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# Coinfection by the tick-borne pathogens *Babesia microti* and *Borrelia burgdorferi*: ecological, epidemiological and clinical consequences

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## Abstract

*Ixodes* ticks maintain a large and diverse array of human pathogens in the enzootic cycle, including *Borrelia burgdorferi* and *Babesia microti*. Despite the poor ecological fitness of *B. microti*, babesiosis has recently emerged in areas endemic for Lyme disease. Studies in ticks, reservoir hosts and humans indicate that coinfection with *B. burgdorferi* and *B. microti* is common, promotes transmission and emergence of *B. microti* in the enzootic cycle, and causes greater disease severity and duration in humans. These integrative studies may serve as a paradigm for the study of other vector-borne coinfections. Identifying ecological drivers of pathogen emergence and host factors that fuel disease severity will help guide the design of effective curative and prevention strategies.

### Keywords

Babesia; babesiosis; Borrelia; coinfection; Lyme disease; tick

## *Ixodes*-borne pathogens as a model system to study vector-borne coinfection

Wildlife and humans are frequently infected by multiple pathogens or several genotypes of a single pathogen [1, 2]. Coinfecting pathogens can interact among themselves and with host

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symbionts for utilization of host resources or through modulation of host immunity [3]. Positive interactions (facilitation) may favor the emergence of an invading pathogen or increase the prevalence of an established pathogen. Negative interactions (competition) may prevent the establishment of an invading pathogen or favor the extinction of an established pathogen. The emergence of a pathogen often involves multiple positive interactions on several ecological scales, i.e., within the host, between hosts, and across areas and/or regions. The suitability of the host ('niche'), the transmission rates between hosts, and the strength of pathogen propagule pressure (the number of organisms dispersing to a new region), determine the risk for pathogen dispersal, establishment and colonization. The complexity of coinfection with multiple pathogens in multiple hosts is best addressed by modeling the network of pathogen-vector-host interactions [1, 2, 4, 5]. Often incomplete due to the paucity of data on more than a few pathogens, these models have overlooked the role of vectors in co-transmitting pathogens and the importance of spatial and temporal variations in vector-host contact rates [5].

In this review, we focus on human pathogens transmitted by ticks of the *Ixodes ricinus* species complex (thereafter *Ixodes*). These pathogens can serve as a useful model for the study of coinfection because they are commonly found together in reservoir hosts, ticks and humans. These reservoir hosts often are infected with *Borrelia burgdorferi sensu lato*, and therefore constitute an 'infected niche' that is encountered by any of the other *Ixodes*-transmitted pathogens [6, 7]. We review the pathogen combinations found in *Ixodes* ticks and reservoir hosts around the world, but focus on coinfection with *B. burgdorferi sensu stricto* and *Babesia microti*, the major etiologic agents of Lyme disease and human babesiosis in the United States, respectively [8, 9]. Both pathogens impose a significant health burden on human populations. An interdisciplinary approach to *B. burgdorferi* and *B. microti* coinfection has been conducted on multiple levels and provides an opportunity to start exploring how processes that favor emergence of an *Ixodes*-borne pathogen are linked across ecological scales. Specifically, we discuss the importance of this coinfection to the transmission of *B. microti* from reservoir hosts to tick, the emergence of *B. microti* within *B. burgdorferi* endemic areas, and the severity of concurrent tick-borne disease.

## Coinfection with *Ixodes*-borne pathogens is prevalent worldwide

Ticks in the *Ixodes ricinus* species complex have a holoartic distribution but are restricted to the regions with temperate climates [6, 10]. Four species within this complex account for most of the transmission of human *Ixodes*-borne pathogens: *Ixodes pacificus* is primarily found along the Pacific coast of the United States; *Ixodes scapularis* in the Northeast, upper Midwest and South of the United States; *Ixodes ricinus* across Europe, in parts of northern Africa, Turkey and the Caucasus; and *Ixodes persulcatus* from northwestern Russia to the Pacific coast of Asia (Figure 1). *Ixodes* ticks can co-transmit several human pathogens, including spirochetal bacteria (*B. burgdorferi sensu lato* and *B. miyamotoi*), rickettsial bacteria (*Anaplasma phagocytophilum* and *Ehrlichia muris*-like agent), flaviviruses (tick-borne encephalitis virus and deer tick virus) and protozoan parasites (*B. microti*, *Babesia duncani*, *Babesia divergens* and *Babesia venatorum*) [8, 9, 11–15].

Coexistence of multiple pathogens in *Ixodes* ticks and rodent reservoirs has been reported from the United States, Europe and Asia [15–23]. Most coinfections involve two of the three major human pathogens, namely *B. burgdorferi sensu lato*, *A. phagocytophilum* and *Babesia* spp. Such dual coinfections occur in 1% to 28% of ticks in Lyme disease endemic areas in the United States and in up 13% of *Ixodes* ticks in Europe [6]. Coinfection in non-human vertebrate hosts greatly varies by location. Dual coinfections as defined above have been noted in as many as three fourths of rodents in the northeastern United States [6]. In Europe, coinfection with *Francisella tularensis* and either *B. burgdorferi* or *A. phagocytophilum* has been observed in 7% of rodents [6]. A meta-analysis revealed that coinfection with *B. burgdorferi* and *A. phagocytophilum* occurs in as many as two thirds of their vertebrate reservoirs, whether domestic animals or wildlife [21].

As predicted by the geographic distribution of *Ixodes* ticks (Figure 1) and the prevalence of coinfection in *Ixodes* ticks and reservoir hosts, coinfections of humans with *Ixodes*-borne pathogens have been reported from the United States, Europe and Asia [6, 24–26]. In the United States, the most frequent *Ixodes*-borne pathogens are *B. burgdorferi sensu stricto*, *B. microti* and *A. phagocytophilum*, the causative agent of human granulocytic anaplasmosis (HGA). Coinfections with various combinations of two or three of these pathogens have been described. Up to a fifth of Lyme disease patients experience concurrent babesiosis [27–30] and approximately a tenth experience concurrent HGA or hard tick-relapsing fever (caused by *B. miyamotoi*) [6, 27, 31–35]. The incidence of coinfections that involve *Ehrlichia muris*-like agent or deer tick virus (Powassan virus type II) is difficult to assess because case reports have been few [14, 36]. In Europe, cases of Lyme disease with concurrent babesiosis or HGA are much less common than in the United States because single infections with *A. phagocytophilum* or Babesia spp. are less common [24, 25, 37]. Tick-borne encephalitis is prevalent in parts of Central and Eastern Europe, and so is concurrent Lyme disease and tick-borne encephalitis [15, 24, 25]. The remainder of this review will focus on coinfection with *B. burgdorferi* and *B. microti* in the enzootic cycle and in humans.

## Coinfection with *B. burgdorferi* and *B. microti* is prevalent in the Northeast and upper Midwest of the United States

Single and concurrent *B. burgdorferi* and *B. microti* infections have been well documented in ticks and enzootic hosts (Table 1). Among ticks, the prevalence of *B. burgdorferi* and *B. microti* coinfection ranges from 0% to 13% in nymphs and from 2% to 13% in adults [22, 38–46]. The prevalence of coinfection tends to be greater in rodents, ranging from 6% to 41%, because they are exposed to multiple tick bites during their lifetime [40, 47–49]. Whether among *I. scapularis* ticks or enzootic hosts, *B. burgdorferi* is consistently more prevalent than *B. microti*. Of the 20 studies or datasets within studies that we reviewed (Table 1), four reported a greater probability of coinfection with *B. burgdorferi* and *B. microti* than expected based on the individual prevalence of each pathogen whereas none reported a lower probability of coinfection. The other studies did not include a statistical analysis of the frequency of coinfection. The most extensive field study noted that coinfection with *B. burgdorferi* and *B. microti* was 1.83 times more frequent in host-seeking

nymphs than predicted by chance [17]. A positive interaction also was observed for *I. scapularis* larvae that had fed on small mammals but not on meso-mammal, sciurid or avian hosts [17], indicating that coinfection in ticks is most closely associated with coinfection in small mammals such as *Peromyscus leucopus* mice. Overall, these cross-sectional studies suggest that coinfection is common in ticks and enzootic hosts, and that coinfection may provide a survival advantage for both pathogens.

Epidemiological and clinical studies of acute tick-borne illnesses in residents of southern New England and New York have shown that the frequency of Lyme disease patients experiencing concurrent babesiosis ranges from 2% to 19% whereas the percentage of babesiosis patients experiencing concurrent Lyme disease varies between 6% and 23% [27–30, 50]. Studies of coinfection based on serologic evidence provide the advantage of large study populations but usually include subjects with resolved infection and fail to distinguish between concurrent infection and sequential infection. In southern New England, the frequency of sera reactive against *B. burgdorferi* and *B. microti* antigens was 7.5% in Lyme disease patients [51] and 5.3% in patients enrolled with acute febrile illness and suspected anaplasmosis [52]. In the Midwest, a lower seroprevalence of 4.2% against both pathogens was noted in patients experiencing acute Lyme disease [53]. Factors that may contribute to the variation in coinfection frequency include geographic and temporal differences in tick density, prevalence of infected ticks, risk of human exposure to ticks, susceptibility to disease in humans, methodology that identifies concurrent or sequential infection, and case definition [26, 30, 31, 54].

## The delayed emergence of babesiosis occurs within Lyme disease endemic areas

The first case of babesiosis caused by *B. microti* in the United States was identified in 1969 in a immunocompetent individual who had summered on Nantucket Island, Massachusetts [9]. Shortly thereafter, the disease was recognized on other islands off the coast of southern New England and on the mainland, particularly in coastal counties ranging from Massachusetts to New Jersey. Another endemic area was identified in the upper Midwest. In the following two decades, the number of cases reported to local public health departments steadily increased. In 2011, when babesiosis became a nationally notifiable disease, more than 1,100 cases were reported to the CDC [55]. By 2013, this number had reached nearly 1,800. The recent emergence of babesiosis is due to an increased incidence in areas known to be endemic for decades [50, 56] but also results from the geographic spread of *B. microti* into new territories situated on the edge of well-established endemic areas [57–59]. Of note, the geographic expansion of *B. microti* has been restricted to areas already endemic for Lyme disease (Figure 2). Thus, although the recognition of babesiosis in the United States preceded the identification of Lyme disease as a new medical entity by more than a decade, the geographical expansion of babesiosis has lagged behind that of Lyme disease [8, 9].

Approximately 30,000 confirmed and probable cases of Lyme disease are reported annually in the United States, mostly from the Northeast and the upper Midwest [60, 61]. Given that *B. burgdorferi* and *B. microti* are transmitted by the same vector and share a range of vertebrate hosts, the relatively low incidence of babesiosis when compared with that of

Lyme disease is intriguing. Epidemiological biases that contribute to an underestimation of the incidence of babesiosis include the greater difficulty in diagnosing babesiosis due to the lack of a distinctive clinical sign such as the erythema migrans rash and the insufficient awareness and/or reporting of babesiosis by physicians in newly endemic areas [9]. Biases in diagnosis, however, are expected to narrow as clinicians and the public become more aware of the disease. Despite epidemiological biases, the delay in the emergence of babesiosis is most likely explained by ecological bottlenecks that restrict the dispersal of *B. microti* or impede the colonization of host reservoir populations. Identifying the ecological drivers of emergence that overcome these ecological bottlenecks should help us predict the spatial and temporal gains of this epidemic and design interventions to curb its trajectory.

## Emergence of *B. microti* despite low ecological fitness: a paradox?

Ecological factors that contribute to the relatively low incidence of babesiosis include low fitness of *B. microti* in the enzootic cycle, as indicated by poor transmission from *P. leucopus* mice to larval ticks, poor transstadial transmission from larvae to nymphs [54, 62], and a restricted host range when compared with *B. burgdorferi* [40]. The enzootic cycles of *B. burgdorferi* and *B. microti* are shown in Figure 3. Low fitness reduces the probability of *B. microti* establishment in new areas and contributes to low infection prevalence of *B. microti* in ticks and hosts in endemic areas. An integrated measure of pathogen fitness is the basic reproductive number ($R_0$), a parameter which indicates whether a pathogen establishes ($R_0>1$) or fades out ($R_0<1$) when introduced into a population of naive, fully susceptible hosts [63, 64]. The magnitude of $R_0$ also predicts the risk of emergence. The $R_0$ for *B. microti* was estimated to be lower than the $R_0$ for *B. burgdorferi* but also lower than the threshold for pathogen persistence ($R_0 <1$) under ecological conditions identified in long-term field studies [65]. Thus, the poor ecological fitness of *B. microti* appears at odds with the persistence and geographic expansion of this pathogen in the Northeast and upper Midwest of the United States.

Sites that have long been endemic for both *B. burgdorferi* and *B. microti* provide clues to elucidate the ecological factors that have led to the emergence of babesiosis. Long term epidemiological studies at several of these sites revealed a high incidence of babesiosis relative to that of Lyme disease [39, 56]. A 10-year epidemiological study on Block Island documented that the incidence of babesiosis is one third that of Lyme disease [56]. In the towns of Lyme and Old Lyme in southeastern CT, the incidence of babesiosis is half that of Lyme disease whereas the pathogen prevalence in nymphal ticks is similar [39]. The paradox between the low ecological fitness of *B. microti* and the relatively high prevalence of *B. microti* at these long established endemic sites led to the concept that *B. burgdorferi* lowers the ecological threshold for *B. microti* establishment by removing one or several of the ecological bottlenecks.

## Coinfection increases the suitability of *P. leucopus* as reservoir host for *B. microti*

*B. burgdorferi* is highly prevalent among reservoir hosts in endemic areas [7]. As *B. microti* infected nymphs disperse into *B. burgdorferi* endemic areas that are *B. microti* free, they

most likely inoculate *B. microti* into reservoir hosts that are infected with *B. burgdorferi*. Using a tick-host-pathogen laboratory model, Dunn *et al*. [54] observed that coinfection with *B. burgdorferi* and *B. microti* significantly increases *B. microti* parasitemia in *P. leucopus* mice and that larval ticks become infected with *B. microti* in greater numbers when fed on coinfected hosts. Increased *B. microti* transmission was readily observed when the *B. burgdorferi* strain had a disseminating phenotype. A possible explanation is that the host immune response to disseminating spirochetes is not restricted to the skin and therefore may interfere with the splenic immune response required to control and eventually clear *B. microti* infection. It has long been established that the Th1 arm of the host adaptive immune response is critical for host resistance to *B. microti* [66–69]. This concept has been extended from inbred mouse strains to natural reservoir hosts by a recent genetic study of field voles (*Microtus agrestis*) [70]. Using a tick-host-pathogen model, Zeidner et al. [71] observed that the infestation of C3H/HeJ mice with *B. burgdorferi*-infected nymphal ticks severely depresses Th1 cytokine (interferon-γ and IL-2) production while increasing Th2 cytokine (IL-4) production. This shift toward a Th2 profile is not seen in disease-resistant BALB/c mice. Thus, coinfection with *B. burgdorferi* and *B. microti* may create an immunological conflict as the adaptive immune response to *B. burgdorferi*, an extracellular bacterium, hinders the adaptive immune response to *B. microti*, an obligatory intracellular parasite [9, 72]. This conflict may increase *B. microti* fitness, resulting in higher parasitemia and increased transmission to feeding ticks.

## From individual hosts to populations: increased transmission as a driver of pathogen emergence

Successful establishment of *B. microti* requires adequate propagule pressure from *B. microti* infected ticks or hosts dispersing from well-established endemic areas as well as suitable naïve hosts residing within the newly colonized areas. By fitting the coinfection-driven enhancement of *B. microti* transmission in a mathematical model, including field-derived parameters, such as tick feeding behavior and tick burden on hosts, Dunn *et al*. [54] predicted that $R_0$ values for *B. microti* are strongly increased when a large proportion of the reservoir host population is coinfected and when larval and nymphal ticks feed synchronously. Thus, models that integrate data from carefully executed longitudinal studies with data on key ecological parameters, including changes in niche suitability, can predict the likelihood of pathogen emergence. When combined with data on propagule pressure, these models can predict spatial and temporal patterns of pathogen spread. In Box 1, we depict how the likelihood of pathogen emergence ($R_0$) is a function of pathogen interactions within reservoir hosts that affect host-to-tick transmission rates and of ecological parameters such as host community composition, tick abundance and feeding behavior that affect tick-host contact rates.

## Concurrent babesiosis exacerbates and prolongs the symptoms of Lyme disease

Initial case reports suggested that concurrent Lyme disease and babesiosis is associated with severe illness. Each of the first three reported cases of Lyme disease and babesiosis

coinfection was admitted to the hospital [73–75]. One patient required blood transfusion and multiple joint aspirations for a recurrent swollen knee, another developed pulmonary edema despite appropriate antibiotic therapy, and a third died of fatal pancarditis. The first prospective study of Lyme disease and babesiosis coinfection compared the clinical outcome of 214 patients with Lyme disease, 10 with babesiosis, and 26 with both illnesses occurring simultaneously [28]. Patients with these concurrent illnesses experienced a greater number of symptoms for a longer duration than patients with Lyme disease alone. No difference in the number or duration of symptoms was noted between coinfected patients and patients with babesiosis alone. The synergism between *B. burgdorferi* and *B. microti* was limited to the early phase of illness as coinfected patients experienced disseminated disease (arthritic, cardiac, or neurologic symptoms of more than two weeks duration) as often as patients with Lyme disease alone. The same research team used the same inclusion/exclusion criteria and case definitions to enroll 89 Lyme disease patients, 26 babesiosis patients and 64 coinfected patients in a follow-up study [27]. As in the initial study [28], coinfected patients experienced a greater number of symptoms for a longer duration than those with Lyme disease alone. In this follow-up study [27], coinfected patients experienced fewer symptoms for a shorter duration than those with babesiosis alone. In sum, concurrent babesiosis increases the severity of acute Lyme disease whereas concurrent Lyme disease has no effect or even a suppressive effect on the clinical manifestations of babesiosis.

Long-term sequelae of Lyme disease were investigated in a retrospective longitudinal cohort study of 171 patients who experienced Lyme disease, 37 of whom had serologic evidence of previous *B. microti* infection at the time of Lyme disease diagnosis [76]. The latter group of patients may have experienced babesial infection before or after Lyme disease rather than concurrently. At follow-up examination one or more years after acute illness, no differences were found in the frequency of long-term sequelae between the two groups.

## The implications of coinfection in the clinical management of tick-borne diseases

Concurrent babesiosis needs to be considered for any Lyme disease patient who experiences an illness more severe than expected, especially when the patient does not respond well to recommended antibiotic therapy [77]. Antibiotics prescribed for Lyme disease are ineffective in the treatment of babesiosis. Early diagnosis of coinfection with *B. microti* is therefore critical, especially in immunocompromised hosts [9]. Lyme disease patients with concurrent HGA also experience a greater number of symptoms for a longer duration than patients with Lyme disease alone [27, 30], although one study reported no such difference [32]. The retrospective diagnosis of concurrent HGA is less problematic, however, because doxycycline, the antibiotic often prescribed for Lyme disease, is effective against HGA [77]. No studies have demonstrated that coinfection with *B. microti* or *A. phagocytophilum* contributes to the long-term complications associated with Lyme disease [76, 78]. Moderate to severe disease has been described for other coinfections, including those involving three pathogens [27] but such cases have been too infrequent to draw any inference regarding the relationship between pathogen interaction and disease severity in these settings.

## Future Directions

Lyme disease and babesiosis are expected to remain serious health threats and other tick-borne diseases are likely to emerge. Predicting the enzootic emergence of tick-borne pathogens will help anticipate the rise in tick-borne disease incidence and may help develop ecological and public health measures to manage these health threats.

### Modeling the enzootic emergence of tick-borne pathogens

Comprehensive empirical studies and modeling frameworks are needed to investigate how coinfection in reservoir hosts affects pathogen invasion, spread, and persistence in specific ecological settings (Figure 4) [69, 79–81]. Definite evidence for pathogen interactions will be obtained from longitudinal studies of pathogen dynamics and experimental pathogen removal in natural populations [82]. The modeling of $R_0$ and population dynamics should be expanded to incorporate pathogen interactions (facilitative and competitive) and spatially explicit environmental parameters [54, 83–86]. Given that the modeling of enzootic invasion is hindered by sparse historical datasets on vector and reservoir host infection, spread parameters may need to be derived from the phenomenological modeling of human case time series data. Phylogeographic studies enabled by whole genome sequencing [87] can help capture pathogen interactions by modeling differential evolutionary and epidemiological dynamics [88–90]. Lastly, a community ecology network approach should be developed to study multiple pathogens within and between reservoir hosts and ticks [1, 4, 91].

### Assessing the incidence and health burden of concurrent tick-borne diseases

Prospective studies should assess the frequency of tick-borne coinfections, characterize the effect of coinfections on disease severity in the short and long term, develop the most cost effective clinical and laboratory algorithm for the diagnosis of multiple tick-borne infections, and identify antibiotic regimens that are best suited for the treatment of coinfections. These studies should take into account many possible confounding factors including, (i) geographic differences in tick density and infection rates, (ii) pathogen genetic variations that may influence disease severity, as documented for *B. burgdorferi* alone and *B. burgdorferi* and *B. microti* coinfection [54, 92, 93], (iii) age and immune status of the patient population at study sites, (iv) use of antibiotic therapy because the clinical outcome of coinfection will differ if only one of two or more concurrent infections is treated, and (v) case definitions because their stringency may impact the recorded frequencies and clinical outcomes of coinfection and single infection [26, 31].

### Characterizing the immunological conflicts imposed by and beneficial to coinfecting pathogens

The host immune response to coinfection with tick-borne pathogens should be investigated in humans and natural reservoir hosts. Clinical studies should aim (i) to elucidate the pathogenesis of single infection and coinfection, and (ii) to identify biomarkers and predictors of severe illness in both settings. When reagents to characterize the inflammatory and immune response in *P. leucopus* mice become available, studies should be carried out to identify mechanisms by which coinfection with *B. burgdorferi* increases *B. microti*

parasitemia. Given that natural reservoir hosts can become infected with tick-borne pathogens in a sequential manner, studies should address whether *B. microti* parasitemia is increased by *B. burgdorferi* when the latter pathogen is introduced before or after *B. microti*. To identify key factors that underlie host susceptibility to single infection and coinfection, the genetic diversity of wild and laboratory-maintained *P. leucopus* mice should be assessed and linkage studies conducted. Nutritional and social stressors should be accounted for in wild and laboratory *P. leucopus* populations, respectively [94, 95].

## Concluding Remarks

The geographic expansion of *Ixodes scapularis* has greatly contributed to increase the incidence of tick-borne diseases in the United States. Of the six human pathogens that *Ixodes scapularis* transmits separately or in combination [9, 11, 14, 27, 28, 33, 56, 96], we focused on *B. burgdorferi and B. microti* because coinfection with these two pathogens is frequent in the enzootic cycle and causes severe disease in humans. The emergence of babesiosis on the heels of Lyme disease is explained, in part, by the effect of coinfection on pathogen transmission and emergence, thereby validating the relevance of complex models to predict the trajectory of epidemics. Multiple pathogens transmitted by a single vector but residing in a community of wildlife hosts can be engaged in a web of interactions that facilitate emergence and spillover to humans. Disease caused by coinfection often is difficult to manage and may impose a serious health burden. Many unknowns remain (see Outstanding Questions Box), in part because progress has been hindered by a single or a dual pathogen approach rather than a ‘systems’ oriented approach. As additional tick-borne pathogens emerge, coinfections will become increasingly common, thereby justifying the need for large, multidisciplinary teams that tackle the many facets of emergence and possibly co-emergence of infectious pathogens.

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## GLOSSARY

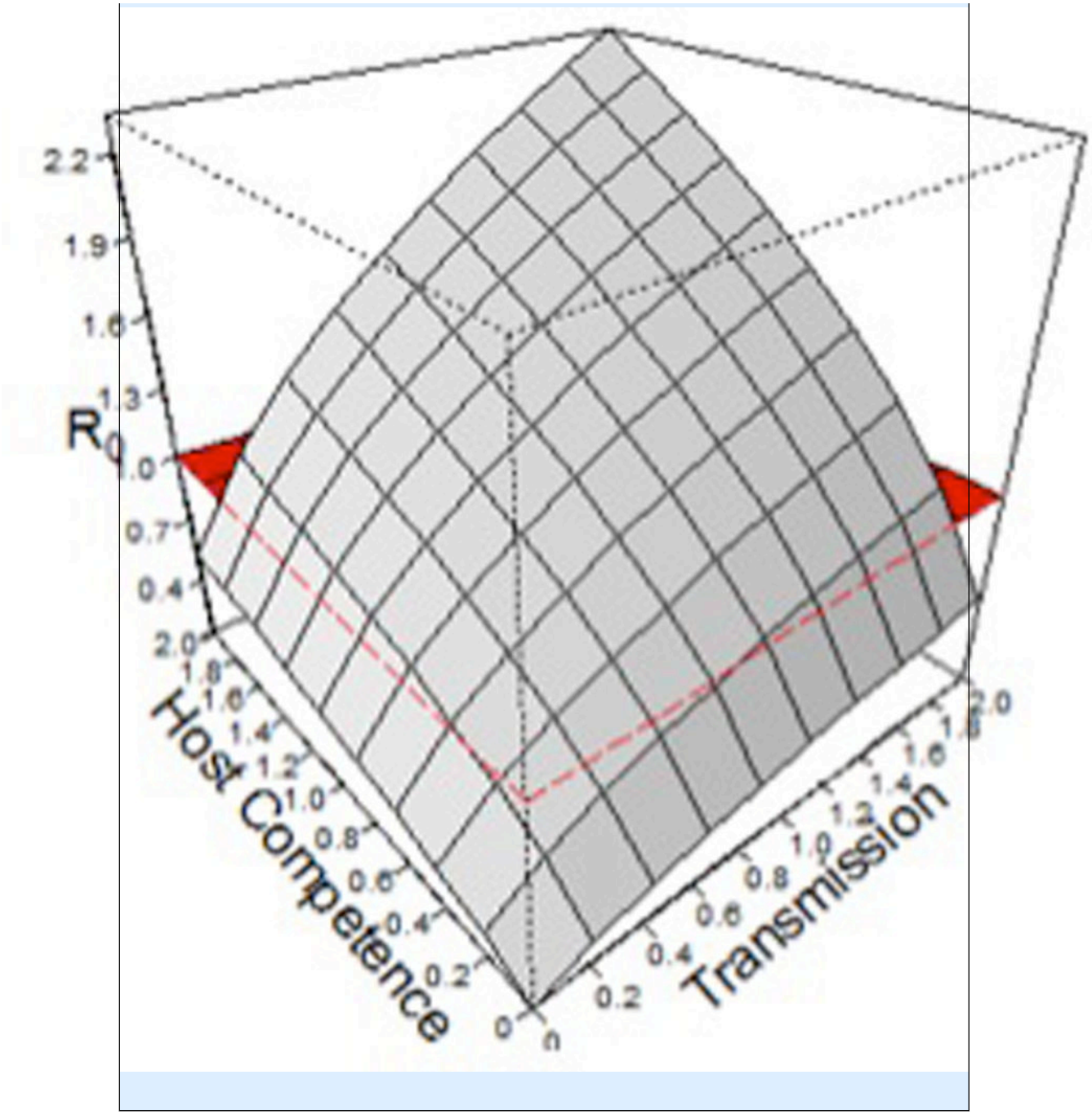
| | |
|---|---|
| **Basic reproductive number** | A standard measure of pathogen transmissibility. The basic reproductive number ($R_0$) measures the average number of secondary cases caused by a pathogen in a completely susceptible population. $R_0$ is also considered a measure of population fitness and it can be interpreted as the likelihood that a pathogen will become established ($R_0 > 1$) or become extinct ($R_0 < 1$) when introduced into a population of naive, fully susceptible hosts. |
| **Coinfection** | Simultaneous infection of a host by two or more pathogen species or strains. |
| **Ecological fitness** | Individual reproductive success. It equals the average contribution to the gene pool of the next generation. |
| **Emerging infectious disease** | An infectious disease whose incidence has increased in the past twenty years or threatens to emerge in the near future. Emerging infections may spread to new geographic areas or host populations. |
| **Host niche** | Vertebrate hosts are considered as niches for pathogens. We use the Hutchinsonian niche definition, i.e., an n-dimensional hypervolume for which the dimensions are environmental conditions and resources (in this case, within the host), that define the requirements of an individual or a species to practice "its" way of life, more particularly, for its population to persist. |
| **Propagule pressure** | A composite measure of the number of organisms of a species released into a region to which they are not native. |
| **Transmission** | A population-level process that integrates the susceptibility (likelihood of becoming infected by a pathogen, given exposure to a potentially infectious dose), infectiousness (likelihood of transmitting the pathogen to other host individuals) and contact rates between pathogens and hosts. |

## Box 1

### Coinfection in reservoir hosts alters the odds of emergence of *Ixodes*-borne pathogens

Establishment of a dispersing *Ixodes*-borne pathogen can be facilitated or compromised by the presence of another pathogen maintained in the same enzootic cycle. In laboratory experiments, infection of *P. leucopus* mice with *B. burgdorferi* increases transmission of *B. microti* [54] but decreases transmission of *A. phagocytophilum* from mice to feeding *I. scapularis* ticks [99]. At the population level, establishment of a pathogen depends on ecological factors such as the timing of tick activity, the diversity and competence of the host community, and the presence of other pathogens in reservoir hosts. The basic reproduction number, $R_0$, is an integrated measure of the likelihood of pathogen spread and establishment into a population of naïve, fully susceptible hosts. $R_0$ values >1 predict pathogen persistence or establishment whereas $R_0$ values <1 predict extinction or failure to establish (Figure I). The next-generation matrix approach represented a significant advance in the modeling of $R_0$ for tick-borne infections [63]. This model has been extended to include tick seasonal activity patterns [65] and host-to-tick infection dynamics [100].

**Figure I. The likelihood of establishment by a pathogen into a new area ($R_0$) is determined by the probability of a tick feeding on a competent host ('host competence') and the efficacy of pathogen transmission from host to tick ('transmission').** 'Host competence' captures the ecological context by integrating the composition of the host community and the intensity and timing of tick-host contact rates [54]. Baseline values for host competence and host-to-tick transmission are derived from those reported in [54] and set to 1 in this figure. The slopped surface of $R_0$ values was generated by calculating $R_0$ for all combinations of host competence and transmission values, above and below the defined baselines, using the formulation as described in [54] and fitted to field data obtained from an endemic site (Block Island, RI). The red surface represents the set of $R_0$ values equal to 1, which is the threshold above which the pathogen is expected to emerge and below which the pathogen is expected to go extinct. The outcome of coinfection depends on the direction of pathogen interaction within hosts and on the ecological setting. For example, enhanced transmission due to coinfection may not suffice for pathogen emergence in a low host competence setting. Conversely, even if coinfection reduces pathogen transmission, pathogen establishment may occur in areas with high host competence.



**TRENDS BOX**

- Coinfection by *Borrelia burgdorferi*, the primary agent of Lyme disease and *Babesia microti*, the primary agent of babesiosis is a useful model to study vector-borne pathogen interaction.
- *B. burgdorferi* increases *B. microti* transmission from *Peromyscus leucopus* mice to ticks. Coinfection likely contributes to the emergence of babesiosis in areas endemic for Lyme disease.
- The frequency of concurrent Lyme disease and babesiosis varies temporally and geographically. The number and duration of symptoms are greater in coinfected patients than in those experiencing Lyme disease alone.
- Modeling the emergence of tick-borne infections should incorporate pathogen interactions within realistic epidemiological and ecological contexts.

**Box 2**

### Outstanding questions

- Which coinfection combinations favor pathogen survival and persistence and which favor pathogen decline in reservoir hosts?
- Which mechanisms account for the increase (or decrease) in tick-to-host and host-to-tick transmission?
- How do pathogen interactions among themselves or with symbionts in reservoir hosts affect pathogen emergence and geographic spread?
- Can the emergence of a tick-borne pathogen in the enzootic cycle signify the decline of another?
- Which modeling approaches are best to capture the effect of pathogen/vector/host interactions on pathogen transmission, emergence and geographic spread?
- Is the illness caused by coinfection usually more severe than the illness caused by single infection? Is the effect of coinfection limited to a subset of symptoms? Does coinfection promote long term manifestations of disease?
- What are the immune and non-immune mechanisms that underlie the effects of coinfection on pathogen survival and disease severity?
- What is the optimal algorithm for the diagnosis of multiple tick-borne infections?
- What are the appropriate treatments for multiple tick-borne diseases?

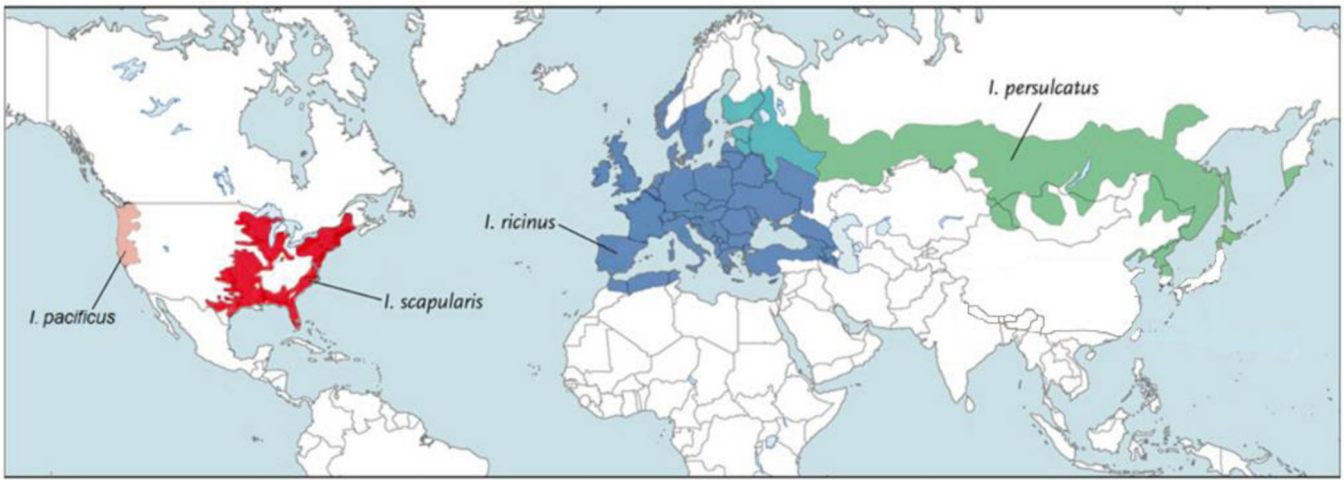



**Figure 1. Geographic distribution of *Ixodes* ticks involved in human disease**

The vast majority of *Ixodes*-borne human diseases are transmitted by four species of the *Ixodes ricinus* species complex [10]. In the United States, *Ixodes pacificus* is found along the Pacific coast (orange) whereas *Ixodes scapularis* is encountered along the eastern seaboard and inland into the upper Midwest and across the South down to the Gulf of Mexico (red). *I. pacificus* is a competent vector for *Borrelia burgdorferi* but its role in the transmission of *Babesia duncani* is uncertain. *I. scapularis* maintains three major human pathogens in their enzootic cycle, namely *B. burgdorferi, Babesia microti* and *Anaplasma phagocytophilum*. Other human pathogens of lesser incidence include *Borrelia miyamotoi, Ehrlichia muris*-like agent and deer tick virus (Powassan virus type II). In most of Europe (blue), *I. ricinus* is the major *Ixodes* vector for transmission of human pathogens, including several Borrelia spp. (notably *B. burgdorferi*, *B. afzelli* and *B. garini*), *A. phagocytophilum*, several *Rickettsia* spp. (notably *R. helvetica* and *R. monacensis*), three Babesia spp. (*B. divergens*, *B. venatorum* and *B. microti*) and tick-borne encephalitis virus (TBEV) [15]. *I. ricinus* also is found in parts of North Africa, Turkey and the Caucasus. *Ixodes persulcatus* is sympatric with *I. ricinus* around the Baltic Sea and in northwestern Russia (turquoise), but is the predominant vector across southern Russia into the Far East (green). *I. persulcatus* is well known as a vector for TBEV, but also can transmit *Borrelia burgdorferi sensu lato, A. phagocytophilum, Ehrlichia muris*, and several Babesia species including *B. divergens, B. venatorum* and *B. caucasica* [23, 97]. Adapted from Brown et al. [98].

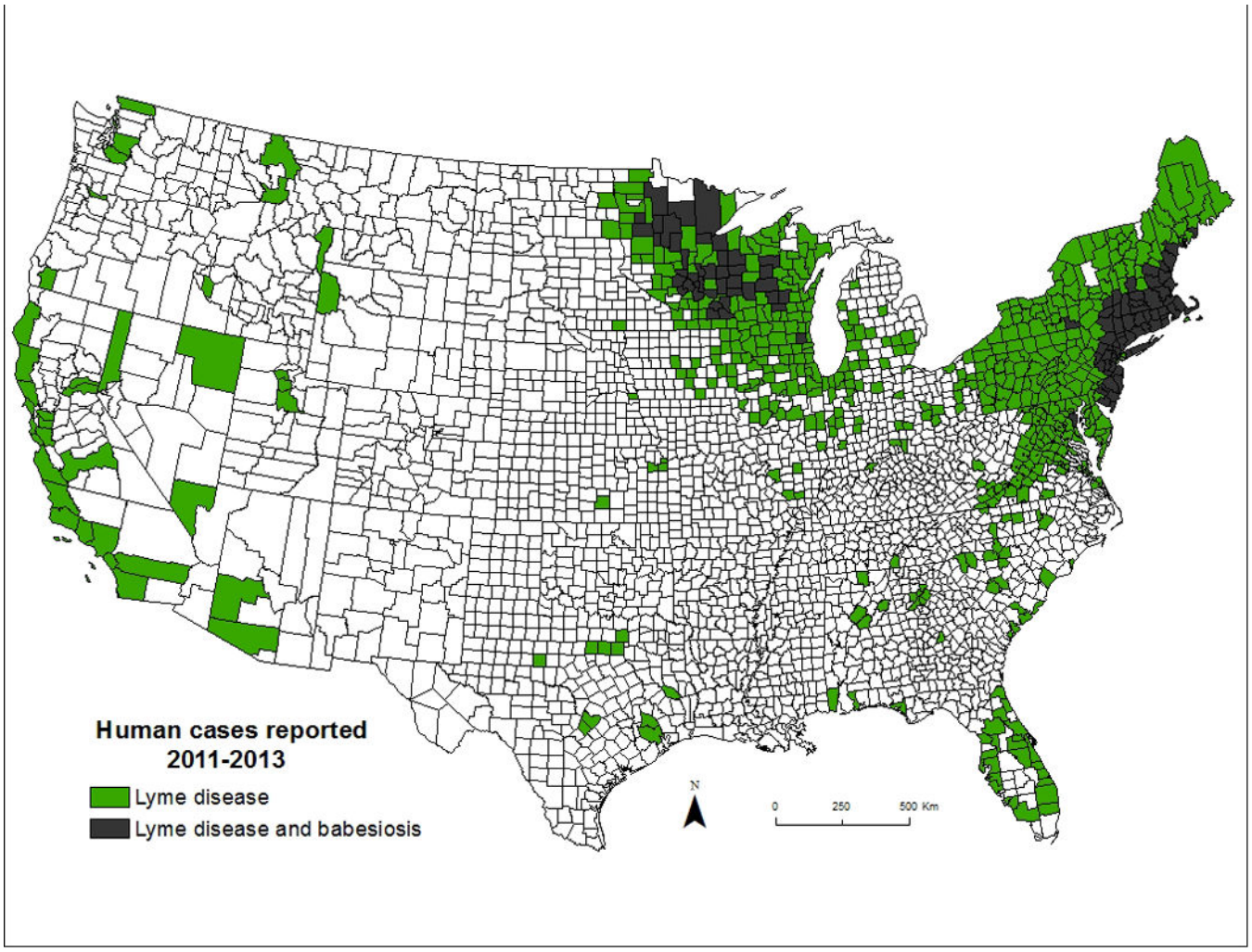



**Figure 2. Human babesiosis is emerging in areas endemic for Lyme disease**

Lyme disease and human babesiosis have been nationally notifiable conditions since 1991 and 2011, respectively. The names of counties that reported cases of Lyme disease and/or babesiosis from 2011 to 2013 were obtained from the Centers for Disease Control and Prevention. Counties with three or more cases of Lyme disease but fewer than three cases of babesiosis are depicted in green. Counties with three or more cases of Lyme disease and three or more cases of babesiosis are depicted in gray. No county reported three or more cases of babesiosis but fewer than three cases of Lyme disease.

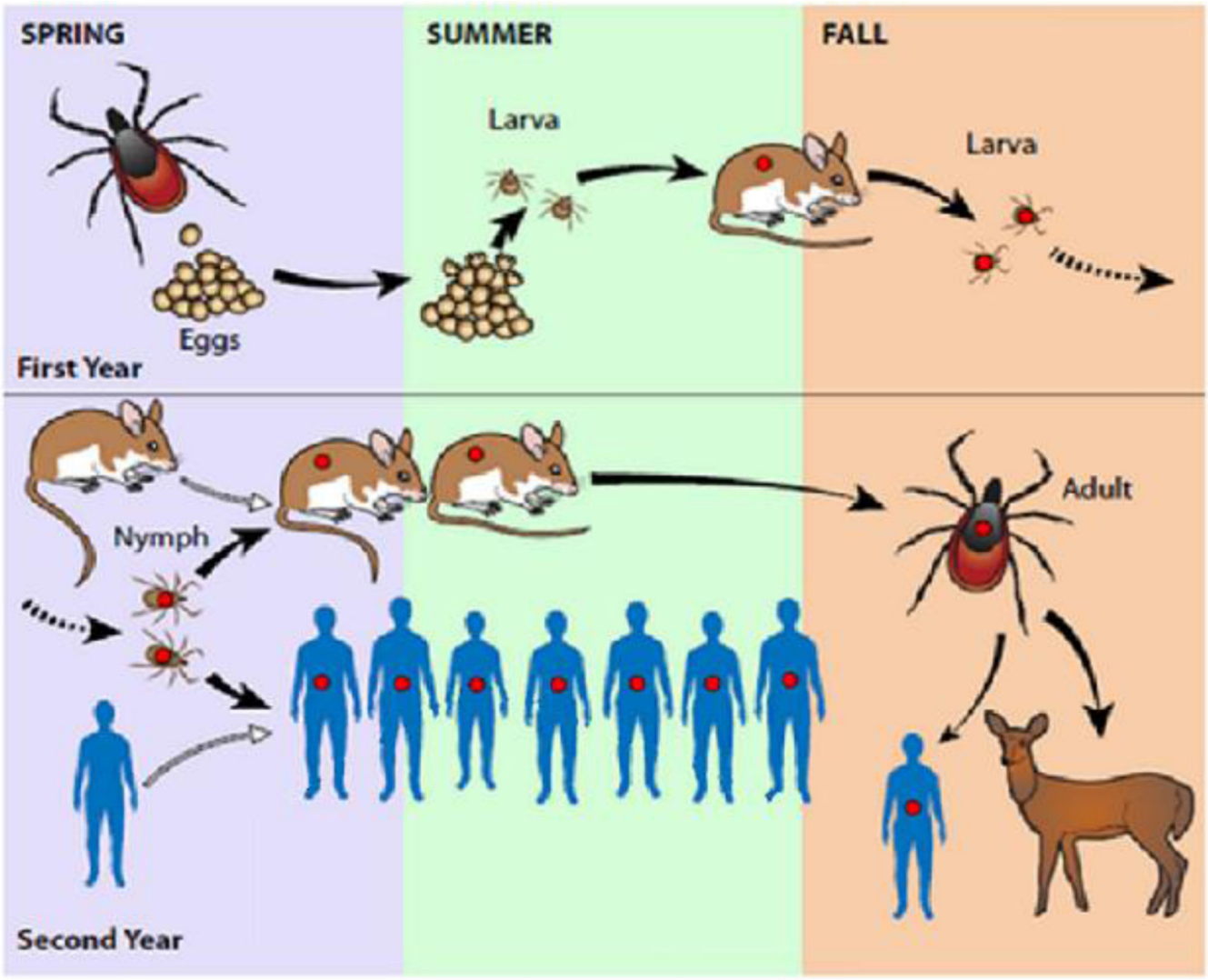



**Figure 3. The enzootic cycle of *Borrelia burgdorferi* and *Babesia microti* in the Northeast and upper Midwest of the United States**

*Ixodes scapularis* ticks undergo a three-stage life cycle. Adult female ticks lay eggs in the spring (first year, top left panel). Although adult females may carry *Borrelia burgdorferi* and/or *Babesia microti*, their eggs do not because these agents do not reach the tick ovaries (no transovarial transmission). Larvae hatch in the early summer and become infected with *B. burgdorferi* and/or *B. microti* (red circle) as they take a blood meal from infected white-footed mice (*Peromyscus leucopus*) in late summer. White-footed mice are the primary reservoir host, but other small- and medium-sized mammals can harbor these agents. Birds also are reservoirs but more so for *B. burgdorferi* than for *B. microti*. Once the blood meal is completed, *B. microti* gametocytes fuse to form zygotes that cross the tick midgut epithelium and become ookinetes. Ookinetes eventually reach the salivary glands where they differentiate into dormant sporoblasts. *B. burgdorferi* spirochetes replicate but remain in the tick midgut where they lose motility, although they are not dormant. Larvae molt to nymphs in the fall or the following spring (second year, bottom left panel). When nymphs

feed in late spring or early summer, reservoir hosts may become infected. Humans are accidental hosts; most cases occur from late spring through summer (as illustrated by the row of infected people). Of all tick stages, the nymph is the primary vector for transmission of *B. burgdorferi* and *B. microti* to humans. Following nymphal tick attachment, sporogony is initiated and leads to the accumulation of *B. microti* sporozoites in the tick salivary glands. *B. burgdorferi* spirochetes that had remained in the midgut undergo replication and progress toward the basement membrane of the gut epithelium. Some spirochetes reach the basement membrane, become motile and reach the salivary glands. By 48–72 hours after attachment, *B. burgdorferi* and *B. microti* are deposited in the dermis. Some *B. burgdorferi* strains remain at the bite site whereas other strains disseminate to various organs, including heart, joints and central nervous system. *B. microti* sporozoites reach the bloodstream where they readily invaded red blood cells. In the fall, nymphs molt to adults that feed on white-tailed deer (*Odocoileus virginianus*) but rarely on humans. White-tailed deer do not become infected with *B. burgdorferi* or *B. microti* but amplify the tick population by providing a blood meal for adult ticks. The following spring, adult female ticks lay eggs and the cycle is repeated. *Adapted from Vannier and Krause* [9].

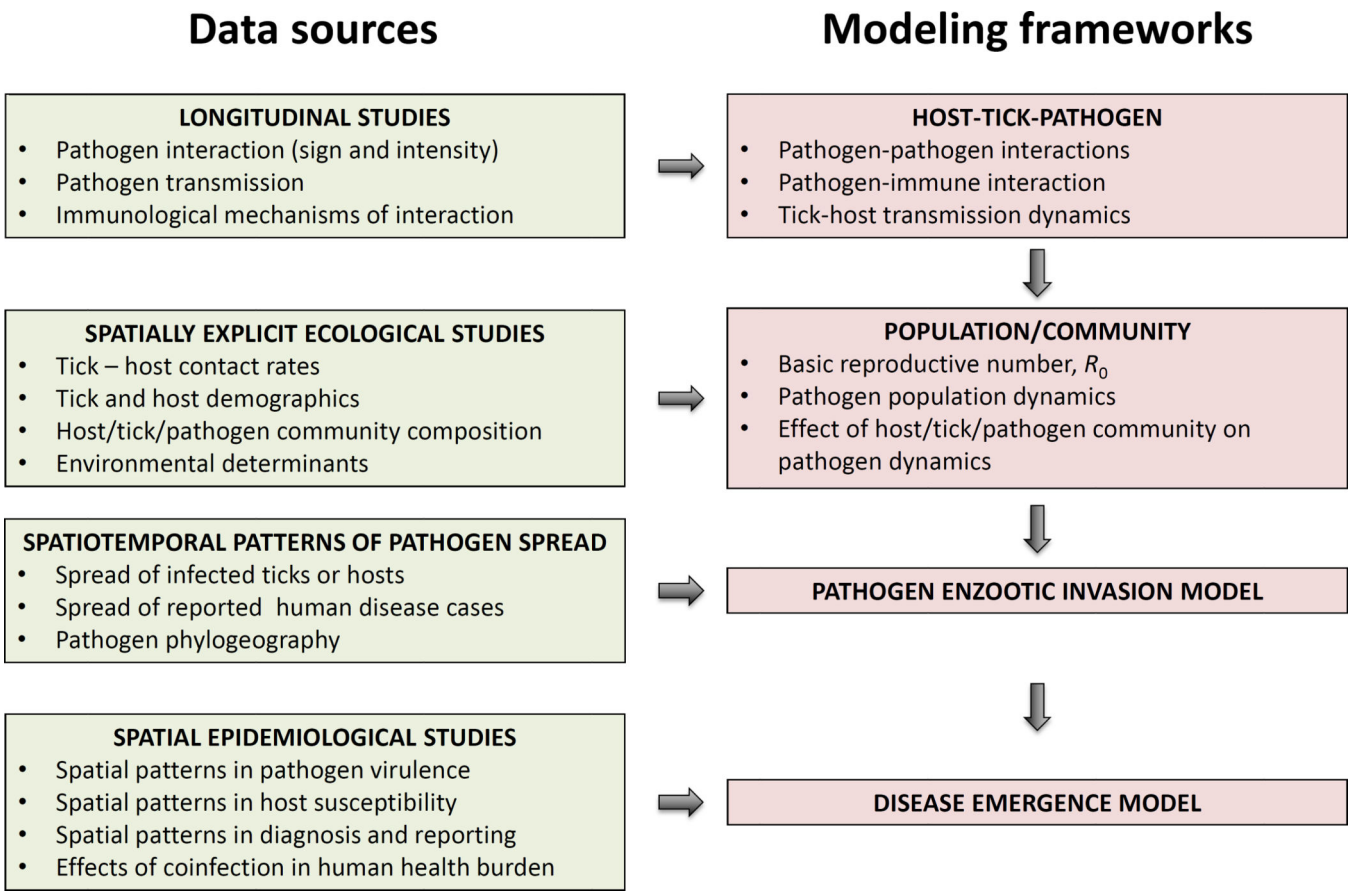



**Figure 4. Hierarchy of frameworks and data sources to predict the effect of coinfection on the emergence of tick-borne human disease**

Green boxes highlight the sources of key model parameters that are measured in laboratory or field studies or derived from ancillary models. Pink boxes highlight hierarchy of modeling frameworks in which coinfection should be incorporated to predict the emergence of tick-borne human disease. At the individual host level, longitudinal laboratory and field studies provide information on how coinfection affects pathogen-pathogen interactions, pathogen interactions with host immune mechanisms and the effect of these interactions on pathogen persistence and transmission duration and intensity to ticks. At the population or community level, ecological contextual parameters measured at various geographic locations are integrated into (i) basic reproduction number, $R_0$, models to assess the probability of pathogen establishment given coinfection, (ii) pathogen population dynamics once coinfecting pathogens become established and (iii) effects of coinfection in the context of a network of pathogen-tick-host interactions. For regional models of pathogen enzootic invasion, pathogen spread can be inferred from ancillary models that estimate rates and patterns of spread using historical datasets of infection in ticks, reservoir hosts, and/or humans (disease cases) and from pathogen phylogeographic studies. Models of disease emergence integrate pathogen invasion models with epidemiological data to assess the likelihood of spillover to humans and the human health burden (disease incidence and

severity), accounting for spatiotemporal heterogeneities in pathogen virulence, host susceptibility and reporting biases.

## Table 1

Prevalence of coinfection with *Borrelia burgdorferi* and *Babesia microti* in *Ixodes scapularis* ticks and small mammalian hosts in the Northeast and upper Midwest of the United States.

| | | | | Infection Prevalence[b] | | | | |
|---|---|---|---|---|---|---|---|---|
| **Study location** | **Screening Method** | **Host[a]** | **Sample #** | ***B. burgdorferi*** | ***B. microti*** | **Coinfection** | **Interaction[c]** | **Reference** |
| MA | Microscopy | Pl and Mp | 14 | 0.714 | 0.571 | 0.357 | | [47] |
| MA | Microscopy | Is nymphs | 102 | 0.314 | 0.422 | 0.186 | + | [42] |
| MA | Microscopy | Is nymphs | 293 | 0.259 | 0.164 | 0.082 | + | [42] |
| CT | Microscopy | Pl | 59 | 0.644 | 0.458 | 0.424 | | [48] |
| WI and MN | Microscopy | Pl | 81 | 0.457 | 0.123 | 0.123 | | [49] |
| NJ | PCR | Is adults | 100 | 0.430 | 0.050 | 0.020 | | [45] |
| NJ | PCR | Is | 107 | 0.355 | 0.103 | 0.019 | | [38] |
| ME | PCR | Is | 394 | 0.223 | 0.008 | 0.005 | | [41] |
| NH | PCR | Is adults | 509 | 0.601 | 0.090 | 0.061 | | [46] |
| NY | PCR | Is adults | 286 | 0.636 | 0.203 | 0.168 | | [44] |
| NJ | PCR | Is nymphs | 478 | 0.100 | 0.040 | 0.029 | | [43] |
| NJ | PCR | Is adults | 610 | 0.452 | 0.082 | 0.062 | | [43] |
| NY | PCR | Is nymphs | 4368 | 0.291 | 0.138 | 0.067 | + | [17] |
| NY | PCR | Pl | 3275 | 0.449 | 0.211 | 0.127 | + | [17] |
| EHR[d], NY (2003–2004) | PCR | Is nymphs | 1481 | 0.190 | 0.050 | 0.016 | | [22] |
| EHR[e], NY (2005–2006) | PCR | Is nymphs | 1272 | 0.060 | 0.010 | 0.005 | | [22] |
| WHR[e], NY (2003–2004) | PCR | Is nymphs | 171 | 0.190 | 0.010 | 0.000 | | [22] |
| Southeastern CT | PCR | Is nymphs | 274 | 0.146 | 0.166 | 0.049 | | [39] |
| Northeastern CT | PCR | Is nymphs | 836 | 0.260 | 0.047 | 0.029 | | [39] |
| MA | PCR | Is nymphs | 87 | 0.207 | 0.092 | 0.011 | | [39] |

[a] Infected vertebrate reservoir host or tick vector species and life stage. Pl = *Peromyscus leucopus;* Mp = *Microtus pennsylvanicus*; Is = *Ixodes scapularis*

[b] Proportion of hosts or vectors infected with a single pathogen or both (coinfected)

[c] Statistical assessment of pathogen interaction as performed in original publication. + = statistically significant positive association (alpha=0.05)

[d] EHR = East of the Hudson River

[e] WHR = West of the Hudson River