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Molecular detection of *Coxiella burnetii* in ticks collected from animals and the environment in Uganda

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Abstract

Aims: *Coxiella burnetii* is a highly infectious organism that is easily spread through aerosols causing Q fever in humans. Ticks can harbour and transmit *C. burnetii* to animals, contributing to disease maintenance. Our aim was to examine the presence of *C. burnetii* in ticks in Uganda.

Methods and Results: In this study, ticks were collected from five Ugandan districts and tested by real-time PCR for *C. burnetii* (*Coxiella* outer membrane protein 1 gene). A total of 859 tick pools (9602 individual ticks) were tested, and pool positivity for *C. burnetii* was 5.5% ($n=47$). Pooled prevalence differed by district; the highest was Luwero (7.3%), then Gulu (6.6%), and Kasese had the lowest (1.3%). However, district variation was not statistically significant (Fisher's exact = 0.07). Ticks collected from dogs and cats had the highest positivity rates [23/47, (48.9%)] followed by livestock (cattle, goats, sheep, and pigs) [18/47, (38.3%)] and vegetation [6/47, (12.8%)]. *Haemaphysalis elliptica* had the highest infection rates, followed by *Rhipicephalus appendiculatus*, *Amblyomma variegatum* and *Rhipicephalus decoloratus* had similar prevalence.

Conclusions: Although ticks are not the primary transmitters of *C. burnetii* to humans, pathogen detection in ticks can be an indirect indicator of risk among animal hosts. Vulnerable populations, including occupations with close animal contact such as farming, butchery, and veterinary practice, have an increased risk of *C. burnetii* exposure. Veterinarians and clinicians should be aware that *C. burnetii* may cause human and animal illness in these regions.

KEYWORDS
febrile illnesses, Q-fever, tick-borne diseases, Uganda, Zoonoses

1 | INTRODUCTION

Coxiella burnetii, an obligate intracellular spore-forming bacteria, causes Q fever in humans and coxiellosis in animals worldwide (Raoult & Marrie, 1995). Livestock, mainly sheep, cattle, and goats, are the primary reservoir in many countries, but companion animals including cats and dogs have been implicated as reservoirs (Duron et al., 2015; Marrie et al., 2008). Animal reservoirs of *C. burnetii* vary by country affecting the local epidemiology. Infected animals are often asymptomatic, but coxiellosis may manifest as abortions in ruminants. Clinical signs of Q fever in humans vary considerably, ranging from subclinical to acute or chronic (Bwatota et al., 2022). Common acute symptoms include fever, malaise, headache, hepatitis, and interstitial lung disease. Pregnant women are at an increased risk for abortion or preterm delivery. Chronic disease, although rare, can manifest as endocarditis, hepatitis, or osteomyelitis. Due to non-specific symptoms, clinical diagnosis is difficult and is confirmed by serology often combined with isolation of the organism and/or polymerase chain reaction (PCR). These tests are not readily available for routine healthcare in developing countries including Uganda. Early diagnosis allows for effective treatment with doxycycline.

Coxiella burnetii is highly infectious; a single aerosol particle can cause human infection (Petri, 2022). *C. burnetii* is environmentally stable, remaining viable in dust and stool for months. Transmission is airborne by inhaling contaminated aerosols or foodborne by consuming infected animal products and rarely from person to person (Eldin et al., 2017). Although ticks have been experimentally proven to transmit *C. burnetii* in the laboratory, natural tick transmission is uncertain (Duron et al., 2015). Detection of *C. burnetii* in ticks collected from animals or their grazing fields can be an indirect indicator of the presence in animals posing a risk to humans. There is an occupational risk of infection among those working in animal rearing or encountering animal yards (Duron et al., 2015). Clinical awareness of Q fever is needed among healthcare providers. Despite reports of disease detection in returning travellers from Uganda and ticks in the region, there are minimal data about Q fever in the Ugandan population and ticks in the environment. In this study, we screened ticks collected from livestock, cats, dogs, and vegetation for *C. burnetii* using real-time PCR targeting the *com*1 gene.

Impacts

- There is a lack of data about *Coxiella burnetii* in ticks in Uganda. These findings detected *C. burnetii* in four tick species.
- *Haemaphysalis elliptica* had the largest MLE values in all districts combined.
- Further monitoring of *C. burnetii* should be used as indirect indicator of risk in animal hosts given that pool positivity was highest among ticks collected from dogs and cats.

2 | MATERIALS AND METHODS

2.1 | Study location and tick collection

Ticks were collected from livestock (cattle, sheep, goats, and pigs), a chicken, companion animals (cats and dogs), and environment (grass) on farmsteads in five Ugandan districts (Gulu, Jinja, Kampala, Kasese, and Luwero) from April 2017 to September 2018 (Figure 1). Ticks were collected and preserved as previously described (Boehnke et al., 2015; Eneku, Erima, Byaruhanga, Atim, et al., 2023). The general climate of Uganda is tropical with high annual humidity. The country has an annual bimodal rainfall pattern, a short rainy season during March to May and long rainy season from September to December. Kasese district lies in the Ugandan cattle corridor and has a National game park with wild animals, with possible crossover of ticks from wild to domestic animals.

2.2 | Tick pools

Morphologic taxonomic keys were used to identify the tick species under a stereomicroscope (Walker et al., 2003). Pooling by tick species, host, date of collection, geographical location, and developmental stage, a total of 859 tick pools were generated from 9602 individual ticks. Questing ticks and those collected from animals were pooled separately even when collected from the same location. The average number of ticks per pool was 11.2 and range of 1–200 ticks per pool. Of all the pools, 523 pools (7272 ticks) were from livestock, 1 pool (single tick) from a chicken, 253 pools (2124 ticks) from companion animals, and 82 pools (205 ticks) were collected from vegetation. Tick pools were added to Eppendorf tubes with RNA later (Sigma Life Science, Darmstadt, Germany). The tick pools were fragmented into coarse particles in a biosafety cabinet using sterile disposable pestles attached to a motorized grinder (HLD-12, Ryobi, China). The coarse particles were passed through a 20-gauge needle at least five times for homogenization and then stored at −80°C.

2.3 | DNA extraction and molecular testing

Following the manufacturer's instructions, total DNA was extracted from the tick homogenates using the Qiagen DNeasy Blood and Tissue kit (Qiagen, Hilden, Germany). DNA extracts from the 859 tick pools were tested for *C. burnetii* by qPCR targeting the *Coxiella* outer membrane protein 1 (*com1a*) gene (91 base pairs) (Howe et al., 2009). The primers used were Forward (5-3); CAGCCGCTAAACAAGGAAAATATT (concentration = 0.5 μM), Reverse (5-3); GCGGTTTGAAGGGTGATTTG (concentration = 0.5 μM), and the Probe (5-3); 6FAM-TGCTTTCCAC GACGCG – MGBNFQ (concentration = 0.1 μM) utilizing the Platinum Quantitative PCR SuperMix-UDG (Thermo Fisher Scientific) PCR kit

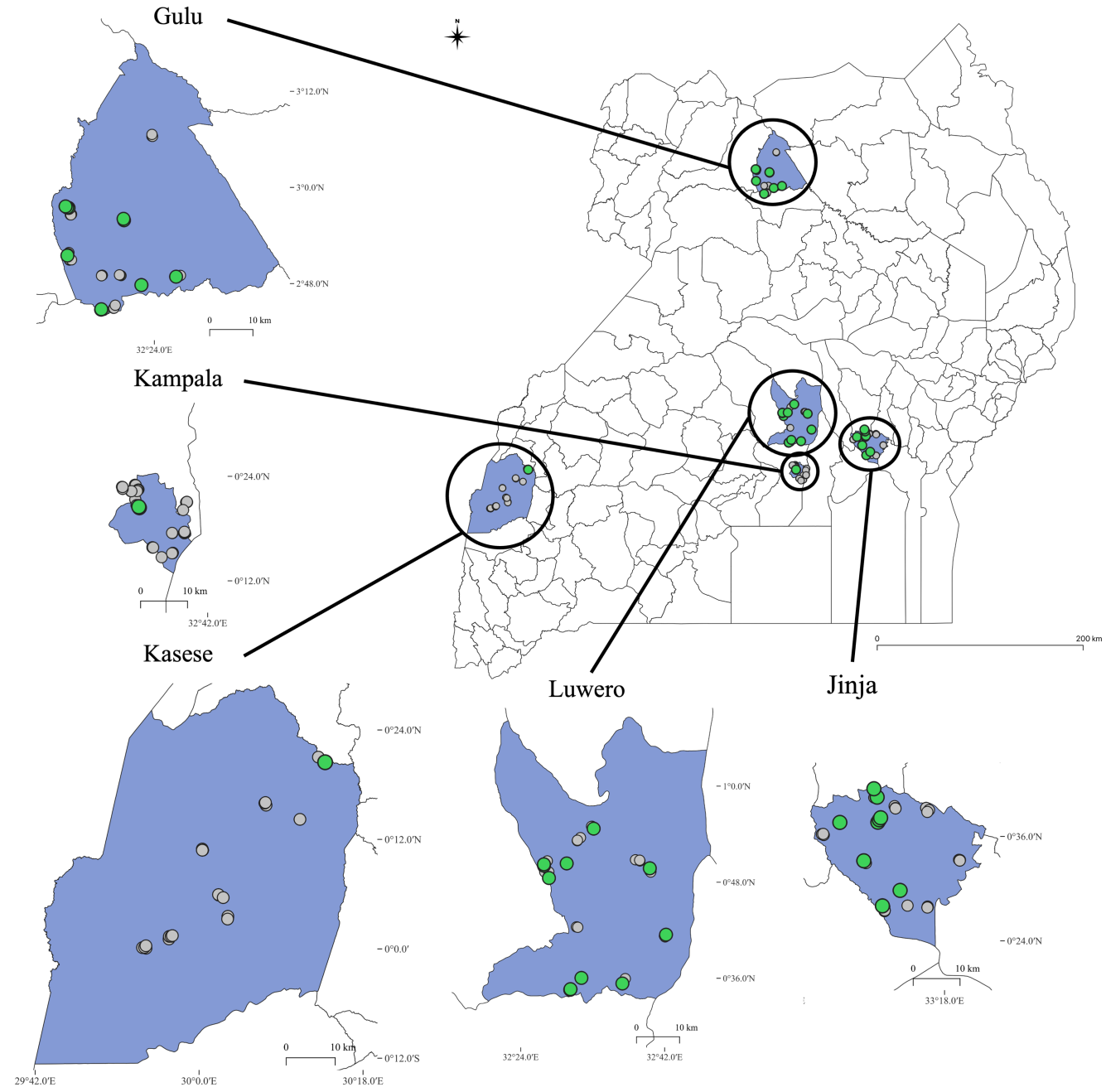

FIGURE 1 Tick collection locations by district. All circles represent sample location coordinates. Grey circles represent collection sites where no *Coxiella burnetii* was detected and green circles represent collection sites where *C. burnetii* was detected.

and a 7500 Real-Time PCR System (Applied Biosystems, US). The qPCR cycling conditions were 50°C for 2 min, 94°C for 2 min, and 45 cycles of both 94°C for 15 s and 60°C for 1 min. Two positive controls (*C. burnetii* DNA) and two negative controls (ultra-pure water) were used for every PCR run.

2.4 | Mapping

Maps illustrating the location of tick collection sites and positive detection locations were generated using QGIS 3.28 with the shapefile for districts of Uganda available at https://data.unhcr.org/en/documents/details/83043 (QGIS.org, 2022).

2.5 | Statistical analysis

The likelihood of *C. burnetii* detection from the tick pools was estimated using pool positivity rates, minimum infection rate (MIR) and maximum likelihood estimation (MLE) by district of collection and tick species. The MIR, MLE, and the corresponding 95% confidence intervals accounting for pool sizes were calculated in Excel using the

CDC's Mosquito Surveillance Software available at https://www.cdc.gov/westnile/resourcepages/mosqSurvSoft.html. Variation in tick species distribution by district was assessed using a Pearson chi-squared test, with significance set at <0.05. Data analysis was performed using STATA software, version 16.1 (StataCorp, College Station, TX).

2.6 | Ethical approval statement

This study was performed under Protocol "Acute Febrile Illness Surveillance in Uganda" approved by Makerere University School of Public Health Research and Ethics Committee (MakSPH-REC #369), Uganda National Council for Science and Technology (UNSCT # 2029) and Walter Reed Army Institute of Research (WRAIR #2327). The local government administration cleared the data collection in their areas of jurisdiction and each household head consented before arthropod vectors were collected from their animals and homesteads.

3 | RESULTS

3.1 | Geographical distribution of tick species

Out of the ticks collected from the five districts, five species were identified. The majority (>50%) of the ticks from each district belonged to the *Rhipicephalus* genus. The highest proportion of tick pools were composed of *Rhipicephalus appendiculatus*, which accounted for 34.5% of all tick pools. *Rhipicephalus decoloratus* formed the second highest tick pools, at 23.4%, while *Rhipicephalus sanguineus* was the least frequently collected tick pool, at 0.3%. *Rh. sanguineus* was only found on dogs in Gulu, Jinja, and Kampala. The other species (*Rh. appendiculatus*, *Rh. decoloratus*, *Amblyomma variegatum*, and *H. elliptica*) were collected from a variety of animals and in the environment in every district (Figure 2). Tick species did not significantly vary by district ($\chi^2 = 26.41$, $df = 20$, $p = 0.153$). One hundred thirty-one tick pools (larvae and nymphs) were not included as they had insufficient body parts for full identification.

3.2 | Pooled prevalence of *C. burnetii*

Maximum likelihood estimates (MLE) and minimum infection rates (MIR) of *C. burnetii* are presented in Table 1. Total pool positivity rate for all districts was 5.5% (47/859). By district, Luwero had the highest of 7.3%, and Kasese had the lowest with 1.4%. The highest pool positivity was among ticks from companion animals (dogs and cats) at 9.1% (95% CI 5.6, 12.6), followed by vegetation at 7.3% (95% CI 1.7, 13.0) and livestock (cattle, goats, sheep, and pigs) at 3.4% (95% CI 1.9, 5.0). *H. elliptica* had the highest positive pool rate (12.0%; 18/150), followed by *A. variegatum* (7.7%; 6/78) and the least was *Rh. decoloratus* (3.0%; 6/201). All the positive *H. elliptica* tick pools were collected from companion animals. Negative pools included

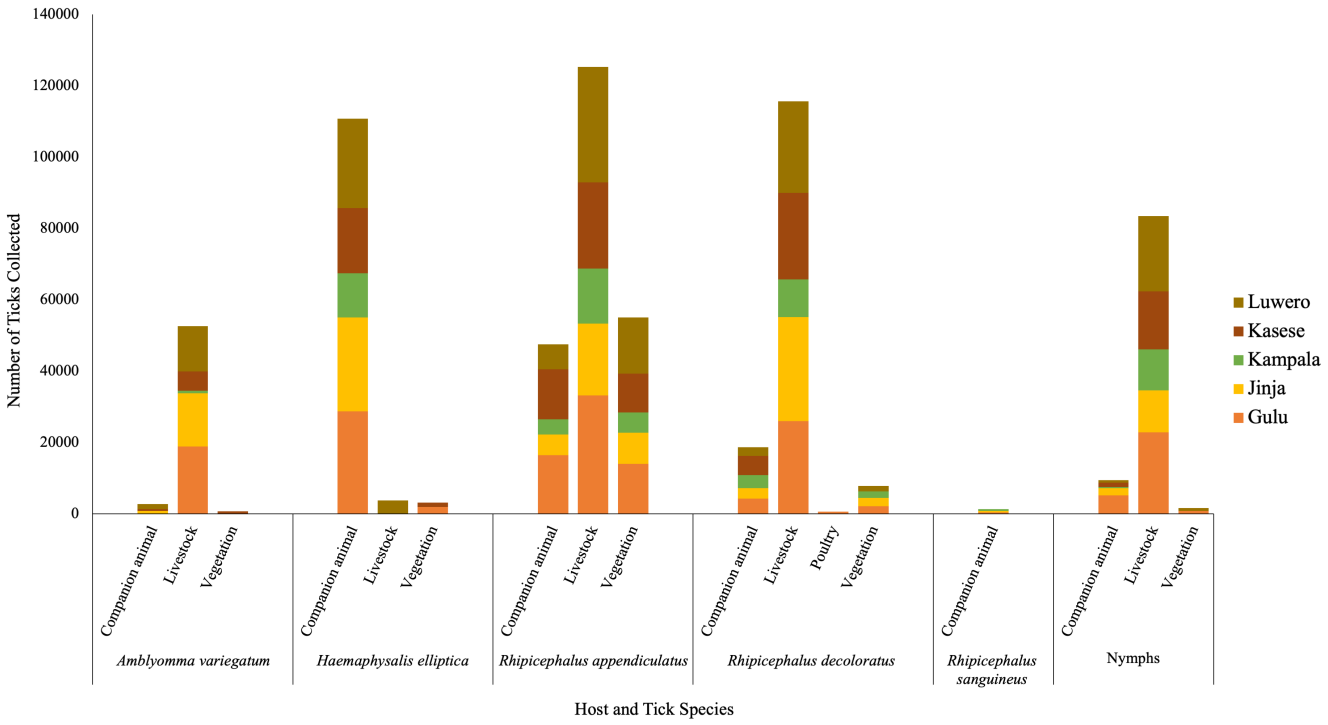

FIGURE 2 Tick distribution by host and district. Bar height represents the total number of ticks collected, and the colour corresponds to their respective district of collection. Companion animals include cats and dogs, livestock includes cattle, goats, sheep and pigs, and poultry includes one chicken. Vegetation represents ticks collected from the environment.

TABLE 1 Detection rates of *Coxiella burnetii* in tick pools including Maximum Likelihood Estimates (MLE) and Minimum Infection Rate (MIR) and corresponding 95% confidence intervals.

					MLE			MIR		
District	Tick species	Positive pools (%)		Total ticks	Point	Low	High	Point	Low	High
Gulu	*A. variegatum*	1/25	(4.0%)	178	5.44	0.33	25.85	5.62	0.00	16.60
	H. elliptica	6/41	(14.6%)	403	19.30	7.85	42.06	14.89	3.06	26.71
	Rh. appendiculatus	6/78	(7.7%)	618	9.87	4.15	20.12	9.71	1.98	17.44
	Rh. decoloratus	1/43	(2.3%)	428	2.27	0.14	10.85	2.34	0.00	6.91
	Rh. sanguineus	0/1	(0%)	2	0.00	0.00	545.52	0.00	–	–
	Nymphs	1/38	(2.6%)	226	4.27	0.26	20.21	4.42	0.00	13.08
	Total	15/226	(6.6%)	1855	8.58	5.04	13.77	8.09	4.01	12.16
Kasese	*A. variegatum*	1/10	(10.0%)	121	8.63	0.51	45.88	8.26	0.00	24.40
	H. elliptica	1/21	(4.8%)	351	2.83	0.17	14.08	2.85	0.00	8.42
	Rh. appendiculatus	0/57	(0%)	675	0.00	0.00	5.21	0.00	–	–
	Rh. decoloratus	0/37	(0%)	685	0.00	0.00	5.07	0.00	–	–
	Nymphs	0/21	(0%)	185	0.00	0.00	17.38	0.00	–	–
	Total	2/146	(1.4%)	2017	1.00	0.18	3.30	0.99	0.00	2.37
Kampala	*A. variegatum*	0/2	(0%)	23	0.00	0.00	88.94	0.00	–	–
	H. elliptica	0/16	(0%)	46	0.00	0.00	68.32	0.00	–	–
	Rh. appendiculatus	1/38	(2.6%)	225	4.52	0.26	22.20	4.44	0.00	13.14
	Rh. decoloratus	2/31	(6.5%)	446	4.59	0.85	15.15	4.48	0.00	10.69
	Rh. sanguineus	0/1	(0%)	1	0.00	0.00	793.45	0.00	–	–
	Nymphs	1/19	(5.3%)	64	16.28	0.92	79.98	15.63	0.00	46.01
	Total	4/107	(3.7%)	805	5.18	1.70	12.44	4.97	0.11	9.83
Jinja	*A. variegatum*	2/20	(10.0%)	64	31.74	5.87	100.50	31.25	0.00	73.88
	H. elliptica	3/32	(9.4%)	335	10.05	2.68	27.97	8.96	0.00	19.04
	Rh. appendiculatus	3/44	(6.8%)	332	8.94	2.47	23.55	9.04	0.00	19.22
	Rh. decoloratus	2/45	(4.4%)	414	4.86	0.88	15.84	4.83	0.00	11.51
	Rh. sanguineus	0/1	(0%)	1	0.00	0.00	793.45	0.00	–	–
	Nymphs	0/18	(0%)	111	0.00	0.00	28.27	0.00	–	–
	Total	10/160	(6.3%)	1257	8.36	4.31	14.80	7.96	3.04	12.87
Luwero	*A. variegatum*	2/21	(9.5%)	88	23.30	4.38	74.24	22.73	0.00	53.87
	H. elliptica	8/40	(20.0%)	584	17.00	8.07	32.87	13.70	4.27	23.13
	Rh. appendiculatus	3/79	(3.8%)	1585	1.85	0.51	4.89	1.89	0.00	4.03
	Rh. decoloratus	1/45	(2.2%)	1117	0.87	0.05	4.16	0.90	0.00	2.65
	Nymphs	2/35	(5.7%)	294	6.74	1.25	21.68	6.80	0.00	16.20
	Total G	16/220	(7.3%)	3668	4.49	2.74	7.00	4.36	2.23	6.49
	Overall total	47/859	(5.5%)	9602	5.10	3.83	6.68	4.89	3.50	6.29

three *Rh. sanguineus* pools from dogs and one *Rh. decoloratus* tick pool from one chicken. The MLE for *C. burnetii* varied by district with the highest in Gulu of 8.6% (95% CI 5.0, 13.8) with a corresponding MIR of 8.1% (95% CI 4.0, 12.2). Kasese had the lowest MLE of 1.0% (95% CI 0.2, 3.3) with a MIR of 1.0% (95% CI 0.1, 2.4). The genera of the *C. burnetii*-positive tick pools were *Haemaphysalis* (12.0%), *Amblyomma* (7.7%), and *Rhipicephalus* (3.8%). The largest MLE and MIR was among *H. elliptica* while for other tick species MLE varied by their collection district (Table 1).

4 | DISCUSSION

Q fever, a zoonotic disease, affects public health and livestock productivity. This is the first detection of *C. burnetii* in ticks in Uganda in the past three decades. A pool positivity rate (PPR) of 5.5% for *C. burnetii* was detected in this study, comparable to studies in Kenya and Ethiopia that demonstrated 5.5% and 6.4% *C. burnetii* prevalence, respectively, in tick pools gathered in pastoral communities from livestock including sheep, goats, and cattle (Koka et al., 2018; Kumsa et al., 2015). Our

study setting was similar to Ethiopia with both pastoral and mixed crop-livestock production where livestock are open grazed or tethered in pastures with companion animals, particularly dogs often accompanying herders to grazing areas. The PPR in our study was slightly higher than the 2.5% in western Kenya, near the eastern Ugandan border where animals are not grazed (Knobel et al., 2013).

Haemaphysalis elliptica had the highest pool positivity rate in our study collected from dogs and cats. In contrast to several studies that suggest *Rhipicephalus evertsi* and other *Rhipicephalus* ticks are the most widespread and infected tick in the region (Koka et al., 2018; Walker et al., 2003). Dogs and cats have been confirmed reservoirs of *C. burnetii* linked to human Q fever cases (Marrie et al., 2008). The detection of *C. burnetii* with higher frequency in *H. elliptica* from dogs and cats in this study could suggest these companion animals are potential reservoirs of Q fever. We recently reported a 7.6% seroprevalence rate to Q fever in febrile patients who visited acute febrile illness surveillance hospitals in the same districts where the ticks were collected during the same period (Eneku, Erima, Byaruhanga, Cleary, et al., 2023). It is likely that Q fever patients were exposed to infected animal excretions/secretions or infected through contaminated dust from animal yards, although this was not confirmed. *C. burnetii* is present in two antigenic phase variations, Phase I and II. Phase I is the variant with smooth surface and most pathogenic form; found in infected animals or in nature. Although ticks are not potent vectors of *C. burnetii* to humans, ticks and animal hosts are natural hosts for Phase 1, the most virulent form of *C. burnetii* (Raoult & Marrie, 1995). *C. burnetii* has been detected in *A. variegatum* and *Rhipicephalus* spp. in the East African region, which could explain the higher pool positivity in *A. variegatum* (7.7%) and *Rh. appendiculatus* (4.4%) (Knobel et al., 2013; Koka et al., 2018; Kumsa et al., 2015). This study confirms that *C. burnetii* is found in areas with animals and in tick species that are known to feed on livestock and ruminants, threatening the livelihood of over half of Ugandans who rely on animals (Bwatota et al., 2022; Byaruhanga et al., 2015; Waiswa et al., 2021). Of the five districts included, *C. burnetii* was detected in ticks from all districts. However, certain areas are likely at higher risk for *C. burnetii* including Gulu and Jinja which both had higher MLEs and Luwero which had the highest total positivity from pools. Surprisingly, from our seroprevalence study, the highest positivity rates (12.5%) were observed in Bwera Hospital, Kasese district, which in this study had lowest pool positivity rate (1.4%) in ticks (Eneku, Erima, Byaruhanga, Cleary, et al., 2023). This could be explained by the predominant animal-owning population as pastoral communities in Kasese district compared to the other study sites and sick patients would likely seek care from the nearest hospital.

5 | CONCLUSIONS

This study used real-time PCR for *C. burnetii* detection across a wide variety of ecosystems in Uganda. The widespread detection of *C. burnetii* signifies the importance of continuous monitoring and providing rapid, accurate information to veterinarians and public health officials. Veterinarians should be informed of *C. burnetii* as a potential cause of abortions in ruminants while clinicians must be aware of the occupational risk of patients with febrile illnesses in Uganda.

AUTHOR CONTRIBUTIONS

DKB, JWK, HK, EM, RT, and WE: Conceived and designed the study. WE, BE, AMB, GA, TT, QAU, and NGC: performed the experiments. WE, NGC, MEvF, and DKB: analysed the data. All the authors participated in drafting and writing the manuscript.

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CONFLICT OF INTEREST STATEMENT

The authors have declared that no competing interests exist.

DATA AVAILABILITY STATEMENT

The data used to support the findings of this study are available from the corresponding author upon request.

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