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Vectors of Babesiosis

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Abstract

Babesiosis, caused by piroplasmid protozoans in the genus *Babesia*, is arguably the most important vector-borne disease of livestock and companion animals and is growing in importance as a zoonosis. Ixodid ticks were identified as vectors more than a hundred years ago, but the particular tick species transmitting some significant pathogens are still unknown. Moreover, it is only recently that the complexity of the pathogen–tick relationship has been revealed as a result of studies enabled by gene expression and RNA interference methodology. In this article, we provide details of demonstrated and incriminated vectors, maps of the current knowledge of vector distribution, a summary of established features of the pathogen life cycle in the vector, and an outline of molecular research on pathogen–tick relationships. The article concludes with a discussion of vector ecology and disease epidemiology in a global-change context and with suggestions for future research.

INTRODUCTION

Babesiosis is caused by an infection of erythrocytes by tick-borne protozoan members of the phylum Apicomplexa, order Piroplasmida, and genus *Babesia*. The vector role of ticks for these parasites was discovered by Smith & Kilbourne (90) in 1893, who were the first to demonstrate arthropod transmission of a disease agent.

Babesia spp. are predominantly parasites of mammals. While the best-known species cause disease in companion and food animals, many have been found in wild hosts, and a few are zoonotic. Descriptions of so-called new *Babesia* species are often based chiefly or solely on a fragment of the 18S ribosomal RNA gene (87). However, this locus can be an unreliable speciation diagnostic tool (4, 46, 80, 87) and, in the absence of accompanying biological data, can lead to incorrect reporting of *Babesia* spp. in new hosts, vectors, or geographical areas. In **Supplemental Table 1**, the identities of *Babesia* spp. that cause diseases of veterinary or medical importance have been based on both molecular taxonomy and biological characteristics, which are essential criteria for reliable speciation. Of the two species of equine piroplasm, *Babesia caballi* and *Theileria equi*, the latter is more widespread and economically important. *Theileria equi* was formerly known as *Babesia equi*; however, certain features of its life cycle resulted in its eventual transfer to the genus *Theileria* (100), and it is not considered further in this review.

The economic impact of babesiosis is considerable, particularly in bovines (including buffalo), but equines, small ruminants, and companion animals are also affected. Furthermore, there is growing interest in *Babesia* spp. as zoonotic agents. Disease occurs when the rate of erythrocyte infection and loss exceeds the rate of their replacement, giving rise to anemia with its attendant health issues. Additionally, debris and toxins released as a result of erythrocyte destruction may adversely affect organ systems. Erythrocytes infected by some species (*B. bovis*, *B. canis*) can be sequestered in brain capillaries, causing cerebral babesiosis. The host immune response plays an important part in the pathogenesis of babesiosis through immune-mediated erythrocyte lysis and overproduction of pharmacologically active agents, especially cytokines. These can cause many circulatory effects, including vasodilatation, vascular stasis, lowered blood pressure, edema, and intravascular coagulation (12, 55, 94, 102). Surviving hosts tend to become carriers and a source of infection for ticks, and, in the case of *B. microti*, some zoonotic strains can be transmitted from human carriers both congenitally and via blood transfusions.

This review focuses on *Babesia* species of veterinary and medical importance and addresses vector identity, the biology of transmission, tick–parasite relations, the geographical distribution of vectors, and the role of vectors in the epidemiology of babesiosis in a globally changing environment.

IDENTITY OF THE VECTORS

Numerous ixodid tick species have been listed as vectors in the literature (37), but many of these reports were based on a perceived association with the disease rather than on an objective demonstration of transmission. Sources of inaccuracy also include misidentifications of vectors and parasites, strain differences in vector competence (53, 81), and an overreliance on molecular tools. In this review, the vector status of a tick species is accepted only if there is epidemiological relevance (e.g., correlation of tick presence with disease occurrence) as well as supporting experimental transmission studies. Reports of pathogen DNA in whole ticks, whether unfed or engorged, are not acceptable on their own as evidence of vector capacity. Demonstrations of *Babesia* DNA in salivary glands, eggs, or unfed larvae (9, 88), while more convincing, also require confirmation.

There are 22 confirmed vectors of 18 *Babesia* species that infect agricultural or companion animals or humans (**Supplemental Table 2**). All ixodid genera are represented, with the conspicuous

DEFINITION OF MULTIPLE FISSION

Multiple Fission is cell division in which the nucleus first divides into several equal parts, followed by cytoplasmic division into as many cells as there are nuclei. It is a form of cell division that characterizes members of the phylum Apicomplexa, to which *Babesia* spp. belong.

exception of *Amblyomma*. Most tick species transmit just one economically or medically important *Babesia* species, and most *Babesia* spp. are transmitted by relatively few tick species; although *B. caballi*, the cause of equine babesiosis, has 7 confirmed vectors. Although humans can be accidental hosts of ixodid ticks (31), very few species (all of them *Ixodes* spp.) transmit zoonotic *Babesia* spp. Thirty additional *Babesia*–vector associations, with epidemiological transmission credibility but without confirmation, have been reported (**Supplemental Table 3**).

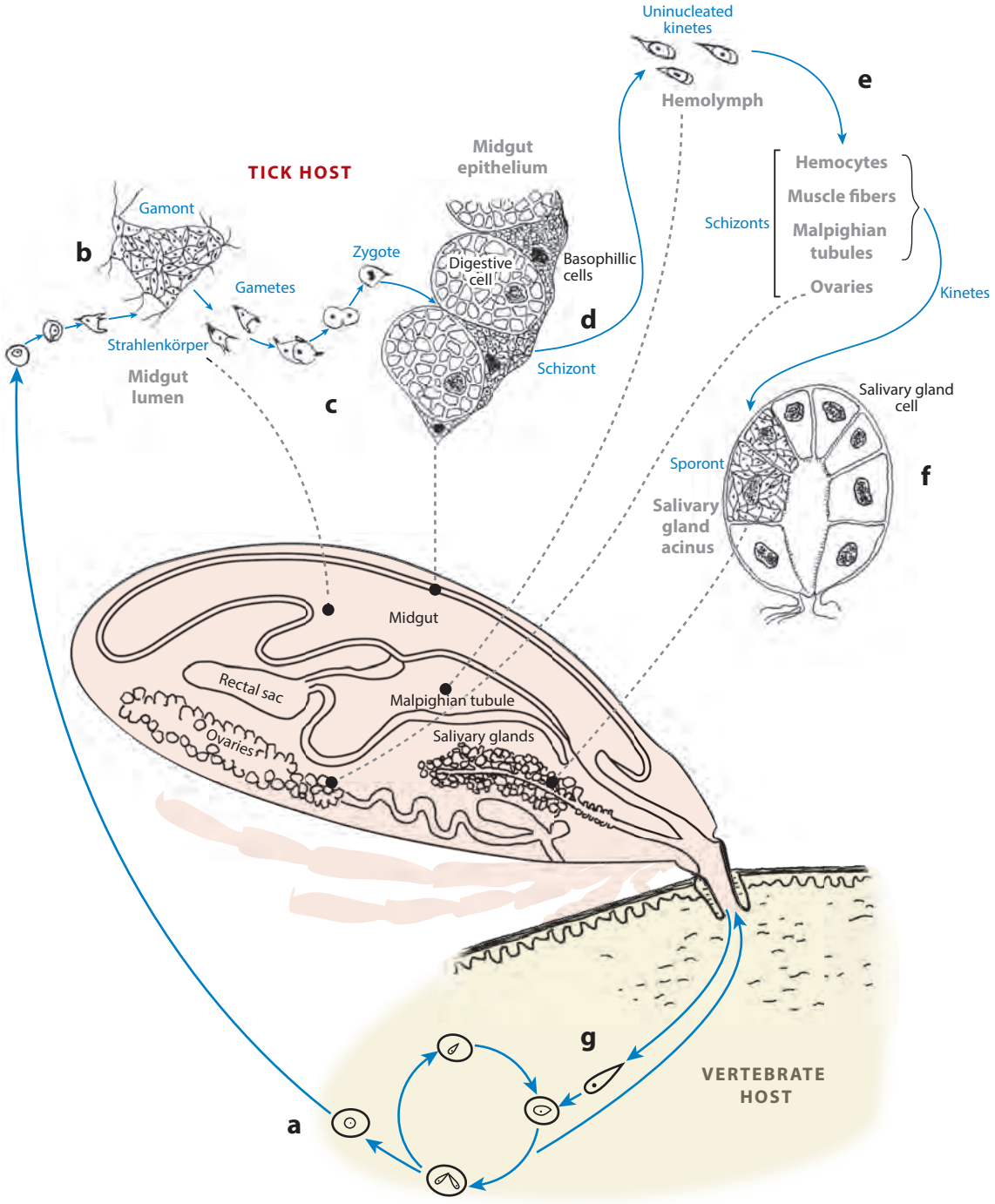
TRANSMISSION OF *BABESIA* SPP.

Mechanics of Transmission

Knowledge of the series of events in the transmission of *Babesia* parasites to and from vertebrate hosts by ixodid ticks emerged many years after the basic facts were known about *Plasmodium* spp. in mosquitoes. As suspected, they are very similar, consisting of the development of sexual stages in the vertebrate bloodstream and the formation of zygotes in the gut of the vector, followed by multiple fission (see the sidebar titled Definition of Multiple Fission) in various tick tissues, and culminating in the development of infective stages in the salivary glands. There is still discussion about variation in the cycle across the genus, but a basic scheme (**Figure 1**) based on many studies (40, 54, 63, 64, 73, 74, 81, 82, 86, 89) is now generally accepted.

Most *Babesia* species can invade the ovaries of the tick and persist in the resultant larvae so that the infection is transmitted vertically (transovarial transmission), whereas in *B. microti*, no transovarial transmission takes place (45, 92). In this case, the parasites are acquired from hosts by larvae or nymphs and can be transmitted only transstadially. For the *Babesia* species in which transovarial transmission occurs, it has long been asserted that the infection can be acquired only by female ticks. However, several tick species feed as immatures on the relevant *Babesia* hosts and thus in principle have the opportunity to acquire infections. In fact, de Waal & Potgieter (27) demonstrated nymphal *Rhipicephalus evertsi* acquisition of *B. caballi*, Higuchi et al. (50) reported sexual reproduction of *B. ovata* in nymphal *Haemaphysalis longicornis*, and Bonnet et al. (14) showed that *Ixodes ricinus* larvae and nymphs can become infected with *B. divergens* in an artificial feeding system. Immature stages are possibly less likely to become infected because they ingest smaller volumes of blood. However, their reduced number and size of midgut epithelial basophilic cells (**Figure 1**), thought to be important for parasite development, may also be a factor (1).

Although transstadial survival occurs in most *Babesia* spp., not all stages can transmit the parasite. For example, only larvae transmit *B. bovis*, while transmission of *B. bigemina* does not occur until the nymphal and adult stages. In other cases, immature stages do not feed on hosts that are susceptible to the relevant *Babesia* species (e.g., *Dermacentor reticulatus*, some *Hyalomma* spp.) (see **Supplemental Table 2**). *Babesia microti* can survive transstadially in both larvae and nymphs, but persistence does not exceed one molt (45). In contrast, many *Babesia* spp. (*B. divergens*, *B. major*, *B. ovata*, *B. motasi*, *B. rossi*, *B. vogeli*, *B. venatorum*) can apparently persist from the larval to the adult stage of their vectors for at least one generation without reinfection (see **Supplemental Table 2**).



(*Caption appears on following page*)

Figure 1 (*Figure appears on preceding page*)

Generalized *Babesia* life cycle. (*a*) Gametocytes (thought to be rounded in appearance) develop within erythrocytes in the host blood stream. (*b*) Gametocytes enter the tick midgut lumen, emerge from erythrocytes, and develop into polymorphic (spherical to pyramidal) Strahlenkörper that go on to divide by multiple fission to form gamonts. (*c*) Gamonts produce gametes that fuse to form zygotes. (*d*) Zygotes invade basophilic midgut epithelial cells and undergo multiple fission, forming schizonts, which produce kinetes. (*e*) Kinetes infect numerous organs and tissues, where they undergo more multiple fission (in many *Babesia* spp., the ovaries are also infected, leading to transovarial transmission). (*f*) In the salivary glands, kinetes undergo sporogony, forming sporonts, ultimately producing sporozoites. (*g*) Infective sporozoites are injected into the bloodstream of a vertebrate host during tick feeding and invade erythrocytes, where they initiate cycles of asexual reproduction by asynchronous binary fission. Some elements of the figure have been adapted, with permission, from Bock et al. (12).

Molecular Aspects of Transmission

The development of new tools such as tick cell lines and RNA interference methodology (RNAi) in gene expression studies now makes it possible to investigate factors determining vector competence at the molecular level (6, 22). An early study that used RNAi in *Ha. longicornis* infected with *B. gibsoni* showed that a specific parasite ligand might bind to the vitellogenin receptor on the surface of tick oocytes, whereby vitellogenin gains entry to oocytes, thus enabling parasite invasion and subsequent transovarial transmission (13). More recent studies suggested that a homolog of TROSPA, a receptor for outer surface proteins of *Borrelia burgdorferi* sensu lato (s.l.) (70), might also serve as a receptor for ligands of *B. bigemina* (5, 66). Other molecules thought to facilitate infection of ticks with *Babesia* spp. include homologs of subolesin (57, 65, 108), serum amyloid A, and calreticulin (5).

TICK–PATHOGEN RELATIONS AND COINFECTIONS

Pathological Effects

Babesia spp. invade a range of tissues in their tick vectors, and deleterious effects might therefore be expected. Indeed, there are several accounts of tick mortality resulting from infection with *Babesia* spp. (25, 26, 41, 44, 53, 81, 104). However, it is notable that all these examples involved artificial infections, and field studies suggest that under natural circumstances, an adaptive tolerance usually exists between vector ticks and *Babesia* spp. (17, 18, 33).

Resistance of Ticks to Infection

Unlike insects, in which nutrient digestion takes place in the midgut lumen, enzyme digestion of nutrients in ticks occurs in the gut epithelial cells, which may favor the survival and multiplication of ingested microorganisms and explain why ticks transmit a greater variety of pathogens than any other group of arthropods (91). Nevertheless, various innate immune mechanisms, consisting of both cellular and humoral components, protect ticks from invasion by microbes. The most important cellular components are the hemocytes, which are responsible for phagocytosis, nodule formation, and encapsulation and which come into play once the microbes have penetrated the gut epithelium and entered the hemocoel (95). However, hemocytes are not known to control infections of *Babesia* spp.; rather, they seem to facilitate the transport and multiplication of *Babesia* kinetes, because cultures of *R. microplus* hemocytes can be used as cell lines for the cultivation of *B. bigemina* (23). Humoral components—specifically, antimicrobial molecules (47)—are more effective against *Babesia* spp. For example, cystatin, an inhibitor of cysteine proteases, obtained from the tick *Ha. longicornis* arrested the growth of *B. bovis* in vitro (105), and an antimicrobial

Table 1 Molecular factors produced by ixodid ticks in response to infection with *Babesia* spp.

Tick species	Protein/peptide	Produced in response to	Effect on *Babesia*[a]	Reference(s)
Haemaphysalis longicornis	Longicin, longipain	*Babesia gibsoni*	Reduces transmission	98, 99
Ha. longicornis	HlLRR	*B. gibsoni*	Inhibits growth in vitro	61
Ha. longicornis	Cystatin	*B bovis*	Inhibits growth in vitro	105
Ha. longicornis	Longicin	*B. microti*	Inhibits parasites in mouse model	98
Rhipicephalus annulatus R. microplus	Ricinusin, microplusin	*B. bigemina*	Inhibition? (Genes overexpressed in infected ticks)	5
Ha. longicornis	Vitellogenic receptor	*B. gibsoni*	Facilitates transovarial transmission	13
Ha. longicornis	BmVDAC	*B. gibsoni*	Enhances infection? (Gene overexpressed in infected ticks)	84
R. annulatus	Calreticulin, serum amyloid A, TROSPA	*B. bigemina*	Increases pathogen load in ticks	5, 66
R. microplus	Subolesin, Q38	*B. bigemina*	Increases pathogen load in ticks	65
Hyalomma lusitanicum	Subolesin	*B. bigemina*	Increases pathogen load in ticks	108
R. haemaphysaloides	Subolesin	*B. microti*	Enhances infection? (Gene overexpressed in infected ticks)	57

[a]In most cases, the evidence for observed effects on the parasites is indirect and is derived from vaccine, RNA interference methodology, or gene expression experiments.

peptide (longicin), excreted by the *Ha. longicornis* midgut, significantly reduced parasitemias of *B. microti* in mice (98). Furthermore, RNAi silencing of the gene expressing longicin in the tick resulted in increased acquisition of *B. gibsoni* from infected dogs (98). Similar results were obtained with longipain, a cysteine protease, also secreted by the midgut of *Ha. longicornis* (99). Antunes et al. (5), comparing gene expression in ticks infected with *B. bigemina* with uninfected ones, found that the defensin ricinusin was overexpressed in infected *R. annulatus* and *R. microplus*. However, because gene knockdown did not have any effect on pathogen levels, this protein does not seem to have a role in controlling *B. bigemina* infections in these particular tick species.

Infection Enhancement

As discussed in the section titled Transmission of *Babesia* Spp. above, several proteins expressed by *Babesia*-infected ticks have a role in amplifying infections. Further molecules are down- or upregulated in infected ticks, with as yet unknown functions (5, 48, 49, 76, 77). To date, there are no reports of specific interactions between *Babesia* spp. and salivary gland proteins, as there are for some other tick-borne pathogens (78). A summary of the proteins and peptides involved both in the transmission of *Babesia* spp. and in tick resistance to these parasites is presented in **Table 1**.

In addition to the cross-talk between pathogen and vector, the process of tick transmission can be affected by interaction with the vertebrate host immune system. Such immunomodulatory effects may be induced by the feeding tick or by the infecting pathogen. Ixodid ticks secrete a variety of molecules into the feeding lesion in order to maintain the flow of blood and tissue fluids over several days, and some of these molecules may also facilitate the invasion, survival, and proliferation of tick-borne pathogens, including *Babesia* spp. (103). There is also evidence that immunosuppression induced by the pathogen may increase the success of tick feeding, thus enhancing additional pathogen transmission. This situation has been described for *B. bovis* and

R. australis in cattle (16) and for *B. microti* and *I. trianguliceps* in bank voles (*Myodes glareolus*) (79). In contrast, there are indications that in cattle that have developed resistance to ticks, transmission of *B. bovis* and *B. bigemina* is compromised (35). While resistance to ticks curtails tick feeding, thus reducing transmission of pathogens, host resistance to the pathogens themselves may also increase in this situation (10, 56).

Coinfections

Ticks harbor a large and diverse microbiome, and the constituent microbes probably act as a defense against pathogens (19). By the same token, it is possible that certain tick-borne pathogens may affect the susceptibility of ticks to others. The much-studied castor bean tick, *I. ricinus*, transmits at least ten different pathogens of note in Europe (83), at least four of which are babesias (*B. microti*, *B. divergens*, *B. venatorum*, and *B. capreoli*). Theoretically, these species can all coinfect an individual tick, though *B. divergens* is associated with agricultural habitats, and the others with woodland. To date, there is no information on possible interactions between these species within the vector.

Whereas *R. microplus* and *R. annulatus* are capable of transmitting both *B. bigemina* and *B. bovis*, the closely related *R. decoloratus* transmits only *B. bigemina*, despite being exposed to both parasites. This might be because *B. bovis* has appeared within the *R. decoloratus* geographical range relatively recently as a result of the spread of *R. microplus* and has not coevolved sufficiently with *R. decoloratus* for transmission to occur (24, 97). A similar situation may occur in West Africa, where both *B. bigemina* and *B. bovis* are reportedly present and where *R. microplus* has recently been introduced (21). The indigenous species in the region, *R. geigyi*, is suspected of transmitting both *B. bigemina* and *B. bovis* (2, 3).

The interaction of pathogens within the vertebrate host is beyond the scope of this review, except insofar as the nature of any interaction may be determined by tick transmission. For example, if two pathogens occur in ticks at different infection rates, then, in theory, the development of disease may be regularly affected by the superimposition of one infection on the other. This mechanism was suggested for the apparent immunosuppressive effect of *Anaplasma phagocytophilum* on subsequent *B. divergens* infections, resulting in acute babesiosis in cattle (107). Although some observations support this concept (75, 96), there is as yet no reliable experimental evidence.

VECTOR ECOLOGY

Current Distribution

The tick vectors of *Babesia* spp. occur in the tropics, Mediterranean, and Holarctic regions. The distribution of proven vectors is shown in **Figure 2** (vectors of the two most economically important forms of the disease—bovine and canine babesiosis) and **Supplemental Figure 1** (vectors of equine, small ruminant, and human babesiosis). In some cases, vector distribution is far more extensive than that of the relevant babesiosis—for example, *I. scapularis* and *B. microti* babesiosis (**Supplemental Figure 1*c***). The reverse is true for canine *B. gibsoni* babesiosis, which has a wider distribution than the confirmed vector, *Ha. longicornis*, although *R. sanguineus* s.l. is probably another vector; additionally, many cases apparently result from direct transmission in fighting dog breeds (55).

The one-host cattle ticks, formerly in the genus *Boophilus* and now *Rhipicephalus*, are all vectors of pathogenic *Babesia* spp. *Rhipicephalus microplus* is evidently a species of Asian origin (59) but now also colonizes Central and South America and parts of Africa. *Rhipicephalus australis*, previously

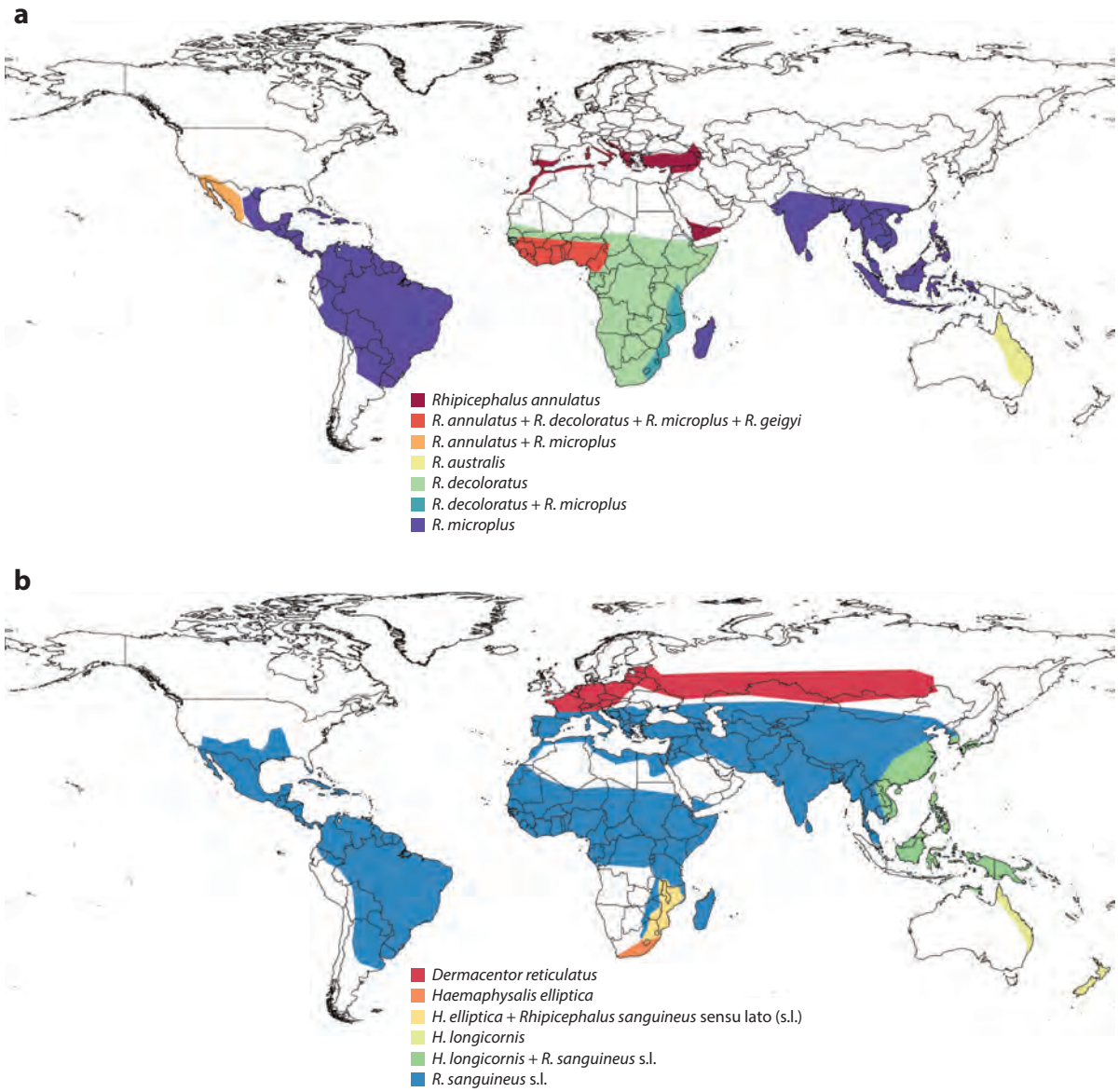


Figure 2

Geographical distributions of tick vectors of (*a*) bovine and (*b*) canine babesiosis. Panel *a* displays only the distribution of boophilid ticks, which transmit the most economically important bovine species, *Babesia bigemina* and *B. bovis*. The distributions of other bovine babesiosis vectors are shown in vector distribution maps for equine, small ruminant, and human babesiosis in **Supplemental Figure 1*a–c***. The relevant bovine babesiosis vectors may be identified from **Supplemental Table 2**.

confused with *R. microplus* but recently reinstated as a species (32), occurs in Australia, New Caledonia, and countries in Southeast Asia. In view of the long-lasting confusion between *R. microplus* and *R. australis*, reexamination of the ecological requirements of these two species is essential. The related *R. decoloratus* is exclusively African and occurs south of the Sahara in woodland with montane vegetation, while *R. geigyi* is reported primarily in the western part of a strip

extending between parallels 5°N and 18°N and is associated with woodland and lowland rain forest with secondary grassland but is absent from deserts and the most humid parts of Africa. *Rhipicephalus annulatus* colonizes semiarid areas in most countries of the Mediterranean basin, with a broken distribution in Sahelian Africa (30). This species was introduced into Central America, where it colonizes dry zones of Mexico and localized regions in the southern United States, replacing *R. microplus* in drier areas. Several of these boophilid species occur sympatrically with one another, mainly as a result of introductions.

The Mediterranean region consists of a complex assemblage of vegetation and climate, but the *Babesia* vector species that occur here have broadly similar ecological preferences and commonly coexist on the same host, often with overlapping periods of activity. They include *R. bursa*, *R. sanguineus* s.l., *Hyalomma marginatum*, and *Ha. punctata*. *Haemaphysalis punctata* also occurs in parts of the central and western Palearctic. *Rhipicephalus bursa*, *R. sanguineus* s.l., and *Hy. marginatum* transmit pathogenic *Babesia* spp. to small ruminants, horses, and dogs, respectively, whereas *Ha. punctata* transmits a relatively nonpathogenic species (*B. major*) to cattle. *Haemaphysalis elliptica* from southern Africa occupies more tropical habitats and is the vector of the canine pathogen *B. rossi*. *Haemaphysalis elliptica* was previously known as *Ha. leachi*, a name now applied to a more northern species (7). The vector competence of *Ha. leachi* for *B. rossi* is unknown. It should also be noted that not all members of the *R. sanguineus* s.l. species complex are necessarily competent vectors of canine *Babesia* spp.

Several *Babesia* vectors occur in the temperate Holarctic and are characterized by life cycles with marked seasonality imposed by climate. The most common tick in Europe is *I. ricinus*, a vector of both bovine and human babesiosis, and is distributed from Ireland to the Urals and from northern Sweden to northern Spain and the Atlantic coast of Portugal (62). It has also been recorded in Northern Africa, but this may be a sister species. *Ixodes ricinus* requires a relative humidity of at least 80% to survive during its off-host periods and is therefore restricted to areas with vegetation that retain a high humidity. Typical habitats vary across Europe and include deciduous and coniferous woodland, heathland, moorland, and rough pasture (42). The second most frequently reported tick species in Europe is *D. reticulatus* [a vector of both canine and equine babesiosis (63)], which occurs in the Eurasian part of the temperate and boreal biomes, from Portugal to the east of Kazakhstan (85). Preferred habitats are open areas and meadows with shrub or forest, often close to periodically flooded areas (34). *Dermacentor reticulatus* has a highly focal distribution divided into a western (France to eastern Germany) and an eastern (eastern Poland and Belarus to the Central Siberian Plateau) population divided by a region in central Europe extending from the Baltic Sea coast to central Germany at 12–13°E, to western Poland at 19°E, and to the southern border of Hungary, where *D. reticulatus* is absent (85).

In the Nearctic, *I. scapularis*, the main vector of human babesiosis caused by *B. microti*, is associated with forests in the northern United States and southern Canada. It also occurs in the southeastern United States, where its role as a vector of zoonotic pathogens seems to be limited, probably because of reduced questing above the leaf litter due to higher temperatures than further north (39). Other Nearctic *Babesia* vectors include *R. sanguineus* s.l., a vector of canine babesiosis, and *D. nitens*, *D. albipictus*, and *D. variabilis*, all of which transmit equine babesiosis.

Epidemiology and Global Change

There are many global drivers, such as uncontrolled movements of livestock and wildlife, deforestation, uneven application of tick control measures, and a warmer climate, that can influence the distribution range of *Babesia* vectors, thus altering the epizootiology of the transmitted diseases. Rates of tick development, regulated by temperature, and the periods of questing for hosts,

determined by endogenous and exogenous mechanisms such as diapause and weather, respectively, are key to tick life cycles (43, 69) and can affect the number of generations per year, the abundance of ticks, and thus the potential *Babesia* infection rates of hosts. Anthropogenic factors underlie most changes in disease epidemiology of domestic animals, including tick control measures, the consequences of their relaxation or breakdown, and the introduction of vectors and hosts into new regions. For example, large-scale disruption to compulsory tick control dipping schemes occurred in Zimbabwe during the country's civil war in the 1960s and 1970s and resulted in the death of an estimated one million cattle. However, by the end of the war, 15 years later in 1980, enzootic stability had emerged in some areas, with the majority of surviving cattle immune to babesiosis (68). This observation led to a reassessment of the value of radically reducing tick populations (unless the aim is eradication) and to the integration of strategic dipping with vaccination and the use of tick-resistant cattle (72).

A successful eradication scheme in the southern states of the United States, based on dipping and targeting *R. microplus* and *R. annulatus* (introduced vectors of *B. bigemina* and *B. bovis*), was concluded in 1943, but there are still periodic incursions from endemic areas in Mexico of these tick species on exotic ungulates [e.g., nilgai (*Boselaphus tragocamelus*)] escaped from game ranches and on increasingly abundant wild deer (*Odocoileus virginianus*) (15, 38, 71).

The factors and mechanisms responsible for more recent changes in the distributions of *Babesia* vectors are not so clear-cut. For example, upon introduction to a new region, *R. microplus* tends to replace the local boophilid fauna, thus affecting the epidemiology of bovine babesiosis. This tick was first discovered in Ivory Coast in 2007 (60) and later recorded in northern Benin (21), which has relatively drier conditions. In less than a decade, it colonized more than half of Benin. A similar spread of *R. microplus* has been observed in South Africa (97), Zambia (11), and East Africa (58). Tønnesen et al. (97) speculated that the shorter cycle of *R. microplus* and assortative mating (which reduces the sterilizing effects of hybridization) might give *R. microplus* an advantage over *R. decoloratus* in southern Africa. Other factors could include a higher population growth potential in warm areas of high rainfall and the tendency of *R. microplus* to induce cattle resistance to the indigenous species (93).

Ixodes ricinus, the vector of *B. divergens* in Europe, has increased in abundance and extended its geographical range over the last few decades (43). Notwithstanding, a dramatic decline in the incidence of bovine babesiosis has occurred during the same period. For example, in Ireland, annual incidence declined progressively from an estimated 1.7% in 1985 to 0.45% in 1994 to 0.06% in 2013. Similar trends have been observed elsewhere in Europe, such as in Norway and Hungary (106). This apparent contradiction may be due to the fact that two habitats are involved; while the primary habitat of *I. ricinus* throughout Europe is woodland, the ticks transmitting bovine babesiosis are maintained by cattle at pasture. The observed decline in disease is likely to be due chiefly to a reduction in farmland tick habitat, as well as changes in the nature of the cattle industry, particularly in Ireland. Additionally, the cattle-dependent tick population may also have been affected by the widespread use of macrocyclic lactone anthelmintics with some acaricidal activity, although as yet, there is no scientific evidence to support this hypothesis.

Ixodes ricinus transmits *B. divergens* to humans, but the disease is too rare (<50 cases) for any epidemiological trends to be discerned, other than the preponderance of cases occurring in immunocompromised patients (46). *Ixodes ricinus*–transmitted zoonotic *B. microti* babesiosis cases are even less common, with just two confirmed autochthonous European records (8, 51). In contrast, *B. microti* transmitted by *I. scapularis* causes several hundred cases annually on the Eastern Seaboard and in the U.S. Midwest. The incidence of this disease has increased exponentially over the last five decades (101), apparently owing to the spread of the vector, which in turn is generally

attributed to the increased abundance of white-tailed deer (*O. virginianus*) (102). However, white-footed mice (*Peromyscus leucopus*), not deer, are the main competent reservoirs for the parasite and, since *B. microti* is not transmitted transovarially, deer are most likely not responsible for the introduction of the parasite into new areas. At present, there is little understanding of the mechanisms involved in the spread of zoonotic *B. microti*, but a study published in 2014 suggests that it is facilitated in some way by prior establishment of the Lyme borreliosis spirochete *Borrelia burgdorferi* sensu stricto, which utilizes the same vector and reservoir host(s) (28).

The expansion of *D. reticulatus* into new regions has been well documented in Europe (34), and various explanations include transportation by increasing numbers of terrestrial hosts such as deer (*Cervus elaphus*, *Capreolus capreolus*) and wild boar (*Sus scrofa*) and changing agricultural practices that create suitable tick habitats (67). However, while these mechanisms are likely to be significant, the most important factor is probably rising temperatures facilitating completion of the tick life cycle in a single year, which is essential for survival of this tick species (43). New foci of canine and equine babesiosis are also regularly reported, but these foci are not always mirrored by those of the vector, because subclinical infections are common and the parasites can survive in ticks in the absence of dogs or horses (34).

CONCLUSIONS

The basic life cycles of *Babesia* species in their ixodid tick vectors are now understood, and major advances have been made in elucidating the molecular details of transmission, with exciting implications for new control technologies (22). Progress has also been made with predictive models that attempt to determine tick-borne disease outcomes in various environmental change scenarios (20, 29, 43, 52). However, a mechanistic understanding of the observed changes in babesiosis epidemiology remains limited (34, 94, 97, 101, 106).

In view of the increasing detection of so-called new *Babesia* species in ticks and hosts by molecular means, resolving the confusion surrounding aspects of *Babesia* molecular taxonomy is essential

FREDERICKS AND REHLMAN'S MODIFIED KOCH'S POSTULATES

1. A nucleic acid sequence belonging to a putative pathogen should be present in most cases of an infectious disease. Microbial nucleic acids should be found preferentially in those organs or gross anatomic sites known to be diseased, and not in those organs that lack pathology.
2. Fewer, or no, copy numbers of pathogen-associated nucleic acid sequences should occur in hosts or tissues without disease.
3. With resolution of disease, the copy number of pathogen-associated nucleic acid sequences should decrease or become undetectable. With clinical relapse, the opposite should occur.
4. When sequence detection predates disease, or sequence copy number correlates with severity of disease or pathology, the sequence-disease association is more likely to be a causal relationship.
5. The nature of the microorganism inferred from the available sequence should be consistent with the known biological characteristics of that group of organisms.
6. Tissue-sequence correlates should be sought at the cellular level: efforts should be made to demonstrate specific in situ hybridization of microbial sequence to areas of tissue pathology and to visible microorganisms or to areas where microorganisms are presumed to be located.
7. These sequence-based forms of evidence for microbial causation should be reproducible.

(4, 87). For most of these poorly defined *Babesia* spp., as well as for many recognized pathogenic species, knowledge of vector identity is incomplete. The main reason for this knowledge gap is the widespread overreliance on uncritical detection of pathogen DNA in ticks, while properly controlled transmission studies are rarely if ever carried out owing to logistical difficulties and/or lack of funding. New rules for the identification of *Babesia* vectors are therefore required—possibly, a version of Koch's postulates modified for the twenty-first century (36) (see the sidebar titled Fredericks and Rehlman's Modified Koch's Postulates).

FUTURE ISSUES

1. Vector competence of ticks for *Babesia* spp. should be determined by properly controlled experimental transmission studies, following, where possible, the (modified) principles of Koch's postulates.
2. Studies that aim to assess the (changing) epidemiology and distribution of babesiosis should ensure unambiguous identification of pathogens and vectors by DNA sequence analysis of multiple loci of sufficient length.
3. Predictive mapping of disease prevalence should develop methods to include the presence and overall movements of wild reservoir hosts of both the pathogens and their vector ticks.
4. In vitro tick culture and artificial feeding systems should be further developed and refined to elucidate species-specific details of parasite development in the vector tick.
5. Gene expression and RNAi studies should be pursued to explore details of vector–pathogen relationships, particularly in relation to novel control methods.

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