Current debates and advances in tick microbiome research

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Keywords: Tick microbiome, Tick-borne pathogens, Anti-tick microbiota vaccines, Tick-microbiome interaction

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
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; Glow et al., 2018; Gotton et al., 2018; Díaz-Sánchez et al., 2019a; Yan et al., 2019; Chandra & Slapeta, 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., 2016). 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 microbiome 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 ensembles. 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 (Jouselin et al., 2016; Callahan et al., 2017a; Larson 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 holobionome (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* (Ponusamy et al., 2014) female ticks. Other studies reported that *I. scapularis* females were also dominated by *Rickettsia* (Hlavena 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               |
|---------------------|----------------------|-------------------------|---------------------------------------------|---------------------------------|-------------------------------------------------|---------------------------------|-------------------------|
| *Dermacentor andersoni* | Lab-reared ticks     | Adult males             | Midgut and salivary glands                  | Idaho (USA)                     | V4 region of 16S rRNA gene                      | Roche 454 GS FLX               | 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 | Adult females           | Saliva and midgut                           | Guyuan (China)                  | V3-V4 region of 16S rRNA gene                   | IonS5™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               | 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                 | Gow 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 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. pacifica, 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 adults       | Whole ticks                                  | New Brunswick, Ontario, Alberta British Columbia, Nova Scotia (Canada); Amberiit (USA); Novosibirsk (Russia) | 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                                  | V3-V5 regions of 16S rRNA gene | Illumina MiSeq | Kurilshikov et al. (2015) |

(continued on next page)
| Tick                                                                 | Origin                  | Developmental stage/Sex | Tissue                      | Location                  | Target gene                  | Approach                        | Reference                  |
|---------------------------------------------------------------------|-------------------------|-------------------------|-----------------------------|---------------------------|-----------------------------|---------------------------------|-----------------------------|
| *Ixodes pacificus*, *I. angustus*, *Dermacentor variabilis*, *D. occidentalis*, *D. albipictus*, *Haemaphysalis leporipalpis* | 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)      | RNA-seq data                | Metatranscriptomics and metaproteomics | Hernández-Jarguin et al. (2018)|
| *Ixodes venaltloi*                                                 | 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          | V4 region of 16S rRNA gene | Metatronascriptomics and metaproteomics | Hernández-Jarguin et al. (2018)|
| *Ixodes ricinus*                                                    | Field-collected ticks  | Nymphs and adults       | Whole tick                  | Swiss Alps                | Illumina MiSeq                | 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                | Illumina MiSeq                | Guizzo et al. (2020)        |
| *Amblyomma longirostre*, *A. nodatum*, *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                | 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, Bodotriaon aurugnatae*, *R. concord*, *Haemaphysalis bancrofti*, *H. bremieri*, *H. hamerosa*, *H. longicornis*, *Ixodes antechini*, *Ixodes australiensis*, *I. fenticus*, *I. holocyclus*, *I. myrmecobi*, *I. ornithorhynchi*, *I. tasmani*, *I. trichotauri* | Field-collected ticks  | Whole tick               | Australia                  | V1-V2 region of 16S rRNA gene | Illumina MiSeq                | Illumina MiSeq                | Egan et al. (2020)          |
| Tick                                                                 | Origin                          | Developmental stage/Sex | Tissue | Location               | Target gene              | Approach                  | Reference                  |
|----------------------------------------------------------------------|---------------------------------|--------------------------|--------|------------------------|--------------------------|---------------------------|----------------------------|
| *Amblyomma auriculatum*, A. diximile, A. guayi, A. longirostre, A. mixtum, A. naponense, A. oblongopunctatum, A. ovale, A. pacas, A. sabenerae, A. tapirellum, A. varium, Haemaphysalis juxtakochi, Ixodes affinis, Ornithodorus puertoricensis | Field-collected ticks           | Larvae, nymphs, adults  | Whole tick | Central Panama          | V1–V3 region of 16S rRNA gene | Illumina MiSeq             | Kueeneman et al. (2021)   |
| *Haemaphysalis wellingtoni*, H. hystricis, H. bispinosa              | Field-collected ticks           | Larvae, nymphs, adults  | 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*, D. anatolicum, A. macropterus, 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 tapiridum, A. oblongopunctatum | 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, Gujanwala, Sherikupura (Pakistan) | V1–V3 region of the 16S rRNA | 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., Ornithodorus 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, Drome, 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*, Dermacontor 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 haemaphysalioides                                       | 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) |
| Ornithodorus turcata                                                | Field-collected ticks           | Adults                    | Whole tick | Mapimi Biosphere Reserve (Mexico) | V3–V4 region of 16S rRNA gene | Illumina MiSeq            | Barraza-Guerrero et al. (2020) |
| Bothriocotus 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)        |
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), 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 ticks, such as D. albipictus, D. variabilis and H. leporispalustris, 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 & Slapeta, 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, 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 (i.e. Haemaphysalis leporispalauris 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.

Table 1

| Tick | Origin | Developmental stage/SEX | Tissue | Location | Target gene | Approach | Reference |
|------|--------|-------------------------|--------|----------|------------|---------|-----------|
| Boophilus americanus | 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 scapularis | 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. trichomani, I. tasmani, Haemaphysalis banroqui | Field-collected ticks | Nymphs and adult females | Whole tick | New South Wales (Australia) | V1-V3 and V3-V4 16S rRNA gene | Illumina MiSeq | Chandra & Slapeta. (2020) |

* Only papers published in 2015 or after were included in the table. For manuscripts on tick microbiome published before 2015, the reader is referred to a previous review (Narasimhan & Fikrig, 2015).
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; Gula-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 |
|-----------------------------|------|---------|---------------|-----------|
| **Amblyomma 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), and lipoic acid | Smith et al. (2015) |
| **Amblyomma americanum** | Coxiella sp. | • Treatment of engorged females with rifampicin or tetracycline was associated with reduced reproductive fitness; | 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; | Tsementzi et al. (2018) |
| **Rhipicephalus sanguineus** | Coxiella-like endosymbiont (CLE) | • Genome of CLE encoded multiple copies of the proline/betaine transporter, prop gene | 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 embryo development; | Guizzo et al. (2017) |
| **Rhipicephalus haemaphysaloides** | Coxiella-like endosymbiont (Coxiella-LE) | • Treatment of engorged females with kanamycin or tetracycline was associated with decreased hatching rates of eggs; | Li et al. (2018a,b) |
| **Haemaphysalis longicornis** | Coxiella-like endosymbiont (CLS-HI) | • Reduced density of CLE-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; | Kurlovs et al. (2014) |
| **Ixodes pacificus** | Rickettsia species phylotype G021 | • No differences in the incubation period, egg hatching rate, and the number of larval offspring between groups antibiotic-treated and control groups | |
| **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; | Obrejon et al. (2019) |
| **Ornithodoros moubata** | Franciscella type F-Om | • Elimination of Franciscella symbiont hampers ticks’ growth and molting to adulthood, deficiencies that were restored with an oral supplement of B vitamins with reduced motility | Duron et al. (2018) |
| **Amblyomma americanum, Dermacentor variabilis, Ixodes scapularis** | Arsenophonus and Rickettsia | • Rickettsia was associated with increased motility while Arsenophonus with decreased motility | Kagemann & Clay (2013) |
| **Amblyomma maculatum** | Franciscella-like endosymbiont (FLE-Am) | • FLE-Am possess extensive metabolic capabilities including production of cofactors, amino acids and bicine | Gerhart et al. (2016) |
| **Amblyomma maculatum, Ornithodoros moubata** | Franciscella-like endosymbiont (FLE) | • FLEs encode complete pathway for the synthesis of several B vitamins and cofactors such as biotin (B7), folate (B9), riboflavin (B2), pantothenic acid and FAD, denoting the possible function of FLE as nutrient-provisioning endosymbionts | Gerhart et al. (2018) |
| **Dermacentor andersoni** | Franciscella-like endosymbiont (FLE) | • Offspring of oxytetracycline-treated ticks presented significant reductions of fitness: lower larval survival, reduced mean larval weight and survival after larval-metamorphosis molt | Clayton et al. (2015) |
| **Ixodes ricinus** | Escherichia coli | • Anti-E. coli and anti-o-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; | Mateos-Hernández et al. (2020) |
| **Ixodes ricinus** | Escherichia coli | • Nymphs that fed on C57BL/6 immunized with E. coli had higher weight | 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 anti-microbial 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 (Narasinhan et al., 2014). Particularly, Narasinhan 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 dysbiozed fed larvae had a higher abundance of bacteria of the genera Delftia, Acidovorax, and Ricetttsia compared to normal larvae, and a lower abundance of bacteria of the genera Comamonas, Chryseobacterium, Lactobacillus, and Paenibacillus in comparison to normal fed larvae (Narasinhan 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 (Narasinhan 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 contributions of endosymbionts to ticks is the nutritional complementation 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 anti-microbial 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 (Narasinhan et al., 2014). Particularly, Narasinhan 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 dysbiozed fed larvae had a higher abundance of bacteria of the genera Delftia, Acidovorax, and Ricetttsia compared to normal larvae, and a lower abundance of bacteria of the genera Comamonas, Chryseobacterium, Lactobacillus, and Paenibacillus in comparison to normal fed larvae (Narasinhan 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 (Narasinhan 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.

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. (Narasinhan et al., 2014, 2017; Sterling et al., 2020; Hamilton et al., 2021). A study conducted by Narasinhan 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, Thioclastic and Delftia and decreased relative abundance of Aquabacterium, Brevibacterium and Novosphingibium. The alteration of the bacterial assemblage 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 (Sterling 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 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).
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 (Strada-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 glycans-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

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### Table 3

| 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; Enteroctococcus and *Borrelia* were decreased whereas *Pseudomonas* was increased; dysbiosis enhanced *A. phagocytophilum* colonization;  
• A. phagocytophilum induced changes in the gut barrier (decrease of peritrophic genes expression and thickness of the peritrophic matrix) via the anti-freeze 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 *Beijerinckia* were under-represented in *Borrelia*-infected ticks | Chauhan et al. (2020) |
| *Ixodes scapularis* | *Anaplasma phagocytophilum* | • *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 burgdorferi* | • *Borrelia*-positive ticks were positively associated the bacterial genera *Tepidomonas*, *Luteibacter*, *Francisella* and *Fibrimonas* | 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 burgdorferi* | • *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 heli* 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 increased level of *Rickettsia heli* in the microbiome was negatively correlated to *A. marginale* levels in ticks; | Gurfeld et al. (2017) |
| *Amblyomma americanum* | *Anaplasma/Ehrlichia* | • An increased level of *Rickettsia* heli and produced overexpression of *Francisella endosymbionts* | Trout Fryxell & Delfryn (2016) |
| *Amblyomma maculatum* | *Rickettsia parkeri* | • In *R. parkeri*-infected tick cells, FLE numbers decreased while “Can didatus Midichloria mitochondrion” increased when compared to uninfected tick cells;  
• *R. parkeri* modulated host’s defenses by upregulating tick selenoproteins | Budacheti 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 Costella-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 | Adogoe et al. (2020) |
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 metabolite 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, 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-ocurrence networks, but have little impact on the functional profile of the tick microbiome (Estrada-Pena 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). Gurfeld 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 (Gurfeld 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-ocurrence 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, 2016; Banerjee et al., 2019). 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 & Cattaneo, 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 competence 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 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.

Funding

UMR BIPAR is supported by the French Government’s Investissement d’Avenir Program, Laboratoire d’Excellence “Integrative Biology of Emerging Infectious Diseases” (grant no. ANR-10-LABX-62-BEID). Alejandra Wu-Chuang is supported by Programa Nacional de Becas de Postgrado en el Exterior “Don Carlos Antonio López” (grant no. 205/2018).

CRediT author statement

Alejandra Wu Chuang: Writing - Original Draft, Writing - Review & Editing. Adnan Hodzic: Writing - Original Draft, Writing - Review & Editing. Lourdes Mateos-Hernández: Writing - Review & Editing. Agustín Estrada-Peña: Writing - Review & Editing. Daniil Obregon: Supervision, Writing - Review & Editing. Alejandro Cabezas-Cruz: Conceptualization, Visualization, Writing - Original Draft, Supervision, Funding acquisition.

Declaration of competing interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper. Given his role as Co-Editor, Agustín Estrada-Peña had no involvement in the peer-review of this article and has no access to information regarding its peer-review. Full responsibility for the editorial process for this article was delegated to Editor-in-Chief Aneta Kostadinova.
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