Chapter

Rickettsial Endosymbionts of Ticks

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Abstract

Rickettsiae are widely known to be human bacterial pathogens transmitted by blood-sucking ectoparasites, such as ticks, fleas, and lice. However, most rickettsial species are nonpathogenic endosymbionts with various groups of organisms, such as arthropods, protists, and other eukaryotes. While attention has been given to rickettsial endosymbionts of insects, rickettsial endosymbionts of ticks have been less well studied. Tick hosts are found across the phylogeny of *Rickettsiae*; hence, the tick was the most probable ancestral host of *Rickettsiae* associated with arthropods. Here, we focus on rickettsial endosymbionts of ticks, describing their role in association with ticks and comparing them to tick-borne vertebrate pathogens.

Keywords: *Rickettsia*, endosymbiont, tick, host-parasite interactions, tick microbiome, tick-borne pathogens, tick-borne diseases

1. Introduction

Rickettsiae are human bacterial pathogens transmitted by blood-sucking ectoparasites, such as ticks, fleas, and lice. Many studies have shown that rickettsioses belong to the oldest known zoonoses. Since they have no pathognomonic signs [1], the association between the disease, the vector, and the causative agent has been described decades apart [2]. With the advent of molecular methods, rickettsial agents are being constantly discovered; however, it is not clear whether these novel tick-borne diseases escaped the attention of physicians or they did not exist [2].

Molecular approaches have also revealed the remarkable diversity of *Rickettsiae* and their host associations ranging from arthropods to plants [3]. Nowadays, the majority of the members of the genus *Rickettsia* are considered nonpathogenic endosymbionts [4]. Multiple serological studies suggest that vertebrates may be possible reservoirs of *Rickettsiae* in nature; however, confirmation of these hypothesized reservoirs requires further study [5]. Nevertheless, in all cases, humans are accidental hosts of tick-borne *Rickettsiae* [6].

While pathogenic *Rickettsiae* have been extensively studied, less attention has been given to nonpathogenic endosymbionts [4, 7]. This chapter presents the current state of knowledge relative to tick rickettsial endosymbionts and focuses on tick-*Rickettsia* interactions and their relationship to tick-borne human pathogens.

2. *Rickettsiae*

2.1 Taxonomy

Rickettsiae are Gram-negative obligately intracellular coccobacilli belonging to the family *Rickettsiaceae* and order *Rickettsiales* in the alpha subdivision of the class *Proteobacteria*. They can be found in the cytoplasm or nucleus of eukaryotic host cells [8].

The term "rickettsia" historically denoted small intracellular bacteria, which could not be identified by cultivation in axenic media due to their obligate intracellular nature [9]. The order *Rickettsiales* contains the families *Rickettsiaceae*, *Bartonellaceae*, and *Anaplasmataceae*. The family *Rickettsiaceae* contains the tribes *Rickettsiae*, *Ehrlichia*, and *Wolbachia*; the tribe *Rickettsiae* includes the genera *Coxiella*, *Rickettsia*, and *Rochalimaea*. The advent of molecular taxonomic methods including *16S rRNA* gene analysis resulted in reclassification of rickettsial taxa, and several genera (e.g., *Coxiella*, *Bartonella*, and *Rochalimaea*) have been removed from the order *Rickettsiales* [10, 11]. Currently, the family *Rickettsiaceae* contains genera *Rickettsia* and *Orientia*. The genus *Rickettsia* traditionally contained two groups of pathogenic *Rickettsiae*: the typhus group and the spotted fever group (SFG). The latter included approximately 20 species, mostly transmitted by ticks. Over several years, a remarkable diversity of *Rickettsiae* in arthropods has been found, which led to a new description of the ancestral group and includes *Rickettsia bellii* and *Rickettsia canadensis*, which are clearly distinct from other *Rickettsiae* [3]. Subsequently, a transitional group containing *Rickettsia felis* and *Rickettsia akari* was established, since these species share molecular features with both the typhus group and the SFG [12]. A recent phylogenetic study, based on whole-genome data, provided a single tree topology that well describes the evolutionary history of the core genome and is, in general, consistent with previous studies [13].

2.2 Endosymbiotic lifestyle of *Rickettsiae*

Rickettsiae are endosymbionts (i.e., organisms living within a host cell), and the level of their dependence on the host is variable. An obligate symbiont is, according to the definition, present in most individuals of a given host species, and the mutualistic relationship is crucial for the survival of both organisms. In such associations, co-cladogenesis between symbiont and host is typical. Facultative symbionts are not essential for the host and vice versa and have variable frequencies of prevalence.

2.3 Host diversity of *Rickettsiae*

A remarkable host diversity has been revealed for *Rickettsiae* [3]. Their common ancestor was presumptively free-living. The estimated transition to an intracellular niche took place 775–525 million years ago. The genus *Rickettsia* appears to have originated more recently, approximately 150 million years ago [3]. Presumably, the primary host for *Rickettsiae* was an arthropod, with some species later shifting to other eukaryotes, such as protists and leeches. It has been estimated that 24% of arthropod species harbor *Rickettsiae* [14].

2.4 *Rickettsiae* associated with ticks

Interestingly, hard tick (Ixodidae) hosts are found across the phylogeny of *Rickettsiae*, and related rickettsial species tend to share related tick host species. This suggests a tick was the most plausible ancestral host for rickettsial species associated with arthropods [14].

Of the approximately 900 known tick species, 81 nonrandomly selected species were tested for the presence of bacterial endosymbionts and 55.6% harbored *Rickettsiae* [15]. The most prevalent endosymbiont in arthropods is *Wolbachia* and in ticks *Coxiella*-LE (*Coxiella*-like endosymbiont) with 52.0 and 60.5% of the known species being infected, respectively. However, these results may be biased by uneven sample collections, e.g., in the study by Weinert et al. [14], the vectors of rickettsial diseases were highly overrepresented, and in the study by Duron et al. [15], the available tick species varied widely.

2.4.1 Perpetuation of Rickettsiae in nature

There are two types of *Rickettsiae* transmission in ticks—vertical and horizontal.

Vertical transmission takes place from female to offspring via egg cytoplasm or from one arthropod stage to another after molting (i.e., from larva to nymph, from nymph to adult) [7, 16]. *Rickettsiae* capable of invading ovarian tissues during oogenesis develop in the interstitial cells of tick ovaries and within oogonia and oocytes. Other tissues of rickettsial endosymbionts of ticks are rarely infected, as reported for *Rickettsia peacockii* and *Rickettsia buchneri* [17]. It has been documented in several pathogenic *Rickettsiae* that bacteria can negatively interfere with tick reproduction. Species reported to use transovarial transmission are shown in **Table 1**.

Horizontal transmission, i.e., transfer among host individuals, may involve several mechanisms. Co-feeding (i.e., several ticks feeding close to each other on the same host individual) seems to be one mode of accidental horizontal transmission of tick rickettsial endosymbionts [18]. Sexual transmission (via copulation) has been reported but probably does not play a significant role in perpetuation of *Rickettsiae* in tick populations.

For successful horizontal transfer of *Rickettsiae* from a vertebrate host under natural conditions, a host must develop rickettsemia with sufficient levels of bacteria in the blood and for a sufficient duration. Since some *Rickettsiae* negatively impact the health of their tick hosts (which is more evident for pathogenic species), a vertebrate host must maintain such *Rickettsiae* in nature (e.g., capybara for *Rickettsia rickettsii* in South America [19]). However, the role of vertebrates in perpetuation of tick-borne *Rickettsiae* remains largely unknown [5].

Rickettsial endosymbionts of ticks are mainly transmitted vertically, while pathogenic Rickettsiae are typically transmitted horizontally [17]. *Occas*ional horizontal transfer allows symbionts to disperse beyond their primary host species, which leads to limited phylogenetic congruence between tick hosts and rickettsial symbionts [14, 20].

2.4.2 Infection of Rickettsiae-free ticks

The initially infected site of a *Rickettsia*-free tick may be the gut when feeding on a *Rickettsiae*-infected vertebrate host [7]. The first interaction with tick cells after *Rickettsiae* ingestion occurs in the midgut, the storage organ [21]. *Rickettsiae* pass through the midgut barrier and escape the ticks' immune response by entering hemocytes present in the hemolymph, then enter the epithelial cells, and replicate. After that, bacteria invade tissues and organs, where they replicate and persist [7].

2.4.3 Strict blood diet of ticks and rickettsial endosymbionts

For decades, it was not fully understood why ticks harbor rickettsial endosymbionts. It was previously suggested that some endosymbionts may manipulate

Species	Pathogenicity	Host species	TOT reported	Ref.
R. aeschlimannii	Spotted fever	*A. variegatum*, *Rhipicephalus* spp., *Hyalomma* spp., *Hae. punctata*	No	[46]
R. africae	African tick bite fever	*Amblyomma* spp., *Rhipicephalus* spp., *Hyalomma* spp.	Yes	[47]
R. amblyommatis	Unknown	*Amblyomma* spp., *Rhipicephalus* spp., *D. nitens*	Yes	[48]
R. argasii	Unknown	*A. dewae*	No	[49]
R. asembonensis	Unknown	*Rh. sanguineus* (mostly associated fleas)	No	[50]
R. asiatica	Unknown	*Ixodes* spp.	No	[51]
R. australis	Queensland tick typhus	*Ixodes* spp.	No	[52]
R. bellii	Unknown	*Amblyomma* spp., *Dermacentor* spp., *Haemaphysalis* spp., *I. loricatus*, *O. concanensis*, *C. capensis*	Yes	[53]
R. buchneri[*]	Unknown	*I. scapularis*	Yes	[17]
R. canadensis	Unknown	*Hae. leporispalustris*	No	[54]
R. conorii subsp. *caspia*	Astrakhan fever	*Rhipicephalus* spp.	No	[55]
R. conorii subsp. *conorii*	Mediterranean spotted fever	*Rhipicephalus* spp., *Haemaphysalis* spp.	Yes	[56]
R. conorii subsp. *indica*	Indian tick typhus	*Rh. sanguineus*	No	[55]
R. conorii subsp. *israelensis*	Israeli spotted fever	*Rh. sanguineus*	Yes	[57]
R. felis	Flea-borne spotted fever	*Hae. flava*, *Rh. sanguineus*, *I. ovatus*, *C. capensis* (mostly associated with fleas)	Yes	[48]
R. gravesii	Unknown	*A. triguttatum*	No	[58]
R. heilongjiangensis	Far Eastern spotted fever	*Haemaphysalis* spp., *D. silvarum*	No	[59]
R. helvetica	Unnamed rickettsiosis	*Ixodes* spp.	Yes	[60]
R. honei	Flinders Island spotted fever, Australian spotted fever (*str. marmionii*)	*B. hydrosauri*, *Ixodes* spp. (str. RB), *Hae. novaeguineae (str. marmionii)*	Yes	[61]
R. hoogstraalii	Unknown	*Haemaphysalis* spp., *Carios* spp., *Arg. persicus*	No	[62]
R. japonica	Japanese spotted fever	*Haemaphysalis* spp., *I. ovatus*, *D. taiwanensis*	Yes	[63]
R. lusitaniae	Unknown	*Ornithodoros* spp.	No	[64]
R. massiliae	Unnamed rickettsiosis	*Rhipicephalus* spp., *I. ricinus*, *Hae. paraleachi*	Yes	[65]
R. monacensis	Spotted fever	*Ixodes* spp.	Yes	[66]

Species	Pathogenicity	Host species	TOT reported	Ref.
R. montanensis	Unknown	*Dermacentor* spp., *A. americanum*	Yes	[48]
R. monteiroi	Unknown	*A. incisum*	No	[67]
R. parkeri	Mild rickettsiosis	*Amblyomma* spp., *D. variabilis*	Yes	[48]
R. peacockii[*]	Unknown	*D. andersoni*	Yes	[17]
R. raoultii	SENLAT	*Dermacentor* spp., *I. ricinus*, *Haemaphysalis* spp., *A. testudinarium*	Yes	[7]
R. rhipicephali	Unknown	*Rhipicephalus* spp., *Dermacentor* spp., *Hae. juxtakochi*	Yes	[7]
R. rickettsii	Rocky Mountain spotted fever (*str. Iowa avirulent*)	*Dermacentor* spp., *Rh. sanguineus*, *Amblyomma* spp., *Hae. leporispalustris*	Yes	[68]
R. sibirica subsp. *mongolitimoniae*	Lymphangitis-associated rickettsiosis	*Hyalomma* spp., *Rh. pusillus*	No	[6]
R. sibirica subsp. *sibirica*	Siberian tick typhus	*Dermacentor* spp., *Haemaphysalis* spp., *I. persulcatus*	Yes?	[7]
R. slovaca	SENLAT	*Dermacentor* spp.	Yes	[69]
R. tamurae	Spotted fever	*A. testudinarium*	No	[70]
R. vini[*]	Unknown	*Ixodes* spp.	Yes	[37]

[*]Obligate endosymbiont.
Abbreviations: A., Amblyomma; Arg., Argas; B., Bothriocroton; D., Dermacentor; H., Hyalomma; Hae., Haemaphysalis; I., Ixodes; O., Ornithodoros; R., Rickettsia; Ref., reference; Rh., Rhipicephalus; SENLAT, scalp eschar and neck lymphadenopathy after a tick bite; spp., species (plural); str., strain; TOT, transovarial transmission.

Table 1.
Valid and published Rickettsial species associated with ticks [71].

reproduction or enable survival in changing environments [22]; however, specific reasons remained unclear until recently.

Some of arthropod endosymbionts became obligate mutualists that adapted to host specialization to a restricted diet, e.g., blood or plant sap [22]. It had been found that the rickettsial endosymbiont of *Ixodes scapularis*, *R. buchneri*, was presented only in females of this tick species. As males do not feed with blood, a possible relationship of the rickettsial endosymbiont and the tick blood diet had been suggested [23].

This hypothesis has been confirmed by metabolic reconstructions derived from rickettsial endosymbiont genomes of *R. buchneri* and *Rickettsia* species phylotype G021, which showed that they contain all the genes required for folate (vitamin B9) biosynthesis [24]. This is in accordance with the expected nutritional compounds required for strict hematophagy [15]. Vitamin B9 is not present in a restricted blood diet in sufficient amounts. Moreover, *Rickettsia* species phylotype G021 was shown to massively proliferate after a tick blood meal in all stages [25].

2.4.4 Insights into rickettsial genomes

In the last decade, whole-genome sequences of several rickettsial species (including obligate endosymbionts) were published, which allows detailed analyses of their evolution and host associations [26].

The recurrent biphasic model described in parasitic and symbiotic organisms is characterized by longer phases of genome reduction and simplification, interrupted by shorter phases of episodic expansion [27]. Rickettsial chromosomes and plasmids are in progressive degradation and size reduction and contain numerous laterally acquired genes that display evidence of horizontal transfer between *Rickettsia*, other *Rickettsiae* and bacterial endosymbionts (such as *Cardinium*), and even eukaryotes [9, 13, 28]. For instance, rickettsial plasmids have gained novel metabolic functions that are missing in rickettsial chromosomes and which may fill host-metabolic gaps [29].

A convergent reductive pattern has led to relatively small rickettsial genomes, ranging from 1.1 Mb for pathogenic *Rickettsia prowazekii* and *Rickettsia typhi* to 2.1 Mb for the obligate endosymbiotic *R. buchneri* [26].

2.4.5 Are pathogenic and endosymbiotic Rickettsiae two separate groups?

The phylogenetic position does not define the pathogenicity since tick rickettsial endosymbiotic and vertebrate pathogen species are dispersed along the phylogeny [3]. In the most recent review on tick-borne rickettsioses, it was stated that every member of the SFG should be considered a potential pathogen [6]. Numerous pathogenic tick-borne *Rickettsiae* are vertically transmitted [7]; hence, transovarial transmission is not a sign of nonpathogenicity. The ability of *Rickettsiae* to invade tick host cells seems to be the crucial feature that was lost by endosymbionts. *R. peacockii*, in *Dermacentor andersoni*, is not able to enter hemocytes and salivary gland tissues, which establishes its obligate endosymbiotic nature and prevents infection of vertebrates [30]. The borderline between pathogens and endosymbionts is not sharp since there are avirulent strains of pathogenic *Rickettsiae* that retain the ability to persist in ticks and can be transmitted transovarially, such as *R. rickettsii* strain Iowa [31].

The pathogenic and endosymbiotic lifestyle could probably evolve via various scenarios: First, loss of pathogenicity, as described for strictly endosymbiotic *R. peacockii*, which is closely related to the most clinically severe *R. rickettsii*. The genome of *R. peacockii* contains various deletions and mutations caused by a recombination of transposon copies that extinguished its ability to cause cytopathic effects [32, 33]; a similar situation exists with nonpathogenic *R. buchneri*, which is closely related to pathogenic *Rickettsia monacensis* [17]. However, since rickettsial phylogeny shows repeated occurrences of horizontal transfer, this may lead to the appearance of novel bacterial phenotypes as described in Q fever cases caused by *Coxiella burnetii*, which probably originated from a *Coxiella*-LE that infected vertebrate cells [15]. *Rickettsia vini*, an obligate endosymbiont of ornithophilic *Ixodes arboricola* and *Ixodes lividus* ticks, has repeatedly been detected in *Ixodes ricinus* ticks, which may illustrate horizontal transmission of endosymbiotic *Rickettsiae* via co-feeding [34–36]. Since this species is a member of the SFG and was successfully isolated in vertebrate Vero cells, it may represent a potential candidate for a vertebrate pathogen [37].

2.4.6 Rickettsial endosymbionts in relationship to other maternally inherited bacteria within ticks

Ten distinct genera of maternally inherited bacteria have been recently described in ticks (e.g., [23, 38, 39]). Based on a recent study by Duron, the most prevalent bacterial genera in ticks are *Coxiella*-LE (60.5%) and *Rickettsia* (55.6%), both of which have been identified in more tick species than any other genera [15]. While 43.2% of tested tick species harbored one bacterium, 56.3% were infected

with two or more bacterial genera. *Rickettsia* has also been found to nonrandomly aggregate with *Midichloria*. Such fixed multiple endosymbiotic associations may imply that, collectively, the bacteria can synthesize all the components needed for certain essential pathways and hence are interdependent [15].

Only 2 out of 81 tick species (2.5%) did not harbor any maternally inherited bacteria [15]. In some filarial nematodes, symbiont genes acquired from bacteria via lateral gene transfer have been found in the host chromosome [40]. This could explain why Duron did not detect any bacterial endosymbiont in two tick species. However, such horizontal gene transfer has yet to be reported in ticks [15].

Infection frequencies vary among different geographical populations of a given tick species [4]. Combining maternal inheritance with horizontal transfer allows unrelated bacteria to coinfect one individual host and to form an endosymbiotic community with complex interactions resulting in phenotypic differentiation within tick populations [41]. Recent studies have revealed that relationships among bacterial communities within ticks are more complex than had been previously assumed [4].

2.4.7 Interaction of nonpathogenic rickettsial endosymbionts and pathogenic bacteria

Ticks are exposed to various *Rickettsiae* while feeding on multiple hosts [38]. However, typically only one rickettsial species is observed per individual tick [42]. Transovarial transmission of more than one rickettsial species from the SFG has not been proven. It is believed that infection of tick ovaries could induce a specific molecular response that results in a second infection being blocking [43]. However, the coexistence of *R. bellii*, which belongs to the ancestral group, with SFG *Rickettsiae* has been described [28]. Additionally, interactions of *Rickettsiae* with other pathogens have been reported. The occurrence of *R. bellii* in *D. andersoni* ticks precludes infection of *Anaplasma marginale* [44]. Males of *I. scapularis* infected by *R. buchneri* were significantly protected against infection by *Borrelia burgdorferi* compared to *R. buchneri*-free males [45].

3. Conclusion

Non-pathogenic rickettsial endosymbionts of ticks appear to interact with ticks in complex ways. While some of them are essential for tick survival and reproduction, others may impact multiple tick features, e.g., rickettsial endosymbionts may significantly influence the abundance of tick-borne pathogens, which may help reduce the health risk to humans. The boundaries between categories, such as vertically transmitted pathogen and maternally inherited endosymbiont, are not terribly sharp since transitional states occasionally arise [4].

Acknowledgements

This work was supported by the Grant Agency of the Czech Republic 17-25455S (to D.S.) and by funds from the Faculty of Medicine MU to junior researcher (to M.N.). We thank Thomas Secrest (Secrest Editing, Ltd.) for his assistance with the English revision of the chapter.

Conflict of interest

The authors declare no conflicts of interest.

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