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The Cold Zone: A Curious Convergence of Tick-Transmitted Diseases

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In recent years, investigators have gained an increasing appreciation of the complexity of the Lyme disease transmission cycle with regard to the number of pathogens involved. *Babesia microti*, a blood parasite that is related to the organism that causes malaria, frequently accompanies the Lyme disease spirochete in the mouse reservoir. Recently, a newly described *Ehrlichia* species related to *Ehrlichia equi* has been found to be transmitted by the deer tick. Human infections with these agents alone and in combination are now being described, and the successful treatment of these infections may depend on proper diagnosis. The convergence of these and other organisms on the Lyme disease transmission cycle provides a unique opportunity to study pathogen-pathogen interactions in a naturally occurring model.

Although significant progress has been made over the past two decades in understanding the immunobiology of Lyme disease and in increasing awareness that this disease is an important public health problem, much about Lyme disease still remains puzzling [1–6]. Its pathogenesis is still poorly understood, and the interdependent problems of diagnosing the disease accurately and assessing therapeutic outcomes confound one another. One of the most curious aspects of Lyme disease in humans has been its inconsistent presentation, in terms of both disease severity and organ system involvement [1–3]. By far, the most consistent finding is erythema migrans, often accompanied by a nonspecific febrile illness. However, even these findings may be absent or unrecognized in many cases, and subclinical or self-limiting infections occur in a substantial proportion of exposed persons [7].

Within a few weeks to months of infection, an astonishing array of signs may appear that affect certain subsets of infected patients. These signs (in roughly descending order) include arthritis, lymphadenopathy, meningitis, cranial neuritis (including Bell's palsy), myopericarditis, nonexudative sore throat, mild transient or recurrent hepatitis, and, less commonly, pancarditis, ocular involvement, and adult respiratory distress syndrome. Years after the onset of disease, occasional patients may develop migratory musculoskeletal disorders along with persistent malaise and fatigue (fibromyalgia-like symptoms) or chronic encephalomyelitis [8, 9]. Indeed, the focus of much of the literature on Lyme disease has been on subsets of these extreme or exceptional cases, many of which may or may not be linked by objective criteria to active infection with *Borrelia burgdorferi*.

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In contrast to Lyme disease in humans, Lyme disease in animal models is generally more predictable. Animal models of *B. burgdorferi* infection have been established in dogs, primates, rabbits, gerbils, and hamsters; resemblance to the human disease has varied [10–13]. The C3H mouse model of the disease (the model that has been best defined microbiologically and immunogenetically) shares some characteristics with the disease in humans (mainly arthritis and carditis), but neurological involvement has never been demonstrated in this model [11, 12]. In contrast, most other mouse strains are not nearly as susceptible to the disease [14].

Lyme disease may be more faithfully reproduced in primate models [10], but primates often harbor endogenous infections that have the potential to alter host immune responses [15–17]. Approximately 40% of baboons residing in primate colonies throughout the United States are chronic carriers of an endemic *Babesia* species that is genetically related to *Babesia microti* and *Babesia rodhaini*, both of which have immunosuppressive effects in laboratory models [18]; foamy virus, simian Epstein-Barr virus, simian cytomegalovirus, and simian T cell lymphotropic viruses are also endemic in most primate colonies in the United States [15–17].

One of the most interesting contrasts in disease susceptibility, relative to microbial definition, is in the canine model of Lyme disease; needle inoculation of dogs with cultured *B. burgdorferi* does not result in significant disease, but inoculation of dogs with *B. burgdorferi* via infected ticks collected from areas of endemicity results in severe acute and chronic polyarthritis [13]. Thus, at least on the surface, it seems that microbial definition and/or route of inoculation may have an impact on biological variation.

There are additional explanations for the biological variability of Lyme disease in humans and animals. First, in most animal models of the disease that have been established to date, "mainstream" isolates of *B. burgdorferi* (i.e., isolates resembling the archetypal laboratory strains) have been used. The use of common laboratory strains of *B. burgdorferi* could limit biological variation because of limited genetic variation of the spirochete. In contrast, human exposure likely involves

a far wider variety of spirochetal subtypes or even mixtures of subtypes, and studies of the genetic diversity of *B. burgdorferi* isolates from the United States support this notion [19].

Second, host immunogenetic variability may be involved. An increased frequency of HLA (human leukocyte antigen) Dr4 specificity is found among a small number of patients who have had joint inflammation continuously for >1 year [20, 21], and an increased frequency of HLA Dr4 alleles along with antibody to OspA and OspB is found among an even smaller number of patients who do not respond to antibiotic therapy [20]. However, patients with less continuous or prolonged Lyme arthritis apparently do not have an overrepresentation of particular class I or class II alleles [22].

Coinfection as a Confounding Variable

Yet another possible explanation for biological variation in the expression of Lyme disease might be inapparent coinfection with other known or yet unknown pathogens. The likelihood of coinfection increases if the agents involved are transmitted by the same arthropod vector. In the United States, the apicomplexan blood parasite *B. microti* and the recently recognized agent of human granulocytic ehrlichiosis (HGE) are both transmitted by the deer tick [23–26]. Both infections appear to be most intensely enzootic in established Lyme disease–endemic habitats. Coinfection is probably not limited to the "cold zones" of the United States; serological evidence of exposure to the HGE agent has been described for a substantial percentage of Lyme disease patients from Switzerland, Germany, and Denmark [27, 28]. In addition, transmission of tick-borne-encephalitis virus by *Ixodes ricinus,* the same tick that transmits Lyme disease in Europe, has been recognized for many years [29].

In general, the cardinal symptoms of the "companion infections" of babesiosis and ehrlichiosis are usually absent in coinfected patients, such that coinfected patients are often difficult to distinguish objectively from patients with uncomplicated Lyme disease [7, 23, 30]. However, because the three infections may require different approaches to management, an understanding of the natural history of infection with *Babesia* and *Ehrlichia* may become critical for understanding the biological variation of human Lyme disease.

At the least, the presence of concurrent infection with two or more pathogens should be ruled out in studies of biological variation because the presence of such infection may serve as a confounding variable. For these and other reasons, considerable effort in several laboratories has gone into further defining the transmission cycles of these agents [23, 26], developing serological and molecular markers of infection for all three pathogens [23, 31–35], propagating all three pathogens from human sources [36–40], and defining the extent of human exposure to these pathogens [23, 30, 41–44].

B. microti Infection

B. microti infection has been diagnosed in residents of many areas in the northeastern and northern midwestern United States where Lyme disease is prevalent [7, 45]. It is interesting to note that *B. microti* was once considered to be a possible agent of Lyme disease, since many patients with babesiosis presented with an erythema migrans–like rash at the site of a deer tick bite. The initial symptoms of both illnesses somewhat overlap: like Lyme disease, babesiosis in humans presents as nonspecific symptoms including fever, fatigue, and other flu-like symptoms [46, 47]. Hemolytic anemia, which lasts from several days to a few months, may occur in patients with clinically severe cases, most commonly asplenic or elderly patients. However, most cases of human babesiosis in normosplenic, immunocompetent patients are probably subclinical and occur as a self-limiting illnesses [7, 48].

Seroepidemiological data suggest that ~10% of Lyme disease patients in Connecticut and perhaps even higher proportions of such patients in other areas have been exposed to *B. microti* [45, 49]. However, these two studies did not provide proof of coinfection (vs. sequential infection) with both agents, because they were conducted retrospectively, and specimens for the direct demonstration of both pathogens were not collected. In an important recent study, Krause et al. [7] provided more convincing evidence of simultaneous infection with *B. burgdorferi* and *B. microti* in substantial numbers of patients with presumptive Lyme disease from coastal New England. Although not usually recognized as such on clinical grounds, patients coinfected with both organisms often had more severe disease and persistent postinfection fatigue [7].

Since antibiotic therapy for Lyme disease is unlikely to be effective against an underlying infection with *B. microti,* it is easy to envision a scenario in which underlying babesiosis is responsible for the persistence of symptoms after therapy for early Lyme disease has been administered. However, it is important to note that the converse scenario does not appear to be true: among patients with chronic fatigue and no history of antecedent Lyme disease, there is no evidence supporting a major role of coinfection with *B. burgdorferi* and/or *B. microti* [50].

HGE

In 1994, Bakken et al. [51] described an outbreak of ehrlichiosis in the Upper Midwest that was characterized by a nonspecific febrile illness, thrombocytopenia, and neutrophilic inclusions (morulae) [51]. Genetic and serological analyses of patients' blood samples indicated that the agent of HGE is closely related to *Ehrlichia equi* and *Ehrlichia phagocytophila* [52] and genetically distinct from the only previously known domestic cause of HGE (*Ehrlichia chaffeensis*), which was first identified in cases that occurred at Fort Chaffee, Arkansas, in 1987 [53, 54]. Since then, ehr-

lichial infections caused by a similar or identical agent have been described in many states including New York, Connecticut, Massachusetts, Rhode Island, Minnesota, Wisconsin, and California [26, 42, 51, 55–57].

The report of a case of HGE that occurred following a deer tick bite [58], together with recognition of the apparent overlap between areas where Lyme disease and HGE are endemic, prompted an investigation into whether the deer tick could also transmit HGE. *Ixodes* ticks collected from fields in several locations in Wisconsin where HGE cases have been described were analyzed retrospectively; the collection obtained from Wisconsin in 1993 and an earlier collection stored in alcohol since 1982 both contained PCR-positive specimens. *Dermacentor* ticks collected from nearby locations were all PCR-negative for the agent of HGE.

In a subsequent study, Telford et al. [26] showed that the deer tick is competent to transmit an agent of HGE that was recovered from a patient on Nantucket Island. *Ixodes pacificus* may be the vector of HGE in the western United States; this tick is the primary vector of *E. equi,* which is closely related to the agent of HGE [52].

A recently described patient with HGE from Northern California [57] was infected with an organism with a ribosomal signature sequence characteristic of *E. equi,* and an *E. equi*–like organism has now been recovered by inoculation of mice with ticks collected in the yard of a second patient (S. Telford, et al., unpublished observations). In summary, all evidence gathered to date implicates the *Ixodes* tick as a vector of HGE; the vector competence of other ticks has not yet been established.

In a first effort to ascertain the extent of human exposure to the agent of HGE, we analyzed serum specimens from Lyme disease patients from the Upper Midwest; these specimens had been stored since 1987 [23, 30]. They were identified as positive with use of EIA and western blot assay and were stored at the Mayo Clinic (Rochester, MN) from the time serological testing for *B. burgdorferi* was first offered. A total of 179 positive serum samples were obtained from patients with serologically confirmed Lyme disease who resided in several different counties in the Great Lakes region. These sera were tested for the presence of polyclonal IgG antibody responses to the HGE agent by indirect immunofluorescence with use of *E. equi* as a substrate [23, 30, 32, 33].

On the basis of phylogenetic analysis, *E. equi* is predicted to cross-react with the HGE agent, and its diagnostic usefulness has been proven in several confirmed cases of HGE because a four-fold or greater increase in titer was detected during the course of infection [32, 33].

Samples collected from healthy blood donors at the Mayo Clinic and a collection of serum specimens from our normal-value control-patient collection were also included in this analysis. The latter group of specimens was obtained from paid participants in a study of normal laboratory parameters. Healthy participants provided serum and whole blood, and medical records were made available for clinical correlation studies. The results of this analysis are shown in table 1. When a conservative cutoff titer of 1:64 was used to indicate past exposure, an overall seroprevalence rate of 7% was observed for this group, with substantial regional variation; higher seroprevalence rates were observed with a cutoff titer of 1:32 [30].

Table 1. *Ehrlichia equi* seroreactivity in *Borrelia burgdorferi*–seroreactive specimens from patients with Lyme disease from the Upper Midwest.

Geographic location	No. with positive sera/total no. tested (%)*
Duluth, Minnesota	15/91 (16%)
Grand Rapids, Minnesota	1/20 (5%)
Marshfield, Wisconsin	1/25 (4%)
Stevens Point, Wisconsin	2/28 (7%)
Eau Claire, Wisconsin	18/307 (6%)
Crosby, Minnesota	2/15 (13%)
Mayo Clinic (Rochester, MN)	6/115 (5%)
Total	46/654 (7%)

NOTE. Of 185 serum samples collected from healthy volunteers, none was positive for the agent of human granulocytic ehrlichiosis (HGE).

* Reciprocal serum titers of antibody to the agent of HGE that were ≥1:64 were considered positive. (The presence of antibody does not necessarily imply active infection.)

Of course, the mere presence of serological reactivity to *E. equi* antigen, even if specific, does not necessarily imply concurrent infection. In many patients, especially those living in areas of endemicity, infections may have been acquired sequentially. To complicate matters further, ehrlichial infection may be associated with false-positive serological tests for *B. burgdorferi* infection [59], which has since been shown to occur with a defined animal model of infection with the HGE agent [60]. Given the potential unreliability of serological testing in the context of coinfection, dependence on other direct detection methods such as PCR and culture may necessarily increase.

It is of interest that some of the HGE-positive serum samples were from patients with presumed cases of Lyme disease that had been identified in 1987, several years before the disease was described. Furthermore, analysis of the archived ticks that had been collected in 1982 showed that the HGE agent was present in a competent vector over a decade before HGE itself was described [23]. Just as retrospective epidemiological studies of *B. burgdorferi* have demonstrated the presence of the pathogen in suitable vectors [61] or reservoirs [62] from antiquity, the existence of the agent of HGE clearly preceded the first descriptions of the disease itself. Indeed, the increased incidence of HGE is likely to be due, in part, to the emergence of its recognition.

Immunosuppressive Effects of Infection with *Babesia* and *Ehrlichia* Species

Animal models have clearly shown that a period of immunosuppression relative to superimposed antigens or infections is

characteristic of a number of parasitic infections including malaria [63], trypanosomiasis [64, 65], and toxoplasmosis [66]. Several lines of evidence exist to indicate that *B. microti* and its relatives are also immunosuppressive. Mice infected with *Trichuris muris,* a nematode, show impaired ability to reject the worms after having been infected with *B. microti* or *Babesia hylomysci* [67]. Infections with *Trypanosoma musculi* that are superimposed on *B. microti*–infected mice are prolonged and enhanced, and production of antibodies to *Trypanosoma* is decreased [68].

Purvis [69] demonstrated that infection with an "avirulent" strain of *B. microti* in mice was accompanied by a marked depression in the ability of the mice to mount an immune response to sheep RBCs. More recently, a similar finding was described in *B. rodhaini*–infected mice [70]. Adachi and Makimura [71] demonstrated immunosuppression in *Babesia gibsoni*–infected dogs. It is of interest that in this study, markedly reduced lymphocyte proliferation and glucose consumption in response to phytohemagglutinin stimulation (ranging from 0.5-fold to 10-fold) were detected in both chronic relapsers and chronic carriers with nearly undetectable parasitemias.

Ehrlichial infections, like babesial infections, have also been associated historically with immunosuppression. The scientific experience documented within the veterinary community again proves to be an invaluable resource. Indeed, the major manifestations of granulocytic ehrlichiosis in sheep do not appear to be due to the hematologic abnormalities wrought directly by the infection but by secondary infections that are activated or exacerbated [72, 73]. Disseminated bacterial infections may occur in the context of ehrlichial coinfection [74]. Likewise, louping ill, an encephalitis of sheep and goats caused by a member of the tick-borne-encephalitis virus family, is more severe in sheep coinfected with *E. phagocytophila,* a member of the genus that is closely related to the human agent [51, 52].

In patients with HGE, direct evidence of immunosuppression is less obvious; although several patients with fatal cases of HGE have been found to be immunosuppressed [51, 56], most of these patients were immunosuppressed before they were exposed to HGE. Thus, on the surface it seems possible that simultaneous infection with *B. burgdorferi* and *Babesia* or *Ehrlichia* species could produce antagonistic immunologic responses or other immunologic effects that affect the outcome of one or both diseases.

The elucidation of the immunologic interplay of the microbial agents that coevolve during the transmission cycle of Lyme disease is likely to yield a better understanding of what can be expected in occasional human cases. In the case of multiple pathogens that coexist within a rodent reservoir involved in overlapping transmission cycles (as is the case for *B. burgdorferi, B. microti,* the HGE agent, and perhaps other organisms), it is reasonable to hypothesize that immune responses to one organism may have an impact on concurrent infections, especially if the underlying infection is associated with immune suppression, as described above.

In studies of what could be relevant mechanisms, investigators have focused on the unique immunologic effects of infection with intracellular pathogens on the role of helper T cell differentiation into Th1 subsets, which promote cytotoxic immunity, and Th2 subsets, which promote antibody development [75]. The results of recent studies suggest that effective control of *B. burgdorferi* infection depends on a regional or systemic Th2 $CD4^+$ T cell response [76–78]. C3H mice, which have an inherited tendency to develop Th1 responses to a wide variety of infectious challenges, develop severe Lyme arthritis and carditis and have elevated numbers of *B. burgdorferi* spirochetes within tissues.

In Balb/C mice, however, Th2 responses dominate; these mice in turn typically harbor low numbers of spirochetes and are relatively disease resistant. The inappropriate Th1 response of the C3H mouse apparently reduces the efficacy of the primary response and may actually further the disease process by increasing nonspecific cytotoxic T cell responses. Thus, the very property that makes Balb/C mice highly susceptible to intracellular pathogens of many types appears to provide an inherent resistance to *B. burgdorferi* infection.

Evidence that global T cell differentiation in mice can be polarized toward either Th1 or Th2 subsets by a preexisting infection or a superinfection is now emerging from experimental studies. Mice infected with *T. muris* exhibit more-efficient clearance of the intestinal roundworm in the presence of a superimposed infection with *Schistosoma mansoni* or after inoculation with killed *S. mansoni* eggs [79–81]. The proposed mechanism of this improved resolution of an unrelated infection is the stimulation of a strong Th2 response via the "adjuvant effect" of *S. mansoni* infection, which indirectly affects the development of *T. muris*–specific T cells toward a Th2 response. Since a Th2 response is required for resolution of *T. muris* infection, it was proposed that "trickle-down" effects of the *S. mansoni* infection facilitate a more appropriate response for clearance of *T. muris.* Similar adjuvant effects have now been documented in experiments on the effects of underlying *S. mansoni* infection on T helper cell responses to a purified protein antigen [82].

Coinfection by the agent of HGE and *B. burgdorferi* or by *B. microti* and *B. burgdorferi* could likewise have substantial immunologic effects on the mammalian host. Concurrent ehrlichiosis or babesiosis could theoretically influence T cell development toward a Th1 response or away from an appropriate Th2 response, thus decreasing antibody production and allowing higher numbers of spirochetes to exist in the host. The above-mentioned finding of decreased antibody production in *B. microti*–infected mice challenged with superimposed agents or antigens is consistent with this hypothesis and would also be consistent with the finding that spirochetal DNA was detected more often in the blood of patients coinfected with *B. microti* and *B. burgdorferi* than in the blood of patients with Lyme disease and no evidence of coinfection [7].

The competence of *Peromyscus leucopus* as a reservoir of *B. burgdorferi* could also be affected such that it becomes more "C3H-like" under the influence of coinfection; increasing the numbers of spirochetes in tissues and blood of the reservoir could likewise influence tick infection rates. Perhaps the best approach to studying the possible interaction of T cell responses may be with use of a microbiologically defined mouse model of coinfection, which has now been established [83].

Clinical Evaluation of Tick-Borne Diseases and Impact on Therapeutic Approaches

For patients exposed to ticks in areas where multiple tick-borne pathogens are endemic, it seems reasonable for clinicians to be aware of clinical signs that may be consistent with each infection alone or in combination, especially when patients with Lyme disease fail to respond promptly to antibiotic therapy. Unfortunately, since the ability to recognize these infections has not been possible until fairly recently, only a few studies have been done.

Symptoms including nausea and/or vomiting, chills, sweats, severe malaise, and a delayed clinical response to antibiotic therapy for presumptive early Lyme disease were characteristic of *Babesia*-coinfected patients [7]. Fever, chills, myalgias, and severe headache are characteristic of granulocytic ehrlichiosis [33]. Laboratory evaluations to be considered include examination of blood smears for the presence of intraerythrocytic inclusions (merozoites) typical of babesial infection and granulocytic morulae typical of HGE. However, the sensitivity of blood smear evaluations for immunocompetent, normosplenic patients has not been firmly established and may be relatively low for both diseases.

In one study [7], most patients coinfected with *Borrelia* and *Babesia* species were smear negative for babesiosis at the time of presentation and thereafter, and although blood smear evaluation has been advocated for the diagnosis of HGE when the index of suspicion is high [33], visual inspection of blood films for the length of time required to find a single morula may be impractical. False-positive results are also possible; artifacts such as platelets superimposed on red cells can appear like *Babesia,* and to an untrained eye, a well-separated nuclear segment within a neutrophil may give the appearance of an ehrlichial morula.

The presence of elevated liver enzymes or other hematologic abnormalities may be especially useful in identifying coinfected patients, since both babesiosis and ehrlichiosis have been associated with increases in the level of alanine aminotransferase. Thromocytopenia may also be present in patients with either disease but is rarely a feature of uncomplicated Lyme disease. Sensitive PCR assays for both *Babesia* and the agent of HGE have been described [7, 23, 31, 34, 84] but currently are not rapid or widely available.

Information regarding the likelihood of coinfection in areas where Lyme disease is endemic, along with the selective use of diagnostic tests for patients with symptoms compatible with coinfection, can form the basis of effective treatment approaches. For instance, treatment with β-lactam antibiotics would likely be effective against *B. burgdorferi* but would have little, if any, effect against intracellular rickettsia-like agents or piroplasms. A regimen of oral tetracycline or doxycycline, which is effective against both *Borrelia* and *Ehrlichia* species, should thus be favored for the treatment of early Lyme disease in geographic areas in which infection due to both organisms has been described.

In the case of concurrent infection with *B. microti* and *B. burgdorferi,* the treatment options are more complicated. Coinfection with these pathogens might be suspected if unusually severe symptoms of early Lyme disease are encountered or if symptoms characteristic of *Babesia* coinfection (nausea, sweats, and chills) are present [7]. Doxycycline could again be given for treatment of *B. burgdorferi* infection, with the possible benefit of a limited effect on babesial infection as well (doxycycline is effective against some piroplasmid infections in cattle and can be used for malaria prophylaxis).

The currently recommended therapy for acute babesiosis is an intravenous regimen of clindamycin plus quinine [47], which is not recommended for treatment of Lyme disease. This combination is not recommended for mild or subclinical cases of babesiosis because of potentially severe side effects from one or both drugs; thus, its use should be limited to laboratory-confirmed, clinically severe cases.

Oral regimens for the treatment of babesiosis are currently under investigation. In conclusion, oral tetracycline or doxycycline should be the drugs of choice for treatment of tick-transmitted diseases in areas where Lyme disease is endemic; the potential benefits to the patient probably well outweigh the associated risk of drug-induced photosensitivity.

The Future of Pathogen Discovery Techniques as Applied to Tick-Borne Diseases

Are there other, yet-to-be-recognized pathogens involved in the transmission cycle of Lyme disease, and if so, how might these pathogens influence the expression of disease? Given the experience of the past few years, the best overall approach might be to first define the array of pathogens that exist in reservoir mice and in ticks and then to apply these findings to the study of unexplained human disease in areas where Lyme disease is endemic. New methods for pathogen identification and discovery, which are based on nucleic acid amplification (i.e., PCR) and direct DNA sequence analysis, have been described recently [36, 85].

The use of broad-range PCR and representational difference analysis (RDA) for detection of pathogen-specific nucleic acids has led to the discovery of many culture-resistant organisms that cause significant human disease. These systems for broad-range detection are generally based on amplification of highly conserved elements within genes encoding rRNA or other con-

served sequence motifs. Sequence analysis of the amplified segments, followed by comparison of these segments to a database of known organisms, allows for identification of the agent, often at a level of precision that cannot be matched by conventional methods.

RDA is a newly described technique for pathogen discovery and for detection of genetic rearrangements associated with neoplastic disease and developmental disorders [86]. For pathogen discovery, RDA serves as the genetic equivalent of a digital background subtraction technique, in which pathogen-specific nucleic acids are recovered by PCR-based kinetic enrichment, while amplification of host sequences is attenuated by competitive hybridization. This technique has been used to detect novel infectious agents of several types [87–89], and it is likely that the use of this approach will lead to the discovery of many new pathogens.

Application of broad-range PCR and other pathogen discovery techniques has the potential to expand the litany of pathogens transmitted by deer ticks, with the ultimate goal of clarifying the role of the known and perhaps soon-to-be-known cold-zone pathogens involved in the transmission cycle of Lyme disease.

Conclusion

Infections with pathogens transmitted by the deer tick constitute an emerging threat to public health in areas where Lyme disease is endemic. The white-footed mouse appears to be a reservoir for all three of the known pathogens (*Borrelia, Ehrlichia,* and *Babesia* species) and is commonly coinfected. In humans, infections due to each of these pathogens often occur alone and can occasionally occur in combination, with possible effects on disease outcome.

Recognition of these infections represents a significant challenge to clinicians and public health professionals, as it relies to a great extent on laboratory testing (evaluation of hematologic parameters, liver function tests, and pathogen-specific tests); this recognition should also rely heavily on epidemiological information regarding case distribution in areas of exposure. Although there are few clinical features of these diseases that appear to be pathognomonic, the failure of antibiotic therapy for Lyme disease might be considered a clue that evaluation of secondary causes is needed. In the meantime, until proven otherwise, doxycycline should be considered strongly as the drug of choice for treatment of tick-borne diseases in nonpregnant patients >8 years of age.

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