Animal Microbiome

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The Bacterial and pathogenic landscape of African buffalo (*Syncerus caffer*) whole blood and serum from Kenya

Richard Nyamota[1,9*], Earl A. Middlebrook[2], Hussein M. Abkallo[1], James Akoko[1], Francis Gakuya[3], Lillian Wambua[4], Bernard Ronoh[5], Isaac Lekolool[5], Athman Mwatondo[1,6,7], Mathew Muturi[1,6,8], Bernard Bett[1], Jeanne M. Fair[2] and Andrew W. Bartlow[2]

Abstract

Background African buffalo (*Syncerus caffer*) is a significant reservoir host for many zoonotic and parasitic infections in Africa. These include a range of viruses and pathogenic bacteria, such as tick-borne rickettsial organisms. Despite the considerations of mammalian blood as a sterile environment, blood microbiome sequencing could become crucial for agnostic biosurveillance. This study investigated the blood microbiome of clinically healthy wild buffaloes in Kenya to determine its applicability in agnostic testing for bacteria in apparently healthy wild animals.

Methods Whole blood and serum samples were collected from 46 wild African buffalos from Meru National Park (30), Buffalo Springs (6) and Shaba (10) National Reserves in upper eastern Kenya. Total deoxyribonucleic acid (DNA) was extracted from these samples and subjected to amplicon-based sequencing targeting the 16 S rRNA gene. The bacteria operational taxonomic units (OTU) were identified to species levels by mapping the generated V12 and V45 regions of 16 S rRNA gene to the SILVA database. These OTU tables were used to infer the microbial abundance in each sample type and at the individual animal level. The sequences for the corresponding OTUs were also used to generate phylogenetic trees and thus infer evolution for the OTUs of interest.

Results Here, we demonstrate that buffaloes harbor many bacteria in their blood. We also report a diversity of 16 S rRNA gene sequences for *Anaplasma* and *Mycoplasma* from individual animals. By sequencing both whole blood and serum in triplicate for each animal, we provide evidence of the differences in detecting bacteria in both sample types.

Conclusions Diverse bacteria, including some potential pathogens, can be found in the blood of clinically healthy wild African buffalo. Agnostic surveillance for such pathogens can be achieved through blood microbiome sequencing. However, considerations for the question being asked for the blood microbiome in wildlife will impact the choice for using whole blood or serum for sequencing.

Keywords Blood microbiome, 16S rRNA sequencing, Anaplasma, Mycoplasma, Buffalo

*Correspondence:
Richard Nyamota
richard_nyamota@yahoo.com

Full list of author information is available at the end of the article

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Background

The African buffalo (*Syncerus caffer*) is a large wild bovid that is widely distributed across Sub-Saharan Africa [1, 2]. There are five subspecies of African buffalo widely distributed in Africa with the largest being the Cape buffalo (*Syncerus caffer caffe*r) found in Southern and East Africa [2]. The African buffalo is a significant species in the epidemiology of zoonotic infections and parasitic diseases in Africa given their reservoir host status for various wildlife and livestock pathogens [3, 4]. A wide range of these economically important pathogens such as *Mycobacterium bovis*, Foot-and-mouth disease virus, Rift Valley fever virus, *Theileria sp*, and *Bacillus anthracis* have been reported in the African buffalo [5–10]. The reservoir status of buffalo for these pathogens has been compounded by the lack of clinical symptoms; thus, their role as asymptomatic reservoir hosts cannot be overlooked [3]. Additionally, the close interaction between African buffalo and livestock, particularly cattle, further enhances the potential for disease transmission and overall agricultural risk. Cross-species transmission of pathogens between buffalo and domestic animals is a significant concern, as evidenced by outbreaks of foot-and-mouth disease [11]. Furthermore, the high genetic diversity of viruses found in African buffalo populations adds to the challenge of controlling these diseases, as different strains may exhibit varying levels of virulence and transmission potential [12].

Besides viral pathogens, high infection levels with rickettsial organisms such as *Anaplasma* spp. and *Ehrlichia* spp. have been reported in buffaloes [13, 14]. Apart from the widely reported bovine anaplasmosis, *Anaplasma* spp. also causes disease in humans thereby qualifying as significant zoonotic pathogens [15–17]. Elsewhere, *Mycoplasma* that causes bovine hemoplasmosis has been reported from hematophagous arthropods associated with buffalo [18, 19]. Heavy buffalo infestation with different ectoparasites that could be vectors of various bacterial pathogens including *Anaplasma* and *Mycoplasma* implies a critical role of this association in the disease epidemiology and transmission cycle [20]. Despite immense evidence of individual pathogen infection within a host, co-infection with multiple pathogens has been understudied, thus calling for metagenomic pathogen surveillance.

Pathogen detection in wildlife has largely relied on classical methods such as microscopy, serology, and basic molecular methods such as polymerase chain reaction (PCR) [21, 22]. However, rapid tests such as microscopy and serology have been hampered by low sensitivity and specificity, despite their dependability [23]. Thus, molecular assays have proved reliable for definitive diagnostics. However, these methods are not exhaustive since they rely on the elimination of probable causes to attribute an infection to a particular pathogen. To further improve sensitivity, reverse line blot hybridization (RLB) has been coupled with PCR in the diagnostics of *Anaplasma* and *Theileria* in different wildlife species by targeting 18 S and 16 S rRNA gene sequences [13, 24, 25]. The genus-specific 16 S rRNA gene marker has tremendously revolutionized the investigation of bacterial communities [26]. This has enabled comparison of the microbial communities between different samples thereby enhancing the attribution of an infection to synergistic microbes rather than individual organisms.

The microbiome in blood is a relatively new area of research in wildlife and livestock, but there is growing evidence that bacteria in blood may play an important role in animal health [27]. In the recent decade, microbiome studies have been explored in different wildlife species to understand the microbial community diversity across varying environmental conditions [28]. In animals, the blood microbiome is also thought to play an important role in health and be used as a diagnostic tool for pathogens in the blood or bacteria that lead to sepsis [27, 29, 30]. Investigating the blood microbiome of wildlife and other animals could become a crucial method for biosurveillance [31, 32]. Several diagnostic methods can be used to find pathogens in the blood or serum. The most common are culture-based methods that involve growing the bacteria in a laboratory environment so that they can be identified. However, this method is unreliable for pathogen detection, especially in wildlife, because many of these bacteria cannot be easily cultured or grow too slowly on traditional media [33]. Alternatively, sequencing-based methods involve sequencing the DNA of the bacteria in the blood to identify the species of bacteria present, as well as to track the genetic changes that occur in bacteria over time and space. Currently, there are few published studies on blood microbiomes in wildlife, especially in the interest of pathogen abundance in potential reservoir hosts such as the African buffalo. Next-generation sequencing can potentially speed up the detection and identification of zoonotic pathogens in wild animals.

African buffalo play a crucial role in the transmission of zoonotic infectious diseases to humans and livestock. Understanding the disease transmission dynamics of this wildlife species is essential for safeguarding public health, protecting animal welfare, and ensuring sustainable agriculture. In this study, we investigated the diversity of the blood microbiome in wild African buffalo in Kenya by sequencing the bacterial 16 S rRNA genes. To distinguish between closely related pathogens, which cannot be easily differentiated using the standard V3-V4 region, we sequenced the V1-V2 and V4-V5 variable regions. Specifically, we aimed to determine whether these regions can be used to reliably identify pathogens in the blood

of wildlife. To inform future biosurveillance studies, we compared the microbial communities in whole blood and serum to evaluate the utility of each sample type for microbiome analysis. Specifically, we aimed to characterize the taxa occurring in both serum and blood, as well as those taxa more likely to be present in either sample type. To the best of our knowledge, this is the first study to document the blood microbiome in a wildlife species.

Results

Our findings demonstrate the presence of many bacterial taxa in the blood of wild African buffaloes. As shown in Fig. 1, more bacteria diversity was observed in whole blood for V12 amplicon compared to V45 which depicts uniform OTU distribution in both blood and serum. Secondly, fewer OTUs were detected for V45 than V12 based on the peak distributions shown in Fig. 1.

Amplicon sequencing of the bacterial V12 and V45 variable regions reveals diverse bacterial communities in both whole blood and serum samples from African buffalo as demonstrated in Fig. 2 where bacterial OTUs from each replicate are presented. *Rickettsiales* and *Mycoplasmatales* were the most abundant bacterial orders detected across a significant proportion of the samples. *Mycoplasmatales* sequences were dominant in the serum samples (124/132 replicates) while *Rickettsiales* sequences were dominant in whole blood (129/131 replicates) for amplicons corresponding to V12 variable regions. Amplicons from V45 variable regions dominantly fell under the *Rickettsiales* order in serum (131/134 replicates) and whole blood (132/132 replicates) samples.

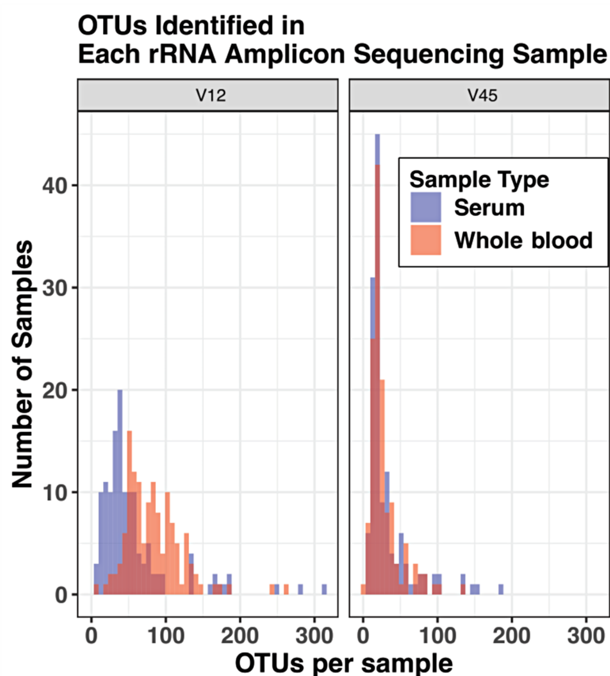

Fig. 1 Histogram showing the numbers of OTUs identified per sample. The left and right figures show numbers for rRNA amplicons V12 and V45, respectively. Colors indicate the sample type of serum or whole blood from which the DNA was extracted

Several bacterial taxa show high abundance and high prevalence among samples. As expected, both *Mycoplasmatales* and *Rickettsiales* occurred with high confidence in the amplicons V12 variable region on both whole blood and serum sample types (Fig. 2). Within the *Rickettsiales* order, the most prevalent OTU was *Anaplasma* which occurred in whole blood from 29 individuals and serum from two individuals (Supplemental Table 1). Interestingly, one variant of *Mycoplasma* OTU was enriched in whole blood (10) compared to serum (2) while another one appeared to be more prevalent in serum (22) than whole blood (2). For the V45 variable region, several variants of *Anaplasma* sequences dominated in both serum (22, 14, 3 samples) and whole blood (38, 16, and 16 samples) results. One *Mycoplasma* OTU was detected with high confidence in 7 serum samples and 0 whole blood samples, while another OTU was found in 2 whole blood samples and 0 serum samples. (Supplemental Table 2). This mirrors, to a lesser degree, the pattern in the V12 results. In addition to *Mycoplasma* and *Anaplasma*, other OTUS identified from V12 variable region sequenced from serum samples included *Kocuria*, *Bacillus*, *Lysinibacillus*, *Pseudarthrobacter*, *Brachybacterium*, *Microbacterium*, *Actinoplanes*, *Pseudomonas*, and *Acinetobacter*. Conversely, besides *Mycoplasma* and *Anaplasma*, *Acinetobacter* were the only OTUs identified with high confidence from V12 variable regions sequences from whole blood. Other bacterial genera detected with high confidence by V45 in serum are *Cutibacterium*, *Terribacillus*, *Haemophilus*, *Microbacterium*, *Pseudomonas*, *Acinetobacter*, *Rhizobium complex*, *Sphingobium*, and *Stenotrophomonas*, while *Cutibacterium*, *Mycoplasma* and *Anaplasma* were the OTUs identified OTUs in whole blood.

Bacterial composition is dependent on the sampling location of buffalo and sex. We tested for compositional differences according to sampling location and sex. The data was stratified by amplicon (V12 or V45) and sample type (whole blood or serum), into four groups for comparison. To visualize sample comparisons by sampling location, non-metric multidimensional scaling (NMDS) plots were generated for V12 and V45 amplicons and the different sample types (Fig. 3).

The results illustrate a significant interaction between sampling location of the animals and their strong effect on the microbial composition (Adonis2: Table 1). To test if differences in microbial composition are driven by constituents of the population or their variances, we tested the effect of sample area on microbial dispersion.

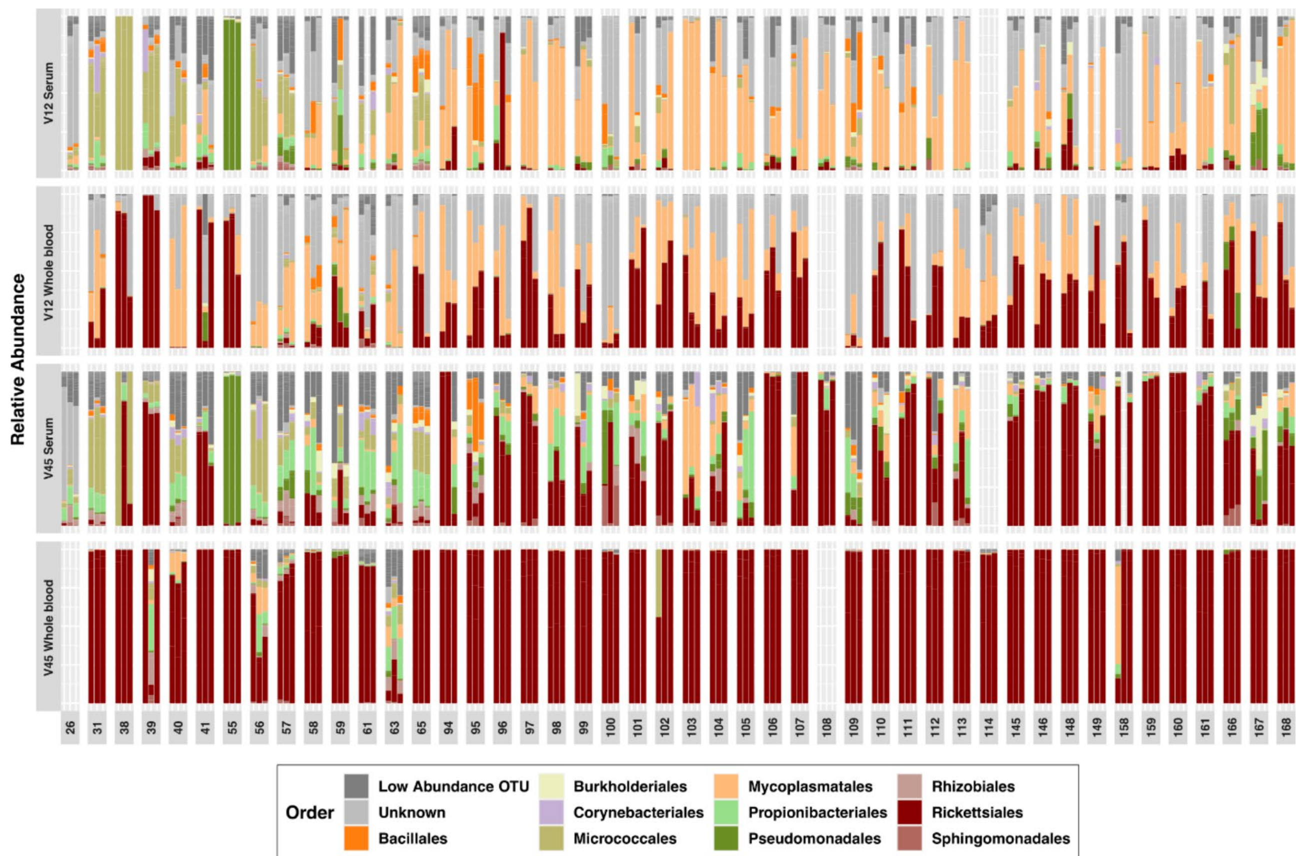

Fig. 2 The relative abundance of bacterial taxon groups per sample. The stacked bars are colored by bacterial OTU's as inferred by SINTAX [34]. Sample replicates are grouped together while blank results are those that were filtered out due to low quality/coverage sequencing data. Dark grey color indicates OTUs with relative abundances less than 0.6%. Sample technical replicates are grouped together. Sample numbers are labeled on the x-axis and amplicon/sample-type are on the y-axis

A permutation test of beta dispersion measures supports that sampling location has a strong effect on sample variability with a p-value of <0.001 for all comparisons and R^2 values of 0.365, 0.269, 0.714, and 0.313 for V12 serum, V12 whole blood, V45 serum, and V45 whole blood, respectively. Full beta dispersion results are available in Supplementary Results.

From our analysis, all Cohen's kappa (κ) values were negative except for two V45 OTUs, both assigned to be in the genus *Anaplasma* (Supplemental Fig. 1). The majority of OTUs having negative κ values indicate disagreement between whole blood and serum tests is greater than expected by chance. To test for statistical difference between OTU counts (and thus detection ability) in blood versus serum, a multivariate negative binomial model was fitted to the OTU counts data using DeSeq2. For the V12 and V45 amplicons, 104 and 11 OTUs, respectively, were enriched in the whole blood, while 29 and 32 were enriched in the serum (Fig. 4). Interestingly, there were many OTUs slightly enriched in serum over whole blood. Several OTUs correspond to common environmental or skin associated genera, perhaps indicating contamination. As expected, this model indicates that *Anaplasma* and several *Mycoplasma* OTUs are strongly enriched in the whole blood samples for V12 and V45 amplicons. However, OTUs identified as *Bartonella* were also enriched in whole blood for both amplicons and one *Ehrlichia* V45 OTU was enriched in whole blood only (Fig. 4). Unexpectedly, the *Mycoplasma* OTU with the highest prevalence and infection intensity by V12 and V45 detection was enriched in the serum. Numbers of animals positive for high confidence OTUs in V12 and V45 sequencing of serum and whole blood are outlined in Supplementary Tables 1 and 2, respectively.

Diverse *Anaplasma* and *Mycoplasma* OTUs were identified from the sequenced 16 S amplicons corresponding to V12 and V45 variable regions. Phylogenetic trees incorporating the V12 OTUs and *Anaplasma* and *Mycoplasma* sequences from the NCBI 16 S and/or SILVA v138 illustrate the diverse occurrence of these bacterial strains in buffalo (Fig. 5A and B). Although the trees based on the V12 variable region lack resolution for many clusters, several interesting relationships are well supported. For instance, *Anaplasma* OTU13 was closely

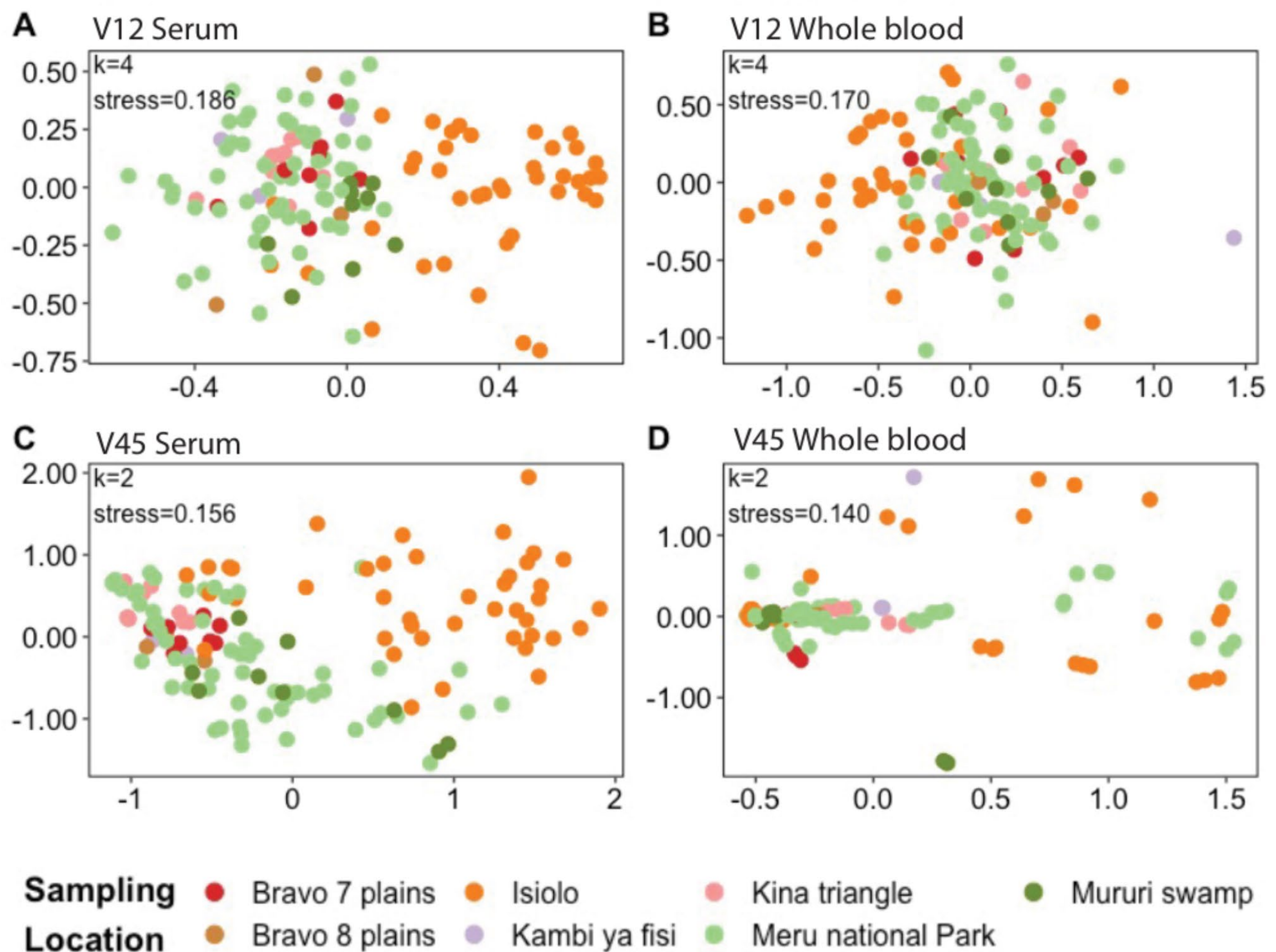

Fig. 3 Non-metric multidimensional scaling (NMDS) of species composition using Bray–Curtis distances. Each amplicon/sample-type dataset was ordinated separately: (**A**) V12 serum, (**B**) V12 whole blood, (**C**) V45 serum, and (**D**) V45 whole blood. Points are colored by the 7 sampling locations. The k and stress for ordinations are provided in each plot

Table 1 Results of the non-metric multidimensional scaling (NMDS) adonis tests for sex and sampling location for both sample types (serum and whole blood) and both 16 S rRNA gene regions that were sequenced

		Sex	Location	Sex * Location
V12 serum	R2	0.013	0.15	0.046
	P-value	**0.021**	**< 0.001**	**< 0.001**
V12 whole blood	R2	0.010	0.080	0.045
	P-value	0.12	**< 0.001**	**< 0.001**
V45 serum	R2	0.018	0.192	0.042
	P-value	**0.004**	**< 0.001**	**< 0.001**
V45 whole blood	R2	0.021	0.131	0.130
	P-value	**0.015**	**< 0.001**	**< 0.001**

Bold P-values denote statistical significance at 0.05

related to an uncultured *Anaplasma* strain reported from cattle in Ethiopia (KY924885), whereas there was 95% support for OTU12 clusters' relatedness to *Anaplasma platys* associated with canine anaplasmosis. Another OTU appears to be closely related to *Anaplasma boleense*, which was reported from the *Anopheles sinensis* mosquito in China. Finally, despite the most prevalent OTU (OTU1) clustering within a clade that included *A. centrale* and *A. marginale*, there was low internal branch support possibly due to short internal branch lengths along a star-like phylogeny.

Likewise, the *Mycoplasma* phylogenetic tree (Fig. 5B) revealed the presence of several distinct *Mycoplasma* species in the buffalo samples. Generally, the splits in *Mycoplasma* tree had a greater resolution compared to the *Anaplasma* tree. The most striking result is that all the high confidence *Mycoplasma* OTUs cluster closely together. This includes OTUs 6 and 4, which were most identified both in serum and whole blood, respectively. Whether this cluster is the result of standing diversity in the local *Mycoplasma* populations or caused by multiple copies of 16 S sequences per genome remains to be resolved. Some OTUs cluster with *M. parvum* (OTU93)

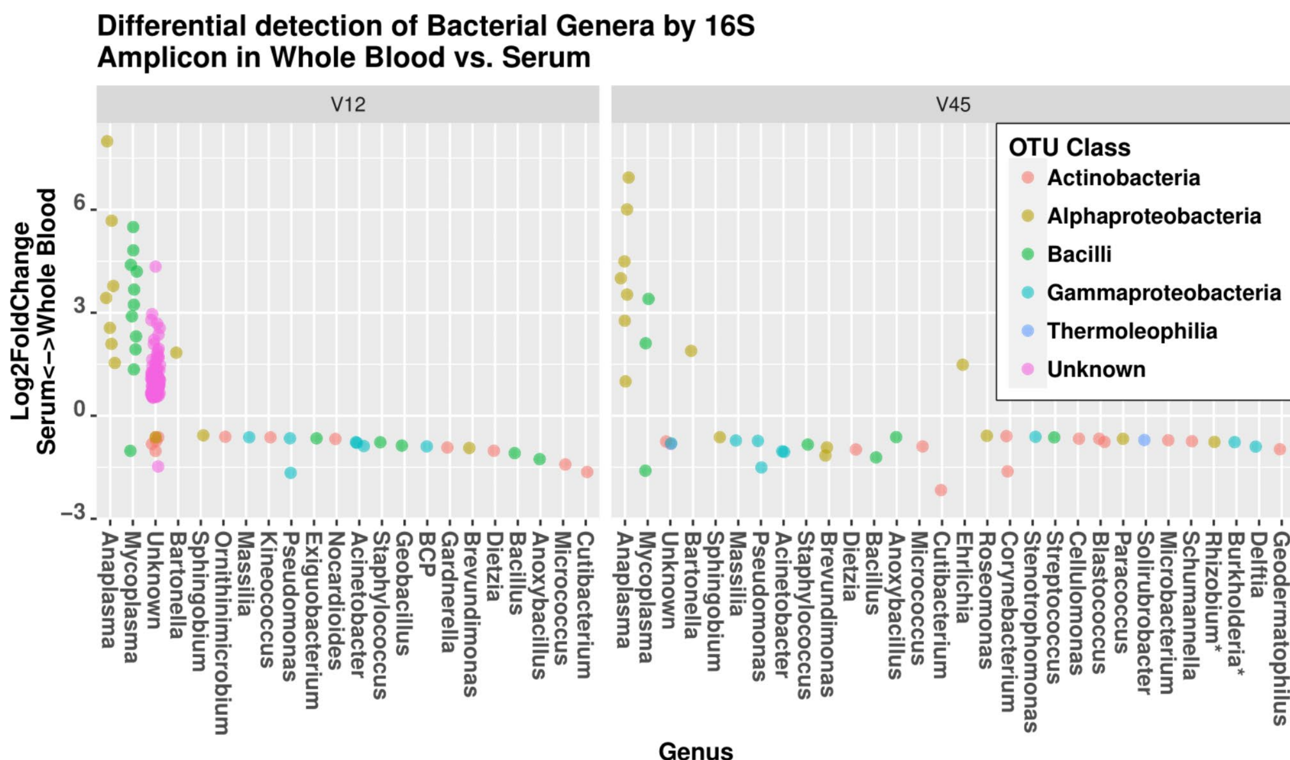

Fig. 4 Differentially identified taxa in whole blood or serum. Each point shows the log2 fold enrichment in whole blood (positive) or serum (negative) of a single OTU. Points are colored based on inferred bacterial class. Points are grouped by their genus classification. *Burkholderia* = Burkholderia, Caballeronia, Paraburkholderia and Rhizobium* = Allorhizobium, Neorhizobium, Pararhizobium, Rhizobium*

and *M. suis* (OTU83), commonly associated with swine whereas one OTU clustered with *M. haemobovis (haemobos)*, associated with cattle. Trees generated with the V45 amplicons largely show the same patterns, but with lower bootstrap support (see Supplemental Fig. 2A-D).

Discussion

The African buffalo is known to be an important reservoir for zoonotic and other pathogens that infect livestock [3, 4, 12]. As sequencing and the ability to agnostically detect microbes in blood increases, it remains unknown if whole blood or serum would be better for surveillance of blood microbiomes in wildlife. Here, we demonstrate the high diversity of blood microbial communities in African buffalo, including significant pathogens, and notably, the high abundance of *Mycoplasma* and *Anaplasma* OTUs. Notably, no *Brucella* spp. reads were identified in the samples, previously considered an important pathogen of risk in African buffaloes [12].

The highest bacterial diversity was from the V12 region in whole blood samples which implies its utility in the interest of detecting the wider bacteria diversity in blood. By sequencing whole blood and serum separately, we found certain taxa that are more likely to be found in either of the sample types. Sequencing the V45 region is more likely to detect *Anaplasma* (*Rickettsiales*) in serum and whole blood compared to the V12 region sequences in which larger proportions of reads were *Mycoplasma* (*Mycoplasmatales*). Depending on the taxa of interest in blood, our results suggest that V12 can be used to detect both *Anaplasma* and *Mycoplasma*, while V45 should be used to detect *Anaplasma*. For the differential detection in serum vs. whole blood, whole blood appears to be the better option for the V12 and V45 regions. It is unclear if other genera in the *Rickettsiales* and *Mycoplasmatales* would follow the same patterns of being more dominant in whole blood compared to serum. We suggest sequencing the whole blood and serum of other animal species to discern whether similar or other biases exist in detecting taxa in different sample types.

In addition to the sample type and variable region sequenced, there were differences in blood microbiome according to sampling location. These results are mainly driven by differences between Meru and Isiolo, the two most sampled locations (Isiolo, $n = 14$; Meru, $n = 32$). These locations also had significantly higher variances in their microbiomes than the other regions, which again, is due to larger sample sizes. The interaction between sex and location may have been driven by the unequal sampling of males and females in these two locations. Isiolo samples were 35.7% female, while Meru samples were 60% female.

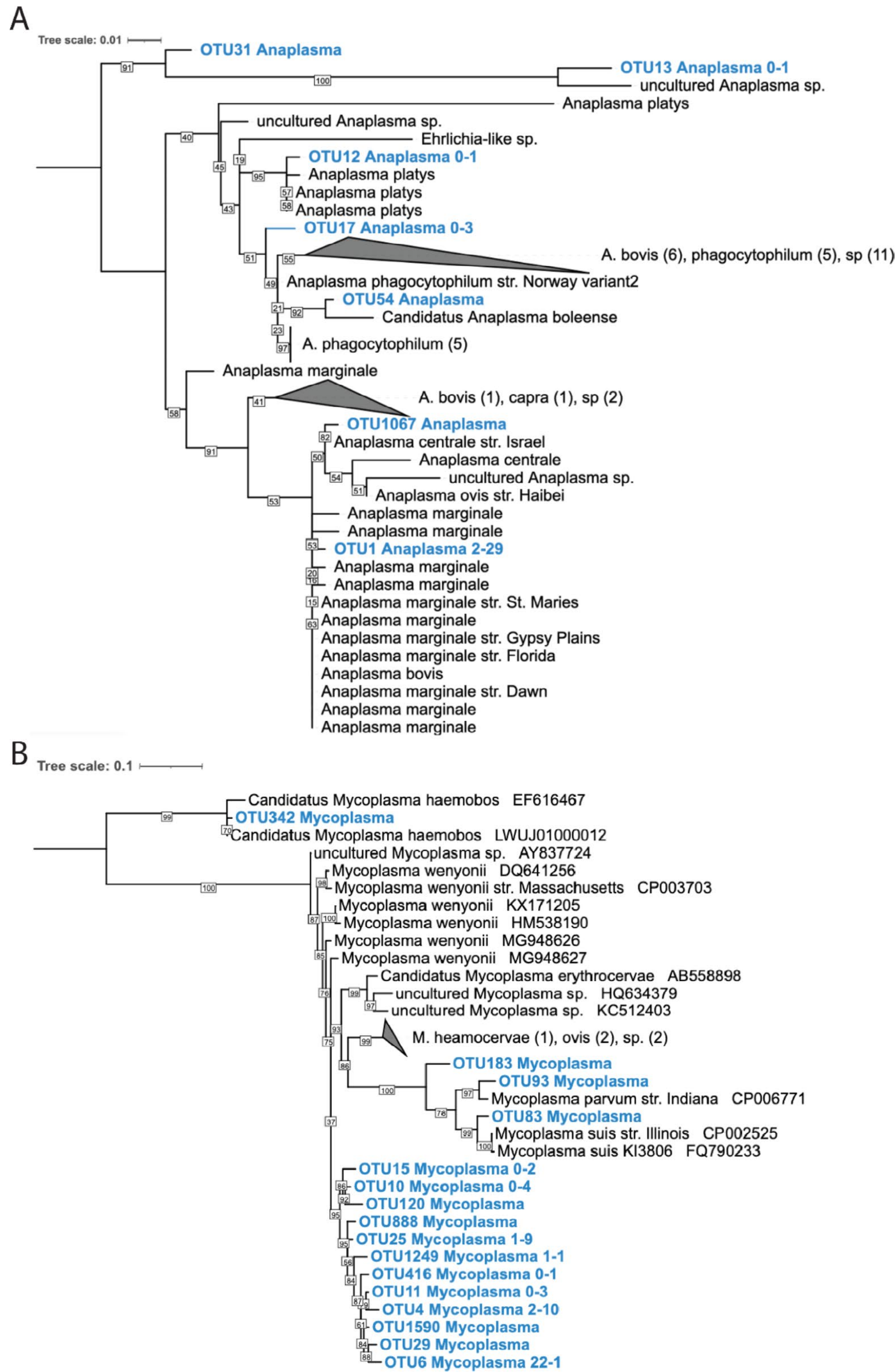

Fig. 5 Phylogenetic trees of (**A**) *Anaplasma* and (**B**) *Mycoplasma* V12 amplicons with related sequences from NCBI and/or SILVA 138.1. Both trees were midpoint rooted and the *Mycoplasma* tree was trimmed to the minimum sub-tree capturing OTU leaves. Placement of OTU sequences are labeled in blue with their inferred genus followed by the number of samples positive with high confidence in whole blood and serum, respectively. Sequences from databases are labeled in black with genus, species, and strain (if available from metadata) followed by sequence database accessions. Several clades were collapsed for plotting. The species labels within collapsed clades are provided with the number of occurrences. Both trees were rendered with iTOL

Significant differences in the microbiomes of buffalo from Isiolo and Meru may also be due to sampling different seasons and the different ecologies of Meru and Isiolo. All samples from Isiolo were collected during the dry season, while those from Meru were collected during the wet season. Additionally, the Meru National Park ecosystem comprises a bushland with open savannah areas which is generally wetter than the dryer savannah habitat characteristic of Isiolo. Arguably, these differences in habitats have been attributed to the differential prevalence of vectors and associated pathogens in wild animal species [35]. Further, the animals in Meru are more contained, while those in Isiolo have more opportunities to interact with livestock [36]. Because of these confounding factors, it is difficult to tease apart how location, seasonality, and host factors contribute to these differences. Future work should investigate sex and seasonal differences in blood microbiomes in wild buffalo across Kenya. It is also worth examining these groups of buffalo in association with their habitat and the consequences of interacting with livestock.

While other studies have found *Mycobacterium* in African buffalo [12], this is the first incidence of *Mycoplasma* in African buffaloes. However, the identification of *Mycoplasma* in African Buffalo is not unexpected because many studies have found varying species and strain in cattle and domestic water buffalo [18, 37–39]. *Mycoplasma* spp. can be a major pathogen in livestock. For instance, *Mycoplasma bovis* has been reported to cause not only bronchopneumonia and mastitis but also affect other major organs in cattle [40]. In the last decade, *M. bovis* infections have spread worldwide and are now responsible for substantial economic impacts on livestock. According to our phylogenetic analysis, the species identified here are most closely related to *M. haemobovis*, *M. parvum*, *M. suis*, and *M. wenyonii. Mycoplasma haemobovis*, *M. parvum*, *M. suis* are established pathogens known to cause disease [41, 42], while *M. wenyonii* is a suspected emerging pathogen [43]. We recommend that additional sequencing and phylogenetic analysis be performed using longer alignments to confirm these results. Additionally, *Mycoplasma* specific cultures could be performed to generate isolates for further differentiation into species.

Anaplasma is a genus of bacteria constituting several species known to be pathogenic in animals and humans. Infected ticks, particularly *Ixodes* species, are the main vectors for *Anaplasma* sp [44]. These *Anaplasma* species include *Anaplasma phagocytophilum*, which causes anaplasmosis in various domestic and wild animals [45], and *Anaplasma marginale*, the most common etiologic agent of bovine anaplasmosis in bovine and ruminant species across Africa [45, 46]. Further, several pathogenic *Anaplasma* species have been reported in camels from Kenya [47–49]. The prevalence for the *A. marginale*-like OTU in our samples according to whole blood high confidence detections are 67 and 86% for V12 and V45 amplicons, respectively. This corroborates findings from previous studies in Africa where up to 70% prevalence of *A. marginale* has been reported in buffalo [13, 50, 51]. This underscores the need for additional studies into *A. marginale* prevalence and, importantly, the impacts on host health.

Serum, and to a lesser degree, whole blood may be susceptible to contamination due to low bacterial DNA content in samples. Here, we show that serum can be used, with caution, to identify some blood microbiome constituents, particularly those at high relative abundances. However, many OTUs were enriched in whole blood, suggesting an underestimation of the prevalence and relative abundances of these (and other) bacteria if only serum samples were sequenced. This might be expected, particularly for intracellular pathogens such as *Anaplasma* and *Mycoplasma* that are depleted in serum. Interestingly, one *Mycoplasma* OTU was strongly enriched in serum samples, both in raw data (Fig. 4) and when filtering for high confidence detections (Supplemental Tables 1 and 2). The reason for this is unknown, but hosts may be mounting active immune responses leading to high amounts of free bacterial DNA in the serum. Regardless, this indicates that sequencing both serum and whole blood might be necessary to get a full picture of the blood microbiome.

We sequenced two variable regions of the 16 S rRNA gene that are typically sequenced for microbiome studies. Mounting evidence suggests that multiple marker genes are required to adequately profile the microbial diversity in individual hosts and environments [50, 52], with different variable regions being recommended for different taxa [53]. Our results provide information on alternative variable regions, as alternative markers for the detection of bacterial pathogens in African buffaloes. It is still unknown whether certain variable regions, or sets of regions, give the most accurate representation of taxa in a sample. Despite these issues, long read sequencing technology may negate the need to choose one or a set of regions for microbiome analysis [54, 55].

One limitation to this study is that the results from the V1-V2 and V4-V5 regions were not compared to the standard V3-V4 region that is typically sequenced. Therefore, we cannot determine whether these two regions provide better resolution of these taxa compared to V3-V4. Additionally, our uneven sampling of buffalo from wet and dry seasons, and the fact that all dry season samples were from one location, did not allow us to reliably compare blood microbiomes between seasons. Additional work should focus on the seasonality of pathogens in buffalo to help tease apart transmission and how the risk to livestock and humans may shift as seasons change.

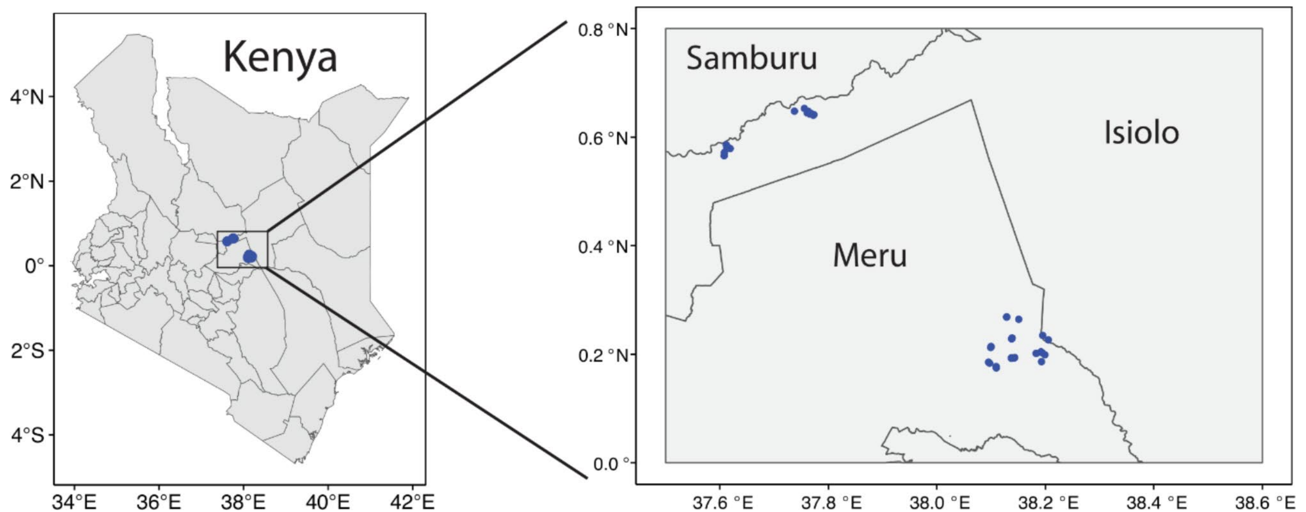

Fig. 6 Map of Africa showing the location of Kenya, and a blown-out map showing the seven-sampling location (shown as blue dots) of the buffalos in Meru and Isiolo counties

Blood contains fewer microbes in apparently healthy animals (or humans) than other body sites such as the gastrointestinal tract or skin. Therefore, DNA or RNA in blood will be lower and the sensitivity of the molecular detection technique or sequencing platform will be important. Although a concept that may not be fully accepted yet, there is accumulating evidence for the existence of a healthy blood microbiome [56]. Since blood has traditionally been seen as a sterile environment, comprising only blood-cells, platelets and plasma, the detection of microbes in blood was consistently interpreted as an indication of infection or sepsis. Despite the growing acceptance of blood microbiome in healthy individuals, there are still uncertainties about whether these bacteria occupy a stable ecological niche or are merely transient residents [56]. For instance, some researchers have suggested that bacteria in the blood may be moving between other sites in the body, particularly the gastrointestinal tract [57]. We hypothesized that wild buffaloes would have both non-pathogenic and pathogenic bacteria in their blood due to wildlife in general being more exposed to microbes in the environment and through vectors.

Conclusions

The findings from this study support the hypothesis that African buffalo have a diverse bacterial blood microbiome. Furthermore, diverse *Anaplasma* and *Mycoplasma* species may reduce livestock productivity and pose zoonotic risks to humans, particularly at the intersections of human, livestock, and wildlife populations. Thus, we recommend microbiome profiling in invertebrate vectors such as ticks that could play a role in the transmission of these pathogens across various vertebrate species. This will be crucial in informing the transmission dynamics and lead to formulation of adequate disease control measures.

Methods

Study site

This study targeted African buffalo (*Syncerus caffer caffer*) populations from protected wildlife areas in Meru National Park, Buffalo Springs and Shaba National Reserves in upper eastern Kenya (Fig. 6). Both the targeted sampling areas are characterized by large populations of wild herbivores that include African elephants, zebra, and diverse antelope species [58, 59]. They also border agro-pastoral areas thus implying livestock-wildlife interactions when herders often drive their animals into the parks in search of pasture during the dry seasons [36]. Sampling was done during the dry season ($n = 14$) in November 2021 and the wet season ($n = 32$) in April 2022.

Sample collection

The animals were sampled by veterinarians from the Kenya Wildlife Service (KWS). The animals were sedated using a combination of Etorphine (9.8 mg/mL) and Azaperone (100 mg/mL). Blood and serum samples collected from 46 buffaloes included 22 female and 24 male animals. About 15 mL of blood was drawn from the jugular vein into sterile EDTA vacutainer tubes (10 mL BD vacutainer© K2E [EDTA, BD]), while 4 ml blood for serum was collected into plain tubes (BD vacutainer©, BD). The anesthesia was reversed using Naltrexone (50 mg/mL). The serum was processed by centrifuging the blood at 1500 g for 15 min and transferred into barcoded 1.5 ml cryovials. Data on geographic coordinates of sampling, age, sex, herd sizes and sample barcodes were collected using Open Data Kit (ODK) database systems (San Diego, USA). The samples were preserved in motorized

freezers at -20 °C before being transported to International Livestock Research Institute (ILRI) laboratories in Nairobi for analysis.

DNA extraction and NGS sequencing

Total deoxyribonucleic acids (DNA) were extracted from each sample using TANBead OptiPure Blood DNA Auto Kit on Maelstrom TANBead Automated DNA extractor (TANBead, Taoyuan, Taiwan). The ready-to-sequence 16 S rRNA gene amplicon library was generated using 16 S Microbial ID Kit (BioID Genomics, Inc. USA) as previously described [60]. The generated library was quantified using Qubit dsDNA HS Assay (ThermoFisher Scientific, USA) while the average size of the library was determined by Agilent High Sensitivity DNA Kit (Agilent Technologies, USA) before pooling. The pooled library was sequenced using MiSeq Reagent kit v2 (500 cycles) on the Illumina MiSeq platform (Illumina Inc., USA) to generate paired-end 250 bp reads. Each sample was sequenced in triplicate where each plate included a no template negative control and ZymoBIOMICS microbial community DNA standard (Zymo, USA) as a positive control.

Bioinformatics and statistical analysis

Demultiplexing, paired-end merging, trimming and analysis of the generated FASTQ files were done on the Rapid Infectious Disease Identification (RIDI) system (Fry Laboratories, LLC. USA) [61]. Reads for V12 and V45 variable regions were extracted from RIDI results files and analyzed separately with a *usearch*-based pipeline [62]. Briefly, unique sequences from concatenated V12 or V45 variable regions were identified from the fastq files using "usearch -fastx_uniques" function. The function *-sortbysize* was used to filter out unique sequences found only once and to sort by length. The operational taxonomic units (OTUs) were generated using the *cluster_otus* method. First, *-cluster_otus* generated OTUs from clusters of 97% identity. Reads were mapped back to both OTUs with *-otutab.* Finally, OTU taxonomies were inferred with *-sintax* using the SILVA version 138.1 small subunit database available at https://www.arb-silva.de/no_cache/download/archive/release_138_1/Exports/SILVA_138.1_SSURef_NR99_tax_silva.fasta.gz [63].

OTU abundance tables and taxonomy were analyzed in R (version 4.1.1), using phyloseq (version 1.40.0) [64], tidyverse (version 2.0.0) [65] and DESeq2 (version 1.36.0) [66]. For all analyses, samples with less than 20 OTU hits and those with less than 5 total reads were filtered out. For high-confidence detection 16 S sequences from corresponding serum and blood, technical replicates were only tagged as positive for an OTU if the relative read abundance was greater than 5%, and an animal was only tagged as positive if all the technical replicates were positive.

To test for agreement between serum and whole blood sequencing, Cohen's Kappa (κ) values were calculated directly for each high confidence OTU positive designation according to [67]. V12 and V45 amplicon agreement was tested similarly except the OTU presence data from above was translated into their corresponding genera according to their SILVA database hits then Cohen's (κ) was calculated. This was required because the V12 and V45 OTUs from the same organism are different sequences and unlinked. Thus, they must be analyzed based on taxonomic assignment instead of the OTU sequence itself.

The V12 and V45 amplicons data and analyzed separately to test for OTU enrichment in either sample type. Sequences from whole blood and serum from each buffalo were paired and analyzed using DESeq2 to build linear models for differential abundances of each detected OTU in whole blood versus serum. These models attempted to detect consistent differences between abundances of a particular OTU in blood or serum while accounting for between replicate variance and the effects of competing amplicons in each reaction. For these models, we chose to use the animal ID as a random effect, with the assumption that each animal has a "true" infectious load that should be reflected in blood and serum equally if there is no differential presence in the two sample types.

Phylogenetic trees were inferred for *Mycoplasma* and *Anaplasma* rRNA amplicons. V12 and V45 amplicons identified as *Mycoplasma* or *Anaplasma* were extracted from the OTU files and aligned to publicly available 16 S sequences. For Anaplasma, only sequences labeled as Anaplasma from the SILVA 138.1 SSURef NR99 database were used. Because the SILVA database contains numerous 16 S sequences of *Mycoplasma*, we first build a tree using curated sequences from NCBI (nucleotide search "(txid2093[ORGN] AND (33175[Bioproject] OR 33317[Bioproject]))". The OTU sequences were contained on a long branch, so additional sequences were deemed necessary. We ran a *Blastn* search of the SILVA database to identify sequences related to the *Mycoplasma* OTUs. Briefly, *Mycoplasma* V12 and V45 OTUs were used as queries separately and the 15 highest scoring sequences for each were retained. These sequences were concatenated together, along with the NCBI 16 S and OTU sequences to make the final dataset for analysis. Alignments were performed with mafft (version v7.520) [68] using default parameters. Poorly aligned regions and those covered by less than 10% of sequences ("-gt 0.9") were removed using Trimal (version v1.4.rev15) [69]. Phylogenetic trees were inferred with IQTree2 (version 2.2.5) [70] using IQtree's internal build of modelfinder

[71] for automatic model selection. Four trees were inferred: *Anaplasma* V12, *Mycoplasma* V12, *Anaplasma* V45, and *Mycoplasma* V45 with TPM3 + I + R2, TVMe + G4, TIM2e + R2, and GTR + F + R3 models, respectively, based on BIC. Support values were calculated from 1000 ultrafast bootstrap replicates. Trees were visualized on iTOL (accessed 4-15-2024) [34]. The V12 and V45 trees were largely the same, so we included the V45 trees with the supplemental information.

To compare bacterial species composition among sampling locations, vegan R package (version 2.5-6) [72] was used to generate non-metric multidimensional scaling (NMDS) of Bray–Curtis distances using the non-rarefied dataset. We transformed the abundance data into proportions for the ordination. We compared species composition between the seven sampling locations and animal sex. To determine significant differences for each variable, we used permutational ANOVAs (PERMANOVAs) using the adonis2 function in the vegan package with 10,000 permutations and included an interaction term between location and sex. We also tested for differences in the dispersion (i.e., variances) between the various locations and sex using the betadisper function also from the vegan package. We tested for dispersion differences based on sample location with the centroid option with 9,999 permutations.

Abbreviations

ANOVA	Analysis of variance
DNA	Deoxyribonucleic acid
EDTA	Ethylenediaminetetraacetic Acid
ILRI	International Livestock Research Institute
KWS	Kenya Wildlife Service
NMDS	Non-metric multidimensional scaling
ODK	Open Data Kit
OTU	Operational taxonomic units
PCR	Polymerase chain reaction
RIDI	Rapid Infectious Disease Identification
RLB	Reverse line blot hybridization

Supplementary Information

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Supplementary Material 1

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Author contributions

AB, FJ and BB conceptualized the study and designed the methodology approach, RN, EM, HA, JA, FG, LW, BR, IL, AM, MM, BB, JF, AB conducted the investigations. RN, EM, AB, JF undertook the formal analysis, BB, AM, JF and AB acquired the resources and funding, RN, EM, JF and AB, wrote the original draft, EM and AB prepared the figures. All authors reviewed and edited the manuscript.

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Data availability

The sequence data from this study will be deposited in the NCBI database (The accession numbers will be provided by the time of manuscript acceptance).

Declarations

Ethics approval and consent to participate

Ethical approval for this work was obtained from the Institutional Research Ethics Committee at the International Livestock Research Institute (reference number: ILRI-IREC2020-07) and Kenya Wildlife Services (permit number WRTI-0092-09-21).

Competing interests

The authors declare no competing interests.

Author details

[1]International Livestock Research Institute, Nairobi, Kenya
[2]Genomics & Bioanalytics, Los Alamos National Laboratory, Los Alamos, NM 87506, USA
[3]Wildlife Research and Training Institute, Naivasha, Kenya
[4]World Organization for Animal Health, Sub-Regional Representation for Eastern Africa, Nairobi, Kenya
[5]Kenya Wildlife Service, Nairobi, Kenya
[6]Zoonotic Disease Unit, Nairobi, Kenya
[7]Department of Medical Microbiology and Immunology, Faculty of Health, University of Nairobi, Nairobi, Kenya
[8]Department of Veterinary Medicine, Dahlem Research School of Biomedical Sciences (DRS), Freie Universität Berlin, Berlin, Germany
[9]Present address: KEMRI-Wellcome Trust Research Programme, Kilifi, Kenya

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References

1. Kingdon J. East African mammals: an atlas of evolution in Africa, volume 3, Part A: carnivores. University of Chicago Press; 1988.
2. Winterbach H. Research review: the status and distribution of Cape buffalo Syncerus caffer caffer in southern Africa. South Afr J Wildl Research-24-month Delayed open Access. 1998;28(3):82–8.
3. Michel AL, Bengis RG. The African buffalo: a villain for inter-species spread of infectious diseases in southern Africa. Onderstepoort J Vet Res. 2012;79(2):26–30.
4. Woodburn DB, Steyl J, Du Plessis EC, Last RD, Reininghaus B, Mitchell EP. Pathological findings in African buffaloes (Syncerus caffer) in South Africa. J S Afr Vet Assoc. 2021;92(1):1–11.
5. Chaisi ME, Sibeko KP, Collins NE, Potgieter FT, Oosthuizen MC. Identification of Theileria parva and theileria sp.(buffalo) 18S rRNA gene sequence variants in the African Buffalo (Syncerus caffer) in southern Africa. Vet Parasitol. 2011;182(2–4):150–62.
6. Collins NE, Allsopp MT, Allsopp BA. Molecular diagnosis of theileriosis and heartwater in bovines in Africa. Trans R Soc Trop Med Hyg. 2002;96:S217–24.
7. Jori F, Alexander KA, Mokopasetso M, Munstermann S, Moagabo K, Paweska JT. Serological evidence of Rift Valley fever virus circulation in domestic cattle and African buffalo in Northern Botswana (2010–2011). Front Vet Sci. 2015;2:63.
8. Michel AL, Bengis RG, Keet D, Hofmeyr M, De Klerk L, Cross P, Jolles AE, Cooper D, Whyte I, Buss P. Wildlife Tuberculosis in South African conservation areas: implications and challenges. Vet Microbiol. 2006;112(2–4):91–100.

9. Omondi GP, Gakuya F, Arzt J, Sangula A, Hartwig E, Pauszek S, Smoliga G, Brito B, Perez A, Obanda V. The role of African buffalo in the epidemiology of foot-and-mouth disease in sympatric cattle and buffalo populations in Kenya. Transbound Emerg Dis. 2020;67(5):2206–21.
10. Palmer M. Mycobacterium bovis: characteristics of wildlife reservoir hosts. Transbound Emerg Dis. 2013;60:1–13.
11. Vosloo W, Bastos A, Sangare O, Hargreaves S, Thomson G. Review of the status and control of foot and mouth disease in sub-saharan Africa. Rev Sci Tech off Int Epizoot. 2002;21(3):437–45.
12. Caron A, Miguel E, Gomo C, Makaya P, Pfukenyi DM, Foggin C, Hove T, de Garine-Wichatitsky M. Relationship between burden of infection in ungulate populations and wildlife/livestock interfaces. Epidemiol Infect. 2013;141(7):1522–35.
13. Eygelaar D, Jori F, Mokopasetso M, Sibeko KP, Collins NE, Vorster I, Troskie M, Oosthuizen MC. Tick-borne haemoparasites in African buffalo (Syncerus caffer) from two wildlife areas in Northern Botswana. Parasites Vectors. 2015;8(1):1–11.
14. Sisson D, Beechler B, Jabbar A, Jolles A, Hufschmid J. Epidemiology of Anaplasma marginale and Anaplasma centrale infections in African buffalo (Syncerus caffer) from Kruger National Park, South Africa. Int J Parasitology: Parasites Wildl. 2023;21:47.
15. Dumic I, Jevtic D, Veselinovic M, Nordstrom CW, Jovanovic M, Mogulla V, Veselinovic EM, Hudson A, Simeunovic G, Petcu E. Human granulocytic anaplasmosis—a systematic review of published cases. Microorganisms. 2022;10(7):1433.
16. Kim Y-J, Seo JY, Kim SY, Lee HI. Molecular detection of Anaplasma phagocytophilum and Ehrlichia species in ticks removed from humans in the Republic of Korea. Microorganisms. 2022;10(6):1224.
17. Li H, Zheng Y-C, Ma L, Jia N, Jiang B-G, Jiang R-R, Huo Q-B, Wang Y-W, Liu H-B, Chu Y-L. Human infection with a novel tick-borne Anaplasma species in China: a surveillance study. Lancet Infect Dis. 2015;15(6):663–70.
18. Erol U, Sahin OF, Altay K. Molecular prevalence of bovine hemoplasmosis in Turkey with first detection of Mycoplasma wenyonii and Candidatus Mycoplasma haemobos in cattle and water buffalo. Vet Res Commun. 2023;47(1):207–15.
19. Thongmeesee K, Chonglomkrod B, Srisakdi C, Saributr M, Suksai P, Kamkong P, Tiawsirisup S. Molecular detection of Mycoplasma wenyonii and its closely related hemotropic Mycoplasma sp. in blood-sucking flies from a buffalo farm in Chachoengsao province. Thail Acta Trop. 2022;235:106647.
20. Anderson K, Ezenwa VO, Jolles AE. Tick infestation patterns in free ranging African buffalo (Syncercus Caffer): effects of host innate immunity and niche segregation among tick species. Int J Parasitology: Parasites Wildl. 2013;2:1–9.
21. Gilbert AT, Fooks A, Hayman D, Horton D, Müller T, Plowright R, Peel A, Bowen R, Wood J, Mills J. Deciphering serology to understand the ecology of infectious diseases in wildlife. EcoHealth. 2013;10:298–313.
22. Michel AL, Van Heerden H, Crossley BM, Al Dahouk S, Prasse D, Rutten V. Pathogen detection and disease diagnosis in wildlife: challenges and opportunities. Revue scientifique et technique. (International Office Epizootics). 2021;40(1):105–18.
23. Ayogu E, Ukwe C, Nna E. Assessing the reliability of microscopy and rapid diagnostic tests in malaria diagnosis in areas with varying parasite density among older children and adult patients in Nigeria. J Postgrad Med. 2016;62(3):150.
24. Bishop RP, Odongo DO, Dolan TT, Dolan RB, Skilton RA, Sayer PD. Theileriosis in mountain bongo repatriated to Kenya: a clinical and molecular investigation. J Zoo Wildl Med. 2019;50(2):342–9.
25. Githaka N, Konnai S, Bishop R, Odongo D, Lekolool I, Kariuki E, Gakuya F, Kamau L, Isezaki M, Murata S. Identification and sequence characterization of novel Theileria genotypes from the waterbuck (Kobus Defassa) in a Theileria parva-endemic area in Kenya. Vet Parasitol. 2014;202(3–4):180–93.
26. Razzauti M, Galan M, Bernard M, Maman S, Klopp C, Charbonnel N, Vayssier-Taussat M, Eloit M, Cosson J-F. A comparison between transcriptome sequencing and 16S metagenomics for detection of bacterial pathogens in wildlife. PLoS Negl Trop Dis. 2015;9(8):e0003929.
27. Williams CL, Caraballo-Rodríguez AM, Allaband C, Zarrinpar A, Knight R, Gauglitz JM. Wildlife-Microbiome interactions and disease: exploring opportunities for disease mitigation across ecological scales. Drug Discovery Today: Disease Models. 2018;28:105–15.
28. Zhu L, Wang J, Bahrndorff S. The wildlife gut microbiome and its implication for conservation biology. Frontiers Media SA; 2021. p. 697499.
29. Tilahun Y, Pinango JQ, Johnson F, Lett C, Smith K, Gipson T, McCallum M, Hoyt P, Tritt A, Yadav A. Transcript and blood-microbiome analysis towards a blood diagnostic tool for goats affected by Haemonchus Contortus. Sci Rep. 2022;12(1):5362.
30. Tsafarova B, Hodzhev Y, Yordanov G, Tolchkov V, Kalfin R, Panaiotov S. Morphology of blood microbiota in healthy individuals assessed by light and electron microscopy. Front Cell Infect Microbiol. 2023;12:1091341.
31. Hao R, Liu Y, Shen W, Zhao R, Jiang B, Song H, Yan M, Ma H. Surveillance of emerging infectious diseases for biosecurity. Sci China Life Sci. 2022;65(8):1504–16.
32. Parker K, Wood H, Russell JA, Yarmosh D, Shteyman A, Bagnoli J, Knight B, Aspinwall JR, Jacobs J, Werking K. Development and optimization of an unbiased, metagenomics-based pathogen detection workflow for infectious disease and biosurveillance applications. Trop Med Infect Disease. 2023;8(2):121.
33. Damerum A, Malka S, Lofgren N, Vecere G, Krumbeck JA. Next-generation DNA sequencing offers diagnostic advantages over traditional culture testing. Am J Vet Res. 2023;84(8).
34. Letunic I, Bork P. Interactive tree of life (iTOL) v5: an online tool for phylogenetic tree display and annotation. Nucleic Acids Res. 2021;49(W1):W293–6.
35. Sehgal RN. Manifold habitat effects on the prevalence and diversity of avian blood parasites. Int J Parasitology: Parasites Wildl. 2015;4(3):421–30.
36. Lengoiboni M, van der Molen P, Bregt AK. Pastoralism within the cadastral system: seasonal interactions and access agreements between pastoralists and non-pastoralists in Northern Kenya. J Arid Environ. 2011;75(5):477–86.
37. Díaz-Sánchez AA, Corona-González B, Meli ML, Álvarez DO, Cañizares EV, Rodríguez OF, Rivero EL, Hofmann-Lehmann R. First molecular evidence of bovine hemoplasma species (Mycoplasma spp.) in water buffalo and dairy cattle herds in Cuba. Parasites Vectors. 2019;12:1–9.
38. Su Q, Song H, Lin R, Yuan Z, Yang J, Zhao G, Huang W, Zhu XQ. The detection of Candidatus Mycoplasma haemobos in cattle and buffalo in China. Trop Anim Health Prod. 2010;42:1805–8.
39. Ybañez AP, Ybañez RHD, Armonia RKM, Chico JKE, Ferraren KJV, Tapdasan EP, Salces CB, Maurillo BCA, Galon EMS, Macalanda AMC. First molecular detection of Mycoplasma wenyonii and the ectoparasite biodiversity in dairy water buffalo and cattle in Bohol, Philippines. Parasitol Int. 2019;70:77–81.
40. Dudek K, Nicholas RA, Szacawa E, Bednarek D. Mycoplasma bovis infections—Occurrence, diagnosis and control. Pathogens (Basel Switzerland). 2020;9(8):640.
41. Meli ML, Willi B, Dreher UM, Cattori V, Knubben-Schweizer G, Nuss K, Braun U, Lutz H, Hofmann-Lehmann R. Identification, molecular characterization, and occurrence of two bovine hemoplasma species in Swiss cattle and development of real-time TaqMan quantitative PCR assays for diagnosis of bovine hemoplasma infections. J Clin Microbiol. 2010;48(10):3563–8.
42. Petri FAM, Sonalio K, de Souza Almeida HM, Ferraz MES, Storino GY, de Souza MR, André MR, de Oliveira LG. Porcine hemothropic mycoplasmas infection associated with productive impact in intensive pig production. Porcine Health Manage. 2020;6:1–8.
43. Kim Y, Kim H, Choi J-H, Cho H-C, Ji M-J, Park Y-J, Park J, Choi K-S. Preliminary report of Mycoplasma Wenoynii and Candidatus Mycoplasma haemobos infection in Korean native cattle. BMC Vet Res. 2024;20(1):121.
44. Rymaszewska A, Grenda S. Bacteria of the genus Anaplasma–characteristics of Anaplasma and their vectors: a review. Vet Med. 2008;53(11):573–84.
45. Said MB, Belkahia H, Messadi L. Anaplasma spp. in North Africa: a review on molecular epidemiology, associated risk factors and genetic characteristics. Ticks tick-borne Dis. 2018;9(3):543–55.
46. Kocan KM, De la Fuente J, Guglielmone AA, Meléndez RD. Antigens and alternatives for control of Anaplasma marginale infection in cattle. Clin Microbiol Rev. 2003;16(4):698–712.
47. Getange D, Bargul JL, Kanduma E, Collins M, Bodha B, Denge D, Chiuya T, Githaka N, Younan M, Fèvre EM. Ticks and tick-borne pathogens associated with dromedary camels (Camelus dromedarius) in northern Kenya. Microorganisms. 2021;9(7):1414.
48. Kidambasi KO, Masiga DK, Villinger J, Carrington M, Bargul JL. Detection of blood pathogens in camels and their associated ectoparasitic camel biting keds, Hippobosca camelina: the potential application of keds in xenodiagnosis of camel haemopathogens. AAS Open Res. 2019;2.
49. Selmi R, Belkahia H, Sazmand A, Said MB, Messadi L. Epidemiology and genetic characteristics of tick-borne bacteria in dromedary camels of the world. Acta Trop. 2022;234:106599.
50. Kameoka S, Motooka D, Watanabe S, Kubo R, Jung N, Midorikawa Y, Shinozaki NO, Sawai Y, Takeda AK, Nakamura S. Benchmark of 16S rRNA gene amplicon sequencing using Japanese gut microbiome data from the V1–V2 and V3–V4 primer sets. BMC Genomics. 2021;22(1):527.

51. Makgabo SM, Brayton KA, Oosthuizen MC, Collins NE. Unravelling the diversity of Anaplasma species circulating in selected African wildlife hosts by targeted 16S microbiome analysis. Curr Res Microb Sci. 2023;5:100198.
52. Sperling JL, Silva-Brandão K, Brandão M, Lloyd V, Dang S, Davis C, Sperling F, Magor K. Comparison of bacterial 16S rRNA variable regions for microbiome surveys of ticks. Ticks tick-borne Dis. 2017;8(4):453–61.
53. Kim M, Morrison M, Yu Z. Evaluation of different partial 16S rRNA gene sequence regions for phylogenetic analysis of microbiomes. J Microbiol Methods. 2011;84(1):81–7.
54. Combrink L, Humphreys IR, Washburn Q, Arnold HK, Stagaman K, Kasschau KD, Jolles AE, Beechler BR, Sharpton TJ. Best practice for wildlife gut microbiome research: a comprehensive review of methodology for 16S rRNA gene investigations. Front Microbiol. 2023;14:1092216.
55. Sharpton TJ. An introduction to the analysis of shotgun metagenomic data. Front Plant Sci. 2014;5:209.
56. Castillo DJ, Rifkin RF, Cowan DA, Potgieter M. The healthy human blood microbiome: fact or fiction? Front Cell Infect Microbiol. 2019;9:449041.
57. Païssé S, Valle C, Servant F, Courtney M, Burcelin R, Amar J, Lelouvier B. Comprehensive description of blood microbiome from healthy donors assessed by 16 S targeted metagenomic sequencing. Transfusion. 2016;56(5):1138–47.
58. Georgiadis NJ, Olwero JN, Romañach SS. Savanna herbivore dynamics in a livestock-dominated landscape: I. Dependence on land use, rainfall, density, and time. Biol Conserv. 2007;137(3):461–72.
59. Litoroh M, Ihwagi FW, Mayienda R, Bernard J, Douglas-Hamilton I. Total aerial count of elephants in Laikipia-Samburu ecosystem in November 2008. Nairobi, Kenya: Kenya Wildlife Service; 2010.
60. Bartlow AW, Moser SK, Ellis JE, Hathcock CD, Fair JM. Comparing western (Megascops kennicottii) and whiskered (M. trichopsis) screech-owl microbiomes in southern Arizona using a novel 16S rRNA sequencing method. Anim Microbiome. 2022;4(1):45.
61. Ellis JE, Missan DS, Shabilla M, Martinez D, Fry SE. Rapid infectious disease identification by next-generation DNA sequencing. J Microbiol Methods. 2017;138:12–9.
62. Edgar R. Usearch. Lawrence Berkeley National Lab.(LBNL). Berkeley, CA: United States); 2010.
63. Cole JR, Wang Q, Fish JA, Chai B, McGarrell DM, Sun Y, Brown CT, Porras-Alfaro A, Kuske CR, Tiedje JM. Ribosomal database project: data and tools for high throughput rRNA analysis. Nucleic Acids Res. 2014;42(D1):D633–42.
64. McMurdie PJ, Holmes S. Phyloseq: an R package for reproducible interactive analysis and graphics of microbiome census data. PLoS ONE. 2013;8(4):e61217.
65. Wickham H, Averick M, Bryan J, Chang W, McGowan LDA, François R, Grolemund G, Hayes A, Henry L, Hester J. Welcome to the Tidyverse. J open Source Softw. 2019;4(43):1686.
66. Love M, Anders S, Huber W. Differential analysis of count data–the DESeq2 package. Genome Biol. 2014;15(550):10–1186.
67. Cohen J. A coefficient of agreement for nominal scales. Educ Psychol Meas. 1960;20(1):37–46.
68. Katoh K, Standley DM. MAFFT multiple sequence alignment software version 7: improvements in performance and usability. Mol Biol Evol. 2013;30(4):772–80.
69. Capella-Gutiérrez S, Silla-Martínez JM, Gabaldón T. trimAl: a tool for automated alignment trimming in large-scale phylogenetic analyses. Bioinformatics. 2009;25(15):1972–3.
70. Nguyen L-T, Schmidt HA, Von Haeseler A, Minh BQ. IQ-TREE: a fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. Mol Biol Evol. 2015;32(1):268–74.
71. Kalyaanamoorthy S, Minh BQ, Wong TK, Von Haeseler A, Jermiin LS. ModelFinder: fast model selection for accurate phylogenetic estimates. Nat Methods. 2017;14(6):587–9.
72. Oksanen J, Blanchet FG, Friendly M, Kindt R, Legendre P, McGlinn D, Minchin PR, O'Hara R, Simpson GL, Solymos P. vegan: Community Ecology Package. R package version 2.5-6. 2019. Community Ecol. 2020;8:732 – 40.

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