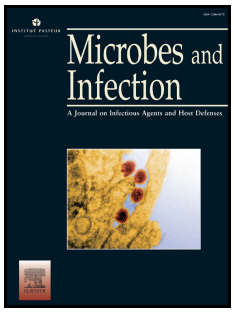
Tick-borne pathogen detection: what's new?

Alejandro Cabezas Cruz, Muriel Vayssier Taussat, Gilbert Greub

Accepted Manuscript



Tick-borne pathogen detection: what's new?

Alejandro Cabezas-Cruz, Muriel Vayssier-Taussat, Gilbert Greub

PII: S1286-4579(18)30004-2

DOI: 10.1016/j.micinf.2017.12.015

Reference: MICINF 4552

To appear in: *Microbes and Infection*

Received Date: 6 October 2017

Revised Date: 15 December 2017

Accepted Date: 20 December 2017

Please cite this article as: A. Cabezas-Cruz, M. Vayssier-Taussat, G. Greub, Tick-borne pathogen detection: what's new?, *Microbes and Infection* (2018), doi: 10.1016/j.micinf.2017.12.015.

Tick-borne pathogen detection: what's new?

Alejandro Cabezas-Cruz[1,2,3], Muriel Vayssier-Taussat[1], Gilbert Greub[4,5]*

[1]UMR BIPAR, INRA, ANSES, Ecole Nationale Vétérinaire d'Alfort, Université Paris-Est, Maisons-Alfort, 94700, France.

[2]Faculty of Science, University of South Bohemia, 37005 České Budějovice, Czech Republic.

[3]Institute of Parasitology, Biology Center, Czech Academy of Sciences, 37005 České Budějovice, Czech Republic.

[4]Center for Research on Intracellular Bacteria, Institute of Microbiology, Faculty of Biology and Medicine, University of Lausanne and University Hospital, 1011 Lausanne, Switzerland.

[5]Infectious Disease Service, University Hospital, 1011 Lausanne, Switzerland.

* **Corresponding author**: Institute of Microbiology, University of Lausanne, Bugnon 48, 1011 Lausanne, Switzerland. Tel:+ (00) 41-21-314-49-79; Fax:+ (00) 41-21-314-40-60; E-mail: gilbert.greub@chuv.ch

Abstract

Ticks and the pathogens they transmit constitute a growing burden for human and animal health worldwide. Traditionally, tick-borne pathogen detection has been carried out using PCR-based methods that rely in known sequences for specific primers design. This approach matches with the view of a 'single-pathogen' epidemiology. Recent results, however, have stressed the importance of coinfections in pathogen ecology and evolution with impact in pathogen transmission and disease severity. New approaches, including high-throughput technologies, were then used to detect multiple pathogens, but they all need *a priori* information on the pathogens to search. Thus, those approaches are biased, limited and conceal the complexity of pathogen ecology. Currently, next generation sequencing (NGS) is applied to tick-borne pathogen detection as well as to study the interactions between pathogenic and non-pathogenic microorganisms associated to ticks, the pathobiome. The use of NGS technologies have surfaced two major points: (i) ticks are associated to complex microbial communities and (ii) the relation between pathogens and microbiota is bidirectional. Notably, a new challenge emerges from NGS experiments, data analysis. Discovering associations among a high number of microorganisms is not trivial and therefore most current NGS studies report lists of microorganisms without further insights. An alternative to this is the combination of NGS with analytical tools such as network analysis to unravel the structure of microbial communities associated to ticks in different ecosystems.

Keywords: ticks, pathogen detection, next generation sequencing, network analysis

Tick-borne pathogens: "One health" concern

Ticks are hematophagous ectoparasites of vertebrates and approximately 10% of the 900 currently known tick species are of significant medical or veterinary importance. Besides causing direct damage associated with blood feeding and in some cases through the excretion of toxins within their saliva [1], the main relevance of ticks lies in the wide variety of pathogens they can transmit, including bacteria, viruses, protozoa and [2-4]. After hatching from the eggs, the life cycle of ticks includes three developmental stages (larvae, nymphs and adults) that in most cases (i.e. three-host ticks) feed on different hosts. Potentially, while feeding on a host, each of these stages can transmit and acquire new pathogens [5]. Thus, ticks are 'hubs' in pathogen's circulation cycles. Major tick-borne pathogens are transmitted by hard ticks (Ixodidae) and include *Anaplasma phagocytophilum*, *Borrelia burgdorferi sensu lato*, Crimean-Congo hemorrhagic fever virus (CCHFV), tick-borne encephalitis virus (TBEV), *Rickettsia* spp. and *Babesia* spp., [4]. These pathogens cause the most prevalent tick-borne diseases such as human granulocytic anaplasmosis (*A. phagocytophilum*), Lyme diseases (*B. burgdorferi*), Crimean-Congo hemorrhagic fever (CCHFV), tick-borne encephalitis (TBEV), spotted fever (*Rickettsia* spp.) and babesiosis (*Babesia* spp.). Other major human pathogens may occasionnally be transmitted by ticks, including *Francisella tularensis* and *Coxiella burnetii*. Importantly, the circulation of tick-borne pathogens in nature involves wildlife and livestock which pose a twofold risk for animal and human health [6].

Single-pathogen models

Our current experimental and theoretical models of pathogen transmission by ticks are limited because they frequently include single pathogen species [7-9]. Despite their limits, single-

pathogen models have allowed for the systematic discovery of tick-borne microorganisms with pathogenic effects in humans and livestock. Detection and identification of single pathogens is not technically demanding and relies mostly on PCR [10, 11]. After amplification of some taxonomically relevant genes by PCR, sequencing is followed by BLAST search and, in some cases, phylogenetic analysis for pathogen classification [10-13]. A major limitation of this approach is that it is extremely biased towards known pathogens as species-specific primers for PCR are designed based on known sequences. As a consequence, pathogen detection within the same geographic region will be strongly influenced by particular research interests. Another limitation of one-pathogen models is that they do not explain the impact of coinfections on pathogen transmission, on the spread of diseases and on the clinical presentation.

Why coinfections are important?

Coinfections, when multiple pathogen species coexist within an individual, are very common in nature [14, 15] and are a major public health concern. Coinfections occur in humans, such as by the malaria parasite, *Plasmodium* [16], in the setting of sexually-transmitted infections or mixed abdominal infections. It may also occur in a wide range of other organisms, from bacteria infected by a mixture of bacteriophages [17] to plants [18] and animals [19]. When pathogens share a host, they can interact, with consequences for individual pathogen fitness [14, 20]. Individual pathogens can adapt and increase their fitness in response to coinfections if pathogens facilitate each other's establishment [21]. Alternatively, it has been shown that individual infection rates can be reduced if pathogens directly compete via resources or toxin-production, or indirectly interact via host immune-mediation, whereby one pathogen primes the host immune response against the other (e.g. cross-reactivity) [15]. Epidemiological

studies in natural populations have provided compelling evidence that within-host interactions are so strong that the dynamics of one pathogen, within a host and within a host population, cannot be understood without knowledge of other pathogens [14, 22].

Probably, one of the best studied examples of tick-borne pathogen coinfection is that of *A. phagocytophilum* and *B. burgdorferi*. These two pathogens have been systematically reported in the literature [23], as well as in clinical cases of humans [24] as occurring together more often than expected by chance. In USA, coinfection with *B. burgdorferi* and *A. phagocytophilum* have been reported in *Ixodes scapularis* [23], as well as in humans [24] and wild animal hosts [25]. Tick infection and colonization by *A. phagocytophilum* and *B. burgdorferi* occurs firstly in the tick gut cells and subsequently in other tissues, including the salivary glands from where transmission occurs during feeding. Thus, these pathogens coexist and potentially interact within the same tissues for long periods of time. Empirical work has shown that coinfection with these two pathogens can enhance pathogen colonization in tick larvae [26], and significantly increase the potential for the spread of Lyme disease. Coinfections also elicit different immune system responses within mice hosts – the antibody response to *A. phagocytophilum* was decreased during coinfection, but antibodies produced in response to *B. burgdorferi* increased in coinfected mice [27] – as well as pathological processes – *A. phagocytophilum*-infected neutrophils enhance the transmigration of *B. burgdorferi* across the human blood brain barrier [28]. All this suggests that coinfection has a major impact on the fitness, transmission and pathology of these two pathogens.

In another study, natural populations of field voles (n=5981), *Microtus agrestis*, were followed for 6 years and coinfections with cowpox virus, *Bartonella* spp., *A. phagocytophilum* and *Babesia microti* were recorded [14]. This impressive field experiment revealed that except for cowpox, infection with other parasite species explained more

variation in infection risk than factors related to exposure risk and host condition, such as age and season [14]. Interestingly, voles with ongoing *A. phagocytophilum* infections were less likely to become infected with *B. microti*, but risk was not reduced in animals that had recently cleared an infection [14].

Currently, coinfections are routinely included in tick-borne pathogen screenings [29-31]. Thus, the Swiss national center for tick-borne diseases sequentially tested the same 8'000 batch of ticks for the presence of the agent of tick-borne encephalitis [32], for chlamydiae [13] as well as *Anaplasma* and *Coxiella* (Pilloux et al, unpublished). Not only different pathogen species were found coinfecting ticks and hosts, but also coinfections with multiple strains of the same pathogen have been reported [33, 34]. Detection of coinfections can be achieved following standard PCR or more demanding technologies such as microfluidic high-throughput real-time PCR. This nanotechnology is a powerful tool capable of performing parallel real-time PCRs using 96x96 chips resulting in 9216 individual reactions in one run [35]. Recently, Michelet and colleagues [29] applied this technology for a large (n=7050 ticks) and rapid screening of tick-borne pathogens in *Ixodes ricinus*, the most common tick in Europe. These authors successfully detected expected pathogens (*B. burgdorferi* sensu lato, *A. phagocytophilum*, *Rickettsia helvetica*, *Candidatus* Neoehrlichia mikurensis, *Babesia divergens*, *Babesia venatorum*), as well as unexpected pathogens (*Borrelia miyamotoi*), and rare (*Bartonella henselae*) pathogens in France, Denmark, and the Netherlands [29]. This technology can be easily adapted to detect 'single pathogens' or 'multiple pathogens' infections. However, despite the leap of this technology compared to standard PCR, both have the same limitation, this is, to be highly biased towards known pathogens as species-specific primers have to be designed based on known sequences.

Despite this review focuses on microorganisms detected in ticks, it is noteworthy that coinfections with different tick-borne pathogens are frequently reported in humans [36-38]. Strikingly, the majority of patients with chronic Lyme disease reported at least one coinfection with another tick-borne pathogen. In particular, 32.3% reported laboratory confirmed diagnosis with *Babesia*, 28.3% with *Bartonella* (note that only *B. henselae* is suspected to be transmitted by ticks), 14.5% with *Ehrlichia*, 4.8% with *Anaplasma*, 5.6% with Rocky Mountain spotted fever (caused by *Rickettsia rickettsii*), and 0.8% with tularemia [36]. An interesting example is that of the human coinfection of *B. burgdorferi* with *B. microti* in the United States [38]. The emergence of *B. microti* has become difficult to explain because this pathogen has a low ecological fitness characterized by poor transmission from *Peromyscus leucopus* mice to larval ticks and poor transstadial transmission from larvae to nymphs [38]. Interestingly, recent studies show that human babesiosis is emerging in areas endemic for Lyme disease. The current hypothesis is that *B. burgdorferi* increases *B. microti* transmission from *P. leucopus* mice to ticks [38]. The current model that explains the epidemiology of *B. microti* in the United States demonstrates that the emergence of tick-borne infections should be studied within realistic epidemiological and ecological contexts. Selected examples of relevant tick-borne pathogen coinfections are provided in Table 1.

Understanding the tick pathobiome

Recent advances in next generation sequencing (NGS) technologies applied to explore the tick microbiome revealed an astonishing diversity of microorganisms associated to these arthropods [39-43]. These studies using NGS have shown that specific tick-borne pathogens are frequently found together with other pathogens, symbionts and commensals. This was described as a technology-driven revolution of tick-borne pathogen's vision and the concept

of pathiobiome was proposed [3]. This theoretical framework recognizes that the pathogen is integrated within its abiotic and biotic (i.e. including other pathogens, commensals and symbionts) environment [3]. Different NGS technologies have been utilized to define the microbiomes of various tick species: Sanger sequencing of full-length 16S rDNA, 454-pyrosequencing, Ion torrent, or Illumina-based sequencing of 16S rDNA hypervariable regions, as well as a whole genome shotgun [41]. A major strength of NGS compared to PCR-based approaches is that NGS is not biased towards the detection of specific microorganisms.

There is functional evidence that the relation between pathogen and microbiome is bidirectional. For example, in the tick *I. scapularis*, the gut microbiota composition influences *B. burgdorferi* colonization of tick guts [40]. A tick gut microbiota composed by high abundance of bacteria of the genera *Rickettsia*, *Thioclava*, and *Delftia*, and low abundance of bacteria of the genera *Aquabacterium*, *Brevibacterium*, and *Novosphingobium* did not favor *B. burgdorferi* colonization of tick guts [40]. This microbiota composition, which was recovered from ticks reared and maintained under ‘‘sterile’’ conditions, decreased the expression of the transcription factor signal transducer and activator of transcription (STAT). Lower STAT expression correlated with diminished expression of peritrophin, a component of the tick peritrophic matrix, which in turn decreased the ability of *B. burgdorferi* to colonize the gut epithelium [40]. Another interesting example showed that tick colonization by *A. phagocytophilum* perturbs the tick gut microbiota by decreasing the relative abundance of *Enterococcus* and *Rickettsia* whereas increasing the abundance of *Pseudomonas* [42]. *Anaplasma phagocytophilum* induces *I. scapularis* to express antifreeze glycoprotein, which encodes a protein that modulates the peritrophic matrix and binds Gram-positive bacteria decreasing their ability for biofilm formation [42]. Thus, by inducing antifreeze glycoprotein expression, *A. phagocytophilum* modifies tick microbiota and tick peritrophic matrix which

may jeopardize *B. burgdorferi* colonization [40, 42]. While these studies provided some functional basis of pathogen-microbiome interactions, a major challenge remains to understand the pathobiome at the bacterial community level. The analysis of bacterial communities as a whole may be challenging and specific analytical tools are needed to this aim.

Network analysis is a suitable tool that has been used to unravel complex microbial communities as those present in soil [44], water [45] or animal microbiota [46, 47]. Recently, ecological networks methodology was applied to unravel the complex interactions between ticks, their vertebrate hosts and pathogens in the western Palearctic [6]. Using data mining, more than 14,000 interactions were quantified among ticks, vertebrates, and pathogens in the western Palearctic [6]. The use of this approach, allowed concluding that ticks and vertebrates interact along the shared environmental gradient, while pathogens are linked to groups of phylogenetically close reservoirs [6]. Another report using networks methodology revealed a prominent role for birds in the dissemination of *B. burgdorferi* and *A. phagocytophilum*, with little contribution to the possible dissemination of other tick-borne pathogens [48]. This was in agreement with the fact that *B. burgdorferi* (*s.l.*) complex circulation is supported by a highly redundant network where few host genera have high centrality values (i.e. high relative importance for pathogen circulation) [49]. NGS projects, such as those performed currently to study the ecology of tick-associated microorganisms [50, 51], generate large data sets that can be combined with networks analysis.

Finally, NGS studies have revealed that ‘single or multiple-pathogens infection’ are both idealized scenarios that do not reflect a more complex reality where ‘pathogen transmission’ appear to be a limited conception of a broader phenomenon, i.e. microorganisms; including pathogens, symbionts and commensals; migrate across biological systems. In fact, several

symbionts can transmit horizontally when their hosts interact through mating, feeding or egg laying [52, 56]. For example, the male-killing heritable symbiont *Arsenophonus nasoniae* is transmitted horizontally when their parasitoid wasp host share oviposition patches with uninfected conspecifics, a phenomenon called superparasitism [55, 56]. Interestingly, *Candidatus* Midichloria mitochondrii, a tick endosymbiont, was proposed to be transmitted both vertically and horizontally [57, 58]. Further NGS studies should evaluate the hypothesis of the transmission of microbial communities in vector-host systems (i.e. between ticks and between ticks and hosts).

Concluding remarks

In the last twenty years, tick-borne pathogen detection have improved dramatically from 'single' and 'multiple' pathogens detection to the elucidation of the pathobiome. The 'single pathogen' view is still widely used and indeed is a necessary 'reduction' that should be integrated to the studies addressing the complexity of the pathobiome. Combining NGS projects with network analysis will provide new insights into the structure of microbial communities associated to ticks and their impact on pathogen circulation.

References

[1] Cabezas-Cruz A, Valdés JJ. Are ticks venomous animals? Front Zool 2014;11:47.

[2] Jongejan F, Uilenberg G. The global importance of ticks. Parasitol 2004;129:S3-14.

[3] Vayssier-Taussat M, Kazimirova M, Hubalek Z, Hornok S, Farkas R, Cosson JF, et al. Emerging horizons for tick-borne pathogens: from the 'one pathogen-one disease' vision to the pathobiome paradigm. Future Microbiol 2015;10:2033-43.

[4] de la Fuente J, Antunes S, Bonnet S, Cabezas-Cruz A, Domingos AG, Estrada-Peña A, et al. Tick-pathogen interactions and vector competence: identification of molecular drivers for tick-borne diseases. Front Cell Infect Microbiol 2017;7:114.

[5] Parola P, Raoult D. Ticks and tickborne bacterial diseases in humans: an emerging infectious threat. Clin Infect Dis 2001;32:897–928.

[6] Estrada-Peña A, de la Fuente J, Ostfeld RS, Cabezas-Cruz A. Interactions between tick and transmitted pathogens evolved to minimise competition through nested and coherent networks. Sci Rep 2015;5:10361.

[7] Rego RO, Bestor A, Stefka J, Rosa PA. Population bottlenecks during the infectious cycle of the Lyme disease spirochete *Borrelia burgdorferi*. PLoS One 2014;9:e101009.

[8] Saito TB, Walker DH. A tick vector transmission model of monocytotropic *Ehrlichiosis*. J Infect Dis 2015;212:968-77.

[9] Lou Y, Wu J. Modeling Lyme disease transmission. Infect Dis Model 2017;2:229–43.

[10] Zweygarth E, Cabezas-Cruz A, Josemans AI, Oosthuizen MC, Matjila PT, Lis K, et al. *In vitro* culture and structural differences in the major immunoreactive protein gp36 of geographically distant *Ehrlichia canis* isolates. Ticks Tick Borne Dis 2014;5:423-31.

[11] Almazán C, González-Álvarez VH, Fernández de Mera IG, Cabezas-Cruz A, Rodríguez-Martínez R, de la Fuente J. Molecular identification and characterization of *Anaplasma platys* and *Ehrlichia canis* in dogs in Mexico. Ticks Tick Borne Dis 2016;7:276-83.

[12] Cabezas-Cruz A, Zweygarth E, Ribeiro MF, da Silveira JA, de la Fuente J, Grubhoffer L, Valdés JJ, Passos LM, et al. New species of *Ehrlichia* isolated from *Rhipicephalus (Boophilus) microplus* shows an ortholog of the *E. canis* major immunogenic glycoprotein gp36 with a new sequence of tandem repeats. Parasit Vectors 2012;5:291.

[13] Pilloux L, Aeby S, Gaümann R, Burri C, Beuret C, Greub G. The high prevalence and diversity of *Chlamydiales* DNA within *Ixodes ricinus* ticks suggest a role for ticks as reservoirs and vectors of Chlamydia-related bacteria. Appl Environ Microbiol 2015;81:8177-82.

[14] Telfer S, Lambin X, Birtles R, Beldomenico P, Burthe S, Paterson S, et al. Species interactions in a parasite community drive infection risk in a wildlife population. Science 2010;330:243-6.

[15] Johnson PT, Preston DL, Hoverman JT, LaFonte BE. Host and parasite diversity jointly control disease risk in complex communities. Proc Natl Acad Sci USA 2013;110:16916-21.

[16] Lord CC, Barnard B, Day K, Hargrove JW, McNamara JJ, Paul RE, et al. Aggregation and distribution of strains in microparasites. Philos Trans R Soc Lond B Biol Sci 1999;354:799-807.

[17] Hall AR, De Vos D, Friman VP, Pirnay JP, Buckling A. Effects of sequential and simultaneous applications of bacteriophages on populations of *Pseudomonas aeruginosa* in vitro and in wax moth larvae. Appl Environ Microbiol 2012;78:5646-52.

[18] Malpica JM, Sacristán S, Fraile A, García-Arenal F. Association and host selectivity in multi-host pathogens. PLoS One 2006;1:e41.

[19] Seppälä O, Karvonen A, Rellstab C, Louhi KR, Jokela J. Reciprocal interaction matrix reveals complex genetic and dose-dependent specificity among coinfecting parasites. Am Nat 2012;180:306-15.

[20] Rigaud T, Perrot-Minnot MJ, Brown MJ. Parasite and host assemblages: embracing the reality will improve our knowledge of parasite transmission and virulence. Proc Biol Sci 2010;277:3693-702.

[21] Reece SE, Drew DR, Gardner A. Sex ratio adjustment and kin discrimination in malaria parasites. Nature 2008;453:609-14.

[22] Cattadori IM, Boag B, Hudson PJ. Parasite co-infection and interaction as drivers of host heterogeneity. Int J Parasitol 2008;38:371-80.

[23] Nieto NC, Foley JE. Meta-analysis of coinfection and coexposure with *Borrelia burgdorferi* and *Anaplasma phagocytophilum* in humans, domestic animals, wildlife, and *Ixodes ricinus*-complex ticks. Vector Borne Zoonotic Dis 2009;9:93-102.

[24] Bakken JS, Dumler JS. Human granulocytic anaplasmosis. Infect Dis Clin North Am 2015;29:341-55.

[25] Nieto NC, Leonhard S, Foley JE, Lane RS. Coinfection of western gray squirrel (*Sciurus griseus*) and other sciurid rodents with *Borrelia burgdorferi* sensu stricto and *Anaplasma phagocytophilum* in California. J Wildl Dis 2010;46:291-6.

[26] Thomas V, Anguita J, Barthold SW, Fikrig E. Coinfection with Borrelia burgdorferi and the agent of human granulocytic *ehrlichiosis* alters murine immune responses, pathogen burden, and severity of Lyme arthritis. Infect Immun 2001;69:3359-71.

[27] Holden K, Hodzic E, Feng S, Freet KJ, Lefebvre RB, Barthold SW. Coinfection with *Anaplasma phagocytophilum* alters *Borrelia burgdorferi* population distribution in C3H/HeN mice. Infect Immun 2005;73:3440-4.

[28] Nyarko E, Grab DJ, Dumler JS. *Anaplasma phagocytophilum*-infected neutrophils enhance transmigration of *Borrelia burgdorferi* across the human blood brain barrier in vitro. Int J Parasitol. 2006; 36(5):601-5.

[29] Michelet L, Delannoy S, Devillers E, Umhang G, Aspan A, Juremalm M, Chirico J, van der Wal FJ, Sprong H, Boye Pihl TP, Klitgaard K, Bødker R, Fach P, Moutailler S, et al. High-throughput screening of tick-borne pathogens in Europe. Front Cell Infect Microbiol. 2014; 4:103.

[30] Moutailler S, Valiente MC, Vaumourin E, Michelet L, Tran FH, Devillers E, et al. Co-infection of ticks: The rule rather than the exception. PLoS Negl Trop Dis 2016;10:e0004539.

[31] Raileanu C, Moutailler S, Pavel I, Porea D, Mihalca AD, Savuta G, Vayssier-Taussat M. *Borrelia* diversity and co-infection with other tick borne pathogens in ticks. Front Cell Infect Microbiol 2017;7:36.

[32] Gäumann R, Mühlemann K, Strasser M, Beuret CM. High-throughput procedure for tick surveys of tick-borne encephalitis virus and its application in a national surveillance study in Switzerland. Appl Environ Microbiol. 2010; 76:4241-9.

[33] Castañeda-Ortiz EJ, Ueti MW, Camacho-Nuez M, Mosqueda JJ, Mousel MR, Johnson WC, Palmer GH. Association of *Anaplasma* marginale strain superinfection with infection prevalence within tropical regions. PLoS One. 2015; 10:e0120748.

[34] Ybañez AP, Ybañez RH, Yokoyama N, Inokuma H. Multiple infections of *Anaplasma* platys variants in Philippine dogs. Vet World. 2016; 9:1456-1460.

[35] Liu J, Hansen C, Quake SR. Solving the "world-to-chip" interface problem with a microfluidic matrix. Anal Chem 2003;75:4718-23.

[36] Johnson L, Wilcox S, Mankoff J, Stricker RB. Severity of chronic Lyme disease compared to other chronic conditions: a quality of life survey. PeerJ. 2014; 2:e322.

[37] Vaumourin E, Vourc'h G, Gasqui P, Vayssier-Taussat M. The importance of multiparasitism: examining the consequences of co-infections for human and animal health. Parasit Vectors. 2015;8:545.

[38] Diuk-Wasser MA, Vannier E, Krause PJ. Coinfection by ixodes tick-borne pathogens: ecological, epidemiological, and clinical consequences. Trends Parasitol 2016;32:30-42.

[39] Nakao R, Abe T, Nijhof AM, Yamamoto S, Jongejan F, Ikemura T, Sugimoto C, et al. A novel approach, based on BLSOMs (Batch Learning Self-Organizing Maps), to the microbiome analysis of ticks. ISME J 2013;7:1003-15.

[40] Narasimhan S, Rajeevan N, Liu L, Zhao YO, Heisig J, Pan J, et al. Gut microbiota of the tick vector *Ixodes scapularis* modulate colonization of the Lyme disease spirochete. Cell Host Microbe 2014;15:58-71.

[41] Narasimhan S, Fikrig E. Tick microbiome: the force within. Trends Parasitol 2015;31:315-23.

[42] Abraham NM, Liu L, Jutras BL, Yadav AK, Narasimhan S, Gopalakrishnan V, et al. Pathogen-mediated manipulation of arthropod microbiota to promote infection. Proc Natl Acad Sci USA 2017;114:E781-E90.

[43] Swei A, Kwan JY. Tick microbiome and pathogen acquisition altered by host blood meal. ISME J 2017;11:813-6.

[44] Barberán A, Bates ST, Casamayor EO, Fierer N. Using network analysis to explore co-occurrence patterns in soil microbial communities. ISME J 2012;6:343-51.

[45] Zancarini A, Echenique-Subiabre I, Debroas D, Taïb N, Quiblier C, Humbert JF. Deciphering biodiversity and interactions between bacteria and microeukaryotes within epilithic biofilms from the Loue River, France. Sci Rep 2017;7:4344.

[46] Faust K, Sathirapongsasuti JF, Izard J, Segata N, Gevers D, Raes J, et al. Microbial co-occurrence relationships in the human microbiome. PLoS Comput Biol 2012;8:e1002606.

[47] Sung J, Kim S, Cabatbat JJT, Jang S, Jin YS, Jung GY, Chia N, Kim PJ. Global metabolic interaction network of the human gut microbiota for context-specific community-scale analysis. Nat Commun. 2017; 8:15393.

[48] de la Fuente J, Estrada-Peña A, Cabezas-Cruz A, Brey R. Flying ticks: Anciently evolved associations that constitute a risk of infectious disease spread. Parasit Vectors 2015 ;8:538.

[49] Estrada-Peña A, Sprong H, Cabezas-Cruz A, de la Fuente J, Ramo A, Coipan EC. Nested coevolutionary networks shape the ecological relationships of ticks, hosts and the Lyme disease bacteria, *Borrelia burgdorferi*. Parasit Vectors 2016;9:517.

[50] Vayssier-Taussat M, Moutailler S, Michelet L, Devillers E, Bonnet S, Cheval J, et al. Next generation sequencing uncovers unexpected bacterial pathogens in ticks in western Europe. PLoS One 2013;8:e81439.

[51] Bonnet S, Michelet L, Moutailler S, Cheval J, Hebert C, Vayssier-Taussat M, et al. Identification of parasitic communities within European ticks using next-generation sequencing. PLoS Negl Trop Dis 2014;8:e2753.

[52] Moran NA, Dunbar HE. Sexual acquisition of beneficial symbionts in aphids. Proc Natl Acad Sci USA 2006;103:12803–6.

[53] Gehrer L, Vorburger C. Parasitoids as vectors of facultative bacterial endosymbionts in aphids. Biol Lett 2012;8:613–5.

[54] Ahmed MZ, Li SJ, Xue X, Yin XJ, Ren SX, Jiggins FM, Greeff JM, Qiu BL. The intracellular *bacterium Wolbachia* uses parasitoid wasps as phoretic vectors for efficient horizontal transmission. PLoS Pathog. 2015; 10:e1004672.

[55] Gonella E, Pajoro M, Marzorati M, Crotti E, Mandrioli M, Pontini M, et al. Plant-mediated interspecific horizontal transmission of an intracellular symbiont in insects. Sci Rep 2015;5:15811.

[56] Parratt SR, Frost CL, Schenkel MA, Rice A, Hurst GD, King KC. Superparasitism drives heritable symbiont epidemiology and host sex ratio in a wasp. PLoS Pathog 2016; 12:e1005629.

372 [57] Epis S, Sassera D, Beninati T, Lo N, Beati L, Piesman J, Rinaldi L, McCoy KD, Torina
373 A, Sacchi L, Clementi E, Genchi M, Magnino S, Bandi C, et al. *Midichloria mitochondrii* is
374 widespread in hard ticks (*Ixodidae*) and resides in the *mitochondria* of phylogenetically
375 diverse species. Parasitology 2008;135:485-94.

376 [58] Montagna M, Sassera D, Epis S, Bazzocchi C, Vannini C, Lo N, Sacchi L, Fukatsu T,
377 Petroni G, Bandi C, et al. "*Candidatus Midichloriaceae*" fam. nov. (*Rickettsiales*), an
378 ecologically widespread clade of intracellular *alphaproteobacteria*. Appl Environ Microbiol
379 2013; 79:3241-8.

Table 1. Selected exemples of ticks co-infection significantly modifying the biology of another microbe co-occuring in ticks.

Co-infections	Effect
Anaplasma/Borrelia	Decreased antibody response towards *A. phagocytophilum*
Borrelia/Anaplasma	Increased transmigration of *B. burgdoreferi* across the human blood brain barrier
Borrelia/Babesia microti	Increased transmission from mice to ticks