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Ticks and tick-borne bacterial pathogens found on hard ticks (Acari: Ixodidae) on cattle in the Central River region of The Gambia

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Abstract

Ticks are significant vectors of pathogens affecting both animals and humans, with the climate and environment of Sub-Saharan Africa providing ideal conditions for their growth. However, there are limited data on ticks and tick-borne pathogens (T&TBPs) in cattle in The Gambia. This study aimed to identify tick species on cattle and conduct molecular screening for T&TBPs. A total of 92 ticks were collected from 306 indigenous cattle. Ticks were first identified morphologically using taxonomic keys and then confirmed molecularly through DNA sequencing. DNA was extracted from the right fourth leg of six representative ticks for species confirmation, while 77 whole adult ticks were used for screening T&TBPs. Screening polymerase chain reaction (PCR) assays targeted *Anaplasma marginale msp1β* gene, *Ehrlichia* spp. *dsb* gene and hemotropic *Mycoplasma* spp. 16S rRNA gene. *Ehrlichia*-positive samples underwent additional assays targeting the *sodB*, 16S rRNA and *groEL* genes, followed by Sanger sequencing and phylogenetic analyses. A total of 92 (53 M, 37 F and two nymphs) ticks were collected from 30/306 (9.8%; 95% confidence interval [CI]: 5.6%–12.2%) cattle. Adult ticks were identified as *Hyalomma marginatum* (73/92; 79.3%; 45 M and 28 F), *Amblyomma variegatum* (8/92;

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8.7%; 8 M), *Hyalomma rufipes* (4/92; 4.3%; 4 F) and *Rhipicephalus evertsi* (1/92; 1.1%; one F). The 16S rRNA sequences of six (four engorged female and two nymphs) ticks showed 98.6–100% identity with reference sequences from *Rhipicephalus geigyi*. Twelve out of 77 (15.6%) ticks tested positive for at least one TBP. Eight *H. marginatum* (six M and two F) (10.4%) were positive for *Ehrlichia* spp. *dsb* gene, three *H. marginatum* (two M and one F) (3.9%) for *A. marginale* and two (one *H. marginatum* F and one *A. variegatum* M) (2.6%) for hemotropic *Mycoplasma* spp. All *Ehrlichia*-positive samples showed 100% detection for the 16S rRNA gene and 62.5% for the *sodB* gene. BLASTn analysis revealed 99.3%–99.7% identity with *Ehrlichia* sp. from Brazil and 98.2%–99.3% identity with *E. minasensis* from Panama and Pakistan. Phylogenetic analysis grouped the sequences from this study with *Ehrlichia* spp. and *E. minasensis* from ticks in the Czech Republic and Brazil. This study identified various tick species and pathogens in cattle from The Gambia, including the first report of *E. minasensis*, *A. marginale* and hemotropic *Mycoplasma* spp. in the country. These findings highlight the importance of ongoing surveillance and research on tick-borne diseases in the region.

KEYWORDS

Amblyomma variegatum, *Anaplasma marginale*, *Ehrlichia minasensis*, hemoplasmas, *Hyalomma marginatum*, molecular screening, The Gambia, ticks

INTRODUCTION

Ticks are parasitic arachnids belonging to the phylum Arthropoda and class Arachnida. They feed exclusively on the blood of vertebrates, including mammals, birds, reptiles and amphibians (Guglielmone et al., 2010). There are currently three recognised tick families: Ixodidae (hard ticks), Argasidae (soft ticks) (Guglielmone et al., 2010) and Nuttalliellidae, which contains only a single species, *Nuttalliella namaqua* (Latif et al., 2012). Ticks are a major global threat to human and animal health, primarily due to their role as vectors and reservoirs for numerous protozoan, bacterial and viral pathogens (Madison-Antenucci et al., 2020). These pathogens can cause a spectrum of illnesses, from mild to life-threatening, affecting humans, domestic animals and wildlife (Anderson & Magnarelli, 2008; Swanson et al., 2006). Additionally, ticks significantly impact cattle production by transmitting various pathogens, leading to an estimated annual economic loss of USD 13.9–18.7 billion worldwide (Hurtado & Giraldo-Ríos, 2018; Jongejan & Uilenberg, 2004). With a broad host range, ticks' preference for hosts varies by species and geographical region, with cattle being a predominant host for many tick species, affecting around 80% of the global cattle population and resulting in significant health and economic burdens (Hurtado & Giraldo-Ríos, 2018; Moyo & Masika, 2009).

Tick-borne pathogens (TBPs) are currently among the most critical emerging infectious diseases worldwide, due to their significant impact on animal health, economic losses and their potential for zoonotic transmission. In Africa, ticks are a major barrier to livestock industry growth (Walker et al., 2003), playing a key role in the persistence and transmission of TBPs (Jafar Bekloo et al., 2018). Tick-borne diseases (TBDs) severely hinder the development of the livestock sector, imposing considerable challenges on livestock health and management in tropical and subtropical regions globally (Jongejan & Uilenberg, 2004).

The genera *Anaplasma* and *Ehrlichia* consist of obligate intracellular Gram-negative bacteria that reside in membrane-bound structures or vacuoles within the host cell cytoplasm (Dumler et al., 2001). These bacteria are transmitted to various animals, including humans, by multiple tick species (Battilani et al., 2017), resulting in considerable economic losses in livestock and posing significant public health risks (Kivaria, 2006). *Anaplasma* species that infect domestic ruminants, such as cattle, include *Anaplasma marginale*, *A. centrale*, *A. ovis*, *A. bovis*, *A. phagocytophilum* and the recently identified *A. platys* (Dahmani et al., 2015; Park et al., 2018). Within the genus *Ehrlichia*, *Ehrlichia minasensis* and *E. ruminantium* are notable, with the latter being the only species known to naturally infect cattle, especially in Africa (Cabezas-Cruz et al., 2019).

Hemotropic *Mycoplasma* species, or hemoplasmas, are small, pleomorphic bacteria without a cell wall that parasitise the surface of red blood cells (RBCs) (Messick, 2004). In cattle, two hemoplasma species have been identified so far: *Mycoplasma wenyonii* and '*Candidatus* Mycoplasma haematobovis' (formerly *Candidatus* Mycoplasma haemobos) (Oren et al., 2020). Although the transmission routes of bovine hemoplasmas are not yet fully understood, ticks are considered potential vectors (Shi et al., 2019).

Understanding the diversity of tick species, their infection rates and the genetic variability of circulating pathogens is essential for understanding the epidemiology and control of TBDs. In Africa, TBDs are especially challenging for smallholder farmers (Jongejan & Uilenberg, 2004; Young et al., 1988). In The Gambia, equatorial climate conditions and the environment support livestock production

but also sustain high tick populations, which intensify TBD transmission. Unfortunately, available data on veterinary relevant TBPs in The Gambia are limited mainly to *E. ruminantium* (Faburay et al., 2007; Faburay et al., 2008). Therefore, gathering data on tick species and TBPs present in the country is crucial to enhance our understanding of TBD epidemiology and assess disease risk. This study aims to identify tick species infesting cattle and to screen and characterise *A. marginale*, *Ehrlichia* spp. and hemotropic *Mycoplasma* spp. in ticks collected from The Gambia.

MATERIALS AND METHODS

Study area and tick collection

The Gambia is bordered by Senegal to the east, north and south, with the Atlantic Ocean to the west. Located on Africa's West Coast, it is the smallest nation in sub-Saharan Africa (Figure 1). The country features a tropical savannah climate, characterised by a long dry season from October to June and a short rainy season from July to September (Pinchbeck et al., 2008), with annual rainfall ranging from 850 to 1200 mm. In the Central River region, temperatures fluctuate significantly, reaching a low of 15°C in January and a peak of 45°C in April, reflecting pronounced seasonal variations. A cross-sectional study was carried out between December 2018 and March 2022 in the Central River region in The Gambia (Figure 1). The study took place within the country's traditional extensive pastoral system, where cattle are raised. A non-probabilistic convenience sampling method was employed to examine 306 indigenous N'Dama cattle for tick infestation. Only animals for which sampling permission was granted were included. Sampling was conducted across 32 herds from eight communities, with four herds selected from each community. While herd sizes varied, approximately 5–15 animals per herd were examined. Each animal underwent a systematic visual inspection, starting from the head and neck and moving systematically to the tail, perianal area, forelegs, axillary region, ventral body, hindlegs, inguinal area and external ears (Duell et al., 2013). Ticks found were carefully removed with tweezers, and ticks from each animal were placed in separate, labelled tubes containing 70% ethanol. Identification of ticks was performed using morphological taxonomic keys (Apanaskevich & Horak, 2008; Apanaskevich & Horak, 2009; Walker, 2003; Walker et al., 2000). Due to challenges in morphological identification, six ticks (four engorged females and two nymphs) were selected for molecular characterisation. Thereafter, tick DNA samples were subjected to a PCR targeting the mitochondrial 16S rRNA gene of ticks (Fukunaga et al., 2001).

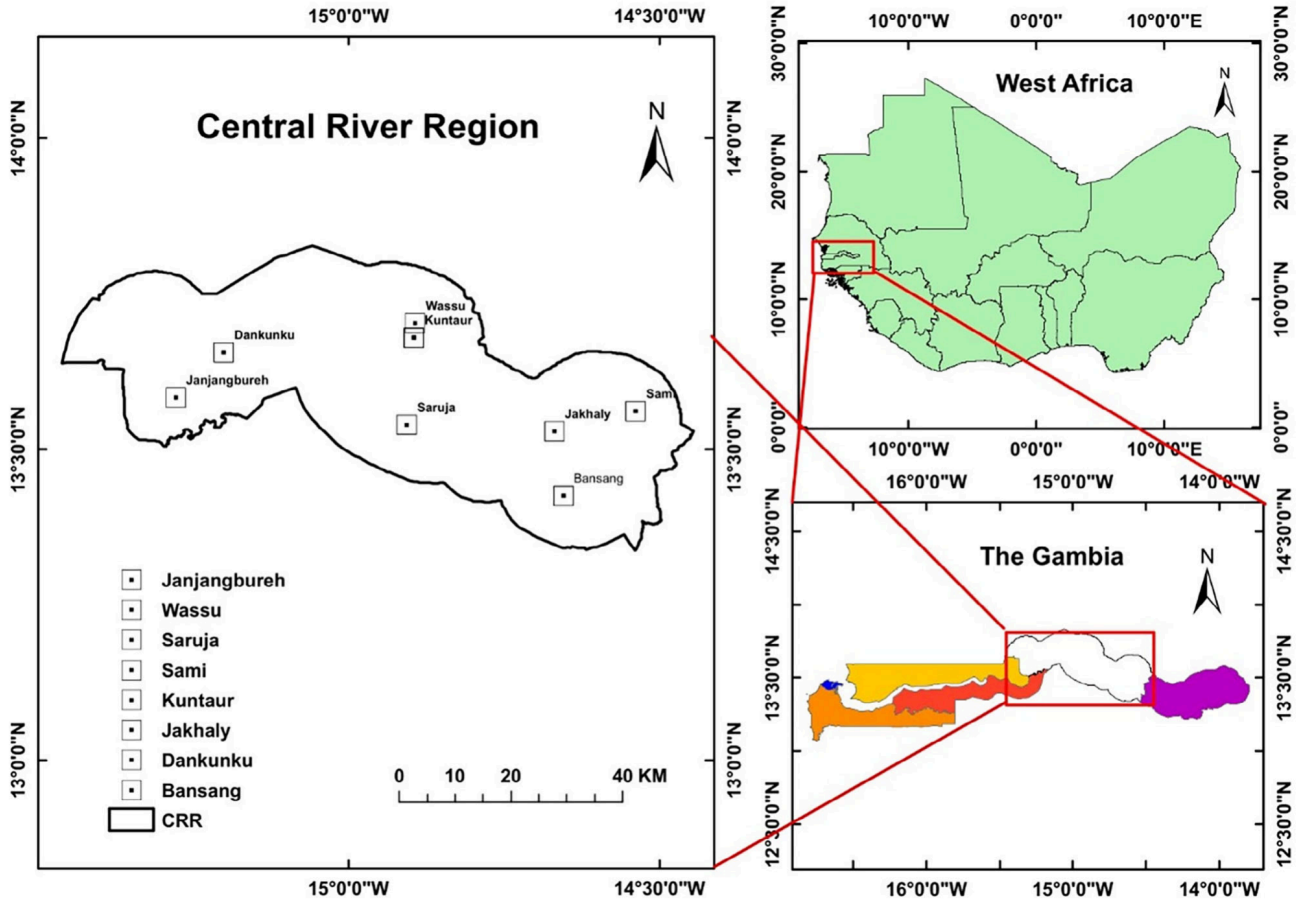

FIGURE 1 Map of The Gambia showing the sampling sites. The figure was generated and modified using QGIS software version 3.26.0.

DNA extraction and molecular assays for TBPs

Ticks were removed from the 70% ethanol, air-dried and rinsed twice in distilled water before drying on filter paper. Genomic DNA was extracted from whole ticks using a commercial kit (MagaZorb® DNA Mini-Prep Kit, Promega, Madison, Wisconsin, USA) per the manufacturer's protocol. Out of the 92 ticks collected, 77 were selected for individual DNA extraction for pathogen screening based on their size, condition and representation of the tick population.

To verify DNA extraction, tick DNA samples were analysed via conventional PCR targeting the mitochondrial 16S rRNA gene (Fukunaga et al., 2001), with positive (known tick isolate) and negative (ultrapure nuclease-free water) controls included in each reaction. DNA samples that tested positive were further screened with a nested-PCR (nPCR) assay targeting a 349 bp fragment of the *Ehrlichia* spp. *dsb* gene (Almeida et al., 2013). To evaluate genetic diversity, PCR-positive samples were tested with additional assays targeting the Anaplasmataceae 16S rRNA (835 bp), *Ehrlichia groEL* (445 bp) and *sodB* (445 bp) genes (Kim et al., 2017; Qurollo et al., 2013). *Ehrlichia canis* DNA (Vieira et al., 2013) used as a positive control in all reactions.

Tick DNA samples were also screened by real-time qPCR for *A. marginale* targeting the *msp1β* gene (Carelli et al., 2007), with goat samples infected by *A. marginale* (da Silva et al., 2018) as positive controls and nuclease-free water (Promega) as a negative control. Additionally, SYBR green-based qPCR targeting the 16S rRNA gene of hemoplasmas was used, as previously described (Willi et al., 2009).

Amplicons were separated by electrophoresis within 1.5% agarose gels for 50 min at 100 V, followed by SYBR safe staining (6 μg/mL; SYBR® Safe DNA Gel Stain, Invitrogen, CA, USA). The resulting agarose gels were then exposed under ultraviolet light using Image Lab Software (Thermo Fisher Scientific® Waltham, Massachusetts, USA).

DNA sequencing, BLASTn and phylogenetic analyses

Amplicons from four *Ehrlichia*-positive *dsb* gene samples were purified using a commercial kit (Promega Wizard® PCR and Gel Clean-Up, Promega) and sequenced bidirectionally with the same PCR primers (forward and reverse) through Sanger sequencing (Sanger et al., 1977). Sequence quality was initially assessed using Geneious 11.1.3 software, and further analysis was performed with BLASTn (National Center for Biotechnology Information, Bethesda, MD, USA). Partial nucleotide sequences of the *Ehrlichia dsb* gene obtained in this study have been deposited in GenBank® under accession numbers PQ280989, PQ280990, PQ280991 and PQ280992. Additionally, partial sequences of the mitochondrial 16S rRNA gene from six ticks were deposited in GenBank® with accession numbers PQ288673, PQ288674, PQ288675, PQ288676, PQ288677 and PQ288678.

In this study, a Maximum Likelihood tree was constructed using sequences from this study along with relevant GenBank® sequences (http://www.ncbi.nlm.nih.gov/genbank). Consensus sequences were generated with Geneious 11.1.3, followed by multiple sequence alignment in MAFFT (https://mafft.cbrc.jp/alignment/server/). The optimal model for nucleotide substitution was identified with jModeltest v.2.1.10 (Darriba et al., 2012) and set to GTR + I + G. Finally, the phylogenetic tree was visualised with FigTree version 1.4.4.

Data analysis

Data analyses were conducted using SPSS® Statistics software (IBM Corp, Armonk, NY, USA, version 26). For each variable, 95% confidence intervals (CIs) and P-values were calculated. The CI provide an upper and lower range within which the actual values are expected to fall with 95% certainty, reflecting the uncertainty in our prevalence. Data compilation and analysis were also carried out in Epi Info™ version 7.2.3.1 (Centers for Disease Control and Prevention, CDC, USA).

RESULTS

Tick species identification

A total of 92 (53 M, 37 F and two nymphs) ticks were collected from 30/306 (9.8%; 95% CI: 5.6%–12.2%) cattle. Ticks were identified as *Hyalomma marginatum* (73/92; 79.3%; 45 M and 28 F), *Amblyomma variegatum* (8/92; 8.7%; 8 M), *Hyalomma rufipes* (4/92; 4.3%; 4 F) and *Rhipicephalus evertsi* (1/92; 1.1%; 1 F). The 16S rRNA gene sequences of all 6 ticks (4 engorged female and 2 nymphs) obtained in this study showed identities ranging from 98.6% to 100% with *Rhipicephalus geigyi* from Mali and Ghana (GenBank® KF569942, OQ337991, respectively).

Pathogens detected in the ticks and infection rates

A total of 77 randomly selected ticks were analysed. PCR targeting the mitochondrial 16S rRNA gene successfully amplified DNA from all tick samples. Twelve out of 77 (15.6%, 95% CI: 8.3%–25.6%) tested positive for at least one pathogen. Specifically, eight (*H. marginatum* six M and two F) ticks (10.4%, 95% CI: 4.6%–19.45%) tested positive for the *Ehrlichia* spp. *dsb* gene. Subsequent testing of these *Ehrlichia*-positive samples revealed that all eight samples (100%) also tested positive for the 16S rRNA-PCR assay, while five/eight (62.5%, 95% CI: 24.5%–91.5%) tested positive for the *sodB*-PCR assay. Notably, all eight *dsb-Ehrlichia*-positive samples tested negative for the *groEL* gene.

Three out of 77 (3.9%; 95% CI: 0.8%–10.9%) ticks (*H. marginatum*, two M and one F) tested positive for *A. marginale*, while two/77 (2.6%; 95% CI: 0.3%–9.1%) (one *H. marginatum* F and one *A. variegatum* M) tested positive for hemotropic *Mycoplasma* spp. One *H. marginatum* tick was co-infected with *Ehrlichia* sp. and *A. marginale* (Table 1).

TABLE 1 Percentage of BLASTn-associated identity of sequences of *Ehrlichia* spp. detected in ticks collected in this study.

Agents	Sample ID	Target gene	Query cover	E-value	Identity	GenBank® accession number
Ehrlichia sp.	*Hyalomma_marginatum2*	*dsb*	100%	2×10^{-158}	100%	*E. minasensis* collected from *Rhipicephalus microplus* from Czech Republic (JX629808)
Ehrlichia sp.	*Hyalomma_marginatum3*	*dsb*	100%	2×10^{-159}	100%	*Uncultured Ehrlichia* sp., collected from *Rhipicephalus microplus* from Brazil (MT135769)
Ehrlichia sp.	*Hyalomma_marginatum4*	*dsb*	100%	2×10^{-142}	100%	*Ehrlichia* sp., collected from cattle from Brazil (KF621012)
Ehrlichia sp.	*Hyalomma_marginatum5*	*dsb*	100%	9×10^{-121}	100%	*E. minasensis* collected from *Bradypus variegatus* from Brazil (MT212416)

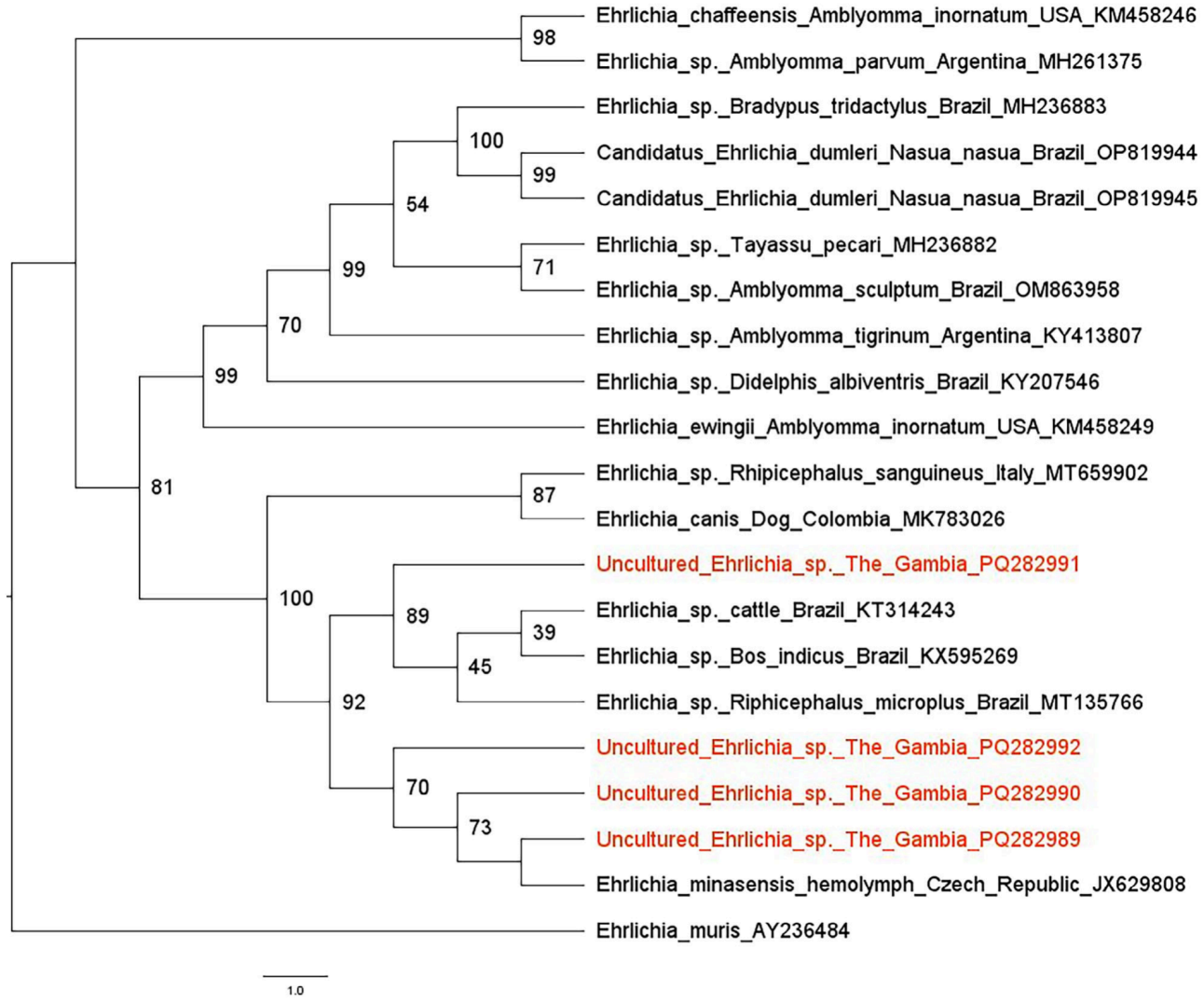

FIGURE 2 A phylogenetic tree was constructed using Maximum Likelihood inference and the GTR + I + G evolutionary model, based on a 390 bp alignment of the *dsb* gene. The sequences identified in this study are highlighted in red. The numbers at the nodes represent posterior probability values greater than 50%, obtained from 1000 bootstrap replicates. *Ehrlichia muris* was used as the outgroup.

BLASTn and phylogenetic analyses

The partial *Ehrlichia dsb* gene sequences obtained from four ticks are shown in Table 1. Multiple attempts to sequence *Ehrlichia* 16S rRNA and *sodB* gene failed due to the occurrence of faint and unspecific bands in the agarose gel electrophoresis. Additionally, sequencing for *Anaplasma* and hemoplasma was not pursued, as high Cq values from qPCR indicated low DNA concentrations that could compromise

sequencing reliability. The phylogenetic analysis based on the *dsb* gene (390 bp alignment) was performed with sequences detected in the present study and selected sequences of *Ehrlichia* sp. obtained from the GenBank® database. The obtained sequences detected in adult specimens of *H. marginatum* herein have shown to be phylogenetically grouped with *Ehrlichia* spp. (GenBank® KT314243, MT135766, KX595269) and *E. minasensis* (GenBank® JX629808) sequences detected in ticks from the Czech Republic and Brazil (Figure 2).

DISCUSSION

Data on the distribution of TBPs are essential for developing effective control strategies and advancing comprehensive surveillance efforts (Gondard et al., 2017). In The Gambia, research on T&TBPs of veterinary and public health significance has been limited (Faburay et al., 2007; Faburay et al., 2008). This study utilised PCR/qPCR assays to survey *Ehrlichia*, *A. marginale* and hemoplasmas in ticks parasitising cattle in The Gambia.

Morphological analysis of the collected ticks revealed that cattle were primarily infested with ticks from the genera *Hyalomma*, *Amblyomma* and *Rhipicephalus*. The majority of infestations were caused by *H. marginatum* (79.3%) and *A. variegatum* (8.7%). These species have been previously reported in other African countries, such as Kenya (Peter et al., 2021), Tanzania (Swai et al., 2008), Uganda (Byaruhanga et al., 2015), Ivory Coast (Boka et al., 2017) and Somalia (Collere et al., 2024; Ferrari et al., 2022). In The Gambia, previous studies have documented the presence of *A. variegatum*, *R. senegalensis*, *Rhipicephalus* spp., *R. decoloratus*, *Hyalomma* sp., *H. truncatum* and *H. rufipes* (Faburay et al., 2007; Mattioli et al., 1997, 1998). However, our study is the first to report *R. evertsi* in The Gambia. This tick species is also present in West Africa, where it was likely introduced through the movement of domestic livestock from East Africa. *R. evertsi* ticks are of significant veterinary importance as a vector of *Anaplasma* spp. and *Babesia* spp. (Abdela et al., 2018), highlighting the need for further research and ongoing surveillance to better understand the country's tick diversity and associated pathogens. These tick species' ability to spread pathogens might increase the danger of TBP transmission and raise disease incidence in both livestock and human populations due to their vectorial capabilities. Interestingly, some of the tick-borne bacteria identified in this study were detected in tick species that are not recognised as their biological vectors. However, it is crucial to note that the presence of TBP DNA in certain tick species does not always indicate that they are capable of spreading the agents.

In this study, ticks were primarily identified based on morphological characteristics. Specimens identified as *R. evertsi* were classified as *R. evertsi evertsi*, distinguished from *R. e. mimeticus* by the presence of uniformly orange-coloured legs, whereas *R. e. mimeticus* typically exhibits yellow rings on the legs, as outlined in Walker et al. (2003). While we acknowledge the morphological similarities between these subspecies, this diagnostic trait enabled confident differentiation. For other tick species, including *Hyalomma* nymphs, which are known to be challenging to identify, specimens were examined using high-resolution microscopy and established taxonomic keys to assess key morphological features. We recognise the inherent limitations of morphological identification, particularly for immature stages, but the process was conducted by experienced personnel, ensuring reliable classification. Future studies integrating molecular tools are recommended to complement and confirm these morphological findings.

The molecular analysis of the 16S rRNA gene from the collected ticks revealed identities ranging from 98.6% to 100% with *R. geigyi* reported in Mali and Ghana (GenBank: KF569942, OQ337991, respectively). This species has previously been documented in West African countries, including Nigeria (Kamani et al., 2017) and Mali (McCoy et al., 2014). *Rhipicephalus geigyi* is genetically related to *R. decoloratus* and *R. annulatus* and shares overlapping distribution patterns (McCoy et al., 2014). It is primarily restricted to the warmer, more humid regions of West Africa (Estrada-Peña et al., 2006).

The prevalence of the *Ehrlichia* spp. *dsb* gene in ticks found herein (10.4%) differs from that reported in other regions, such as ticks collected from cattle in South Africa (2.1%) (Iweriebor et al., 2017) and Ethiopia (4.1%) (Tufa et al., 2021). Notably, our findings are comparable to studies conducted in ticks collected from cattle in Kenya (8.5%) (Peter et al., 2021) and Brazil (9.24%) (Santana et al., 2022). It is essential to highlight that a study in Angola did not detect *Ehrlichia* spp. in ticks collected from cattle (Barradas et al., 2021). While our study detected *E. minasensis* and *Ehrlichia* sp. in ticks collected from cattle, *E. chaffeensis*, *E. canis*, *E. muris* and *Ehrlichia* spp. UFMG-EV and *Ehrlichia* spp. UFMT were identified in ticks collected from cattle in South Africa (Iweriebor et al., 2017). *E. minasensis* and *E. ruminantium* were identified in ticks collected from cattle in Ethiopia (Tufa et al., 2021), and *E. canis*, *E. ruminantium* and *Ehrlichia* spp. were reported in Kenya (Peter et al., 2021). The relatively high prevalence observed in our study may be influenced by several ecological and environmental factors that vary across regions. Differences in host community composition, particularly the presence and density of reservoir-competent wildlife, may significantly influence pathogen transmission dynamics. Climatic factors, such as higher humidity and milder seasonal variations, could favour tick survival and enhance host-seeking behaviour, thereby increasing opportunities for pathogen acquisition. Additionally, variations in land cover, such as fragmented landscapes and forest edge habitats, may increase host–tick encounter rates. These variations highlight the complexity and diversity of TBPs across different geographical regions and warrant further investigation into their prevalence and impact on livestock health.

In The Gambia, *A. marginale* has been documented in only one study, which reported a low prevalence (3.2%) in N'Dama cattle using blood smear microscopy, a diagnostic method with low sensitivity (Mattioli et al., 1997). This study also reported *R. geigyi*, *R. senegalensis* and *R. decoloratus* as the primary tick vectors, with peak infestations occurring during the rainy season. However, no molecular studies have been conducted in The Gambia, resulting in significant gaps in characterization and prevalence. Our study provides the first

molecular detection of *A. marginale* in The Gambia. In the qPCR analysis, 3.9% of ticks tested positive for the *A. marginale msp1β* gene. This observed prevalence aligns closely with rates reported in other countries, such as Cameroon (4.3%) (Ngnindji-Youdje et al., 2022), South Africa (3.8%) (Guo et al., 2019) and Ethiopia (4.47%) (Teshale et al., 2015). However, it is worth noting that a study conducted in Ethiopia did not detect *A. marginale* in ticks collected from cattle (Tufa et al., 2021). Differences in the sensitivity and specificity of the molecular assays used may explain these findings.

Anaplasma marginale has been reported in 17 tick species across Africa, including 11 *Rhipicephalus* spp., four *Amblyomma* spp. and one *Hyalomma* sp., excluding the central part of the continent (Cossu et al., 2023). In our study, we extend these findings by confirming the presence of *A. marginale* in *H. marginatum* collected from cattle in The Gambia. This consistent distribution pattern has been well-documented across Africa (Cossu et al., 2023). The persistent detection of *A. marginale* underscores its broad distribution and emphasises the importance of comprehending its prevalence within diverse tick species in the African context. Further studies focusing on other livestock species are needed for The Gambia, since *A. marginale* has been shown to infect goats co-grazed with cattle (Barbosa et al., 2021; da Silva et al., 2018).

Herein, 2.6% of ticks tested positive for hemoplasmas using the qPCR assay. While *Mycoplasma haemocanis* has been experimentally transmitted by *Rhipicephalus sanguineus* sensu lato ticks in dogs (Seneviratna et al., 1973), DNA of '*Candidatus* Mycoplasma haematohydrochoerus' has been identified in *Amblyomma sculptum* ticks from wild and synanthropic mammals in Brazil (Gonçalves et al., 2020). Additionally, *A. sculptum* and *Amblyomma ovale* ticks from wild boars (*Sus scrofa*) and the salivary glands of *Amblyomma dubitatum* ticks from capybaras (*Hydrochoerus hydrochaeris*) have also been found to carry hemoplasma DNA (Santana et al., 2022; Vieira et al., 2021). Furthermore, '*Ca.* M. haematobovis' has been experimentally transmitted by *Rhipicephalus microplus* ticks in murine models (Shi et al., 2019).

In our study, both *H. marginatum* and *A. variegatum* ticks tested positive for hemoplasmas. A previous study on ticks from cattle in Ethiopia also found *A. variegatum* to be hemoplasma-positive (Hornok et al., 2014). However, the transmission dynamics of hemotropic *Mycoplasma* spp. through hard ticks remain unclear and require further investigation to understand their role and potential impact on disease transmission.

Co-infections of epidemiologically important pathogens in hard ticks have been documented, with the prevalence of these co-infections varying based on geographic location and the extent of pathogen screening (Rocha et al., 2022). Herein, 1.3% of ticks showed DNA of *Ehrlichia* spp. and *A. marginale*, which aligns with previous reports (Haji et al., 2023; Klitgaard et al., 2019; Moutailler et al., 2016; Ngnindji-Youdje et al., 2022; Santana et al., 2022) that also identified co-infections in adult questing ticks. Ticks carrying multiple pathogens pose an increased risk of co-infections in vertebrate hosts, which can result in more complex clinical manifestations and a higher chance of misdiagnosis.

The phylogenetic analysis presented (Figure 2); some branches were supported by relatively low bootstrap values. This is a known limitation in phylogenetic studies, particularly when dealing with closely related *Ehrlichia* species or when relying on short or conserved gene regions with limited phylogenetically informative sites. In our case, while several clades such as those including *Ehrlichia* spp. from this study showed moderate to high bootstrap support, others exhibited weaker support, likely reflecting low sequence divergence and the evolutionary proximity of the taxa involved. Despite these constraints, the overall topology is consistent with previously reported *Ehrlichia* phylogenies. Future analyses incorporating longer sequences or multi-locus approaches may enhance phylogenetic resolution and provide further clarity on the evolutionary relationships within the *Ehrlichia* genus.

A limitation of this study is its focus solely on the dry season, which may not fully capture the diversity of tick species and associated pathogens that could be present throughout the year. Future research encompassing both dry and wet seasons will likely provide a more comprehensive picture of tick and pathogen diversity in the region. Additionally, this analysis did not evaluate the occurrence of tick pathogens in relation to variables such as breed, sex and age. Incorporating these factors in future research will offer a deeper understanding of TBD epidemiology in the region. Furthermore, as the ticks subjected to PCR for pathogen identification also contained host blood, there is a possibility that the detected pathogens originated from the host rather than the tick itself. Therefore, the detection of a pathogen in these ticks does not necessarily indicate the vector competence of the tick species.

In conclusion, this study advances our knowledge of the occurrence and genetic diversity of *Ehrlichia* spp., *A. marginale* and hemotropic *Mycoplasma* spp. in ticks from cattle in The Gambia. Notably, this is the first report of *E. minasensis*, *A. marginale* and hemotropic *Mycoplasma* spp. in ticks from this country. Further research is needed to assess the epidemiology and clinical and economic impacts of TBPs on The Gambia's livestock industry.

AUTHOR CONTRIBUTIONS

Alpha Kargbo: Conceptualization; funding acquisition; writing – review and editing. **Aamir M. Osman:** Methodology; data curation; investigation; validation; formal analysis; writing – original draft; writing – review and editing. **Edrisa Jawo:** Conceptualization; methodology. **Lamin K. M. Fatty:** Conceptualization; methodology. **Flavia C. M. Collere:** Investigation; writing – review and editing. **Marcos R. André:** Conceptualization; methodology; validation; supervision; funding acquisition; writing – review and editing. **Ahmed A. Hassan-Kadle:** Conceptualization; methodology; funding acquisition; writing – review and editing. **Thállitha S. W. J. Vieira:** Conceptualization; methodology; formal analysis; validation; funding acquisition; writing – review and editing. **Rosangela Z. Machado:** Conceptualization; methodology; validation; supervision; funding acquisition; writing – review and editing; project administration. **Rafael F. C. Vieira:** Conceptualization; methodology; data curation; validation; formal analysis; supervision; project administration; funding acquisition; writing – review and editing; writing – original draft.

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CONFLICT OF INTEREST STATEMENT

The authors have no competing financial interests to declare.

DATA AVAILABILITY STATEMENT

Partial nucleotide sequencing data from this study are available in GenBank https://www.ncbi.nlm.nih.gov/ under the following accession numbers: *Ehrlichia dsb* gene (PQ280989–PQ280992) and mitochondrial 16S rRNA gene from six ticks (PQ288673–PQ288678). Additional datasets generated and analyzed are openly accessible via the Dryad repository at https://doi.org/10.5061/dryad.34tmpg4v6.

ETHICS STATEMENT

This study was approved by the Ethics Committee of the Department of Livestock Services, Ministry of Agriculture, The Gambia (reference number: DLS 91/562/109). All cattle owners consented prior to the examination of their animals in the study. All animal handling and tick collection procedures were conducted by trained personnel in accordance with established ethical guidelines to ensure animal welfare and minimise stress. Tick collection was performed using non-invasive methods that did not cause harm or distress to the animals, adhering to recognised standards of veterinary care and animal ethics.

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