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Current debates and advances in tick microbiome research

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ABSTRACT

The main importance of ticks resides in their ability to harbor pathogens that can be transmitted to terrestrial vertebrates including humans. Recently, studies have focused on the taxonomic and functional composition of the tick microbiome, its microbial diversity and variation under different factors including tick species, sex, and environment among others. Of special interest are the interactions between the tick, the microbiome and pathogens since tick microbiome can influence pathogen colonization within the tick vector, and potentially, transmission to the vertebrate host. In this review, we tackled a synthesis on the growing field of tick microbiomes. We focus on the current state of tick microbiome research, addressing controversial and hotly debated topics and advances in the precise manipulation of tick microbiome. Furthermore, we discuss the innovative anti-tick microbiota vaccines as a possible tool for microbiome modulation and thus, control of tick-borne diseases. Deciphering tick-microbiome pathogen interactions can spur new strategies to control tick-borne diseases *via* modulation of tick microbiome.

1. Introduction

The first study on the tick microbiome was published in 2011 by Andreotti et al. (2011). In their study, the authors used bacterial *16S* tag-encoded FLX-titanium amplicon pyrosequencing to characterize the bacterial diversity of the cattle tick *Rhipicephalus microplus* (Andreotti et al., 2011). They showed that the tick microbiome consists of a variety of bacterial genera whose origin could be tracked to the host and the environment. Since then, an increasing number of studies have employed next-generation sequencing technologies to characterize tick microbiome composition allowing for a wider view of its different components. Several factors shaping the bacterial composition of the tick microbiome have been identified and they include abiotic (e.g. temperature) and biotic factors (e.g. tick species, host blood-meal, and tick-developmental stages). Beyond bacteria, it has been shown that tick microbiota is formed also by protists, nematodes, archaea, fungi, and viruses (Nakao et al., 2013; Landesman et al., 2019; Vandegrift & Kapoor, 2019).

Efforts have been also concentrated on understanding the impact of the microbiome on tick biology. Several studies show that ticks are associated with bacterial symbionts that can influence tick survival, fitness, reproduction, nutritional adaptation, and immunity (Bonnet et al., 2017; Bonnet & Pollet, 2021; Narasimhan et al., 2021). In addition to endosymbionts and commensals, ticks harbor multiple pathogenic microorganisms of medical and veterinary importance, including *Borrelia burgdorferi*, *Anaplasma phagocytophilum*, Spotted Fever Group Rickettsia, among others (Bonnet & Pollet, 2021). These pathogens and the other microorganisms coexist within the ticks (Bonnet & Pollet, 2021), and bacteria residing the tick gut can modulate tick vector capacity by affecting pathogen colonization of tick tissues (Narasimhan et al., 2014, 2017; Abraham et al., 2017). These findings provided the basis for developing new strategies to interrupt pathogen transmission *via* modulation of the tick microbiota. However, to reach this goal, comprehension of the regulation of tick microbiome and the biological interactions between the tick, its microbiome and tick-borne pathogens is needed. Progress in this area is limited by technical difficulties in manipulating the microbiome with precision. In this review, we will discuss the current state of tick microbiome research, controversial and hotly debated topics and advances in the precise manipulation of tick microbiome. Within the

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text, "microbiome" refers to the microorganisms and their genes whereas "microbiota" only refers to the microbes themselves.

2. Current debates on tick microbiome diversity

An interesting finding of the pioneer study by Andreotti et al. (2011) was the high number of bacterial genera associated with adult ticks, gut tissue, and tick eggs, in contrast to ovaries that exhibited a relatively lower bacterial diversity. To date, the tick microbiome composition in several tick species has been published (Table 1). These include major vectors of the genera *Ixodes*, *Dermacentor*, *Amblyomma* and *Rhipicephalus*. Following the study by Andreotti et al. (2011) on *R. microplus* microbiome, a high bacterial diversity has been reported in several tick species (Table 1, Nakao et al., 2013; Budachetri et al., 2014; Budachetri et al., 2016; Budachetri et al., 2017; Karim et al., 2017; Panetta et al., 2017; Clow et al., 2018; Gofton et al., 2018; Díaz-Sánchez et al., 2019a; Yan et al., 2019; Chandra & Šlapeta, 2020). Also, of 126 bacterial genera identified in the microbiome of *I. ricinus*, and the spleen of one of its main hosts, the vole *Myodes glareolus*, the communities of co-occurring bacteria were always more phylogenetically diverse in ticks than in voles (Rynkiewicz et al., 2015; Estrada-Peña et al., 2018). These early discoveries suggested that ticks are associated with highly diverse microbial communities. However, the idea of highly diverse tick microbiomes has been recently challenged by several studies reporting that bacterial diversity in tick microbiomes is not as high as initially thought. For example, it has been reported that tick microbiome of several ticks including *Ixodes pacificus*, *I. scapularis*, *I. ricinus*, *R. microplus* and *Dermacentor* spp. were dominated by a few core species, likely endosymbionts (Ross et al., 2018; Chicana et al., 2019; Couper et al., 2019; Guizzo et al., 2020). Furthermore, the loss of genes involved in interbacterial interaction pathways in *Borrelia* has been suggested to be an indirect evidence of a limited tick microbiome diversity (Ross et al., 2018). Similarly, the genomes of tick-transmitted intracellular pathogens such as *Rickettsia*, *Coxiella*, *Anaplasma* and *Ehrlichia* also lack interbacterial effector immunity genes involved in bacteria-bacteria interactions (Ross et al., 2018). O'Keeffe et al. (2020) proposed that the negative selection of the effector genes may be explained by low selective pressure on interbacterial competition pathways by a poor microbiota. The idea of loss of effector genes as evidence of poor tick microbiome is based on the assumption that competition and/or bacteria-bacteria protein-mediated interactions predates microbiome-pathogen ensambles. However, host microbiota can also facilitate pathogen infection and microbiome-pathogen interactions go well beyond protein-mediated interactions (Stevens et al., 2021). For example, pathogens can exploit microbiota metabolites, or can take advantage of a depletion in host defences to cause infection (Stevens et al., 2021).

Other authors reported that up to 50.9% of the bacterial diversity identified in the tick microbiome could be due to contamination at different steps of the DNA extraction, purification and amplification process (Lejal et al., 2020). Some of the studies reporting low bacterial diversity in the tick microbiome eliminated operational taxonomic units (OTUs) that were detected in negative controls (e.g. Ross et al., 2018). Filtering and removal of taxa found in the negative controls should be done with caution because cross-contamination between samples often causes abundant true sequences to be detected in negative controls (Jousselin et al., 2016; Callahan et al., 2017a; Larsson et al., 2018). Also, the removal of sequences below a relative abundance threshold removes rare features truly present in the sample (Davis et al., 2018). Decontam is one of the alternatives proposed to account for the biased removal of taxa in microbiome studies (Davis et al., 2018). Decontam is an open-source R package for statistical classification that identifies contaminants that appear at higher frequencies in low-concentration samples and in negative controls of metagenomic sequencing studies (Davis et al., 2018). To the best of our knowledge, decontam has not been applied to the unbiased removal of taxa in tick microbiome studies.

The use of different units for marker gene analysis, such as OTUs or amplicon sequence variants (ASVs), also has a great impact on microbiome diversity measures. For example, the taxonomic analysis by the assembly of OTUs (i.e. clusters of sequencing reads that differ by less than a fixed dissimilarity threshold; see Callahan et al., 2017b), skews diversity measures since unrepresented data in the reference database are removed (Callahan et al., 2017b). In contrast to OTUs, ASVs (i.e. single DNA sequences recovered from a high-throughput marker gene analysis) can resolve sequence variants to the level of single-nucleotide differences over the sequenced gene region (Callahan et al., 2017b). The finer resolution has the benefit of ASVs as consistent labels with intrinsic biological meaning identified independently from a reference database (Callahan et al., 2017b). Considering the improvements in reusability, reproducibility and comprehensiveness of ASVs compared to OTUs, Callahan et al. (2017b) proposed that ASVs should replace OTUs as the standard unit of marker-gene analysis and reporting. Except for few studies that consider the ASVs (Estrada-Peña et al., 2020a,b), most studies on the tick microbiome use OTUs for taxonomic classification, which may have concealed an even broader bacterial diversity. Whether the consistency of the diversity pattern observed in tick microbiomes concerns the biology or the methodologies used for *16S* rRNA sequencing, analysis of amplicon sequencing data and assess contamination, remains an open question.

3. Factors influencing tick microbiome composition and diversity

Amid the current debate on tick microbiota diversity, experiments in the field and under controlled conditions demonstrated that the tick microbiome is under the influence of several factors including the tick species, physiological stress by environmental traits, blood-meal, host species, tick immunity and developmental stage. Despite the taxonomic variability observed across microbiomes of different tick species, comparative studies suggested that tick microbiome assemblages are not stochastic (Cabezas-Cruz et al., 2018). Rather, the phylogenetic structure of ixodid tick microbial communities supports the existence of a species-specific tick holobiont (Díaz-Sánchez et al., 2019b). The influence of the hologenome (i.e. the collective genomes of the holobiont) on tick fitness and vector competence is largely unknown.

The impact of tick genetic traits on microbiome composition remains also poorly characterized. However, the unequal distribution of the bacterial diversity among ticks collected within the same site suggests that some *I. ricinus* strains are highly permissive to polymicrobial challenges and harbor diverse microbial communities, while others are not (Estrada-Peña et al., 2018). Specifically, Estrada-Peña et al. (2018) reported that approx. 80% of bacterial phylogenetic diversity was carried by approx. 20% of ticks, regardless of the sampling sites. In agreement with an unequal permissiveness to polymicrobial challenge, Ross et al. (2018) showed that the majority of field-collected adult *I. scapularis* harbor limited internal microbial communities, while a minority of ticks harbors abundant midgut bacteria. Genetic traits may determine the permissiveness of ticks to polymicrobial colonization. Whether polymicrobial permissiveness concerns only the microbiome, or also multi-pathogen infections also remains an open question.

Microbiome analyses in different tick species showed that the bacterial community composition differed by sex (van Treuren et al., 2015; Thapa et al., 2019). Analysis of *I. scapularis* and *Ixodes affinis* microbiomes by 454 pyrosequencing and Illumina sequencing showed that microbiomes of adult female ticks were significantly less diverse than those of male ticks (van Treuren et al., 2015). Frequently, the microbiota of female ticks is dominated by a single taxon with a high relative abundance. For example, a high relative abundance of *Rickettsia* has been observed in *I. affinis* (van Treuren et al., 2015) and *A. americanum* (Ponnusamy et al., 2014) female ticks. Other studies reported that *I. scapularis* females were also dominated by *Rickettsia* (Hawlena et al., 2013; Jory Brinkerhoff et al., 2020) or by an unknown genus in the family *Enterobacteriaceae* (van Treuren et al., 2015). The high prevalence of *Rickettsia* in females could be explained by the high rate of transovarial

Table 1
Microbiome studies in different tick species

Tick	Origin	Developmental stage/Sex	Tissue	Location	Target gene	Approach	Reference[a]
Dermacentor andersoni	Lab-reared ticks	Adult males	Midgut and salivary glands	Idaho (USA)	V4 region of *16S* rRNA gene	Roche 454 GS FLX Titanium pyrosequencing	Clayton et al. (2015)
Dermacentor andersoni	Field-collected and lab-reared ticks	Adult males	Midgut and salivary glands	Oregon and Montana (USA)	Nearly full-length *16S* rRNA gene	Pacific Biosciences CCS	Gall et al. (2017)
Dermacentor silvarum	Field-collected ticks	Adults	Whole tick	Jiagedaqi (China)	*16S* rRNA gene	Pyrosequencing	Wang et al. (2018)
Dermacentor silvarum	Lab-reared ticks	Eggs, larvae, nymphs, adults	Whole tick	Shandong (China)	V3–V4 region of *16S* rRNA gene	Illumina MiSeq	Zhang et al. (2020)
Dermacentor silvarum	Field-collected ticks	Adut females	Saliva and midgut	Guyuan (China)	V3–V4 region of *16S* rRNA gene	IonS5^{TM}XL	Duan et al. (2020)
Dermacentor albipictus	Field-collected ticks	Nymphs, adult males and females	Whole tick	Alberta (Canada)	V4 region of *16S* rRNA gene	Ion PGM	Ben-Yosef et al. (2020)
Dermacentor marginatus, D. reticulatus	Field-collected ticks	Adult males and females	Whole tick	Slovak Karst (Slovakia)	V3–V4 region of *16S* rRNA gene	Illumina MiSeq	Zhang et al. (2019a)
Dermacentor variabilis, Ixodes scapularis	Field-collected ticks	Larvae and nymphs	Whole tick	Southern Indiana (USA)	V1–V3 region of *16S* rRNA gene	Roche 454 GS FLX Titanium pyrosequencing	Rynkiewicz et al. (2015)
Dermacentor variabilis, Ixodes scapularis	Field-collected ticks	Nymphs and adults	Whole tick	Ontario (Canada)	V4 region of *16S* rRNA gene	Illumina MiSeq	Clow et al. (2018)
Ixodes scapularis, I. affinis	Field-collected ticks	Adult males and females	Whole tick	South Carolina, North Carolina, Virginia, Connecticut, New York (USA)	V1–V3 region of *16S* rRNA gene	454 pyrosequencing; Illumina MiSeq	van Treuren et al. (2015)
Ixodes scapularis	Field-collected and lab-reared ticks	Larvae, nymphs, adults	Midgut and salivary glands	New York (USA)	V3–V4 region of *16S* rRNA gene	Illumina MiSeq	Zolnik et al. (2016)
Ixodes scapularis	Lab-reared ticks	Adult males and females	Whole tick	Texas (USA)	V4 region of *16S* rRNA gene	Illumina MiSeq	Thapa et al. (2019)
Ixodes scapularis	Field-collected ticks	Nymphs and adults	Whole tick	New York (USA)	V3–V4 region of *16S* rRNA gene	Illumina	Zolnik et al. (2018)
Ixodes scapularis	Field-collected ticks	Nymphs	Whole nymph	Vermont (USA)	*16S* rRNA gene	Illumina HiSeq	Landesman et al. (2019)
Ixodes scapularis	Field-collected ticks	Adult males and females	Whole tick	Pennsylvania (USA)	V4/V6 region of *16S* rRNA gene	Illumina MiSeq	Sakamoto et al. (2020)
Ixodes scapularis, Ixodes sp.	Field-collected ticks	Adult females	Whole tick	Alberta (Canada)	V2, V3, V4, V6-7, V8, V9 region of *16S* rRNA gene	Ion Personal Genome Machine PGM™	Sperling et al. (2020)
Ixodes scapularis, I. pacificus, Amblyomma maculatum, Dermacentor spp.	Field-collected ticks	Adult males and females	Midgut, reproductive tissues and salivary glands	Washington, Illinois, Minnesota, Wisconsin, Oklahoma (USA)	V3–V4 region of *16S* rRNA gene	Illumina MiSeq	Ross et al. (2018)
Ixodes scapularis, I. angustus	Field-collected ticks	Nymphs and adult females	Whole ticks	New Brunswick, Ontario, Alberta British Columbia, Nova Scotia (Canada); Amherst (USA)	V2, V3, V4, V6-7, V8, V9 region of *16S* rRNA gene	Ion Torrent PGM	Sperling et al. (2017)
Ixodes persulcatus, I. pavlovskyi, Dermacentor reticulatus	Field-collected ticks	Adult males and females	Whole tick	Novosibirsk (Russia)	V3–V5 regions of *16S* rRNA gene	Illumina MiSeq	Kurilshikov et al. (2015)

(*continued on next page*)

Table 1 (*continued*)

Tick	Origin	Developmental stage/Sex	Tissue	Location	Target gene	Approach	Reference[a]
Ixodes pacificus, I. angustus, Dermacentor variabilis, D. occidentalis, D. albipictus, Haemaphysalis leporispalustris	Field-collected ticks	Larvae, nymphs, adults	Whole tick	California, San Francisco (USA)	V3–V4 region of *16S* rRNA gene	Illumina MiSeq	Chicana et al. (2019)
Ixodes pacificus	Field-collected ticks	All stages	Whole tick	San Francisco (USA)	V3–V4 region of *16S* rRNA gene	Illumina MiSeq	Swei & Kwan (2017)
Ixodes pacificus	Field-collected and lab-reared ticks	Larvae, nymphs, adults	Whole tick	San Francisco (USA)	V3–V4 region of *16S* rRNA gene	Illumina MiSeq	Kwan et al. (2017)
Ixodes persulcatus	Field-collected and lab-reared ticks	Adult females	Whole tick	Heilongjiang (China)		Illumina Hiseq	Sui et al. (2017)
Ixodes ventalloi	Field-collected ticks	Adult females	Whole tick	Sicily (Italy)	Whole genome	Shotgun-metagenomic sequencing	Díaz-Sánchez et al. (2019a)
Ixodes ricinus	Lab-reared ticks	Larvae and adult females	Whole internal tissues and salivary glands	Czech Republic	RNA-seq data	Metatranscriptomics and metaproteomics	Hernández-Jarguín et al. (2018)
Ixodes ricinus	Field-collected ticks	Nymphs and adults	Whole tick	Swiss Alps	V4 region of *16S* rRNA gene	Illumina MiSeq	Aivelo et al. (2019)
Ixodes ricinus, Rhipicephalus microplus	Field-collected and lab-reared ticks		Midgut and ovaries	Ceske Budejovice (Czech Republic)	V6–V8 region of *16S* rRNA gene	Illumina MiSeq	Guizzo et al. (2020)
Amblyomma longirostre, A. nodosum, A. maculatum, Haemaphysalis juxtakochi	Field-collected ticks	Larvae and nymphs	Whole tick	Louisiana (USA)	V1–V3 region of *16S* rRNA gene	454 pyrosequencing	Budachetri et al. (2017)
Amblyomma maculatum	Field-collected ticks	Adults	Whole tick	Mississippi (USA)	V4 region of *16S* rRNA gene	Illumina MiSeq	Varela-Stokes et al. (2018)
Amblyomma tuberculatum	Field-collected ticks	Adult females	Whole tick and midguts	Mississippi (USA)	*16S* rRNA gene	454 pyrosequencing	Budachetri et al. (2016)
Amblyomma cajennense (*sensu stricto*)	Field-collected ticks	Adult females	Whole tick without the gut and midgut	Piste de La Mirande (French Guiana)	V4 region of *16S* rRNA gene	Illumina GenSeq	Binetruy et al. (2019)
Amblyomma gemma	Field-collected ticks	Adults	Whole tick	Tanzania	V3–V4 region of *16S* rRNA gene	Illumina MiSeq	Lee et al. (2019)
Amblyomma sp.	Lab-reared and field-collected ticks		Whole tick	America and Africa	V4 region of *16S* rRNA gene	Illumina MiSeq	Binetruy et al. (2020)
Amblyomma americanum	Field-collected ticks	Adult females	Midgut, salivary glands and ovaries	Kansas (USA)	V3–V4 region of *16S* rRNA gene	MiSeq Next Generation	Maldonado-Ruiz et al. (2021)
Amblyomma americanum, Ixodes scapularis	Field-collected ticks	Eggs, larva, nymph, adults	Whole tick	Virginia (USA)	V3–V4 region of *16S* rRNA gene	Illumina MiSeq	Jory Brinkerhoff et al. (2020)
Amblyomma sculptum, A. aureolatum	Lab-reared ticks	Adult females	Midgut	São Paulo (Brazil)	V3–V4 region of *16S* rRNA gene	Illumina MiSeq	Pavanelo et al. (2020)
Amblyomma triguttatum, Bothriocroton auruginans, B. concolor, Haemaphysalis bancrofti, H. bremneri, H. humerosa, H. longicornis, Ixodes antechini, Ixodes australiensis, I. fecialis, I. holocyclus, I. myrmecobii, I. ornithorhynchi, I. tasmani, I. trichosuri	Field-collected ticks		Whole tick	Australia	V1–V2 region of *16S* rRNA gene	Illumina MiSeq	Egan et al. (2020)

Table 1 (*continued*)

Tick	Origin	Developmental stage/Sex	Tissue	Location	Target gene	Approach	Reference[a]
Amblyomma auricularium, A. dissimile, A. geayi, A. longirostre, A. mixtum, A. naponense, A. oblongoguttatum, A. ovale, A. pacae, A. sabanerae, A. tapirellum, A. varium, Haemaphysalis juxtakochi, Ixodes affinis, Ornithodoros puertoricensis	Field-collected ticks	Larvae, nymphs, adults	Whole tick	Central Panama	V1–V3 region of *16S* rRNA gene	Illumina MiSeq	Kueneman et al. (2021)
Haemaphysalis wellingtoni, H. hystricis, H. bispinosa	Field-collected ticks	Larvae, nymphs, adult females	Whole tick	Perak (Malaysia)	V6 region of *16S* rRNA gene	Ion Torrent PGM	Khoo et al. (2016)
Haemaphysalis flava	Field-collected ticks	Egg, larvae, nymphs, adults	Whole tick	Henan (China)	V3 region of *16S* rRNA gene	Illumina MiSeq	Duan & Cheng (2017)
Haemaphysalis lemuris	Field-collected ticks	Nymphs and adults	Whole tick	Mahajanga, Betampona, Analamazoatra, Ambatovy, Kianjavato (Madagascar)	V4 region of *16S* rRNA gene	Illumina MiSeq	Lado et al. (2018)
Haemaphysalis longicornis	Field-collected ticks	Adult males and females	Whole tick	Shandong (China)	V3–V4 region of *16S* rRNA gene	Illumina MiSeq	Zhang et al. (2019b)
Haemaphysalis hystricis, Dermacentor atrosignatus, D. compactus, D. steini, Amblyomma testudinarium	Field-collected ticks	Adults	Whole tick	Selangor (Malaysia)	V6 region of *16S* rRNA gene	Ion Torrent PGM	Lim et al. (2020)
Haemaphysalis juxtakochi, Amblyomma tapirellum, A. oblongoguttatum	Field-collected ticks	Nymphs and adults	Whole tick	Panama Canal Zone (Panama)	V4 region of the *16S* rRNA	Illumina	Bennett et al. (2019)
Hyalomma anatolicum, Rhipicephalus microplus	Field-collected ticks	Adults	Whole tick	Sialkot, Gujrat, Gujranwala, Sheikhupura (Pakistan)	V1–V3 region of the *16S* rRNA gene	Illumina MiSeq	Adegoke et al. (2020)
Hyalomma dromedarii	Field-collected ticks	Adults	Whole tick	Al-Ain (UAE)	V3–V4 region of *16S* rRNA gene	Illumina MiSeq	Perveen et al. (2020)
Hyalomma lusitanicum	Field-collected ticks	Adult males	Whole tick	Cáceres (Spain)	V4 region of *16S* rRNA gene	Illumina MiSeq	Díaz-Sánchez et al. (2021)
Rhipicephalus sp., *Haemaphysalis* sp., *Hyalomma* sp., *Ornithodoros* sp., *Argas sp.*	Field-collected ticks	Larvae, nymphs, adults	Whole tick	Pakistan	V1–V3 region of *16S* rRNA gene	454 pyrosequencing	Karim et al. (2017)
Rhipicephalus sanguineus (*sensu lato*)	Field-collected ticks	Nymphs and adults	Whole tick	Corsica, Drôme, Gard and Var (France); Dakar (Senegal); Arizona (USA)	V5–V6 region of *16S* rRNA gene	Illumina MiSeq	René-Martellet et al. (2017)
Rhipicephalus sanguineus (*sensu lato*), *Haemaphysalis punctata, Dermacentor marginatus, Ixodes ricinus*	Field-collected ticks	Nymphs and adults	Whole tick	La Rioja (Spain)	V3–V4 region of *16S* rRNA gene	Illumina MiSeq	Portillo et al. (2019)
Rhipicephalus haemaphysaloides	Lab-reared ticks	Adult males and females	Whole tick	Yunnan (China)	V3–V4 region of *16S* rRNA gene	Illumina MiSeq	Li et al. (2018a,b)
Rhipicephalus microplus	Field-collected ticks	Adult females	Salivary glands and gut	Antioquia (Colombia)	V3–V4 region of *16S* rRNA gene	Illumina MiSeq	Segura et al. (2020)
Argas japonicus	Field-collected ticks	Nymphs and adults	Whole tick	Inner Mongolia Autonomous Region (China)	*16S* rRNA gene	PacBio RSII	Yan et al. (2019)
Ornithodoros turicata	Field-collected ticks	Adults	Whole tick	Mapimi Biosphere Reserve (Mexico)	V3–V4 region of *16S* rRNA gene	Illumina MiSeq	Barraza-Guerrero et al. (2020)
Bothriocroton auruginans, Haemaphysalis bancrofti, H. longicornis, Ixodes tasmani, I. holocyclus	Field-collected ticks	Larvae, nymphs, adults	Whole tick	Eastern Australia	V3–V4 region of *16S* rRNA gene	Illumina	Beard et al. (2021)

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Table 1 (*continued*)

Tick	Origin	Developmental stage/Sex	Tissue	Location	Target gene	Approach	Reference[a]
Bothriocroton undatum	Field-collected ticks	Adult females	Whole tick	New South Wales (Australia)	V1–V3 and V3–V4 *16S* rRNA gene	Illumina MiSeq	Panetta et al. (2017)
Ixodes ornithorhynchi	Field-collected ticks	Larvae, nymphs and adult females	Whole tick	Queensland and Tasmania (Australia)	V1–V2 region of *16S* rRNA gene	Illumina MiSeq	Gofton et al. (2018)
Ixodes holocyclus, I. trichosuri, I. tasmani, Haemaphysalis bancrofti	Field-collected ticks	Nymphs and adult females	Whole tick	New South Wales (Australia)	V1–V3 and V3–V4 *16S* rRNA gene	Illumina MiSeq	Chandra & Šlapeta. (2020)

[a] Only papers published in 2015 or after were included in the table. For manuscripts on tick microbiome published before 2015, the reader is refereed to a previous review (Narasimhan & Fikrig, 2015).

transmission of these bacteria, which have been reported in several tick species (Macaluso et al., 2001; Moore et al., 2018; Hauck et al., 2020). Considering that infection by *Rickettsia montana* and *Rickettsia rhipicephali* inhibits transovarial transmission of the heterologous *Rickettsia* sp. (i.e. *R. rhipicephali* and *R. montana*, respectively) (Macaluso et al., 2002), it is expected that some *Rickettsia* OTUs may dominate over others depending on who arrives first. It was proposed that *Rickettsia* colonization of tick ovaries modulate gene expression of the oocytes, making them resistant to a secondary infection with other rickettsiae (Macaluso et al., 2002). Interestingly, the loss of the first *Rickettsia* sp. (*R. montana* or *R. rhipicephali*) in the offspring allowed infection with the second heterologous *Rickettsia* sp. (*R. rhipicephali* or *R. montana*, respectively), which was then able to transmit to the tick progeny (Macaluso et al., 2002). This suggests that the association between the tick and specific *Rickettsia* endosymbionts is transgenerationally unstable and several *Rickettsia* lineages may colonize a single tick lineage across generations.

The concept of transgenerational microbiome was studied by Jory Brinkerhoff et al. (2020) in *I. scapularis* ticks. They showed that the microbiome richness, diversity and composition were similar in adult females and their eggs, with *Rickettsia* being the dominant genus, suggesting the vertical transmission of the endosymbiont. Supporting this idea, Zhang et al. (2020) demonstrated that the microbiota of *Dermacentor silvarum* females and eggs exhibit high similarity. In contrast to the former study, the dominant genus here was *Coxiella.* Interestingly, *Coxiella* and *Rickettsia* were identified as nutritional endosymbionts (Hunter et al., 2015; Smith et al., 2015). Thus, we can hypothesize that transgenerational microbiome inheritance includes bacteria that are indispensable for early tick development.

Tick microbiome also changes with the progression of the life-cycle and developmental stages. Several studies have shown that microbiome species richness and diversity are higher in the larval stage and decrease as the tick ages. This finding was observed in different tick species such as *I. pacificus* (Kwan et al., 2017; Swei & Kwan, 2017; Chicana et al., 2019), *Dermacentor albipictus* (Chicana et al., 2019), *D. silvarum* (Zhang et al., 2020) and *A. americanum* (Menchaca et al., 2013). The mechanisms ruling microbiome diversity changes through the tick ontogeny are not clear, but it has been hypothesized that the loss of diversity could be associated with competitive interactions between tick microbiome bacteria or could be the result of a gradual loss of unstable microbes through the tick development (Chicana et al., 2019). Bacterial community structure and tick microbiome functionality can also differ between life stages. For example, it has been shown that *A. americanum* and *I. ricinus* nymphs have significant differences in the microbial structure when compared to adults (Carpi et al., 2011; Williams-Newkirk et al., 2014). In the functional aspect, Zhang et al. (2020) demonstrated that sequences associated with the biosynthesis of amino acids and purine metabolism pathways were overrepresented in *D. silvarum* nymphs compared to other stages. This finding suggests that the functional differences between life stages could explain the variation of microbiome diversity and structure associated with different developmental stages (Zhang et al., 2020). Furthermore, the study of Chicana et al. (2019) found that predicted gene function was similar at the larval stage across all studied species of tick and begin to change at ticks nymphal stage suggesting that tick age or host blood-meal could be implicated in observed microbiome differences.

The influence of host blood-meal in tick microbiome has also been studied in different tick species (Egyed & Makrai, 2014; Rynkiewicz et al., 2015; Swei & Kwan, 2017; Chicana et al., 2019). For example, Jory Brinkerhoff et al. (2020) reported that engorged *I. scapularis* females presented lower microbial richness compared to unfed males and nymphs suggesting an impact of blood-feeding on tick microbiome diversity. However, considering that the feeding status of compared tick stages was not same (i.e. fed females *vs* unfed males and nymphs), the study by Jory Brinkerhoff et al. (2020) makes it difficult to distinguish the impact of feeding from that of different developmental stages on tick microbiota composition. Others showed that the microbiome of *I. pacificus* nymphs fed on western fence lizards (*Sceloporus occidentalis*) presented significantly lower species richness when compared to the microbiome of nymphs fed on mice (Swei & Kwan, 2017). Chicana et al. (2019) further demonstrated that ticks that feed predominantly on a single or limited range of hosts (e.g. *Haemaphysalis leporispalustris* and *D. albipictus* ticks), have lower microbiome species richness and diversity compared to ticks, such as *I. pacificus* or *D. variabilis*, that feed on several host species. Altogether, these results show that feeding contributes substantially to variation in tick microbiota composition.

Environmental factors were also considered as a possible factor of tick microbiome variation. Two studies (Zolnik et al., 2016; Kwan et al., 2017) found that laboratory-reared or field-collected larvae and nymphs possess different microbiome composition, and Narasimhan et al. (2014) found that laboratory-reared ticks have different microbiomes compared to ticks reared in "sterile" containers, suggesting that environmental factors, and/or host availability, have an impact on tick microbiome. An experimental trial studied the effect of temperature on tick bacterial community and showed that the bacterial community composition and diversity of *I. scapularis* ticks changed at 30 °C and 37 °C in contrast to the group incubated at 4 °C and 20 °C demonstrating the impact of temperature on tick microbiome (Thapa et al., 2019). Several studies also compared the microbiome of ticks collected in different geographical sites and showed that bacterial community or structure changes according to collection site (Carpi et al., 2011; van Treuren et al., 2015; Trout Fryxell & DeBruyn, 2016; Gall et al., 2017; Chandra & Šlapeta, 2020). We can speculate that tick microbiome variation across different sampling sites could be the result of acquisition, by the ticks, of microbes present in the soil. Indeed, Zolnik et al. (2016) showed the existence of soil-associated bacteria in *I. scapularis* microbiome. Furthermore, Rynkiewicz et al. (2015) reported that *Lactobacillus*, a diverse group of bacteria found in soil, can be detected not only in *D. variabilis* and *I. scapularis* but also in their rodent host. It is noteworthy that other studies (Hawlena et al., 2013; Jory Brinkerhoff et al., 2020) did not find an association between the collection site and variation in tick microbiome. Indeed, Hawlena et al. (2013) reported that arthropod traits as life stages or tick

species, and not environmental factors, determined the bacterial community. Furthermore, they proposed the existence of dominant species-specific endosymbionts that exclude other bacteria masking possible environmental effects.

4. Role of tick immunity in shaping tick microbiome dynamics

Several signaling pathways such as the immune deficiency (IMD), the Janus kinase (JAK), signal transducer and activator of transcription (STAT) and Toll receptor signaling pathway have been described as important components of the tick immune system (Smith & Pal, 2014; Gulia-Nuss et al., 2016). In *Drosophila*, activation of these pathways by recognition of pathogen-associated molecular patterns (PAMPs) and activation of the Toll receptor ligand Spaetzle triggers the production of antimicrobial peptides (AMPs), which contributes to controlling infection by invading bacteria, viruses or fungi (Hoffmann & Reichhart, 2002). Despite missing several canonical components of immune signaling pathways, notably in the IMD pathway, ticks develop effective immune responses against invading pathogens (Rosa et al., 2016; Shaw et al., 2017). For example, lipids that make up the bacterial membrane activate the IMD pathway of ticks and RNAi knockdown of genes involved in IMD signaling resulted in increased *B. burgdorferi* burden in ticks (Shaw et al., 2017). Notably, activation of JAK-STAT signaling pathway by *A. phagocytophilum* infection was linked to the expression of specific tick AMPs (Liu et al., 2012). The role of pathogen-induced AMPs on the tick microbiome composition remains poorly characterized.

Table 2
Tick-microbiome interactions

Tick	Microbe	Main findings	Reference
Amblyoma americanum	*Coxiella*-like endosymbiont of *A. americanum* (CLEAA)	‣ CLEAA genome encodes most major vitamin and cofactor biosynthesis pathways including folic acid (vitamin B9), riboflavin (B2), pantothenic acid (B5), nicotinamide (B3), pyridoxine (B6), thiamine (B1), biotin (B7), and lipoic acid	Smith et al. (2015)
Amblyoma americanum	*Coxiella* sp.	‣ Treatment of engorged females with rifampicin or tetracycline was associated with reduced reproductive fitness; ‣ Direct correlation between reduced number of *Coxiella* sp. and measures of reproductive fitness was found	Zhong et al. (2007)
Rhipicephalus turanicus	*Coxiella*-like symbiont	‣ *Coxiella*-like symbiont genome encodes for at least five vitamins (B2, B5, B6, B7, B9)	Gottlieb et al. (2015)
Rhipicephalus sanguineus, R. turanicus	*Coxiella*-like endosymbiont (CLE)	‣ *In silico* flux balance metabolic analysis revealed an excess production of L-proline in the genome of CLE; ‣ Genome of CLE encoded multiple copies of the proline/betaine transporter, *prop* gene	Tsementzi et al. (2018)
Rhipicephalus sanguineus	*Coxiella*-like endosymbiont (CLE)	‣ Treatment of engorged nymphs with ofloxacin reduced the bacterial load and CLE numbers in subsequent life stages; ‣ Symbiont suppression was associated with fitness reduction throughout the tick's life-cycle	Ben-Yosef et al. (2020)
Rhipicephalus microplus	*Coxiella* endosymbiont from *R. microplus* (CERM)	‣ Treatment of tick or vertebrate host with tetracycline reduced bacterial load in progeny (eggs and larvae) with no impact in reproductive fitness of the adult female or on embryon development; ‣ Antibiotic treatment of engorged females blocked development at the metanymph stage	Guizzo et al. (2017)
Rhipicephalus haemaphysaloides	*Coxiella*-like endosymbiont (*Coxiella*-LE)	‣ Treatment of engorged female ticks with kanamycin or tetracycline was associated with decreased hatching rates of eggs; ‣ The reduced hatching rates were associated with the density of *Coxiella*-LE	Li et al. (2018a,b)
Haemaphysalis longicornis	*Coxiella*-like endosymbiont (CLS-HI)	‣ Reduced density of CLS-HI, obtained after treatment with tetracycline, was associated with decreased reproductive fitness in ticks	Zhang et al. (2017)
Ixodes pacificus	*Rickettsia* species phylotype G021	‣ Decrease in rickettsial density of *I. pacificus* by antibiotic treatment had no significant effect on the preoviposition period or the number of offspring; ‣ No differences in the incubation period, egg hatching rate, and the number of larvae were found between antibiotic-treated and control groups	Kurlovs et al. (2014)
Ixodes pacificus	*Rickettsia* species phylotype G021	‣ *Rickettsia* species phylotype G021 genomes encode all folate genes	Hunter et al. (2015)
Ixodes ovatus, I. persulcatus, Amblyomma variegatum		‣ Functional metagenomics analysis showed differences in taxonomic and functional profiles (abundance of genes involved in carbohydrate, aminoacid, lipid and vitamin B metabolism) between sexes of the same species; ‣ The majority of genes and functions were found in different bacteria of the microbiota indicating functional redundancy	Obregón et al. (2019)
Ornithodoros moubata	*Francisella* type F-Om	‣ Elimination of *Francisella* symbiont hampers ticks' growth and molting to adulthood, deficiencies that were restored with an oral supplement of B vitamins	Duron et al. (2018)
Amblyomma americanum, Dermacentor variabilis, Ixodes scapularis	*Arsenophonus* and *Rickettsia*	‣ *Rickettsia* was associated with increasing motility while *Arsenophonus* with decreased motility	Kagemann & Clay (2013)
Amblyomma maculatum	*Francisella*-like endosymbiont (FLE-Am)	‣ FLE-Am possess extensive metabolic capabilities including production of cofactors, amino acids and heme	Gerhart et al. (2016)
Amblyomma maculatum, Ornithodoros moubata	*Francisella*-like endosymbiont (FLE)	‣ FLEs encode complete pathway for the synthesis of several B vitamins and cofactors such as biotin (B7), folate (B9), riboflavin (B2), lipoic acid and FAD, denoting the possible function of FLE as nutrient-provisioning endosymbionts	Gerhart et al. (2018)
Dermacentor andersoni		‣ Offspring of oxytetracycline-treated ticks presented significant reductions of fitness: lower larval survival, reduced mean larval weight and survival after larva-nymphal molt	Clayton et al. (2015)
Ixodes ricinus	*Escherichia coli*	‣ Anti-*E. coli* and anti-α-Gal IgM and IgG, produced after immunization of α1,3-galactosyltransferase-deficient-C57BL/6 (α1,3 GT KO) with live *E. coli* vaccine, was associated with high mortality of nymphs; ‣ Nymphs that fed on C57BL/6 immunized with *E. coli* had higher weight	Mateos-Hernández et al. (2020)
Ixodes ricinus	*Escherichia coli*	‣ Anti-*E. coli* IgM and IgG, produced after immunization of C57BL/6 immunized with *E. coli*, was associated with modulation of the tick microbiome.	Mateos-Hernández et al. (2021)

In the arthropod model *Drosophila melanogaster*, immune pathways are induced in response to both commensal and pathogenic microbes, and these pathways are important to regulate the location, density, and diversity of the host microbiome (Lesperance & Broderick, 2020). Toll and IMD pathways recognize cell wall components in Gram-positive and Gram-negative bacteria, respectively (Hanson & Lemaitre, 2020). Pathway stimulation by PAMPs leads to the activation of the transcription factors NF-κB (Toll) and Relish (IMD), which results in the expression of different AMPs (Hanson & Lemaitre, 2020). Promoting colonization by beneficial microbes from the environment, these antimicrobial molecules shape the host microbiome in legume plants, other insects and protists (Mergaert, 2018). How AMPs modulate the microbiome in ticks (Smith & Pal, 2014; Kurokawa et al., 2020) and *Drosophila* (Hanson & Lemaitre, 2020) has not been characterized to the same extent. In one study, alterations to the tick microbiota were shown to decrease immune activation through the JAK-STAT pathway in fed ticks (Narasimhan et al., 2014). Particularly, Narasimhan et al. (2014) observed that rearing and maintaining *I. scapularis* larvae under "sterile" conditions induced dysbiosis in the gut microbiome, and decreased expression of STAT in fed larvae compared to fed larvae maintained under normal conditions. The gut microbiome of dysbiosed fed larvae had a higher abundance of bacteria of the genera *Delftia*, *Acidovorax*, and *Rickettsia* compared to normal larvae, and a lower abundance of bacteria of the genera *Comamonas*, *Chryseobacterium*, *Lactobacillus*, and *Paenibacillus* in comparison to normal fed larvae (Narasimhan et al., 2014). Changes in the microbiota composition associated with JAK-STAT pathway modulation were linked to lower expression of peritrophin genes, decreased thickness of the peritrophic matrix (PM), and reduced *B. burgdorferi* colonization (Narasimhan et al., 2014). Further studies are needed to unravel the association between activation of JAK-STAT, Toll and IMD pathways and the expression of AMP genes in response to microbiota modulation and their influence on pathogen colonization. Interestingly, transcriptome analyses have shown that the microbiota triggers the expression of several AMP genes (e.g. *Drosomycin-like* 2 and 3) regulated by JAK-STAT in *Drosophila*. The decrease in bacterial diversity in adult ticks compared to larvae supports the notion of microbiome selection through the tick ontogeny, a process in which tick immunity may play an important role.

5. Tick-microbiome interactions

The role of non-pathogenic microbes in the tick biology have been the focus of several investigations (Table 2). One of the best-characterized contributions of endosymbionts to ticks is the nutritional complementation. Because of their restrictive, blood-based diet, ticks lack important nutrients like B vitamins and other cofactors, deficiencies that are countered by ticks *via* their association with symbiotic bacteria (Duron et al., 2018). For example, the genome of *Coxiella*-like endosymbiont, an obligate intracellular bacterium (Bonnet and Pollet, 2021), encodes for cofactor and vitamins including riboflavin (B2), pantothenic acid (B5), pyridoxine (B6), biotin (B7) and folic acid (B9) in *A. americanum* (Smith et al., 2015) and *R. turanicus* (Gottlieb et al., 2015). The complete pathway for B vitamins and cofactors synthesis is also encoded in the genome of *Francisella*-like endosymbiont (FLE) present in *A. maculatum* and *Ornithodoros moubata* (Gerhart et al., 2018), and the endosymbiont has the capability for production of amino acids and heme (Gerhart et al., 2016). Further examples are *Rickettsia* endosymbionts that have the genetic capacity for *de novo* folate synthesis in *I. pacificus* (Hunter et al., 2015). Endosymbionts may also affect the development, reproduction fitness or the behavior of their hosts. Antibiotic-based elimination of *Francisella* symbiont in *O. moubata* nymphs hampers its growth and molting to adults. Interestingly, these deficiencies were restored with an oral supplementation of B vitamins underlying the crucial role of *Francisella* symbiont as an obligate nutritional mutualist (Duron et al., 2018). Several other experiments have demonstrated an association between the reduction of *Coxiella*-like endosymbiont numbers and a decreased reproductive fitness (Zhong et al., 2007; Zhang et al., 2017; Li et al., 2018a,b; Ben-Yosef et al., 2020), or impairment in development to adult stage (Guizzo et al., 2017). Microbial infection can also impact tick motility. One study demonstrated that *Rickettsia* and *Arsenophonus* were associated with increased and decreased tick larvae locomotion, respectively (Kagemann & Clay, 2013).

The role of specific commensals is not as well characterized as that of endosymbionts in ticks. The majority of the available studies have associated one symbiont with one role in tick's biology, but it is noteworthy that Obregon et al. (2019) demonstrated that the tick microbiome has genes involved in different metabolic pathways such as carbohydrate, aminoacid, lipid and B vitamin metabolism. Notably, these genes were not identified in one but in different bacteria of the tick microbiota. Similarly, Estrada-Peña et al. (2020b) reported the existence of functional redundancy (i.e. the presence of the same genes and/or functional categories in different microbes) in the tick microbiome. A remarkable example of functional redundancy is that up to 198 bacterial genera could contribute to a single pathway in *I. scapularis* microbiome. Such functional redundancy suggests that ticks evolved mechanisms to modulate their microbiome selecting multiple bacteria that contribute to a functional profile and hence, may provide ecological advantages to the ticks (Obregón et al., 2019; Estrada-Peña et al., 2020a, b). The functional redundancy can contribute to the microbiome stability in stressful conditions that could otherwise disturb the functional composition of the bacterial community (Estrada-Peña et al., 2020b). The resistance of the tick microbiome to disturbing factors such as anti-tick vaccines, pathogen infection and peptides with antimicrobial activity was tested in *I. scapularis* (Estrada-Peña et al., 2020a). The results showed that pathogen infection and peptides affect the taxonomic composition and taxa co-occurrence networks, but had limited impact on the functional traits of the tick microbiome. In contrast, immunization with tick proteins increased both the taxonomic and pathways diversity (Estrada-Peña et al., 2020a). These results suggest that functional redundancy prevents pathways depletion and contributes to the resistance of the tick microbiome to disturbance.

6. Tripartite interactions between the tick, microbiome and transmitted pathogens

Mounting evidence suggests that the contributions of the tick microbiota to tick physiology and pathogen life-cycle are so relevant that tick biology and vector capacity cannot be understood without considering tick microbial communities (Table 3). A growing body of research indicates the possible associations between non-pathogenic components of tick microbiome and pathogens such as *Borrelia* spp. (Narasimhan et al., 2014, 2017; Sperling et al., 2020; Hamilton et al., 2021). A study conducted by Narasimhan et al. (2014) showed that the gut microbiome of *I. scapularis*, a major vector of Lyme borreliosis in North America, has an important role in spirochete colonization. Unfed larval ticks raised under "sterile" conditions had increased relative abundance of *Rickettsia*, *Thioclava* and *Delftia* and decreased relative abundance of *Aquabacterium*, *Brevibacterium* and *Novosphingobium*. The alteration of the bacterial assembly resulted in increased tick engorgement weights and a decreased ability of *B. burgdorferi* to colonize the larvae gut after feeding on *Borrelia*-infected mice. In line with the evidence supporting *Borrelia*-microbiome interactions, *Borrelia*-positive *I. scapularis* ticks collected from the field had significantly greater bacterial diversity than *Borrelia*-negative ticks (Sperling et al., 2020). Bacterial β-diversity also varied based on *B. burgdorferi* presence/absence status in *I. scapularis* (Landesman et al., 2019). An additional study by Hamilton et al. (2021) showed that depletion of the bacterial microbiome in larval ticks has no effect on *Borrelia afzelii* acquisition during blood-feeding on infected mice, but exposure to this *Borrelia* sp. changed the tick microbiome by decreasing bacterial abundance, shifting bacterial community composition, and increasing bacterial diversity. However, two recent epidemiological studies suggested that infection with *B. burgdorferi* does not influence the

Table 3
Tick-microbiome-pathogen interactions

Tick	Pathogen	Findings	Reference
Ixodes scapularis	*Borrelia burgdorferi*	‣ Dysbiosed larvae of *I. scapularis* increased engorgement weights and decreased *B. burgdorferi* colonization; ‣ Dysbiosed tick larvae presented decreased expression of STAT and peritrophin resulting in altered tick gut peritrophic membrane integrity; ‣ Altered integrity of the peritrophic matrix decreased epithelium-bound spirochetes	Narasimhan et al. (2014)
Ixodes scapularis	*Anaplasma phagocytophilum*	‣ *A. phagocytophilum* changed tick microbiota: *Enterococcus* and *Rickettsia* were decreased whereas *Pseudomonas* was increased; dysbiosis enhanced *A. phagocytophilum* colonization; ‣ *A. phagocytophilum* induced changes in the gut barrier (decrease of *peritrophin* genes expression and thickness of the peritrophic matrix) *via* the antifreeze glycoprotein IAGFP; ‣ IAGFP bound to the D-alanine residue of bacterial peptidoglycan which results in altered permeability and the capacity of bacteria to form biofilms	Abraham et al. (2017)
Ixodes scapularis	*Borrelia burgdorferi*	‣ *B. burgdorferi* infection induced PIXR expression which facilitates pathogen colonization in tick gut and larval molting; inhibits bacterial biofilm formation and affects gut microbiome and metabolome composition	Narasimhan et al. (2017)
Ixodes scapularis	*Borrelia burgdorferi*	‣ After computational removal of the dominant rickettsial endosymbiont, *B. burgdorferi*-infected ticks presented lower microbiome diversity, particularly species evenness compared to uninfected field-collected ticks	Kwan et al. (2017)
Ixodes scapularis	*Borrelia burgdorferi*	‣ *B. burgdorferi* infection in ticks was associated with increased abundance of *Bacillus*, *Enterobacteriaceae* and *Pseudomonas* within the midgut	Ross et al. (2018)
Ixodes scapularis	*Borrelia burgdorferi*	‣ *B. burgdorferi* presence/absence was correlated with bacterial β-diversity, specifically in the differences in the relative abundance of taxa; ‣ *B. burgdorferi*-negative nymphs presented higher levels of *Pseudomonas* ASV and *Staphylococcus* while *B. burgdorferi*-positive nymphs were associated with higher levels of *Sphingomonas*	Landesman et al. (2019)
Ixodes scapularis	*Borrelia burgdorferi*	‣ No association between microbiome diversity and *B. burgdorferi* was found in field-collected *I. scapularis* ticks; ‣ The abundance of reads from *Cutibacterium* and *Borrelia burgdorferi* was over-represented while *Rickettsia*, *Diplorickettsiaceae* and *Beijerinckiaceae* were under-represented in *Borrelia*-infected ticks	Chauhan et al. (2020)
Ixodes scapularis		‣ *Anaplasma phagocytophilum* infection and antifreeze glycoprotein treatment affected taxonomic composition and co-occurrence network; ‣ Anti-tick immunity to PIXR impacted microbial diversity and functional profile and produced over-representation of pathways involved in biofilm formation	Estrada-Peña et al. (2020b)
Ixodes scapularis	*Borrelia* spp.	‣ *Borrelia*-positive ticks were positively associated the bacterial genera *Tepidomonas*, *Luteibacter*, *Francisella* and *Fibriimonas*	Jory Brinkerhoff et al. (2020)
Ixodes scapularis	*Borrelia burgdorferi*	‣ Interference with Peritrophic Membrane Chitin Binding Protein (PM_CBP) expression reduced thickness of the peritrophic matrix, impacted its integrity and affected tick feeding; ‣ Passive transfer of anti-PM_CBP antibodies to ticks impaired the survival and transmission of *B. burgdorferi* and altered the microbial diversity in tick gut	Yang et al. (2021)
Ixodes scapularis	*Borrelia* spp.	‣ *Borrelia*-positive ticks presented greater bacterial diversity compared to *Borrelia*-negative ticks	Sperling et al. (2020)
Ixodes scapularis	*Borrelia burgdorferi*	‣ Microbiome of *Borrelia*-infected larvae presented lower occurrence and diversity of bacteria, lower functional redundancy and a lack of coherence in the network built around co-occurring taxa compared to uninfected nymphs	Estrada-Peña et al. (2020a)
Dermacentor andersoni	*Anaplasma marginale; Francisella novicida.*	‣ An increased level of *Rickettsia belli* in the microbiome was negatively correlated to *A. marginale* levels in ticks; ‣ A decreased level of *Francisella* endosymbionts was associated with lower *F. novicida* infection levels	Gall et al. (2016)
Dermacentor occidentalis	*Rickettsia*	‣ An inverse relationship was observed between *Rickettsia* and FLE infection that is consistent with partial interference between FLE and Spotted Fever Group *Rickettsia* infecting ticks	Gurfield et al. (2017)
Amblyomma americanum	*Anaplasma/Ehrlichia*	‣ No significant differences in the overall microbial community structure were found between *Anaplasma/Ehrlichia*-infected and uninfected ticks	Trout Fryxell & DeBruyn (2016)
Amblyomma maculatum	*Rickettsia parkeri*	‣ In *R. parkeri*-infected tick cells, FLE numbers decreased while "*Candidatus* Midichloria mitochondrii" increased when compared to uninfected tick cells; ‣ *R. parkeri* modulated host's defenses by upregulating tick selenoproteins	Budachetri et al. (2018)
Amblyomma aureolatum; A. sculptum	*Rickettsia rickettsii*	‣ *R. rickettsii*-infected *A. aureolatum* presented significant reduction of bacterial load in the midgut while *R. rickettsii*-infected *A. sculptum* had higher bacterial load	Pavanelo et al. (2020)
Rhipicephalus haemaphysaloides	*Babesia microti*	‣ Reduced density of *Coxiella*-like endosymbiont in larval ticks was associated with higher prevalence of *B. microti* among nymphs	Li et al. (2018a,b)
Rhipicephalus microplus	*Theileria* sp.	‣ Presence of *Theileria* sp. in *R. microplus* ticks was associated with reduced microbial diversity, richness and evenness	Adegoke et al. (2020)

overall diversity or richness of the *I. scapularis* microbiome, but they revealed significant associations between the persistence of spirochetes and the occurrence of specific microbial taxa (Chauhan et al., 2020; Jory Brinkerhoff et al., 2020). These results suggest that *B. burgdorferi* requires a specific gut microbial environment for successful colonization, but the mechanisms underlying these complex networks of interaction are not fully elucidated (Kurokawa et al., 2020).

Mechanistically, it was shown that interactions between *B. burgdorferi* and the microbiome is mediated by tick gut proteins. RNA interference-mediated silencing of the gene encoding PIXR, a secreted gut protein of *I. scapularis* with a Reeler domain, and anti-PIXR immunity in mice significantly decreased *B. burgdorferi* colonization in the tick gut, suggesting that the bacterium induces PIXR to enhance its colonization in the tick (Narasimhan et al., 2017). The microbiome of ticks fed on PIXR-immunized mice had increased taxonomic and functional pathways diversity (Estrada-Peña et al., 2020b). Both *in vitro* and *in vivo* experiments showed that PIXR inhibits bacterial biofilm formation and it is, therefore, possible that alteration of biofilm formation could affect the spirochete adherence to the gut epithelium (Narasimhan et al., 2017). Dysbiosis of the tick gut microbiome interrupts the formation of the PM, a glycan-rich structure that separates the gut lumen from the epithelial cells, by diminishing the STAT-mediated expression of a key structural component of PM known as peritrophin. The changes in the structural integrity of the PM also reduced *B. burgdorferi* colonization and its

adherence to the gut lumen (Narasimhan et al., 2014). These data indicate that bacterial components of the tick gut microbiome are critical for the maintenance of PM integrity and that functional integrity is essential for efficient *B. burgdorferi* colonization of the gut epithelium likely because it protects the spirochetes from toxic constituents of the tick guts (Narasimhan et al., 2014).

While the above studies provide some functional basis of the tripartite interactions between the tick, the microbiome and the spirochete, the tick microbiome could also influence *B. burgdorferi* persistence in the gut through other possible ways that are yet to be explored and understood. For instance, the genome of *B. burgdorferi* lacks several genes required for the synthesis of amino acids, fatty acids, nucleotides, and vitamins, and thus the bacterium is dependent on its tick vector and vertebrate host for many essential nutrients and metabolic products (Kurokawa et al., 2020). Some gut endosymbionts or commensals could thus play an important role in the survival of spirochetes in the tick vectors by providing deficient nutrients. On the other hand, *Borrelia* spirochetes may actively alter the microbial structure to generate an environment that is favorable for its colonization (Narasimhan et al., 2017). The infection may increase the expression of specific genes coding for antimicrobial peptides to modulate the composition of the tick microbiome, favoring the establishment of spirochetes in the tick gut. In this sense, *I. scapularis* ticks employ an antimicrobial molecule, called domesticated amidase effector 2 (Dae2) that selectively kills harmful mammalian skin microbes while having no intrinsic ability to kill *B. burgdorferi* (Hayes et al., 2020).

Another example is the obligate intracellular bacterium *A. phagocytophilum* that perturbs the gut microbiome of *I. scapularis* and, in contrast to *Borrelia*, requires a thin and permeable PM for successful colonization as it rapidly passes from the tick guts to the salivary glands (Abraham et al., 2017). Infection with this zoonotic bacterium induces the expression of tick antifreeze glycoprotein (IAFGP), which has antibacterial properties. Mechanistically, IAFGP binds the peptidoglycan of Gram-positive bacteria, resulting in altered permeability and the capacity of bacteria to form biofilms. The antimicrobial activity of IAFGP concurs with a reduced abundance of Gram-positive biofilm-forming taxa in the tick microbiome upon *A. phagocytophilum* colonization. These results suggest that *A. phagocytophilum* induces IAFGP expression to modulate the tick gut microbiome and decrease the structural integrity of the PM and gut barrier, facilitating gut colonization by this bacterium (Abraham et al., 2017). A recent metagenomics study of the resistance of the tick gut microbiome to biological disturbance showed that both *A. phagocytophilum* infection and IAFGP affect the taxonomic composition and bacterial co-occurrence networks, but have little impact on the functional profile of the tick microbiome (Estrada-Peña et al., 2020b). This could be considered an example of tick-microbiome-pathogen coevolution in which *A. phagocytophilum* hijacks a tick protein to apply selective pressure on the tick microbiome which in turn influences pathogen fitness in the vector.

Only a few studies have addressed the interactions between the microbiota and pathogenic bacteria in ticks other than *Ixodes*. For example, Gall et al. (2016) have demonstrated that microbiome disruption with antibiotics can impact pathogen susceptibility in *D. andersoni*. Specifically, they showed a negative correlation between the burden of *Rickettsia bellii* and *Anaplasma marginale* and a positive correlation between *Francisella* endosymbionts and *Francisella novicida* infection levels (Gall et al., 2016). Gurfield et al. (2017) also showed a negative relationship between the levels of FLE and Spotted Fever Group Rickettsia (SFGR) in *D. occidentalis* suggesting interference between FLE and SFGR in this tick species (Gurfield et al., 2017). Further example is the study of Budachetri et al. (2018) which demonstrated that decreased levels of FLE and increased levels of "*Candidatus* Midichloria mitochondrii" were associated with *R. parkeri* infection in *A. maculatum* (Budachetri et al., 2018). The mechanism by which endosymbiont bacteria could regulate pathogen infection had not been well elucidated, but it has been hypothesized that endosymbionts can directly or indirectly impact pathogen growth. The direct mechanism could include the secretion of molecules by endosymbionts that can either enhance or limit pathogen replication while the indirect mechanisms include the competition for host resources that are essential for pathogen growth limiting their replication or the inhibition of immune factors that hampers the pathogenic bacteria enhancing their growth (Gall et al., 2016). For example, the tick immune system has been associated with the lower susceptibility of *Amblyomma sculptum* to the infection of *Rickettsia rickettsii*, the causative agent of Rocky Mountain spotted fever (Martins et al., 2017). Indeed, transcriptional analysis of the midgut of *A. sculptum* showed that immune factors are mostly upregulated in *R. rickettsii*-infected ticks (Martins et al., 2017). Interestingly, the midgut bacterial load is higher in these ticks (Pavanelo et al., 2020). Thus, Pavanelo et al. (2020) have hypothesized that microbiota components can regulate immune factors of *A. sculptum* to create a more efficient immune system resulting in a lower susceptibility.

7. Emerging tools for the precise manipulation of the tick microbiome

Despite recent advances in defining the taxonomic and functional composition of the tick microbiome, mechanistic insights into the role of the microbiome on tick homeostasis and/or vector competence requires the use of precise microbiology tools to manipulate the tick microbiome in a taxon-specific manner. Antimicrobiota vaccines were recently introduced as a precision microbiology tool to target specific taxa in tick microbiomes (Mateos-Hernández et al., 2020, 2021). Combining *16S* rRNA amplicon sequencing and network analysis, highly relevant bacteria for the tick microbiome (i.e. keystone taxa) were identified and used as a live bacteria vaccine to target the microbiome of ticks fed on immunized mice. Based on the ubiquitousness (i.e. ubiquitous presence of bacteria in all the samples tested), high eigenvector-centrality (i.e. indicates the connectivity of the node with other well connected nodes in the network), and high relative abundance, four bacterial families (i.e. *Enterobacteriaceae*, *Corynebacteriaceae*, *Pseudomonadaceae* and *Sphingomonadaceae*) were identified as keystone taxa in the microbiome of *I. ricinus* and *I. scapularis* (Mateos-Hernández et al., 2020). *Enterobacteriaceae* was among the ubiquitous bacterial families with the highest relative abundance and eigenvector-centrality in the microbiota of *I. ricinus* and *I. scapularis* (Mateos-Hernández et al., 2020). Within the family *Enterobacteriaceae*, the bacterial genus *Escherichia-Shigella* was the second most represented taxon in *I. scapularis* and the only taxon represented in *I. ricinus* (Mateos-Hernández et al., 2020). Immunization of C57BL/6 mice with a vaccine formulation containing live *Escherichia coli* (as a representative of *Escherichia-Shigella*) induced the production of anti-*E. coli* IgM and IgG, which were associated with decreased abundance of the genus *Escherichia-Shigella* in the tick microbiome (Mateos-Hernández et al., 2021) and increased tick engorgement (Mateos-Hernández et al., 2020, 2021). In addition, microbiome modulation by antimicrobiota vaccines was associated with decreased tick microbiome diversity (Mateos-Hernández et al., 2021), a restructuration in the hierarchy of microbial community members and decreased keystoneness of *Escherichia-Shigella* in the co-occurrence networks (Mateos-Hernández et al., 2021).

Keystone taxa have a great explanatory power of the community structure and functioning irrespective of their abundance (Banerjee et al., 2018). These highly connected taxa drive community composition and function and can be identified using co-occurrence networks (Weiss et al., 2016; Herren & McMahon, 2018; Banerjee et al., 2018). Accordingly, removal or addition of keystone taxa may be associated with major shifts in the whole community structure. Despite alterations of tick microbiomes are expected to be a potentially fruitful avenue for disrupting pathogen transmission (Shaw & Catteruccia, 2019), progress in molecular and mechanistic insights into the tick microbiome has been hindered by technical difficulties in manipulating the microbiome in a taxon-specific manner. The results by Mateos-Hernández et al. (2020, 2021) opened up the possibility of using antimicrobiota vaccines to

manipulate the tick microbiome and possibly block tick-borne pathogen transmission (Wu-Chuang et al., 2021).

8. Conclusions and perspectives

The number of studies dealing with tick microbiota has risen in the last years, allowing for a deeper understanding of a highly complex structure composed of a diverse assembly of bacteria including commensals, endosymbionts and pathogens, that interact between them and with the tick. Despite plenty of unanswered questions remain, the study of these biological interactions has revealed that tick microbiome can impact tick biology and more importantly, pathogen colonization and transmission (Narasimhan et al., 2014, 2017; Abraham et al., 2017; Mateos-Hernández et al., 2020, 2021; Hamilton et al., 2021). Modulation of the tick microbiome has emerged as a new strategy to impair tick vector capacity and therefore, control tick-borne diseases (Shaw & Catteruccia, 2019). Recently, anti-tick microbiota vaccines have been proposed as a potential powerful tool for manipulation of the tick microbiome (Mateos-Hernández et al., 2020, 2021). As anti-tick microbiota vaccine offers the possibility to target a specific microorganism by injecting live bacteria into the tick's host and subsequently modulate tick microbiome *via* antibodies acquired during feeding, it allows to study the function that selected bacteria have in the tick. Therefore, anti-tick microbiota vaccine can be used as a precision tool to establish the contribution of single bacterial taxa in tick biology and vector competence. Moreover, anti-tick microbiota vaccine can be employed as a tool for tick microbiome engineering. We can foresee that targeting keystone taxa that have a central role in microbial networks would result in homeostasis perturbation of the tick microbiome which could affect tick performance and also vectorial capacity. In this sense, anti-tick microbiota vaccine can be used to weaponise the microbiome against pathogenic microorganisms by targeting bacterial taxa that facilitate pathogen colonization or that are important producers of indispensables elements for pathogen survival in ticks. This could result in a perturbed and harmful environment for pathogens that could stop their spreading and subsequently their transmission to the vertebrate host.

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References

Abraham, N.M., Liu, L., Jutras, B.L., Yadav, A.K., Narasimhan, S., Gopalakrishnan, V., et al., 2017. Pathogen-mediated manipulation of arthropod microbiota to promote infection. Proc. Natl. Acad. Sci. U.S.A. 114, E781–E790. https://doi.org/10.1073/pnas.1613422114.

Adegoke, A., Kumar, D., Bobo, C., Rashid, M.I., Durrani, A.Z., Sajid, M.S., Karim, S., 2020. Tick-borne pathogens shape the native microbiome within tick vectors. Microorganisms 8, 1299. https://doi.org/10.3390/microorganisms8091299.

Aivelo, T., Norberg, A., Tschirren, B., 2019. Bacterial microbiota composition of *Ixodes ricinus* ticks: the role of environmental variation, tick characteristics and microbial interactions. PeerJ 7, e8217. https://doi.org/10.7717/peerj.8217.

Andreotti, R., Pérez de León, A.A., Dowd, S.E., Guerrero, F.D., Bendele, K.G., Scoles, G.A., 2011. Assessment of bacterial diversity in the cattle tick *Rhipicephalus* (*Boophilus*) *microplus* through tag-encoded pyrosequencing. BMC Microbiol. 11, 6. https://doi.org/10.1186/1471-2180-11-6.

Banerjee, S., Schlaeppi, K., van der Heijden, M.G.A., 2018. Keystone taxa as drivers of microbiome structure and functioning. Nat. Rev. Microbiol. 16, 567–576. https://doi.org/10.1038/s41579-018-0024-1.

Barraza-Guerrero, S.I., Meza-Herrera, C.A., De la Peña, C.G., González-álvarez, V.H., Vaca-Paniagua, F., Díaz-Velásquez, C.E., et al., 2020. General microbiota of the soft tick *Ornithodoros turicata* parasitizing the bolson tortoise (*Gopherus flavomarginatus*) in the Mapimi Biosphere Reserve, Mexico. Biology 9, 275. https://doi.org/10.3390/biology9090275.

Beard, D., Stannard, H.J., Old, J.M., 2021. Morphological identification of ticks and molecular detection of tick-borne pathogens from bare-nosed wombats (*Vombatus ursinus*). Parasit. Vectors 14, 60. https://doi.org/10.1186/s13071-020-04565-6.

Ben-Yosef, M., Rot, A., Mahagna, M., Kapri, E., Behar, A., Gottlieb, Y., 2020. *Coxiella*-like endosymbiont of *Rhipicephalus sanguineus* is required for physiological processes during ontogeny. Front. Microbiol. 11, 493. https://doi.org/10.3389/fmicb.2020.00493.

Bennett, K.L., Almanza, A., McMillan, W.O., Saltonstall, K., Vdovenko, E.L.P., Vinda, J.S., et al., 2019. Habitat disturbance and the organization of bacterial communities in Neotropical hematophagous arthropods. PLoS One 14, e0222145. https://doi.org/10.1371/journal.pone.0222145.

Binetruy, F., Buysse, M., Lejarre, Q., Barosi, R., Villa, M., Rahola, N., et al., 2020. Microbial community structure reveals instability of nutritional symbiosis during the evolutionary radiation of *Amblyomma* ticks. Mol. Ecol. 29, 1016–1029. https://doi.org/10.1111/mec.15373.

Binetruy, F., Dupraz, M., Buysse, M., Duron, O., 2019. Surface sterilization methods impact measures of internal microbial diversity in ticks. Parasit. Vectors 12, 268. https://doi.org/10.1186/s13071-019-3517-5.

Bonnet, S.I., Binetruy, F., Hernández-Jarguín, A.M., Duron, O., 2017. The tick microbiome: why non-pathogenic microorganisms matter in tick biology and pathogen transmission. Front. Cell. Infect. Microbiol. 7, 236. https://doi.org/10.3389/fcimb.2017.00236.

Bonnet, S.I., Pollet, T., 2021. Update on the intricate tango between tick microbiomes and tick-borne pathogens. Parasite Immunol. 43, e12813. https://doi.org/10.1111/pim.12813.

Budachetri, K., Browning, R.E., Adamson, S.W., Dowd, S.E., Chao, C.C., Ching, W.M., Karim, S., 2014. An insight into the microbiome of the *Amblyomma maculatum* (Acari: Ixodidae). J. Med. Entomol. 51, 119–129. https://doi.org/10.1603/ME12223.

Budachetri, K., Gaillard, D., Williams, J., Mukherjee, N., Karim, S., 2016. A snapshot of the microbiome of *Amblyomma tuberculatum* ticks infesting the gopher tortoise, an endangered species. Ticks Tick Borne Dis. 7, 1225–1229. https://doi.org/10.1016/j.ttbdis.2016.07.010.

Budachetri, K., Kumar, D., Crispell, G., Beck, C., Dasch, G., Karim, S., 2018. The tick endosymbiont *Candidatus* Midichloria mitochondrii and selenoproteins are essential for the growth of *Rickettsia parkeri* in the Gulf Coast tick vector. Microbiome 6, 141. https://doi.org/10.1186/s40168-018-0524-2.

Budachetri, K., Williams, J., Mukherjee, N., Sellers, M., Moore, F., Karim, S., 2017. The microbiome of Neotropical ticks parasitizing on passerine migratory birds. Ticks Tick Borne Dis. 8, 170–173. https://doi.org/10.1016/j.ttbdis.2016.10.014.

Cabezas-Cruz, A., Pollet, T., Estrada-Peña, A., Allain, E., Bonnet, S.I., Moutailler, S., 2018. Handling the microbial complexity associated to ticks. In: Abubakar, M., Perera, P.K. (Eds.), Ticks and Tick-Borne Pathogens. IntechOpen, London. www.intechopen.com.

Callahan, B.J., DiGiulio, D.B., Aliaga Goltsman, D.S., Sun, C.L., Costello, E.K., Jeganathan, P., et al., 2017a. Replication and refinement of a vaginal microbial signature of preterm birth in two racially distinct cohorts of US women. Proc. Natl. Acad. Sci. U.S.A. 114, 9966–9971. https://doi.org/10.1073/pnas.1705899114.

Callahan, B.J., McMurdie, P.J., Holmes, S.P., 2017b. Exact sequence variants should replace operational taxonomic units in marker-gene data analysis. ISME J. 11, 2639–2643. https://doi.org/10.1038/ismej.2017.119.

Carpi, G., Cagnacci, F., Wittekindt, N.E., Zhao, F., Qi, J., Tomsho, L.P., et al., 2011. Metagenomic profile of the bacterial communities associated with *Ixodes ricinus* ticks. PLoS One 6, e25604. https://doi.org/10.1371/journal.pone.0025604.

Chandra, S., Šlapeta, J., 2020. Biotic factors influence microbiota of nymph ticks from vegetation in Sydney, Australia. Pathogens 9, 566. https://doi.org/10.3390/pathogens9070566.

Chauhan, G., McClure, J., Hekman, J., Marsh, P.W., Bailey, J.A., Daniels, R.F., et al., 2020. Combining citizen science and genomics to investigate tick, pathogen, and commensal microbiome at single-tick resolution. Front. Genet. 10, 1322. https://doi.org/10.3389/fgene.2019.01322.

Chicana, B., Couper, L.I., Kwan, J.Y., Tahiraj, E., Swei, A., 2019. Comparative microbiome profiles of sympatric tick species from the far-western United States. Insects 10, 353. https://doi.org/10.3390/insects10100353.

Clayton, K.A., Gall, C.A., Mason, K.L., Scoles, G.A., Brayton, K.A., 2015. The characterization and manipulation of the bacterial microbiome of the Rocky Mountain wood tick, *Dermacentor andersoni*. Parasit. Vectors 8, 632. https://doi.org/10.1186/s13071-015-1245-z.

Clow, K.M., Weese, J.S., Rousseau, J., Jardine, C.M., 2018. Microbiota of field-collected *Ixodes scapularis* and *Dermacentor variabilis* from eastern and southern Ontario, Canada. Ticks Tick Borne Dis. 9, 235–244. https://doi.org/10.1016/j.ttbdis.2017.09.009.

Couper, L.I., Kwan, J.Y., Ma, J., Swei, A., 2019. Drivers and patterns of microbial community assembly in a Lyme disease vector. Ecol. Evol. 9, 7768–7779. https://doi.org/10.1002/ece3.5361.

Davis, N.M., Di Proctor, M., Holmes, S.P., Relman, D.A., Callahan, B.J., 2018. Simple statistical identification and removal of contaminant sequences in marker-gene and metagenomics data. Microbiome 6, 226. https://doi.org/10.1186/s40168-018-0605-2.

Díaz-Sánchez, S., Estrada-Peña, A., Cabezas-Cruz, A., de la Fuente, J., 2019b. Evolutionary insights into the tick hologenome. Trends Parasitol. 35, 725–737. https://doi.org/10.1016/j.pt.2019.06.014.

Díaz-Sánchez, S., Hernández-Jarguín, A., Torina, A., de Mera, I.G.F., Blanda, V., Caracappa, S., et al., 2019a. Characterization of the bacterial microbiota in wild-caught *Ixodes ventalloi*. Ticks Tick Borne Dis. 10, 336–343. https://doi.org/10.1016/j.ttbdis.2018.11.014.

Díaz-Sánchez, S., Fernández, A.M., Habela, M.A., Calero-Bernal, R., de Mera, I.G.F., de la Fuente, J., 2021. Microbial community of *Hyalomma lusitanicum* is dominated by *Francisella*-like endosymbiont. Ticks Tick Borne Dis. 12, 101624. https://doi.org/10.1016/j.ttbdis.2020.101624.

Duan, D., Cheng, T., 2017. Determination of the microbial community features of *Haemaphysalis flava* in different developmental stages by high-throughput sequencing. J. Basic Microbiol. 57, 302–308. https://doi.org/10.1002/jobm.201600557.

Duan, D.Y., Liu, G.H., Cheng, T.Y., 2020. Microbiome analysis of the saliva and midgut from partially or fully engorged female adult *Dermacentor silvarum* ticks in China. Exp. Appl. Acarol. 80, 543–558. https://doi.org/10.1007/s10493-020-00478-2.

Duron, O., Morel, O., Noël, V., Buysse, M., Binetruy, F., Lancelot, R., et al., 2018. Tick-bacteria mutualism depends on B vitamin synthesis pathways. Curr. Biol. 28, 1896–1902. https://doi.org/10.1016/j.cub.2018.04.038 e5.

Egan, S.L., Loh, S.M., Banks, P.B., Gillett, A., Ahlstrom, L., Ryan, U.M., et al., 2020. Bacterial community profiling highlights complex diversity and novel organisms in wildlife ticks. Ticks Tick Borne Dis. 11, 101407. https://doi.org/10.1016/j.ttbdis.2020.101407.

Egyed, L., Makrai, L., 2014. Cultivable internal bacterial flora of ticks isolated in Hungary. Exp. Appl. Acarol. 63, 107–122. https://doi.org/10.1007/s10493-013-9762-y.

Estrada-Peña, A., Cabezas-Cruz, A., Obregón, D., 2020a. Resistance of tick gut microbiome to anti-tick vaccines, pathogen infection and antimicrobial peptides. Pathogens 9, 309. https://doi.org/10.3390/pathogens9040309.

Estrada-Peña, A., Cabezas-Cruz, A., Obregón, D., 2020b. Behind taxonomic variability: the functional redundancy in the tick microbiome. Microorganisms 8, 1829. https://doi.org/10.3390/microorganisms8111829.

Estrada-Peña, A., Cabezas-Cruz, A., Pollet, T., Vayssier-Taussat, M., Cosson, J.-F., 2018. High throughput sequencing and network analysis disentangle the microbial communities of ticks and hosts within and between ecosystems. Front. Cell. Infect. Microbiol. 8, 236. https://doi.org/10.3389/fcimb.2018.00236.

Gall, C.A., Reif, K.E., Scoles, G.A., Mason, K.L., Mousel, M., Noh, S.M., Brayton, K.A., 2016. The bacterial microbiome of *Dermacentor andersoni* ticks influences pathogen susceptibility. ISME J. 10, 1846–1855. https://doi.org/10.1038/ismej.2015.266.

Gall, C.A., Scoles, G.A., Magori, K., Mason, K.L., Brayton, K.A., 2017. Laboratory colonization stabilizes the naturally dynamic microbiome composition of field collected *Dermacentor andersoni* ticks. Microbiome 5, 133. https://doi.org/10.1186/s40168-017-0352-9.

Gerhart, J.G., Auguste Dutcher, H., Brenner, A.E., Moses, A.S., Grubhoffer, L., Raghavan, R., 2018. Multiple acquisitions of pathogen-derived *Francisella* endosymbionts in soft ticks. Genome Biol. Evol. 10, 607–615. https://doi.org/10.1093/gbe/evy021.

Gerhart, J.G., Moses, A.S., Raghavan, R., 2016. A *Francisella*-like endosymbiont in the Gulf Coast tick evolved from a mammalian pathogen. Sci. Rep. 6, 33670. https://doi.org/10.1038/srep33670.

Gofton, A.W., Loh, S., Barbosa, A.D., Paparini, A., Gillett, A., Macgregor, J., Oskam, C.L., Ryan, U.M., Irwin, P.J., 2018. A novel *Ehrlichia* species in blood and *Ixodes ornithorhynchi* ticks from platypuses (*Ornithorhynchus anatinus*) in Queensland and Tasmania, Australia. Ticks Tick Borne Dis. 9, 435–442. https://doi.org/10.1016/j.ttbdis.2017.12.011.

Gottlieb, Y., Lalzar, I., Klasson, L., 2015. Distinctive genome reduction rates revealed by genomic analyses of two *Coxiella*-like endosymbionts in ticks. Genome Biol. Evol. 7, 1779–1796. https://doi.org/10.1093/gbe/evv108.

Guizzo, M.G., Neupane, S., Kucera, M., Perner, J., Frantová, H., da Silva Vaz, I., et al., 2020. Poor unstable midgut microbiome of hard ticks contrasts with abundant and stable monospecific microbiome in ovaries. Front. Cell. Infect. Microbiol. 10, 211. https://doi.org/10.3389/fcimb.2020.00211.

Guizzo, M.G., Parizi, L.F., Nunes, R.D., Schama, R., Albano, R.M., Tirloni, L., et al., 2017. A *Coxiella* mutualist symbiont is essential to the development of *Rhipicephalus microplus*. Sci. Rep. 7, 17554. https://doi.org/10.1038/s41598-017-17309-x.

Gulia-Nuss, M., Nuss, A.B., Meyer, J.M., Sonenshine, D.E., Roe, R.M., Waterhouse, R.M., et al., 2016. Genomic insights into the *Ixodes scapularis* tick vector of Lyme disease. Nat. Commun. 7, 10507. https://doi.org/10.1038/ncomms10507.

Gurfield, N., Grewal, S., Cua, L.S., Torres, P.J., Kelley, S.T., 2017. Endosymbiont interference and microbial diversity of the Pacific coast tick, *Dermacentor occidentalis*, in San Diego County, California. PeerJ 2017, e3202. https://doi.org/10.7717/peerj.3202.

Hamilton, P.T., Maluenda, E., Sarr, A., Belli, A., Hurry, G., Duron, O., Plantard, O., Voordouw, M.J., 2021. *Borrelia* infection in rodent host has dramatic effects on the microbiome of ticks. BioRxiv. https://www.biorxiv.org/content/10.1101/2021.03.15.435198v1.

Hanson, M.A., Lemaitre, B., 2020. New insights on *Drosophila* antimicrobial peptide function in host defense and beyond. Curr. Opin. Immunol. 62, 22–30. https://doi.org/10.1016/j.coi.2019.11.008.

Hauck, D., Jordan, D., Springer, A., Schunack, B., Pachnicke, S., Fingerle, V., Strube, C., 2020. Transovarial transmission of *Borrelia* spp., *Rickettsia* spp. and *Anaplasma phagocytophilum* in *Ixodes ricinus* under field conditions extrapolated from DNA detection in questing larvae. Parasit. Vectors 13, 176. https://doi.org/10.1186/s13071-020-04049-7.

Hawlena, H., Rynkiewicz, E., Toh, E., Alfred, A., Durden, L.A., Hastriter, M.W., et al., 2013. The arthropod, but not the vertebrate host or its environment, dictates bacterial community composition of fleas and ticks. ISME J. 7, 221–223. https://doi.org/10.1038/ismej.2012.71.

Hayes, B.M., Radkov, A.D., Yarza, F., Flores, S., Kim, J., Zhao, Z., et al., 2020. Ticks resist skin commensals with immune factor of bacterial origin. Cell 183, 1562–1571. https://doi.org/10.1016/j.cell.2020.10.042.

Hernández-Jarguín, A., Díaz-Sánchez, S., Villar, M., de la Fuente, J., 2018. Integrated metatranscriptomics and metaproteomics for the characterization of bacterial microbiota in unfed *Ixodes ricinus*. Ticks Tick Borne Dis. 9, 1241–1251. https://doi.org/10.1016/j.ttbdis.2018.04.020.

Herren, C.M., McMahon, K.D., 2018. Keystone taxa predict compositional change in microbial communities. Environ. Microbiol. 20, 2207–2217. https://doi.org/10.1111/1462-2920.14257.

Hoffmann, J.A., Reichhart, J.M., 2002. *Drosophila* innate immunity: an evolutionary perspective. Nat. Immunol. 3, 121–126. https://doi.org/10.1038/ni0202-121.

Hunter, D.J., Torkelson, J.L., Bodnar, J., Mortazavi, B., Laurent, T., Deason, J., et al., 2015. The *Rickettsia* endosymbiont of *Ixodes pacificus* contains all the genes of de novo folate biosynthesis. PLoS One 10, e0144552. https://doi.org/10.1371/journal.pone.0144552.

Jory Brinkerhoff, R., Clark, C., Ocasio, K., Gauthier, D.T., Hynes, W.L., 2020. Factors affecting the microbiome of *Ixodes scapularis* and *Amblyomma americanum*. PLoS One 15, e0232398. https://doi.org/10.1371/journal.pone.0232398.

Jousselin, E., Clamens, A.-L., Galan, M., Bernard, M., Maman, S., Gschloessl, B., et al., 2016. Assessment of a *16S* rRNA amplicon Illumina sequencing procedure for studying the microbiome of a symbiont-rich aphid genus. Mol. Ecol. Resour. 16, 628–640. https://doi.org/10.1111/1755-0998.12478.

Kagemann, J., Clay, K., 2013. Effects of infection by *Arsenophonus* and *Rickettsia* bacteria on the locomotive ability of the ticks *Amblyomma americanum, Dermacentor variabilis*, and *Ixodes scapularis*. J. Med. Entomol. 50, 155–162. https://doi.org/10.1603/ME12086.

Karim, S., Budachetri, K., Mukherjee, N., Williams, J., Kausar, A., Hassan, M.J., et al., 2017. A study of ticks and tick-borne livestock pathogens in Pakistan. PLoS Negl. Trop. Dis. 11, e0005681. https://doi.org/10.1371/journal.pntd.0005681.

Khoo, J.J., Chen, F., Kho, K.L., Ahmad Shanizza, A.I., Lim, F.S., Tan, K.K., et al., 2016. Bacterial community in *Haemaphysalis* ticks of domesticated animals from the Orang Asli communities in Malaysia. Ticks Tick Borne Dis. 7, 929–937. https://doi.org/10.1016/j.ttbdis.2016.04.013.

Kueneman, J.G., Esser, H.J., Weiss, S.J., Jansen, P.A., Foley, J.E., 2021. Tick microbiomes in Neotropical forest fragments are best explained by tick-associated and environmental factors rather than host blood source. Appl. Environ. Microbiol. 87, e02668-20. https://doi.org/10.1128/aem.02668-20.

Kurilshikov, A., Livanova, N.N., Fomenko, N.V., Tupikin, A.E., Rar, V.A., Kabilov, M.R., et al., 2015. Comparative metagenomic profiling of symbiotic bacterial communities associated with *Ixodes persulcatus, Ixodes pavlovskyi* and *Dermacentor reticulatus* ticks. PLoS One 10, e0131413. https://doi.org/10.1371/journal.pone.0131413.

Kurlovs, A.H., Li, J., Cheng, D., Zhong, J., 2014. *Ixodes pacificus* ticks maintain embryogenesis and egg hatching after antibiotic treatment of *Rickettsia* endosymbiont. PLoS One 9, e104815. https://doi.org/10.1371/journal.pone.0104815.

Kurokawa, C., Lynn, G.E., Pedra, J.H.F., Pal, U., Narasimhan, S., Fikrig, E., 2020. Interactions between *Borrelia burgdorferi* and ticks. Nat. Rev. Microbiol. 18, 587–600. https://doi.org/10.1038/s41579-020-0400-5.

Kwan, J.Y., Griggs, R., Chicana, B., Miller, C., Swei, A., 2017. Vertical *vs.* horizontal transmission of the microbiome in a key disease vector, *Ixodes pacificus*. Mol. Ecol. 26, 6578–6589. https://doi.org/10.1111/mec.14391.

Lado, P., Qurollo, B., Williams, C., Junge, R., Klompen, H., 2018. The microbiome of *Haemaphysalis lemuris* (Acari: Ixodidae), a possible vector of pathogens of endangered lemur species in Madagascar. Ticks Tick Borne Dis. 9, 1252–1260. https://doi.org/10.1016/j.ttbdis.2018.05.003.

Landesman, W.J., Mulder, K., Page Fredericks, L., Allan, B.F., 2019. Cross-kingdom analysis of nymphal-stage *Ixodes scapularis* microbial communities in relation to *Borrelia burgdorferi* infection and load. FEMS Microbiol. Ecol. 95, fiz167. https://doi.org/10.1093/femsec/fiz167.

Larsson, A.J.M., Stanley, G., Sinha, R., Weissman, I.L., Sandberg, R., 2018. Computational correction of index switching in multiplexed sequencing libraries. Nat. Methods 15, 305–307. https://doi.org/10.1038/nmeth.4666.

Lee, S., Kim, J.Y., Yi, M.-H., Lee, I.Y., Fyumagwa, R., Yong, T.S., 2019. Comparative microbiomes of ticks collected from a black rhino and its surrounding environment. Int. J. Parasitol. Parasites Wildl. 9, 239–243. https://doi.org/10.1016/j.ijppaw.2019.05.008.

Lejal, E., Estrada-Peña, A., Marsot, M., Cosson, J.-F., Rué, O., Mariadassou, M., et al., 2020. Taxon appearance from extraction and amplification steps demonstrates the value of multiple controls in tick microbiota analysis. Front. Microbiol. 11, 1093. https://doi.org/10.3389/fmicb.2020.01093.

Lesperance, D.N., Broderick, N.A., 2020. Microbiomes as modulators of *Drosophila melanogaster* homeostasis and disease. Curr. Opin. Insect Sci. 39, 84–90. https://doi.org/10.1016/j.cois.2020.03.003.

Li, L.H., Zhang, Y., Zhu, D., 2018b. Effects of antibiotic treatment on the fecundity of *Rhipicephalus haemaphysaloides* ticks. Parasit. Vectors 11, 242. https://doi.org/10.1186/s13071-018-2807-7.

Li, L.H., Zhang, Y., Zhu, D., Zhou, X.N., 2018a. Endosymbionts alter larva-to-nymph transstadial transmission of *Babesia microti* in *Rhipicephalus haemaphysaloides* ticks. Front. Microbiol. 9, 1415. https://doi.org/10.3389/fmicb.2018.01415.

Lim, F.S., Khoo, J.J., Tan, K.K., Zainal, N., Loong, S.K., Khor, C.S., AbuBakar, S., 2020. Bacterial communities in *Haemaphysalis, Dermacentor* and *Amblyomma* ticks collected from wild boar of an Orang Asli community in Malaysia. Ticks Tick Borne Dis. 11, 101352. https://doi.org/10.1016/j.ttbdis.2019.101352.

Liu, L., Dai, J., Zhao, Y.O., Narasimhan, S., Yang, Y., Zhang, L., Fikrig, E., 2012. *Ixodes scapularis* JAK-STAT pathway regulates tick antimicrobial peptides, thereby controlling the agent of human granulocytic anaplasmosis. J. Infect. Dis. 206, 1233–1241. https://doi.org/10.1093/infdis/jis484.

Macaluso, K.R., Sonenshine, D.E., Ceraul, S.M., Azad, A.F., 2001. Infection and transovarial transmission of rickettsiae in *Dermacentor variabilis* ticks acquired by artificial feeding. Vector Borne Zoonotic Dis. 1, 45–53. https://doi.org/10.1089/153036601750137660.

Macaluso, K.R., Sonenshine, D.E., Ceraul, S.M., Azad, A.F., 2002. Rickettsial infection in *Dermacentor variabilis* (Acari: Ixodidae) inhibits transovarial transmission of a second *Rickettsia*. J. Med. Entomol. 39, 809–813. https://doi.org/10.1603/0022-2585-39.6.809.

Maldonado-Ruiz, L.P., Neupane, S., Park, Y., Zurek, L., 2021. The bacterial community of the lone star tick (*Amblyomma americanum*). Parasit. Vectors 14, 49. https://doi.org/10.1186/s13071-020-04550-z.

Martins, L.A., de Melo Galletti, M.F.B., Ribeiro, J.M., Fujita, A., Costa, F.B., Labruna, M.B., et al., 2017. The distinct transcriptional response of the midgut of *Amblyomma sculptum* and *Amblyomma aureolatum* ticks to *Rickettsia rickettsii* correlates to their differences in susceptibility to infection. Front. Cell. Infect. Microbiol. 7, 129. https://doi.org/10.3389/fcimb.2017.00129.

Mateos-Hernández, L., Obregón, D., Maye, J., Borneres, J., Versille, N., de la Fuente, J.L., et al., 2020. Anti-tick microbiota vaccine impacts *Ixodes ricinus* performance during feeding. Vaccines 8, 702. https://doi.org/10.3390/vaccines8040702.

Mateos-Hernández, L., Obregón, D., Wu-Chuang, A., Maye, J., Borneres, J., Versille, N., et al., 2021. Anti-microbiota vaccines modulate the tick microbiome in a taxon-specific manner. BioRxiv. https://www.biorxiv.org/content/10.1101/2021.05.12.443756v1.

Menchaca, A.C., Visi, D.K., Strey, O.F., Teel, P.D., Kalinowski, K., Allen, M.S., Williamson, P.C., 2013. Preliminary assessment of microbiome changes following blood-feeding and survivorship in the *Amblyomma americanum* nymph-to-adult transition using semiconductor sequencing. PLoS One 8, e67129. https://doi.org/10.1371/journal.pone.0067129.

Mergaert, P., 2018. Role of antimicrobial peptides in controlling symbiotic bacterial populations. Nat. Prod. Rep. 35, 336–356. https://doi.org/10.1039/c7np00056a.

Moore, T.C., Pulscher, L.A., Caddell, L., von Fricken, M.E., Anderson, B.D., Gonchigoo, B., Gray, G.C., 2018. Evidence for transovarial transmission of tick-borne rickettsiae circulating in northern Mongolia. PLoS Negl. Trop. Dis. 12, e0006696. https://doi.org/10.1371/journal.pntd.0006696.

Nakao, R., Abe, T., Nijhof, A.M., Yamamoto, S., Jongejan, F., Ikemura, T., Sugimoto, C., 2013. A novel approach, based on BLSOMs (Batch Learning Self-Organizing Maps), to the microbiome analysis of ticks. ISME J. 7, 1003–1015. https://doi.org/10.1038/ismej.2012.171.

Narasimhan, S., Rajeevan, N., Liu, L., Zhao, Y.O., Heisig, J., Pan, J., et al., 2014. Gut microbiota of the tick vector *Ixodes scapularis* modulate colonization of the Lyme disease spirochete. Cell Host Microbe 15, 58–71. https://doi.org/10.1016/j.chom.2013.12.001.

Narasimhan, S., Fikrig, E., 2015. Tick microbiome: the force within. Trends Parasitol. 31, 315–323. https://doi: 10.1016/j.pt.2015.03.010.

Narasimhan, S., Schuijt, T.J., Abraham, N.M., Rajeevan, N., Coumou, J., Graham, M., et al., 2017. Modulation of the tick gut milieu by a secreted tick protein favors *Borrelia burgdorferi* colonization. Nat. Commun. 8, 184. https://doi.org/10.1038/s41467-017-00208-0.

Narasimhan, S., Swei, A., Abouneameh, S., Pal, U., Pedra, J.H.F., Fikrig, E., 2021. Grappling with the tick microbiome. Trends Parasitol. https://doi.org/10.1016/j.pt.2021.04.004. S1471-4922(21)00084-2.

O'Keeffe, K.R., Oppler, Z.J., Brisson, D., 2020. Evolutionary ecology of Lyme *Borrelia*. Infect. Genet. Evol. 85, 104570. https://doi.org/10.1016/j.meegid.2020.104570.

Obregón, D., Bard, E., Abrial, D., Estrada-Peña, A., Cabezas-Cruz, A., 2019. Sex-specific linkages between taxonomic and functional profiles of tick gut microbiomes. Front. Cell. Infect. Microbiol. 9, 298. https://doi.org/10.3389/fcimb.2019.00298.

Panetta, J.L., Šíma, R., Calvani, N.E.D., et al., 2017. Reptile-associated *Borrelia* species in the goanna tick (*Bothriocroton undatum*) from Sydney, Australia. Parasit. Vectors 10, 616. https://doi.org/10.1186/s13071-017-2579-5.

Pavanelo, D.B., Schröder, N.C.H., Pin Viso, N.D., Martins, L.A., Malossi, C.D., Galletti, M.F.B.M., et al., 2020. Comparative analysis of the midgut microbiota of two natural tick vectors of *Rickettsia rickettsii*. Dev. Comp. Immunol. 106, 103606. https://doi.org/10.1016/j.dci.2019.103606.

Perveen, N., Muzaffar, S. Bin, Vijayan, R., Al-Deeb, M.A., 2020. Microbial communities associated with the camel tick, *Hyalomma dromedarii: 16S* rRNA gene-based analysis. Sci. Rep. 10, 17035. https://doi.org/10.1038/s41598-020-74116-7.

Ponnusamy, L., Gonzalez, A., Van Treuren, W., Weiss, S., Parobek, C.M., Juliano, J., et al., 2014. Diversity of Rickettsiales in the microbiome of the lone star tick, *Amblyomma americanum*. Appl. Environ. Microbiol. 80, 354–359. https://doi.org/10.1128/AEM.02987-13.

Portillo, A., Palomar, A.M., de Toro, M., Santibáñez, S., Santibáñez, P., Oteo, J.A., 2019. Exploring the bacteriome in anthropophilic ticks: to investigate the vectors for diagnosis. PLoS One 14, e0213384. https://doi.org/10.1371/journal.pone.0213384.

René-Martellet, M., Minard, G., Massot, R., Van Tran, V., Valiente Moro, C., Chabanne, L., Mavingui, P., 2017. Bacterial microbiota associated with *Rhipicephalus sanguineus* (*s.l.*) ticks from France, Senegal and Arizona. Parasit. Vectors 10, 416. https://doi.org/10.1186/s13071-017-2352-9.

Rosa, R.D., Capelli-Peixoto, J., Mesquita, R.D., Kalil, S.P., Pohl, P.C., Braz, G.R., et al., 2016. Exploring the immune signalling pathway-related genes of the cattle tick *Rhipicephalus microplus*: from molecular characterization to transcriptional profile upon microbial challenge. Dev. Comp. Immunol. 59, 1–14. https://doi.org/10.1016/j.dci.2015.12.018.

Ross, B.D., Hayes, B., Radey, M.C., Lee, X., Josek, T., Bjork, J., et al., 2018. *Ixodes scapularis* does not harbor a stable midgut microbiome. ISME J. 12, 2596–2607. https://doi.org/10.1038/s41396-018-0161-6.

Rynkiewicz, E.C., Hemmerich, C., Rusch, D.B., Fuqua, C., Clay, K., 2015. Concordance of bacterial communities of two tick species and blood of their shared rodent host. Mol. Ecol. 24, 2566–2579. https://doi.org/10.1111/mec.13187.

Sakamoto, J.M., Diaz, G.E.S., Wagner, E.A., 2020. Bacterial communities of *Ixodes scapularis* from central Pennsylvania, USA. Insects 11, 718. https://doi.org/10.3390/insects11100718.

Segura, J.A., Isaza, J.P., Botero, L.E., Alzate, J.F., Gutiérrez, L.A., 2020. Assessment of bacterial diversity of *Rhipicephalus microplus* ticks from two livestock agroecosystems in Antioquia, Colombia. PLoS One 15, e0234005. https://doi.org/10.1371/journal.pone.0234005.

Shaw, D.K., Wang, X., Brown, L.J., Chávez, A.S.O., Reif, K.E., Smith, A.A., et al., 2017. Infection-derived lipids elicit an immune deficiency circuit in arthropods. Nat. Commun. 8, 14401. https://doi.org/10.1038/ncomms14401.

Shaw, W.R., Catteruccia, F., 2019. Vector biology meets disease control: using basic research to fight vector-borne diseases. Nat. Microbiol. 4, 20–34. https://doi: 10.1038/s41564-018-0214-7.

Smith, A.A., Pal, U., 2014. Immunity-related genes in *Ixodes scapularis* - perspectives from genome information. Front. Cell. Infect. Microbiol. 4, 116. https://doi.org/10.3389/fcimb.2014.00116.

Smith, T.A., Driscoll, T., Gillespie, J.J., Raghavan, R., 2015. A *Coxiella*-like endosymbiontis a potential vitamin source for the lone star tick. Genome Biol. Evol. 7, 831–838. https://doi.org/10.1093/gbe/evv016.

Sperling, J.L.H., Fitzgerald, D., Sperling, F.A.H., Magor, K.E., 2020. Microbiome composition and *Borrelia* detection in *Ixodes scapularis* ticks at the northwestern edge of their range. Trav. Med. Infect. Dis. 5, 173. https://doi.org/10.3390/tropicalmed5040173.

Sperling, J.L., Silva-Brandão, K.L., Brandão, M.M., Lloyd, V.K., Dang, S., Davis, C., et al., 2017. Comparison of bacterial *16S* rRNA variable regions for microbiome surveys of ticks. Ticks Tick Borne Dis. 8, 453–461. https://doi.org/10.1016/j.ttbdis.2017.02.002.

Stevens, E.J., Bates, K.A., King, K.C., 2021. Host microbiota can facilitate pathogen infection. PLoS Pathog. 17, e1009514. https://doi.org/10.1371/journal.ppat.1009514.

Sui, S., Yang, Y., Sun, Y., Wang, X., Wang, G., Shan, G., et al., 2017. On the core bacterial flora of *Ixodes persulcatus* (Taiga tick). PLoS One 12, e0180150. https://doi.org/10.1371/journal.pone.0180150.

Swei, A., Kwan, J.Y., 2017. Tick microbiome and pathogen acquisition altered by host blood meal. ISME J. 11, 813–816. https://doi.org/10.1038/ismej.2016.152.

Thapa, S., Zhang, Y., Allen, M.S., 2019. Effects of temperature on bacterial microbiome composition in *Ixodes scapularis* ticks. Microbiol. Open 8, e00719. https://doi.org/10.1002/mbo3.719.

Trout Fryxell, R.T., DeBruyn, J.M., 2016. The microbiome of *Ehrlichia*-infected and uninfected lone star ticks (*Amblyomma americanum*). PLoS One 11, e0146651. https://doi.org/10.1371/journal.pone.0146651.

Tsementzi, D., Castro Gordillo, J., Mahagna, M., Gottlieb, Y., Konstantinidis, K.T., 2018. Comparison of closely related, uncultivated *Coxiella* tick endosymbiont population genomes reveals clues about the mechanisms of symbiosis. Environ. Microbiol. 20, 1751–1764. https://doi.org/10.1111/1462-2920.14104.

van Treuren, W., Ponnusamy, L., Brinkerhoff, R.J., Gonzalez, A., Parobek, C.M., Juliano, J.J., et al., 2015. Variation in the microbiota of *Ixodes* ticks with regard to geography, species, and sex. Appl. Environ. Microbiol. 81, 6200–6209. https://doi.org/10.1128/AEM.01562-15.

Vandegrift, K.J., Kapoor, A., 2019. The ecology of new constituents of the tick virome and their relevance to public health. Viruses 11, 529. https://doi.org/10.3390/v11060529.

Varela-Stokes, A.S., Park, S.H., Stokes, J.V., Gavron, N.A., Lee, S.I., Moraru, G.M., Ricke, S.C., 2018. Tick microbial communities within enriched extracts of *Amblyomma maculatum*. Ticks Tick Borne Dis. 9, 798–805. https://doi.org/10.1016/j.ttbdis.2018.02.022.

Wang, M., Zhu, D., Dai, J., Zhong, Z., Zhang, Y., Wang, J., 2018. Tissue localization and variation of major symbionts in *Haemaphysalis longicornis, Rhipicephalus haemaphysaloides*, and *Dermacentor silvarum* in China. Appl. Environ. Microbiol. 84, e00029-18. https://doi.org/10.1128/AEM.00029-18.

Weiss, S., Van Treuren, W., Lozupone, C., Faust, K., Friedman, J., Deng, Y., et al., 2016. Correlation detection strategies in microbial data sets vary widely in sensitivity and precision. ISME J. 10, 1669–1681. https://doi.org/10.1038/ismej.2015.235.

Williams-Newkirk, A.J., Rowe, L.A., Mixson-Hayden, T.R., Dasch, G.A., 2014. Characterization of the bacterial communities of life stages of free living lone star ticks (*Amblyomma americanum*). PLoS One 9, e102130. https://doi.org/10.1371/journal.pone.0102130.

Wu-Chuang, A., Obregon, D., Mateos-Hernández, L., Cabezas-Cruz, A., 2021. Anti-tick microbiota vaccines: how can this actually work? Biologia. https://doi.org/10.1007/s11756-021-00818-6.

Yan, P., Qiu, Z., Zhang, T., Li, Y., Wang, W., Li, M., et al., 2019. Microbial diversity in the tick *Argas japonicus* (Acari: Argasidae) with a focus on *Rickettsia* pathogens. Med. Vet. Entomol. 33, 327–335. https://doi.org/10.1111/mve.12373.

Yang, X., Koči, J., Smith, A.A., Zhuang, X., Sharma, K., Dutta, S., et al., 2021. A novel tick protein supports integrity of gut peritrophic matrix impacting existence of gut microbiome and Lyme disease pathogens. Cell Microbiol. 23, e13275. https://doi.org/10.1111/cmi.13275.

Zhang, C.M., Li, N.X., Zhang, T.T., Qiu, Z.X., Li, Y., Li, L.W., Liu, J.Z., 2017. Endosymbiont CLS-HI plays a role in reproduction and development of *Haemaphysalis longicornis*. Exp. Appl. Acarol. 73, 429–438. https://doi.org/10.1007/s10493-017-0194-y.

Zhang, R., Huang, Z., Yu, G., Zhang, Z., 2019b. Characterization of microbiota diversity of field-collected *Haemaphysalis longicornis* (Acari: Ixodidae) with regard to sex and blood meals. J. Basic Microbiol. 59, 215–223. https://doi.org/10.1002/jobm.201800372.

Zhang, Y.-K., Yu, Z.-J., Wang, D., Víchová, B., Peťko, B., Liu, J.-Z., 2019a. The bacterial microbiome of field-collected *Dermacentor marginatus* and *Dermacentor reticulatus* from Slovakia. Parasit. Vectors 12, 325. https://doi.org/10.1186/s13071-019-3582-9.

Zhang, R., Yu, G., Huang, Z., Zhang, Z., 2020. Microbiota assessment across different developmental stages of *Dermacentor silvarum* (Acari: Ixodidae) revealed stage-specific signatures. Ticks Tick Borne Dis. 11, 101321. https://doi.org/10.1016/j.ttbdis.2019.101321.

Zhong, J., Jasinskas, A., Barbour, A.G., 2007. Antibiotic treatment of the tick vector *Amblyomma americanum* reduced reproductive fitness. PLoS One 2, e405. https://doi.org/10.1371/journal.pone.0000405.

Zolnik, C.P., Falco, R.C., Daniels, T.J., Kolokotronis, S.O., 2018. Transient influence of blood meal and natural environment on blacklegged tick bacterial communities. Ticks Tick Borne Dis. 9, 563–572. https://doi.org/10.1016/j.ttbdis.2018.01.007.

Zolnik, C.P., Prill, R.J., Falco, R.C., Daniels, T.J., Kolokotronis, S.O., 2016. Microbiome changes through ontogeny of a tick pathogen vector. Mol. Ecol. 25, 4963–4977. https://doi.org/10.1111/mec.13832.