The importance of ticks in Q fever transmission: what has (and has not) been demonstrated?

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Opinion

The Importance of Ticks in Q Fever Transmission: What Has (and Has Not) Been Demonstrated?

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Q fever is a widespread zoonotic disease caused by *Coxiella burnetii*, a ubiquitous intracellular bacterium infecting humans and a variety of animals. Transmission is primarily but not exclusively airborne, and ticks are usually thought to act as vectors. We argue that, although ticks may readily transmit *C. burnetii* in experimental systems, they only occasionally transmit the pathogen in the field. Furthermore, we underscore that many *Coxiella*-like bacteria are widespread in ticks and may have been misidentified as *C. burnetii*. Our recommendation is to improve the methods currently used to detect and characterize *C. burnetii*, and we propose that further knowledge of *Coxiella*-like bacteria will yield new insights into Q fever evolutionary ecology and *C. burnetii* virulence factors.

Trends

Q fever is a widespread zoonotic disease caused by *Coxiella burnetii*, an intracellular bacterium that infects humans and a wide range of vertebrates. Domestic ruminants are the main reservoir.

C. burnetii is frequently detected in ticks, and laboratory experiments have revealed that at least some tick species are competent vectors. However, under natural conditions, Q fever is far more frequently airborne than vector-borne.

Many *Coxiella*-like bacteria, closely related to but genetically distinct from *C. burnetii*, have been described in ticks and, very occasionally, in vertebrates. They likely behave as non-virulent tick symbionts. Their pathogenicity for vertebrates is largely unknown.

Coxiella-like bacteria may have been misidentified as *C. burnetii* in past field studies. New means of detecting and characterizing tick-borne *C. burnetii* and *Coxiella*-like bacteria are needed.

Q Fever: An Airborne Zoonotic Disease

Q fever is a zoonosis (see Glossary) found worldwide that is caused by the obligate intracellular bacterium *Coxiella burnetii*. This pathogen can infect a wide range of vertebrates, including livestock (which are thought to be the primary reservoir), a variety of wild species, and humans [1]. The clinical signs of Q fever vary dramatically (Box 1). In animals, infections are usually asymptomatic and are not considered to be a veterinary problem, except in ruminants where Q fever is a well-recognized cause of abortion [2,3]. In humans, *C. burnetii* infections vary from self-limiting to severe [4,5]. The acute form ranges from causing mild flu-like symptoms to provoking pneumonia or hepatitis, which may require hospitalization. The disease can become chronic and result in endocarditis, aneurysmal, valvular, or vascular infections, chronic fatigue syndrome, premature birth, or abortion, and particularly for individuals with risk factors of severity. Though rarely fatal, Q fever remains highly debilitating, even when treated with antibiotics. Many sporadic cases in humans occur annually worldwide, and occasional outbreaks are also common. The 2007–2010 Q fever epidemic in The Netherlands attracted attention because of its exceptional magnitude and duration: more than 4000 human cases were reported [6,7].

C. burnetii produces spore-like small cell variants that are able to resist harsh environmental conditions and are thus more likely to persist in the environment for long periods of time. For this reason, and because public health measures are particularly difficult to implement given that the disease is aerially transmitted, highly morbid, and difficult to diagnose, *C. burnetii* is classified as a category B potential aerosolized biological weapon by the United States [8]. Infection commonly occurs via the inhalation of barnyard dust contaminated with the excreta of infected

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Box 1. Q Fever: A Challenging Disease for Public and Animal Health

Q fever is a zoonotic disease that has a high socioeconomic burden [6] and is difficult to diagnose, prevent, and treat in both humans and animals [2,89]. It therefore presents significant challenges for both public and animal health.

Challenges for Public Health

In humans, initial exposure to *C. burnetii* may result in asymptomatic or mild infection but also in acute or chronic disease [4,5]. The clinical diagnosis can be very difficult. The reasons for this high clinical polymorphism are largely unknown, even if risk factors of severity (e.g., pregnancy, immunosuppression, preexisting cardiac valvulopathy, vascular grafts, and aneurysms) have been described. Although rarely fatal, the disease may lead to substantial morbidity and can be highly debilitating, even under treatment. Most human cases result from the inhalation of dust particles contaminated by infected livestock or animal products [2,4,89]. *C. burnetii* is also a potential agent of bioterrorism [8]. Prevention is difficult to put in place because transmission is essentially airborne. Although an effective vaccine (Q-VAX, CSL Limited, Parkville, Victoria, Australia) may be used in Australia for at-risk professions, its use in endemic areas is difficult because it has significant side effects in persons who have been exposed to *C. burnetii*, thus requiring pre-vaccination screening [8].

Challenges for Animal Health

In domestic animals, Q fever is mostly associated with abortion peaks in small ruminants and sporadic abortions in cattle [90]. The differential diagnosis with other infectious and non-infectious causes of abortion may be difficult. Within herds with Q fever abortions, the bacterium is excreted in large quantities in the placenta and fetal membranes of females, whether they have aborted or not, and excretion in vaginal mucus and feces may further last several months, which results in massive environmental contamination [2,91]. Disease management is extremely difficult: antibiotic treatment is inefficient and no environmental decontamination procedures have been validated. Long-term control options include segregated birthing areas, removal of abortion/birth material, manure management, and vaccination of primiparous females [2]. Disease management is further complicated by the fact that Q fever transmission is essentially, but not only, airborne, and also that many animal reservoirs may be involved in the epidemiological cycle.

animals, such as birth products, which may contain high quantities of *C. burnetii*; other infection pathways (e.g., sexual, oral, or congenital) are thought to be rare [2,4].

Q Fever: A Tick-Borne Zoonosis?

The importance of ticks in Q fever epidemiology remains controversial even though major pioneering studies have focused on *C. burnetii* in ticks [1,9]. It is noteworthy that the highly-virulent reference strain, Nine Mile, was isolated from a guinea pig upon which *Dermacentor andersoni* ticks had fed [10]. Furthermore, many early microscopic morphological observations suggested that over 40 tick species carry *C. burnetii* [11]. Nowadays, ticks are still the focus of many field studies of Q fever epidemiology (Table S1 in the supplementary material online). The occasional reports of unexpectedly high levels of *C. burnetii* infection in ticks [12,13] raise questions of whether ticks play an important role in Q fever transmission.

In this article we review the available literature to assess the importance of ticks in natural cycles of Q fever transmission. First, we examine the ability of ticks to readily transmit *C. burnetii* in both experimental and field systems. Second, we highlight recent findings that reveal the diversity of *Coxiella*-like bacteria, which are genetically related to, but distinct from, *C. burnetii*, and we explore the reliability of the screening methods commonly used for ticks. We further argue that future research must focus on developing methods that better detect and characterize tick-borne *Coxiella*.

Ticks Are Competent Vectors for *C. burnetii* in Experimental Systems

The role of ticks in Q fever transmission has been extensively studied ever since the Nine Mile strain was isolated from *D. andersoni* in the 1930s [10]. At least seven hard and soft tick species, including *D. andersoni*, have formally been shown to be competent vectors of *C. burnetii* (Table 1). For each species, three major traits related to **vector competence** have been experimentally confirmed: (i) the ability to acquire *C. burnetii* from an infected animal, (ii) the **trans-stadial** transmission of infection from larvae to nymphs and from nymphs to adults, and

Table 1. List of Studies Investigating the Transmission of *Coxiella burnetii* by Arthropods in Experimental Conditions[a]

Tick (or other arthropod) species	Competent vector	Trans-stadial transmission		Transmission to vertebrate host (species)			Infection by engorgement (species)	Methods to confirm tick infection			*C. burnetii* strain	Refs
		L to N	N to Ad	Bite	Feces	Other		Engorgement	Inoculation	Detection		
Rhipicephalus microplus[b]	?	–	–	–	Yes[c]	Yes[d]	Yes (calf)	–	Yes (GP)	Yes[h]	Australian[e]	[92]
Dermacentor andersoni	Yes	–	–	Yes (GP)	–	–	Yes[f]	Yes (GP)	–	–	Nine Mile[g]	[93]
		Yes	Yes	Yes (GP)	–	–	Yes (GP)	Yes (GP)	Yes (GP)	–	Nine Mile	[15]
		–	–	–	Yes[c]	–	Yes (GP)	–	–	–	Nine Mile	[14]
Haemaphysalis bispinosa	?	Yes	No	–	–	–	Yes (GP)	–	Yes (GP)	Yes[h]	Australian	[94]
Haemaphysalis humerosa	Yes	Yes	Yes	Yes[i] (GP)/No[j]	Yes[c,k]/No[l]	No[m]	Yes (GP)	Yes (GP)	–	–	Australian	[16]
Hyalomma aegyptium	Yes	Yes	Yes	Yes (GP)	–	–	Yes (GP)	Yes (GP)	–	Yes[n]	Nine Mile	[95]
Hyalomma asiaticum	Yes	Yes	Yes	Yes (GP)	–	No[o]	Yes (GP)	Yes (GP)	Yes (GP)	Yes[h]	Ixodes II Luga[p]	[96]
Ixodes holocyclus	Yes	Yes	Yes[i]/No[j]	Yes (GP/bandicoot)	–	–	Yes (GP/bandicoot)	Yes (GP/bandicoot)	Yes (GP)	Yes[h]	Australian	[97]
Rhipicephalus sanguineus	?	Yes	Yes	–	–	–	Yes (GP)	–	Yes (GP)	Yes[h]	Australian	[98]
		–	–	–	–	Yes[d,q]	Yes (dog)	–	Yes (GP)	–	Unknown[r]	[99]
Ornithodoros canestrinii	?	–	–	–	–	Yes[d]	No[s]	–	Yes (GP)	–	Grit	[100]
Ornithodoros gurneyi	No	No	No	No (GP)	–	No[t]	Yes (GP)	Yes (GP)	Yes (GP)	–	Australian	[94]
Ornithodoros hermsi	Yes	–	Yes	Yes (GP)	–	–	Yes (GP)	Yes (GP)	Yes (GP)	–	Nine Mile	[17]
Ornithodoros lahorensis	?	–	–	–	–	Yes[d]	No[s]/Yes[u]	–	Yes (GP)	–	M44 and Grit	[101]
Ornithodoros moubata	Yes	–	Yes	Yes (GP)	–	–	Yes (GP)	Yes (GP)	Yes (GP)	–	Nine Mile	[17]
		–	–	–	–	Yes[d]	No[s]	–	Yes (GP)	–	M44 and Grit	[101]
Ornithodoros papillipes	?	–	–	–	–	Yes[d]	Yes[u]	–	Yes (GP)	–	M44 and Grit	[101]
		–	–	–	–	Yes[d]	Yes[u]	–	Yes (mice)	–	Ixodes IV Luga[p]	[102]
Ornithodoros turicata	No	–	No	No (GP)	Yes[c]	Yes[d]	Yes (GP)	Yes (GP)	Yes (GP)	–	Nine Mile	[103]

Glossary

Enzootic: refers to a disease that is constantly present in an animal population with local transmission events; analogous to 'endemic' in humans.

Epizootic: refers to a disease that rapidly spreads in an animal population within a short period of time; analogous to 'epidemic' for human diseases.

Serological test: a diagnostic test based on the detection of antibodies in the serum of patients or animals; specific antibodies are synthesized in response to an infection against a given microorganism.

Transovarial: transmission of a microorganism from a female tick to its progeny through its ovary and eggs.

Trans-stadial: transmission of a microorganism from one tick stage to the next.

Vector capacity: a measure of the transmission potential of a vector population in field conditions; it corresponds to the number of secondary cases arising per day from one infective host in a susceptible host population exposed to the vector.

Vector competence: the ability of a vector to acquire, maintain, and transmit a microbial agent in laboratory conditions.

Zoonosis: a disease of animals that can be naturally transmitted to humans. Zoonotic diseases can be either enzootic or epizootic (see above).

Table 1. (continued)

Tick (or other arthropod) species	Competent vector	Trans-stadial transmission		Transmission to vertebrate host (species)			Infection by engorgement (species)	Methods to confirm tick infection			*C. burnetii* strain	Refs
		L to N	N to Ad	Bite	Feces	Other		Engorgement	Inoculation	Detection		
Ctenocephalides felis (flea)	No	No	na	–	–	–	Yes (mice)	–	Yes (mice)	–	Australian	[104]
Aedes aegypti (mosquito)	No	No	na	No (GP)	–	No[v]	Yes (GP)	Yes (GP)	Yes (GP)	–	Nine Mile	[14]

[a]Abbreviations: –, not tested; Ad, Adults; GP, guinea pig L, Larvae; N, Nymphs; na, not applicable.
[b]Recently assigned to the genus *Rhipicephalus* from the genus *Boophilus*.
[c]Inoculation to guinea pig.
[d]Intraperitoneal inoculation of tick homogenates.
[e]Australian: *Coxiella burnetii* isolated from *Hae. humerosa*, successive passages in guinea pig (Queensland, Australia).
[f]Natural infection.
[g]Nine Mile Strain: *Coxiella burnetii* isolated from *D. andersoni*, successive passages in guinea pig (Montana, USA).
[h]Smears.
[i]Female.
[j]Male.
[k]Deposition of feces on abraded skin.
[l]Deposition of feces on unabraded skin.
[m]Cutaneous transmission by deposition of tick homogenates on unabraded skin.
[n]PCR.
[o]Transpermal transmission from male to female during tick mating.
[p]Ixodes II Luga: *C. burnetii* isolated from *Ixodes ricinus* in Leningrad during Q fever epidemic.
[q]Intraperitoneal inoculation of crushed tick eggs.
[r]Fetal membrane from infected sheep.
[s]Intracoelomic inoculation of tick homogenates.
[t]Cutaneous transmission by deposition of tick coxal fluid on unabraded skin.
[u]Engorgement on artificial membrane.
[v]Percutaneous transmission during tick bite with interrupted feeding.

(iii) the ability to transmit infectious *C. burnetii* to an uninfected animal. Obviously, many more tick species may be competent vectors of Q fever. Other tick species have been found to transmit the pathogen, but their vector competence has not been fully demonstrated (for instance, transstadial transmission has not been shown; Table 1). Of all the tick species examined thus far, only two have been experimentally shown to be incompetent vectors (Table 1). Consequently, in experimental systems, most tick species seem to be able to transmit *C. burnetii* to uninfected animals.

In laboratory-infected ticks, infection is typically systemic; *C. burnetii* has been detected in the midgut, hemolymph, Malpighian tubules, salivary glands, and ovaries [1]. Ticks have also been found to excrete large numbers of infectious *C. burnetii* in their body fluids and feces – up to 10^{10} organisms per gram of feces [14]. This finding underscores the potential risk of tick-borne infection posed by tick excreta, through inhalation (e.g., during sheep shearing), direct contact (e.g., while crushing a tick with one's bare hands), or tick bites. Furthermore, **transovarial** transmission – the transmission of *C. burnetii* from a female tick to her offspring – has also been observed in three tick species [15–17], which shows that *C. burnetii* can be maintained by tick hosts across several generations without needing to infect vertebrates. As a result, this pathogen may be transmitted both transovarially and via blood meals in several tick species.

The Vector Capacity of Ticks in the Field Remains Unknown

Field studies are essential to evaluating the potential of ticks to vector pathogens under natural conditions. The natural ability of a tick to transmit *C. burnetii* (i.e., its vector capacity) depends on several factors besides vector competence, including tick population density, host preference, biting rate, and ecological constraints. Consequently, even if ticks are competent vectors under laboratory conditions, they may inefficiently transmit disease in nature if their **vector capacity** is low. This might be the case for *C. burnetii*.

To date, most field studies examining the role of ticks in Q fever epidemiology have restricted themselves to describing *C. burnetii* prevalence. The observed percentage of *C. burnetii*-positive ticks is typically low (<5%) but prevalence levels greater than 5% or even 10% are also reported (Table S1). These levels are consistent with those observed for strictly tick-borne pathogens, such as bacteria from the genus *Anaplasma* [18]. Therefore, a sylvatic cycle based on *C. burnetii* tick-borne transmission seems to be sustainable. The fact that *C. burnetii* has occasionally been isolated from ticks sampled from wildlife [12] or wildlife burrows [19] supports this hypothesis. However, direct transmission likely also takes place among wildlife species because *C. burnetii* has been reported in the feces [20–22], placenta [23–25], and vaginal mucus [26] of diverse wildlife species. Interestingly, within or in the vicinity of farms where Q fever has been known to circulate, *C. burnetii* prevalence in ticks may be low or seemingly absent [27,28]. Conversely, a strong correlation between the seropositivity in domestic ruminants and their infestation with *C. burnetii*-infected ticks has been reported [29], and several studies have identified the presence of ticks as a risk factor for seropositivity in livestock [30–32]. Thus, the vector capacity of ticks to transmit *C. burnetii* remains unclear. In humans, limited data support the occurrence of tick-borne *C. burnetii* transmission, including occasional reports of *C. burnetii* infections in patients bitten by ticks [33–37], or concomitantly infected with tick-borne pathogens [38,39], or positive for Q fever by **serology** [40]. However, in these cases, exposure to infection sources other than ticks (particularly via the aerial route) generally cannot be excluded.

Overall, therefore, the ability of ticks to vector *C. burnetii* seems limited: although they may occasionally transmit the bacterium to vertebrate animals and humans, this route is clearly secondary compared to airborne transmission. Nonetheless, ticks may serve as an ecological bridge for *C. burnetii* transmission between wild and domestic animal hosts [12,41,42]. In crossing these species barriers, *C. burnetii* may be experiencing increased selection for genomic

plasticity and enhanced genetic diversity, promoting its diversity of virulence and resistance factors [43,44].

Coxiella-like Bacteria Are Common in Ticks

C. burnetii is the only species that has been formally described in the *Coxiella* genus [45], although another putative species (*C. cheraxi*) has been reported in crayfishes [46]. Interestingly, in the mid-1990s, the advent of simple PCR assays, together with extensive 16S rRNA gene sequencing, led to the description of *Coxiella*-like bacteria in three tick species [47]. These novel *Coxiella*-like bacteria were closely related to (but genetically distinct from) *C. burnetii*, revealing that an overlooked degree of diversity may actually exist within the *Coxiella* genus [48]. We now know that *Coxiella*-like bacteria are exceptionally diverse and widespread in ticks. In a recent study, Duron *et al*. [49] identified *Coxiella*-like bacteria from 40 of 58 examined tick species, suggesting that more than two-thirds of tick species may be infected. Overall, molecular evidence based on 16S rRNA gene sequences showed that at least 52 tick species are infected, with an infection frequency close to 100% in many cases (Table 2). In addition, a few other *Coxiella*-like bacteria have sporadically been found in domestic birds [46,50–52]. In most cases, these newly described bacteria have been characterized solely by their 16S rRNA gene sequences. Therefore, although other genes may prove to discriminate well between *C. burnetii* and *Coxiella*-like bacteria, they are currently mainly defined based on phylogenetic analyses considering the 16S rRNA gene (Figure 1). Multilocus DNA sequencing further indicates that the *Coxiella* genus is subdivided into four highly-divergent genetic clades (A–D; Figure 1), with *C. burnetii* belonging to the A clade [49]. Remarkably, phylogenetic investigations also converge to support the hypothesis that one of the *Coxiella*-like bacteria, belonging to the A clade and primarily hosted by soft ticks, has served as the progenitor of *C. burnetii* [49].

Despite their genetic relatedness, tick-borne *Coxiella*-like bacteria and *C. burnetii* are ecologically distinct from each other. In particular, some *Coxiella*-like bacteria, such as those detected in *Amblyomma americanum*, *A. cajennense*, and *Ornithodoros rostratus*, display prevalences of 100% in all the life-stages of their hosts; the infection is maternally transmitted, via the egg cytoplasm, and maintained trans-stadially, rather than being acquired through blood feeding on infected vertebrates [49,53–55]. Accordingly, when the *Coxiella*-like bacterium found in *A. americanum* was recently sequenced [56], no recognizable virulence genes were found, which indicates that this bacterium is likely not a pathogen. By contrast, its genome encodes major vitamin and cofactor biosynthesis pathways, which suggests that it may be a vitamin-provisioning endosymbiont instead. Remarkably, eliminating this bacterium from *A. americanum* ticks using antibiotics reduced tick fecundity and viability [57], which further supports the hypothesis that *Coxiella*-like bacteria are engaged in mutualistic symbioses in this tick species (Box 2).

Coxiella-like Bacteria May Be Commonly Misidentified as *C. burnetii*

The discovery that ticks carry both *C. burnetii* and *Coxiella*-like bacteria underscores the need to be able to clearly distinguish between the two. Numerous *C. burnetii* detection methods are in use (Table S1) and, in some cases, they produce clear evidence that ticks are infected by *C. burnetii* rather than by *Coxiella*-like bacteria, as shown in Figure 1 for the bacteria detected in the tick species *D. andersonii* and *A. trigrinum*. For instance, in the noteworthy case-study by Pacheco *et al.* (2013), *C. burnetii* infection in ticks was confirmed using an impressive array of detection methods, including hemolymph tests, isolation in Vero cells, and multilocus DNA sequencing. However, many other studies aiming to estimate *C. burnetii* prevalence in ticks have not been as rigorous, and may have misidentified *Coxiella*-like bacteria as *C. burnetii*.

Historically, and until the late 1990s, ticks were essentially screened for *C. burnetii* using morphological observations, staining, and immunodetection techniques because this obligate intracellular bacterium is difficult to culture. However, the recent discovery of so many tick-borne

Table 2. List of Tick Species Infected by *Coxiella*-like Bacteria

Tick species	Countries or regions	Prevalence of *Coxiella*-like bacteria	Targeted genes by molecular assays	Infected stages	Examined organs	Refs
Hard ticks (Ixodidae)						
Amblyomma americanum	USA	75–100%	16S rRNA gene, *rpsF*, *rpsG*, *dnaK*, and *FusA*	Eggs, larvae, nymphs, adults	Midgut, ovaries, salivary glands	[49,55,60–62, 105,106]
Amblyomma cajennense	Brazil	100%	16S and 23S rRNA genes, *groEL*, *rpoB*, and *dnaK*	Eggs, larvae, nymphs, adults	Midgut, ovaries, salivary glands	[49,54]
Amblyomma loculosum	Indian Ocean	64–100%	16S and 23S rRNA genes, *groEL*, *rpoB*, and *dnaK*	n.d.[a]	n.d.	[49,107]
Amblyomma variegatum	Indian Ocean	n.d.	16S and 23S rRNA genes, *groEL*, *rpoB*, and *dnaK*	Adults	n.d.	[49]
Bothriocroton auruginans	Australia	100%	16S rRNA gene and *IS1111*	Adult females	n.d.	[67]
Dermacentor silvarum	China	100%	16S and 23S rRNA genes, *groEL*, *rpoB*, and *dnaK*	Eggs, larvae, nymphs, adults (males and females)	Ovaries, malpighian tubes	[49,108]
Dermacentor marginatus	France	100%	16S and 23S rRNA genes, *groEL*, *rpoB*, and *dnaK*	Adults	n.d.	[49]
Haemaphysalis hystricis	Thailand	17%	16S rRNA gene	Adults	n.d.	[109]
Haemaphysalis concinnae	Russia	100%	16S rRNA gene, *gltA* and *ompA*	Adult females	n.d.	[110]
Haemaphysalis falva	Japan	100%	16S rRNA gene and *IS1111*	Adults	Salivary glands	[63,111]
Haemaphysalis lagrangei	Thailand	39%	16S rRNA gene	Eggs, larvae, nymphs, adults	n.d.	[109,112]
Haemaphysalis longicornis	Korea, Japan	2%	16S and 23S rRNA genes, *Com1*	Adults	Ovaries, malpighian tubes	[47,65]
Haemaphysalis obesa	Thailand	47%	16S rRNA gene	Adults	n.d.	[109]
Haemaphysalis shimoga	Thailand	58%	16S rRNA gene	Eggs, larvae, nymphs, adults	n.d.	[109,112]

Table 2. (continued)

Tick species	Countries or regions	Prevalence of *Coxiella*-like bacteria	Targeted genes by molecular assays	Infected stages	Examined organs	Refs
Haemaphysalis punctata	England	100%	16S and 23S rRNA genes, *groEL*, *rpoB*, and *dnaK*	Adults	n.d.	[49]
Ixodes hexagonus	France	100%	16S and 23S rRNA genes, *groEL, and rpoB*	Adults	n.d.	[49]
Ixodes ovatus	Japan	95%	16S rRNA gene	Adults	Salivary glands	[63]
Ixodes persulcatus	Japan	20%	16S rRNA gene	Adults	Salivary glands	[63]
Ixodes ricinus	France, Austria	n.d.	16S and 23S rRNA genes, *groEL, and rpoB*	Adults	n.d.	[49]
Ixodes uriae	Canada	0–50%	16S and 23S rRNA genes, *groEL, and rpoB*	Adults	n.d.	[49,113]
Ixodes sp. 1	Ivory Coast	100%	16S and 23S rRNA genes, *groEL*, *rpoB*, and *dnaK*	Adults	n.d.	[49]
Ixodes sp. 2	Ivory Coast	100%	16S and 23S rRNA genes, *groEL*, *rpoB*, and *dnaK*	Adults	n.d.	[49]
Rhipicephalus annulatus	Burkina-Faso, Benin	100%	16S and 23S rRNA genes, *groEL*, *rpoB*, and *dnaK*	Adults	n.d.	[49]
Rhipicephalus australis	New Caledonia	100%	16S and 23S rRNA genes, *groEL*, *rpoB*, and *dnaK*	Adults	n.d.	[49]
Rhipicephalus bursa	Italia	100%	16S and 23S rRNA genes, *groEL*, *rpoB*, and *dnaK*	Adults	n.d.	[49]
Rhipicephalus decoloratus	Africa	100%	16S and 23S rRNA genes, *groEL*, *rpoB*, and *dnaK*	Adults	n.d.	[49]
Rhipicephalus evertsi	Zimbabwe	100%	16S and 23S rRNA genes, *groEL*, *rpoB*, and *dnaK*	Adults	n.d.	[49]
Rhipicephalus geigyi	Burkina-Faso, Benin	100%	16S and 23S rRNA genes, *groEL*, *rpoB*, and *dnaK*	Adults	n.d.	[49]

Table 2. (continued)

Tick species	Countries or regions	Prevalence of *Coxiella*-like bacteria	Targeted genes by molecular assays	Infected stages	Examined organs	Refs
Rhipicephalus microplus	USA, Africa	100%	16S and 23S rRNA genes, *groEL*, *rpoB*, and *dnaK*	Eggs and adults	Ovaries	[49,64]
Rhipicephalus pusillus	France	n.d.	16S and 23S rRNA genes, *groEL*, *rpoB*, and *dnaK*	Adults	n.d.	[49]
Rhipicephalus sanguineus	Switzerland, France, USA	12–100%	16S and 23S rRNA genes	Eggs, larvae, nymphs, adults	Ovaries, malpighian tubes	[47,49,106, 114–116]
Rhipicephalus turanicus	Europe	23–100%	16S rRNA gene	Nymphs and adults	Ovaries, malpighian tubes	[49,114–116]
Rhipicephalus sp. 1	Ivory Coast	100%	16S and 23S rRNA genes, *groEL*, *rpoB*, and *dnaK*	Adults	n.d.	[49]
Rhipicephalus sp. 2	Ivory Coast	100%	16S and 23S rRNA genes, *groEL*, *rpoB*, and *dnaK*	Adults	n.d.	[49]
Soft ticks (Argasidae)						
Argas monolakensis	USA	53%	16S rRNA gene, *mucZ*, and *gltA*	n.d.	n.d.	[70]
Argas monachus	Argentina	100%	16S and 23S rRNA genes, *groEL*, *rpoB*, and *dnaK*	Adults	n.d.	[49]
Ornithodoros amblus	Peru	100%	16S and 23S rRNA genes, *groEL*, *rpoB*, and *dnaK*	Adults	n.d.	[49]
Ornithodoros brasiliensis	Brazil	100%	16S and 23S rRNA genes, *groEL*, *rpoB*, and *dnaK*	Adults	n.d.	[49]
Ornithodoros capensis	Various tropical and temperate regions	46–100%	16S rRNA gene, *icd*, *sod*, *pyrG*, *gltA*, *mucZ*, *groEl* (*htpB*), etc	Eggs, nymphs, adults	n.d.	[49,68,69, 107,117]
Ornithodoros denmarki	Unknown	100%	16S and 23S rRNA genes, *groEL*, *rpoB*, and *dnaK*	Adults	n.d.	[49]

Table 2. (continued)

Tick species	Countries or regions	Prevalence of *Coxiella*-like bacteria	Targeted genes by molecular assays	Infected stages	Examined organs	Refs
Ornithodoros erraticus	North Africa	100%	16S and 23S rRNA genes, *groEL*, *rpoB*, and *dnaK*	Adults	n.d.	[49]
Ornithodoros kairouanensis	North Africa	100%	16S and 23S rRNA genes, *groEL*, *rpoB*, and *dnaK*	Adults	n.d.	[49]
Ornithodoros maritimus	Mediterranean Islands	100%	16S and 23S rRNA genes, *groEL*, *rpoB*, and *dnaK*	Eggs, adults	n.d.	[49]
Ornithodoros marocanus	North Africa	100%	16S and 23S rRNA genes, *groEL*, *rpoB*, and *dnaK*	Adults	n.d.	[49]
Ornithodoros moubata	n.d.	n.d.	16S and 23S rRNA genes	n.d.	Ovaries, malpighian tubes	[47]
Ornithodoros occidentalis	North Africa	100%	16S and 23S rRNA genes, *groEL*, *rpoB*, and *dnaK*	Adults	n.d.	[49]
Ornithodoros peruvianus	Chile	100%	16S and 23S rRNA genes, *groEL*, *rpoB*, and *dnaK*	Adults	n.d.	[49]
Ornithodoros rostratus	Brazil	100%	16S rRNA gene, *pyrG, and Cap*	Eggs, nymphs, adults	n.d.	[49,53]
Ornithodoros rupestris	North Africa	100%	16S and 23S rRNA genes, *groEL*, *rpoB*, and *dnaK*	Adults	n.d.	[49]
Ornithodoros sonrai	North Africa	100%	16S and 23S rRNA genes, *groEL*, *rpoB*, and *dnaK*	Adults	n.d.	[49]
Ornithodoros spheniscus	Chile	100%	16S and 23S rRNA genes, *groEL*, *rpoB*, and *dnaK*	Adults	n.d.	[49]
Ornithodoros sp.	Cape Verde	100%	16S and 23S rRNA genes, *groEL*, *rpoB*, and *dnaK*	Adults	n.d.	[49]

[a]Abbreviation: n.d., not defined.

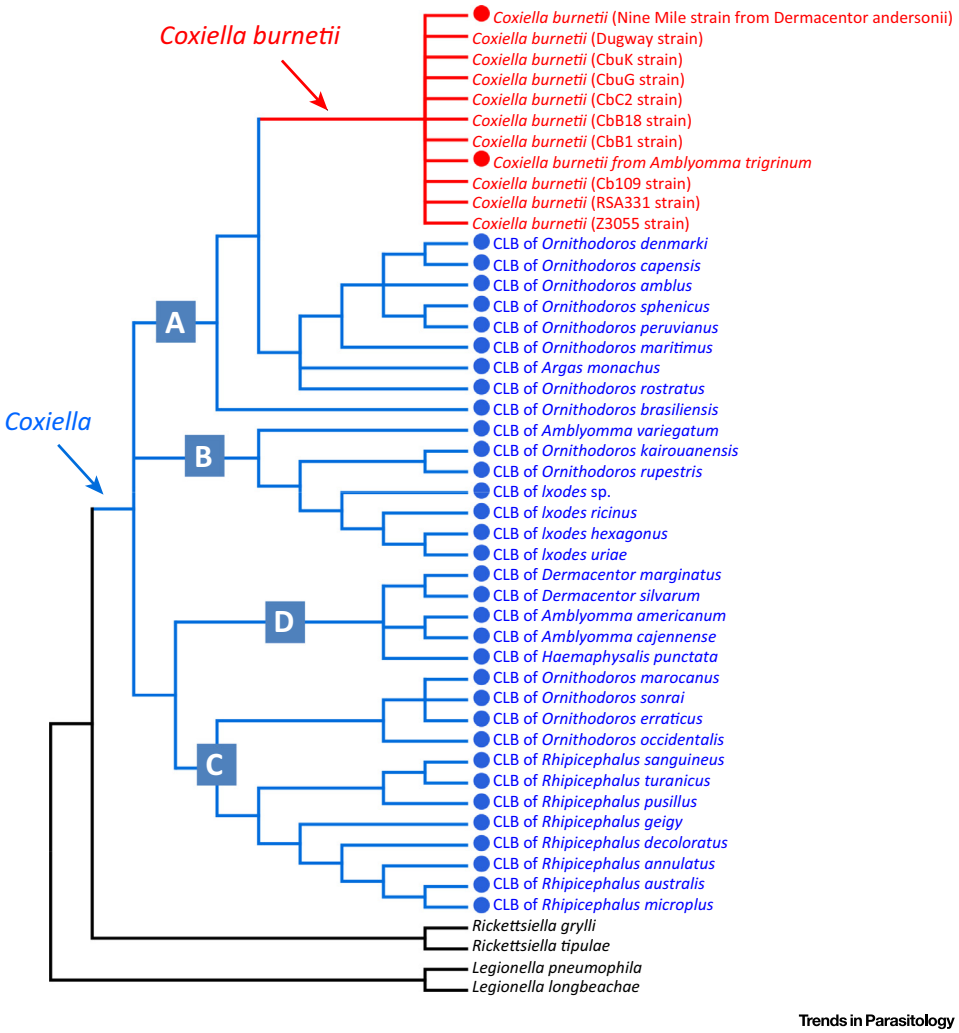

Figure 1. Cladogram of the *Coxiella* Genus Based on Representative DNA Sequences Available in the GenBank Database (adapted from [49] and [117]). Members of the two sister-genera of *Coxiella* (*Rickettsiella* and *Legionella*) have been added to delineate the *Coxiella* genus. The four *Coxiella* clades are labeled A–D. CLB, *Coxiella*-like bacteria; circles, *Coxiella* strains primarily characterized in ticks; blue, *Coxiella*-like bacteria; red, *C. burnetii*; Black, bacteria belonging to bacterial genera other than *Coxiella*.

Coxiella-like bacteria using molecular techniques puts the results of these past studies into question. The case of *A. americanum* is illustrative: while *C. burnetii* is repeatedly reported to occur in this species in the older literature [58,59], recent studies using sequence-based methods have found that *A. americanum* actually harbors a *Coxiella*-like bacterium [60,61]. Next-generation sequencing (NGS) approaches, which provide new means to exhaustively describe the bacterial communities found in ticks, have also revealed that *Coxiella*-like bacteria, and not *C. burnetii*, predominate in most tick species investigated thus far [62–64]. It therefore seems reasonable to assume that some of the strains initially identified visually as *C. burnetii* will be reclassified as *Coxiella*-like bacteria. Hence, the historic and dogmatic assertion that over 40 tick species are infected by *C. burnetii* [11] may be erroneous, and should be reevaluated when appropriate molecular data become available.

At present, there is still a substantial risk of misidentification given that the screening of ticks for *C. burnetii* frequently relies on the detection of a single gene, based on diagnostic PCR assays,

Box 2. Insights on Maternally Inherited Bacteria in Arthropods

Symbiosis, in which different species engage in long-term and intimate associations, is a ubiquitous feature of life. Arthropods, in particular, are known to engage in exceptionally diverse associations with specific bacterial endosymbionts that live exclusively within their cells and undergo maternal (transovarial) transmission to their offspring [118,119]. These heritable bacteria use specific adaptive strategies to spread and persist within arthropod populations, either providing fitness benefits to female hosts or subtly manipulating host reproduction. Two categories of endosymbioses are usually recognized, although intermediates and transitions are frequent. The first category consists of obligate (primary) mutualistic symbionts that are necessary to support normal host development and assist their host in various functions such as complementation of the diet. For example, most blood-feeding insects (e.g., bedbugs, kissing bugs, tsetse flies...) harbor obligate symbionts that provide B vitamins, which are necessary to complete their life cycle [118]. Many *Coxiella*-like bacteria of ticks seem to belong to this category. The second category consists of facultative (secondary) symbionts that are not required for host survival. Some protect against certain environmental stresses, such as heat or attack by parasitoids and pathogens [80,120]. Others are reproductive parasites that spread by increasing host reproduction through daughters (the transmitting sex) at the expense of reproduction through sons [121,122].

Overall, heritable endosymbiotic bacteria are of ecological and evolutionary importance to the particular arthropod species that are infected because they potentially mediate the acquisition of important ecological traits or drive changes in reproductive traits [118,122,123].

without confirmation by sequencing that the obtained PCR products are specific for *C. burnetii*. Indeed, while sequencing has revealed the presence of mutations specific to *Coxiella*-like bacteria, it has also highlighted genetic similarities between *Coxiella*-like bacteria and *C. burnetii*. As detailed in Table S1, the most routinely targeted genes are *IS1111* (a transposase insertion element for which PCR kits are commercially available), *sod* (superoxide dismutase), *icd* (isocitrate dehydrogenase) and *com1* (encoding a 27 kDa outer membrane protein). Interestingly, in studies in which several of these genes are amplified from the same tick samples, amplification may take place for a specific gene whereas another is not amplified (e.g., [27,42,65,66]), and this may suggest that the detected bacteria is not *C. burnetii*. Accordingly, a *Coxiella*-like bacterium found in *Bothriocroton auruginans* was shown to harbor an *IS1111*-like element 90% identical to the *IS1111* insertion sequence of *C. burnetii* [67]; as a result, the detection of *IS1111* may reveal infection by this *Coxiella*-like bacterium rather than by *C. burnetii*. Similarly, Reeves *et al*. [68] showed that a *C. burnetii sodB* gene amplified from the *Coxiella*-like bacteria found in *Carios capensis* displayed >92% identity with the *sodB* gene from *C. burnetii*; however, they did not detect *IS1111* nor *com1*. Conversely, high differences exist between *sod* gene sequences from *C. burnetii* and from the *Coxiella* endosymbiont of *A. americanum* (Genbank accession number CP007541). Taken together, these results suggest that *Coxiella*-like bacteria share genetic features with *C. burnetii*, but that the sequences in common are variable.

These methodological problems may be encountered with other supposedly *C. burnetii*-specific genetic markers because several genes used to detect *C. burnetii* have now been found in tick-borne *Coxiella*-like bacteria [53,65,69,70]. In recent years, remarkable progress has been made in designing new PCR-based techniques to detect *C. burnetii*. These promising methods include multiple-locus variable number tandem repeat (MLVA) analysis, multispacer sequence typing (MST), and SNP genotyping [71–75]. They can be used to rapidly and sensitively detect *C. burnetii* in a variety of clinical and environmental samples. These methods were developed using a broad panel of *C. burnetii* strains, but *Coxiella*-like bacteria, whose genotype profiles remain entirely uncharacterized, were not included. Consequently, the ability of these techniques to distinguish between *C. burnetii* and *Coxiella*-like bacteria needs to be further tested before they can be applied to tick samples.

Overall, PCR-based screening that does not use complementary PCR-product sequencing may not be specific enough to unambiguously identify *C. burnetii*, and may thus overestimate the prevalence of the pathogen in ticks.

Concluding Remarks and Future Perspectives

There is no doubt that ticks may be infected by *C. burnetii* in nature and that they may act as competent vectors (Figure 2, Key Figure). However, further field studies are needed to evaluate their vector capacity for *C. burnetii* under natural conditions. Generally, Q fever is probably far more frequently transmitted to humans and domestic ruminants via the airborne route than via ticks. Nevertheless, because ticks can parasitize a broad diversity of hosts that potentially disperse over large distances, they may act as major drivers of the heterospecific transmission and spatial dispersal of Q fever among vertebrates. Unfortunately, because *Coxiella*-like bacteria

Key Figure

Transmission Routes of *Coxiella burnetii* and *Coxiella*-like Bacteria.

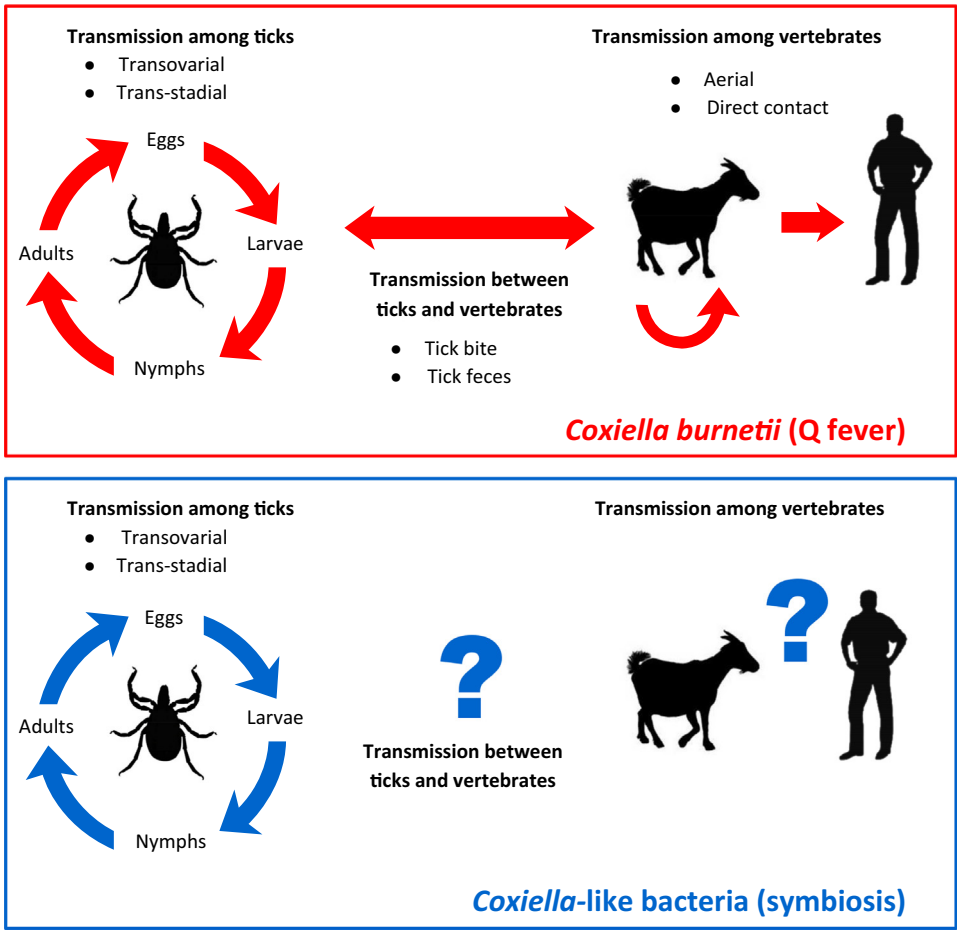

Figure 2. *C. burnetii* is a zoonotic pathogen responsible for Q fever. Infection results most commonly from the inhalation of aerosols or dust particles contaminated by small cell variants of *C. burnetii* produced from infected animals; however, in some cases the disease is also tick-borne, and *C. burnetii* transovarial and/or trans-stadial transmission has been described for some tick species. By contrast, *Coxiella*-like bacteria are almost exclusively found in ticks and likely behave as non-virulent tick symbionts; however, their pathogenicity for vertebrates remains to be formally tested. *Coxiella*-like bacteria are maternally transmitted in ticks, via the egg cytoplasm, and seem to be consistently maintained trans-stadially rather than through blood feeding on vertebrates.

Outstanding Questions

What is the specificity of the PCR-based techniques currently used to detect *C. burnetii*?

(i) How frequently does misidentification between *C. burnetii* and *Coxiella*-like bacteria occur?

(ii) Which genetic markers should be used to unambiguously differentiate *C. burnetii* from *Coxiella*-like bacteria?

Are *Coxiella*-like bacteria transmitted to vertebrates during the tick blood meal? If yes:

(i) Are they misidentified as *C. burnetii* when vertebrates are screened for Q fever infection using either direct or indirect (serological) tests?

(ii) Are they pathogenic for vertebrates?

How has *C. burnetii* evolved from a *Coxiella*-like ancestor?

(i) How did it acquire its virulence genes and its ability to infect vertebrate cells?

(ii) How did it become able to survive in the environment and be aerially transmitted?

Do *Coxiella*-like bacteria present in ticks interact with tick-borne pathogens?

(i) In particular, do they reduce the replication of tick-borne pathogens?

(ii) If yes, can they be used as biological tools to limit the vector competence of ticks for these pathogens?

are likely to have been misidentified as *C. burnetii* in past field studies, our knowledge of *C. burnetii* infection patterns in ticks has become unreliable (see Outstanding Questions Box). New means of detecting and characterizing tick-borne *C. burnetii* and *Coxiella*-like bacteria are clearly necessary to improve our understanding of Q fever epidemiology and evolutionary history.

Future studies should additionally focus on improving the specificity of the diagnostic tests used in vertebrates, including humans (see Outstanding Questions Box). Indeed, the presence of *Coxiella*-like bacteria in the salivary glands of ticks (Table 2) suggests that diverse *Coxiella* antigens could be inoculated into the vertebrate host during the tick bite. As a result, these antigens may prompt a cross-reactive serological response [76], and therefore lead to an overdiagnosis of Q fever in vertebrates. Both cases would lead to an overdiagnosis of Q fever in vertebrates. This point can be illustrated by recent work involving *Midichloria mitochondrii*, another maternally inherited endosymbiont of ticks. It is present in the salivary glands of the ticks, and is thus released during tick bites; consequently, seropositivity against *M. mitochondrii* is highly prevalent in humans bitten by ticks [77]. Future research about potential crossreactivity between *C. burnetii* and *Coxiella*-like bacteria will be necessary to better assess the specificity of diagnostic methods and screening tools currently used in vertebrates.

In addition, because *Coxiella*-like bacteria are present in tick salivary glands, they may be transmitted during blood meals and therefore directly represent an infection risk for vertebrates, including humans (see Outstanding Questions Box; Figure 2, Key figure). The overall probability that such tick-to-vertebrate transfers of *Coxiella*-like bacteria occur is high because ticks are found worldwide and feed on many different hosts. However, apart from occasional reports in pet birds [50–52], most *Coxiella*-like bacteria described to date are confined to ticks. The fact that these bacteria pose a much lower infection risk to vertebrates than does *C. burnetii* is supported by the fact that the genome of the symbiont of *A. americanum*, which is the only *Coxiella*-like bacteria genome available to date, is extremely reduced and devoid of known virulence genes [56]. Nonetheless, future research will be necessary to describe the diversity of *Coxiella*-like bacteria, characterize more fully their genetic relatedness, and assess their potential to cause infections in vertebrates.

Furthermore, the presence of *Coxiella*-like endosymbionts in ticks raises a series of exciting questions regarding their role in pathogen transmission (see Outstanding Questions Box). Interestingly, these bacteria may enhance or reduce the probability of not only *C. burnetii* infections but also that of other tick-borne pathogens. Some other maternally inherited bacteria (e.g., *Wolbachia* spp. and *Regiella insecticola*) have recently been found to act as defensive endosymbionts: they interfere with the replication and transmission of a wide range of pathogens in diverse arthropod hosts, including mosquitoes, flies, and aphids [78–80]. It has been suggested that they could eventually be used to limit the vector competence of blood-feeding arthropods [81,82]. In ticks, new symbiont-based approaches to controlling pathogen transmission may thus become feasible using *Coxiella*-like endosymbionts, which means current research efforts in this direction should be supported [83].

In conclusion, we propose that the study of *Coxiella*-like bacteria can advance our understanding of Q fever. Although *Coxiella*-like bacteria and *C. burnetii* are closely related, they vary in their ecology, as illustrated by the differences observed in transmission routes and infectiousness (Figure 2, Key Figure). This phenotypic diversity makes evolution in the genus *Coxiella* a topic of special interest, as it is also for the genus *Francisella* [84,85], because there are clearly transitions between pathogenic and non-pathogenic members. Recent investigations based on multilocus phylogenetic analyses and whole-genome sequencing data revealed that all known *C. burnetii* strains originated within the vast group of *Coxiella*-like endosymbionts and are the descendants of a *Coxiella*-like progenitor hosted by ticks [49]. Several evolutionary pathways may explain the acquisition of the genetic material necessary for this major lifestyle transition; this includes

spontaneous genetic mutations in the genome of a *Coxiella*-like ancestor, or the transfer and integration of virulence genes from a coinfecting pathogen. Some *Coxiella*-like organisms may have dynamic genomes as observed in many arthropod symbionts: although they reside in confined intracellular environments, arthropod symbionts commonly experience variable degrees of recombination and gene transfer with coinfecting bacteria [86–88]. These gene transfers may serve as immediate and powerful mechanisms of rapid adaptation and explain the evolutionary transition from a *Coxiella* tick-symbiont to the vertebrate pathogen *C. burnetii* [49]. In this context, comparative genomic approaches will be highly valuable in enhancing understanding of the evolutionary ecology of both *C. burnetii* and *Coxiella*-like bacteria and in identifying genes involved in virulence and tick symbiosis.

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Supplemental Information

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