and methodological factors. Serological positivity often reflects previous exposure or infection that has already been cleared bacteriologically, resulting in the persistence of antibodies despite the absence of detectable bacteria in circulation (34). In addition, during chronic or latent stages of brucellosis, bacteraemia is typically intermittent or absent, and *Brucella* organisms tend to localize in reproductive organs, lymph nodes, or bone marrow rather than in peripheral blood (35). The possibility of intermittent bacteremia, particularly in chronic infections, further reduces the likelihood of detecting *Brucella* DNA in single blood samples and may partly explain the PCR negativity observed in this study. Therefore, blood may not always be the most suitable specimen for molecular detection of *Brucella* DNA, particularly when bacterial loads are low or the infection is inactive at the time of sampling (36). Indeed, reviews have reported that brucellosis in equines (horses, donkeys, and mules) presents as abscesses in tendons, bursae, and joints, while rreproductive disorders seen in other livestock are rare occurrence (10). The limited sensitivity of conventional PCR assays, coupled with low bacterial concentrations in peripheral blood, likely contributed to the absence of detectable DNA in this study. Future investigations should therefore include alternative specimens such as reproductive tissues, milk, vaginal swabs, and lymph nodes and employ more sensitive diagnostic approaches, including real-time PCR or culture-based techniques, to improve detection and confirm active infection (33). But it is also argued that tests currently used in the diagnosis of brucellosis in other livestock have not been validated for equines (10). Globally, donkey brucellosis seroprevalence varies from 0% to 65%, with a pooled mean of approximately 10.3% (17). Our ELISA-derived prevalence aligns with reports from Nigeria (2.5–11.4%) (37, 38) but is lower than values reported in other African contexts such as Ethiopia (14.5%) (39). These differences likely reflect variation in diagnostic approaches, ecological conditions, animal movement, and national control measures (40).

Age was a significant independent predictor of seropositivity, with donkeys under three years old being over thirteen times more likely to test positive than adults (aOR = 13.21, p = 0.008). This observation, consistent with findings by Jansen et al. (41), differs from the classical pattern in many livestock species, where seroprevalence typically rises with sexual maturity and reproductive activity (42). Several explanations may account for this deviation. Younger donkeys may experience early exposure through communal grazing and shared watering points, which are well-known pathways for cross-species transmission of *Brucella* in pastoral systems (43). For example, in Nigeria, A study done to determine prevalence of equine brucellosis reported a higher seroprevalence of 9.6% for donkeys' aged 4–6 years which could explain the high rate of infection in young donkeys; this was in comparison to 6.8% for pregnant donkey's and 3.8% for non-pregnant ones (44). Therefore, young donkeys grazed within infected environments may be exposed to infections by *Brucella* organisms. Infection may also occur through ingestion of contaminated maternal milk or close nursing contact, since *Brucella* spp. can be shed in milk and persist within dairy secretions (41). Additionally, variation in maternal antibody transfer and its gradual decline can affect serological detection, as foals acquire antibodies solely through colostrum and these wane over time, complicating interpretation of seropositivity in very young animals (45). Management practices such as young donkeys accompanying their dams and other livestock to communal grazing areas may further increase their contact with infectious materials (46). Given the small number of iELISA-positive animals, the wide 95% confidence interval around the odds ratio suggests statistical instability, and the observed association should therefore be interpreted with caution. To clarify these uncertainties, larger age-stratified and longitudinal studies combining serology with culture or molecular testing of milk, reproductive tissues, and environmental samples are recommended, alongside measurements of maternal antibody dynamics to distinguish passive immunity from true infection. This pattern mirrors findings in goats in Qatar (47) but contrasts with reports in Nigerian donkeys, where older animals showed higher prevalence (38). A possible explanation is that younger animals may be more susceptible due to immature immune systems or acute infections generating stronger antibody responses, whereas older animals may have declining antibody titre following past exposure (34, 35).

Interestingly, donkeys with moderate body condition had significantly lower odds of seropositivity compared to those in good condition (aOR = 0.081, 95% CI: 0.011–0.57, p = 0.012). Although counterintuitive, similar associations have been attributed to differences in mobility, workload, and exposure opportunities (48). However, the small number of seropositive cases within these subgroups may have influenced the association, and the finding should therefore be interpreted with caution until verified in larger studies.

Geographical clustering of iELISA-positive cases in Turkana (3.96%), Narok (4.84%), and Nairobi (3.45%) suggests spatial heterogeneity in transmission risk. Such patterns have also been reported in Algeria, where pastoral and peri-urban environments with intense interspecies interactions were linked to higher prevalence (40). In our study, over 80% of donkey owners reported shared grazing and watering points with cattle, goats, and sheep; well-recognized risk factors for cross-species transmission of *Brucella* spp. (49–51). However, the relatively small and uneven sample sizes across counties particularly in Nairobi (n = 29) limit the robustness of inter-county comparisons and may exaggerate apparent clustering. Larger, more evenly distributed sampling will be needed to confirm true geographical patterns.

The KAP survey revealed critical public health gaps. Most respondents (91.1%) assisted donkeys during foaling without protective equipment, and 19.4% consumed donkey products often raw, mirroring risky practices reported in Ethiopia and northern Kenya (29, 39, 52). Awareness of brucellosis was low, with only 25.3% having heard of the disease and 20.7% recognizing its zoonotic potential, similar to patterns reported across pastoral regions in Africa and the Middle East (53, 54). Notably, 19.9% of respondents reported vaccinating donkeys against brucellosis, despite no official vaccination ever recorded for the donkeys in Kenya, suggesting confusion with cattle vaccination or misinterpretation of the question. Such findings underscore possible biases in self-reported data, including recall errors, misunderstanding of questions, and social desirability bias, which should be considered when interpreting the results.

In Kenya, brucellosis surveillance has predominantly focused on ruminants, with prevalence estimates reaching 19.5% in camels in Isiolo County (29), while donkeys remain largely excluded from national disease reporting frameworks. Yet, their close and frequent contact with humans, particularly women and youth engaged in transport and animal husbandry, suggests that donkeys could represent overlooked link in the epidemiology of brucellosis. Although equids are generally regarded as incidental hosts for *Brucella* spp., the concurrent circulation of *B. abortus* and *B. melitensis* among multiple livestock species and humans in Kenyan pastoral systems, coupled with shared grazing areas, water sources, and close interspecies interactions, makes it plausible that donkeys may function as transient carriers or "bridge" hosts facilitating spill-over between species. Direct evidence demonstrating that donkeys independently maintain *Brucella* transmission is, however, still lacking (10, 55a; 12). *Brucella* in equines has previously been reported in cattle farms in Minas Gerais, a state in Brazil where bovine brucellosis was widespread (56). This infection of donkeys could support the hypothesis of cross infection especially if the environment is contaminated birth products by the *Brucella* spp. However, other studies conducted to test this hypothesis that presence of other farm animals that commingled with herds and/or flocks are potential hosts that could maintain the disease in the farm. In this study, 10 donkeys and 2 dogs which were in close proximity to cattle herds raised in areas which was known to have endemic brucellosis for ruminants did not show evidence of cross infection between donkeys dogs and the other ruminants (57). To date, there is also no evidence of direct donkey-to-human transmission of *Brucella* spp., which underscores their likely incidental rather than reservoir role and defines the current epidemiological limits of transmission (58). Targeted research incorporating bacterial isolation and molecular typing from reproductive tissues, milk, and environmental samples is therefore needed to determine whether donkeys can sustain local transmission cycles or simply serve as occasional spill-over hosts within multi-host ecosystems.

From a diagnostic perspective, our findings highlight the limitations of relying solely on blood-based PCR in donkeys. Incorporating more sensitive real-time PCR assays, reproductive tissue sampling, or bacteriological culture could improve detection, particularly in chronic infections (35, 36).

This study reinforces the need to include donkeys in brucellosis surveillance and control programs. Targeted public health messaging, improved diagnostic strategies, and community engagement should form part of integrated One Health interventions to mitigate both animal health and zoonotic risks.

5 Conclusion

This study provides the first integrated assessment of serological, molecular, and behavioural aspects of donkey brucellosis in Kenya. We report a low seroprevalence of 2.0% by iELISA, with RBPT screening indicating a higher but less specific estimate of 10.7%. Confirmed seropositive cases were clustered in Turkana, Narok, and Nairobi. No *Brucella* DNA was detected in blood samples by PCR, likely reflecting chronic or past infections with minimal or absent bacteraemia. Younger donkeys and those in good body condition had higher odds of seropositivity though these associations should be interpreted with caution given wide confidence intervals and small subgroup sizes. Widespread high-risk practices, such as unprotected foaling assistance and consumption of raw donkey products, were documented, yet awareness of the zoonotic potential of brucellosis remained critically low.

These findings provide evidence of past exposure to *Brucella* spp. in donkeys rather than confirmed active infection. They underscore the need to include donkeys in national brucellosis surveillance systems, strengthen community education, and expand diagnostic capacity. Embedding these measures within a coordinated One Health framework will be essential to sustain progress and to reduce both the animal health burden and the risk of zoonotic transmission.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material. Further inquiries can be directed to the corresponding author.

Ethics statement

The animal studies were approved by Ethical clearance for this study was obtained from the Biosafety, Animal Use, and Ethics Committee of the Faculty of Veterinary Medicine, University of Nairobi, under reference number BAUEC/2024/549 (Appendix 9); National Commission for Science, Technology and innovation Licence No: NACOSTI/P/24/36654 (Appendix 10). Additional ethical clearance was obtained from Brooke's Animal Welfare Ethical Review Board (Appendix 11), and formal permission to carry out the study was granted by the respective County Directors of Veterinary Services and local administrative authorities in each study area. The studies were conducted in accordance with the local legislation and institutional requirements. Written informed consent was obtained from the owners for the participation of their animals in this study.

Author contributions

JK: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Visualization, Writing – original draft, Writing – review & editing. TW: Conceptualization, Methodology, Project administration, Supervision, Validation, Writing – review & editing. JO: Conceptualization, Data curation, Methodology, Project administration, Supervision, Validation, Visualization, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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