



OPEN Exploring the microbiomes of camel ticks to infer vector competence: insights from tissue-level symbiont-pathogen relationships

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Ticks are blood-feeding ectoparasites that harbor diverse pathogens and endosymbionts. Their microbial communities vary based on tick species, stage, sex, geographical location, surrounding environment, and tissue type. Understanding tick microbiota at the tissue level is crucial for unraveling how microbiomes are distributed in tick tissues and influence pathogen transmission. We used V1-V2 16 S rRNA gene sequencing to analyze tissue-specific bacterial compositions (hemolymph, saliva, salivary glands, and midgut) of *Amblyomma gemma*, *Rhipicephalus pulchellus*, *Hyalomma dromedarii*, and *Hyalomma rufipes* ticks collected from camels in Marsabit County, northern Kenya. The V1-V2 region of the 16 S rRNA gene effectively differentiated 43 *Rickettsia africae* and 16 *Rickettsia aeschlimannii* tick samples from other rickettsial species, as well as *Coxiella* endosymbionts from *Coxiella burnetii*. In contrast, the V3-V4 region sequences of these species could not be clearly distinguished. *Coxiella* endosymbionts were most common in *Am. gemma* and *Rh. pulchellus*, while *Francisella* endosymbionts predominated in *Hyalomma* ticks; both were primarily localized in the salivary glands. High abundances of *Coxiella* endosymbionts, as well as *Pseudomonas*, were associated with the absence or low abundance of *Rickettsia* pathogens in both *Am. gemma* and *Rh. pulchellus*, suggesting competitive interactions between these microbes. Additionally, *Proteus mirabilis*, an opportunistic pathogen of the urinary tract in humans, was found predominantly in *Hyalomma* ticks, except for the salivary glands, which were most abundant with *Francisella* endosymbionts. Furthermore, we detected the *Acinetobacter*, *Pseudomonas*, and *Corynebacterium* genera in all the tick tissues, supporting the hypothesis that these bacteria might circulate between camel blood and ticks. Saliva and hemolymph generally harbored more extracellular bacteria than the salivary glands and midgut. This study provides a new approach to unravel tick-endosymbiont-pathogen interactions by examining the tissue localization of tick-borne pathogens and symbionts in *Am. gemma*, *Rh. pulchellus*, *Hy. dromedarii*, and *Hy. rufipes* from camels in northern Kenya. Our findings establish a baseline for developing an understanding of the functional capacities of symbionts and for designing symbiont-based control strategies.

Keywords Tick tissues, *Amblyomma gemma*, *Rhipicephalus pulchellus*, *Hyalomma dromedarii*, *Hyalomma rufipes*, Endosymbionts, *Rickettsia*, *Proteus mirabilis*, Tick-borne pathogens

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Ticks are obligate blood-sucking ectoparasites widely known to transmit pathogens to animals and humans¹. In addition to tick-borne pathogens (TBPs), ticks host a diverse array of microorganisms, both commensal and symbiotic, constituting the microbiome². The tick microbiome comprises bacteria, Archaea, viruses, protozoa, nematodes, and fungi³. The functional properties of microbiomes are still being investigated^{4–6}. Recent studies have provided evidence that the microbiome contributes significantly to the overall fitness and survival of ticks^{3,4,7,8}. Infection can also influence the likelihood of ticks becoming infected with pathogens by modulating the tick immune response^{3,7,8}. Characterizing tick microbiota is crucial for obtaining a better understanding of tick-microbiome interactions^{4,9–11}. Most related studies have focused on identifying the microbiomes of whole ticks¹². However, there is limited understanding of how different microbial communities are distributed across tick tissues¹³. Although microbial communities are found not only in the mucosal organs of ticks¹⁴, only one study has investigated tick saliva microbiomes¹⁵, and no studies have examined the microbiomes of tick hemolymph. Saliva and hemolymph are crucial for the spread of pathogens to mammalian hosts during the process of feeding and are also involved in modulating tick immune responses^{16,17}. Investigating microbial communities at the organ level is essential for unraveling pathogen transmission mechanisms and understanding the influence of the microbiome on this process¹⁸. Additionally, this approach can elucidate potential targets of anti-tick microbiota vaccines aimed at manipulating tick microbiota and blocking pathogen transmission in tick tissues^{3,19–21}.

Understanding how microbiomes interact with TBPs is particularly important for devising strategies to reduce tick and TBP burdens in regions heavily reliant on livestock, such as camels in northern Kenya, which play a crucial role in regional economies. Kenya's camel population was 4.7 million in 2020, approximately 6% of the African camel population^{1,22,23}. Annual camel meat and milk production in Kenya is estimated to be approximately US\$ 11 million²³. In northern Kenya, camels are infested mainly by four tick species, *Rhipicephalus pulchellus*, *Amblyomma gemma*, *Hyalomma dromedarii*, and *Hyalomma rufipes*²⁴. Camels in Kenya are associated with several bacterial and viral TBPs that can significantly impact both human and veterinary health. These include bacteria, such of the genera *Rickettsia*, *Ehrlichia*, and *Coxiella*^{25–27}, and viruses such as the Ifit tick virus, Mbalambala tick virus, Bangali torovirus, Bole tick virus 4, Liman tick virus²⁸, and Crimean-Congo hemorrhagic fever virus²⁹. Knowledge on the bacterial microbiomes of ticks infesting livestock in Africa is limited at present. However, some studies have begun to shed light on tick microbiomes. For instance, the bacterial community structures of *Amblyomma variegatum*, *A. hebraeum*, and *Hyalomma truncatum* ticks infesting cattle in South Africa have been characterized^{1,30,31}. Furthermore, Lee et al. (2019)³² identified the microbial community of *Am. gemma* collected from black rhinos, *Diceros bicornis*, in Tanzania. Moreover, the bacterial microbiota of *Rhipicephalus sanguineus (sensu lato)* in Senegal was identified by René-Martellet et al.³³. In Kenya, *Coxiella* endosymbionts, which play a pivotal role in tick physiology and reproduction, have been reported for various tick species, including *Am. gemma*, *Am. variegatum*, *Rh. evertsi*, and *Rh. appendiculatus*^{26,29,34–36}. Additionally, *Francisella* endosymbionts (FEs), known for their involvement in tick nutrition³⁷, have been associated with the camel ticks *Hy. dromedarii* in Egypt³⁸ and *Hy. rufipes* in Ethiopia³⁹. These studies underscore the necessity of further research to elucidate symbiotic relationships and their impact on tick vector competence.

Next-generation sequencing techniques enable the identification of vector microbiomes through various platforms, such as Illumina MiSeq, which is known for its cost-effectiveness and accuracy^{40,41}. Amplicon sequencing targeting the 16 S rRNA gene is widely used, due to its conserved and variable regions, to analyze microbiota by next-generation sequencing⁴². Recently, 16 S rRNA metabarcoding techniques have provided new insights into the diversity of dissected organs from engorged *Ixodes ricinus* ticks⁷. The bacterial community sizes of tick midguts are overall reduced in comparison to those of other hematophagous arthropods⁴³. Evidence indicates that different tick tissues represent different microbial environments, yet the impact of such variation remains unclear^{13,44}. The microbiota in tick tissues can influence their immunity and pathogen transmission competency⁴⁵. A broader understanding of microbial diversity will be required for the manipulation of these microbes as a new strategy for blocking pathogen transmission. Here, we employed metabarcoding sequencing to characterize the bacterial communities within tick tissues (saliva, hemolymph, salivary glands and midgut) of tick species infesting camels in northern Kenya.

Materials and methods

Study area

This study was conducted in November 2022 in Marsabit County, northern Kenya. Marsabit County is the largest county in Kenya, with an area of ~ 66,923 km², and is located between longitudes 37°57' and 39°21' E and latitudes 02°45' and 04°27' N. It borders Wajir and Isiolo counties to the east⁴⁶. Marsabit County is located approximately 550 km north of Nairobi⁴⁷. Marsabit County is home to nomadic pastoralists who mainly keep camels, cattle, sheep, and goats and rely on mobile livestock production for their livelihoods. In Marsabit, the camel population is estimated to be 132,215⁴⁸. We collected ticks from dromedary camels (*Camelus dromedarius*) in Laisamis and from Saku in Marsabit County (Fig. 1).

Ethical approval

We obtained ethical approval from the Pwani University Ethics Review Committee (Ref: ERC/EXT/002/2020E) and research permit from the National Commission for Science Technology and Innovation (NACOSTI) under (Ref: NACOSTI/P/22/16467). Verbal consent was obtained from the camel farmers before sampling, as most of them could not read or write. We carried out the sampling process with the assistance of veterinarians and field assistants from each community. The field assistants explained the purpose of the study and assisted with translations.

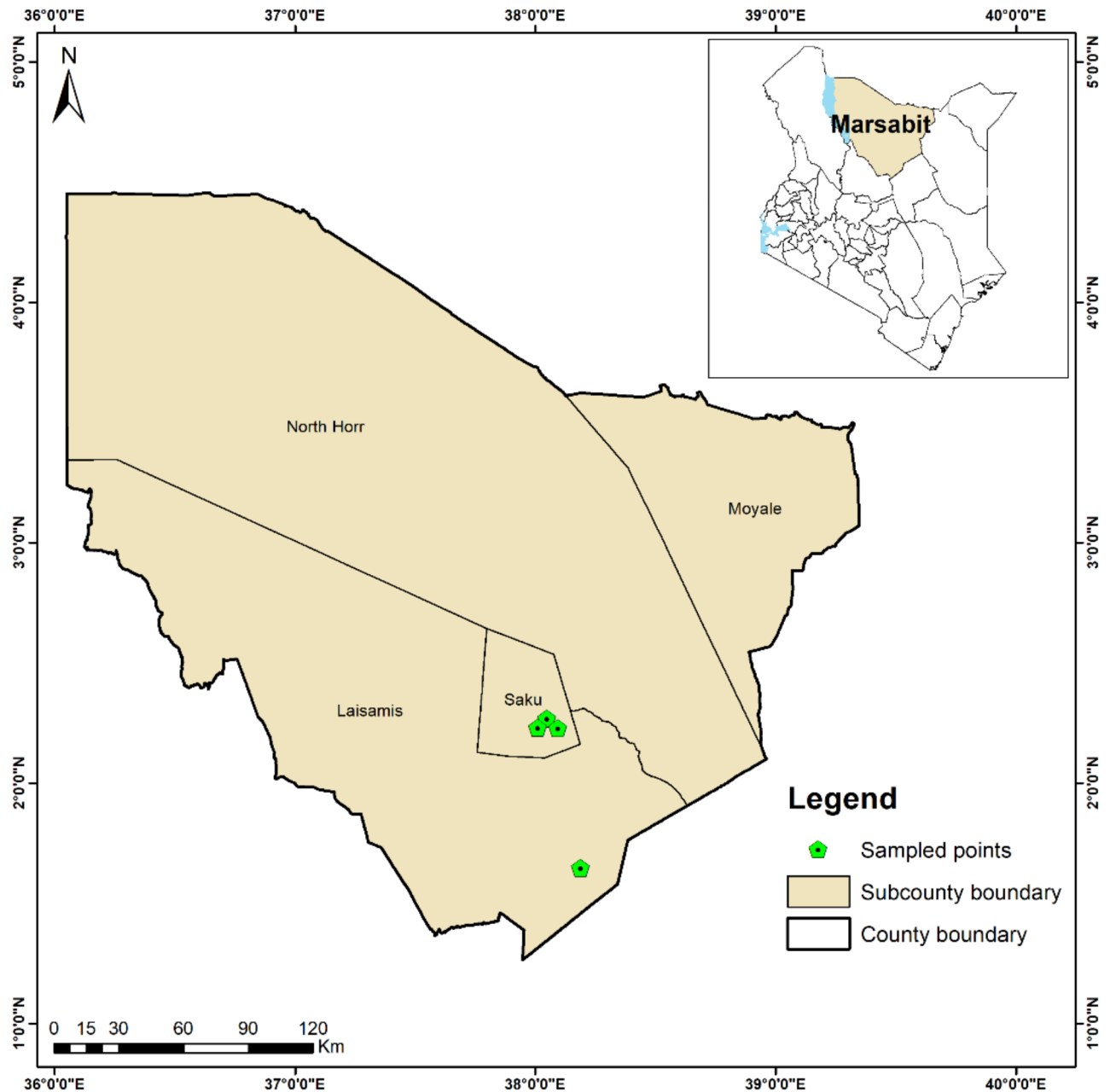


Fig. 1. Map of Kenya showing the sampling sites in Saku and Laisamis in Marsabit County where camel ticks were collected. The map was generated using the open-source software QGIS v.3.28.4.

Tick collection, transportation, identification, and dissection

Out of 1778 adult ticks collected from 278 one-humped camels (*Camelus dromedarius*), during a larger, ongoing cross-sectional study on ticks and tick-borne pathogens, we selected 96 tick specimens from 18 camels to be representative of the four tick species. We transported the ticks alive to the Martin Lüscher Emerging Infectious Diseases (ML-EID) laboratory at the International Centre of Insect Physiology and Ecology (*icipe*) in Nairobi, where tissue collection and tick dissection were performed using a Stemi 2000-C microscope (Zeiss, Oberkochen, Germany) according to the methods described by Khogali et al.²⁴. Within the constraints of efficient dissection and storage of tick tissues, we aseptically harvested 70 samples corresponding to four tick tissue sample types, viz. saliva (SL), hemolymph (HL), salivary glands (SGs), and midgut (MG). First, we sterilized the tick mouthparts and whole body using 1% bleach followed by 70% ethanol. To collect SL, we injected the tick with 2% Pilocarpine HCl behind coxa four. Saliva was then collected from the mouthparts using a 10- μ L pipette^{49,50}. For HL collection, a tick leg was carefully severed using a scalpel blade, and HL was extracted using a 10- μ L pipette²⁴. Before the dissection, according to Binetruy et al.⁵¹ ticks were dipped three times in 1% bleach solution followed by a final rinse in nuclease-free water. The tick was subsequently dissected using paraffin wax melted in petri dishes⁵² to isolate the SGs and MG. These tissues were rinsed in five sequential droplets of phosphate-buffered saline (PBS)

on a glass slide to prevent contamination by HL. Each tissue (SL and HL) required separate sterile 10- μ L pipettes and different scalpel blades. The paraffin wax, PBS, petri dishes, and glass slides were autoclaved prior to use. Each tick was dissected in a separate, sterilized dish. Stainless steel sharp forceps, sterilized by immersion in 1% bleach before reuse on a different tick, which were used to pick the tissues. The tissues were individually placed into well-labeled cryovials, labelling tissue type, tick species, tick sex, sampling site on the camel host, camel host ID, and storage location. All samples were stored at -80 °C to preserve their integrity.

All ticks were identified to the species level using taxonomic keys^{53,54}.

DNA extraction

The SGs and MG homogenates as well as the SL and HL were processed for extraction. Total DNA was extracted from the tick samples using HighPrep™ Viral DNA/RNA kits (Magbio, Gaithersburg, USA) according to the manufacturer's instructions.

PCR, library preparation and sequencing

To determine the bacterial microbiome profiles, we amplified the hypervariable regions, V1-V2 and V3-V4, of the 16 S ribosomal RNA (rRNA) gene from tick tissues (SL, HL, SGs and MG). This was achieved using primers targeting the 16 S rRNA V1-V2 (27 F: 5'-GAGTTTGATCMTGGCTCAG-3'; 388R: 5'-GCTGCCTCCCGTAGG AGT-3') and V3-V4 (341 F: 5'-CCTACGGGNGGCWGCAG-3'; 805R: 5'-GACTACHVGGGTATCTAATCC-3') regions. DNA amplicons were shipped to Macrogen Europe (The Netherlands) for sequencing, and the library was prepared according to the Illumina standard protocol for MiSeq. Overhang adapters were incorporated into the primers using 2x KAPA HiFi HotStartReadyMix. AMPure XP beads were utilized to purify the amplicons from primers and primer dimers. Dual indices and Illumina sequencing adapters were attached using the Hercules II Fusion DNA Polymerase Nextera XT Index V2 Kit. Sequencing was performed with 300-bp paired-end reads in the V3 chemistry MiSeq instrument. Quality control was performed using FastQC, and the primers were identified and trimmed using Cutadapt (v4.1).

According to the Illumina MiSeq protocol, a two-step PCR amplification was conducted. In the first PCR, the DNA template was amplified using forward and reverse primers at a concentration of 1 μ M. The thermal cycling conditions included initial denaturation at 95 °C for 3 min, then 25 cycles consisting of denaturation at 95 °C for 30 s, annealing at 55 °C for 30 s, and extension at 72 °C for 30 s; and a final extension at 72 °C for 5 min. In the second PCR, known as the index PCR, dual indices and Illumina sequencing adapters were attached to the amplified DNA using index primers N7xx and S5xx. The thermal cycling conditions for this step included an initial denaturation at 95 °C for 3 min; followed by 8 cycles of denaturation at 95 °C for 30 s, annealing at 55 °C for 30 s, and extension at 72 °C for 30 s; and a final extension at 72 °C for 5 min.

Bioinformatics and phylogenetic analysis

The raw amplicon sequences obtained from the Illumina MiSeq platform were preprocessed using the nf-core/ampliseq (v2.6.1) pipeline. This pipeline was implemented using Nextflow (v21.10.3)⁵⁵ and Singularity (v3.6.3) for optimal processing efficiency. The Divisive Amplicon Denoising Algorithm 2 (DADA2; v1.26.0)⁵⁶ analysis workflow generated amplicon sequence variant (ASV) abundance and taxonomic classification tables. The DADA2 preprocessing parameters used were Filter and Trim, Denoise, Chimera removal, Dereplication, Read Merge, ASV inference, and Taxonomy Assignment. Taxonomic assignment utilized the "Silva = 138" database⁵⁷ and the Basic Rapid Ribosomal RNA Predictor (Barrnap, v0.9). Subsequently, the filtered ASV abundance matrix, taxonomy table, and metadata were merged into a phyloseq R object to determine differential microbiota abundance among tick species using the phyloseq (v1.41) package⁵⁸. For the heatmap, data normalization was performed through log10 transformation using GraphPad Prism version 0.8⁵⁹. The metagMisc (v0.04) package facilitated the manipulation and visualization of the phyloseq object, offering insights into microbiome abundances and percentages across various factors, such as tick tissues (SL, HL, SGs, and MG), and tick species (*Am. gemma*, *Hy. dromedarii*, *Hy. rufipes*, and *Rh. pulchellus*). The Microbiota Process function (v1.9.3) was used to assess prokaryotic species richness and diversity based on ASV read counts. Alpha diversity metrics, including the Chao1, Pielou evenness, and Shannon indices were calculated using the phyloseq (v1.42.0) and vegan (v2.6-4) to analyze the microbial diversities within the tick microbiomes. For beta diversity analysis, principal coordinate analyses (PCoA) were conducted on the phyloseq R object using Bray-Curtis dissimilarity to identify factors contributing to bacterial diversity variation. Permutational multivariate analysis of variance (PERMANOVA) was used to compare bacterial populations across tick tissues (SL, HL, SGs, and MG) and tick species (*Am. gemma*, *Hy. dromedarii*, *Hy. rufipes*, and *Rh. pulchellus*). We extracted, edited, trimmed, and aligned the sequences of the V1-V2 and V3-V4 16 S rRNA genes using Geneious Prime software v. 2020.2.2 (created by Biomatters, Auckland, New Zealand)⁶⁰. We obtained the V1-V2 and V3-V4 sequences from the four species of ticks, and aligned them using the MAFFT plugin within the Geneious software. Nucleotide sequences were queried against known annotated sequences in the GenBank nr database (<http://www.ncbi.nlm.nih.gov>) using BLAST nucleotide search⁶¹ to confirm their identity and relation to homologous reference sequences. Maximum-likelihood phylogenies were constructed using PhyML version 3.0, using the Akaike information criterion for automatic model selection⁶². Tree visualization was performed with FigTree version 1.4.4⁶³. All V1-V2 and V3-V4 region sequences were submitted to the SRA database of NCBI (<http://www.ncbi.nlm.nih.gov>) under the BioProject accession: PRJNA1134173 (<https://www.ncbi.nlm.nih.gov/sra/PRJNA1134173>); V1-V2 16 S rRNA gene sequences (SRA accessions: SRX25265166 to SRX25265249); and V3-V4 16 S rRNA gene sequences (SRA accessions: SRX25266559 to SRX25266669).

Results

For each of the four tick species, the following tissue samples were obtained: *Am. gemma* ($n=19$; HL = 5, SL = 4, SGs = 6, and MG = 4; ten females and nine males), *Rh. pulchellus* ($n=20$; HL = 5, SL = 4, SGs = 6, and MG = 5; 11 females and 9 males), *Hy. dromedarii* ($n=18$; HL = 5, SL = 4, SGs = 5, and MG = 4; 11 females and 7 males), and *Hy. rufipes* ($n=13$; HL = 3, SL = 3, SGs = 4, and MG = 3; nine females and four males).

Sequence analyses

Out of 96 samples, only 70 were successfully sequenced. after preprocessing 4,944,524 16 S V1-V2 rRNA amplicon reads, we obtained 4,060,361 quality reads from the four tick species, which generated a total of 3,742 ASVs. A total of 18 phyla, 29 classes, 161 families, and 450 genera were recovered from the 16 S rRNA ASVs. Similarly, from 4,584,849 raw reads of the V3-V4 16 S rRNA variable region, we obtained 3,317,439 filtered reads after quality control, filtering, denoising, and chimera removal, with 9,241 ASVs generated. A total of 11 phyla, 34 classes, 150 families, and 546 genera were obtained from the V3-V4 region. Unlike those of the V3-V4 sequences, the V1-V2 sequences were effective at differentiating *Rickettsia africae* and *Rickettsia aeschlimannii* pathogens from the other rickettsial species as well as *Coxiella* endosymbionts (CEs) from *Coxiella burnetii* (Supp. Figure 1A). Therefore, we henceforth only report the V1-V2 16 S rRNA microbial diversity of the tick samples.

Bacterial microbial composition and relative abundance

Composition and abundance of the microbial communities associated with tick species

Taxonomic assignment based on the V1-V2 16 S rRNA regions revealed notable similarities in the abundances of some bacteria between *Am. gemma* and *Rh. pulchellus*, and between *Hy. dromedarii* and *Hy. rufipes* (Fig. 2). *Coxiella* endosymbionts were abundant in *Rh. pulchellus* (16.3%) and *Am. gemma* (11.6%). In contrast, *Francisella* was the predominant endosymbiont, especially in the salivary glands, in *Hy. rufipes* (42.5%) and *Hy. dromedarii* (33.5%), whereas *Proteus* was predominant in the other tissues. *Acinetobacter* was the most abundant bacterial genus in *Rh. pulchellus*, especially in the hemolymph and saliva. Remarkably, *R. africae* was the most abundant genus in *Am. gemma* (36.6%). *Candidatus* Midichloria, though less abundant, was found mainly in the midgut and saliva of *Hy. rufipes* but absent in *Am. gemma* (Supp. Table 1; Fig. 2). BLAST analysis confirmed the ASV identities of *Coxiella* endosymbionts, FEs, *Proteus mirabilis* and *R. africae* in all four tick species. In addition, we found *Ehrlichia ruminantium* in *Am. gemma* and *R. aeschlimannii* in *Rh. pulchellus*, *Hy. rufipes*, and *Hy. dromedarii*.

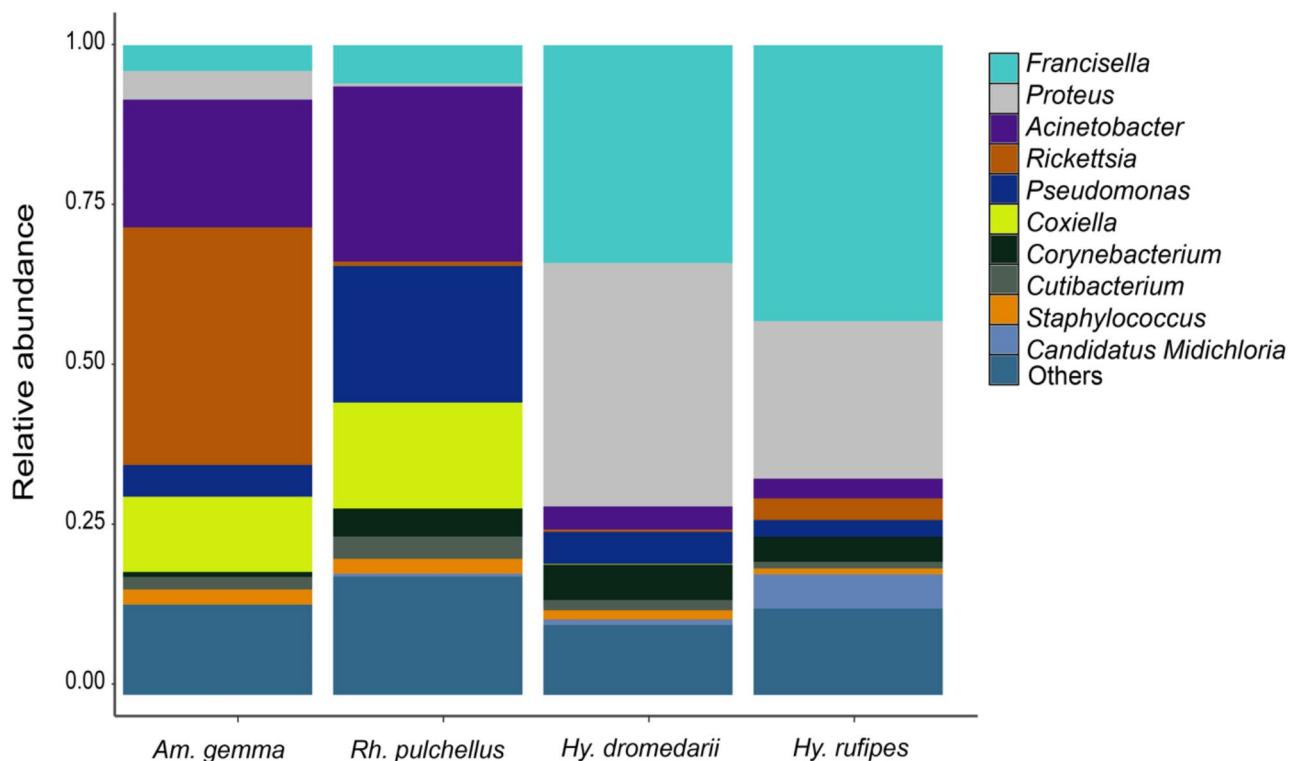


Fig. 2. Relative abundance of bacterial genera in *Amblyomma gemma*, *Rhipicephalus pulchellus*, *Hyalomma dromedarii*, and *Hyalomma rufipes* ticks.

Bacterial compositions and relative abundances in tick tissues

We observed that low abundances of CEs correlated with high levels of *Rickettsia* in the HL, SGs, and MG of *Am. gemma*. In contrast, there were high abundances of CEs in the SGs and MG of *Rh. pulchellus*, coinciding with low levels of *Rickettsia*. *Ehrlichia ruminantium* was detected in extracts from only two different *Am. gemma* ticks (one from an MG and one from SGs). We observed that *Acinetobacter* was more abundant in the HL and SL of *Rh. pulchellus* and *Am. gemma*, while *Pseudomonas* was more abundant in the SGs and MGs of *Rh. pulchellus*. Notably, there was also a negative correlation between *Pseudomonas* and *Rickettsia* in both *Am. gemma* and *Rh. pulchellus* (Supp. Table 2, Fig. 3A and B). *Francisella* endosymbionts were predominantly found in the SGs of both *Hy. rufipes* and *Hy. dromedarii*. Intriguingly, a negative correlation was observed between the abundance of FEs and that of *Proteus*. Among *Hyalomma* spp., *Proteus* was highly prevalent in the HL, MG, and SL samples, whereas the abundance of FEs was low. *Cutibacterium*, *Staphylococcus*, *Pseudomonas* and other bacteria were highly concentrated in the HL and SL and less so in the SGs and MG of *Hyalomma* spp. (Supp. Table 2, Fig. 3C and D).

Alpha diversities

Tick species

We assessed the alpha diversities of the four tick species, *Am. gemma*, *Rh. pulchellus*, *Hy. dromedarii*, and *Hy. rufipes*, using three diversity metrics (Chao1, Shannon, and Evenness) (Fig. 4). The Shannon index revealed that *Rh. pulchellus* had the most bacterial diversity and richness. The *Rh. pulchellus* bacterial diversity was significantly greater than that of *Hy. dromedarii* ($P < 0.01$), and the bacterial richness index indicated that *Rh. pulchellus* also had higher bacterial species richness than *Hy. rufipes* and *Hy. dromedarii* ($P < 0.01$) (Supp. Table 3).

Comparing the alpha diversities of tick tissues within tick species

In *Am. gemma*, we observed that the bacterial richness and diversity were significantly greater in the HL and SL than in the SGs ($P < 0.01$). These bacteria were more evenly distributed in the HL than in the SGs ($P < 0.05$). In contrast, the bacterial diversity was greater in the MG than in the HL ($P < 0.05$) of *Rh. pulchellus*. Furthermore, in *Hy. dromedarii*, the bacterial diversity and richness in the SL was greater than those in the SGs and MG ($P > 0.01$), and these bacteria were more evenly distributed in the SL than in the SGs ($P > 0.05$) and MG ($P > 0.01$). The bacterial richness in the SL of *Hy. rufipes* was significantly greater than that in the SGs and MG ($P < 0.01$). These bacteria were significantly more diverse in the SL than in the SGs ($P < 0.01$), and the bacteria were more evenly distributed in the SL than in the SGs ($P < 0.05$) and in the MG ($P < 0.01$) (Fig. 5 and Supp. Table 4).

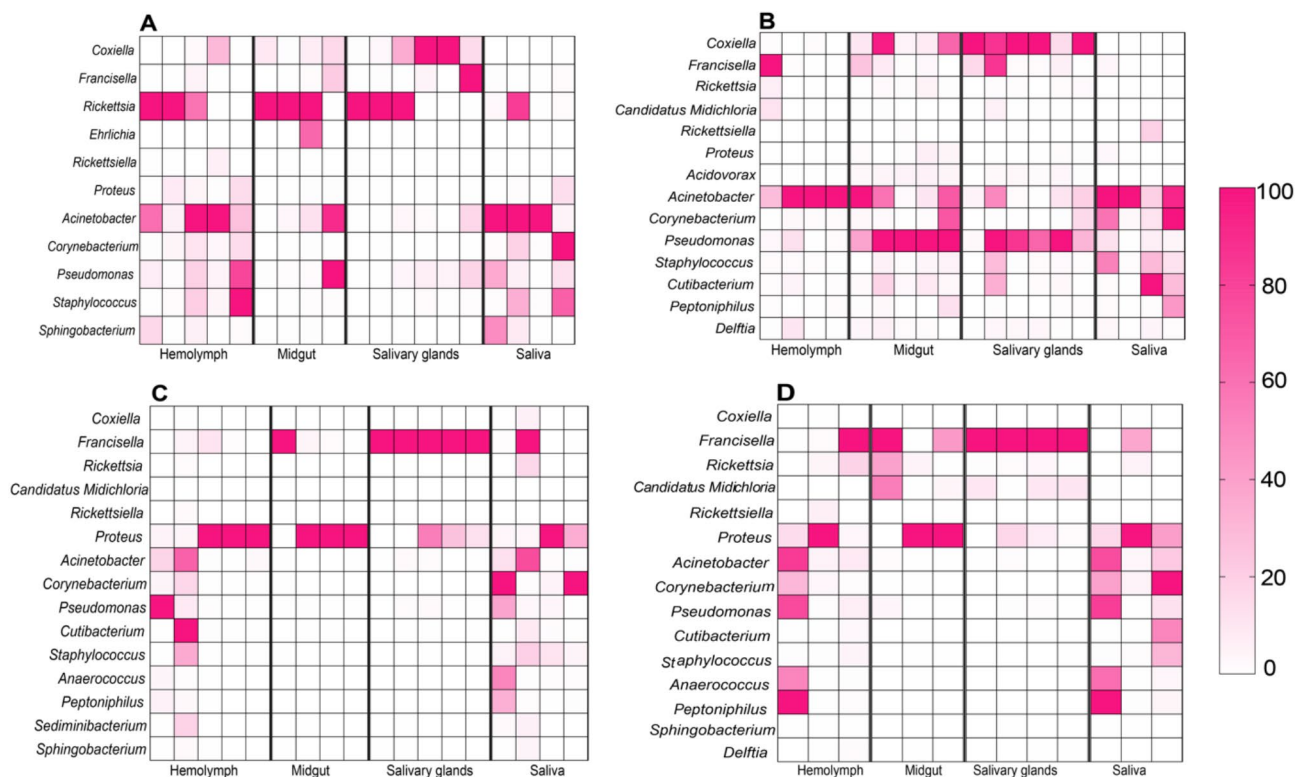


Fig. 3. Heatmap of the normalized amplicon sequence variants (ASVs) of several bacterial species across various tick tissues (hemolymph, midgut, salivary glands, and midgut) of four tick species: (A) *Amblyomma gemma*, (B) *Rhipicephalus pulchellus*, (C) *Hyalomma dromedarii*, and (D) *Hyalomma rufipes*. The color gradient bar indicates relative abundances.

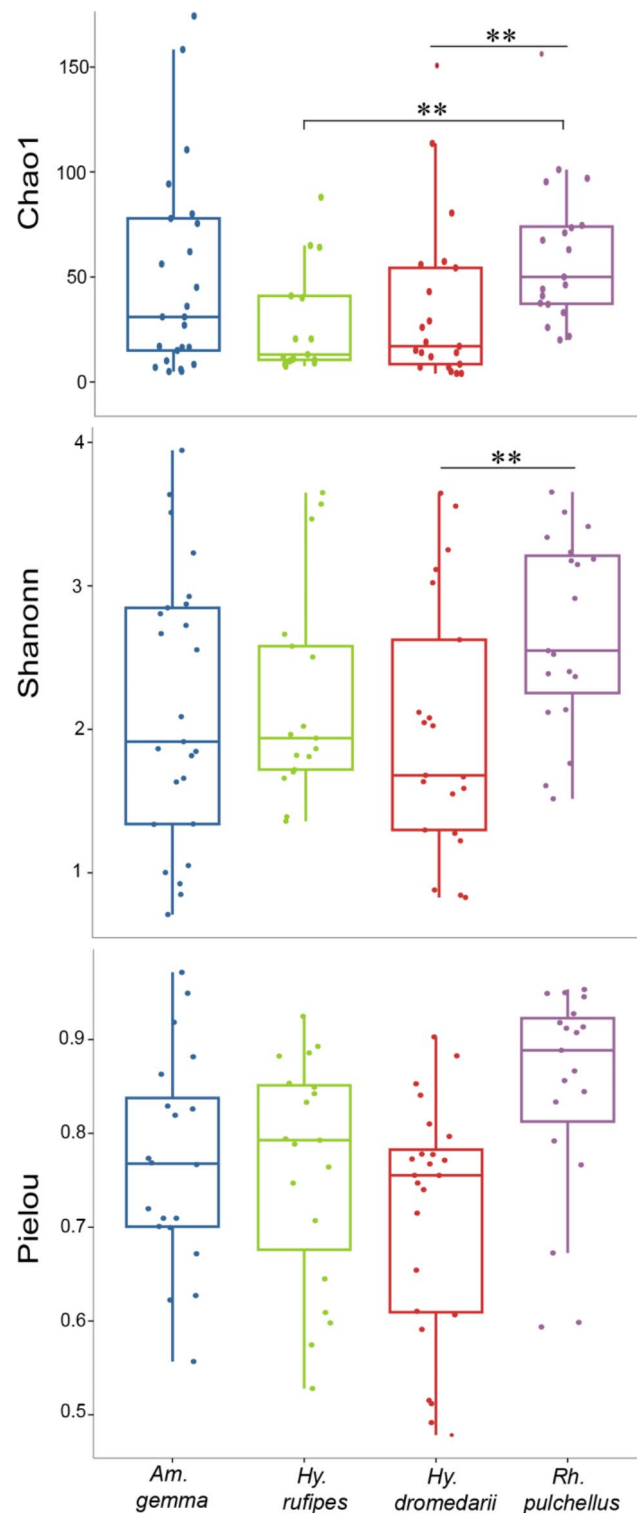


Fig. 4. Boxplot showing alpha diversity in *Amblyomma gemma*, *Rhipicephalus pulchellus*, *Hyalomma dromedarii*, and *Hyalomma rufipes* using three diversity metrics, Chao1, Shannon, and Pielou evenness. P-value (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$).

Comparing the alpha diversities of the different tick species considering tick tissues

There were no significant differences in the alpha diversities of the HL and SL samples among the four tick species. However, the alpha diversity of the MG of *Rh. pulchellus* was greater than that of the MG of *Hy. dromedarii* ($P < 0.01$), *Hy. rufipes* ($P < 0.05$), and *Am. gemma* ($P < 0.05$), and its bacterial richness was also greater than that

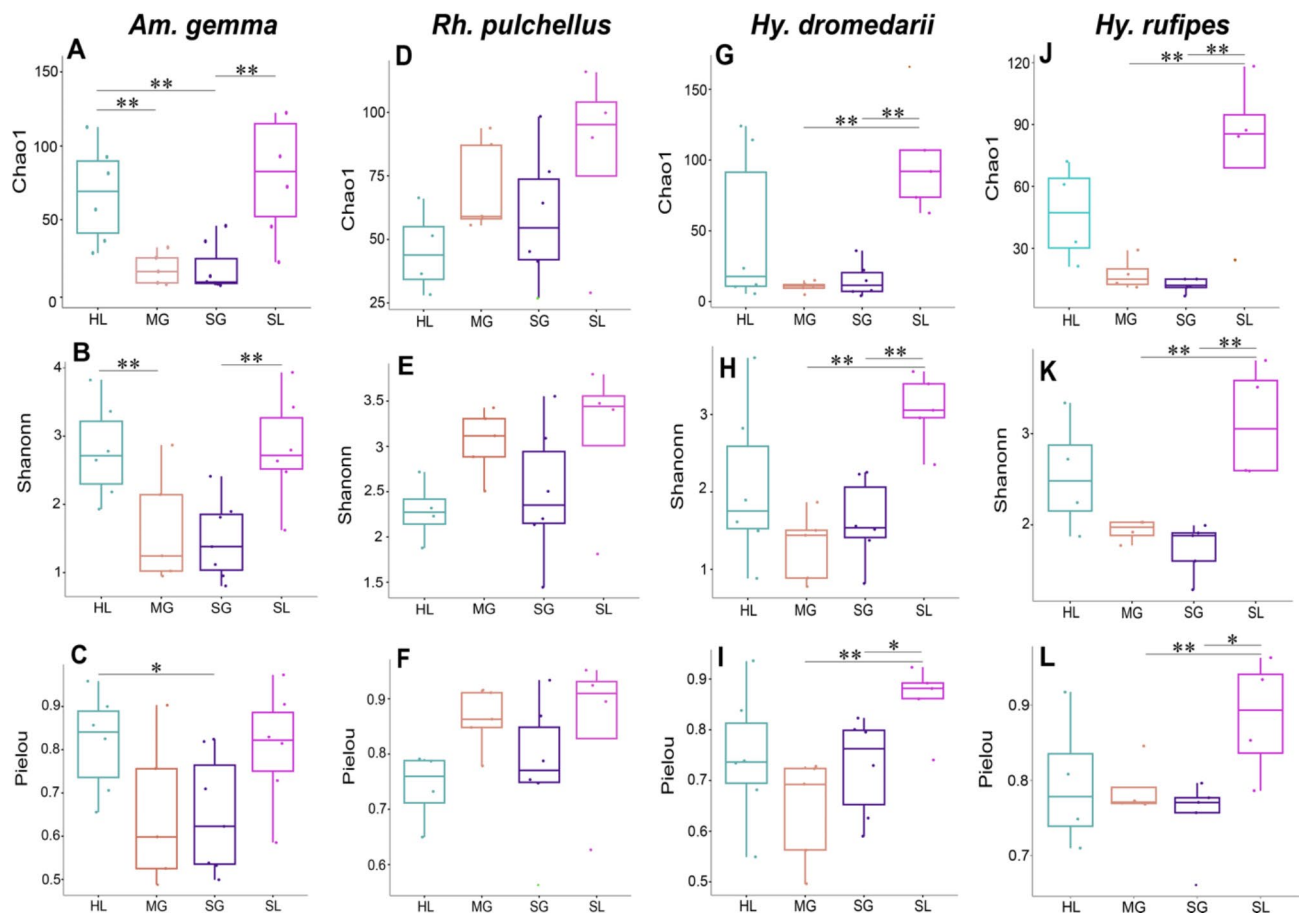


Fig. 5. Boxplots showing comparison of Chao1, Shannon and Pielou evenness alpha diversity analyses of bacterial microbiomes in tissues (saliva SL, hemolymph HL, salivary glands SGs, and midgut MG) of *Amblyomma gemma* (A, B, C), *Rhipicephalus pulchellus* (D, E, F), *Hyalomma dromedarii* (G, H, I), and *Hyalomma rufipes* (J, K, L). P- value (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$).

in *Hy. rufipes* and *Hy. dromedarii* ($P < 0.01$). Additionally, the SGs of *Rh. pulchellus* showed greater bacterial richness than those of *Hy. dromedarii* and *Hy. rufipes* ($P < 0.05$) (Fig. 6 and Supp. Table 5).

Beta diversity

We assessed beta diversity using PCoA based on the Bray-Curtis dissimilarity metric to determine the dissimilarity and diversity of bacterial communities between the tick species and tick tissues using Venn diagrams of core taxa to show the percentages of the shared and unique bacteria. We found that the bacterial composition was significantly different among the four tick species (PERMANOVA, $P < 0.001$); *Hy. dromedarii* and *Hy. rufipes* samples were clustered together, and *Am. gemma* and *Rh. pulchellus* formed discrete clusters (Fig. 7A). The Venn diagram shows that *Hy. dromedarii* and *Hy. rufipes* harbored fewer unique bacterial species than *Rh. pulchellus* and *Am. gemma*, which exhibited greater diversities of unique bacteria (Fig. 7B). Additionally, the PCoA showed that the SL samples clustered significantly with the HL samples and that the SG samples clustered significantly with the MG samples; this was more apparent in *Rh. pulchellus* ($P < 0.001$) (Fig. 8B) than in *Am. gemma* (Fig. 8A), *Hy. dromedarii* (Fig. 8C), and *Hy. rufipes* ($P < 0.05$) (Fig. 8D). Interestingly, the Venn diagram shows that, among the four tick species, the SL and HL exhibited greater numbers of unique bacterial species and shared more bacterial species than did the SGs and MG (Fig. 8).

Discussion

Using 16 S rRNA metabarcoding sequencing, we characterized the bacterial composition of the tissues (SL, HL, SGs, and MG) of four tick species (*Am. gemma*, *Rh. pulchellus*, *Hy. dromedarii*, and *Hy. rufipes*) collected from camels in northern Kenya. The phylogenetic analysis of ASV sequences with reference sequences showed that, unlike the V3-V4 region, the V1-V2 16 S rRNA sequences could clearly distinguish *R. africae* from the other rickettsial species, as well as CEs from the *C. burnetii* pathogen.

We identified *Coxiella* endosymbionts (CEs) predominantly in *Rh. pulchellus* and *Am. gemma*, while FEs were primarily found in *Hyalomma* ticks. These endosymbionts produce B vitamins, which are crucial for cell growth and energy metabolism and compensate for nutrient deficiencies in blood meals³⁵. Similarly, CEs were previously identified as the predominant endosymbionts associated with *Rh. sanguineus*, *Rh. turanicus* and *Rh.*

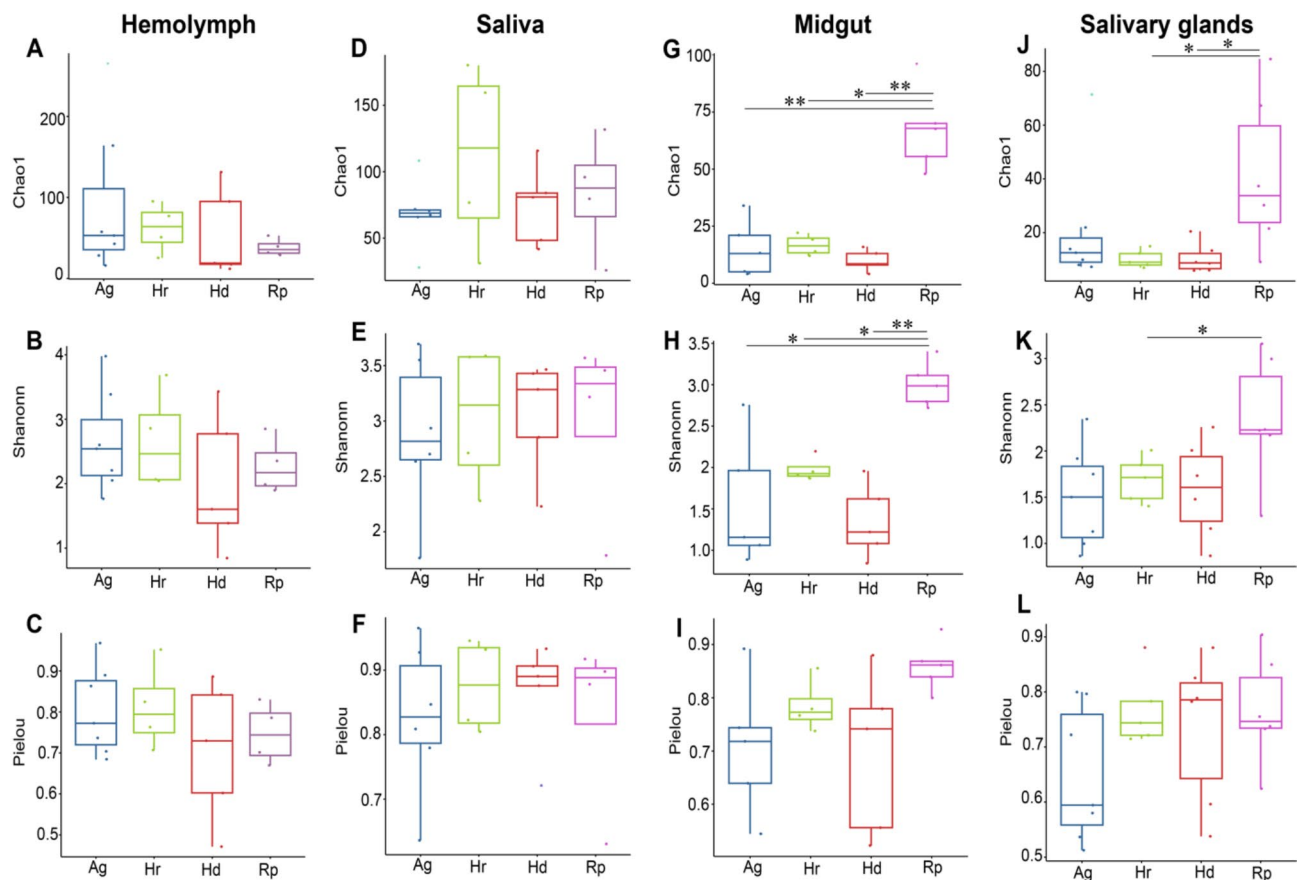


Fig. 6. Boxplots showing the Chao1, Shannon and Pielou evenness alpha diversity analyses of tick species; *Amblyomma gemma* (Ag), *Rhipicephalus pulchellus* (Rp), *Hyalomma dromedarii* (Hd), and *Hyalomma rufipes* (Hr) in different tick tissues; hemolymph (A, B, C); saliva (D, E, F); midgut (G, H, I); and salivary glands (J, K, L). P value (*P < 0.05, **P < 0.01, ***P < 0.001).

microplus^{64,65}. We found that CEs and FEs were most highly concentrated in the SGs, followed by the MG, and less abundant in HL and SL samples. Our findings are congruent with the fact that CEs are predominantly concentrated in SGs of *Am. americanum*, suggesting that they play a significant role in blood-sucking during which they interact with other microbes⁶⁶. It may be attributed to the metabolic activity that facilitating cell multiplication within the SGs acini, and the replication of these bacteria that colonize the same acini^{67,68}. Other observations suggest that CEs may aid tick feeding and infestation by promoting the expression of DA-P36 proteins, which help inhibit blood-meal host immune responses. These proteins, identified in the SGs of *Rh. microplus* in the presence of CEs⁶⁶, play a crucial role in inhibiting T-lymphocyte proliferation, disrupting the host immune response to tick infestation^{69,70}. Moreover, a high prevalence of *R. africae* linked with a low prevalence of CEs was previously reported in *Am. gemma*³⁶, and in *Rh. sanguineus*, *Rh. appendiculatus*, *Dermacentor andersoni*, and *Dermacentor variabilis*^{64,71–73}. Buysse et al.⁷⁴ hypothesized that CEs might protect ticks against TBP. However, the ability of CEs to block *R. africae* transmission needs further investigation.

The presence of CEs in the HL is likely due to their role in recycling metabolites to synthesize essential compounds like B vitamins⁷⁵. *Rickettsia africae* was highly abundant in the HL of *Am. gemma*. The ability of *Amblyomma* species to transmit *R. africae* is well known, and its high abundance in the HL supports the competency of *Am. gemma* to transmit this pathogen²⁴. The transmissibility of other bacteria from ticks to camels, which were highly concentrated in the HL, needs to be investigated. To date, most studies have focused on whole-tick microbiome analysis. However, our study provides a comprehensive approach to better understand the interactions between TBPs and endosymbionts within specific tissue environments.

Francisella endosymbionts (FEs) were found in high abundance in *Hyalomma* ticks, with lower levels in *Am. gemma* and *Rh. pulchellus*. The obligatory symbiotic relationship between FEs and *Hyalomma* spp. was previously shown by Azagi et al.⁷⁶. Consistent with the findings of Ravi et al.⁷⁷, Elbir et al.⁷⁸, and Perveen et al.⁴¹, this study reports the highest prevalence of FEs in *Hy. dromedarii*. We observed the highest concentrations of FEs in the SGs of the two *Hyalomma* species. These results concur with those from previous studies^{76,79}, which showed that FEs exhibited predominant localization in the SGs of *Hyalomma* spp. The high occurrence of FEs in the SGs suggests that their potential role in supplying B vitamins could be important for cell growth and potentially saliva production^{37,80}. As we mentioned above, due to high SG cell multiplication during blood feeding, high FE replication tends to occur. The colonization of SGs by FEs also suggests that FEs might play a

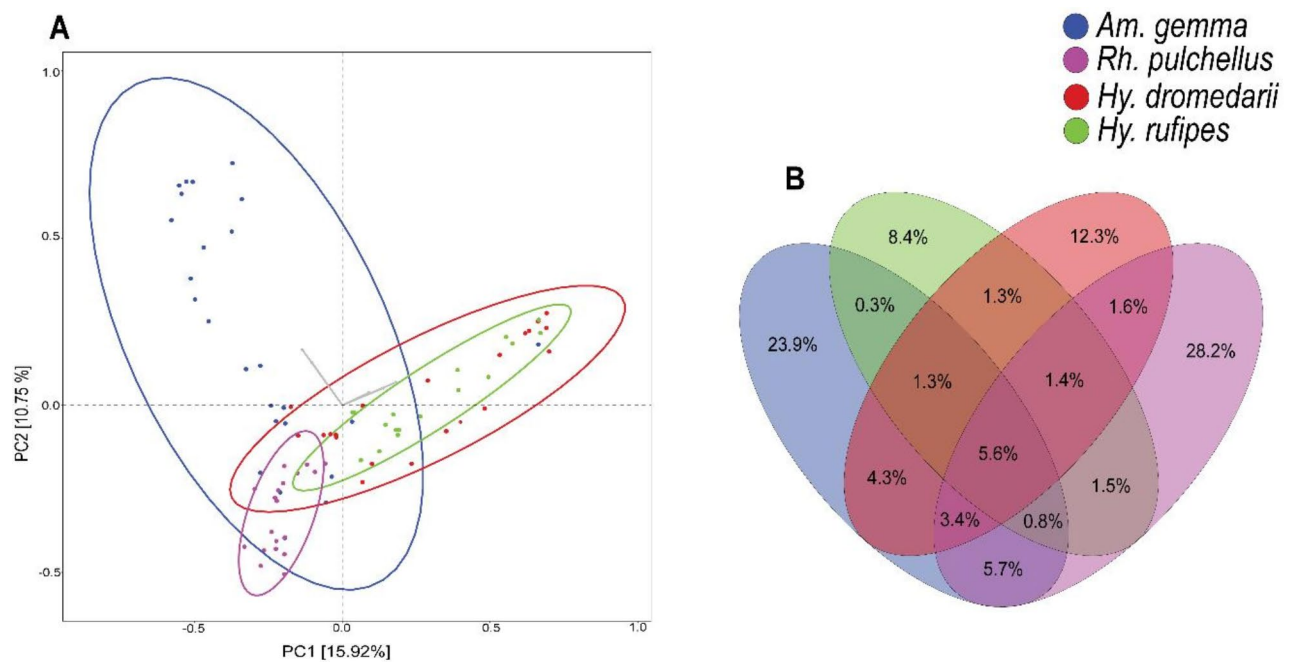


Fig. 7. Beta diversity of bacterial communities; **(A)** Principal coordinate analysis (PCoA) plot of the bacterial communities of four tick species, *Amblyomma gemma*, *Rhipicephalus pulchellus*, *Hyalomma dromedarii* and *Hyalomma rufipes*. **(B)** Venn diagram showing the percentages of different common and unique bacteria between *Amblyomma gemma*, *Rhipicephalus pulchellus*, *Hyalomma dromedarii*, and *Hyalomma rufipes*.

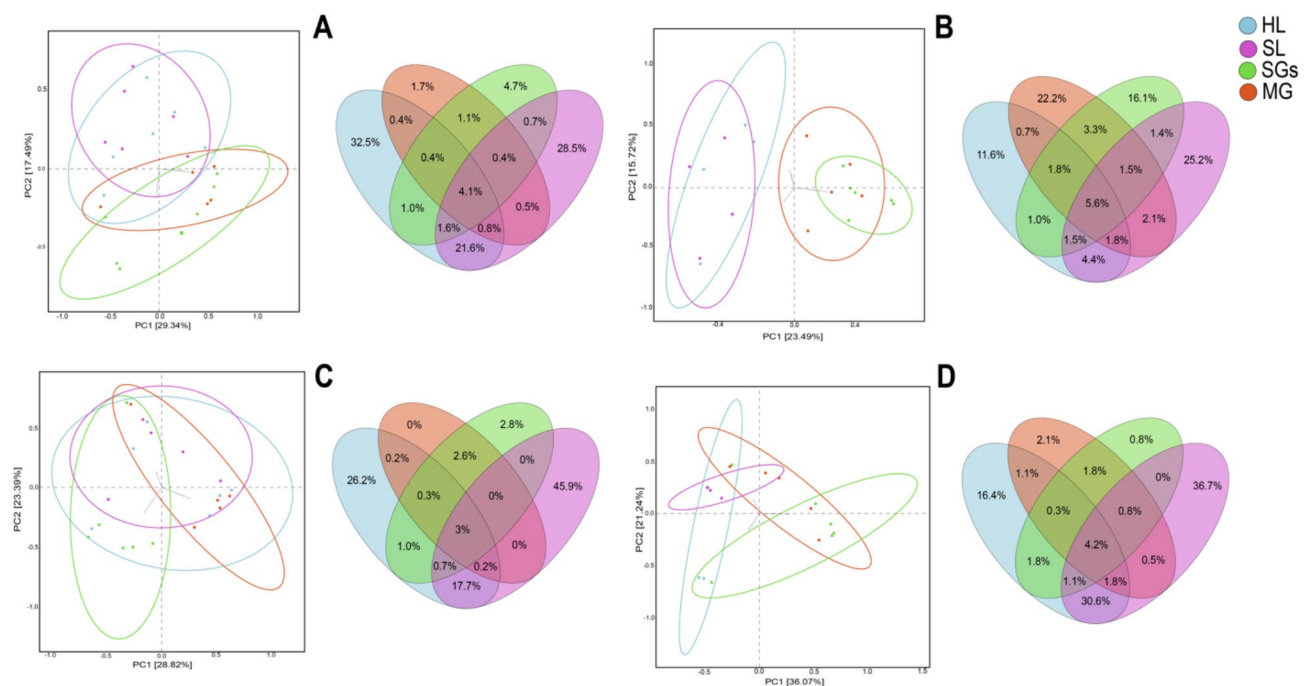


Fig. 8. Beta diversity of bacterial species communities; Principal coordinate analysis (PCoA) plot and Venn diagrams of the bacterial communities of the tissues (HL: hemolymph, SL: saliva, SGs: salivary glands, and MG: midgut) in different tick species: **(A)** *Amblyomma gemma*, **(B)** *Rhipicephalus pulchellus*, **(C)** *Hyalomma dromedarii*, and **(D)** *Hyalomma rufipes*.

key role in horizontal transmission of microbes to vertebrate hosts. Furthermore, FEs may interact with various pathogens inside the SGs, potentially facilitating pathogen transmission⁸¹. We hypothesize that FEs may play a key role in modulating microbiomes and optimizing conditions for effective saliva production during blood-feeding. However, additional studies are needed to understand the interactions between FEs and pathogens transmitted by *Hyalomma* spp.

We identified *Proteus mirabilis* in all tick species and tissues; it was most abundant in *Hyalomma* spp., particularly in *Hy. dromedarii*. This bacterium, recognized as an opportunistic pathogen, was previously identified in *Dermacentor andersoni*⁸². *Proteus mirabilis* has also been identified in *Amblyomma* spp. and *Rhipicephalus* spp. in Kenya⁸³. *Proteus mirabilis* has been associated with urinary tract infection in humans and animals^{84,85} and detected in camels with conjunctivitis infections⁸⁶. Our findings revealed high abundances of *P. mirabilis* across all tick tissues, particularly in the HL, SL, and MG, with the lowest abundances in the SGs. The high abundance in the MG could be due to the symbiotic relationship between the ticks and *P. mirabilis*, which is reflected by hemolytic activity, contributing to efficient blood digestion in the MG⁸⁷. While it is high concentration in the HL and SL could be explained by its extracellular nature and swarming motility of *P. mirabilis*⁸⁸. Given the circulation of *P. mirabilis* in all tissues and the high concentration in the HL, further investigations are needed to confirm the transmission of this bacterium by *Hyalomma* species. As hypothesized by Khogali et al.²⁴ the presence of pathogens in the HL may indicate their ability to evade the tick's immune system, allowing them to persist within the HL.

We identified *Acinetobacter* spp., *Pseudomonas* spp., and *Corynebacterium* spp. among all the tick species. While some studies suggest that these bacteria might be contaminants⁸⁹, others indicate that they could be environmental bacteria acquired by ticks and maintained throughout the tick life cycle^{90–92}. Notably, Mohamed et al.⁹³ reported the presence of these bacteria in camel blood. Along with our finding of these bacteria in different tick tissues, we suggest that these bacteria circulate between camel blood and ticks. These three bacterial genera were also identified at tick bite sites⁹⁴. They are likely to influence pathogen transmission by modulating inflammation and the host's response to tick bites⁹⁵. Interestingly, we noticed an inverse correlation between the abundance of *R. africae* and *Pseudomonas* spp. in *Am. gemma*, which requires further investigation.

According to the alpha diversity analysis, *Rh. pulchellus* had a greater bacterial composition and diversity than did the other tick species, and its MGs had more diverse bacteria than did the other organs. This could be due to its diverse range of host animals²⁴, including humans⁹⁶; *Rh. pulchellus* questing ticks opportunistically attach to hosts as they pass by. Generally, as we observed for the other tick species, the bacterial diversity of their MGs were lowest. In ixodid ticks in particular, the bacterial diversity of MG was reduced after a blood meal^{43,97}. This bacterial reduction could be due to bacterial digestion within tick digestive cells through endocytosis⁹⁸ or the influence of tick immunity on bacterial populations in tick MG, facilitated by antimicrobial peptides and reactive oxygen species^{99,100}. Remarkably, we identified higher abundances of *Pseudomonas* in the MG of *Rh. pulchellus* than in other organs. *Pseudomonas* species have been found to detoxify phytotoxins such as terpene. For instance, *Pseudomonas* in the mountain pine beetle (*Dendroctonus ponderosae*) was found to be rich in genes encoding terpene-degrading enzymes, which are frequently detected in the gut metagenome^{101–103}. The high abundance of *Pseudomonas* in *Rh. pulchellus* could potentially be attributed to the coevolution of the bacterium, due to the detoxification mechanisms of ticks while questing on plants.

In general, we found greater bacterial diversity and richness in the SL and HL than in the MG and SGs in the *Am. gemma*, *Hy. dromedarii*, and *Hy. rufipes*. Similarly, greater diversity was observed in the SL than in the MG of *Dermacentor silvarum*¹⁵. According to Itoh et al.¹⁰⁴, symbiotic bacteria in insects generally exhibit tissue tropism and are localized in symbiotic organs. These bacteria can range from extracellular, within the body cavity, to intracellular, within specialized cells. Beta diversity analysis (PCoA) indicated that the SL and HL shared similar bacterial compositions, as did the SGs and MG. This is largely explained by the fact that we identified many extracellular genera concentrated in the SL and HL, while intracellular bacteria such as *Coxiella*, *Francisella*, and *Rickettsia* were found mainly in the SGs and MG. Nonetheless, extracellular bacteria, including *Acinetobacter*, *Pseudomonas*, *Staphylococcus*, *Corynebacterium*, and *Sphingobacterium*, were found mainly in the MG of *Rh. pulchellus*, indicating that if the gut lumen harbors such extracellular bacteria, the bacteria must be capable of surviving the heme-filled lumen, the toxic reactive oxygen species from neutrophils and macrophages, and proteases in blood meals⁴. Narasimhan et al.¹⁰⁵ identified a similar complement of extracellular bacteria in the gut of questing *Ixodes scapularis* tick nymphs. The most detoxifying symbiosis, referred to as 'gut symbiosis', involves bacteria that are extracellularly localized in the lumen of the gastrointestinal tract in various insects, such as *Acinetobacter* and *Pseudomonas*¹⁰⁴. Mota et al.¹⁰⁶ found that *Rickettsia* spp. and *Pseudomonas* spp., as well as with the other environmental bacteria (*Acinetobacter*, *Staphylococcus*, *Corynebacterium*), were associated with broader competent vector ticks. We found *Rickettsia* spp. and *Pseudomonas* spp. to be highly abundant in *Am. gemma* and *Rh. pulchellus*. The physiology of these ticks could be participating in the infection of several TBPs. These bacteria could be used to reduce the vector competence of these ticks¹⁰⁶.

Understanding the mechanisms by which endosymbionts are distributed in different tick tissues could reveal new strategies for tick control. For instance, the predominance of CEs and FEs in SGs and their association with TBPs suggests that these endosymbionts are critical for tick physiology and pathogen transmission. Vaccines targeting these endosymbionts could disrupt their metabolic contributions, such as B-vitamin synthesis, which are essential for tick survival and reproduction. Anti-microbiota vaccines have shown promise in reducing vector competence by targeting symbiotic bacteria essential for host fitness, as demonstrated in recent studies (Mateos-Hernández et al.²⁰; Wu-Chuang et al.³; Cano-Argüelles et al.²¹). By immunizing the hosts, such vaccines could impair the fitness of ticks feeding on these hosts and reduce the transmission of TBPs like *R. africae*.

While our study provides significant insights into the interactions between TBPs and endosymbionts within specific tissue environments, the relatively small sample size may limit the generalizability of our findings across broader tick populations and geographic regions. Further studies are needed to explore the microbiome pattern in

unfed ticks and different developmental stages of the four tick species. Although, we used a stringent sterilization method, cross-contamination cannot be entirely ruled out. Future work could benefit from additional procedures such as spatially separated processing environments, to further reduce this risk and employing bioinformatic tools to filter the contaminants. Additionally, though we used short 16 S rRNA V1-V2 region sequences that could clearly distinguish *R. africae* and *R. aeschlimannii* from other rickettsial and CE from *C. burnetii*, the resolution for certain other taxa may not be robust. Other techniques such as Oxford Nanopore Technology or shotgun sequencing could provide higher taxonomic and functional resolution.

Conclusions

Our study provides new insights into the tissue-specific localization of symbionts in four tick species infesting camels in northern Kenya, thus enhancing our understanding of their functional roles and interactions in different tissue environments. This study represents the first bacterial microbiome characterization of four tick tissues (hemolymph, saliva, salivary glands, and midgut) in *Am. gemma*, *Rh. pulchellus*, *Hy. dromedarii*, and *Hy. rufipes*. This work contributes to the understanding of the interactions between TBPs and endosymbionts in specific tissues, thereby enhancing our knowledge of vector competence. Additionally, we highlight the advantage of using the V1-V2 region, rather than the more commonly used V3-V4 region, for 16 S rRNA metabarcoding of tick microbiomes. The ASVs generated from the V1-V2 16 S sequences clearly differentiated CEs from the *C. burnetii* pathogen and *R. africae* and *R. aeschlimannii* from other rickettsial species. We show that both tick species and their specific tissues are determining factors for the colonization of microbial communities. For instance, the HL and SL tend to select for extracellular bacteria, while SGs and MG contain most intracellular bacteria. Further investigations are needed to understand how the distinct tick tissue microbiomes affect tick immunity and pathogen transmission. The interactions between *Coxiella* endosymbionts, *Pseudomonas*, and *R. africae* warrant further investigations. Understanding the relationships between ticks and their bacterial endosymbionts is a crucial step toward developing symbiont-based tick and TBP control strategies.

Data availability

The datasets utilized in this study are available in online repositories. The names of the repositories and their respective accession numbers are provided as follows: <https://www.ncbi.nlm.nih.gov/genbank> under the BioProject accession: PRJNA1134173 (<https://www.ncbi.nlm.nih.gov/sra/PRJNA1134173>); SRX25265166 -SRX25265249 (<https://www.ncbi.nlm.nih.gov/sra/PRJNA1134173>), SRX25266559-SRX2526669 (<https://www.ncbi.nlm.nih.gov/sra/PRJNA1134173>).

Received: 8 October 2024; Accepted: 26 November 2024

Published online: 15 February 2025

References

- de la Fuente, J. et al. Tick-host-pathogen interactions: conflict and cooperation. *PLoS Pathog.* **12** (4), e1005488. <https://doi.org/10.1371/journal.ppat.1005488> (2016).
- Greay, T. L. et al. Recent insights into the tick microbiome gained through next-generation sequencing. *Parasit. Vectors.* **11** (1), 12. <https://doi.org/10.1186/s13071-017-2550-5> (2018).
- Wu-Chuang, A. et al. Current debates and advances in tick microbiome research. *Curr. Res. Parasitol. Vector Borne Dis.* **1** (10036). <https://doi.org/10.1016/j.crpvbd.2021.100036> (2023).
- Narasimhan, S. & Fikrig, E. Tick microbiome: the force within. *Trends Parasitol.* **31** (7), 315–323. <https://doi.org/10.1016/j.pt.2015.03.010> (2015).
- Duron, O. et al. Tick-bacteria mutualism depends on B vitamin synthesis pathways. *Curr. Biol.* **28** (12), 1896–1902e5. <https://doi.org/10.1016/j.cub.2018.04.038> (2018).
- Zhong, Z. et al. Symbiont-regulated serotonin biosynthesis modulates tick feeding activity. *Cell. Host Microbe.* **29** (10), 1545–1557e4. <https://doi.org/10.1016/j.chom.2021.08.011> (2021).
- Grandi, G. et al. Characterization of the bacterial microbiome of Swedish ticks through 16S rRNA amplicon sequencing of whole ticks and of individual tick organs. *Parasit. Vectors.* **16** (1), 39. <https://doi.org/10.1186/s13071-022-05638-4> (2023).
- Narasimhan, S. et al. Grappling with the tick microbiome. *Trends Parasitol.* **37** (8), 722–733. <https://doi.org/10.1016/j.pt.2021.04.004> (2021).
- Swei, A. & Kwan, J. Y. Tick microbiome and pathogen acquisition altered by host blood meal. *ISME J.* **11** (3), 813–816. <https://doi.org/10.1038/ismej.2016.152> (2017).
- Cabezas-Cruz, A. et al. Handling the microbial complexity associated to ticks. *Ticks Tick. Borne Path.* <https://doi.org/10.5772/intechopen.80511> (2018).
- Estrada-Peña, A., Cabezas-Cruz, A., Pollet, T., Vayssier-Taussat, M. & Cosson, J. F. High throughput sequencing and network analysis disentangle the microbial communities of ticks and hosts within and between ecosystems. *Front. Cell. Infect. Microbiol.* **8**, 236. <https://doi.org/10.3389/fcimb.2018.00236> (2018).
- Gall, C. A., Scoles, G. A., Magori, K., Mason, K. L. & Brayton, K. A. Laboratory colonization stabilizes the naturally dynamic microbiome composition of field collected *Dermacentor andersoni* ticks. *Microbiome* **5** (1), 133. <https://doi.org/10.1186/s40168-017-0352-9> (2017).
- Wiesinger, A. et al. Revealing the tick microbiome: insights into midgut and salivary gland microbiota of female *Ixodes ricinus* ticks. *Int. J. Mol. Sci.* **24** (2), 1100. <https://doi.org/10.3390/ijms24021100> (2023).
- Fogaça, A. C. et al. Tick immune system: what is known, the interconnections, the gaps, and the challenges. *Front. Immunol.* **12**, 628054. <https://doi.org/10.3389/fimmu.2021.628054> (2021).
- Duan, D. Y., Liu, G. H. & Cheng, T. Y. Microbiome analysis of the saliva and midgut from partially or fully engorged female adult *Dermacentor silvarum* ticks in China. *Exp. Appl. Acarol.* **80** (4), 543–558. <https://doi.org/10.1007/s10493-020-00478-2> (2020).
- Liu, X. Y. & Bonnet, S. I. Hard tick factors implicated in pathogen transmission. *PLoS Negl. Trop. Dis.* **8** (1), e2566. <https://doi.org/10.1371/journal.pntd.0002566> (2014).
- Nuttall, P. A. Tick saliva and its role in pathogen transmission. *Wien Klin. Wochenschr.* **135** (7–8), 165–176. <https://doi.org/10.1007/s00508-019-1500-y> (2023).
- Pollet, T. et al. The scale affects our view on the identification and distribution of microbial communities in ticks. *Parasit. Vectors.* **13** (1), 36. <https://doi.org/10.1186/s13071-020-3908-7> (2020).

19. Piloto-Sardiñas, E. et al. Comparison of salivary gland and midgut microbiome in the soft ticks *Ornithodoros erraticus* and *Ornithodoros moubata*. *Front. Microbiol.* **14**, 1173609. <https://doi.org/10.3389/fmicb.2023.1173609> (2023).
20. Mateos-Hernández, L., Obregón, D., Maye, J., Borneres, J. & de la Versille, N. Fuente et. Al. Anti-tick Microbiota Vaccine impacts *Ixodes ricinus* performance during feeding. *Vaccines (Basel)*. **8** (4), 702. <https://doi.org/10.3390/vaccines8040702> (2020).
21. Cano-Argüelles, A. L. et al. Microbiota-driven vaccination in soft ticks: implications for survival, fitness and reproductive capabilities in *Ornithodoros moubata*. *Mol. Ecol.* **18**, e17506. <https://doi.org/10.1111/mec.17506> (2024).
22. Hughes, L., Anderson, I. & Moffat, J. Correlating analytical microscopy reveals quantitative alterations to the structure, chemistry and materials properties of tooth enamel exposed to acidic solutions. *Microsc Microanal.* **27**, 2582–2583. <https://doi.org/10.1017/S143192762100917X> (2021).
23. Collins, M. et al. Detection of antibodies to *Ehrlichia* spp. in dromedary camels and co-grazing sheep in northern Kenya using an *Ehrlichia ruminantium* polyclonal competitive ELISA. *Microorganisms* **10** (5), 916. <https://doi.org/10.3390/microorganisms10050916> (2022).
24. Khogali, R. et al. Tissue-specific localization of tick-borne pathogens in ticks collected from camels in Kenya: insights into vector competence. *Front. Cell. Infect. Microbiol.* **14**, 1382228. <https://doi.org/10.3389/fcimb.2024.1382228> (2024).
25. Koka, H., Sang, R., Kutima, H. L. & Musila, L. The detection of spotted fever group *Rickettsia* DNA in tick samples from pastoral communities in Kenya. *J. Med. Entomol.* **54** (3), 774–780. <https://doi.org/10.1093/jme/tjw238> (2017).
26. Getange, D. et al. Ticks and tick-borne pathogens associated with dromedary camels (*Camelus dromedarius*) in northern Kenya. *Microorganisms* **9** (7), 1414. <https://doi.org/10.3390/microorganisms9071414> (2021).
27. Younan, M. et al. *Ehrlichia* spp. close to *Ehrlichia ruminantium*, *Ehrlichia canis*, and *Candidatus Ehrlichia regneri* linked to heartwater-like disease in Kenyan camels (*Camelus dromedarius*). *Trop. Anim. Health Prod.* **53** (1), 147. <https://doi.org/10.1007/s11250-020-02524-y> (2021).
28. Zhang, Y. et al. Viromes and surveys of RNA viruses in camel-derived ticks revealing transmission patterns of novel tick-borne viral pathogens in Kenya. *Emerg. Microbes Infect.* **10** (1), 1975–1987. <https://doi.org/10.1080/22221751.2021.1986428> (2021).
29. Chiuya, T. et al. Tick-borne pathogens, including Crimean-Congo haemorrhagic fever virus, at livestock markets and slaughterhouses in western Kenya. *Transbound. Emerg. Dis.* **68** (4), 2429–2445. <https://doi.org/10.1111/tbed.13911> (2021).
30. Chigwada, A. D., Mapholi, N. O., Ogola, H. J. O., Mbizeni, S. & Masebe, T. M. Pathogenic and endosymbiotic bacteria and their associated antibiotic resistance biomarkers in *Amblyomma* and *Hyalomma* ticks infesting Nguni cattle (*Bos* spp.). *Pathogens* **11** (4), 432. <https://doi.org/10.3390/pathogens11040432> (2022).
31. Kisten, D., Brinkerhoff, J., Tshilwane, S. I. & Mukaratirwa, S. A pilot study on the microbiome of *Amblyomma hebraeum* tick stages infected and non-infected with *Rickettsia africae*. *Pathogens* **10** (8), 941. <https://doi.org/10.3390/pathogens10080941> (2021).
32. Lee, S. et al. Comparative microbiomes of ticks collected from a black rhino and its surrounding environment. *Int. J. Parasitol. Parasites Wildl.* **9**, 239–243. <https://doi.org/10.1016/j.iippaw.2019.05.008> (2019).
33. René-Martellet, M. et al. Bacterial microbiota associated with *Rhipicephalus sanguineus* (s.l.) ticks from France, Senegal and Arizona. *Parasit. Vectors.* **10** (1), 416. <https://doi.org/10.1186/s13071-017-2352-9> (2017).
34. Mwamuye, M. M. et al. Novel *Rickettsia* and emergent tick-borne pathogens: a molecular survey of ticks and tick-borne pathogens in Shimba Hills National Reserve, Kenya. *Ticks Tick. Borne Dis.* **8** (2), 208–218 (2017). Epub 2016 Sep 5.
35. Chiuya, T. et al. Molecular prevalence and risk factors associated with tick-borne pathogens in cattle in western Kenya. *BMC Vet. Res.* **17** (1), 363. <https://doi.org/10.1186/s12917-021-03074-7> (2021).
36. Oundo, J. W. et al. Biological tick control: modeling the potential impact of entomopathogenic fungi on the transmission of East Coast fever in cattle. *bioRxiv* 2024–2007. <https://doi.org/10.1101/2024.07.08.602534> (2024).
37. Hodosi, R., Kazimirova, M. & Soltys, K. What do we know about the microbiome of *I. ricinus*? *Front. Cell. Infect. Microbiol.* **12**, 990889. <https://doi.org/10.3389/fcimb.2022.990889> (2022).
38. Ghoneim, N. H., Abdel-Moein, K. A. & Zaher, H. M. Molecular detection of *Francisella* spp. among ticks attached to camels in Egypt. *Vector Borne Zoonotic Dis.* **17** (6), 384–387. <https://doi.org/10.1089/vbz.2016.2100> (2017).
39. Szigeti, A., Kreizinger, Z., Hornok, S., Abichu, G. & Gyuranecz, M. Detection of *Francisella*-like endosymbiont in *Hyalomma rufipes* from Ethiopia. *Ticks Tick. Borne Dis.* **5** (6), 818–820. <https://doi.org/10.1016/j.ttbdis.2014.06.002> (2014).
40. Kulski, J. K. Next-generation sequencing—an overview of the history, tools, and omic applications. *Next Generation Sequencing-Advances Appl. Challenges.* **10**, 61964. <https://doi.org/10.5772/61964> (2016).
41. Perveen, N., Bin Muzaffar, S. & Al-Deeb, M. A. Population dynamics of *Hyalomma dromedarii* on camels in the United Arab Emirates. *Insects* **11** (5), 320. <https://doi.org/10.3390/insects11050320> (2020).
42. Couper, L. & Sweil, A. Tick microbiome characterization by next-generation 16S rRNA amplicon sequencing. *J. Vis. Exp.* (138), 58239. <https://doi.org/10.3791/58239> (2018).
43. Guizzo, M. G. et al. Poor unstable midgut microbiome of hard ticks contrasts with abundant and stable monospecific microbiome in ovaries. *Front. Cell. Infect. Microbiol.* **10**, 211 (2020).
44. Paulson, A. R., Loughheed, S. C., Huang, D. & Colautti, R. I. Multiomics reveals symbionts, pathogens, and tissue-specific microbiome of blacklegged ticks (*Ixodes scapularis*) from a Lyme disease hot spot in southeastern Ontario, Canada. *Microbiol. Spectr.* **11** (3), e0140423. <https://doi.org/10.1128/spectrum.01404-23> (2023).
45. Caragata, E. P. & Short, S. M. Vector Microbiota and immunity: modulating arthropod susceptibility to vertebrate pathogens. *Curr. Opin. Insect Sci.* **50**, 100875. <https://doi.org/10.1016/j.cois.2022.100875> (2022).
46. Siciliano, G. et al. Comparison of multiple maximum and minimum temperature datasets at local level: the case study of North Horr Sub-county, Kenya. *Climate* **9**, 62. <https://doi.org/10.3390/cli9040062> (2021).
47. Kanyina, E. W. Characterization of visceral leishmaniasis outbreak, Marsabit County, Kenya, 2014. *BMC Public Health.* **20** (1), 446. <https://doi.org/10.1186/s12889-020-08532-9> (2020).
48. Kasivalu, J. K., Omwenga, G. I. & Aboge, G. O. Molecular detection and characterization of *Pasteurella multocida* infecting camels in Marsabit and Turkana Counties, Kenya. *Int J Microbiol.* ; 2022:9349303. doi: (2022). <https://doi.org/10.1155/2022/9349303>
49. Patton, T. G. et al. Saliva, salivary gland, and hemolymph collection from *Ixodes scapularis* ticks. *J. Vis. Exp.* (60), 3894. <https://doi.org/10.3791/3894> (2012).
50. Tirloni, L., Calvo, E., Konnai, S. & da Silva Vaz, I. Jr. Editorial: the role of saliva in Arthropod-Host-Pathogen relationships. *Front. Cell. Infect. Microbiol.* **10**, 630626. <https://doi.org/10.3389/fcimb.2020.630626> (2021).
51. Binetruy, F., Dupraz, M., Buysse, M. & Duron, O. Surface sterilization methods impact measures of internal microbial diversity in ticks. *Parasit. Vectors.* **12** (1), 268. <https://doi.org/10.1186/s13071-019-3517-5> (2019).
52. Edwards, K. T., Goddard, J. & Varela-Stokes, A. S. Examination of the internal morphology of the ixodid tick, *Amblyomma maculatum* Koch, (Acari: Ixodidae); a How-to. *Pict. Dissection Guide Midsouth Entomol.* **2**, 28–39 (2009). https://midsouthentomologist.org.msstate.edu/Volume2/Vol2_1_html_files/vol2-1_004.html
53. Estrada-Peña, A., Bouattour, A. J., Camicas, J. L. & Walker, A. R. *Ticks of Domestic Animals in the Mediterranean Region* 131 (University of Zaragoza, 2004).
54. Walker, A. R. *Ticks of Domestic Animals in Africa: A Guide to Identification of Species* (Bioscience Reports, 2003).
55. Di Tommaso, P. et al. Nextflow enables reproducible computational workflows. *Nat. Biotechnol.* **35** (4), 316–319. <https://doi.org/10.1038/nbt.3820> (2017).
56. Callahan, B. J. et al. DADA2: high-resolution sample inference from Illumina amplicon data. *Nat. Methods.* **13** (7), 581–583. <https://doi.org/10.1038/nmeth.3869> (2016).

57. Quast, C. et al. The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. *Nucleic Acids Res.* **41** (Database issue), D590–D596. <https://doi.org/10.1093/nar/gks1219> (2013).
58. McMurdie, P. J. & Holmes, S. Phyloseq: an R package for reproducible interactive analysis and graphics of microbiome census data. *PLoS One*. **8** (4), e61217. <https://doi.org/10.1371/journal.pone.0061217> (2013).
59. GraphPad Software. *GraphPad Prism version 8 for Windows*. GraphPad Software, San Diego, California, USA. (2018). <https://www.graphpad.com>
60. Kearse, M. et al. Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics* **28** (12), 1647–1649. <https://doi.org/10.1093/bioinformatics/bts199> (2012).
61. Altschul, S. F., Gish, W., Miller, W., Myers, E. W. & Lipman, D. J. Basic local alignment search tool. *J. Mol. Biol.* **215** (3), 403–410. [https://doi.org/10.1016/S0022-2836\(05\)80360-2](https://doi.org/10.1016/S0022-2836(05)80360-2) (1990).
62. Guindon, S. et al. New algorithms and methods to estimate maximum-likelihood phylogenies: assessing the performance of PhyML 3.0. *Syst. Biol.* **59** (3), 307–321. <https://doi.org/10.1093/sysbio/syq010> (2010).
63. Rambaut, A. *FigTree; Version 1.4.4* (University of Edinburgh), (2020).
64. Lalzar, I., Friedmann, Y. & Gottlieb, Y. Tissue tropism and vertical transmission of *Coxiella* in *Rhipicephalus sanguineus* and *Rhipicephalus turanicus* ticks. *Environ. Microbiol.* **16** (12), 3657–3668. <https://doi.org/10.1111/1462-2920.12455> (2014).
65. Guizzo, M. G. et al. A *Coxiella* mutualist symbiont is essential to the development of *Rhipicephalus microplus*. *Sci. Rep.* **7** (1), 17554. <https://doi.org/10.1038/s41598-017-17309-x> (2017).
66. Guizzo, M. G. et al. *Coxiella* Endosymbiont of *Rhipicephalus microplus* modulates tick physiology with a major impact in blood feeding capacity. *Front. Microbiol.* **13**, 868575. <https://doi.org/10.3389/fmicb.2022.868575> (2022).
67. Šimo, L., Kazimirova, M., Richardson, J. & Bonnet, S. I. The essential role of Tick Salivary glands and Saliva in Tick Feeding and Pathogen Transmission. *Front. Cell. Infect. Microbiol.* **7**, 281. <https://doi.org/10.3389/fcimb.2017.00281> (2017).
68. Neelakanta, G. & Sultana, H. Tick Saliva and Salivary glands: what do we know so far on their role in Arthropod Blood Feeding and Pathogen Transmission. *Front. Cell. Infect. Microbiol.* **11**, 816547. <https://doi.org/10.3389/fcimb.2021.816547> (2022).
69. Anatriello, E. et al. An insight into the sialotranscriptome of the brown dog tick, *Rhipicephalus sanguineus*. *BMC Genom.* **11**, 1–17. <https://doi.org/10.1186/1471-2164-11-450> (2020).
70. Ribeiro, C. M. et al. Prevalence of *Rickettsia rickettsii* in ticks: systematic review and meta-analysis. *Vector Borne Zoonotic Dis.* **21** (8), 557–565. <https://doi.org/10.1089/vbz.2021.0004> (2021).
71. de Castro, M. H. et al. De novo assembly and annotation of the salivary gland transcriptome of *Rhipicephalus appendiculatus* male and female ticks during blood feeding. *Ticks Tick. Borne Dis.* **7** (4), 536–548. <https://doi.org/10.1016/j.ttbdis.2016.01.014> (2016).
72. Niebyski, M. L., Peacock, M. G., Fischer, E. R., Porcella, S. F. & Schwan, T. G. Characterization of an endosymbiont infecting wood ticks, *Dermacentor andersoni*, as a member of the genus *Francisella*. *Appl. Environ. Microbiol.* **63** (10), 3933–3940. <https://doi.org/10.1128/aem.63.10.3933-3940> (1997).
73. Macaluso, K. R., Sonenshine, D. E., Ceraul, S. M. & Azad, A. F. Rickettsial infection in *Dermacentor variabilis* (Acari: Ixodidae) inhibits transovarial transmission of a second *Rickettsia*. *J. Med. Entomol.* **39** (6), 809–813. <https://doi.org/10.1603/0022-2585-39.6.809> (2002).
74. Buysse, M., Plantard, O., McCoy, K. D., Duron, O. & Menard, C. Tissue localization of *Coxiella*-like endosymbionts in three European tick species through fluorescence in situ hybridization. *Ticks Tick. Borne Dis.* **10** (4), 798–804. <https://doi.org/10.1016/j.ttbdis.2019.03.014> (2019).
75. Cibichakravarthy, B. et al. Comparative proteomics of *Coxiella* like endosymbionts (CLEs) in the symbiotic organs of *Rhipicephalus sanguineus* ticks. *Microbiol. Spectr.* **10** (1), e0167321. <https://doi.org/10.1128/spectrum.01673-21> (2022).
76. Azagi, T. et al. *Francisella*-like endosymbionts and *Rickettsia* species in local and imported *Hyalomma* ticks. *Appl. Environ. Microbiol.* **83**, e01302–e01317. <https://doi.org/10.1128/AEM.01302-17> (2017).
77. Ravi, A. et al. Metagenomic profiling of ticks: identification of novel rickettsial genomes and detection of tick-borne canine parvovirus. *PLoS Negl. Trop. Dis.* **13** (1), e0006805. <https://doi.org/10.1371/journal.pntd.0006805> (2019).
78. Elbir, H., Almathen, F. & Alhumam, N. A. A glimpse of the bacteriome of *Hyalomma dromedarii* ticks infesting camels reveals human *Helicobacter pylori* pathogen. *J. Infect. Dev. Ctries.* **13** (11), 1001–1012. <https://doi.org/10.3855/jidc.11604> (2019).
79. Clayton, K. A., Gall, C. A., Mason, K. L., Scoles, G. A. & Brayton, K. A. The characterization and manipulation of the bacterial microbiome of the Rocky Mountain wood tick, *Dermacentor andersoni*. *Parasit. Vectors.* **8**, 632. <https://doi.org/10.1186/s13071-015-1245-z> (2015).
80. Zhong, J. *Coxiella*-like endosymbionts. *Adv. Exp. Med. Biol.* **984**, 365–379. https://doi.org/10.1007/978-94-007-4315-1_18 (2012).
81. Al-Khafaji, A. M. et al. *Rickettsia* symbiont of the deer tick *Ixodes scapularis*, can colonise the salivary glands of its host. *Ticks Tick. Borne Dis.* **11** (1), 101299. <https://doi.org/10.1016/j.ttbdis.2019.101299> (2020).
82. Brown, R. S., Reichelderfer, C. F. & Anderson, W. R. An endemic disease among laboratory populations of *Dermacentor andersoni* (= *D. Venustus*) (Acarina: Ixodidae). (1970). [https://doi.org/10.1016/0022-2011\(70\)90220-X](https://doi.org/10.1016/0022-2011(70)90220-X)
83. Kimemia, B. B. et al. Detection of pathogenic bacteria in ticks from Isiolo and Kwale counties of Kenya using metagenomics. *PLoS One*. **19** (4), e0296597. <https://doi.org/10.1371/journal.pone.0296597> (2024).
84. Drzewiecka, D. Significance and roles of *Proteus* spp. bacteria in natural environments. *Microb. Ecol.* **72** (4), 741–758. <https://doi.org/10.1007/s00248-015-0720-6> (2016).
85. Nardoni, S., Ebani, V. V., D'Ascenzi, C., Pistelli, L. & Mancianti, F. Sensitivity of entomopathogenic fungi and bacteria to plants secondary metabolites, for an alternative control of *Rhipicephalus (Boophilus) microplus* in cattle. *Front. Pharmacol.* **9**, 937. <https://doi.org/10.3389/fphar.2018.00937> (2018).
86. Shawaf, T. & Hussen, J. Cytological and microbiological evaluation of conjunctiva in camels with and without conjunctivitis. *Vet. Ophthalmol.* **26**(1):39–45. doi: (2023). <https://doi.org/10.1111/vop.13000>. Epub 2022 Jun 3. PMID: 35657141.
87. Kotelko, K. et al. Some biological features of *Proteus* bacilli. 1. Comparison of *Proteus mirabilis* strains provided from various sources. *Acta Microbiol. Pol.* **32** (4), 339–344 (1983).
88. Potter, R. F. et al. Uncharacterized and lineage-specific accessory genes within the *Proteus mirabilis* pan-genome landscape. *mSystems* **8** (4), e0015923. <https://doi.org/10.1128/msystems.00159-23> (2023).
89. Lejal, E. et al. Taxon appearance from extraction and amplification steps demonstrates the value of multiple controls in tick microbiota analysis. *Front. Microbiol.* **11**, 1093. <https://doi.org/10.3389/fmicb.2020.01093> (2020).
90. Hernández-Jarguín, A., Díaz-Sánchez, S., Villar, M. & de la Fuente, J. Integrated metatranscriptomics and metaproteomics for the characterization of bacterial microbiota in unfed *Ixodes ricinus*. *Ticks Tick. Borne Dis.* **9** (5), 1241–1251. <https://doi.org/10.1016/j.ttbdis.2018.04.020> (2018).
91. Díaz-Sánchez, S., Estrada-Peña, A., Cabezas-Cruz, A. & de la Fuente, J. Evolutionary insights into the tick hologenome. *Trends Parasitol.* **35** (9), 725–737. <https://doi.org/10.1016/j.pt.2019.06.014> (2019).
92. Li, S. S. et al. Bacterial microbiota analysis demonstrates that ticks can acquire bacteria from habitat and host blood meal. *Exp. Appl. Acarol.* **87** (1), 81–95. <https://doi.org/10.1007/s10493-022-00714-x> (2022).
93. Mohamed, W. M. A. et al. Exploring prokaryotic and eukaryotic microbiomes helps in detecting tick-borne infectious agents in the blood of camels. *Pathogens* **10** (3), 351. <https://doi.org/10.3390/pathogens10030351> (2021).
94. Zhang, J. et al. Skin infectome of patients with a tick bite history. *Front. Cell. Infect. Microbiol.* **13**, 1113992. <https://doi.org/10.3389/fcimb.2023.1113992> (2023).
95. Bernard, Q., Grillon, A., Lenormand, C., Ehret-Sabatier, L. & Boulanger, N. Skin interface, a key player for *Borrelia* multiplication and persistence in Lyme borreliosis. *Trends Parasitol.* **36** (3), 304–314. <https://doi.org/10.1016/j.pt.2019.12.017> (2020).

96. Keesing, F., Ostfeld, R. S., Young, T. P. & Allan, B. F. Cattle and rainfall affect tick abundance in central Kenya. *Parasitology* **145** (3), 345–354 (2018). Epub 2017 Nov 8.
97. Stafford, K. C. 3rd, Molaei, G., Williams, S. C. & Mertins, J. W. Introduction of the ectoparasite *Rhipicephalus pulchellus* (Ixodidae: Ixodidae) into Connecticut with a human traveler from Tanzania, and a review of its importation records into the United States. *J. Med. Entomol.* **60** (6), 1426–1432. <https://doi.org/10.1093/jme/tjad109> (2023).
98. Ross, B. D. et al. *Ixodes scapularis* does not harbor a stable midgut microbiome. *ISME J.* **12** (11), 2596–2607. <https://doi.org/10.1038/s41396-018-0161-6> (2018).
99. Lara, F. A., Lins, U., Bechara, G. H. & Oliveira, P. L. Tracing heme in a living cell: hemoglobin degradation and heme traffic in digest cells of the cattle tick *Boophilus microplus*. *J. Exp. Biol.* **208** (Pt 16), 3093–3101. <https://doi.org/10.1242/jeb.01749> (2005).
100. Isogai, E. et al. Tertiary structure-related activity of tick defensin (persulcatusin) in the taiga tick, *Ixodes persulcatus*. *Exp. Appl. Acarol.* **53** (1), 71–77. <https://doi.org/10.1007/s10493-010-9379-3> (2011).
101. Perner, J. et al. RNA-seq analyses of the midgut from blood- and serum-fed *Ixodes ricinus* ticks. *Sci. Rep.* **6**, 36695. <https://doi.org/10.1038/srep36695> (2016).
102. Agwunobi, D. O. et al. The toxicity of the monoterpenes from lemongrass is mitigated by the detoxifying symbiosis of bacteria and fungi in the tick *Haemaphysalis longicornis*. *Ecotoxicol. Environ. Saf.* **247**, 114261. <https://doi.org/10.1016/j.ecoenv.2022.114261> (2022).
103. Xavier, M. A. et al. Interfering with cholesterol metabolism impairs tick embryo development and turns eggs susceptible to bacterial colonization. *Ticks Tick. Borne Dis.* **12** (6), 101790. <https://doi.org/10.1016/j.ttbdis.2021.101790> (2021).
104. Itoh, H., Tago, K., Hayatsu, M. & Kikuchi, Y. Detoxifying symbiosis: microbe-mediated detoxification of phytotoxins and pesticides in insects. *Nat. Prod. Rep.* **35** (5), 434–454. <https://doi.org/10.1039/c7np00051k> (2018).
105. Narasimhan, S. et al. Gut microbiota of the tick vector *Ixodes scapularis* modulate colonization of the Lyme disease spirochete. *Cell. Host Microbe.* **15** (1), 58–71. <https://doi.org/10.1016/j.chom.2013.12.001> (2014).
106. Mota, T. F. et al. Another tick bites the dust: exploring the association of microbial composition with a broad transmission competence of tick vector species. *Microbiol. Spectr.* **11** (6), e0215623. <https://doi.org/10.1128/spectrum.02156-23> (2023).

Acknowledgements

We thank Marsabit County Veterinary Services Department staff and all camel owners and herdsmen who consented to our request to collect ticks from their camels. We are also grateful to David Wainaina at icipe for technical support.

Author contributions

RK: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing original draft, Writing – review & editing. AB: Conceptualization, Supervision, Writing – review & editing. DG: Data curation, Investigation, Methodology, Writing – review & editing. JB: Investigation, Writing review & editing, SK: Supervision, Writing – review & editing. NO: Data curation, Bioinformatic analysis. JTPV: Data curation, Bioinformatic analysis. JK: Investigation, Methodology, Writing review & editing. JN: Investigation and Methodology. DM: Conceptualization, Supervision, Writing review & editing. JV: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing.

Funding

The authors gratefully acknowledge the financial support for this research by the following organizations and agencies: European Research Council (ERC) under the European Union's Horizon 2020 Research and Innovation Program under grant agreement No. 101000365 (PREPARE4VBD); the Swedish International Development Cooperation Agency (Sida); the Swiss Agency for Development and Cooperation (SDC); the Australian Centre for International Agricultural Research (ACIAR); the Government of Norway; the German Federal Ministry for Economic Cooperation and Development (BMZ); and the Government of the Republic of Kenya. RK was supported by a German Academic Exchange Service (DAAD) through an icipe ARPPIS-DAAD scholarship and through a UP post-graduate bursary. The views expressed herein do not necessarily reflect the official opinion of the donors.

Declarations

Competing interests

The authors declare no competing interests.

Ethics statement

The animal studies were approved by the Pwani University Ethics Review Committee (Ref: ERC/EXT/002/2020E), a license from the National Commission for Science Technology and Innovation (NACOSTI) under (Ref: NACOSTI/P/22/16467). The studies were conducted in accordance with local legislation and institutional requirements. Written informed consent was not obtained from the owners for the participation of their animals in this study prior to livestock sampling. However, verbal and non-written consent was obtained from the livestock keepers, as most of the participants were unable to read or write. Field assistants from the community assisted in translating the language from English to the local language spoken by the community members to ensure that they understood the purpose of the study and how it would benefit them.

Additional information

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1038/s41598-024-81313-1>.

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