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Bacteriome in Ticks Collected from Domestic Livestock in Kenya

Beth Mutai[1,2], Kariuki Njaanake[2], Kimita Gathii[1], Benson B. Estambale[2,3], John N. Waitumbi[1*]

[1]US Army Medical Research Directorate—Africa/Kenya Medical Research Institute, Kisumu, Kenya
[2]Department of Medical Microbiology, University of Nairobi, Nairobi, Kenya
[3]Jaramogi Oginga Odinga University of Science and Technology, Bondo, Kenya
Email: *john.waitumbi@usamru-k.org

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Abstract

Background: Metagenomics approaches are increasingly being utilized as "dipstick" for microbial carriage. In this study, 16S rRNA metagenomics was used to probe for microbial community that resides in the ticks, those they pick from the environment, wildlife and livestock and to identify potential tick borne zoonoses. **Methods:** Tick DNA from 463 tick pools collected from domestic animals between 2007 and 2008 were amplified with primers that target the 16S rRNA V3-V4 domain and then sequenced on Illumina Miseq platform using 300 cycles version 3 kits. Ticks were pooled according to species and animal from which they were collected. A non-target control was used to track laboratory contaminants. Sequence data were analyzed using Mothur v1.3 pipeline and R v3.3.1 software and taxonomy determined using SILVA rRNA database. Shannon diversity index was used to compute bacterial diversity in each tick species before computing the means. **Results:** A total of 645 bacteria genera grouped into 27 phyla were identified. Four phyla contributed 97.4% of the 36,973,934 total sequences. Proteobacteria contributed 61.2% of these sequences that tarried to 33.8% genera, compared to 15.9% (23.4% genera) for Firmicutes, 15.6% (20% genera) for Actinobacteria and 4.7% (11.6% genera) for Bacteroidetes. The remaining 23 phyla only contributed 2.6% of the sequence reads (11.2% genera). Amongst the 645 genera, three groups were discernible, with the biggest group comprised commensals/symbionts that contributed 93.6% of the genera, but their individual sequence contribution was very low. Group two comprised genera that are known to contain pathogenic species, with C*oxiella* contributing 15,445,204 (41.8%) sequences, *Corynebacterium* (13.6%), *Acinetobacter* (4.3%), *Staphylococcus* (3.9%), *Bacillus* (2.7%) and *Porphyromonas* (1.6%), *Ralstonia* (1.5%), *Streptococcus* (1.3%), *Moraxella* (1.3%), amongst others. Group three com-

prised genera known to contain tick borne zoonotic pathogens (TBZ): *Rickettsiae*, *Anaplasma*, *Francisella*, *Ehrlichia*, *Bartonella* and *Borrelia*. Individually the TBZ contributed <1% of the sequences. By Shannon diversity index, *Amblyomma variegatum* carried the least diverse bacteria (mean Shannon diversity index of 2.69 ± 0.92) compared to 3.79 ± 1.10 for *A. gemma*, 3.71 ± 1.32 for *A. hebraeum*, 4.15 ± 1.08 for other *Amblyomma* spp, 3.79 ± 1.37 for *Hyalomma truncatum*, 3.67 ± 1.38 for other *Hyalomma* spp, 3.86 ± 1.27 for *Rhipicephalus annulatus*, 3.56 ± 1.21 for *Rh. appendiculatus*, and 3.65 ± 1.30 for *Rh. Pulchellus*, but the difference was not significant (p = 0.443). **Conclusion:** This study illustrates the utility of 16S rRNA metagenomics in revealing the complexity of bacteria communities that reside and/or transit through the tick having been picked from the environment, livestock and/or wild animals, some with potential to cause zoonoses.

Keywords

Livestock, Ticks, Bacteriome, Tick-Borne Zoonoses, 16S rRNA, Next Generation Sequencing

1. Introduction

Worldwide, ticks are considered second to mosquitoes in their ability to transmit disease causing pathogens, and are the most relevant disease vectors for domestic and wild animals [1]. Among the tick borne zoonotic pathogens (TBZ) vectored by ticks are tick-borne encephalitis virus (TBEV), Crimean Congo hemorrhagic fever virus (CCHFV), *Babesia* spp., *Rickettsia*, *Anaplasma*, *Francisela*, *Borrelia*, *Bartonella*, *Coxiella* and *Ehrlichia* [2], which they can transmit transstadially and in some cases transovarially [3]. In addition, an individual tick can harbor multiple pathogens which could be co-transmitted during feeding [4]. Other microbes co-exist in ticks as endosymbionts or commensals, and have been reported to influence vector survival and pathogen transmission fitness [5]. Until the advent of next generation sequencing (NGS), methods for detection targeted known pathogens [6], making routine pathogen discovery efforts harder. NGS is pathogen agnostic and allows unbiased detection of sequences in a sample that can then be analyzed bioinformatically for matches in microbial gene repositories.

For this study, 16S rRNA gene, which is universally present in all bacteria, was used for identification of bacteria communities in ticks. The gene comprises 9 hyper-variable regions (V1 to V9) that are interspaced by conserved regions [7] [8]. Although the variable regions have considerable sequence diversity, no single region can differentiate all bacteria and a combination of 2 or more regions is recommended [7] [9] [10]. A combination of V3-V4 region that covers 460 base-pairs (bp) increases the accuracy of taxonomic classification to genus level compared to other variable regions [10] [11] [12]. Using these regions, the Illumina 300 cycles sequencing kit can be used to process 460 bp paired-ends reads

[12].

The 16S rRNA approach has been used for detection of pathogenic bacteria and symbionts in ticks [13] [14] [15] [16] [17]. These studies have reported up to 80% dominance of Proteobacteria followed by Actinobacteria, Bacteroidetes or Firmicutes [13] [14]. Potentially pathogenic genera that have been identified in these studies include *Anaplasma*, *Coxiella*, *Ehrlichia*, *Rickettsia*, *Borrelia*, *Acinetobacter*, *Burkholderia* and *Staphylococcus* among others [14] [15] [16]. In addition to harboring bacteria of medical relevance, ticks also carry endosymbionts such as *Midichloria mitochloria*, *Wolbachia* spp., *Neoehrlichia* spp., *Rickettsiella*. Endosymbionts very similar to tick-transmitted pathogens, including *Coxiella*-like, *Rickettsia*-like, or *Francisella*-like [13] [17] [18] that are said to provide additional nutrients lacking in blood meals have also been identified [19] [20]. Thus, 16S rRNA metagenomics is a useful approach for analyzing the complexity of bacteria community in ticks, as well as identifying potential TBZ.

This study used 16S rRNA metagenomics to examine bacterial community in ticks that were collected from livestock. We highlight the complexity of tick microbiome that they can acquire from the environment, livestock, wildlife or humans, some of them with potential to cause zoonoses.

2. Method

2.1. Ethics Statement

This study used archived genomic DNA from ticks that were collected from domestic livestock as detailed before [21]. The tick samples were collected under an animal use protocol SSC#1248 that was reviewed and approved by the Animal Use Committee of the Kenya Medical Research Institute.

2.2. Sample Acquisition and Study Sites

Tick samples had been collected from 333 cattle, 112 Sheep and 18 goats as previously described [21]. Briefly, ticks were collected from domestic animals presented for slaughter to major slaughterhouses in Nairobi (Athi River Kenya Meat Commission [KMC]) and Mombasa (Kibarani, Uwanja wa Ndege, Mariakani and Kasemeni) between November, 2007 and September, 2008. Up to 10 ticks were collected from each animal that was infested with ticks and placed in 1.5-mL vials containing 70% ethanol, then transported to the laboratory. Ticks were identified taxonomically using the standard taxonomic keys [22]. Ticks of the same species and from the same domestic animal were pooled together and placed in a 1.5-mL vial containing 70% ethanol and stored at −80˚C.

2.3. Genomic DNA Isolation from Ticks

Tick pools were re-hydrated, homogenized using a pestle and mortar and then suspended in 1 mL of phosphate buffered saline as described before [20]. DNA was extracted from 200 µl of the homogenate using a Qiagen QIAamp DNA Mini Kit according to manufacturer's instructions (Qiagen Inc., Valencia, CA).

Genomic DNA was eluted in 200 µL and stored at −80˚C until testing was performed.

2.4. Amplification of 16S rRNA V3-V4 Region, Library Preparation and Sequencing

Bacteria DNA in the ticks was amplified with primers targeting 16S rRNA V3-V4 region as described earlier [23]. The primers were tagged with Illumina sequencing adapters. PCR water was used as non–target control and was included in each run to track laboratory contaminants. Briefly, PCR was performed in a total volume of 25 µL that contained 2.5 µL of genomic DNA, 5 µL of forward and reverse primers, each at a final concentration of 1 µM and 12.5 µL of NEBNext High-Fidelity 2X PCR Master Mix (New Englands BioLabs, MA USA) at 95˚C initial denaturation for 3 min, followed by 25 cycles of 95˚C for 30 s, 62.3˚C for 30 s, and 72˚C for 30 s, and a final extension at 72˚C for 5 min. Amplicons were purified with Agencourt AMPure XP beads (Beckman Coulter Genomics, CA USA) according to the manufacturer's protocol.

A dual indexing PCR to allow multiplexing of samples was done using 5 µL of purified amplicons, 5 µL of Nextera XT i7 Index Primer, 5 µL of Nextera XT i5 Index Primer (Illumina, CA USA), 25 µL of NEBNext High-Fidelity 2X PCR Master Mix (New Englands BioLabs, MA USA) and 10 µL of PCR grade water (Thermo Fisher Scientific, MA USA), with thermocycling at 95˚C for 3 min, followed by 12 cycles of 95˚C for 30 s, 55˚C for 30 s, and 72˚C for 30 s, and a final extension at 72˚C for 5 min. Constructed libraries were purified using Agencourt AMPure XP beads (Beckman Coulter Genomics, MA USA) according to manufacturer's protocol. The libraries were quantified on Qubit Flourometer 2.0 using Qubit dsDNA HS Assay kit (ThermoFsher Scientific, MA USA). Libraries were normalized and pooled to 4 nM based on Qubit values. Pooled samples were denatured and diluted to a final concentration of 10 pM and spiked with 10% PhiX (Illumina, CA USA). 96 samples that included appropriate controls were multiplexed in each sequencing run and paired end sequenced using MiSeq Reagent Kit V3 on the Illumina MiSeq System (Illumina, CA USA).

3. Data Analysis

The 16S rRNA MiSeq sequences output were de-multiplexed and adapters trimmed using the MiSeq reporter software version 2.6.3 (Illumina, CA USA). Mothur pipeline (version 1.35) was used for paired end reads contig assembly, sequence quality filtering, chimera removal and taxonomic assignment [24]. In brief, contigs containing ambiguous bases, and those with lengths < 350 bp (bp) or greater than 466 bp were discarded. Sequences were then aligned to a customized V3-V4 region on the SILVA database [25], followed by merging sequences that were not more than 2 bp different from each other using the pre-cluster command in Mothur [24]. The merged sequences were then filtered for chimeras before taxonomic assignment against the customized V3-V4 SILVA database [24] using Bayesian classifier with 80% confidence [24] [25]. Unas-

signed operational taxonomic unit (OTU) and those assigned to *Chloroplast*, *Mitochondria*, *Archaea*, and *Eukaryote* were discarded. Samples with less than 1000 (n = 40) sequences were excluded from downstream analysis as small library sizes often conceal biologically meaningful results [26]. Taxa detected in the non-template control were censored from the tick sample dataset [27].

Statistical analysis and data visualization were done from OTU tables generated by Mothur on R software environment version 4, with Phyloseq, vegan and ggplot2 statistical adds-on [28] [29] [30] [31]. To down sample the data for alpha diversity calculation and account for unequal sequencing between samples, rarefaction was done using *rarefy_even_depth* command in phloseq with replacement [32]. The rarefied data was used to determine Shannon diversity index by first determining bacterial diversity for each tick species before computing the mean [33].

4. Results

4.1. Tick Samples

In total, 463 tick pools belonging to three tick genera, 25% (n = 113) *Amblyomma* (*A. gemma*, *A. hebraeume*, *A. variegatum* and *Amblyomma* spp.), 13% (n = 62) *Hyalomma* (*H. truncatum*, other *Hyalomma* spp.) and 62% (n = 288) *Rhipicephalus* (*Rh. Annulatus*, *Rh. appendiculatus* and *Rh. Pulchellus*) were accessed in this study.

4.2. 16S rRNA Sequencing Results

Of the 463 tick pools, 400 had their paired end sequences assembled and yielded a total of 97,993,917 contigs. The lowest number of sequence contigs in a sample was 237, and the highest was 4,252,150. After quality filtering, collapsing duplicate sequences, removing chimeras and non-bacterial sequences, 41,500,930 unique sequences remained and were used for taxonomic assignment. Using prevalence method in the "decontan" command within R package, 6 OTUs were identified as contaminants and removed from the dataset. 40 tick pools with library size of <1000 sequences were dropped from downstream analysis, leaving a total of 360 tick pools with 36,973,934 sequence reads.

4.3. Bacterial Community Detected in Ticks

645 unique OTUs (genera) were identified in ticks representing over 89% (36,973,934/41,500,930) of the sequences that passed the QC after quality filtering. The bacteria genera grouped into 27 phyla and as shown in **Figure 1**, Proteobacteria contributed the majority (61.2%) of the sequences that tarried to 33.8% OTUs, 15.9% for Firmicutes (23.4% OTUs), 15.6% for Actinobacteria (20% OTUs) and 4.7% for Bacteroidetes (11.6% OTUs). The remaining 23 phyla only contributed 2.6% of the sequence reads (11.2% OTUs) that included Fusobacteria (0.7%), TM7 (Saccharibacteria) (0.5%), Verrucomicrobia (0.3%), Acidobacteria (0.2%), Deinococcus-Thermus (0.2%), Planctomycetes (0.2%),

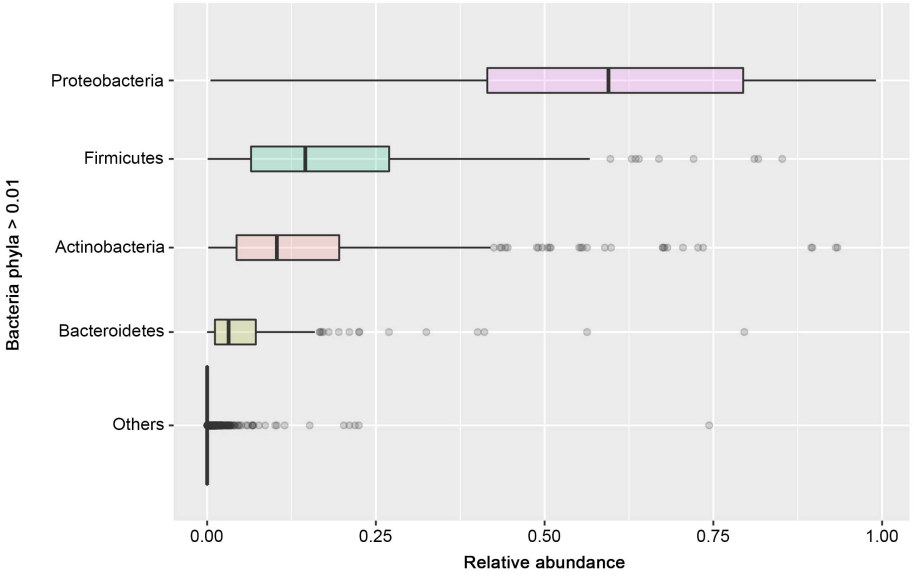

Figure 1. Box plots showing the 27 bacterial phyla detected in ticks with relative abundance >0.01%. Four phyla contributed 97.4% of the sequences, of which Proteobacteria dominated (61.2%) followed by Firmicutes (15.9%), Actinobacteria (15.6%) and Bacteroidetes (4.7%). The remaining 23 phyla are grouped together as "Others" and contributed 2.6 % of the total sequences.

Chloroflexi (0.1%). Others with less than 0.1% sequence reads included OD1 (Parcubacteria), Tenericutes, Gemmatimonadetes, Armatimonadetes, Spirochaetes, Aquificae, SR1 (Absconditabacteria), Lentisphaerae, BRC1, Chlamydiae, Nitrospira, Chlorobi, Synergistetes, Fibrobacteres, WS3 and Elusimicrobia.

Of the 645 genera identified, three groups were discernible (**Figure 2**): commensals/symbionts (**Figure 2**, panel A) that contributed 93.6% of the OTUs, but their individual sequence contributions were very low (25% out of 36,973,934 sequences). Commensals in this group included *Cloacibacterium*, *Aquabacterium*, *Schlegelella*, *Tepidimonas*, *Aerococcus*, *Enhydrobacter*, *Acidovorax*, *Proteus*, *Micrococcus*, *and Fusobacterium* among others. Group two comprised genera known to contain pathogenic species that were probably picked by the ticks from the livestock and/or environment (**Figure 2**, panel B). In this group, C*oxiella* dominated, contributing 41.8% (15,445,204 out of 36,973,934) of the sequences, *Corynebacterium* 13.6%, *Acinetobacter* 4.3%, *Staphylococcus* 3.9%, *Bacillus* 2.7%, *Porphyromonas* 1.6%, *Ralstonia* 1.5%, *Streptococcus* 1.3%, and *Moraxella* 1.3% (**Figure 2** Panel B). Others that contributed <0.1% of total sequences included *Burkholderia*, *Klebsiella*, *Escherichia*, *Shigella*, *Achromobacter*, *Haemophilus*, *Legionella*, *Campylobacter*, *Treponema*, *Elizabethkingia*, *Mycoplasma*, *Bordetella*, *Vibrio and Brucella* (**Figure 2** Panel B). Group three comprised genera known to contain tick borne zoonotic pathogens (TBZ): *Rickettsiae*, *Anaplasma*, *Francisella*, *Erlichia*, *Bartonella* and *Borrelia*. Individually the TBZ contributed <1% of the sequences (**Figure 2** Panel C).

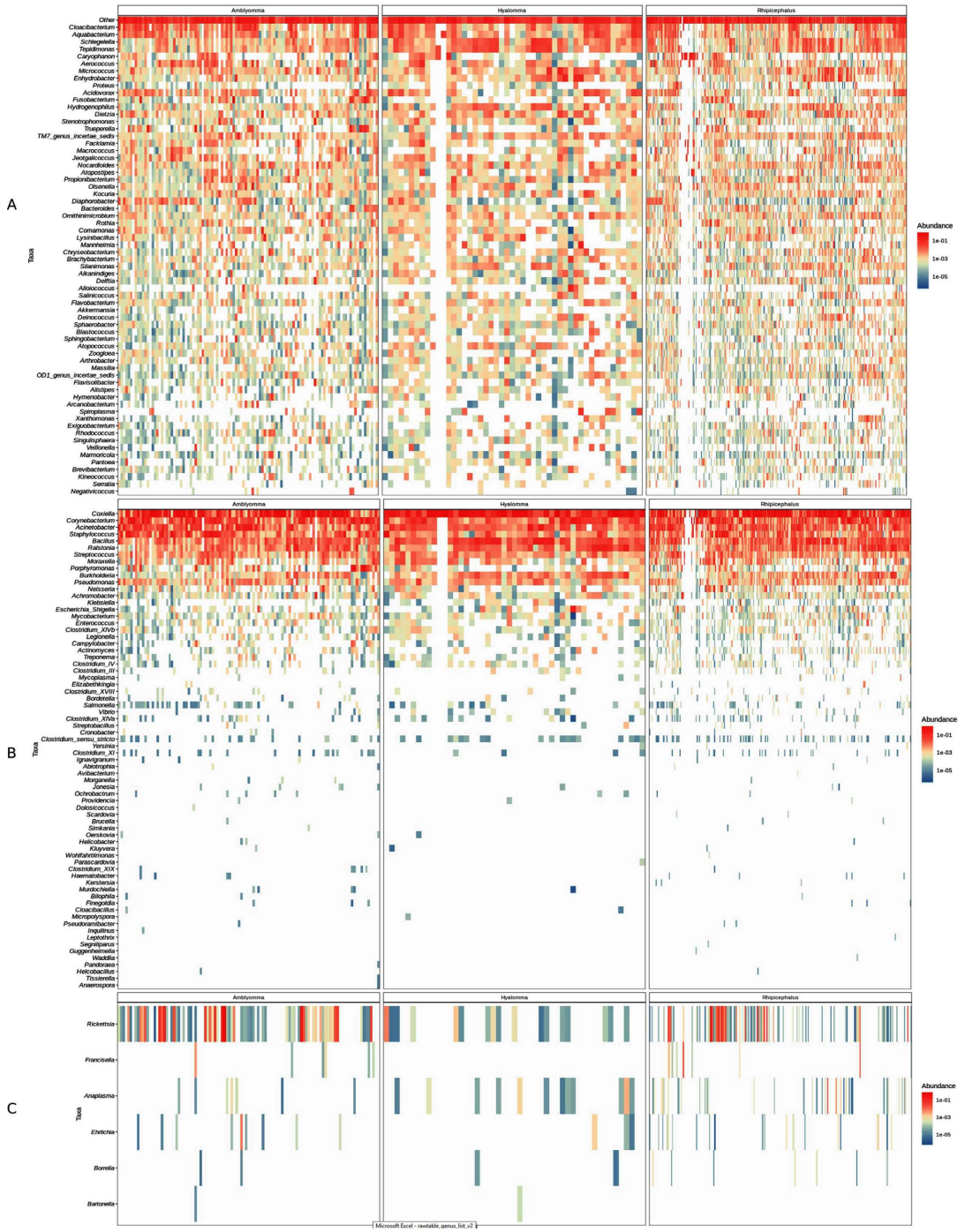

Figure 2. Heat map showing the three groups of bacteria that were identified in different tick genera. Panel A shows taxa that are probably commensals/symbiotic or from the environment appearing at abundance >1%). The commensals/symbionts group dominated, contributing 93.6% of the OTUs, but their individual sequence contribution was very low. Group two comprised genera that are known to have pathogenic species (Panel B): *Coxiella* dominated in this group at 41.8%, followed by *Corynebacterium* (13.6%), *Acinetobacter* (4.3%), *Staphylococcus* (3.9%), *Bacillus* (2.7%) and *Porphyromonas* (1.6%), *Ralstonia* (1.5%), *Streptococcus* (1.3%), *Moraxella* (1.3%), amongst others. Group three comprised tick borne zoonotic pathogens (Panel C): *Rickettsiae*, *Anaplasma*, *Francisella*, *Ehrlichia*, *Bartonella* and *Borrelia* and individually contributed <1% of the sequences. The X-axis represents the samples from the different tick genus. The Y-axis represents microbial taxa at the genus level, ordered by hierarchical clustering. Red color indicates a greater number of reads of that bacterial genus and dark blue color indicates less reads. White boxes indicate samples without sequence reads.

4.4. Diversity of Tick Bacterial Community

A total of 37,344,683 sequences from 360 samples were rarefied to 1220 reads per samples resulting in 381,726 sequences that were used to determine the Shannon diversity index. As shown in **Figure 3**, the diversity index ranged from 2.69 to 4.15. *A. variegatum* showed the least diversity (mean Shannon diversity index of 2.69 ± 0.92) compared to 3.79 ± 1.10 for *A. gemma*, 3.71 ± 1.32 for *A. hebraeum*, 4.15 ± 1.08 for other *Amblyomma* spp, 3.79 ± 1.37 for *Hyalomma truncatum*, 3.67 ± 1.38 for other *Hyalomma* spp, 3.86 ±, 1.27 for *Rhipicephalus annulatus*, 3.56 ± 1.21 for *Rh. appendiculatus*, and 3.65 ± 1.30 for *Rh. Pulchellus*, but the difference was not significant (p = 0.443).

5. Discussion

This study presents data on bacterial communities associated with ticks of the genus *Rhipicephalus*, *Amblyomma* and *Hyalomma* that were collected from domestic animals (cattle, sheep and goats). Ticks belonging to these genera feed on multiple hosts, including wildlife and domestic livestock, in addition to being homophilic, vector multiple zoonotic pathogens [3], and are therefore very important component of "One Health". To the best of our knowledge, our study is the first in Kenya to report on the 16S rRNA approach in tick microbial diversity.

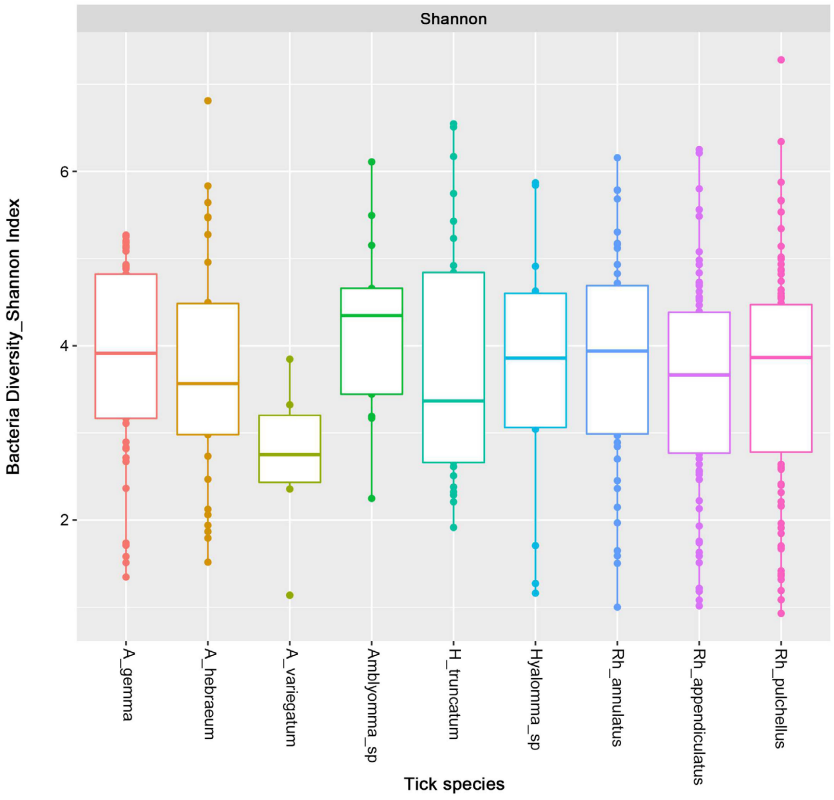

Figure 3. Boxplot showing median bacteria diversity in tick species measured by Shannon diversity index. *A. variegatum* carried less diverse bacteria (mean Shannon diversity index (2.69 ± 0.92 standard deviation) compared to the other tick species, but the difference was not significant (p = 0.443).

Four bacteria phyla carried nearly all the tick microbiome (**Figure 1**). The patterns of bacterial phyla is consistent with findings from other studies that reported over-abundance of Proteobacteria, Firmicutes and Actinobacteria, singly or in different combinations [14] [17] [18] [34] [35]. Of these four, Proteobacteria that comprises Gram negative bacteria, many of them pathogenic to humans and animals [36] [37] accounted for 33.3% of the OTUs. Firmicutes comprise Gram-positive bacteria that include notable pathogens that are found in different environments [38] and were the second abundant phylum at 23.4% of the OTUs. Actinobacteria, Gram-positive bacteria that comprise species that are plant and animal pathogens inhabits diverse environments [39] [40] was the third most abundant and contained 20.0% of the OTUs. Our finding is similar to other reports that indicate Actinobacteria as the third most common phylum after Proteobacteria and Firmicutes [41]. Bacteroidetes was the 4th dominant phylum and constituted 11.6% of the OTUs. Bacteria in this phylum are Gram negative and inhabit many diverse habitats, where they play a beneficial role in the degradation of organic matter. Nevertheless, some members of the phylum are commonly isolated in human and animal clinical samples, including blood, urine, wounds, and feces [42] [43]. The remaining 23 phyla contributed 11.2% of the OTUs. Members of these phyla have also been reported in other tick studies [44].

Amongst the 645 genera, three groups were discernible (**Figure 2**). The biggest group comprised commensals/symbionts (**Figure 2**, Panel A) that contributed 93.6% of the genera, but their individual sequence contribution was very low. Group two comprised genera that are known to contain potentially pathogenic species that were probably picked from the livestock during the ticks' blood meals (**Figure 2**, Panel B). Of these, *Coxiella* was the most abundant and contributed 41.8%% of the total sequence reads. In studies conducted in Malaysia and China, *Haemaphysalis* ticks were found to contain overabundance of *Coxiella* [34] [45]. *Coxiella* also dominated in *Amblyomma* ticks collected in the United States [46]. Due to inability of the 16S rRNA V3-V4 approach to resolve genera to species, the *Coxiella* in ticks could contain pathogenic or symbionts species [11]. We opted to error on the side of caution and referred the genera as potentially pathogenic. *Corynebacterium* in the phylum Actinobacteria, is widely distributed in nature as part of animal and human microbiota, but some species such as *C. diphtheria* cause human infections [40]. This genus was the second most abundant at 13.9%. High abundance of *Corynebacterium* was found in *Hyalomma* ticks from United Arab Emirates (UAE) [47]. Other potentially pathogenic bacteria identified included *Enterococcus*, *Staphylococcus*, *Klebsiella*, *and Acinetobacter* (ESKAPE) that comprise species with high rates of antibiotic resistance [48]. Others included *Acinetobacter* (*4.3%*) *and Staphylococcus* (*3.8%*) and have been reported to occur in *Ixodes*, *Amblyomma* and *Rhipicephalus* ticks [13] [45] [49]. Similarly, *Enterococcus and Klebsiella* have been reported in low prevalence in ticks collected from dogs in China [13]. Although pathogenic, *Burkhoderia*, *Escherichia-Shegella*, *Achromobacter*, *Haemophilus*, *Legionella*, *Campylobacter*, *Treponema*, *Elizabethkingia*, *Mycoplasma*, *Borde-*

tella, *Vibrio*, *and Brucella* were present in low abundance. Multiple reports indicate presence of these genera in ticks [13] [44] [50] [51]. Group three comprised genera known to contain TBZ pathogens (**Figure 2**, Panel B). *Rickettsiae*, *Anaplasma*, *Francisella*, *Erlichia*, *Bartonella* and *Borellia* individually contributed <1% of the sequences (**Figure 2** Panel C). Unlike other tick microbiome studies that have reported dominance of *Rickettsia* and *Borrelia* [14] [18], in this study, these pathogens occurred at <1% relative abundance. As shown in **Figure 2**, panel C, *Rickettsia* was identified in all tick species accessed but notably in lower prevalence in *Hyalomma* compared to *Amblyomma* and *Rhipeciphalus*. Similar studies in the United Arab Emirates (UAE) and Saudi Arabia have reported absence of *Rickettsia* in *Hyalomma* tick collected from camels [47] [52]. Similar to this finding, *Anaplasma* and *Ehrlichia* have been reported in relatively low abundance in Tennessee, USA for example [44]. *Francisella* has also been shown to be dominant in *Hyalomma* ticks comprising up to 99.1% in some locations in the UAE [47], but in our study, *Francisella* was present in much lower abundance (relative abundance of 0.005) and was absent in *Hyalomma* ticks. Similar to studies in Germany and China [13] [53], *Bartonella* was detected in low abundance and only in *Amblyomma spp* and *H. truncatum*. As a caveat, we cannot say with certainty that the *Rickettsia* and *Francisella* identified in this study contained pathogenic species [5] [54] [55]. But, for *Rickettsia*, we know that Kenyan ticks carry a high prevalence of *Rickettsia africae*, *R. aeschlimannii*, *R. mongolotimonae*, *R. conorii subsp. israelensis*), *Candidatus Rickettsia kulagini*, and other unspeciated Rickettsiae [21] [56].

Bacterial community in different tick species varies, probably as a result of environmental factors, blood-meals sources, tick immunity and developmental stages [57]. Contamination at different steps of the DNA extraction, purification and amplification process has been identified as a contributor to the high bacteria diversity seen in ticks [58]. To offset this problem, negative controls are used to track contamination, and if identified, censored from the dataset. Using the "decontam" approached [27], 6 OTUs were dropped from the dataset. Clearly, since the ticks were not surface sterilized before DNA extraction, exo-skeleton associated bacteria contributed to some of the community observed. But, as noted by Narasimhan *et al*., exo-skeleton associated microbes should be considered as being part of the ticks and they probably play a key role in maintaining a healthy physical immune barrier [59].

As shown in **Figure 3**, the aggregated mean Shannon diversity index was low (ranged between 2.69 to 4.15), suggesting that the bacteria genera were shared among the tick species. Similar findings have been reported previously and similar to these studies, a few core bacteria taxa, likely endosymbionts dominate [60] [61] [62].

6. Conclusion

16S rRNA was used to identify bacterial communities associated with different tick species that were collected from domestic livestock in Kenya. There was lit-

tle difference in bacteria diversity between the ticks, probably because of similarity in the environment and the hosts that the ticks interact with. Amidst the hundreds of commensal taxa, six genera known to contain TBZ pathogens, namely *Rickettsiae*, *Anaplasma*, *Francisella*, *Ehrlichia*, *Bartonella* and *Borrelia* were identified and illustrate the central role ticks play in "one health" and their usefulness as bio-indicators of pathogens they likely transmit to humans, domestic livestock and wildlife. Future studies should, in addition to prokaryotic 16S rRNA, include eukaryotic 18S rRNA and viral discovery in order to comprehensively monitor microbial ecosystem in the ticks.

Ethical Statement

The tick samples were collected under protocol SSC#1248 that was reviewed and approved by the Animal Use Committee of the Kenya Medical Research Institute.

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Disclaimer

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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