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Bacterial Microbiota of the Brown Dog Tick (*Rhipicephalus sanguineus*), a Broad Starting Point to Establish Potential Pathogens in Northern Mexico

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Abstract: Ticks are important vectors of pathogenic bacteria that cause diseases in both humans and animals. Analysis of tick microbiota via massive sequencing allows rapid and comprehensive identification of almost all bacteria inhabiting ticks. This has improved the detection of emerging pathogens and has helped define their relationship with public health. In Mexico, the brown dog tick (*Rhipicephalus sanguineus* sensu lato) is a public health problem, especially in northeast Durango. In the present study, the bacterial microbiota of this tick was determined using third-generation massive sequencing (PacBio, V1–V9 region of the 16S rRNA gene); bacteria with pathogenic potential that are transmitted by salivation and those that can be transmitted by accidental regurgitation of the parasite were also identified. In 2024, 60 dogs were searched for unfed ticks; then, 15 groups of female ticks and 15 groups of male ticks were formed, with each group consisting of 30 individuals. DNA was extracted from each tick pool, and the complete 16S rRNA gene was amplified (PacBio). Bioinformatics analysis was performed in QIIME2 (Quantitative Insights into Microbial Ecology) to obtain amplicon sequence variants (ASVs). Alpha and beta diversity metrics, as well as statistical analyses, were performed to test for differences between the microbiota of females and males. The bacterial taxa were classified into 21 phyla, 24 classes, 81 orders, 137 families, 339 genera, and 565 species. The male microbiota presented a significantly greater number of ASVs and a greater phylogenetic diversity index (FaithPD). Additionally, the unweighted UniFrac metric was significantly different between the sexes. The endosymbiont *Coxiella mudrowiae* was significantly more abundant in females, and *Ehrlichia canis* was more abundant in males. The pathogens *E. canis* and *Anaplasma platys* (transmitted by salivation) were detected, as well as 75 species of potential pathogens recorded in this tick that could enter the host in case of accidental regurgitation of the parasite (e.g., *Staphylococcus*, *Streptococcus*, *Acinetobacter*, *Corynebacterium*, *Proteus*, *Clostridum*, *Klebsiella*, *Bacillus*, *Escherichia*, *Fusobacterium*, and *Pasteurella*). It is necessary to continue analyzing the microbiota of ticks through massive sequencing for the benefit of public health and to establish new alternatives for controlling these parasites.

Keywords: PacBio; 16S rRNA; Durango; *Coxiella mudrowiae*; *Ehrlichia canis*; *Anaplasma platys*

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Citation: García-De la Peña, C.; Zamudio-López, A.; Barraza-Guerrero, S.I.; Martínez-Aranda, E.; De la Cruz-Ramos, J.M.; Acosta-Ayala, A.; Siller-Rodríguez, Q.K.; Torres-Delgado, M.G.; Ávila-Rodríguez, V.; Vásquez-Arroyo, J.; et al. Bacterial Microbiota of the Brown Dog Tick (*Rhipicephalus sanguineus*), a Broad Starting Point to Establish Potential Pathogens in Northern Mexico. *Microbiol. Res.* **2024**, *15*, 2507–2521. https://doi.org/10.3390/microbiolres15040167

Academic Editor: Edson Roberto da Silva

Received: 12 November 2024
Revised: 28 November 2024
Accepted: 28 November 2024
Published: 30 November 2024

1. Introduction

Ticks are among the primary vectors of viruses, bacteria, protozoa, fungi, and nematodes worldwide [1] and are ranked second as transmitters of human diseases after

mosquitoes [2]. In recent years, numerous studies have been conducted on the bacterial microbiota of various tick species around the world [3], as this group of microorganisms are agents of serious diseases such as Rocky Mountain spotted fever (*Rickettsia rickettsii*; Ref. [4]) and Lyme disease (*Borrelia burgdorferi*; Ref. [5]). These studies have shown that ticks not only carry bacteria with pathogenic potential but also harbor a bacterial community composed of endosymbionts (e.g., *Midichloria*, *Arsenophonus*, *Coxiella*, *Francisella*, *Rickettsia*), which provide nutritional benefits (vitamins, essential amino acids, sterols, and enzymes absent in their hosts) and play an important role in the transmission of pathogens to hosts [6–8]. This information is ecologically interesting; however, in regions with a high incidence of tick bites, it is essential to have a reference bacterial database that supports public health programs, and that helps to find alternatives to control the transmission of tick-borne diseases. In this context, massive sequencing technologies (next-generation sequencing, third-generation sequencing, and shotgun sequencing) are highly useful, as they allow for the rapid and cost-effective identification of nearly all microorganisms inhabiting ticks [9].

The brown dog tick (*Rhipicephalus sanguineus*) is an ectoparasite distributed worldwide [10] (Figure 1). Although it has a certain specificity for parasitizing dogs, humans, and other mammals can be accidental hosts [11,12]. The endophilic behavior of this tick represents a potential threat to public health. Several studies have used next-generation sequencing (16S rRNA gene regions) to determine the microbiota of *R. sanguineus*. For example, Lalzar et al. [13] used the V4–V6 region in ticks from Israel and reported 90% dominance of the genus *Coxiella*, followed by *Rickettsia* (10%). René-Martellet et al. [14] analyzed the bacterial communities associated with *R. sanguineus* from France, Senegal, and Arizona, USA, using the V5–V6 region and reported high abundances of the bacterial genera *Coxiella*, *Rickettsia*, and *Bacillus*. In Spain, Portillo et al. [15] used the V3–V4 region and reported that the family Coxiellaceae and the genus *Rickettsia* were the most abundant taxa. In Nigeria, Agwunobi et al. [16] amplified the V4 region, reporting 300 bacterial genera in *R. sanguineus*, with *Cetobacterium* being the most abundant.

Figure 1. Adult male (**a**) and adult female (**b**) of the brown dog tick (*Rhipicephalus sanguineus*) from northeast Durango, Mexico. Millimeter ruler is shown below for size reference.

In Brazil, Luzii et al. [17] analyzed the microbiota of female *R. sanguineus* from tropical and temperate lineages using the V4–V5 region and reported that the phylum Proteobacteria and the genus *Coxiella* predominated. Recently, Paez-Triana et al. [18] used the shotgun technique on *R. sanguineus* ticks from Colombia, where the family Coxiellaceae was dominant in all samples. For bacteria with potential pathogenicity to humans, *R. sanguineus* can transmit *Anaplasma phagocytophilum*, *A. platys*, *Bartonella* spp., *Borrelia burgdorferi*, *Coxiella burnetii*, *Ehrlichia canis*, *E. chaffeensis*, *E. ewingii*, *Mycoplasma haemocanis*, *Rickettsia amblyommatis*, *R. conorii*, *R. rickettsii*, *R. rhipicephali*, *R. parkeri*, *R. typhi*, and Candidatus *Neoehrlichia*

mikurensis [11,14,19–24]. However, the risk this tick poses to public health in North America may be increasing due to the evident global warming over the past few decades [25,26]. This is because the increasing temperature trends resulting from climate change are expanding and/or changing the potential distributions of several tick species (including *R. sanguineus*) toward higher latitudes and elevations [27]. This will result in an increased number of people coming into contact with this tick species; therefore, it is essential to investigate its microbiota throughout its distribution and evaluate it over time, seeking significant changes.

In Mexico, this tick inhabits almost the entire territory in the form of two lineages: tropical (*R. sanguineus* sensu lato) and temperate (*R. sanguineus* sensu stricto) [27,28]. The tropical lineage predominates in almost the entire country, whereas the temperate lineage is located in the northern territory along the border with the United States [29]. The state of Durango is located in the north-central region of Mexico. The northeast of the state is a region known as Comarca Lagunera de Durango, where *R. sanguineus* s.l. is currently a public health problem due to the number of people who have fallen ill or died after being bitten by this arthropod [30,31]. To date, there are no exhaustive microbiological studies on this tick species in Mexico; therefore, this study aimed to determine the internal bacterial microbiota of *R. sanguineus* using third-generation massive sequencing (V1–V9 region of the 16S rRNA gene), which can identify more than 98% of the bacterial species in a sample [32]. Likewise, bacteria previously reported as potential pathogens for humans are indicated. This includes those bacteria transmitted through salivation (i.e., bacteria residing in the salivary glands) or those that may be accidentally regurgitated by the tick (i.e., bacteria residing in its midgut). This information will enhance the current microbiological understanding of this tick species globally and simultaneously expand the ability to manage disease risk, benefiting public health in this region of Mexico.

2. Materials and Methods

The study area included the municipalities of Gómez Palacio, Lerdo, Tlahualilo, and Mapimi, which are located in the northeast of the state of Durango, Mexico (Figure 2). This region has a very arid climate, with an average annual temperature of 25.5 °C and an average annual precipitation of 264 mm [33]. From March to April 2024, the streets of the four municipalities were searched for abandoned dogs. In total, 60 dogs were searched for ticks (15 dogs selected at random from each municipality) regardless of age or sex. The search for parasites included the ears, back, chest, and legs of the dogs. Living ticks were collected using tweezers and placed in plastic jars.

The taxonomic identity of the ticks was confirmed using the taxonomic keys of Castillo-Martínez et al. [34] Only unfed *R. sanguineus* ticks (not swollen from drinking dog blood) were selected to avoid recovering bacteria from the dogs' blood. The ticks were washed twice with sterile water and 1% sodium hypochlorite (chlorine) to eliminate bacteria from the exoskeleton [35]. Then, 15 groups of females and 15 groups of males were formed; each group consisted of 30 individuals (a total of 900 ticks were analyzed). Each tick was dissected in a cross-section using a stereoscope and scalpel blades. Each tick pool was placed in a BashingBead tube (Zymo Research®, Irvine, CA, USA) with silicon beads and 750 µL of lysis buffer (Zymo Research®). The tubes were then processed in a cell disruptor (TerraLyzer®, Irvine, CA, USA) for 30 sec for tissue maceration and DNA preservation. DNA extraction from each pool was performed using a ZymoBiomics Kit (Zymo Research®) in a laminar flow hood. The pools were sent to Zymo Research Corp. in the USA, where third-generation massive sequencing was carried out using a PacBio Sequel II Kit [32]. The full-length 16S sequencing library was prepared following PacBio's complete 16S amplification protocol (https://www.pacb.com). The 16S gene was amplified using the barcoded primers 27f (AGRGTTYGATYMTGGCTCAG) and 1492r (RGYTACCTTGTTACGACTT3) with adapters. Two nanograms of DNA were used as a PCR template for each sample. PCR was run with Kapa Hifi HotStart DNA polymerase for 25 cycles following the conditions described in the protocol. After that, the PCR product

from each reaction was cleaned with a Select-a-Size DNA Clean & Concentrator MagBead Kit (Zymo Research), keeping the fragments > 300 bp. The library from each reaction was then quantified using a NanoDrop, and all the DNA samples were combined. The pooled library was processed with an SMRTbell 3.0 Prep Kit for PacBio. Positive and negative controls were used with ZymoBiomics standards (Zymo Research). Sequencing was performed on an 8M SMRT cell on the Sequel II system.

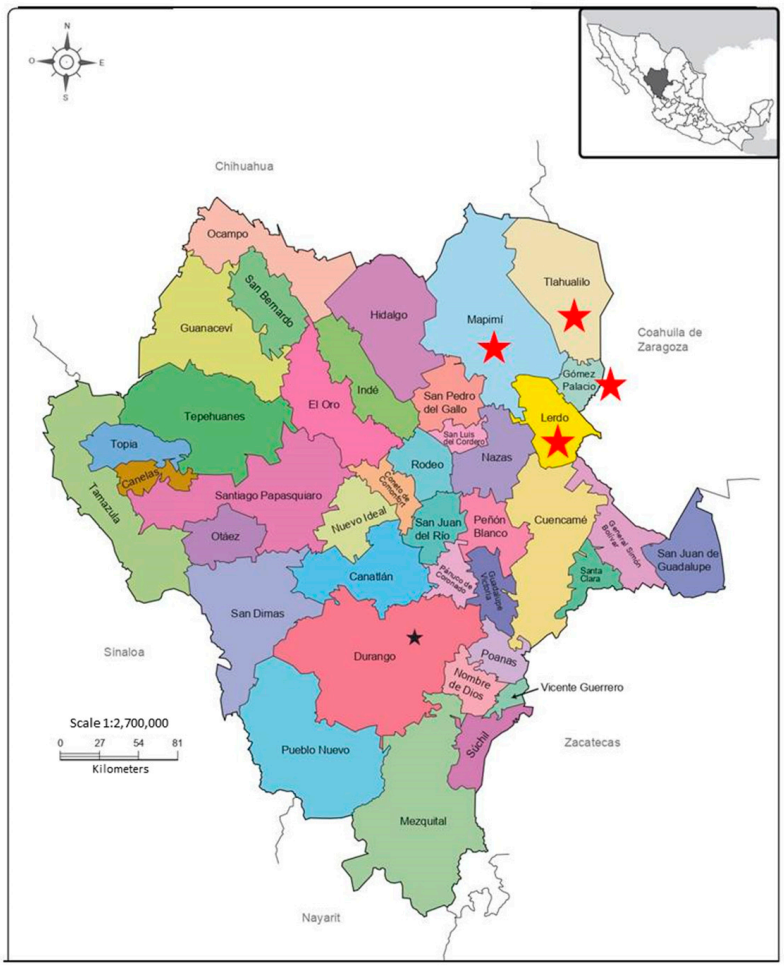

Figure 2. Location of the municipalities of northeast Durango, Mexico, where the collection of *Rhipicephalus sanguineus* (red star) ticks was carried out. Capital city of the state of Durango (black star). INEGI, Geostatistical Framework, December 2018.

Sequence analysis was performed in QIIME2 (Quantitative Insights into Microbial Ecology) in Linux-Ubuntu language [36] using the DADA2 algorithm (Division Amplicon Denoising Algorithm, Ref. [37]) to refine the sequences and obtain amplicon sequence variants (ASVs). Taxonomy assignment was performed with the sklearn method using the Greengenes2 taxonomic database [38]. ASVs at the species level were confirmed in the National Center for Biotechnology Information (NCBI) database using the Basic Local Alignment Search Tool (BLAST) tool, which has an E value of 0.0 and a minimum identity percentage of 97%. The relative abundance of the dominant species was graphed using heatmaps in the Morpheus program (Broad Institute).

To compare the bacterial microbiota between the sexes of *R. sanguineus*, a rarefaction process was performed to unify the number of sequences for all the samples; with this new absolute abundance file, four alpha diversity metrics were obtained (number of ASVs, Shannon index, Pielou evenness and Faith's phylogenetic diversity index), and a Kruskal-Wallis test ($p < 0.05$) was applied to each one to test for significant differences between the sexes. Significant alpha diversity metrics were visualized in boxplots using GraphPad

Prism ver. 8.0.2. Four beta diversity metrics were subsequently obtained: (1) Jaccard [39], a qualitative dissimilarity metric based on the presence/absence of species between communities that varies between 0 (communities have no species in common) and 1 (communities are identical in terms of species present); (2) Bray-Curtis [40], a quantitative dissimilarity metric based on the abundance of each species in the communities, varying between 0 (the two communities are identical in species composition and abundance) and 1 (the two communities do not share species at all); (3) Unweighted UniFrac and (4) weighted UniFrac. Both UniFrac [41] metrics generate a dissimilarity value that varies between 0 and 1 (0 when communities are completely identical in terms of phylogenetic composition (in Unweighted) or composition and abundance (in Weighted), and 1 when communities do not share a phylogeny, and all lineages are exclusive to a community). PERMANOVA tests were applied ($p < 0.05$) to each beta diversity metric between sexes. Significant beta diversity metrics were visualized in PCoA graphs in Emperor [42]. To determine the bacterial taxa causing dissimilarity between sexes, SIMPER analyses [43] were applied at the family and species levels; subsequently, Mann-Whitney tests ($p < 0.05$) were performed between sexes for the taxa that made the greatest contribution to dissimilarity.

3. Results

A total of 42,020 sequences were obtained for females and 35,832 for males; after processing with DADA2, the average number of quality sequences (nonchimeric) for females was 27,994 (66.6%), and that for males was 24,080 (67.2%) (Table S1). The total number of ASVs for both sexes was 1720 (733 for females and 1251 for males), which were classified into 21 phyla, 24 classes, 81 orders, 137 families, 339 genera, and 565 species (Table S2). The sequences of those 565 species were blasted at NCBI, and 290 had a taxonomic name (Table S3). Among the 21 phyla determined, females harbored 10 (Proteobacteria $\bar{x}$ = 81.27%, Firmicutes_D $\bar{x}$ = 13.96%, Actinobacteriota $\bar{x}$ = 4.30%), and males harbored 21 (Proteobacteria $\bar{x}$ = 76.53%, Firmicutes_D $\bar{x}$ = 20.35%, Bacteroidota $\bar{x}$ = 1.57%). At the family level, 74 taxa were classified in females (Coxiellaceae $\bar{x}$ = 70.86%, Staphylococcaceae $\bar{x}$ = 11.96%, Moraxellaceae $\bar{x}$ = 4.79%), and 123 were classified in males (Coxiellaceae $\bar{x}$ = 54.53%, Staphylococcaceae $\bar{x}$ = 17.77%, Anaplasmataceae $\bar{x}$ = 10.68%). Females reported 166 genera and males 288, whereas at the species level, females presented 280 taxa (*Coxiella mudrowiae* $\bar{x}$ = 70.86%, *Staphylococcus pseudintermedius* $\bar{x}$ = 9.85%, *Acinetobacter variabilis* $\bar{x}$ = 3.28%, *Corynebacterium amycolatum* $\bar{x}$ = 3.32%, *Proteus mirabilis* $\bar{x}$ = 3.35%, *Ehrlichia canis* $\bar{x}$ = 0.87%, *Ac. indicus* $\bar{x}$ = 0.86%, *Ac. schindleri* $\bar{x}$ = 0.26%, *Anaplasma platys* $\bar{x}$ = 0.24%), whereas males presented 469 taxa (*C. mudrowiae* $\bar{x}$ = 54.53%, *S. pseudintermedius* $\bar{x}$ = 16.28, *E. canis* $\bar{x}$ = 8.76%, *Ac. variabilis* $\bar{x}$ = 2.12%, *Ac. schindleri* $\bar{x}$ = 2.29%, *A. indicus* $\bar{x}$ = 1.33%, *An. platys* $\bar{x}$ = 1.92%); Figure 3.

Alpha diversity metrics were significantly different between the sexes of *R. sanguineus* in terms of the number of ASVs observed and the Faith index (H = 3.88, $p = 0.048$; H = 11.709, $p = 0.0006$; respectively; Figure 4). Shannon and Pielou's evenness results did not significantly differ (H = 1.39, $p = 0.237$; H = 1.35, $p = 0.210$, respectively). In terms of beta diversity, the unweighted UniFrac metric was significantly different between the sexes (PERMANOVA pseudo-F = 2.173, $p = 0.003$; Figure 5), but the other metrics were not significantly different (Bray-Curtis PERMANOVA pseudo-F = 1.909, $p = 0.093$; Jaccard PERMANOVA pseudo-F = 1.126, $p = 0.165$; weighted UniFrac PERMANOVA pseudo-F = 2.332, $p = 0.064$).

According to the results of the SIMPER analysis, at the family level, there was a global dissimilarity in the microbiota of 49.92% between the sexes of *R. sanguineus*, with Coxiellaceae being significantly more abundant in females ($\bar{x}$ = 20,285.3 sequences) than in males ($\bar{x}$ = 13,170.8 sequences), U = 61, $p = 0.034$, and *E. canis* more abundant in males ($\bar{x}$ = 2023.9 sequences) than in females ($\bar{x}$ = 214.4 sequences), U = 40, $p = 0.002$. Notably, although no significant difference was observed, *Anaplasma platys* tended to be more abundant in males ($\bar{x}$ = 414.8 sequences) than in females ($\bar{x}$ = 70.1 sequences).

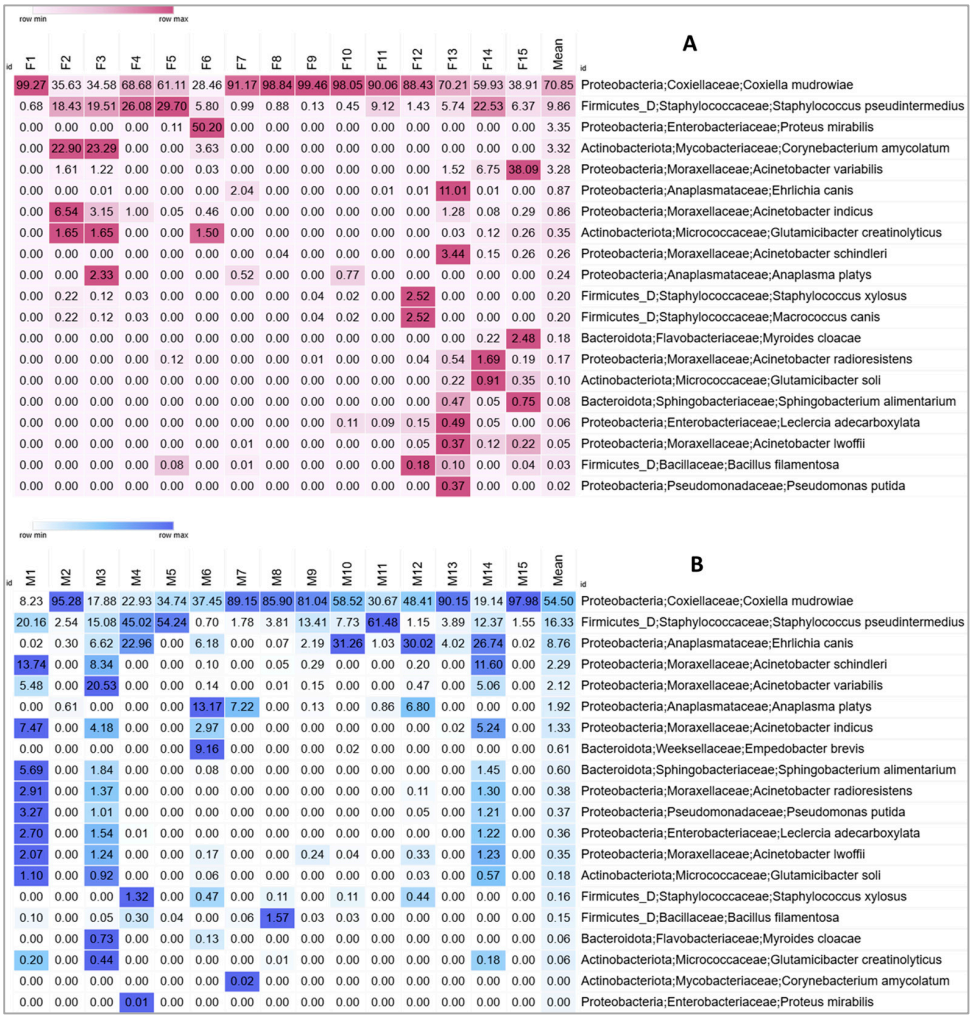

Figure 3. Heatmaps showing the relative abundances of the most abundant taxa in the internal bacterial microbiota of female (**A**) and male (**B**) *Rhipicephalus sanguineus* ticks in northeast Durango, Mexico.

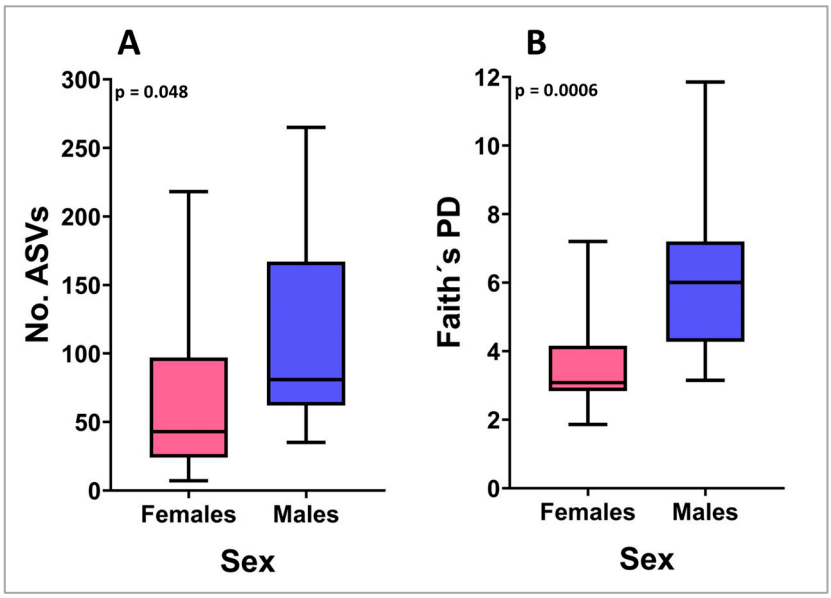

Figure 4. Boxplots of the number of ASVs (**A**) and Faith's phylogenetic diversity index values (**B**) of the bacterial microbiota of both sexes of the tick *Rhipicephalus sanguineus* in northeast Durango, Mexico.

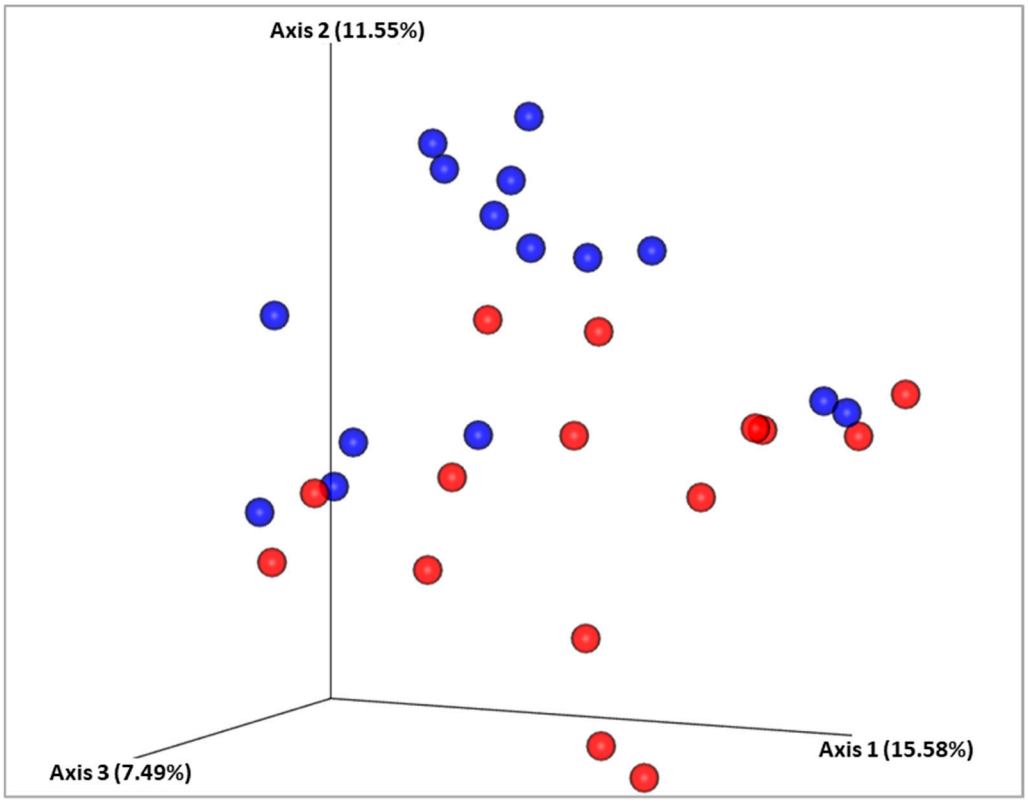

Figure 5. PCoA using the unweighted UniFrac metric, revealing dissimilarity between the female (red dots) and male (blue dots) bacterial microbiotas of the tick *Rhipicephalus sanguineus* in northeast Durango, Mexico.

4. Discussion

The study of tick microbiota is essential for expanding the knowledge of microbial ecology, improving public health programs, and the management of diseases transmitted by these arthropods. This finding also opens the possibility of using symbiotic bacteria in the development of new techniques for blocking pathogens or eliminating ticks.

4.1. Ecological Context of the Rhipicephalus sanguineus Microbiota

The results that were obtained in the present study revealed a high diversity of bacteria that compose the microbiota of brown dog ticks in northeast Durango, Mexico, compared with studies of this tick performed in other countries (Israel [13], France, Senegal, USA [14], Spain [15], Nigeria [16], and Brazil [17]), where the microbiota was dominated (75–99%) by few bacterial genera (such as *Coxiella*, *Rickettsia*, *Bacillus*, and *Cetobacterium*). In a recent study conducted in Colombia, Paez-Triana et al. [18] used the shotgun sequencing technique to characterize the microbiota of *R. sanguineus*; specifically, they identified 807 bacterial genera, including *Coxiella*, *Bacillus*, *Clostridium*, *Pseudomonas*, *Rickettsia*, *Streptomyces*, *Propionibacterium*, *Burkholderia*, *Fusobacterium*, *Lactobacillus* and *Campylobacter*. In the present study, the use of third-generation sequencing (PacBio) considering the entire 16S rRNA gene (V1–V9) and the ASVs approach offered the advantage of achieving species classification in 290 of the 565 features recorded. Importantly, the type of sequencing technology that is used (such as pyrosequencing, Illumina, or PacBio), as well as the regions of the 16S rRNA gene that are analyzed (or the complete DNA as in shotgun sequencing), can influence the observed differences among the different studies. Although PacBio technology is obviously more expensive than those focusing on 16S rRNA single regions, the benefit of accurately determining the bacteria species (ASVs) of the microbiota of this tick is clear. For example, unlike most previous studies of the microbiota of *R. sanguineus*, in the present study, the classification of *Coxiella* species was achieved (*C. mudrowiae*). It

is important to determine the species of *Coxiella* that resides in ticks due to the fact that these bacteria are known to provide considerable benefits to these parasites. Numerous experimental [44] and field studies have demonstrated the *Coxiella* genus (*Coxiella*-like or CLE) as being the dominant or most abundant genus in *R. sanguineus* in Israel [13], France, Senegal, Arizona, USA [14], Nigeria [16], China [45] and India [46]. CLEs offer the following benefits to their host: (1) providing additional nutrients to the tick (amino acid cofactors, rivoflavin (B2), biotin (B7), and folic acid (B9)); (2) increasing blood feeding; (3) increasing resistance to pathogens; (4) promoting nitrogen recycling; and (5) improving reproductive success [47,48]. To date, the benefits that *C. mudrowiae* may be providing to *R. sanguineus* in the population of Durango, Mexico, are unknown. However, these benefits may be very relevant since *C. mudrowiae* was the most abundant bacterial species in both sexes and was significantly dominant in females ($\bar{x}$ = 70.86%). This same situation was reported previously in *R. sanguineus* ticks from Colombia [18] and *Haemaphysalis* spp. in Thailand [49], where *C. mudrowiae* was the most abundant bacterial species in females. Although the particular role that *C. mudrowiae* may play in ticks has not yet been studied, it is likely that this bacterium is essential for obtaining nutrients and promoting successful reproduction in females. In fact, Ben-Yosef et al. [44] demonstrated a high dependence of *R. sanguineus* on CLEs; according to their results, these bacteria predominate in nymphs and adult females. The authors argued that CLEs are more important in females than in males because they proliferate rapidly in adulthood, colonizing the ovaries and transmitting them vertically to progeny. Therefore, a large amount of CLEs in a female can supplement the nutrients she requires for successful reproduction. At the same time, the dominance of *C. mudrowiae* in female ticks from Durango, Mexico, decreases their bacterial diversity with respect to males. In fact, it was recorded that males presented greater diversity than females according to the number of ASVs and the phylogenetic diversity index (Faith). The dominance of *C. mudrowiae* in females likely limits the presence and abundance of other bacteria (including pathogens), which leads to less diversity. In contrast, the lower abundance of this endosymbiont in males allows other species of bacteria to be present in their microbiota, and in the case of some pathogens (e.g., *E. canis*, *A. platys*), their abundance is higher than in females. This was also noted by Paez-Triana et al. [18], who reported an inverse correlation between endosymbionts and pathogens in *R. sanguineus* of Colombia. This difference in the abundance of pathogenic bacteria between male and female ticks probably has several ecological implications involving aspects related to disease transmission and their interaction with hosts. If males carry more species of pathogenic bacteria, they could play a more significant role as vectors in the transmission of diseases. However, females, by spending more time feeding, could favor the development and transmission of other pathogenic species (e.g., *Rickettsia*).

The second most abundant bacterial species in the microbiota of both sexes of *R. sanguineus* was determined to be *Staphylococcus pseudintermedius*. This species is a common (although occasionally pathogenic) commensal microorganism of the skin and mucous membranes of dogs and other animals [50]; moreover, due to the fact that it is so abundant on the skin of dogs, the tick ingests this bacterium during the bite. In fact, *Staphylococcus* spp. are considered to be common in the genera *Rhipicephalus*, *Ixodes*, *Dermacentor,* and *Haemaphysalis* because they are related to the normal microbiota of the skin of their mammalian hosts [51]. However, alternative biocontrol strategies employing entomopathogenic microorganisms have recently been explored. An experiment with cattle ticks (*R. microplus*) demonstrated that some *Staphylococcus* species that are commensal to the skin of these ruminants (such as *S. shinii*, *S. succinus*, and *S. xylosus*) can cause incapacitating or lethal infections in these ticks [52], which can be used to control their populations while reducing the use of acaricides. In the present study, 11 species of *Staphylococcus* were identified in addition to *S. pseudintermedius* (including *S. xylosus*, *S. succinus*, *S. saprophyticus*, *S. gallinarum*, *S. arlettae*, *S. simulans*, *S. felis*, *S. equorum*, *S. hominis*, *S. aureus* and *S. haemolyticus*) but at low abundances (less than 0.20% for each species); however, these species may represent an opportunity for possible applications in the biological control of *R. sanguineus*. Some

other less abundant genera in the microbiota of *R. sanguineus* were *Proteus*, *Corynebacterium*, *Acinetobacter*, *Empedobacter*, *Glutamicibacter*, *Sphingobacterium*, *Pseudomonas*, *Macrococcus*, *Myroides*, *Leclercia* and *Bacillus*. These bacteria have been previously reported in the microbiota of *R. sanguineus* [18] and other tick species [53–56]; moreover, they likely originate from the environment (soil, water, or air) or from the skin of their host, but their function in the tick (if any) remains to be elucidated.

4.2. Public Health Context of the Rhipicephalus sanguineus Microbiota

Climate change is expanding the geographic distribution of ticks, thus increasing the risk of transmission of zoonotic diseases to new areas. Increasing temperatures, shorter winters, and altered precipitation patterns have created favorable conditions for tick survival and reproduction in previously inhospitable regions [57]. In addition, climate change may accelerate the life cycles of ticks, thus increasing their capacity to transmit pathogens and creating challenges for public health surveillance and control systems. This growing risk makes it necessary to explore new and more efficient methods for controlling ticks and their transmission of pathogens to humans. The bacterial microbiota may play an important role in these new strategies. Because *C. mudrowiae* is the most abundant endosymbiont in *R. sanguineus* from Durango and other parts of the world, new "antimicrobiota" vaccine techniques can focus on this species, as proposed by Mateos-Hernández et al. [58,59] with other ixodids. However, the biology, ecology, and pathogenic potential of this endosymbiont are still unknown. Research should prioritize the determination of whether *C. mudrowiae* can cause disease or act as a co-factor in tick-borne illnesses due to the fact that this bacterium belongs to the C lineage of the *Coxiella* genus [60,61]; moreover, several species of this group (such as *C. massiliensis* and *Coxiella*-like species) cause diseases in people who are bitten by ticks [62,63]. Surveillance systems need to monitor cases of atypical febrile illnesses that may involve *C. mudrowiae* and obtain access to PCR assays targeting these species-specific genes to determine the possible presence of this bacterium in tick bite-exposed individuals. Furthermore, studies on the manner in which *C. mudrowiae* interacts with other tick-borne pathogens are needed. If this bacterium suppresses the growth of other harmful pathogens, its dominance could indirectly reduce the risk of diseases such as Rocky Mountain spotted fever. Alternatively, it may facilitate coinfections or modify tick behavior, thus enhancing their vector competence. With respect to tick control measures, strategies that specifically target *C. mudrowiae* may affect tick fitness and could serve as an innovative method for population control. By investing in research, refining diagnostic tools, and incorporating *C. mudrowiae* into existing surveillance frameworks, public health systems can better prepare for potential risks while advancing our understanding of the role of this bacterium in tick-borne disease ecology. On the other hand, *E. canis* and *A. platys* recorded in *R. sanguineus* ticks from Durango have been documented to cause disease in humans. Human infection by *E. canis* has been reported in the United States [64], Venezuela [65], Costa Rica [66], Panama [67], and Mexico [68]. In most cases, the disease is believed to be Rocky Mountain spotted fever caused by *R. rickettsii*; however, some signs and symptoms were different, so immunoassays and PCR tests were performed, which identified *E. canis* infection. This disease differs from Rocky Mountain spotted fever in that most people do not experience skin rashes (i.e., exanthema or red-purple spots on the skin). Regarding *A. platys*, although it is primarily a canine pathogen, there are isolated reports of infections in humans, mainly in immunocompromised people or those who have had close and prolonged contact with infected animals or ticks. These reports correspond to the case of an exotic species veterinarian who worked with animals on the island of Grenada (Caribbean), Ireland, and South Africa [69], as well as two cases of women who lived with dogs in Venezuela [70]. Due to the exposure of these people to the bite of the *R. sanguineus* tick, bacteria transmitted by this vector were suspected. After a blood smear and PCR were performed, the presence of *A. platys* was detected.

In the present study, only unfed ticks were analyzed, which provides important insights into the pathogens that are present in the vector in a latent or active form before

feeding. However, this approach may lead to an underestimation of bacterial prevalence and diversity, thereby omitting bacteria that are reactivated by feeding and excluding those bacteria that are directly acquired from the host. In this context, the presence of *Rickettsia rickettsii* (causing Rocky Mountain spotted fever) and *Anaplasma phagocytophilum* (causing human granulocytic anaplasmosis) was not recorded in the samples from the present study in Durango. Castillo-Martínez et al. [71] and Ortega-Morales et al. [22] confirmed the presence of *R. rickettsii* in a low number of engorged *R. sanguineus* individuals by traditional PCR in the Comarca Lagunera, and Barraza-Guerrero (pers. comm.) recorded *A. phagocytophilum* (V3–V4 sequencing) in the blood of a veterinarian bitten by an *R. sanguineus* tick in this same region. This finding indicates that both species of bacteria are present in dog ticks northeast of Durango; however, their abundance may be very low since they are not beneficial bacteria for ticks [72]. In the case of *R. rickettsii*, infection in *R. sanguineus* ticks has a negative effect on their lifespan, delays their feeding process, and decreases their oviposition capacity [73–75]; likewise, *A. phagocytophilum* can have several negative effects on ticks, including reduced colonization resistance, disrupted community assembly and weaker colonization resistance [76]. Furthermore, when *R. sanguineus* is not fed, *R. rickettsii* bacteria change their morphology and physiology, entering a period of "inactivation," and only when the tick is engorged with blood do the bacteria "reactivate" by increasing their metabolism and multiplication [77,78], which is when transmission to the host takes place. In this context, it is likely that the non-engorged condition of the ticks analyzed in the present study prevented the detection of *R. rickettsii* and *A. phagocytophilum* in the performed sequencing. Thus, for a comprehensive understanding of the role of ticks as vectors, future studies involving engorged ticks are recommended. Additionally, the pooling of ticks for DNA extraction introduces several potential biases that can impact the interpretation of pathogen prevalence and diversity in studies. These biases stem from the aggregation of individuals and the inherent variabilities in their infection status, pathogen load, and DNA quality. Due to the fact that this is the first sequencing study conducted on *R. sanguineus* in Mexico, the approach to its bacterial microbiota and pathogens has become generalized; however, this approach represents the basis for the development of future detailed analyses with respect to individual ticks, different stages, different feeding conditions and different seasons of the year, all of which could cause variations in its bacterial communities.

Bacteria such as *Rickettsia* spp., *Ehrlichia* spp., and *Anaplasma* spp. normally inhabit the tick gut but have an affinity for congregating in the salivary glands, where they remain until the tick begins to feed and are subsequently transmitted to the host through salivation [79]. However, regurgitation is a second form of pathogen transmission. During blood feeding, there is an initial period of slow sucking and a subsequent period of rapid congestion with alternating periods of blood-sucking and salivation observed, with frequent regurgitation [80]. This process (salivation and regurgitation) is highly important for the transmission of pathogens during blood feeding [11]. This means that some intestinal bacteria can enter the host, as has been demonstrated experimentally with *Amblyomma americanum* ticks [81]. Likewise, improper removal of a tick that is attached to a person or animal (i.e., crushing the body of the tick to take it off quickly) can cause further regurgitation and, therefore, accidental inoculation of bacteria that the tick carries only in its midgut. It is also not advisable to cover the tick with substances such as oil, gasoline, alcohol, or Vaseline or to burn it with a cigarette or lighter to remove it from the skin, as the parasite can become stressed, and its reflex response may also be regurgitation [82]. Hence, it is important to know which bacteria, in addition to those already known as pathogens, can enter the human bloodstream via accidental regurgitation and cause infections. In the present study, 75 species were recorded (Table S3) that, according to a review by Bartlett et al. [83], are classified as human pathogens, causing mainly bacteremia, as well as other types of infections. These bacteria are from genera *Staphylococcus*, *Acinetobacter*, *Corynebacterium*, *Proteus*, *Leclercia*, *Exiguobacterium*, *Fusobacterium*, *Klebsiella*, *Psychrobacter*, *Brachybacterium*, *Streptococcus*, *Pantoea*, *Dietzia*, *Bacillus*, *Sphingobacterium*, *Comamonas*, *Morganella*, *Vagococcus*,

Rodococcus, *Shewanella*, *Escherichia*, *Shigella*, *Pasteurella*, *Helcococcus*, *Enterococcus*, *Empedobacter*, *Finegoldia*, *Frederiksenia*, *Williamsia*, *Stenotrophomonas*, *Moraxella*, *Cutibacterium*, *Weissella*, *Dysgonomonas*, *Luteococcus*, *Pseudomonas*, *Massilia*, *Cronobacter*, *Bergeyella*, *Clostridium*. These bacteria are probably transient inhabitants of the tick's internal organs and have reached that place through the blood of their host (mostly dogs) or from the environment; however, it is important to recognize that when a tick bites a person, there is complete exposure to all the bacteria that the parasite carries internally. In the worst-case scenario, a cocktail of bacteria can be inoculated into the person, leading to coinfections with already known pathogens (*Rickettsia*, *Ehrlichia*, *Anaplasma*) but also infections with secondary bacteria that could aggravate the clinical picture. In this context, microbiota studies using massive sequencing allow us to observe the complete image of what can be transmitted during a bite, which should complement the knowledge of clinicians who treat these types of infections to provide more accurate and effective diagnoses and treatments. Interestingly, massive sequencing of human blood has been widely recommended in China for the diagnosis of patients bitten by ticks, offering an encouraging outlook for other countries that are struggling with this type of disease [84]. It is possible that in the near future, this technology can be used daily to support the clinical diagnostic processes currently being applied in hospitals regarding tick bites. Finally, it is essential to continue analyzing the microbiota of ticks in different contexts through massive sequencing for the benefit of public health and to generate new alternatives for controlling these parasites.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/microbiolres15040167/s1, Table S1: V1–V9 (PacBio) amplicon sequencing results for each tick sample (numbers of reads before and after quality filtering for each tick pool).; Table S2: Complete list of the bacterial taxa obtained for females and males, and the associated absolute and relative abundances for each of the tick pools.; Table S3: *Rhipicephalus sanguineus* bacteria that were positively blasted at the species level at the NCBI (National Center for Biotechnology Information).; Table S4: Species of bacteria that were part of the microbiota of *Rhipicephalus sanguineus* in northeast Durango, Mexico, and have been previously reported causing infections to people.

Author Contributions: Conceptualization, C.G.-D.l.P. and S.I.B.-G.; funding acquisition, C.G.-D.l.P.; project administration, E.M.-A., J.M.D.l.C.-R. and J.C.H.-S.; methodology, Q.K.S.-R., V.Á.-R. and M.G.T.-D.; data curation, A.Z.-L. and J.V.-A.; formal analysis, C.G.-D.l.P.; writing—original draft preparation, C.G.-D.l.P.; writing—review and editing, S.I.B.-G., A.A.-A. and L.M.V.-N. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by Consejo de Ciencia y Tecnología del Estado de Durango (COCYTED), Convocatoria mujeres en la ciencia, tecnologías, ingeniería y matemáticas 2023, grant number 1317.

Institutional Review Board Statement: All the methods and activities of this study were in strict accordance with accepted guidelines for ethical use, care, and welfare of animals in research at the international level, and has been approved by the Biological Sciences Faculty UJED ethic committee on 27 November 2023 (ethic code: 0014).

Informed Consent Statement: Not applicable.

Data Availability Statement: The raw data supporting the conclusions of this article will be made available by the authors on request.

Acknowledgments: To Consejo de Ciencia y Tecnología del Estado de Durango (COCYTED), who provided funding for this study. We thank Irene Pacheco-Torres, for their support in the lab work. We deeply appreciate the suggestions of anonymous reviewers for the improvement of this manuscript.

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Jia, N.; Wang, J.; Shi, W.; Du, L.; Sun, Y.; Zhan, W.; Jiang, J.F.; Wang, Q.; Zhang, B.; Ji, P.; et al. Large-scale comparative analyses of tick genomes elucidate their genetic diversity and vector capacities. *Cell* **2020**, *182*, 1328–1340.e13. [CrossRef] [PubMed]
2. De la Fuente, J.; Estrada-Peña, A.; Venzal, J.M.; Kocan, K.M.; Sonenshine, D.E. Overview: Ticks as vectors of pathogens that cause disease in humans and animals. *Front. Biosci.* **2008**, *13*, 6938–6946. [CrossRef] [PubMed]
3. Wu-Chuang, A.; Hodžic, A.; Mateos-Hernández, L.; Estrada-Peña, A.; Obregon, D.; Cabezas-Cruz, A. Current debates and advances in tick microbiome research. *Curr. Res. Parasitol. Vector-Borne Dis.* **2021**, *1*, 100036. [CrossRef]
4. Ammerman, N.C.; Beier-Sexton, M.; Azad, A.F. Laboratory maintenance of *Rickettsia rickettsii*. In *Current Protocols in Microbiology*; Chapter 3, Unit 3A.5.; Wiley: Oxford, UK, 2008. [CrossRef]
5. Barbour, A.G.; Benach, J.L. Discovery of the Lyme Disease Agent. *mBio* **2019**, *10*, e02166-19. [CrossRef] [PubMed]
6. Ahantarig, A.; Trinachartvanit, W.; Baimai, V.; Grubhoffer, L. Hard ticks and their bacterial endosymbionts (or would-be pathogens). *Folia Microbiol.* **2013**, *58*, 419–428. [CrossRef]
7. Bonnet, S.I.; Binetruy, F.; Hernández-Jarguín, A.M.; Duron, O. The tick microbiome: Why non-pathogenic microorganisms matter in tick biology and pathogen transmission. *Front. Cell. Infect. Microbiol.* **2017**, *7*, 236. [CrossRef]
8. Bonnet, S.I.; Pollet, T. Update on the intricate tango between tick microbiomes and tick-borne pathogens. *Parasite Immunol.* **2021**, *43*, e12813. [CrossRef]
9. Greay, T.L.; Gofton, A.W.; Paparini, A.; Ryan, U.M.; Oskam, C.L.; Irwin, P.J. Recent insights into the tick microbiome gained through next-generation sequencing. *Parasit. Vectors* **2018**, *11*, 12. [CrossRef]
10. Amber, N.; Grant, M.W.; Lineberry, K.D.; Allen, S.K.E.; Little, S.E. Geographic distribution and seasonality of brown dog tick lineages in the United States. *J. Med. Entomol.* **2023**, *60*, 102–111. [CrossRef]
11. Dantas-Torres, F. The brown dog tick, *Rhipicephalus sanguineus* (Latreille, 1806) (Acari: Ixodidae): From taxonomy to control. *Vet. Parasitol.* **2008**, *152*, 173–185. [CrossRef]
12. Dantas-Torres, F. Biology and ecology of the brown dog tick, *Rhipicephalus sanguineus*. *Parasites Vectors* **2010**, *3*, 26. Available online: http://www.parasitesandvectors.com/content/3/1/26 (accessed on 20 July 2024). [CrossRef] [PubMed]
13. Lalzar, I.; Harrus, S.; Mumcuoglu, K.Y.; Gottlieb, Y. Composition and seasonal variation of *Rhipicephalus turanicus* and *Rhipicephalus sanguineus* bacterial communities. *Appl. Environ. Microbiol.* **2012**, *78*, 4110–4116. [CrossRef] [PubMed]
14. René-Martellet, M.; Minard, G.; Massot, R.; Van, V.T.; Moro, C.V.; Chabanne, L.; Mavingui, P. Bacterial microbiota associated with *Rhipicephalus sanguineus* (s.l.) ticks from France, Senegal, and Arizona. *Parasites Vectors* **2017**, *10*, 416. [CrossRef] [PubMed]
15. Portillo, A.; Palomar, A.M.; de Toro, M.; Santibañez, S.; Santibañez, P.; Oteo, J.A. Exploring the bacteriome in anthropophilic ticks: To investigate the vectors for diagnosis. *PLoS ONE* **2019**, *14*, e0213384. [CrossRef] [PubMed]
16. Agwunobi, D.O.; Kamani, J.; Zheng, H.; Guo, L.; Yu, Z.; Liu, J. Bacterial diversity in *Rhipicephalus sanguineus* (Acari: Ixodidae) from two states in Nigeria. *J. Entomol. Sci.* **2021**, *56*, 256–271. [CrossRef]
17. Luzzi, M.C.; de Carvalho, L.A.L.; Pinheiro, D.G.; Lima-Duarte, L.; Camargo, J.V.; Kishi, L.T.; Fernandes, C.C.; Machado, R.Z.; Soares, J.F.; André, M.R.; et al. Analysis on the prokaryotic microbiome in females and embryonic cell cultures of *Rhipicephalus sanguineus* tropical and temperate lineages from two specific localities in Brazil. *Braz. J. Vet. Parasitol.* **2021**, *30*, e005721. [CrossRef]
18. Paez-Triana, L.; Herrera, G.; Vega, L.; Garcia-Corredor, D.; Pulido-Medellín, M.O.; Paniz-Mondolfi, A.; Muñoz, M.; Ramirez, J.D. Metagenomic exploration of endosymbionts and pathogens in the tropical lineage of *Rhipicephalus sanguineus* sensu lato (s.l.) ticks in Colombia. *Metabarcoding Metagenomics* **2023**, *7*, e109085. [CrossRef]
19. Rodríguez-Vivas, R.I.; Ojeda-Chi, M.M.; Bolio-González, M.E.; Rosado-Aguilar, J.A. Las garrapatas como vectores de enfermedades zoonóticas en México. *Bioagrociencias* **2019**, *12*, 19–26. [CrossRef]
20. Walker, J.; Keirans, J.; Horak, I. *Genus Rhipicephalus (Acari, Ixodidae): A Guide to the Brown Ticks of the World*; Cambridge University Press: Cambridge, UK, 2000.
21. Kamani, J.; Baneth, G.; Mumcuoglu, K.Y.; Waziri, N.E.; Eyal, O.; Guthmann, Y.; Harrus, S. Molecular detection and characterization of tick-borne pathogens in dogs and ticks from Nigeria. *PLoS Neglected Trop. Dis.* **2013**, *7*, e2108. [CrossRef]
22. Ortega-Morales, A.I.; Nava-Reyna, E.; Ávila-Rodríguez, V.; González-Álvarez, V.H.; Castillo-Martínez, A.; Siller-Rodríguez, Q.K.; Cabezas-Cruz, A.; Dantas-Torres, F.; Almazán, C. Detection of *Rickettsia* spp. in *Rhipicephalus sanguineus* (sensu lato) collected from free-roaming dogs in Coahuila state, northern Mexico. *Parasites Vectors* **2019**, *12*, 130. [CrossRef]
23. Wechtaisong, W.; Bonnet, S.I.; Lien, Y.-Y.; Chuang, S.-T.; Tsai, Y.-L. Transmission of *Bartonella henselae* within *Rhipicephalus sanguineus*: Data on the potential vector role of the tick. *PLoS Negl. Trop. Dis.* **2020**, *14*, e0008664. [CrossRef] [PubMed]
24. Salomon, J.; Fernandez-Santos, N.A.; Zecca, I.B.; Estrada-Franco, J.G.; Davila, E.; Hamer, G.L.; Rodríguez-Pérez, M.A.; Hamer, S.A. Brown dog tick (*Rhipicephalus sanguineus* sensu lato) infection with endosymbiont and human pathogenic *Rickettsia* spp., in northeastern México. *Int. J. Environ. Res. Public Health* **2022**, *19*, 6249. [CrossRef]
25. Bush, E.; Lemmen, D.S. *Canada's Changing Climate Report*; Government of Canada: Ottawa, ON, Canada, 2019; Available online: https://changingclimate.ca/CCCR2019/ (accessed on 15 July 2024).
26. Cuervo-Robayo, A.P.; Ureta, C.; Gómez-Albores, M.A.; Meneses-Mosquera, A.K.; Téllez-Valdés, O.; Martínez-Meyer, E. One hundred years of climate change in Mexico. *PLoS ONE* **2020**, *15*, e0209808. [CrossRef]
27. Alkishe, A.; Cobos, M.E.; Peterson, A.T.; Samy, A.M. Recognizing sources of uncertainty in disease vector ecological niche models: An example with the tick *Rhipicephalus sanguineus* sensu lato. *Perspect. Ecol. Conserv.* **2020**, *18*, 91–102. [CrossRef]

28. Rodríguez-Vivas, R.I.; Flota Burgos, G.J.; Bolio-González, M.E.; Rosado-Aguilar, J.A.; Gutiérrez-Ruiz, E.J.; Torres-Castro, M.; Panti-May, A.; Reyes-Novelo, E. La garrapata café del perro, *Rhipicephalus sanguineus*: Biología y control. *Vanguardia Veterinaria* **2023**, *11*, 10–16.
29. Sánchez-Montes, S.; Salceda-Sánchez, B.; Bermúdez, S.E.; Aguilar-Tipacamú, G.; Ballados González, G.G.; Huerta, H.; Aguilar-Domínguez, M.; Mora, J.D.-d.l.; Licona-Enríquez, J.D.; Mora, D.D.-d.l.; et al. *Rhipicephalus sanguineus* complex in the Americas: Systematic, genetic diversity, and geographic insights. *Pathogens* **2021**, *10*, 1118. [CrossRef] [PubMed]
30. López-Castillo, D.C.; Vaquera-Aparicio, D.; González-Soto, M.A.; Martínez-Ramírez, R.; Rodríguez-Muñoz, L.; Solórzano-Santos, F. Fiebre manchada de montañas rocosas: Experiencia en 5 años de vigilancia activa en un hospital pediátrico de segundo nivel en el noreste de México. *Boletín Médico Del Hosp. Infant. De México* **2018**, *75*, 303–308. [CrossRef]
31. Rodríguez-Muñoz, L.; Barrera-Salinas, R.; Sánchez-García, C.; Solórzano-Santos, F.; Vaquera-Aparicio, D.N.; López-Castillo, D. Rickettsiosis de fiebre manchada: Estudio de casos notificados en un hospital pediátrico de segundo nivel en el noreste de México, 2012–2022. *Gac. Médica De México* **2023**, *159*, 138–144. [CrossRef]
32. Athanasopoulou, K.; Boti, M.A.; Adamopoulos, P.G.; Skourou, P.C.; Scorilas, A. Third-generation sequencing: The spearhead towards the radical transformation of modern genomics. *Life* **2022**, *12*, 30. [CrossRef] [PubMed]
33. García, E. *Modificaciones al Sistema de Clasificación Climática de Köppen*; Universidad Nacional Autónoma de México: Coyoacán, México, 2004.
34. Castillo-Martínez, A.; Cueto-Medina, S.M.; Hernández-Rodríguez, S.; Valdés-Perezgasga, M.T.; Ortega-Morales, A.I. Garrapatas peridomésticas (Acari: Ixodidae, Argasidae) de Matamoros, Coahuila, México. *Entomol. Mex.* **2015**, *2*, 47–51.
35. Binetruy, F.; Dupraz, M.; Buysse, M.; Duron, O. Surface sterilization methods impact measures of internal microbial diversity in ticks. *Parasites Vectors* **2019**, *12*, 268. [CrossRef]
36. Bolyen, E.; Rideout, J.R.; Dillon, M.R.; Bokulich, N.A.; Abnet, C.C.; Al-Ghalith, G.A.; Alexander, H.; Alm, E.J.; Arumugam, M.; Asnicar, F.; et al. Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. *Nat. Biotechnol.* **2019**, *37*, 852–857. [CrossRef]
37. Callahan, B.J.; McMurdie, P.J.; Rosen, M.J.; Han, A.W.; Johnson, A.J.; Holmes, S.P. DADA2: High resolution sample inference from Illumina amplicon data. *Nat. Methods* **2016**, *13*, 581–583. [CrossRef] [PubMed]
38. McDonald, D.; Jiang, Y.; Balaban, M.; Cantrell, K.; Zhu, Q.; Gonzalez, A.; Morton, J.T.; Nicolaou, G.; Parks, D.H.; Karst, S.M.; et al. Greengenes2 unifies microbial data in a single reference tree. *Nat. Biotechnol.* **2024**, *42*, 715–718. [CrossRef] [PubMed]
39. Jaccard, P. The distribution of the flora in the alpine zone. *New Phytol.* **1912**, *11*, 37–50. [CrossRef]
40. Bray, J.R.; Curtis, J.T. An ordination of the upland forest communities of Southern Wisconsin. *Ecol. Monogr.* **1957**, *27*, 325–349. [CrossRef]
41. Lozupone, C.; Knight, R. UniFrac: A new phylogenetic method for comparing microbial communities. *Appl. Environ. Microbiol.* **2005**, *71*, 8228–8235. [CrossRef] [PubMed]
42. Vázquez-Baeza, Y.; Pirrung, M.; Gonzalez, A.; Knight, R. EMPeror: A tool for visualizing high-throughput microbial community data. *GigaScience* **2013**, *2*, 16. [CrossRef]
43. Khomich, M.; Måge, I.; Rud, I.; Berget, I. Analysing microbiome intervention design studies: Comparison of alternative multivariate statistical methods. *PLoS ONE* **2021**, *16*, e0259973. [CrossRef]
44. Ben-Yosef, M.; Rot, A.; Mahagna, M.; Kapri, E.; Behar, A.; Gottlieb, Y. *Coxiella*-like endosymbiont of *Rhipicephalus sanguineus* is required for physiological processes during ontogeny. *Front. Microbiol.* **2020**, *11*, 493. [CrossRef]
45. An, L.; Bhowmick, B.; Liang, D.; Suo, P.; Liao, C.; Zhao, J.; Han, Q. The microbiota changes of the brown dog tick, *Rhipicephalus sanguineus* under starvation stress. *Front. Physiol.* **2022**, *13*, 932130. [CrossRef] [PubMed]
46. Devaraju, P.; Gopal, A.A.; Arif, W.; Srinivasan, L.; Anthony, T.N.; Ashokkumar, M. Detection and genotyping of Coxiella-like endosymbionts in *Rhipicephalus* and *Haemaphysalis* ticks infesting pets and livestock in Puducherry, India. *J. Bacteriol. Virol.* **2024**, *54*, 40–49. [CrossRef]
47. Garcia-Guizzo, M.; Parizi, L.F.; Dutra-Nunes, R.; Schama, R.; Albano, R.M.; Tirloni, L.; Oldiges, D.P.; Pilz-Vieira, R.; Cruz-Oliveira, W.M.; de Souza-Leite, S.A.; et al. A *Coxiella* mutualist symbiont is essential to the development of *Rhipicephalus microplus*. *Sci. Rep.* **2017**, *7*, 17554. [CrossRef]
48. Kolo, A.O.; Raghavan, R. Impact of endosymbionts on tick physiology and fitness. *Parasitology* **2023**, *150*, 859–865. [CrossRef]
49. Arthan, W.; Sumrandee, C.; Hirunkanokpun, S.; Kitthawee, S.; Baimai, V.; Trinachartvanit, W.; Ahantarig, A. Detection of *Coxiella*-like endosymbiont in *Haemaphysalis* tick in Thailand. *Ticks Tick-Borne Dis.* **2015**, *6*, 63–68. [CrossRef]
50. Sawhney, S.S.; Vargas, R.C.; Wallace, M.A.; Muenks, C.E.; Lubbers, B.V.; Fritz, S.A.; Burnham, C.A.D.; Dantas, G. Diagnostic and commensal *Staphylococcus pseudintermedius* genomes reveal niche adaptation through parallel selection of defense mechanisms. *Nat. Commun.* **2023**, *14*, 7065. [CrossRef]
51. Xu, X.L.; Cheng, T.Y.; Yang, H.; Yan, F. Identification of intestinal bacterial flora in *Rhipicephalus microplus* ticks by conventional methods and PCR-DGGE analysis. *Exp. Appl. Acarol.* **2015**, *66*, 257–268. [CrossRef]
52. Cossio-Bayugar, R.; Arreguin-Perez, C.A.; Aguilar-Diaz, H.; Miranda-Miranda, E. Efficacy of entomopathogenic Staphylococcus bacteria as a biocontrol agent against *Rhipicephalus microplus* ticks: Assessing reproductive inhibition and mortality rates. *Microorganisms* **2024**, *12*, 551. [CrossRef]
53. Brinkerhoff, R.J.; Clark, C.; Ocasio, K.; Gauthier, D.T.; Hynes, W.L. Factors affecting the microbiome of *Ixodes scapularis* and *Amblyomma americanum*. *PLoS ONE* **2020**, *15*, e0232398. [CrossRef]

54. Wang, S.; Hua, X.; Cui, L. Characterization of microbiota diversity of engorged ticks collected from dogs in China. *J. Vet. Sci.* **2021**, *22*, e37. [CrossRef]
55. Tahulending, J.; Tulung, M.; Pelealu, J.; Doda, D.V. Molecular detection of pathogenic bacteria in *Rhipicephalus sanguineus* (sensu lato) ticks from Bitung, North Sulawesi, Indonesia. *Biodiversitas J. Biol. Divers.* **2022**, *23*. [CrossRef]
56. Mutai, B.; Njaanake, K.; Gathii, K.; Estambale, B.B.; Waitumbi, J.N. Bacteriome in ticks collected from domestic livestock in Kenya. *Adv. Microbiol.* **2022**, *12*, 67–82. [CrossRef]
57. Alkishe, A.; Raghavan, R.K.; Peterson, A.T. Likely geographic distributional shifts among medically important tick species and tick-associated diseases under climate change in North America: A review. *Insects* **2021**, *12*, 225. [CrossRef] [PubMed]
58. Mateos-Hernández, L.; Obregón, D.; Maye, J.; Borneres, J.; Versille, N.; de la Fuente, J.; Estrada-Peña, A.; Hodžić, A.; Šimo, L.; Cabezas-Cruz, A. Anti-tick microbiota vaccine impacts *Ixodes ricinus* performance during feeding. *Vaccines* **2020**, *8*, 702. [CrossRef] [PubMed]
59. Mateos-Hernández, L.; Obregón, D.; Wu-Chuang, A.; Maye, J.; Bornères, J.; Versillé, N.; de la Fuente, J.; Díaz-Sánchez, S.; Bermúdez-Humarán, L.G.; Torres-Maravilla, E.; et al. Anti-microbiota vaccines modulate the tick microbiome in a taxon-specific manner. *Front. Immunol.* **2021**, *12*, 704621. [CrossRef]
60. Lalzar, I.; Friedmann, Y.; Gottlieb, Y. Tissue tropism and vertical transmission of *Coxiella* in *Rhipicephalus sanguineus* and *Rhipicephalus turanicus* ticks. *Environ. Microbiol.* **2014**, *16*, 3657–3668. [CrossRef]
61. Shi, M.; Qin, T.; Liu, Z.; Feng, H.; Sun, Y. Molecular detection of *Candidatus Coxiella mudorwiae* in *Haemaphysalis concinna* in China. *Zoonoses* **2022**, *2*, 39. [CrossRef]
62. Angelakis, E.; Mediannikov, O.; Jos, S.L.; Berenger, J.M.; Parola, P.; Raoult, D. *Candidatus Coxiella massiliensis* infection. *Emerg. Infect. Dis.* **2016**, *22*, 285–288. [CrossRef]
63. Guimard, T.; Amrane, S.; Prudent, E.; Karkouri, K.E.; Raoult, D.; Angelakis, E. Case report: Scalp eschar and neck lymphadenopathy associated with bacteremia due to *Coxiella*-like bacteria. *Am. J. Trop. Med. Hyg.* **2017**, *97*, 1319–1322. [CrossRef]
64. Conrad, M.E. *Ehrlichia canis*: A tick-borne rickettsial-like infection in humans living in the southeastern United States. *Am. J. Med. Sci.* **1989**, *297*, 35–37. [CrossRef]
65. Perez, M.; Bodor, M.; Zhang, C.; Xiong, Q.; Rikihisa, Y. Human infection with *Ehrlichia canis* accompanied by clinical signs in Venezuela. *Ann. N. Y. Acad. Sci.* **2006**, *1078*, 110–117. [CrossRef] [PubMed]
66. Bouza-Mora, L.; Dolz, G.; Solórzano-Morales, A.; Romero-Zuñiga, J.J.; Salazar-Sánchez, L.; Labruna, M.B.; Aguiar, D.M. Novel genotype of *Ehrlichia canis* detected in samples of human blood bank donors in Costa Rica. *Ticks Tick-Borne Dis.* **2017**, *8*, 36–40. [CrossRef] [PubMed]
67. Daza, T.C.; Osorio, J.; Santamaría, A.M.; Suárez, J.A.; Hurtado, A.; Bermúdez, S. Caracterización del primer caso de infección humana por *Ehrlichia canis* en Panamá. *Rev. Médica Panamá* **2018**, *38*, 63–68.
68. Silva, A.B.; Pina-Canseco, S.; Gabriel-de la Torre, M.P.; Mayoral-Silva, A.; Mayoral, M.A.; Pérez-Campos-Mayoral, L.; López-Martínez, J.; Pérez-Campos, E. Infección humana asintomática por contacto con perros. Un caso de ehrlichiosis humana. *Gac. Médica México* **2014**, *150*, 171–174.
69. Maggi, R.G.; Mascarelli, P.E.; Havenga, L.N.; Naidoo, V.; Breitschwerdt, E.B. Co-infection with *Anaplasma platys*, *Bartonella henselae* and *Candidatus Mycoplasma haematoparvum* in a veterinarian. *Parasites Vectors* **2013**, *6*, 103. [CrossRef]
70. Arraga-Alvarado, C.M.; Qurollo, B.A.; Parra, O.C.; Berrueta, M.A.; Hegarty, B.C.; Breitschwerdt, B.E. Case report: Molecular evidence of *Anaplasma platys* infection in two women from Venezuela. *Am. J. Trop. Med. Hyg.* **2014**, *91*, 1161–1165. [CrossRef]
71. Castillo-Martínez, A.; Cueto-Medina, S.M.; Valdés-Perezgasga, M.T.; Sánchez-Ramos, F.J.; López-Hernández, J.; Hernández-Rodríguez, S.; Ortega-Morales, A.I. Detección de *Rickettsia rickettsii* en la garrapata café del perro *Rhipicephalus sanguineus* en México. *Acta Zoológica Mex. Nueva Ser.* **2017**, *33*, 339–344. [CrossRef]
72. Demma, L.J.; Traeger, M.S.; Nicholson, W.L.; Paddock, C.D.; Blau, D.M.; Eremeeva, M.E.; Dasch, G.A.; Levin, M.L.; Singleton, J.; Zaki, S.R.; et al. Rocky Mountain spotted fever from an unexpected tick vector in Arizona. *N. Engl. J. Med.* **2005**, *353*, 587–594. [CrossRef]
73. Burgdorfer, W.; Brinton, L.P. Mechanisms of transovarial infection of spotted fever rickettsiae in ticks. *Ann. N. Y. Acad. Sci.* **1975**, *266*, 61–72. [CrossRef]
74. Niebylski, M.L.; Peacock, M.G.; Schwan, T.G. Lethal effect of *Rickettsia rickettsii* on its tick vector (*Dermacentor andersoni*). *Appl. Environ. Microbiol.* **1999**, *65*, 773–778. [CrossRef]
75. Santos, A.S.; Bacellar, F.; Santos-Silva, M.; Formosinho, P.; Grácio, A.J.; Franca, S. Ultrastructural study of the infection process of *Rickettsia conorii* in the salivary glands of the vector tick *Rhipicephalus sanguineus*. *Vector-Borne Zoonotic Dis.* **2002**, *2*, 165–177. [CrossRef] [PubMed]
76. Abuin-Denis, L.; Piloto-Sardinas, E.; Maître, A.; Wu-Chuang, A.; Mateos-Hernández, L.; Obregon, D.; Corona-González, B.; Fogaça, A.C.; Palinauskas, V.; Azelyte, J.; et al. Exploring the impact of *Anaplasma phagocytophilum* on colonization resistance of *Ixodes scapularis* microbiota using network node manipulation. *Curr. Res. Parasitol. Vector-Borne Dis.* **2024**, *5*, 100177. [CrossRef] [PubMed]
77. Hayes, S.F.; Burgdorfer, W. Reactivation of *Rickettsia rickettsii* in *Dermacentor andersoni* ticks: An ultrastructural analysis. *Infect. Immun.* **1982**, *37*, 779–785. [CrossRef] [PubMed]
78. Walker, D.H. The rickettsia-host interaction. *Intracell. Parasit.* **1988**, *1*, 79–92.

79. Lejal, E.; Moutailler, S.; Šimo, L.; Vayssier-Taussat, M.; Pollet, T. Tick-borne pathogen detection in midgut and salivary glands of adult *Ixodes ricinus*. *Parasites Vectors* **2019**, *12*, 152. [CrossRef] [PubMed]
80. Parola, P.; Raoult, D. Ticks and tickborne bacterial diseases in humans: An emerging infectious threat. *Clin. Infect. Dis.* **2001**, *32*, 897–928. [CrossRef]
81. Brown, S.J. Evidence for regurgitation by *Amblyomma americanum*. *Vet. Parasitol.* **1988**, *28*, 335–342. [CrossRef]
82. Juárez-Jiménez, M.V.; De La Cruz-Villamayor, J.A.; Baena-Bravo, A.J. Tratamiento de la garrapata en Atención Primaria. *Med. Fam. De Andal.* **2017**, *18*, 74–79.
83. Bartlett, A.; Padfield, D.; Lear, L.; Bendall, R.; Vos, M. A comprehensive list of bacterial pathogens infecting humans. *Microbiology* **2022**, *168*, 001269. [CrossRef]
84. Zhang, X.; Chen, H.; Han, D.; Wu, W. Clinical usefulness of metagenomic next-generation sequencing for *Rickettsia* and *Coxiella burnetii* diagnosis. *Eur. J. Clin. Microbiol. Infect. Dis.* **2023**, *42*, 681–689. [CrossRef]