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REVIEW

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Emerging horizons for tick-borne pathogens: from the 'one pathogen–one disease' vision to the pathobiome paradigm

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Ticks, as vectors of several notorious zoonotic pathogens, represent an important and increasing threat for human and animal health in Europe. Recent applications of new technology revealed the complexity of the tick microbiome, which may affect its vectorial capacity. Appreciation of these complex systems is expanding our understanding of tick-borne pathogens, leading us to evolve a more integrated view that embraces the 'pathobiome'; the pathogenic agent integrated within its abiotic and biotic environments. In this review, we will explore how this new vision will revolutionize our understanding of tick-borne diseases. We will discuss the implications in terms of future research approaches that will enable us to efficiently prevent and control the threat posed by ticks.

Recent applications of next-generation sequencing (NGS) technology revealed the complexity of the tick microbiome, which may impact upon its vectorial capacity and consequently affect vector–reservoir host interactions. Appreciation of these complex systems is increasing our understanding of tick-borne pathogens, leading us to evolve a more integrated view that embraces the 'pathobiome' representing the pathogenic agent integrated within its abiotic and biotic environments including other pathogens, commensals or mutualists. In this review, we will explore how this emerging vision of tick-borne pathogens will revolutionize our understanding of tick-borne diseases, which are a growing concern given their exponential increase since the discovery of the Lyme disease agent. We will discuss the implications in terms of future research approaches that will enable us to efficiently prevent and control the threat posed by ticks.

KEYWORDS

• co-infections • emerging diseases • *Ixodes ricinus* • new paradigm • next-generation sequencing • pathobiome • unknown pathogens • vector competence • zoonoses

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Current state-of-the-art knowledge of tick-borne pathogens using 'conventional vision'

• Expanding horizons of tick-borne pathogens

In Europe, the most prevalent tick-borne disease in humans is Lyme borreliosis (LB), caused by a group of bacteria belonging to the *Borrelia burgdorferi* sensu Iato group with at least five different species infecting humans in Europe [1]. Recently, *B. miyamotoi*, belonging to the more distantly related relapsing fever group, has been detected in patients in USA, Japan, Russia and The Netherlands [2–5], and is transmitted by the tick species involved in LB. Ticks can also be infected with other pathogens that might be transmitted to humans [6] (see **Table 1**). Among them, *Anaplasma phagocytophilum* is responsible for granulocytic anaplasmosis, Candidatus *Neoerhlichia* mikurensis has emerged as a cause of severe febrile illness in immunocompromised patients [7,8], while rickettsiae of the spotted fever group are known (*R. monacenis*, *R. conorii*) or suspected (*R. helvetica*) to cause rickettsioses [9,10]. Other bacterial pathogens such as *Francisella tularensis*, causing tularemia, and the Q fever agent *Coxiella burnetii* have also been detected in *I. ricinus*, but the direct role of this tick species in the epidemiology of these diseases is probably not significant [11,12]. Humans may develop babesiosis following tick-borne transmission of protozoans belonging to the genus *Babesia*, mainly *B. divergens*, however, the virulence of additional members of this genus, such as *B. venatorum*, have recently been confirmed [13]. *Babesia microti*, an emerging human tick-borne pathogen in USA, has also been identified in ticks in Europe, with one single human case to date [14]. Tick species also transmit arboviruses, with the tick-borne encephalitis virus being the most notorious in terms of public health in Europe [15,16]. Beside TBEV, many tick-borne viruses are known to be transmitted by other ticks. Among them, Crimean–Congo hemorrhagic fever virus (CCHFV) is considered to be one of the major emerging disease threats in the European Union following an expanding distribution of its main tick vector, the genus *Hyalomma* [17]. More anecdotally, Omsk virus, an endemic virus from rural regions in Siberia and transmitted by *Dermancentor* species, is expanding its range. This virus caused capillary damage responsible for the hemorrhagic manifestations [15]. Other European tick-borne viruses are less well established as causes of disease but case reports are emerging. Among them, Powassan virus, a member of the genus *Flavivirus*, has been recovered from the brains of patients following fatal infection [15]. Louping ill virus, also member of the genus *Flavivirus*, causes encephalitis in sheep, whereas exposed humans develop asymptomatic infection [15].

An increasing number of new species, strains or genetic variants of other micro-organisms are being detected in ticks, resulting in an ever-increasing list of (potential) pathogens capable of infecting livestock, companion animals and humans. However, it needs to be taken into account that a significant portion of these 'new' species/genotypes are not truly emerging, but rather newly detected. This increasing recognition of pathogen biodiversity is not generating answers, but instead raising rather complex questions regarding ecological cycles of pathogens, their polymicrobial cross-talk and their influence upon infection mechanisms, clinical differential diagnosis and intervention opportunities.

Identification of micro-organisms in ticks has been largely dominated by the use of conventional molecular approaches mostly using specific primers combined with (real-time) PCR, and less frequently by culture-dependent methods. However, pathogen detection in an arthropod is not sufficient to validate its vector competence. This entails use of vector competence studies to establish both the interaction of new or unexpected pathogen with ticks, and to evaluate the risk of exposure for both humans and animals. These types of studies require living ticks raised under controlled conditions. Because of their complex biological cycle and their feeding biology, maintenance of tick colonies and their infection with micro-organisms is not easy. However, several methods have been successfully developed and used to infect hard ticks with pathogens, for example, feeding ticks on infected animals, injecting pathogens through the cuticule, by using thin capillary tubes and feeding ticks on infected blood through artificial or animal-derived membranes [18]. These methods have been successfully employed to validate vector competence for a number of tick-borne pathogens, including Lyme spirochaetes [19], *A. phagocytophilum* [20], *Babesia* sp. EU1 (or *B. venatorum*) [21] *Bartonella* sp. [22,23] and tick-borne encephalitis virus [24,25]. However, for some established tick-borne pathogens, such as *Ca.* N. mikurensis or *R. helvetica* (both of which

Table 1. The predominant tick species present in the northern hemisphere, the pathogens they transmit, associated diseases, animal hosts as well as animal reservoirs of the corresponding pathogens.

Ticks species	Pathogens	Diseases (hosts)	Reservoirs
Ixodes species	*Borrelia burgdorferi* sensu lato	Lyme disease (human, cattle, dog, horse)	Rodent, bird, reptile
	Borrelia miyamotoi	Recurrent fever	Rodent, bird
	Anaplasma phagocytophilum	Granulocytic anaplasmosis (flu-like symptoms in human, cattle, goat, sheep, horse, dog, cat)	Wild ruminants, rodent,
	Babesia divergens	Babesiosis (human, cattle)	Deer, cattle
	Babesia microti	Babesiosis (human)	rodent
	Babesia venatorum		
	Babesia capreoli		
	Coxiella burnetii	Q fever (human, goat, sheep…)	Rodent
	Francisella tularensis	Tularemia (human, rodents, sheep, goat, …)	Hare
	Bartonella henselae	Bartonellosis (human)	Cat
	Bartonella berkhoffii	Bartonellosis (dog, human)	Dog
	Tick-borne encephalitis Virus	TBE (human, dog)	Rodent
	Candidatus Neoehrlichia mikurensis	Fever (human, dogs)	Rodent
	Rickettsia helvetica (suspected)	Fever (human)?	Unknown
	Rickettsia monacensis	Fever (human)	Unknown
	Powassan virus	Fever, neurological signs (human)	Rodent
	Louping hill virus	Encephalitis (human, sheep)	Mountain hare, sheep
Dermacentor spp.	*Anaplasma ovis*	Anaplasmosis (goat, sheep)	Unknown
	Babesia caballi	Babesiosis (horse)	Horse
	Theileria/Babesia equi	Theileriosis (horse)	Horse
	Babesia canis	Canine Babesiois	Dogs
	Rickettsia slovaca	TIBOLA/SENLAT (human)	Unknown
	Rickettsia raoultii	TIBOLA/SENLAT (human)	Unknown
	Anaplasma marginale	Bovine anaplasmosis (cattle)	Cattle
	Francisella tularensis	Tularemia (human, rodents sheep, goat, …)	Hare
	Coxiella burnetii	Q fever (human, goat, sheep, …)	Rodent
	Omsk hemorrhagic virus	Hemorrhagic manifestations (human)	Muskrat
	Powassan virus	Fever, neurological signs (human)	Rodent
Haemaphysalis spp.	*Babesia* spp.	Babesiosis (human, possibly cattle and dog)	Unknown
	Theileria spp.	Theileriosis (cattle)	Unknown
Hyalomma spp.	*Theileria annulata*	Theileriosis (cattle)	Unknown
	Theileria equi		
	Crimean-Congo hemorrhagic fever virus*	Hemorrhagic fever (human)	Rodent, bird?
Rhipicephalus sanguineus	*Rickettsia conorii*	Mediterranean spotted fever (human)	Dog?
	Ehrlichia canis	Ehrlichiosis (dog)	Dog
	Anaplasma platys	Cyclic thrombocytopenia	Dog
	Babesia vogeli/canis	Canine babesiosis	Dog
	Hepatozoon canis	Hepatozoonosis	Dog
	Babesia gibsoni	Canine Babesiosis	Dog

currently lack any cultivable strain), the tick vector competence remains to be proven. These are consequently considered '*de facto*' tick-borne pathogens under more or less strong 'epidemiological evidence'.

• Diagnostic challenges posed by tick-borne pathogens/diseases

Given a clinical history of tick bites, Lyme borreliosis is the primary consideration, but in some this diagnosis remains elusive being unconfirmed

by conventional serological tests [26]. People bitten by ticks can also be infected by tick-borne encephalitis virus (TBEV) causing severe encephalitis, which is readily diagnosed by serological tests [15]. TBE can be successfully prevented by active immunization, but no specific treatment is available [27]. As already mentioned, ticks are capable of transmitting the largest variety of pathogens among arthropod vectors, and pathogens other than the Lyme or tick-borne encephalitis agents might be involved in tick-borne diseases (TBDs). Interestingly, the majority of those pathogens have been discovered during the last 20 years. The symptoms induced by those pathogens are often mild and nonspecific (high fever, fatigue, body aches, chills, etc.) and can be confused with symptoms caused by infection with other agents. This is probably the underpinning reason why these infections are poorly recognized in humans by medical practitioners despite their abundance in ticks and/or reservoir animals. A striking example is that of *B. miyamotoi*. This *Borrelia* species was first isolated from Japanese *Ixodes* ticks in 1995 whereby it was considered a nonpathogenic endogenous tick bacterium until the first human cases of *B. miyamotoi* infection were reported in Russia some 16 years later [2]. Subsequently, human infections have been described in the USA and most recently in The Netherlands [3–5,28]. Circulation of *B. miyamotoi* between *I. ricinus* and wild animals has been confirmed in other European countries such as France, Estonia, Poland and Switzerland [29], which has confirmed that the French genotype is identical to an isolate from a Dutch patient [30]. Despite this apparent absence of human cases of *B. miyamotoi* infections among these countries, this is likely to reflect the absence of serological or molecular tests for *B. miyamotoi* combined with the lack of knowledge of these bacteria among medical practitioners. Thus, it is likely that the absence of human infections is rather due to missed diagnoses than to an actual absence of infection.

Those patients bitten by ticks are additionally at risk for co-infection by several pathogens. For instance, Horowitz *et al.* [31] described co-infection rates ranging from 2 to 5% for *Borrelia* species and *A. phagocytophilum* among patients with erythema migrans, the diagnostic hallmark for Lyme borreliosis. Co-infections between *B. afzelii* and *R. monacensis* were also identified in skin biopsy of erythema migrans patients in The Netherlands [32]. However, co-infections are rarely diagnosed in routine practice, alerting us to the problem that co-infection in humans a relevant, albeit understudied issue, with important implications for public health.

Consequently, individuals infected by pathogens other than Lyme borreliosis spirochaetes or TBEV, are rarely identified. In recent years, unexplained syndromes occurring after tick bites have became an increasingly important issue leading to considerable discord between scientists, patients and institutions of infectious disease.

The technology-driven revolution of tick-borne pathogen's vision

• From pathogen to pathobiome

Until now, most studies detecting pathogens in ticks have used assays that are only able to assess a limited number of agents simultaneously [33,34]. This is partly due to technological limitations making complete screens of micro-organisms in their natural vector/reservoir populations using standard laboratory procedures unachievable. Within the last few years, the rapid development of NGS methods has revolutionized the research field of epidemiology and diagnosis of infectious diseases, thus facilitating complete screening of pathogens within their hosts, discovery of new pathogens or the detection of unexpected ones. NGS has recently been successfully used to identify the bacterial communities associated with *I. ricinus* [35–38] based on the amplification and sequencing of hypervariable regions of the 16S rRNA encoding genes (metagenomic profile), revealing a highly diverse microbial community (108 genera representing all bacterial phyla). As expected, those approaches have allowed detection without *a priori* established tick-borne pathogens, such as the *Borrelia*, *Anaplasma*, *Coxiella*, *Francisella* or *Rickettsia* genus. Those genera, mostly known as pathogenic for vertebrates, while other species are considered as endosymbionts (e.g., the *Rickettsia*-endosymbiont of *I. scapularis*) [39], highlight the challenge of differentiating between pathogens and endosymbionts. Adding further complexity, some authors consider *Rickettsia* species as endosymbionts that are transmitted vertically in arthropods, and only secondarily serve as pathogens of vertebrates [40]. For the *Coxiella* genus, the species *C. burnetii* is mostly considered as a vertebrate pathogen while numerous other *Coxiella* species have been found associated to ticks [41]. Phylogenetic analyses combined with

experimental approaches suggested that these might also be considered as endosymbionts of ticks [11,42]. Thus, the pathogenic nature of *C. burnetii* could be an exception within the genus [43]. Beside the well-known vertebrate pathogenic species, *F. tularensis* (occasionally found in ticks), *Francisella*-like-endosymbionts associated with *Dermacentor* spp. have been described but their potential pathogenic nature remains to be investigated [44]. The *Wolbachia* and *Arsenophonus* genera are also bacteria associated with arthropods (mostly insects) and influence reproduction and/or immunity of their hosts [45,46]. They have also been found associated with ticks [47]. However, a recent study revealed that in *I. ricinus*, the finding of *Wolbachia* is a consequence of parasitism by a parasitoid wasp (*Ixodiphagus hookeri*) [48]. The role of *Arsenophonus* as a tick endosymbiont is still to be demonstrated. Finally, the endosymbiont *Midichloria mitochondrii* was initially observed in tick cells (especially in ovarian cells of *I. ricinus*) [49]. Use of molecular probes specific for this alphaproteobacteria have demonstrated their presence in almost 100% of *I. ricinus* females derived from natural populations [50], but also in other tick species [51]. Furthermore, *M. mitochondri* has recently been implicated as a potential vertebrate pathogen [52].

Use of NGS technology will undoubtedly shed new light on the intriguing bacterial communities associated with ticks [37]. The clear-cut boundaries between the so-called 'vertebrate-pathogens', 'arthropod-pathogens' or 'arthropod-symbionts' may thus fade into a more dynamic and complex vision of bacterial–vector–vertebrate communities. Better knowledge of the role of these bacteria could even constitute useful resources for developing antivectorial control measures.

Besides the known micro-organisms (either belonging to pathogens, endosymbionts or both), NGS also revealed that the majority of RNA/DNA sequences carried by ticks belonged to unknown micro-organisms. For instance, 80% of the viral nucleic sequences detected from tick extracts represented currently unidentified micro-organisms (Vayssier-Taussat *et al.*, Unpublished Data). Among these new viral sequences, we identified genera transmissible to humans and/or animals via arthropods, including Bunyaviridae (Nairovirus and Phlebovirus), Rhabdoviridae (Vesiculovirus) and Reoviridae (Coltivirus) [vayssier-taussat et al., unpublished data]. In the USA, a similar study, undertaken by Lipkin *et al.*, characterized the virome of different tick species. Powassan virus, a well-known human pathogenic tick-borne virus, and eight novel viruses belonging to nairovirus, phlebovirus and mononegavirus genera were identified among the three ticks assessed [53]. New viruses recently identified in ticks by NGS are listed in **Table 2**.

By understanding the entire tick microbial community, we can identify that pathogens are intimately associated to the vast community of micro-organisms (including other pathogens) and by elucidating their influence on tick biology, pathogen persistence, transmission and virulence justifies the need to shift from the study of isolated pathogens to a more integrated approach. Within this context, we define the 'pathobiome' as representing the pathogen within its abiotic and biotic environment [54] (See **Figure 1**). Understanding the multifactorial pathobiome requires comprehensive knowledge of the microbial community comprising the pathobiome, the network of interactions between microbes and the biological relevance of these interactions.

• Deciphering microbial interactions within the tick ecosystem

Microbial interactions have largely been considered on a one-to-one interaction level, where the infection by one pathogen influences the

Table 2. New viruses recently identified in ticks by next-generation sequencing.

Viruses	Diseases	Tick species	Ref.
Nairovirus (South Bay virus)	Unknown	*Ixodes scapularis*	[53]
Blacklegged tick Phlebovirus (BTPV)	Unknown	*Ixodes scapularis*	[53]
American dog tick Phlebovirus (ADTPV)	Unknown	*Ixodes scapularis*/*D. variabilis*	[53]
Monongavirales-like virus	Unknown	*Ixodes scapularis*	[53]
Phlebovirus (Hearltand virus)	Severe febrile illness	*Amblyomma americanum*	[84]
Shibuinji virus (New tick-borne virus phlebovirus)	Unknown	*Rhipicephalus* spp.	[85]

acquisition of and/or dynamics of infection by a second pathogen. However, interactions between sets of pathogens can be considered when different pathogens interact within a network or through 'cascade consequence' [55,56]. In experimental studies, one can investigate how the presence of one pathogen may interfere with infection by another, however, this is not possible using the pathobiome perspective where many pathogens and other micro-organisms are present, including those members that remain poorly understood. In such a scenario, one can use population studies to assess dynamics of change through the probability of finding those pathogens together beyond those which could occur by chance. Seeking microbial congruence initially assesses this, even though this can also result from confounding factors that create statistical associations between pathogens, without true biological interactions. In population studies, longitudinal or time series data are useful for identifying pathogen associations, identifying whether the presence of one pathogen modifies subsequent infection by another [57]. However, such studies are resource intensive. An alternative is to run one-off cross-sectional studies, which are cheaper and less time consuming than longitudinal studies. Cross-sectional studies can easily be used to detect several pathogens and are especially appropriate in the case of emerging or poorly known pathogens or host species. In such cases, numerous approaches are available to detect pathogen associations. Multivariate analyses (e.g., PCA, FCA, DA, CoA) [58] will evaluate which pathogens tend to group together. However, statistical tests associated with these analyses are usually not available [but see, for example, permutation methods, 59,60]. A new modeling approach was to develop 'the association screening approach' to detect the overall and more detailed multipathogen associations [61]. This method is quite powerful but would require over 1000 samples if one were to study over ten micro-organisms. However, strong methodological developments on robust network analytical methods have been made [62] and continue to evolve (e.g., in medicine: metabolic pathways [63,64]; in computer science: peer-to-peer networks [65]; or in social science: scientific collaboration [66]). They also offer an attractive representation of assessing dynamics of multiple pathogen relationships. They provide indices of association such as connectance [67], nestedness [68] or betweeness [69]. However, to date, statistical tests regarding the network parameters have rarely been used, although developments in this field are promising.

• Importance of the pathobiome concept to elucidate competence mechanisms

Microbes present with other pathogens in ticks may interfere with pathogen transmission. For instance, Rickettsial endosymbionts are thought to alter transmission of other rickettsial pathogens, as seen by the inverse relationship between the infection prevalence of *R. rickettsii* (pathogen) and *R. peacockii* (symbiont) in *Dermancentor andersoni* [47,70]. Furthermore, the presence of *Coxiella*-related symbionts in the salivary glands of *Amblyomma* ticks impairs transmission of *Ehrlichia chaffeensis* [71]. In addition to symbionts, ticks are also colonized by a natural bacterial microbiota mainly belonging to the Proteobacteria, Firmicutes and Bacteroides phyla [72]. It has also been demonstrated that these tick microbiomes can interfere with pathogens. For example, when ticks were bred in a sterile environment, the absence of microbiota altered gut integrity and the ability of *B. burgdorferi* to colonize [72]. Microbiome alterations might also result in a modulated immune response which might then interfere with pathogen survival and infection, as shown for other arthropod vectors [73]. Thus taking into account the pathobiome rather than the isolated pathogens, is crucial to understand how pathogens are transmitted and how they survive within ticks.

Pathobiome approach for surveillance, diagnosis & prevention of tick-borne diseases

• Surveillance & diagnosis

Considering the vast number of potential tick-borne pathogens that can result in disease, either alone or in association, there is an urgent need to develop methods that are capable of assessing this diversity, as well as providing insights into the biology of tick-borne pathogens. For instance, many tick-borne pathogens colonize blood (residing within either intra- or extracellular niches) of vertebrate hosts. Thus, it makes sense to detect the presence of their DNA in the blood of infected human patients. However, blood infection does not occur for all tick-borne pathogens. A notable exception is the Lyme spirochete that does not stably infect the blood of human hosts, therefore, detection of DNA in the blood of such a patient bitten by a tick is unhelpful, necessitating the use of more specific

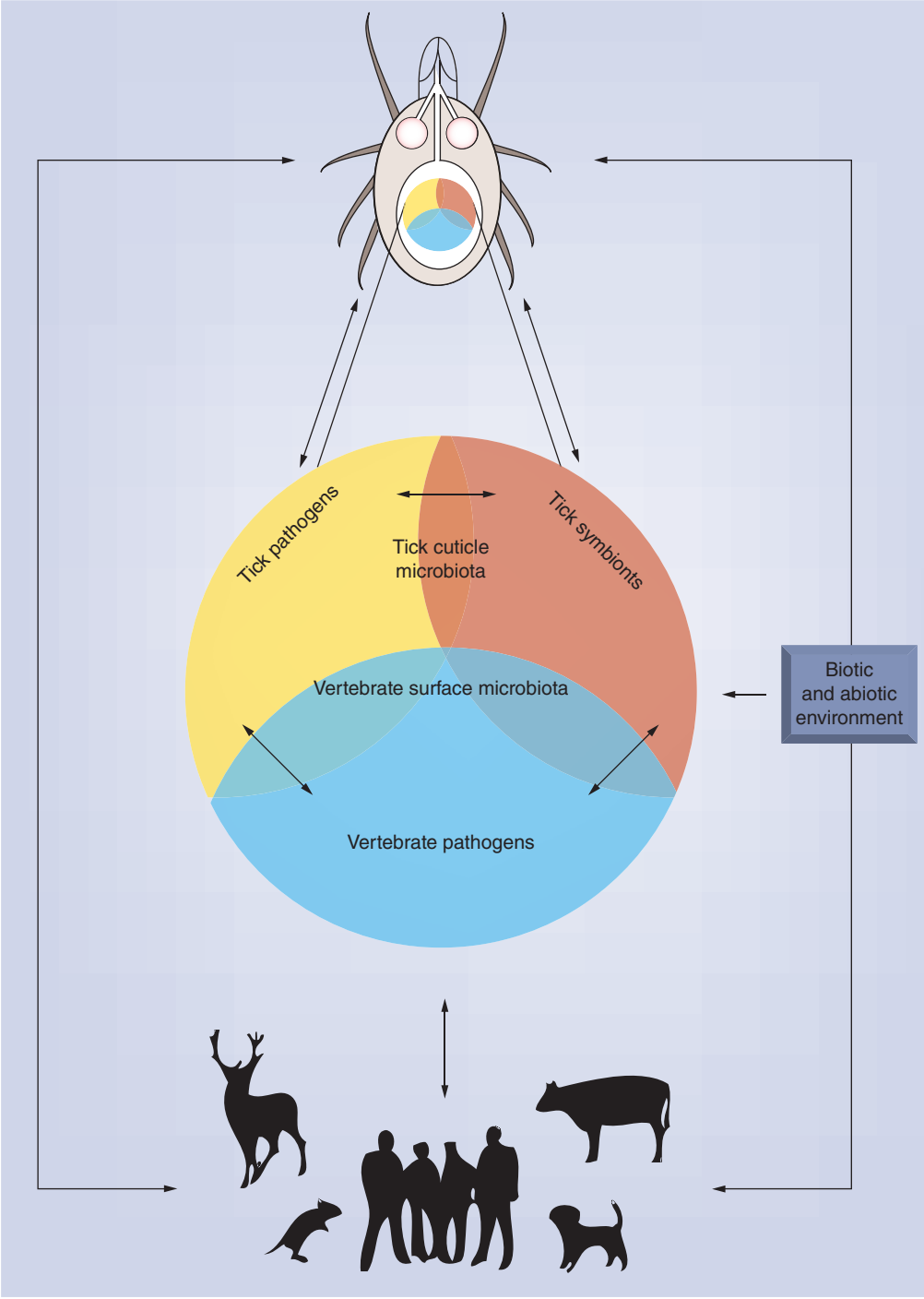

Figure 1. The tick pathobiome concept.

samples (such as skin biopsies) or serological tests, although their specificity and sensitivity are not always optimal. Molecular identification of tick-borne pathogens has mostly been based on the use of specific primers combined with real-time PCR, which can only detect a selected and limited number of species simultaneously. To overcome these limitations, new tools enabling high-throughput monitoring of tick-borne pathogens were an urgent priority. Based upon NGS data on presence of tick-borne pathogens in ticks in different European geographical regions, we developed a microfluidic system allowing multiple parallel real-time PCRs for

TBD surveillance that might be adapted to diagnostic settings [74]. This has the unique ability to simultaneously analyze multiple pathogens (up to 48 different species) in the same sample. This new tool presents the major advantage and can be easily adapted to new or emerging situations as it is entirely possible to remove primers/probe sets in order to modify the panel of targeted pathogens. If developed by private companies, this approach will represent an important improvement for the diagnosis of TBD.

- **Vaccination**

Given the vast number of pathogens/potential pathogens that could be transmitted by the same tick species, deployment of tick vaccines would be both a smart and environmentally friendly alternative to protect human and animal populations against tick-borne diseases. This novel approach for control of vector infestations and thus reducing subsequent pathogen transmission necessitates a deep understanding of microbial interactions within the tick. For that purpose, research on molecular interactions between ticks and pathogens, as well as the identification of suitable targets for vaccine development, are major challenges for the implementation of new TBD control strategies [75]. Among these, target molecules playing key roles in vector capacity are particularly promising [76]. To date, the only commercially available antitick vaccine is based on the *R. microplus* midgut protein BM86 that interferes with tick feeding and subsequent egg production [77]. However, owing to technological advances for tick infection combined with improved resolution of molecular investigative methods, further promising candidates have recently been identified. These include tick proteins derived from *I. ricinus* [78,79], *I. scapularis* [80], *Rhipicephalus microplus* [81,82], as well as candidates common to several hard tick species [83]. Improving our understanding of molecular interactions between ticks and tick-borne pathogens is an essential prerequisite for the conception of future generations of vaccines and for vectors and disease control.

Conclusion

Owing to powerful molecular and technological advancements, the tick pathobiome vision now offers a new vantage point to understand tick-borne pathogens from a more holistic point of view.

Future perspective

Shifting the paradigm from pathogens to pathobiome will have many research consequences; the most important being how to determine the significance of micro-organisms revealed by NGS technology in human and/or animal idiopathic disease following tick bites; and to decipher the impact of complex microbial interactions between pathogens and/or other tick endogenous micro-organisms that might influence pathogen transmission, persistence,

EXECUTIVE SUMMARY

Current knowledge on tick-borne pathogens

- An increasing number of 'new' species, strains or genetic variants of micro-organisms are being detected in ticks, resulting in an ever-increasing list of potential pathogens.
- This increasing recognition of pathogen diversity is raising complex questions regarding ecological cycles of the pathogen, polymicrobial cross-talk, diagnosis and intervention opportunities.

The new vision

- Next-generation technology sheds new lights on bacterial communities associated with ticks.
- The majority of DNA/RNA sequences carried by ticks belong to unknown micro-organisms.
- Pathogens are intimately associated with the tick microbial community.
- This justifies the need to shift the research focus from isolated pathogens to a more integrated pathobiome approach.

Future research directions in term of surveillance, diagnosis & prevention of tick-borne diseases

- New tools enabling high-throughput monitoring of tick-borne pathogens are an urgent priority.
- Given the vast number of pathogens that could be transmitted by the same tick species, deployment of tick vaccines would be a smart and environmentally friendly alternative to protect human and animal populations from tick-borne diseases.

virulence and evolution. Based upon this new knowledge, new research avenues will have to be followed to develop adequate strategies to better diagnose and combat tick-borne diseases

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