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Review

The Importance and Impact of *Francisella*-like Endosymbionts in *Hyalomma* Ticks in the Era of Climate Change

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Abstract: Ticks are obligatory hematophagous parasites that serve as vectors for a large amount of important human and livestock pathogens around the world, and their distribution and incidence of tick-associated diseases are currently increasing because of environmental biomass being modified by both climate change and other human activities. *Hyalomma* species are of major concern for public health, due to their important role as vectors of several diseases such as the Crimea–Congo hemorrhagic fever (CCHF) virus in humans or theileriosis in cattle. Characterizing the *Hyalomma*-associated microbiota and delving into the complex interactions between ticks and their bacterial endosymbionts for host survival, development, and pathogen transmission are fundamental, as it may provide new insights and spawn new paradigms to control tick-borne diseases. *Francisella*-like endosymbionts (FLEs) have recently gained importance, not only as a consequence of the public health concerns of the highly transmissible *Francisella tularensis*, but for the essential role of FLEs in tick homeostasis. In this comprehensive review, we discuss the growing importance of ticks associated with the genus *Hyalomma*, their associated tick-borne human and animal diseases in the era of climate change, as well as the role of the microbiome and the FLE in their ecology. We compile current evidence from around the world on FLEs in *Hyalomma* species and examine the impact of new molecular techniques in the study of tick microbiomes (both in research and in clinical practice). Lastly, we also discuss different endosymbiont-directed strategies for the control of tick populations and tick-borne diseases, providing insights into new evidence-based opportunities for the future.

Keywords: *Francisella*-like endosymbiont; *Hyalomma*; tick microbiome; endosymbiont; tick symbiosis; climate change

1. Introduction

Ticks are obligatory hematophagous ectoparasites that act as vectors for many important human and livestock pathogens worldwide [1]. Like spiders and scorpions, they belong to the class Arachnida. Within the order Ixodida, most species of ticks belong to one of the two main large families, Argasidae or Ixodidae. The latter are known as "hard" ticks since they have a sclerotized dorsal plaque or scutum. In contrast, those belonging to the family *Argasidae* lack this physical feature and are therefore known as "soft ticks" [2]. Tick-borne infectious diseases spread following the bite of infected ticks, which can carry and be infected by bacteria, viruses, or parasites. Some of the most important bacteria-infecting ticks include species of the genera *Rickettsia*, *Borrelia*, *Francisella*, *Anaplasma*, and *Ehrlichia*, as well as viruses such as the Crimea–Congo hemorrhagic fever (CCHF) virus, the West Nile virus, and the tick-borne encephalitis virus, among others.

The population of these parasites has been shown to increase over the last past years, as has the incidence of vector-related animal diseases and human zoonoses [3]. Recently, *Hyalomma* ticks have been more frequently found in areas where they were not previously reported. This genus, its occurrence, and its potential spread are of major concern for public

health due to their important role as vectors of several diseases, such as the CCHF virus in humans or theileriosis in cattle, among others [4].

Characterizing the tick-associated microbiota and delving into the complex interactions between ticks and their bacterial endosymbionts for host survival, development, and pathogen transmission are fundamental. This may give new insights and spawn new paradigms to control tick-borne diseases [5]. Among the different both pathogenic and non-pathogenic bacterial species within the tick microbiome, *Francisella*-like endosymbionts (FLEs) have recently gained importance, not only as a consequence of the public health concerns of the highly transmissible *Francisella tularensis*, but also for the essential role of FLEs in tick homeostasis.

Current evidence on the ecology, biology, and pathogenicity of FLEs is limited, as is evidence on the hosts in which they act as endosymbionts. Therefore, the global aim of this review is to gather state-of-the-art knowledge of FLEs and *Hyalomma* species through a comprehensive analysis of all studies that aim to characterize the endosymbiont–tick relationships, their ecology, and their importance for tick survival, as well as epidemiological evidence based on molecular techniques for species-level identification. Improving our knowledge on these species and their relationship with *Hyalomma* ticks could prove to be highly beneficial for developing strategies to prevent and control tick-borne diseases and new emerging pathogens.

2. The Increase in Ticks and Tick-Borne Diseases in the Era of Climate Change

In recent years, there has been an increase in the population of these ectoparasites, possibly as a result of the modification of the environmental biomass caused by humans, but also due to climate change and global warming, the altered migration patterns of birds, as well as human migrations due to wars and geopolitical conflicts, among others [3,6–9].

There are rising reports that advocate for the northward spread of thermophilic ixodid species, such as *Hyalomma*. In this particular case, there have been many studies that evidence the dwindling success of these Afro-Mediterranean tick species in continental climates in Europe, and with contact with animals and humans.

For example, early in 2012, an adult case of *H. marginatum rufipes* was reported on cattle under a continental climate in Hungary [10]. Since then, many other studies have also identified other *Hyalomma* species in animals and humans in Hungary [11], the Netherlands [12], and Germany [13]; in cattle of Corsica in France [14]; in an English horse [15]; and in a migrant in Malta [6]. Furthermore, the first human exposure to a locally acquired adult *H. marginatum* was recently described in England in 2021 [16]. Most of these reports have detected the successful molting of these *Hyalomma* species in newly colonized regions of Europe.

Both climate change and other human activities (deforestation, urbanization, and international travel) [17] play a decisive role in the increase in infectious pathogens and their transmission vectors. In a recent study, the impact of climate trends in the distribution of *H. marginatum* was explored. The authors generated annual models of environmental suitability for the tick in the period 1970–2018, using harmonic regression-derived data of the daily maximum and minimum temperature, soil moisture, and water vapor deficit. It was concluded that climate change could create new areas in Europe with suitable climates for *H. marginatum*, while keeping its "historical" distribution in the Mediterranean [7]. Moreover, other works have discussed the importance of migratory birds in the international dispersion of *Hyalomma* ticks. They may play an important role in the changing climatic and environmental conditions in Europe because birds that cover long distances over a short time and stay temporarily in different habitats in the context of climate change can introduce tick and pathogen species in areas where they have never been [8].

This appearance of vectors and zoonotic infectious diseases in areas where no disease had been previously reported so far [2,18] reaffirms the prompt needed for further strategies for dispersion control, especially considering both the impact of tick-borne diseases and also their potential social and economic impact [1].

3. The Impact of Ticks of the Genus *Hyalomma* on Human and Animal Health

The genus *Hyalomma* is included in the family *Ixodidae* and is considered one of the main transmission vectors of the CCHF virus in humans and theileriosis in cattle. This genus comprises more than 20 species, mainly distributed across three continents: Europe, Asia, and Africa [19] (Table 1). Up to half of them are capable of transmitting disease-causing pathogens, and due to their adaptability, they are usually found in tropical or subtropical areas, mainly where arid climates predominate [20]. Despite being transmitters of potentially fatal diseases, ticks from this genus are not particularly anthropophilic, and the risk of transmitting diseases to humans is lower than in other genera, such as *Ixodes* or *Dermacentor* [19].

Table 1. Pathogens of human and animal importance transmitted by ticks of the *Hyalomma* species.

Hyalomma Species	Human Pathogens	Animal Pathogens	Continent	Reference
H. aegyptium	− - *Anaplasma phagocitophylum* − - *Ehrlichia canis* − - *Coxiella burnetti* − - *Rickettsia aeschlimannii* − - *Rickettsia africae* − - *Borrelia turcica* − - *Borrelia burgdoferi* − - *Leishmania infantum*	− *Theileria annulata* − *Hepatozoon kisrae* − *Hemolivia mauritanica* − *Theileria lestoquardi* − *Candidatus* − *Midichloria mitochondrii*	Africa Europe	[21,22]
H. albiparmatum	− - *R.conorii*		Africa	[23,24]
H. anatolicum	− - Crimea–Congo hemorrhagic fever (CCHF) virus − - Virus Kadam − - Virus Kundal − - Virus Karyana − - *Rickettsia* spp. − *Ehrlichia* spp. − *Anaplasma* spp. − *Babesia* spp.	− *Theileria annulata* − *Theileria equi* − *Theileria lestoquardi* − *Theileria ovis*	Africa Asia Europe	[25,26]
H. arabica			Asia	[27]
H. brevipunctata	− *Anaplasma marginale* − *Babesia bigemina* − *Bhanja virus*		Asia	[25]
H. detritum	− *Rickettsia aeschlimannii* − *Theileria annulata*		Africa Europe	[28]
H. dromedarii	− CCHF virus − Dhori virus − Kadam virus − Sindilbis virus − Chick Ross virus − Thogoto virus − Bhanja virus − *Coxiella burnetti* − *Rickettsia aeschlimannii* − *Rickettsia rickettsii* − *Babesia* spp.	− *Theileria annulata* − *Theileria camelensis* − *Coxiella burnetti*	África Asia Europa	[29]
H. erythraeum o H. somaliticum			Africa Asia Europe	[30]

Table 1. *Cont.*

Hyalomma Species	Human Pathogens		Animal Pathogens		Continent	Reference
H. excavatum	–	*Rickettsia aeschlimannii*	– – – –	*Theileria lestoquardi* *Anaplasma marginale* *Anaplasma centrale* *Theileria annulata*	Africa Europe	[19]
H. franchinii					Europe	[23]
H. husainii					Asia	[25]
H. impeltatum	– – – –	CCHF virus Dhori virus *Rickettsia aeschlimannii* *Rickettsia africae*	– –	*Babesia ocultans* *Theileria lestoquardi*	Africa Asia Europe	[30,31]
H. impressum			–	*Babesia ocultans*	Africa	[25]
H. kumari						[32]
H. lussitanicum	– – – – –	*Rickettsia* spp. *Anaplasma phagocytophilum* *Borrelia* *Coxiella burnetii* *Francisella tularensis*			Europe	[33,34]
H. marginatum	– – – – – – – –	CCHF virus Dhori virus West Nile virus Bhanja virus *Rickettssia aeschlimannii* *Rickettsia sibirica* *Rickettssia africae* Thogoto virus	– –	*Babesia* spp. *Theileria annulata*	Africa Asia Europe	[16,35,36]
H. nitidum	–	CCHF virus			Africa	[24]
H. punctata					Africa Asia	[37]
H. rhipicephaloides					Africa Asia	[37]
H. rufipes	– – – –	Thogoto virus CCHF virus *Rickettssia aeschlimannii* *R.conorii*	–	*Babesia ocultans*	Africa Asia Europe	[6,38]
H. schulzei	–	Dhori virus	– – –	*Anaplasma ovis* *Anaplasma marginale* *Ehrlichia ewingii*	Asia	[39]
H. scupense	–	*Coxiella burnetii*	–	*Theileria annulata*	Africa Asia Europe	[40,41]
H. truncatum	– – – – – – –	CCHF virus Bhanja virus Rift Valley fever virus *Coxiella burnetti* *Rickettsia aeschlimannii* *R. sibirica* *R. conorii*	– –	Venezuelan equine encephalitis Rift Valley fever virus	Africa Asia Europe	[24,42]
H. turanicum	– –	CCHF virus *R. sibirica*	– – – –	*B. ocultans* *B. caballi* Venezuelan equine encephalitis Rift Valley fever virus	Africa Asia Europe	[38]

Firstly, *Hyalomma aegyptum* constitutes one of the best known species within the genus. This species of tick is abundantly distributed throughout the eastern parts of European territory and the northern parts of the African continent. Characteristically, it has been seen to use camels and tortoises of the genus *Testudo* as hosts [21]. In a study conducted in Qatar, the most frequently identified pathogen in these latter hosts was *Hemolivia mauritanica*.

This justifies the need to increase control over species such as *Testudo* tortoises in order to reduce the spread of infectious diseases caused by H. mauritanica, *Candidatus Midichloria mitochondrii*, or *Ehrlichia* spp. [22]. This same study shows that *H. dromedarii* uses the same intermediate hosts as *H. aegyptum* and is also one of the main vectors of the transmission of Q fever and theileriosis among camels in North Africa [29].

Secondly, *H. albiparmatum, H. nitidum*, and *H. truncatum* are morphologically very similar species that are differentiated by the identification and comparison of somatic primary or secondary sexual characteristics that they develop throughout adult life [23]. Both *H. albiparmatum* and *H. nitidum* extend through the central areas of the African continent (Senegal, Kenya, and the Democratic Republic of Congo), while *H. truncatum* has been found to be widely distributed [24].

H. brevipunctata, together with *H. hussaini, H. anatolicum, H. dromedarii,* and *H. marginatum*, uses the buffalo of northern India as an animal host to transmit diseases such as babesiosis [25]. Babesia has also been identified in other species such as *H. rufipes, H. impressum, H. truncatum, H. marginatum*, and *H. impeltatum*. It is worth mentioning that *H. brevipunctata* is one of the most virulent species, capable of causing otoacariasis and tick paralysis in humans [19]. Surprisingly, despite the fact that *H. marginatum* and *H. impeltatum* act as multi-pathogen reservoirs and have a higher prevalence around the world, it is *H. rufipes* that has the greatest participation and influence in the spread of this disease among Nigerian cattle [38].

H. arabica is the most abundant tick species in the *Hyalomma* genus in Saudi Arabia. It is restricted to the western parts of this country and the Al-Sarawat mountain range in Yemen, and sheep are its main host [27]. It is the closest phylogenetic species to *H. kumari*, which ranges from India to western Iran [32].

H. eryathareum has been taxonomically redefined as *H. somaliticum*. Along with *H. impelatum*, both are included in the *H. asiaticum* group [30]. *H. somaliticum* is one of the main vectors of bovine theileriosis in rural areas of Saudi Arabia [31], whilst *H. detritum* is one of the main vectors in parts of North Africa, such as Morocco [28].

H. franchinii is one of eight tick species of the *Ixodes* group that are restricted to the Mediterranean basin [23]. Along with *H. lussitanicum*, it is one of the species undergoing an explosive population increase and has been identified in Spain as a transmitter of the CCHF virus [33]. Up to 80% of the ticks identified in the Iberian Peninsula belong to the species *H. lusitanum*. Furthermore, *Francisella* were the most common genera in *H. anatolicum*, possibly indicating that some pathogenic species such as *F. tularensis* may be present in the animal population parasitized by this tick species [26].

H. marginatum is one of the most widespread species worldwide. It is the main known vector of the CCHF virus, and, in recent years, its prevalence has increased in areas of northern Europe such as Sweden [35], England [16], and the Czech Republic [36]. Likewise, *H. rufipes* has also recently increased since it was unexpectedly found for the first time in Malta in 2020 [6]. Their increasing incidence evidences the prompt need to epidemiologically control and develop screening strategies for tick-borne infectious diseases in Northern Europe.

H. punctata is predominantly distributed in northern Somalia and Ethiopia, while *H. rhipicephaloides* has been primarily identified round the Dead Sea. Both types of ticks hav goats and gazelles as hosts [37]. *H. schulzei* is known to be one of the leading species in the transmission of ehrlichiosis and anaplasmosis among cattle on the border between Iran and Pakistan. It poses a serious problem for the economy of rural parts of the country, given that livestock accounts for their principal income source [39].

H. scupense, along with *H. anatolicum*, stands out as one of the main vectors of theileriosis transmission in Africa [41]. It is mainly distributed in the African Maghreb area [40]. *Theileriosis* is a disease caused by the apicomplexan protist of the genus *Theileria*. They are obligatory intracellular parasites that use cattle as their main host. The two best known species are *T. parva*, which causes East Coast fever, and *T. annulata*, which causes tropical theileriosis. Only *T. annulata, T. lestoquardi, T. ovis*, and *T. separata* have been identified

in ticks of the *Hyalomma* genus. The East Coast fever is characterized by symptoms of generalized lymphadenopathy, fever, anorexia, the appearance of petechiae or ecchymosis on mucous membranes, and neurological signs. Tropical theileriosis also causes the destruction of red blood cells and therefore also causes jaundice, anemia, and hemoglobinuria [43]. In recent years, various strategies have been developed for the control of theileriosis in African cattle. These include vaccination with inactivated *T. annulata* vaccines or with *H. scupense* antigens, which have been associated with a reduction in tropical theileriosis in North Africa [44] and China [45].

Finally, *H. turanicum* and *H. truncatum* are two species mainly implicated in the spread of the Rift Valley fever virus and the CCHF virus, and the horizontal and vertical transmission of these agents have been previously described. They are distributed worldwide and have been reported as one of the main species that cause this disease in sheep and humans [42].

4. Delving into the Microbiome of Ticks

Since the early 20th century, an association between ticks and non-pathogenic bacteria has been known to exist [46]. Beyond those primitive reports by means of simple histological techniques and light microscopy, currently, molecular and next-generation sequencing methodologies have emerged as important tools to characterize tick microbiome compositions [47]. For some years now, efforts have been focused on determining the role of the microbiome of vector arthropods in pathogen acquisition and transmission, in the life cycle of the host, and its impact on its biology and ecology, but also on delving into both biotic and abiotic factors that model its composition. This growing interest lies in the importance of its understanding in the context of arthropod survival and pathogen transmission for the development of a new generation of arthropod and arthropod-borne pathogen control strategies [46].

Many studies have shown that ticks are closely associated with both pathogenic and non-pathogenic bacterial symbionts, which are tightly implicated in host nutritional adaptation, survival, fitness, vectorial competence, immunity, and reproduction, among others [5,48–50]. These bacteria are hence called endosymbionts and can either be obligatory or facultative, depending on their indispensable role in tick physiology [1]. Obligatory endosymbionts are necessary for the tick's survival, and their depletion can lead to host death; meanwhile, facultative endosymbionts are not obligatory for tick survival but aid in several physiological processes. Almost all endosymbionts are vertically transmitted by the female germ line through the transovarial route [51]. However, evidence on the horizontal transmission of tick symbionts from the genera *Midichloria, Coxiella,* and *Arsenophonus* has also been reported [1,52–54].

Traditionally, the contribution of tick microbiota was assumed to be limited to providing a source of vitamin B thanks to the presence of vitamin B synthesis genes in the symbionts of the *Coxiella* or *Francisella* genera to compensate for its deficiency in the blood [55]. Nevertheless, many genera and species of non-pathogenic bacterial endosymbionts or commensals have been implicated in a wide range of important functions. *Coxiella*-like endosymbionts (CLEs) have been implicated in nutrient synthesis [56], the regulation of feeding activity [57], and reproductive fitness in females [58]. *Rickettsia* symbionts play a vital role in tick physiology, fitness, population dynamics, and pathogen transmission [1]. FLEs are important for tick growth and essential for tick nutrition and their life cycle [59]. *Wolbachia* bacteria are implicated in reproductive functions, vectorial competence, and defense mechanisms [1,60] *Midichloria* endosymbionts are involved in nutrient biosynthesis and play a role in aiding antioxidative defense and hydric balance [61]. Other genera are also present, yet with largely unknown implications, such as *Spiroplasma* and *Arsenophonus*, or even *Escherichia coli, Staphylococcus, Enterococcus,* and *Lysinibacillus*, among others [5].

5. What We Know about *Francisella*-like Endosymbionts

FLEs are facultative intracellular Gram-negative coccobacilli that may be obligatory symbionts of several species of ticks and hematophagous arthropods [62]. *Francisella* species are almost ubiquitous and are present in many ecosystems and hosts, such as lagomorphs, rodents, insectivores, carnivores, ungulates, marsupials, birds, amphibians, and (as stated) invertebrates [1]. The best known species of the genus is *Francisella tularensis*, the highly infectious and zoonotic pathogen that causes tularemia, a potentially fatal disease if untreated. *F. tularensis* as well as other subspecies and even FLEs share a high average nucleotide identity that exceeds 98% similarity [63].

Many species of *Francisella* find their reservoir in several tissues of ticks, such as reproductive tissues, which facilitates transovarial transmission [19,64,65], but also in salivary glands, where they may be transmitted through tick bites [1,66,67]. Furthermore, trans-stadial transmission has also been confirmed [68].

Whilst FLEs are limitedly pathogenic in small animals, it is thought that FLEs are not pathogenic for humans [69]. However, they are frequently found in many genera of human-biting ticks, such as *Amblyomma, Dermacentor*, and *Ixodes*. *Hyalomma* ticks are one of the main reservoirs for these endosymbionts. The lack of a proper species or even subspecies-level identification limits the information on the pathogenicity of FLEs in humans. This is problematic as there are some reports on the potentially virulent FLEs in smaller mammals [69], and identification limitations may restrict our understanding of these species.

6. The Ecological Importance of *Francisella*-like Endosymbionts in Ticks as Zoonotic Vectors

The development of new molecular techniques for the study of arthropod-associated microbiota has opened up a whole new field of research, not only in entomology or microbiology, but also for public health and infectious disease scientists. The study of symbiotic relationships and interactions between ticks and their tissue microbiome opens up new opportunities for disease control, as there is a growing body of knowledge on the impact of these symbiotic interactions on host ecology and physiology.

In this regard, an ecological-to-evolutionary continuum has been previously proposed, by which environmental, free-living, and/or host-associated bacteria are able to colonize tick tissues. Later, under certain conditions, they establish a symbiotic relationship with the tick host [1]. After this coevolutionary adaptation, ticks have evolved to harbor two types of endosymbionts, as previously defined: obligatory mutualistic symbionts (mostly vertically transmitted from mother to offspring) [70] and facultative symbionts [1,71] (which participate in reproductive physiology, defense against infections, or thermal adaptation, among others [72]).

Knowledge on both tick-associated pathogens and both types of symbionts is of medical and veterinary importance for the assessment of health risks and strategies. It is also of evolutionary and ecological interest, as many bacteria and their hosts share several million years of evolutionary history. This history leads to a tight interdependence with shared associated risks for obligatory endosymbionts, while facultative microbiota allow for more adaptable host–symbiont interactions [1,71].

To date, FLEs are present in several species of human-biting ticks, such as *Amblyomma, Dermacentor, Hyalomma*, and *Ixodes*, but also in many other species of tick-infesting animals, as detailed in Table 2. Despite the fact that their prevalence has been diversely reported, FLEs are homogeneously distributed in many other hosts, such as dogs, cats, sheep, and cattle, but also in wild animals, including many species of migratory birds, tortoises, and even snakes. Furthermore, they have also been found in isolates from all continents, except Antarctica (Table 2).

Table 2. Current evidence of *Francisella*-like endosymbionts in ticks and their hosts.

Species of Tick	Region	Host or Environment	Reference
Ixodes ricinus and *Dermacentor reticulatus*	Slovakia	Environment	[73]
Rhipicephalus sanguineus, Hyalomma marginatum, Hyalomma, and *Rhipicephalus bursa*	Sardinia	Environment	[74]
Hyalomma marginatum, Hyalomma rufipes, Hyalomma dromedarii, Hyalomma aegyptium, and *Hyalomma excavatum*	Israel	Environment, camels, horses, tortoises, and migratory birds	[62]
Amblyomma maculatum	Oklahoma, USA	Oklahoma State University Tick Rearing Facility (OSUTRF)	[75]
Ornithodoros moubata and *Argus arboreus*	Czech Republic	Laboratory colony	[76]
Rhipicephalus sanguineus	Southern Thailand	Chicken	[77]
Hyalomma marginatum, Hyalomma aegyptium, Rhipicephalus sanguineus, and *Dermacentor reticulatus*	Bulgaria	Human and animal hosts (not specified)	[78]
Amblyomma americanum, Amblyomma maculatum	Georgia, USA	Environment, human, and dogs	[79]
Ixodes ricinus, Ixodes acuminatus, Dermacentor marginatus, Dermacentor reticulatus, Haemaphysalis inermis, Haemaphysalis concinna, and *Haemaphysalis punctata*	Hungary	Environment, common hamsters, and dogs	[80]
Hyalomma lusitanicum	Spain	Environment	[34]
Hyalomma dromedarii	Saudi Arabia	Camels	[81]
Haemaphysalis longicornis and *Hyalomma asiaticum*	China	Environment	[82]
Dermacentor anderson, Dermacentor variabilis, Dermacentor albipictus, Dermacentor occidentalis, Dermacentor hunteri Dermacentor nitens, Amblyomma maculatum, and *Ornithodoros porcinus*	Various origins	Several collections: environment, horses, coyote, sheep, and humans	[83]
Dermacentor variabilis	USA and Canada	Several collections: environment, dogs, humans, raccoons, and cats	[84]
Dermacentor reticulatus	France	Environment	[85]
Hyalomma rufipes	Ethiopia	Environment	[86]
Dermacentor reticulatus	Portugal	Environment, wolves, and dogs	[87]
Amblyomma varanense, Amblyomma helvolum	Thailand	Snakes	[88]
Amblyomma dubitatum, Dermacentor nitens, and *Rhipicephalus microplus*	Brazil	Horses, cattle, and dogs	[89]
Dermacentor andersoni and *Dermacentor variabilis*	Canada	Environment, humans, dogs, horses, skunks, raccoons, deers, mice, and voles	[90]
Haemaphysalis flava and *Haemaphysalis phasiana*	Korea	Environment	[91]
Amblyomma americanum	USA	Laboratory from OSUTRF and environment	[92]
Dermacentor marginatus and *Dermacentor reticulatus*	Italy	Environment	[93]
Haemaphysalis doenitzi	China	Environment and rabbit	[94]
Dermacentor albipictus	Minnesota (USA)	White-tailed deer	[95]
Hyalomma lusitanicum, Dermacentor reticulatus, and *Ixodes hexagonus*	Spain and Portugal	Environment	[96]
Hyalomma anatolicum, Hyalomma hussaini, Hyalomma scupense, Rhipicephalus microplus, and *Rhipicephalus annulatus*	Pakistan	Buffalo and cattle	[97]
Dermacentor albipictus	Alberta and Canada	Environment	[98]
Hyalomma dromedarii	Egypt	Camels	[99]
Hyalomma rufipes	Spain, Greece, and Israel	Migratory birds	[100]
Dermacentor reticulatus and *Ixodes ricinus*	Poland	Environment	[101]

Table 2. *Cont.*

Species of Tick	Region	Host or Environment	Reference
Rhipicephalus bursa, Rhipicephalus sanguineus, Hyalomma detritum, Hyalomma marginatum, Hyalomma lusitanicum, and *Ixodes ricinus*	Algeria	Environment and cattle	
Rhipicephalus microplus	Galapagos Islands	Cattle	[102]
Hyalomma rufipes	Italy	Migratory birds	[103]
Dermacentor reticulatus and *Haemaphysalis punctata*	France	Environment	[104]
Amblyomma maculatum	Mississippi, USA	Environment	[105]
Hyalomma dromedarii	Palestine	Camels	[106]
Hyalomma marginatum, Hyalomma rufipes, Hyalomma aegyptium, Rhipicephalus sanguineus, Dermacentor occidentalis, and *Dermacentor variabilis*	Anatolia	Environment, dogs, cattle, and goats	[107]
Amblyomma maculatum	Mississippi, USA	Environment	[108]
Ixodes ricinus	Serbia	Humans	[109]
Dermacentor reticulatus	Serbia	Environment	[110]
Dermacentor reticulatus	England, Wales, Belgium, Germany, Netherlands	Environment	[111]
Amblyomma latum	South Africa	Spitting cobra	[112]
Amblyomma tuberculatum	Mississippi, USA	Gopher tortoise	[113]
Dermacentor marginatu and *Dermacentor reticulatus*	Germany	Environment	[114]
Dermacentor andersoni	Canada	Environment and squirrels	[115]
Dermacentor occidentalis	California, USA	Environment	[116]
Dermacentor albipictus	Alberta, Canada	Whitetail and mule deers	

Several works have been devoted to understanding their role in tick physiology. FLEs have been identified as obligatory mutualists in the life cycle of the African hut tampan or *Ornithodoros moubata* by providing B vitamins absent in the blood meal of the tick [1,59]. On the one hand, one *Francisella* type, defined as F-Om by Duron et al., was shown to be maternally transmitted to all maturing ticks' oocytes, and its experimental elimination ended up causing alteration of tick life history traits and physical abnormalities, which were cured by giving oral supplementation of B vitamins to experimental specimens of *O. moubata* [59]. On the other hand, another FLE, termed FLE-Am by Gerhart et al. (2016) [75], was shown to have a more extensive metabolic capacity and biosynthetic capability than CLEs in the Gulf Coast tick or *Amblyomma maculatum*, producing heme, cysteine, tyrosine, tryptophan, phenylalanine, or serine among other cofactors also present in CLE [75].

In both cases, genomic analyses have shown that FLEs likely evolved from a pathogenic strain of *Francisella* that recently transitioned to an endosymbiotic lifestyle as observed in other *Francisella* symbionts both related and not related to ticks [59,76,117]. This fact becomes even more interesting, especially considering that the available molecular evidence suggests that the ancestral *Francisella* species originated in a marine habitat [117]. Further evidence is needed in other species of ticks to keep unraveling their physiological and ecological roles.

7. The Role of Francisella-like Endosymbionts in the Ecology of Ticks of the Genus *Hyalomma*

Due to upcoming evidence, several studies have delved into the analysis of the prevalence and role of FLEs associated with different species of *Hyalomma* worldwide, as well as their associations with other pathogens (Table 3).

Table 3. The epidemiology of *Francisella*-like endosymbionts in *Hyalomma* tick species.

Region	Reference	Total Number of Collected Ticks	*Hyalomma* Ticks	FLE Prevalence
Anatolia (Turkey)	[107]	280	*H. aegyptum* *H. marginatum* *H. excavatum*	25% of the total
Israel	[62]	310	*H.marginatum* *H. excavatum* *H. dromedarii,* *H. aegyptium* *H. rufipes*	90.66% of the total 84.6% *H. marginatum* 90.5% *H.* excavatum 89.8% *H. dromedarii,* 100% *H. aegyptium,* 90.4% *H. rufipes*
Argelia	[101]	235	41 *H. detritum* 15 *H. marginatum* 4 *H. lusitanicum*	88.5% of the total 90.2% *H. detritum* 80% *H. marginatum* 100% *H. lusitanicum*
Bulgaria	[78]	472	*H. marginatum marginatum* (5.9%; *n* = 28) *H. aegyptium* (0.2%; *n* = 1).	2.5% of the total
China	[82]	671	*H. asiaticum (n 88, 13%)*	13% of the total
Italy	[74]	236	*H. marginatum* (8, 3%) *H. lusitanicum* (1, 1%)	1.7% of the total
Turkey	[118]	1115	245 *H. marginatum marginatum* 106 *H. anatolicum anatolicum* 53 *H. anatolicum excavatum* 68 *H. detritum detritum,*	0%
Pakistan	[97]	234	212 *Hyalomma anatolicum* 2 *H. hussaini* 1 *H. scupense*	91.5% of the total 96.2% *H. anatolicum* 0.45% *H. hussaini* 0% *H. scupense*
Occidental Paleartic region	[100]	575	*H. rufipes* *H. marginatum*	77% of the total *H. rufipes*: 76.7% *H. marginatum*: 75%
Ethiopia	[86]	296	*H. rufipes* (1/296)	0,34% of the total *H. rufipes* 0.34%
Egypt	[99]	319	*H. dromedarii* (249/319)	6% of the total 14.94% *H. dromedarii*
Saudi Arabia	[81]	151	*H. dromedari* (148/151) *H. impeltatum* (2/151) *Hyalomma* spp. (1/151)	98.01% of the total *H. dromedari* 100%
Cáceres (Spain)	[34]	48	*H. lusitanicum* (48/48)	52–99% of the total

In a study conducted in the Anatolian region, 280 ticks from 10 different species were analyzed using next-generation sequencing in order to expand current information on tick-associated bacteria and protozoans [107]. Within the 10 selected species, 3 species of the genus *Hyalomma* (*H. aegyptum, H. marginatum*, and *H. excavatum*) were included. Endosymbiont microorganisms were identified in 65% of ticks. The most frequently isolated genus was *Coxiella* spp. (40%), followed by *Francisella* spp. (25%) and *Rickettsia* spp. (17.5%). In their work, most FLEs were associated with the genus *Hyalomma*, while CLEs seemed to be linked with the genus Rickettsia. According to metagenomic sequencing data, these *Francisella* species were different from *F. tularensis*, as has been systematically reported in other similar studies [81,86,99,118]. Furthermore, the FLE sequences formed phylogenetically distinct clusters with a 98% genetic similarity score, which were associated with their tick hosts, suggesting differential evolutionary patterns in various hosts and ecological niches.

These data are supported by the evidence provided by the work of Wang et al. (2018) [84] carried out in China. After analyzing a total of 627 *Haemaphysalis longicornis* and 88 *H. asiaticum* ticks, FLEs were detected in 100% of *Hyalomma* ticks, but in less than 10% of *H. longicornis* individuals. Phylogenetic analysis indicates that the sequences

of FLEs isolated in *H. asiaticum* and *H. longicornis* from three regions of northern China shared a consistent 16S rRNA sequence and differed from known FLEs, suggesting genetic heterogeneity in the group, perhaps allowing adaptations to different ecosystems, as in this case of the cold parts of northern China.

However, contrary to the work by Brinkmann and colleagues (2019) [107], other studies have suggested that the evolution of FLEs could be independent of their tick hosts. In a study carried out in Bulgaria [78], it was revealed that the genetic sequences of FLEs in H. m. marginatum and *R. sanguineus* were identical. Moreover, the identification of two taxa of FLEs was reduced to a fraction of ticks from the same region, which seemed to be facultative secondary endosymbionts of ticks that can adapt to distinct ecological niches and environmental characteristics.

This hypothesis was also posed in an article published in Italy [74], in which the *Francisella* sequences detected in the study were phylogenetically close to others detected in *H. marginatum* and *H. rufipes* from Turkey [118] and Ethiopia [86]. Likewise, the results published by Díaz-Sánchez and colleagues (2021) [34] show that the FLEs identified in *H. lusitanicum* showed similarities of around 97–99% with other FLEs from *H. marginatum*, *H. truncatum,* or *Dermacentor auratus*, but not with the pathogenic species *F. tularensis*. Moreover, in the interesting work conducted by Azagi et al. (2017) [62], it was reported that the FLEs and *Hyalomma* phylogenies show obvious similarities. Through a Procrustes approach to co-phylogeny (PACo) analysis, the apparent dependence of the symbiont phylogeny on the host phylogeny appeared unlikely to be coincidental.

In a study by Szigeti et al. (2014) [86] in Ethiopia, 296 ticks were collected for the genetic sequencing of *F. tularensis* and FLEs. FLEs were found to cross-react with *F. tularensis* in basic screening tests. They proposed that the characterization of the tu4 gene could be useful to discern between FLEs and *F. tularensis*. However, one must take into account that the genome of FLEs includes pseudogenes and inactivated virulence versions of *F. tularensis* genes, suggesting that both endosymbionts and tularemia-causing pathogens have a common ancestor, as stated by Hoffman et al. (2022) [100]. Of the 296 ticks collected, only 1 belonged to the genus *Hyalomma* (*H. rufipes*), which represents a prevalence of 0.34% of the total collection. This prevalence was substantially lower than that reported in articles from North America and Eurasia, but also in Africa. In fact, in a study carried out in Egypt [99], 319 ticks were isolated, of which 249 belonged to the genus *H. dromedarii*. The prevalence of FLEs obtained was 6% within the genus *Hyalomma*, significantly lower than the 100% observed in China [82], but higher than those of Turkey (0%) [118] or Bulgaria (2.5%) [78].

One of the most important concerns regarding FLEs in *Hyalomma* ticks is their participation in the coinfection with pathogens of the genus Rickettsia [62].

Hoffman and colleagues [101] documented the coexistence between *Francisella*- and *Rickettsiae*-transmitting avian hemorrhagic fevers in tick-infecting birds from the Palearctic region of West Africa. The results showed a coinfection rate of 47.1% (271/575) of all selected ticks. Within the genus *Hyalomma*, the prevalence of the coinfection was 48.8% (235/482). Depending on the species, the following results were obtained: *H. rufipes*—50.6% (226/447) and *H. marginatum*—29.2% (7/24). This suggests that the presence of FLEs is not a protective factor against infection by other pathogens, but on the contrary, it may favor the presence of potentially virulent microorganisms. In contrast to these results, in a study published in Algeria, the highest rate of coinfection was identified within the genus *Ixodes* (12/13, 92.3%), followed by *Hyalomma* (91/113, 80.5%) and *Rhipicephalus* (39/109, 35.7%) [101].

Regarding coinfections with other pathogens, in a study carried out in Pakistan [97], it was found that the most frequent coinfection was between FLEs and *Piroplasma* species (15.8%, 37/234), followed by the coexistence of FLEs and *Ehrlichia* species (9.4%). The most frequent triple coinfection consisted of FLE, *Piroplasma*, and *Ehrlichia* spp. (6.8%, 16/234). These coinfections pose a significant risk, especially in veterinary medicine, not only due to the cumulative pathogenic effects of different species, but also due to

the increased complexity of the targeted treatment and management of life-threatening diseases. Understanding this symbiosis between ticks, endosymbionts, and other pathogens is crucial to establishing sanitary control strategies, as the interaction between pathogenic and non-pathogenic microorganisms can affect the host's susceptibility to other bacterial infections.

Finally, the genome of FLEs identified in *H. marginatum* includes genes that participate in the metabolic pathways of folate and riboflavin synthesis, while it lacks genes involved in biotin synthesis [119]. Its authors maintain that this biotin synthesis deficit is compensated by the presence of *Midichloria* in such a way that the coexistence between FLEs and *Midichloria* is a necessary endosymbiosis to meet the vitamin requirements for the host. As is the case in *H. marginatum*, the coexistence of these two endosymbionts has been demonstrated in the rest of the species of the genus *Hyalomma*, except in *H. asiaticum*, which contains a FLE that is capable of autonomously synthesizing biotin [119].

8. Discrimination between *Francisella tularensis* and *Francisella*-like Endosymbionts in Clinical Practice

Tularemia is a potentially fatal disease, and *F. tularensis* is a highly infectious microorganism that is considered a category A agent of bioterrorism [120,121]. However, there is still little data available on the implication of ticks in its transmission, and some studies have suggested that epidemiological studies that aimed to evaluate the prevalence of *F. tularensis* in ticks are strongly required to better understand their role in the transmission of this pathogen [63,85].

Outside the context of wide experimental studies, in the clinical practice of units of infectious diseases and clinical microbiology laboratories, patients are referred after a tick bite with or without the clinical suspicion of infectious disease and a specimen is provided for analysis. This provides an opportunity to perform molecular testing of ticks possibly implicated in disease and also in clinical practice. However, this has crucial implications for public health.

As has been previously described, the genomes of *Francisella* species are highly conserved, and FLEs are closely related to clinically important *F. tularesis* strains, as well as to other species of the genus. With most of the currently available molecular detection techniques based on multiplex panels of PCR, FLEs have been shown to cross-react with *F. tularensis*, leading to misidentification and misinterpretation [85]. This fact is associated with the erroneous activation of public health alarm systems, leading to an unsuitable waste of resources and the assumption that *F. tularensis* strains are present in clinically detected tick specimens that are only colonized by FLEs. Therefore, an accurate discrimination between *F. tularensis* and FLEs is critical, and the interpretation of the results must be reassessed by trained professionals who understand the ecology of ticks and the implication of endosymbionts in their ecology. Furthermore, the used detection methods need to be carefully evaluated to avoid any inaccurate interpretation of epidemiological surveys [85], and positive results in the commercial PCR panels (including tularemia, among other zoonoses) must be further confirmed by molecular techniques that can effectively discriminate between *F. tularensis* and FLEs.

Several studies have been devoted to analyzing and comparing molecular techniques for adequate identification and typing via real-time PCR [85] and differential insertion sequence amplification [63]. These techniques provide new capabilities for epidemiological investigations and characterizations of tularemia source outbreaks and for the discrimination of cross-reactivity in screening molecular panels. Hence, in the presence and suspicion of a positive result for *F. tularensis* in the molecular analyses of ticks in clinical practice, the sample must be adequately interpreted and sent to a microbiology reference lab for confirmation, and ideally for sequencing and typing, in order to characterize the *Francisella* species.

9. Endosymbiont-Directed Strategies for the Control of Ticks and Tick-Borne Diseases

Tick-borne diseases continue to rise and disseminate globally. They are becoming one of the more important threats to human and animal health around the world. Owing to the disruptive impacts of human activity in ecosystems, further biological and regional niches of ticks are growing, and hence the outcomes of management and control programs for tick-borne infectious diseases are difficult to predict. Over the last few years, there has been an increase in the geographical range of *Hyalomma* ticks, which can be attributed to the global climate changes that affect pathogens, vectors, and their hosts (mainly migratory birds) [7–9]. This may increase the incidence and the spread of emerging tick-borne diseases around the world [7–9]. Tick control via the application of acaricides has been the centerpiece of control strategies [122]. However, due to their immediate effect and cost-effective profile, the intensive and repeated use of acaricides has led to the appearance of acaricide-resistant ticks.

Due to these limitations, as well as the complex, and, in many cases, unknown biology and life cycle of ticks, the presence of two or more intermediate hosts for some species, and epidemiological changes, due to climate change among other things, multidisciplinary approaches are urgently needed. Since the 1990s, multimodal interventions, including the application of acaricides [123], autocidal control [124], the improved management of wildlife hosts [125], and especially vaccination [126–129], have proven to be effective.

Targeting vector microbiota with different strategies has been the foundation for several treatments and control measures for arthropod-borne diseases [126], as well as nematode infections such as animal and human filariasis. For instance, in human onchocerciasis and lymphatic filariasis caused by *Onchocerca volvulus* and *Wurchereria bancrofti*, respectively, the elimination of the endosymbiont *Wolbachia* with the use of doxycycline as an anti-*Wolbachia* antibiotic leads to the death of adult parasitic worms. Contrary to other anthelmintic therapies, they are readily available, cheap, and safe to use in adult patients and in individuals with other parasitic infections such as loiasis [130].

However, anti-microbiota vaccines have recently emerged as a promising tool for the control of ticks and tick-borne infections [1]. During feeding, ticks take up functional host plasma antibodies which may interact with their endosymbionts [128,129,131,132]. The utilization of vaccines that target endosymbionts to control tick populations shows that modifications in their microbiome can alter essential physiological functions, especially if the intervention is effective against obligatory endosymbionts, as previously discussed. Furthermore, several authors have emphasized that new vaccine targets should encompass proteins of the tick immune response involved in the tolerance of microbial populations [131,133].

Despite the fact that these anti-microbiota vaccines are still in their infancy, immunization with keystone tick-associated *Enterobacteriaceae* caused significant tick mortality. In this study, the vaccination of mice with a live vaccine containing *Escherichia coli* showed that the production of anti-*E. coli* and anti-α-Gal IgM and IgG (glycan present in tick bacterial microbiota) was associated with the high mortality rates of *I. ricinus* nymphs during feeding [128].

The use of microbiota-directed antibiotics has been shown to affect their bacterial symbionts and is associated with a major negative impact on both tick competence and survival [134]. Nevertheless, this approach is limited due to the risk associated with the emergence of antibiotic resistance, a worldwide emerging menace for healthcare systems, human and animal health, the ecosystem, and the food industry, as well as alterations in human and animal microbiomes [1].

Finally, the genetic manipulation of endosymbionts has also been proposed as a possible approach that is used to control vectors and vector-borne diseases [135]. Genetically transformed symbiotic bacteria are able to express molecules with antiparasitic activity may alter the arthropod's ability to transmit pathogens. This strategy, known as paratransgenesis, has shown promise in controlling the transmission of *Trypanosoma cruzi* by *Rhodnius prolixus* [136].

10. Conclusions

In this review, we discussed the growing importance of ticks of the genus *Hyalomma*, their associated tick-borne human and animal diseases in the era of climate change, as well as the role of the microbiome and FLEs in their ecology. Global current evidence on FLEs in *Hyalomma* species has been compiled, and the impact of new molecular techniques in the study of the tick microbiome (both in research and in clinical practice) has been examined. Lastly, we also discussed different endosymbiont-directed strategies for the control of tick populations and tick-borne diseases, providing insights into new evidence-based opportunities for the future.

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