

Coinfections Acquired from *Ixodes* Ticks

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INTRODUCTION

Ticks have been implicated as a source of disease for >100 years. In 1893 Smith and Kilbourne offered the first description of a tick-borne disease, establishing that the cattle tick (*Boophilus microplus*) transmits the protozoan *Babesia bigemina*, the causative pathogen of Texas cattle fever (182). This dramatic report became the foundation for subsequent work on vertebrate hosts and arthropod vectors. Later work in 1909 by Ricketts recognized the role of ticks as vectors of human disease, with his description of the wood tick, *Dermacentor andersoni*, transmitting Rocky Mountain spotted fever (165). The first recognition of disease caused by *Ixodes* ticks occurred in the early 20th century when a Swedish dermatologist reported that the bite of an *Ixodes ricinus* tick was associated with

a characteristic skin lesion near tick bites, termed erythema chronicum migrans (2). In the 1940s, spirochetes were observed in skin lesions, but only isolated cases of erythema migrans (EM) were reported until 1975, when Steere and colleagues investigated a cluster of children with juvenile rheumatoid arthritis living in Old Lyme, Connecticut (192, 193). They observed that the majority of children had illness onset in the summer or fall, and many recalled an expanding rash before the onset of arthritis. Further epidemiologic investigations strongly implicated *Ixodes scapularis* as the tick vector for Lyme disease (LD) (191). Not until 7 years after the initial recognition was a spirochete (*Borrelia burgdorferi*) finally isolated from *Ixodes* ticks by Burgdorfer and colleagues at the Rocky Mountain Laboratories of the U.S. Public Health Service (31).

Since then, newly recognized pathogens and health hazards associated with *Ixodes* ticks have increased dramatically. We now realize that *B. burgdorferi* is a genogroup of multiple closely related spirochetes, which have been described

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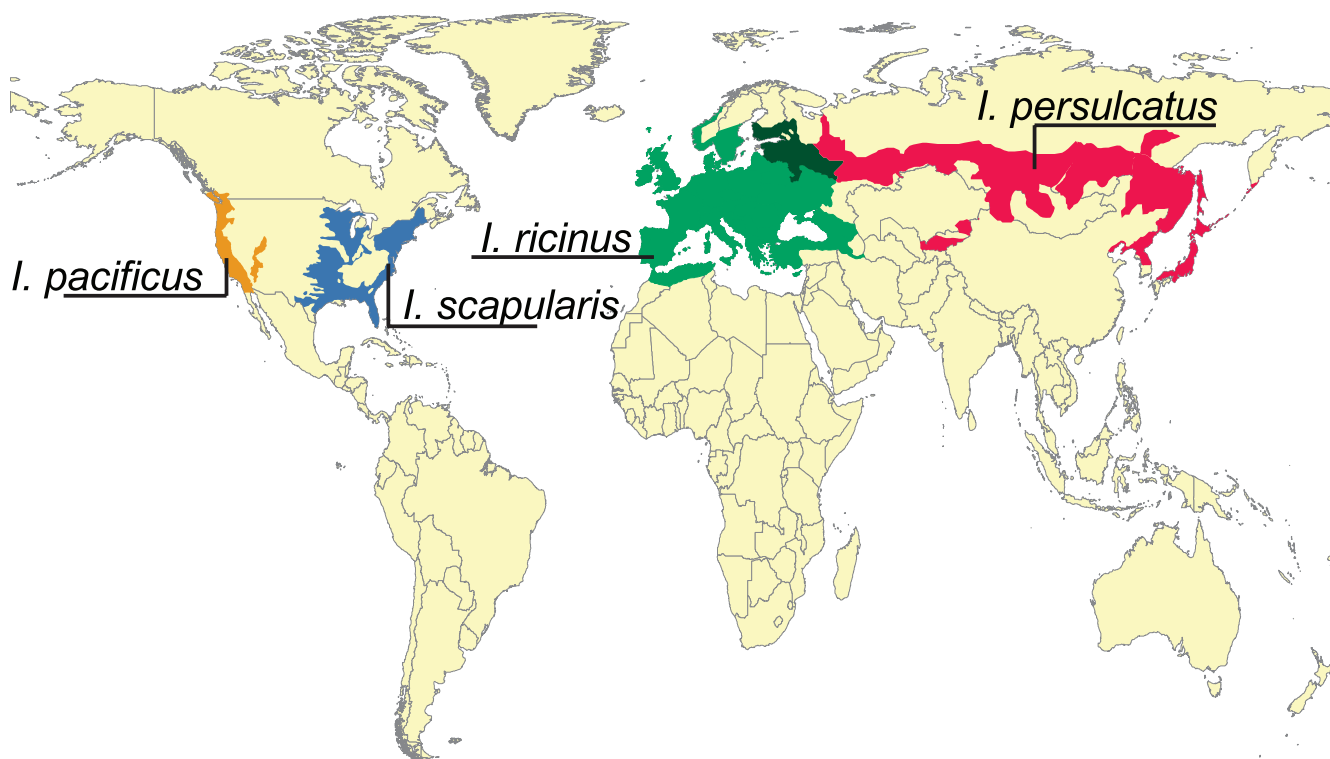


FIG. 1. Approximate geographic distributions of four medically important *Ixodes ricinus* complex ticks. (Adapted from reference 28a with permission.)

throughout the world. The first documented human case of babesiosis occurred in 1957 (181), but only a few isolated cases were reported before 1977, when five cases of *Babesia microti* infection were identified among residents of Nantucket Island (167). In 1979 the vector for *B. microti* was identified as an *Ixodes* tick, and the white-footed mouse (*Peromyscus leucopus*) was thereafter identified as being a common reservoir for both *B. microti* and *B. burgdorferi* (184, 186). Human infections with other *Babesia* species have since been reported, including *Babesia divergens* and the unnamed species WA1, CA1, MO1, and TW1 (82, 150, 160, 177).

Human anaplasmosis (HA; previously known as human granulocytic ehrlichiosis) was first reported among patients from Minnesota and Wisconsin in 1994 (12, 39). The etiologic agent, *Anaplasma phagocytophilum* (previously known as *Ehrlichia equi* and *E. phagocytophila*), was detected in blood samples from 12 patients presenting with fever, headache, and myalgias. Subsequent studies confirmed *I. scapularis* as the vector (147). HA is now known to occur in regions of North America and Europe inhabited by vector-competent species of *Ixodes* (24, 25, 49, 170, 201, 213). Certain species of *Ixodes* ticks in Europe (*I. ricinus* and *I. persulcatus*) are also capable of transmitting tick-borne encephalitis (TBE) virus, a flavivirus that can cause fatal brain infection among humans (47, 142, 226).

Not surprisingly, because all of these agents can coexist in *Ixodes* ticks, coinfections have been reported. However, the epidemiology and natural history of coinfections are not fully understood, and the majority of clinicians have limited experience in recognizing or managing them. The purpose of this

review is to summarize relevant findings from the medical literature on the occurrence, natural history, and outcomes of coinfections acquired from *Ixodes* ticks.

BIOLOGY AND ECOLOGY OF *IXODES* TICKS

Approximately 865 species of ticks exist worldwide (95), of which approximately 650 species are classified in the family Ixodidae, characterized by the presence of a dorsal plate (scutum). The genus *Ixodes* includes approximately 245 species, of which 14 are in the *ricinus* complex (96). This complex includes four species (*I. scapularis*, *I. pacificus*, *I. ricinus*, and *I. persulcatus*) that account for the majority of *Ixodes*-vectored human disease. These species are widely distributed throughout the world (Fig. 1) and serve as primary vectors of LD, HA, and babesiosis. In the northeastern and north central United States, *I. scapularis* is a competent vector of these diseases, able to acquire, transstadially maintain through tick life stages, and subsequently transmit pathogens to susceptible hosts (55, 156, 183, 206, 207). On the West Coast of the United States, the primary vector is a morphologically similar species, *Ixodes pacificus* (107, 163, 164). In Europe, including the British Isles, *I. ricinus* is the primary vector for LD, HA, and probably babesiosis (43, 56, 68, 71, 77, 188, 208), but it is largely replaced by *I. persulcatus* in Eastern Europe and Asia (6, 37, 203).

In many regions, *Ixodes* ticks are found beyond the areas of endemicity of the pathogens they are known to transmit. The discrepancies between tick species and pathogen distribution are not well understood but might be related to habitat needs, feeding behavior, and host-reservoir dynamics. A mid-1990s

review of distribution records in the United States (51) demonstrated the establishment of *I. scapularis* or *I. pacificus* populations in 1,058 of 3,141 (34%) U.S. counties, an area including the West Coast and much of the United States east of the Great Plains. However, only a limited proportion of counties (63, or 2%) accounted for the majority (78%) of nationally reported LD cases in 1995 (38).

The distribution and abundance of *Ixodes* ticks are related to multiple factors, including the presence of suitable wooded or brushy habitat and the abundance of hosts for all life stages of the ticks. The resurgence in white-tailed deer populations during the past 30 years might have allowed *I. scapularis* to expand its range in much of the eastern United States (80, 186, 220). The distributions of tick-borne pathogens and resulting human infections often depend on local tick feeding habits and the distribution and density of small-mammal species that act as competent pathogen reservoirs. For example, the lack of human LD cases in the southern United States might be partially the result of immature *I. scapularis* ticks commonly feeding on lizards (144), which are incompetent reservoir species for *B. burgdorferi* (108, 186); in addition, for unknown reasons, *I. scapularis* ticks in that region do not commonly bite humans (66). Conversely, northern populations of immature *I. scapularis* feed on reservoir-competent small mammals (e.g., *P. leucopus* and eastern chipmunks [*Tamias striatus*]) as well as humans. Reservoir competence, local tick vector feeding habits, and pathogen strain variations each contribute to differences in the geographic distribution of tick-borne diseases.

The risk for tick-borne disease is also closely linked with the life cycle of the *Ixodes* tick and with vector competency at each life stage. This life cycle involves four life stages (egg, larva, nymph, and adult) and spans ≥ 2 years, with tick activity differing dramatically by season and life stage. For example, larval *I. scapularis* ticks often have peaks in seasonal activity during early and late summer, whereas the nymph stage is most active from late spring through midsummer (137, 221). Adult *I. scapularis* ticks are abundant during the early fall and are active again during spring months if they did not feed in the fall. Transmission of LD, HA, and babesiosis usually occurs during the relatively short period of the nymph stage when the tick is active (145). The nymphs' small size (approximately 1 mm) allows them to often feed undetected on humans long enough to transmit these pathogens. Adult ticks are larger and more likely to be detected and removed before disease transmission, whereas host-seeking larvae are uninfected and thus epidemiologically unimportant.

The feeding behavior of *Ixodes* ticks at each life stage has an impact on the risk for tick-borne infection and coinfection among humans. All *Ixodes* species of public health importance are three-host ticks that must find a new host at each life stage. During each life stage after hatching (larva, nymph, and adult), an *Ixodes* tick takes one blood meal, which typically requires 3 to 5 days to complete. Certain *Ixodes* ticks are host specific, whereas others feed on different host species. Those with non-specific feeding habits, (e.g., *I. scapularis*, *I. pacificus*, *I. ricinus*, and *I. persulcatus*) not only feed on species that are reservoirs for multiple tick-borne pathogens (e.g., small mammals) but also will readily bite humans. Therefore, nonspecific feeders might be more important as vectors of human disease than host-specific ticks, which are less likely to bite humans.

When feeding on an infected small-mammal host, tick larvae and nymphs can take up one or more pathogens, which might be transmissible during subsequent blood meals. Larvae are generally not infected with *B. burgdorferi*, *A. phagocytophilum*, or *B. microti* upon hatching; transovarial passage of these pathogens from adult females to eggs has not been consistently demonstrated or is considered insignificant (91, 136, 148, 154, 171, 228). However, transovarial transmission of *B. divergens* from adult *I. ricinus* ticks to larvae does occur (57, 207) and is also believed to be important in maintaining the life cycle of other tick-borne viral and rickettsial pathogens (e.g., TBE virus, spotted fever group rickettsia) (32, 162). Following acquisition of either LD, HA, or *Babesia*, transstadial transmission (i.e., from larva to nymph or from nymph to adult tick) occurs. After molting, nymphs and adult ticks infected in a previous life stage emerge infective and may transmit disease to susceptible hosts during subsequent feedings. Adult female ticks require a blood meal to develop their egg mass and commonly seek a large-mammal host for their third and final blood meal.

COINFECTIONS AMONG *IXODES* TICKS AND MAMMALIAN HOSTS

Prevalence of Coinfecting Pathogens among *Ixodes* Ticks

The risk for human coinfection with multiple pathogens after an *Ixodes* tick bite differs by geographic location and depends on the prevalence of pathogens within the reservoir host and *Ixodes* ticks. The distribution of pathogens within *Ixodes* ticks has been derived largely from epidemiologic reports of human disease. Systematic or large-scale surveys of tick-borne pathogens are lacking. Numerous smaller studies have attempted to identify the prevalence of pathogens among ticks through PCR analysis of DNA isolated from individual ticks. These studies remain difficult to compare because of considerable differences in the methods of tick collection, sample size, specimen preparation, DNA extraction, and selection of nucleic acid probes (primers). Less specific PCR primers potentially yield higher reported prevalence rates among *Ixodes* ticks as a result of the detection of additional strain variants not associated with human illness (120, 178). Thus, the true prevalence of coinfecting human pathogens among *Ixodes* ticks remains largely unknown in the majority of geographic locations. Nonetheless, infection of both ticks and humans with *B. burgdorferi* appears to be substantially more widespread in North America and Europe than infection with *Babesia* or *Anaplasma*, and the reasons for this difference are poorly understood.

North America. Molecular evidence of coinfection with multiple human pathogens has been demonstrated for *Ixodes* ticks sampled from select geographic areas of California, Wisconsin, and the northeastern United States (Table 1). The prevalence of dually infected ticks appears highest among *I. scapularis* ticks from regions of LD endemicity in the northeastern United States, with reported prevalences of $\leq 28\%$. Studies from other North American regions have generally reported lower prevalences of dually infected *Ixodes* ticks. In Wisconsin, 2% of *I. scapularis* adult ticks were coinfecting with *B. burgdorferi* and *A. phagocytophilum* (147). In northern California, approximately 1% of both *I. pacificus* nymph ticks from decidu-

TABLE 1. Prevalences of coinfections of *Ixodes* ticks with *Borrelia burgdorferi*, *Anaplasma phagocytophilum*, and *Babesia microti/divergens* by species and geographic region as determined by PCR^a

Region	Reference	<i>Ixodes</i> species	No. of ticks sampled (population) ^b	% Infection ^c with:			% Coinfection ^d with:	
				<i>B. burgdorferi</i> ^e	<i>A. phagocytophilum</i> ^e	<i>B. microti</i> or <i>B. divergens</i> ^f	Two pathogens	Three pathogens
North America								
California	Holden et al. (86)	<i>I. pacificus</i>	776 (a)	6.7	7.2		1.0	
California	Lane et al. (109)	<i>I. pacificus</i>	158 (n)	3.8	3.2		1.3	
Maine	Holman et al. (88)	<i>I. scapularis</i>	394 (a, n)	22.3	2.8	0.8	1.0	0
Massachusetts	Piesman et al. (155)	<i>I. scapularis</i>	395 (n)	27.3		23.0	10.9	
Massachusetts	Telford et al. (206)	<i>I. scapularis</i>	51 (a)	36.0	11.0	9.0	4.0	0
New Jersey	Adelson et al. (1)	<i>I. scapularis</i>	107	33.6	1.9	8.4	3.7	0
New Jersey	Schulze et al. (174)	<i>I. scapularis</i>	147 (a)	50.3	6.1		2.7	
New Jersey	Varde et al. (213)	<i>I. scapularis</i>	100 (a)	43.0	17.0	5.0	10	0
New York	Schauber et al. (170)	<i>I. scapularis</i>	188 (a)	66.0	42.6		28.2	
New York (1995)	Schwartz et al. (175)	<i>I. scapularis</i>	100 (a)	52.0	53		26	
New York (1984)		<i>I. scapularis</i>	73 (n)	26.0	21		5	
New York (1984)		<i>I. scapularis</i>	100 (a)	45.0	32		19	
Pennsylvania	Courtney et al. (46)	<i>I. scapularis</i>	454 (a)	41.2	17.8		3.5	
Wisconsin	Pancholi et al. (147)	<i>I. scapularis</i>	89 (a)	11.2	7.9		2.2	
Europe								
Bulgaria	Christova et al. (41)	<i>I. ricinus</i>	112 (a)	32.1	33.9		13.4	
France	Halos et al. (77)	<i>I. ricinus</i>	92 (a, n)	3.3		20.6 ^g	2.1 ^g	
Germany	Baumgarten et al. (17)	<i>I. ricinus</i>	275 (a)	21.8	2.2		0.7	
Germany	Fingerle et al. (68)	<i>I. ricinus</i>	401 (a)	37.4	2.0		1.0	
			91 (n)	30.8	0		0	
Germany	Hildebrandt et al. (83)	<i>I. ricinus</i>	62 (a)	21.0	6.5		1.6	
			243 (n)	8.6	1.2		0.4	
Germany	Oehme et al. (141)	<i>I. ricinus</i>	898	20.4	2.9		0.8	
Italy	Cinco et al. (44)	<i>I. ricinus</i>	86 (a, n)	19.8	24.4		8.1	
The Netherlands	Schouls et al. (173)	<i>I. ricinus</i>	121	13.0	28.9		3.3	
Poland	Skotarczak et al. (180)	<i>I. ricinus</i>	550 (a)	12.2		12.5	1.8	
			1,160 (n)	5.8		9.5	0.2	
			385 (l)	5.5		4.2	0	
Poland	Skotarczak et al. (179)	<i>I. ricinus</i>	280 (a)	25.0	10.0	19.3	5.4	1.1
			234 (n)	11.5	4.3	11.1	0.8	0
			19 (l)	42.1	0	21.0	0	0
Poland	Stánczak et al. (189)	<i>I. ricinus</i>	424 (a)	11.6	19.2		5.0	
Poland	Stánczak et al. (188)	<i>I. ricinus</i>	303 (a)	19.5	29.7	3.6	10.6	0
Russia	Alekseev et al. (6)	<i>I. persulcatus</i>	1,282 (a)	29.9	1.0	0.9	1.2 ^h	0 ⁱ
Slovakia	Derdáková et al. (54)	<i>I. ricinus</i>	40 (a)	45.0	20.0		7.5	
Switzerland	Leutenegger et al. (111)	<i>I. ricinus</i>	20 (n)	40.0	0		0	
			100 (a)	49.0	2.0		2.0	
China	Cao et al. (37)	<i>I. persulcatus</i>	1,146 (a)	37.3	4.9		0.5	
			199 (n)	13.6	3.0		0.5	

^a PCR assays differ among studies, and results might include strain variants (e.g., *A. phagocytophilum*) that are potentially nonpathogenic in humans. Microscopy-based detection of infection in ticks occurs in older studies.

^b a, adults; n, nymphs; l, larvae.

^c Prevalence includes totals from coinfecting ticks.

^d Coinfection data overlap with the single-pathogen prevalence percentages.

^e *B. burgdorferi* sensu lato genogroup; European and Asian studies include pathogenic *Borrelia* species, *Borrelia garinii*, and *Borrelia afzelii*.

^f *Babesia odocoilei*, not reported to cause human disease, has since been demonstrated to be prevalent among *I. scapularis* ticks in certain locations in the northeastern and north central United States (9, 172). *I. ricinus* ticks in Europe can also carry species of *Babesia* that are neither *B. microti* nor *B. divergens* (59). Thus, estimates of pathogen prevalence based solely on microscopy or using nonspecific assays may overestimate the risk of human babesiosis.

^g Particular species of *Babesia* not determined.

^h Dual coinfection with TBE virus and *B. burgdorferi* sensu lato was demonstrated for 15 (1.2%) of 1,280 *I. persulcatus* ticks.

ⁱ Triple coinfection with *B. microti*, *B. burgdorferi*, and TBE virus was demonstrated for a single (0.1%) tick.

ous woodlands (109) and *I. pacificus* adult ticks from coastal regions (86) were dually infected with *B. burgdorferi* and *A. phagocytophilum* (Table 1). Fewer studies have attempted to identify simultaneous infection with three tick-borne pathogens, *B. burgdorferi*, *B. microti*, and *A. phagocytophilum*. These studies weakly suggest that molecular evidence from *Ixodes* ticks of dual infection with *B. burgdorferi* and *A. phagocytophi-*

lum appears more common than *B. burgdorferi*-*B. microti* or *B. microti*-*A. phagocytophilum* coinfections, although geographic differences do exist (179, 188, 206, 213). Triple coinfection appears to be even less common among *Ixodes* ticks (Table 1). None of the *I. scapularis* ticks collected in an area of LD endemicity in New Jersey were demonstrated to have triple coinfection with these pathogens, whereas 4% were dually

infected (1). Other researchers have not identified molecular evidence of triple coinfection among *Ixodes* ticks, despite reporting a dual-pathogen prevalence of 1% to 10% (88, 206, 213). Taken together, these few studies indicate that dual infection with any combination of *B. burgdorferi*, *B. microti*, and *A. phagocytophilum* occurs in 1% to 28% of *Ixodes* ticks from regions of LD endemicity in the United States and in <1% to 13% of sampled European *Ixodes* ticks (Table 1). Triple coinfection is rarely detected in geographic regions where all three tick-borne diseases are endemic and likely represents an incident occurrence of <1%.

Europe. European studies have predominantly examined *I. ricinus* for molecular evidence of coinfecting pathogens. Multiple studies have involved ticks collected in Germany and Poland (Table 1); individual studies also exist from geographic areas within Bulgaria, France, Italy, The Netherlands, Slovakia, and Switzerland. The prevalence of dual pathogens in *I. ricinus* ticks differs depending on the geographic site of tick sampling and the methodology, with the highest reported prevalences occurring in Bulgaria (13%) (41) and Poland (2% to 11%) (188). European studies demonstrated DNA evidence of *B. burgdorferi* sensu lato genogroup and *A. phagocytophilum* in 0.5% to 13% of *I. ricinus* adult ticks. The *B. burgdorferi* sensu lato genogroup includes 11 *Borrelia* species worldwide, which can be identified and differentiated by using molecular approaches described elsewhere (216). Of these 11 *B. burgdorferi* sensu lato species, only 3 species (*B. burgdorferi* sensu stricto, *Borrelia afzelii*, and *Borrelia garinii*) are known to cause disease among humans (4, 190). All three pathogenic species inhabit Europe, whereas primarily *B. afzelii* and *B. garinii* are thought to cause disease in Asia; *B. burgdorferi* sensu stricto is the sole pathogenic species identified in the United States (15, 212, 216).

A limited number of studies have attempted to detect molecular evidence of tick coinfection with *Babesia* species. From northwestern Poland, coexistent DNA of *B. burgdorferi* sensu lato species and *B. microti* was identified for 3% of sampled *I. ricinus* female adult ticks but only 0.1% of nymphs (180). Among questing *I. ricinus* ticks collected in northern France, 2% had evidence of coinfection with *B. burgdorferi* sensu lato and *Babesia* species (77). Of note, a limited number of European studies have attempted PCR detection of all three coinfecting pathogens in *I. ricinus* ticks; among these studies, only one report, from northwestern Poland, demonstrated a 1% prevalence of all three pathogens—*B. burgdorferi* sensu lato, *B. microti*, and *A. phagocytophilum*—among *I. ricinus* ticks (179).

Substantially less is known about the prevalence of dual pathogens among *I. persulcatus* ticks. Among *I. persulcatus* adult ticks collected from St. Petersburg, Russia (6), 1% had molecular evidence of coinfection with *B. burgdorferi* sensu lato and either *B. microti* or *A. phagocytophilum*. Triple infection was rarely demonstrated; 0.3% of sampled *I. persulcatus* ticks had evidence by PCR of TBE virus, *B. burgdorferi* sensu lato, and either *B. microti* or *A. phagocytophilum*. None of the *I. persulcatus* ticks had triple coinfection with *B. burgdorferi* sensu lato, *B. microti*, and *A. phagocytophilum*.

Asia and the remainder of the world. Coinfection of *I. persulcatus* ticks has been reported from the forest areas of north-eastern China (37), where LD is highly endemic (5, 205). Of 1,345 adult and nymph *I. persulcatus* ticks, 33.8% were infected

with *B. burgdorferi*, 4.6% with *A. phagocytophilum*, and 0.5% with both pathogens (37). Coexistence of both pathogens had not been previously reported for *I. persulcatus* ticks from Asia. Korenberg and colleagues reported a 6% prevalence of coinfection with TBE virus and *Borrelia* species among *I. persulcatus* Eurasian ticks (99). The prevalences of TBE virus and *Borrelia* in ticks appeared independent, with no apparent effect on each other (98).

Overall, information is limited or nonexistent on the prevalence of pathogens among *Ixodes* ticks in Asia, Central and South America, Oceania, and Africa. Furthermore, despite reports of human babesiosis from countries such as China (71), Taiwan (177), Japan (8, 168), Colombia (166), Mexico (71), Egypt (127), and South Africa (35), coinfection of *Ixodes* ticks with *Babesia* species and *B. burgdorferi* or *A. phagocytophilum* has not been reported outside sampled regions of LD endemicity in Europe and the United States.

Prevalence of Coinfecting Pathogens among Nonhuman Mammalian Hosts

Ticks can become infected with multiple pathogens after a single blood meal from a coinfecting host or by feeding on single infected hosts during sequential life stages (113, 114, 155, 209). Numerous wild rodent species have been demonstrated to be naturally infected with *B. burgdorferi*, *B. microti*, and *A. phagocytophilum*, serving as key reservoirs for *Ixodes* tick species. In focused regions of the northeastern United States where LD is highly endemic, the proportion of rodents infected with either *B. burgdorferi* or *B. microti* differed significantly by season, at times exceeding 75% (7, 185). Antibodies to *A. phagocytophilum* have also been identified among different rodent species in California, Colorado, Connecticut, Florida, New Jersey, New York, Maryland, Minnesota, and Wisconsin (138, 215).

Studies have reported the prevalence of coexisting tick-borne pathogens among nonhuman mammalian hosts. Among white-footed mice (*P. leucopus*) captured in Lyme, Connecticut, 50% had evidence of past or present infection with *B. burgdorferi*, *B. microti*, and *A. phagocytophilum* (187), confirming earlier findings of antibodies to these pathogens among mice from Connecticut (116). *B. burgdorferi* and *A. phagocytophilum* DNAs were simultaneously detected among 7% of *I. scapularis* ticks allowed to feed as nymphs on wild-caught *P. leucopus* in Connecticut (112). Naturally occurring coinfection with *B. burgdorferi* and *B. microti* has also been documented for *P. leucopus* mice captured in the upper Midwest (85). Among these and perhaps other populations of *P. leucopus* mice, *B. microti* infection was strongly associated with concurrent *B. burgdorferi* infection (85). In areas of the western United States, coinfection with *A. phagocytophilum* and *B. burgdorferi* has been demonstrated among additional rodent species, including deer mice (*Peromyscus maniculatus*), Mexican wood rats (*Neotoma mexicana*), and prairie voles (*Microtus ochrogaster*) (224). In Colorado, *B. microti* DNA has been commonly detected among prairie voles as well (33). Both *B. burgdorferi* and *B. microti* are considered to cause long-lived infections among rodent reservoir hosts (153, 185), but less is known about the duration of *A. phagocytophilum* infections among reservoir hosts.

In Europe, additional studies have demonstrated the presence of *Francisella tularensis* as a coinfecting pathogen among reservoir animals. Christova and Gladnishka evaluated captured urban rodents (e.g., *Rattus rattus*, *Mus musculus*, and *Apodemus agrarius*) for infection with *F. tularensis*, *B. burgdorferi* sensu lato, and *A. phagocytophilum* (40). PCR assays yielded evidence of *F. tularensis* in 22% of captured rodents, whereas *B. burgdorferi* and *A. phagocytophilum* DNAs were detected in specimens from 26% and 8% of rodents, respectively. Overall, the prevalence of coinfection with *F. tularensis* and either *B. burgdorferi* or *A. phagocytophilum* was 7%. A similar study of small terrestrial mammals captured from a region of the Austrian and Slovakian borderland where LD and TBE are endemic revealed a coinfection prevalence of 0.5% with *B. burgdorferi* sensu lato and *F. tularensis* (214). Taken together, evidence of coinfection among rodent hosts has increased, yet information on the prevalence, intensity, or duration of dual and triple infections among these and other reservoir hosts remains limited.

Transmission Dynamics of Coinfections among Ticks and Reservoir Hosts

All *Ixodes*-vectored diseases of humans require a vertebrate host reservoir other than humans for maintenance of the pathogen in nature (52). The transmission dynamics are complex, in part because at least three conditions must be met before transmission cycles can be sustained. First, a vertebrate host that is susceptible to infection with the pathogen must be present, and that host must experience a sufficient level of infection in the blood so that the pathogen can be passed on to a tick during bloodfeeding. Second, *Ixodes* ticks that acquire the pathogen must be able to maintain infection for extended periods of nonfeeding, including molting into subsequent life stages, and then pass the infection on to other vertebrate reservoir hosts or humans. Last, sufficient numbers of susceptible vertebrate hosts must be present to maintain enzootic transmission cycles.

Transmission cycles among ticks and vertebrate hosts are perpetuated when ticks transfer pathogens between susceptible hosts (horizontal transmission) but cannot be sustained when transmission is directed toward dead-end hosts incapable of experiencing high levels of the organism in blood (tangential transmission). Reservoir host responses to infection with a tick-borne pathogen differ, depending on the specific agent and host, and this interaction has a direct impact on transmission dynamics. For example, parasites of red blood cells (e.g., *Babesia* spp.) are often associated with long-term, relatively asymptomatic infection of the reservoir host. These chronically infected animals can provide numerous opportunities for feeding ticks to acquire infection. In contrast, viral and bacterial infections often either are fatal or induce an immune response in the reservoir host that limits the time during which the pathogen is circulating in high numbers in the peripheral blood. In those situations where fewer opportunities exist for feeding ticks to acquire infection, the tick becomes the crucial link in maintaining the enzootic cycle in nature, by passing organisms either between different stages of tick development (transstadial maintenance from larva to nymph or from nymph to adult), between generations (transovarial transmission from an

adult female to her eggs), or from one tick to another during cofeeding in close proximity on the same host (149).

Theoretically, coinfection with *Ixodes*-associated pathogens has the potential to modulate transmission dynamics at multiple points in the transmission chain. These include alterations in the efficiency of transmission from rodent to tick or from tick to vertebrate, cooperative or competitive pathogen interactions, and increasing or decreasing disease severity among hosts (210). Several laboratory studies have been used to quantify these potential interactions, and the results have been conflicting.

For example, Levin and Fish investigated whether previous infection of ticks with either *Borrelia* or *Anaplasma* affects the acquisition and transmission of a second pathogen. They fed *Anaplasma*-infected *I. scapularis* nymphs on *Borrelia*-infected mice (and vice versa) and measured the efficiency of previously infected nymphal ticks at acquiring a second pathogen and transmitting one or both agents to susceptible hosts. No evidence of interaction between the agents of LD and human anaplasmosis among *I. scapularis* ticks was found with regard to acquiring or transmitting these infections (113). A murine model of coinfection, however, reveals that dual infection with *B. burgdorferi* and *A. phagocytophilum* alters immune responses and increases the pathogen burden, such that an increased bacterial burden resulted in increased pathogen transmission to the vector (87, 209).

Effects of Strain Diversity

Tick-borne pathogens undergo substantial selection pressures to survive in the different environments of a mammalian host and a tick vector. In the host, pathogens must overcome the inflammatory and immunologic defenses of the mammal (140), and in the tick, pathogens must survive extreme fluctuations in temperature, pH, hemolymph osmotic pressure, and other factors related to the physiological status of the tick (130). Strain diversity has been demonstrated to be a critical outcome of this selective pressure, allowing a pathogen to evade host immune responses and to increase the number of different mammalian host species that can be infected. In the laboratory, different strains of microorganisms are distinguished by identifying differences in immunodominant antigens or by detecting changes in nucleic acid sequences at different gene loci. During the past 2 decades, considerable progress has been made in documenting the diversity of strains among pathogens associated with *Ixodes* ticks, as well as in understanding the genetic mechanisms behind these variations.

Antigenic variation in major surface proteins of tick-borne bacterial pathogens is one of the most important mechanisms for evasion of the host immune response and can result in persistent infection. This can be accomplished by different mechanisms. For example, borreliae generate antigenic diversity of specific coat proteins (*vmp/vls*) through a process of recombination termed gene conversion (16, 227). Gene conversion is usually widespread among tick-borne bacterial pathogens and allows organisms to retain a complete set of variable antigen genes. In selected instances, gene conversion is complete, and all epitopes of an antigen are replaced. On

other occasions, partial replacement occurs at hypervariable regions of proteins.

Antigenic variation can also occur at the level of gene transcripts. Gene expression of a variable antigen can be activated at one locus and inactivated at another. This is a reversible process that does not involve changes in DNA at the loci themselves. Conversely, certain DNA rearrangements involve recombination between short direct repeats common to two or more alleles and result in the loss of an allele in the process. Finally, antigenic variation can be generated by accumulation of point mutations among multiple genes. These mutations, along with recombination or reassortment between two different strains infecting the same host, are essential for generating genetic variation among select tick-borne pathogens.

In animal models, *B. burgdorferi* strain variation has been demonstrated to alter the risk of disease transmission. Derdakova and colleagues investigated the interaction between two strains of *B. burgdorferi* in a laboratory system of *P. leucopus* mice and *I. scapularis* ticks. Two groups of mice were infected with either strain BL206 or strain B348 of *B. burgdorferi*. Two weeks later, experimental mice were challenged with the opposite strain. Transmission of both strains was assessed by xenodiagnosis with uninfected larval ticks at weekly intervals. Fewer dual infections were observed among xenodiagnostic ticks, and BL206 was transmitted more efficiently than B348. These findings suggest that certain *B. burgdorferi* strains (e.g., BL206) might be preferentially maintained in transmission cycles between *Peromyscus* mice and ticks, whereas other strains are maintained in alternate tick-vertebrate host transmission cycles (53). However, whether strain variation in *B. burgdorferi* affects the transmission dynamics of other tick-borne pathogens is unclear.

Strain variation has critical implications for preventing tick-borne infections, including vaccine development and serologic tests. If variable antigens are the intended targets for immune prophylaxis, then certain vaccines for pathogens transmitted by *Ixodes* ticks will need to be multivalent. *B. burgdorferi* strain and genospecies diversity is a more acute issue in Europe than in North America and therefore presents greater challenges for vaccine development. Which epitopes to include or exclude in vaccines might not be obvious; too few antigens might provide insufficient protection, while too many epitopes might render development of an effective vaccine impractical. Furthermore, when different geographic areas require different vaccine formulations, the market might not be sufficiently large to support product development. Similar concerns surround the laboratory diagnosis of tick-borne infections, especially with regard to immunoserologic testing; determining the best combinations of epitopes to include in an enzyme-linked immunosorbent assay (ELISA) or similar assay for optimal sensitivity and specificity is difficult (161).

COINFECTIONS AMONG HUMANS

Human coinfection with tick-borne pathogens can occur after attachment of a single tick infected with multiple pathogens or from concurrent single-pathogen tick attachments. Both of these scenarios potentially can result in human coinfection and might not be easily differentiated from sequential infection by pathogens occurring at different points in time. Individual dif-

ferences in innate and acquired immunity, as well as differences in personal behaviors, occupation, activities, and place of residence, contribute to one's risk for acquiring tick-borne infections. Studies have reported that age-related differences exist among patients with diagnosed babesiosis alone (104), those with HA alone (18), and those at risk for coinfection with LD and HA (3). However, at least one prospective study of tick-borne coinfections demonstrated no substantial differences by age or sex (104).

Epidemiology of Coinfections among Humans

The epidemiology of tick-borne coinfections is ascertained largely from serologic studies of patients with suspected or confirmed LD from limited regions of LD endemicity within the United States and Europe. In many geographic regions (e.g., Africa, Oceania, Central and South America, and large regions of Asia), it is doubtful whether human babesiosis, LD, or HA occurs. In tropical regions, cross-reactivity to *B. burgdorferi* proteins has been observed (34). Antigenic cross-reactivity, combined with the diverse clinical manifestations of LD, likely contributes to an overdiagnosis of LD; this problem is particularly evident in geographic regions where neither competent vectors nor known LD spirochetes have been isolated (197).

Epidemiologic knowledge is further limited in Europe and North America by the common use of seroprevalence data, with little ability to differentiate sequential or past infections from simultaneous infections. Additional limitations of seroprevalence studies exist (e.g., inappropriate cut-off values, false-positive and false-negative reactions, and possible cross-reactivity between tick-borne pathogens such as *A. phagocytophilum* and *B. burgdorferi*) which should be considered in interpreting the epidemiologic conclusions of these studies. In contrast, epidemiologic studies that use prospective seroincidence data or molecular methods of DNA detection provide a more accurate picture of the incidence of coinfections; these studies, however, are less common. Taken together, epidemiologic studies demonstrate that the majority of coinfections acquired from *Ixodes* ticks in North America and Europe include infection with *B. burgdorferi*, for reasons that need further investigation.

Prospective studies. (i) Molecular evidence of coinfection. In prospective studies, the incidence of coinfection appears highest among persons with LD; 4% to 45% of LD patients from regions where LD is endemic are coinfecting with either HA or babesiosis. In a 1997-to-2000 New England study, patients who presented during the summer months with an EM rash or influenza-like illness were prospectively enrolled; they submitted blood samples for tick-borne, pathogen-specific serologic and PCR assays (104). One hundred ninety-two (62%) of 310 patients in this study had at least one tick-borne disease; 75 (39%) of these 192 patients had coinfections. LD and babesiosis accounted for the majority (81%) of tick-borne coinfection scenarios, followed by LD-HA coinfection (9%), triple coinfection (LD, HA, and babesiosis [5%]), and lastly babesiosis-HA coinfection (4%). In this particular study, 161 patients had diagnoses of acute LD; 45% of these LD patients demonstrated simultaneous evidence of coinfection with *B. microti* or

A. phagocytophilum.

Other prospective studies have reported lower rates of acute coinfection. Approximately 10% of 240 LD patients from southern New England had either PCR, serologic, or direct microscopic evidence of coinfection with *B. microti* (106). In a 4-year prospective study in Rhode Island and Connecticut, 2 (2%) of 93 patients with a culture-proven *Borrelia burgdorferi* EM skin lesion had PCR or immunoglobulin G (IgG) seroconversion evidence of coinfection with *B. microti*, and 2 (2%) had evidence of coinfection with *A. phagocytophilum* (194). A prospective Wisconsin study of patients with EM indicated a higher prevalence of coinfection with *A. phagocytophilum*, with 11 (12%) of 94 patients with EM demonstrating laboratory evidence (serologic or molecular) of dual infections (20). Notably, approximately 20% of patients with LD do not develop a rash (195, 200), and these persons were not included in either prospective study.

(ii) Serologic evidence of coinfection. In the only prospective seroincidence study performed to date, 671 persons with high-risk exposures in a region of New York where Lyme borreliosis is endemic participated in a 1-year study (84). Nineteen persons (2.8%) seroconverted to *A. phagocytophilum*, *B. burgdorferi*, *B. microti*, or *Rickettsia rickettsii*. However, incident cases of coinfection were not observed, because no participants seroconverted to dual pathogens during the 1-year follow-up. Five participants (0.7%) had evidence of prior exposure to dual pathogens on their baseline sera. This study suggested that the absolute risk for dual infections is low, even among populations at high risk. Although the absolute risk for coinfection appears to be low, this risk differs by geographic region and by level of human and tick activity. Not surprisingly, when coinfection is reported, it is from regions of Lyme borreliosis endemicity, and coinfection occurs most commonly among patients with LD. This indicates that patients with one documented tick-transmitted infection might be at increased risk for infection with another pathogen. At present, coinfection with *A. phagocytophilum* and *B. microti* and triple coinfections are rarely reported, even in prospective studies.

Serologic studies. (i) Lyme disease-babesiosis coinfection. Geographic areas where LD and babesiosis are endemic, particularly regions of New England and the mid-Atlantic states, have long been associated with reported serologic evidence of both *B. burgdorferi* and *B. microti* among humans. Serologic confirmation of concurrent babesiosis and LD was first reported in 1983 for an asplenic male aged 36 years, from Shelter Island, N.Y., who experienced recurrent fevers, erythema chronicum migrans, and monoarticular arthritis (72). Within 2 years, additional reports confirmed the simultaneous occurrence of Lyme borreliosis and babesiosis (119, 198). In a retrospective study of persons residing in areas of LD endemicity in New York and Massachusetts during 1978 to 1984, approximately 50% of patients with confirmed babesiosis had antibodies to *B. burgdorferi* (22). In the same study, 66% of patients who fulfilled clinical and serologic criteria for LD had IgM and IgG antibodies to *B. microti* (22). Additional studies have reached similar conclusions, namely, that the seroprevalence of *B. microti* is highest among persons with prior or active LD (105, 118, 217). For instance, on Nantucket Island, the estimated population seroprevalence of both *B. burgdorferi* and

B. microti is 3.5%; however, 26% of Nantucket Island residents who were seropositive for LD also had serologic evidence of prior *B. microti* infection (217).

Other studies from regions of *Babesia* and LD endemicity in the northeast and mid-Atlantic United States have also demonstrated serologic evidence of *B. microti* infection among persons with LD, although generally in the 2%-to-12% range (Table 2). Febrile Connecticut residents with hematologic abnormalities and exposure to tick-infested areas were evaluated for antibodies to tick-borne pathogens (118). Twenty-two of 180 (12.2%) seropositive persons had dual antibodies to *B. microti* and *B. burgdorferi*, and 15 (8.3%) had antibodies to *E. equi* (*A. phagocytophilum*) and *B. burgdorferi*. In Wisconsin and Minnesota, 2 (2%) of 96 patients with laboratory-confirmed LD demonstrated immunoserologic evidence of *B. microti* infection (128). On the West Coast, the recently identified *Babesia* species WA-1 was determined in one study to be a coinfecting pathogen; 60 (23.5%) of 255 LD patients tested positive for antibodies to the WA-1 piroplasm (199).

In Europe, a limited number of English-language reports exist on human coinfection, and epidemiologic studies of coinfection with *B. burgdorferi* sensu lato and *B. microti* or *B. divergens* are limited. Most human babesiosis in North America is due to infection with *B. microti*, whereas in Europe *B. microti* infections are rare and *B. divergens* appears to cause most human babesiosis. A single case report of babesiosis (*B. microti*) was described regarding a Swiss adult diagnosed with LD, though sequential infection could not be ruled out (125). Despite molecular evidence of *Babesia* species existing in European *Ixodes* ticks, two European studies involving humans failed to demonstrate evidence of coinfection with *Babesia* species; neither *B. microti* nor *B. divergens* was present among hospitalized patients with LD in Poland (81) or febrile pediatric patients with tick-borne infections in Slovenia (10).

(ii) Lyme disease-HA coinfection. Serosurveys indicate that simultaneous occurrence of antibodies to *B. burgdorferi* and *A. phagocytophilum* is relatively common. In Wisconsin, Minnesota (20, 128), and regions of the northeastern United States (3, 50, 84), seropositivity for both pathogens ranged from <3% to 26% (Table 2). In a serosurvey of residents of Connecticut and Rhode Island performed by an ELISA and Western blotting for *B. burgdorferi* and an ELISA (with a recombinant HGE-44 protein) for *A. phagocytophilum*, 2 (4%) of 52 patients had a positive IgG response to each, and 7 (21%) of 34 patients with a positive IgM response to *B. burgdorferi* also had a positive IgM response to *A. phagocytophilum* (50). In a study from Westchester County, New York, Aguero-Rosenfeld and colleagues demonstrated that 45 (26%) of 175 *B. burgdorferi*-seropositive subjects had antibodies to *A. phagocytophilum* (3). The same study found that 9 (21%) of 42 patients with culture-confirmed Lyme borreliosis were seropositive for *A. phagocytophilum*. It should be noted, however, that this study also demonstrated a 5%-to-11% background rate of seropositivity for *A. phagocytophilum* among healthy *B. burgdorferi*-negative children and adults, suggesting potential limitations of serologic testing (e.g., false-positive reactivity, low cutoff values) (3). False-positive IgM responses to *B. burgdorferi* are now recognized to occur also in response to *A. phagocytophilum* infection (222), such that determining *B. burgdorferi*-*A. phago-*

TABLE 2. Prevalences of reported coinfections of humans with *Borrelia burgdorferi* (LD), HA, and babesiosis by geographic region

Region	Study population characteristics	Reference	No. tested	Method of determination ^a				No. (%) with:				
				LD	Babesia	HA	LD + babesiosis	LD + HA	HA + babesiosis	Triple coinfection		
North America	California	Stricker et al. (199)	255		S				60 (24)			
	Connecticut	LD patients screened for <i>Babesia</i> strain WA-1			S				— ^f (9/5)			
		LD-seropositive persons	Krause et al. (105)	735	S				3 (8)	3 (8)		
		LD-seropositive patients with EM rash	Magnarelli et al. (117)	40	S		S					
	Connecticut, Rhode Island	Persons with positive IgM titers for <i>B. burgdorferi</i>	De Martino et al. (50)	34	S		S			7 (21)		
	Connecticut, Rhode Island	Patients diagnosed with LD	Krause et al. (106)	240	PCR, S	PCR, S			26 (11)			
		4-yr prospective study of patients with culture-proven EM rash	Steere et al. (194)	93	C	PCR, S	PCR, S		2 (2)	2 (2)	0	0
	Nantucket Island	LD patients according to CDC surveillance case definition	Wang et al. (217)	171	D, S	M, S			37 (22)			
	New York	LD-seropositive persons	Aguero-Rosenfeld et al. (3)	175	S		S		45 (26)			
		LD patients with culture-confirmed EM		42	C		S		9 (21)			
New York	LD patients from areas of <i>Babesia</i> endemicity	Benach et al. (22)	30	S		S		20 (67)				
Wisconsin, Minnesota	Patients with EM and laboratory-confirmation of LD	Mitchell et al. (128)	96	C, S	S	S		2 (2)	5 (5)	2 (2)		
Wisconsin, Minnesota	HA patients											
	HA patients positive by PCR	Mitchell et al. (128)	19	S	S	PCR			1 (5)	1 (5)	1 (5)	
	Patients with HA or LD	Belongia et al. (20)	121	S		M, PCR, S		11 (9)				
Wisconsin	HA patients identified through active surveillance	Belongia et al. (19)	142	S	S	M, PCR, S		7 (5)	7 (5)	7/102 (7)		
Connecticut, Nantucket Island, Rhode Island	Symptomatic patients with laboratory evidence of >1 tick-borne pathogen	Other tick-borne illness Krause et al. (104)	192 ^b	PCR, S	M, PCR, S	M, PCR, S		61 (32)	7 (4)	3 (2)	4 (2)	
	Babesiosis patients	Benach et al. (22)	41	S		S		14 (34)				
Connecticut	Febrile patients with tick exposure and leucopenia or thrombocytopenia	Febrile patients Magnarelli et al. (118)	375 ^c	S	S	S		22 (6)	15 (4)	2 (0.5)	2 (0.5)	
Wisconsin	Patients with unexplained febrile illness during tick season	Belongia et al. (21)	62	S	M, S	M, PCR, S		0	2 (3)	0	0	
New York	Prospective seroincidence: 1-yr study of adults with high-risk exposures	Hilton et al. (84)	671	S	S	S		0	0	0	0	
Europe	LD patients											
	Bulgaria	Patients with EM rash	Christova and Dumler (42)	145	D		S		14 (10)			
	Norway	Patients with acute LD	Bakken et al. (13)	58	D, S		S		6 (10)			
	Poland	Hospitalized LD patients	Hermanowska-Szapakowicz et al. (81)	74	D, PCR, S	PCR	PCR		0	8 (11)	0	0
	Switzerland	LD-seropositive persons	Brouqui et al. (28)	70	S		S		12 (17)			
Switzerland	Patients previously diagnosed with LD	Pusterla et al. (159) ^d	149	S		S		19 (13)				

Country	Study description	Sumption et al. (202)	40	S	S	3 (8)
United Kingdom	LD-seropositive persons	Sumption et al. (202)	40	S	S	3 (8)
Slovenia	Other tick-borne illness: febrile pediatric patients with established tick-borne infection ^f	Arnez et al. (10)	28	C, D, S	PCR, S	1 (4)
Sweden	Febrile patients: prospective study of febrile patients following tick exposure	Björnsdörff et al. (24)	27	D, S	PCR, S	3 (11)
Sweden	Community serosurvey: permanent residents of Koster Islands of Sweden; seroprevalence of HA (11%) similar to that of LD (14%)	Dumler et al. (61)	185	S	S	6 (3)

^a C, bacterial culture; D, diagnosed clinically; M, microscopy; PCR, DNA detection; S, serology.
^b A total of 310 participants enrolled with EM rash or influenza-like illness indicative of tick-borne disease; 192 (62%) of 310 had confirmed tick-borne infection. Coinfection was documented for 75 (39%) of 192 subjects.
^c One hundred eighty (48%) of 375 patients tested demonstrated serologic evidence in acute- or convalescent-phase sera of a tick-borne disease; 47 (26.1%) of 180 had antibodies to two or more tick-borne agents.
^d The highest seroprevalence of HA occurred among persons who were seropositive for central European TBE virus (40 of 205 [20%]).
^e Evidence of dual infection with TBE virus and *B. burgdorferi* sensu lato was found for 4 (4%) of 28 pediatric patients.
^f Specific number not reported by authors.

cytophilum coinfection from serology alone is problematic.

In Europe, human HA infection was first reported for a Slovenian woman, aged 70 years, with evidence of potential coinfection with *B. burgdorferi* sensu lato determined through a rise in the IgG antibody titer (151). Serologic evidence of HA infection has since been reported widely across Europe, in more than 17 countries. Seroprevalence rates among examined populations range from zero or low to 28% (201); however, nonstandardized serologic tests for *A. phagocytophilum* and different diagnostic approaches make it difficult to fully interpret and compare these different European studies. The highest number of incident cases of HA has been reported in Central Europe (Slovenia) and Sweden, and seroepidemiologic evidence of HA infection has been reported to be higher among persons frequently exposed to ticks (e.g., forestry workers) and among patients with Lyme borreliosis or TBE. Despite this, well-documented, clinically compatible cases of HA have rarely been reported from Europe, and *A. phagocytophilum* has yet to be isolated from European patients. Potentially infected persons identified by serologic testing often appear to have had asymptomatic infections (48, 67, 146, 158). These factors have led to speculation that European HA might represent a milder illness, possibly related to strain variants, or that serologic testing might be detecting cross-reacting pathogens rather than *A. phagocytophilum* (25, 70).

Evidence of potential coinfection with the pathogens of LD and HA has since been demonstrated in Belgium (73), the Czech Republic (92), Germany (115), Italy (169), Norway (13), Poland (81), Slovenia (10), Switzerland (28), Sweden (24), and the United Kingdom (202) (Table 2). Studies indicate a range of coinfection prevalences, from 3.2% among permanent residents of the Koster Islands in Sweden to 17% among LD-seropositive individuals in Switzerland (28, 61). A serosurvey of 1,515 persons representing different risk categories for tick exposure in Switzerland indicated that the highest HA seroprevalence occurred among persons who were seropositive for *B. burgdorferi* (13%) or central European TBE virus (20%) (159).

(iii) HA, babesiosis, and triple coinfection. Only a limited number of studies have attempted to document either dual infection with HA and *B. microti* or triple coinfection with these two agents and LD. Among 192 patients with confirmed tick-borne illness from Nantucket, Rhode Island, and Connecticut (104) during the months of May through September, 1997 to 2000, dual infection with HA and babesiosis was detected for three (1.6%) persons and triple coinfection for four (2%) persons. In a different study by Magnarelli and colleagues, dual infections with HA and *B. microti* ($n = 1$) and triple coinfections ($n = 2$) were noted for <1% of 375 febrile patients in Connecticut suspected of having a tick-borne illness (118). Within the United States, the highest prevalence of HA-babesiosis dual infections has been reported in Wisconsin, where $\leq 7\%$ of patients with confirmed or probable *A. phagocytophilum* infection demonstrated a fourfold change in antibody titers to *B. microti* on paired sera samples (19). Overall, evidence of triple coinfection is rare, with the majority of studies reporting no patients to 2% of patients with a tick-associated illness demonstrating laboratory evidence of infection with *B. microti*, *A. phagocytophilum*, and *B. burgdorferi* combined (Table 2).

Laboratory Diagnosis of Coinfections

A number of testing methodologies are available in the clinical laboratory to aid in diagnosis and patient management of tick-borne diseases. These methods include light microscopy for detection of organisms in tissues or peripheral blood, measurement of specific antibody responses, culture isolation, and molecularly based assays. Given the differences in geographic distribution and prevalence of tick-borne pathogens, advocating for a single testing algorithm that can be applied universally for the laboratory diagnosis of infections transmitted by *Ixodes* ticks is difficult. However, consideration of disease features in a sequential manner can help narrow the differential diagnosis and effectively guide laboratory diagnosis and treatment.

A common theme is that patients with LD, HA, babesiosis, TBE virus, and other tick-transmitted diseases can all present with relatively nonspecific influenza-like illnesses. In these instances, the choice of laboratory tests should be guided by a thorough patient history and physical examination that documents evidence of tick exposure, place of residence, recent travel, and objective signs and symptoms of tick-borne infection. The decision whether or not to pursue specific laboratory testing can then be based on knowledge of which tick-borne diseases are endemic in a particular area and, more importantly, can be made after thoughtful assessment of the probability that the patient actually has one or more tick-transmitted infections. For example, according to the guidelines of the American College of Physicians, patients with vague subjective complaints (e.g., headache, fatigue, and myalgia) are considered to have a low pretest probability for LD and should not undergo antibody screening by ELISA or immunofluorescence assay (IFA), because the majority of positive results will be false positives as a result of cross-reactivity with other microorganisms or disease conditions (211). This is a critical concept, because an exaggerated perception of risk by patients and health care providers can result in substantial amounts of unnecessary testing and associated expense.

Despite those limitations, after it is established that a patient is at moderate to high risk for having one or more tick-transmitted infections, laboratory testing is indicated with only limited exceptions. Patients presenting with typical primary or secondary EM in areas where LD is endemic can often be treated empirically, and the diagnosis does not require laboratory confirmation. Additionally, when these patients are treated with an antimicrobial agent (e.g., doxycycline), performing laboratory testing to document coinfection with HA is neither cost-effective nor necessary, because therapy is highly effective for both agents. In the majority of other clinical situations, laboratory testing is required for definitive diagnosis and to guide therapy.

Among the most useful laboratory tests for tick-borne infections are complete blood counts and peripheral blood smears along with tests of liver function. For patients with HA, leukopenia, lymphopenia, granulocytopenia, and elevated liver enzymes are commonly observed. Anemia is common in babesiosis, and thrombocytopenia is frequently evident in babesiosis and HA infections. Babesiosis and HA can often be diagnosed directly by observing organisms on Giemsa-stained smears of peripheral blood. For patients with intact spleens, 1% to 10% of erythrocytes might show *B. microti* ring forms on

thin blood smears; this proportion may be as high as 80% for asplenic patients (89, 126). The laboratory should screen multiple slides before considering a smear to be negative. Manual microscopy is a subjective process, and the accuracy of the examination depends on the vigilance and experience of the observer, the intensity of parasitemia, and the timing of evaluation relative to illness onset. Intracellular babesia might be confused with Howell-Jolly bodies (121); conversely, false-positive results can occur when inexperienced observers mistake platelets or staining artifacts for piroplasm within erythrocytes or anaplasma morulae within granulocytes. A minority of HA patients with early, mild infection had intragranulocytic morulae detected on smears (21), in contrast with symptomatic, untreated patients whose smear results were evaluated after several days of fever (12). With development of more sensitive molecularly based techniques for diagnosing *B. microti* and *A. phagocytophilum*, PCR is increasingly relied on to detect infection among patients with low pathogen loads and negative blood smears.

For the majority of tick-borne infections, laboratory diagnosis is often made by detection of IgM antibodies in specimens obtained during acute illness or by observing an increase in IgG antibody titers between acute- and convalescent-phase samples taken 10 to 14 days apart. A significant advantage of this approach is that immunoserologic testing is widely available and is usually cost-effective. When seeking laboratory confirmation of a tick-borne disease, health care professionals should utilize a licensed laboratory that employs strict quality control and is experienced in antibody testing. Multiple testing formats are available, including ELISA, IFA, and immunoblotting. For such diseases as LD, the interpretation of immunoserologic testing has become standardized (29).

In the United States, suspected cases of extracutaneous LD are often evaluated by the CDC's two-step protocol, where positive or equivocal screening results by ELISA or IFA are confirmed by a standardized immunoblot. This approach improves specificity and provides sufficient information to allow rational patient management decisions in the majority of cases. In contrast, for Europe and Asia, development of a uniform approach for the immunoserologic evaluation of LD is complicated, because organisms from three species of the *B. burgdorferi* sensu lato genogroup can cause infections. Within these species, substantial antigenic variation exists (75). For the best performance, immunoserologic assays need to be developed for defined geographic areas on the basis of specific species and strains of *B. burgdorferi* sensu lato genogroup organisms that are endemic to each area.

This review does not discuss the performance characteristics of each of the immunoserologic assays available for *Ixodes*-associated infections, but a number of concerns related to this type of testing warrant emphasis. Clinicians should recognize that performance characteristics for these tests differ, depending on the type and quality of the antigens incorporated into each test. Cutoff values for positive results differ among laboratories; furthermore, the patient population that is being tested and the prevalence of specific infections in a particular geographic area will affect the sensitivity and specificity of the test. Laboratories should provide physicians with detailed information on the performance characteristics for each of the assays they provide.

Culture isolation of *Ixodes*-associated pathogens from clinical specimens provides direct evidence of infection but can be time-consuming and expensive and is usually limited to special circumstances. Examples of this include confirmation of infection caused by pathogens in areas where they have not previously been endemic and diagnosis of reinfection for patients for whom the results of immunoserologic testing might not be interpretable. The techniques involved often require special culture media, cell lines, animal inoculation, and a high level of biocontainment that is not practical for the majority of clinical laboratories. Sample requirements can also be stringent. Clinicians should contact their reference laboratory for guidance if isolation of these pathogens is being considered.

Laboratories have often turned to molecular assays in an attempt to increase sensitivity and specificity and to decrease the turnaround time for laboratory results. PCR assays are available for detecting nucleic acids of the agents for LD, HA, babesiosis, and TBE. An advantage of molecular detection is the ability to diagnose early infections before the appearance of serum antibodies, without the delay associated with culture isolation. However, these assays have limitations as well, and assessing the probability of a tick-borne infection for PCR-based tests is as important as assessment for immunoserologic assays. False-positive and false-negative results can occur for different reasons, and results need to be interpreted in the context of the clinical situation. Transient or limited numbers of tick-borne organisms (e.g., HA or babesiosis) within the sampled material might yield a false-negative test result. Providers should be aware that there has been little standardization of molecular assays for tick-borne infections across laboratories, and performance characteristics are highly variable. Therefore, in the majority of cases, a negative PCR or other molecular test result does not exclude the possibility that an infection is present. One circumstance where PCR has been evaluated extensively and is especially useful is the case of LD-associated arthritis. Determining whether chronic arthritis is caused by persistent infection or a prolonged immunologic response is difficult. A negative PCR result from joint fluid in this instance supports an immune-mediated etiology for persistent arthritis rather than an active infection requiring additional antimicrobial therapy.

Pathogenesis and Immunologic Effects

Uncertainties remain regarding the pathogenesis and immunologic effects of coinfections among humans. No prospective studies have been conducted to assess the immunologic effects on humans, but experimental studies on animals reveal that simultaneous infection with *B. burgdorferi* and *A. phagocytophilum* modulates the immune response and affects the development of arthritis. In a mouse model, coinfection increases the number of CD4⁺ cells and drives cytokine release toward a T helper 1 (Th1) lymphocyte response (elevated interleukin-4 [IL-4] and decreased IL-2 levels) (225). There is also evidence from animal models that coinfection with *B. burgdorferi* and *A. phagocytophilum* leads to increased pathogen loads in blood and tissue, and to more-severe LD-associated arthritis, than single infection with *B. burgdorferi* alone (209). During coinfection, murine levels of IL-12, gamma interferon, and tumor necrosis factor in serum were paradoxically decreased and

levels of IL-6 were elevated. These findings suggest that dual infection may modulate host immune responses, such that an increased spirochete burden results in more-severe Lyme arthritis. A similar study of coinfection among C3H/HeN mice evaluated the population distribution of tick-transmitted *B. burgdorferi* and *A. phagocytophilum* infection by quantitative PCR of multiple organ tissues as well as by serologic responses to both agents (87). Among coinfecting animals, spirochete numbers increased in multiple tissues but *Anaplasma* numbers remained constant. Antibody responses decreased for *A. phagocytophilum* but not for *B. burgdorferi*. The researchers postulated that coinfection modulated pathogen burdens and host antibody responses, possibly by the ability of *A. phagocytophilum* to functionally impair neutrophils in the early defense against *B. burgdorferi* infection.

Coinfection with *B. burgdorferi* and *B. microti* has also been demonstrated to have immunologic effects in animal models, including alteration of the Th1 cell response and increased severity of arthritis (129). In another mouse model, dual infection with *B. burgdorferi* and *B. microti* appeared to follow independent courses (45). When young immunocompetent, young asplenic, young BALB/c, and aged C3H/HeN mice were coinfecting with *B. burgdorferi* and *B. microti*, babesiosis followed its normal course of infection without evidence of increased severity, as determined by the percentage of parasitemia and other clinical and laboratory parameters. LD also followed its usual course and severity among coinfecting mice compared with singly infected control subjects, with no increase in spirochete dissemination or arthritis. In summary, the immunologic and pathological effects in animal models are often not generalizable to humans, and further investigation is needed to determine the clinical implications of these findings.

Clinical Manifestations

Lyme disease and babesiosis. Early reports of babesiosis described the occurrence of initial and secondary EM-like skin lesions and recurring monoarticular arthritis for some patients (23, 72), now widely recognized as clinical manifestations of LD and indicative of undiagnosed coinfection. With the exception of EM, the initial symptoms of LD can overlap with symptoms of babesiosis. However, patients simultaneously infected with *B. burgdorferi* and *B. microti* appear to have more diverse, intense, and persistent symptoms. In a prospective study, coinfecting patients were twice as likely to report influenza-like symptoms (e.g., fever, chills, sweats, headaches, fatigue, and nausea) than patients with LD alone; they also had a higher incidence of splenomegaly (104, 106). EM was more frequent among patients with LD alone (88%; $n = 214$) than among coinfecting patients (62%; $n = 26$) (106). Not surprisingly, concurrent infection often resulted in a longer duration of illness, which is attributable, at least in part, to a delay in diagnosis of, and lack of treatment for, babesiosis (104, 106). Persistent symptoms appear to be more associated with coinfection than with either babesiosis or LD alone, and 50% of coinfecting patients report at least one symptom (most commonly, extreme fatigue) for ≥ 3 months (106).

Persons coinfecting with *B. burgdorferi* and *B. microti* do not appear to be at greater risk for spirochete dissemination or LD complications, despite the fact that *B. burgdorferi* DNA persists

longer in the blood of coinfecting patients after treatment (106). At least one prospective study demonstrated no evidence of increased joint, cardiac, or neurologic disease or hospitalization among coinfecting patients compared with patients with LD alone (104). This study's findings are consistent with a similar lack of increase in musculoskeletal and neurologic effects noted in other published reports of coinfecting patients (106, 217). Case reports have discussed a more dramatic, severe clinical course among coinfecting patients, but these likely reflect a bias toward reporting cases with more severe or unusual manifestations (119, 131, 143, 204). All studies to date have been limited in their power to detect potentially subtle differences in outcomes because of their relatively small numbers of patients. Whether clinical outcomes differ among partially treated or untreated patients with coinfection compared with those with LD alone remains unanswered.

In summary, possible dual infections with *B. burgdorferi* and *B. microti* should be considered for patients with a diagnosed tick-borne illness whose exposure occurred in areas where both diseases are endemic. Hematologic findings, such as anemia and thrombocytopenia, are uncommon among patients with LD alone and can be indicators of *Babesia* coinfection (20, 76, 104). Tick-borne diseases rarely occur during influenza season. Thus, the clinician should consider coinfection for any LD patient complaining of marked influenza-like symptoms, or if the patient demonstrates unexplained splenomegaly, anemia, thrombocytopenia, or a failure to respond to antimicrobial therapy directed against *B. burgdorferi*.

Lyme disease and HA. A limited number of case reports describe *B. burgdorferi*-*A. phagocytophilum* coinfections that have been confirmed by molecular methods or visualization of intragranulocytic morulae. Serologic evidence alone is often insufficient for confirming dual infection, because patients with acute HA infection might have a false-positive LD serology (222). The presence of IgM or IgG antibodies to *B. burgdorferi* might also represent prior asymptomatic infection. In an LD vaccine trial in the United States, 30 (11%) of the 269 study participants who acquired LD had asymptomatic IgG seroconversion to *B. burgdorferi* (196). Several seroprevalence studies in Europe have demonstrated that >50% of *B. burgdorferi*-seropositive persons do not recall having any symptoms of LD (64, 74, 139). Furthermore, levels of IgM and IgG antibodies to *B. burgdorferi* decline slowly after treatment, and these antibodies have been demonstrated to persist for months to years after clinical cure (65, 94). Thus, LD infection might be incorrectly diagnosed on the basis of a positive serologic test and should be considered significant only if accompanied by a characteristic clinical picture.

A small cohort of 7 Rhode Island patients coinfecting with *B. burgdorferi* and *A. phagocytophilum* reported significantly more chills, sweats, headaches, arthralgias, and sore throats than 89 patients with LD alone (104). Coinfecting patients reported a significantly higher mean number of symptoms and a longer duration of symptoms. Although coinfection with *B. burgdorferi* and *A. phagocytophilum* might result in more severe or persistent symptoms (131, 147), no evidence exists of increased dissemination of the LD spirochete among these coinfecting patients (104). One-third of patients with HA might have a cough (12), which is rarely reported in cases of LD (133). Leukopenia and thrombocytopenia are commonly reported in HA (12, 104)

but would be unusual for LD patients (133, 135, 218). Elevated liver transaminase levels can occur in both Lyme borreliosis and HA (12, 133).

As with babesiosis, clinicians should consider additional laboratory tests for HA when LD patients demonstrate a more intense and persistent array of nonspecific, influenza-like symptoms, especially fever, chills, and headache. Coinfection with HA is also suggested when LD patients fail to respond to appropriate β -lactam antimicrobial therapy (amoxicillin, ampicillin, or ceftriaxone) (104, 131) or demonstrate laboratory evidence of neutropenia and thrombocytopenia (14, 104). For suspected coinfection with HA or babesiosis, clinicians might consider a complete blood count with a Giemsa-stained blood smear a reasonable initial evaluation.

Transfusion-Related Tick-Borne Illness

Tick-borne pathogens have been demonstrated to survive for prolonged periods in refrigerated blood components, leading to concern about the possibility of transmission of tick-borne diseases by transfusion (36, 110). More than 50 transfusion-related cases of infection with *Babesia* species (*B. microti*, WA1, MO1) (110, 123) and at least 1 potential case of *A. phagocytophilum* infection have been reported in the United States (62). Somewhat surprisingly, there are no confirmed reports of *B. burgdorferi* transmission by blood transfusions, despite the fact that tens of thousands of cases of LD are reported annually in North America and Europe (110, 123).

Nonetheless, viable *B. burgdorferi* has been identified in refrigerated packed red blood cells, whole blood, and frozen plasma stored for >1 month (11, 63, 134), and public health concern exists regarding the possible risk for transfusion-related transmission of LD. Less documentation exists on the viability of *A. phagocytophilum* in stored blood products; at least one study documented that presumptive *A. phagocytophilum* remained viable in refrigerated blood for ≤ 18 days (93). At present, there are no known case reports on dual tick-borne infections transmitted through transfusion of blood products.

THERAPY

Treatment of HA and LD

Doxycycline, 100 mg twice a day, has been used successfully to treat HA and remains the treatment of choice for the majority of HA infections among both children and adults. Doxycycline is highly active against *A. phagocytophilum* in vitro, and neither acquired nor inherent resistance has been described (90, 97, 122). Doxycycline is typically administered orally for 7 days but can be intravenously administered for more severe cases. Despite concerns about dental staining in children, doxycycline remains the drug of choice for treating HA infections, providing superior therapy for a potentially life-threatening disease; further, available data suggest that short courses of doxycycline (≤ 14 days) do not cause significant discoloration of permanent teeth (152). However, caution should be exercised during pregnancy, because of concerns of possible adverse effects on fetal skeletal development, bone growth, and enamel hypoplasia.

Under special non-life-threatening circumstances, rifampin

can be considered an alternative to doxycycline for treatment of HA (70). Both rifampin and levofloxacin demonstrate excellent in vitro activity against *A. phagocytophilum* (90); however, clinical experience and documentation of effectiveness are limited for rifampin and nonexistent for levofloxacin. The use of rifampin in treating HA during pregnancy (30) and for two pediatric patients (102) has been described; no data exist regarding the effectiveness of levofloxacin in treating HA. Chloramphenicol demonstrates suboptimal in vitro activity against *A. phagocytophilum* (122), and treatment failures with chloramphenicol have been reported (60). *A. phagocytophilum* is resistant to numerous antimicrobials commonly used in clinical settings, including aminoglycosides (gentamicin, amikacin), β -lactam antibiotics (amoxicillin, ampicillin, cefuroxime axetil, and ceftriaxone), macrolides (erythromycin, azithromycin, clarithromycin), clindamycin, and trimethoprim-sulfamethoxazole (90, 122).

Doxycycline has the additional benefit of being highly active against *B. burgdorferi*. Longer courses of doxycycline therapy are required for treatment of LD coinfection, with a 14-day course for early localized infection with *B. burgdorferi* (EM) and longer regimens (≤ 28 days) for disseminated or late complications. Other antibiotics commonly used in treating early and late LD among both pediatric and adult populations (e.g., amoxicillin and ceftriaxone) are ineffective in treating HA. In contrast, doxycycline not only is highly active against both *A. phagocytophilum* and *B. burgdorferi* but also has the advantage of being active against other tick-borne infections, such as ehrlichiosis, Rocky Mountain spotted fever, other spotted fever group rickettsioses, and tick-borne relapsing fever (caused by *Borrelia* species). For patients with HA who have clinical or laboratory features indicative of coinfection with *B. burgdorferi*, a longer course of doxycycline (14 to 21 days) is recommended, according to evidence-based treatment recommendations for LD presented by the Infectious Diseases Society of America (223). The ultimate choice and duration of antimicrobial therapy should be guided by the stage of LD. Clinicians might want to empirically treat HA patients from regions of LD endemicity with 14 days of doxycycline, because *B. burgdorferi* coinfection is difficult to rule out during the acute illness phase, and it has been reported for as many as 10% of HA patients (128).

Treatment of Babesiosis

Patients infected by *B. microti* often remain asymptomatic or experience only mild illness and recover without specific chemotherapy. The combination of oral clindamycin (for adults, 600 mg every 8 h; for children, 20 to 40 mg/kg of body weight/day, divided into three daily doses) with oral quinine (for adults, 650 mg every 8 h; for children, 25 mg/kg/day, divided into three daily doses), taken for 7 to 10 days, appears to be an effective chemotherapeutic regimen for the less severely ill patient, although potential toxicities with this regimen are clinically significant (103, 124). Use of intravenous clindamycin (for adults, 300 to 600 mg every 6 h; for children, 20 to 40 mg/kg/day in three doses) with oral quinine remains the recommended and preferred regimen for the more severely ill patient (101). In treating children, careful attention must be given to the calculation of dosing. The pediatric dose of med-

ication, as a general principle, must not exceed the recommended adult dose.

More recently, combination therapy with atovaquone (for adults, 750 mg every 12 h with meals; for children, 20 mg/kg every 12 h with meals) and azithromycin (for adults, 500 mg on day 1 and 250 mg/day thereafter; for children, 12 mg/kg/day) for 7 days has been demonstrated to be as effective as 7 days of combination therapy with clindamycin-quinine for patients with non-life-threatening *B. microti* infections (103, 124). Higher doses of azithromycin (500 to 1,000 mg daily) and 10 days of total combination therapy with atovaquone have been employed also (124, 219). Fifteen percent of patients on atovaquone-azithromycin reported adverse reactions, most commonly diarrhea and rash; clindamycin-quinine was associated with adverse reactions (tinnitus, diarrhea, and decreased hearing) for 72% of patients (103).

Partial or complete exchange transfusions have been used for severely ill babesiosis patients with substantial hemolysis, renal or pulmonary compromise, or high levels of parasitemia ($\geq 10\%$) (26, 78, 101). When given concurrently with appropriate chemotherapy, whole-blood exchange transfusion might be life-saving (58). Human infections with the bovine pathogen *B. divergens* should also be regarded as a medical emergency, and prompt therapy is indicated. Exchange transfusions in combination with intravenous clindamycin and intravenous or oral quinine have been successful in treating *B. divergens* in Europe (26, 27).

Certain antiprotozoal and antimalarial therapeutic regimens have been largely unsuccessful, including chloroquine, primaquine, pyrimethamine, pyrimethamine-sulfadoxine, tetracycline, minocycline, pentamidine isethionate, and trimethoprim-sulfamethoxazole (100, 101). Clinicians should be aware that treatment for babesiosis will not provide effective treatment for LD or HA. Neither clindamycin, quinine, atovaquone, nor azithromycin provides any activity against *B. burgdorferi* or *A. phagocytophilum*. Under these circumstances, patients with *B. microti/divergens* infections who are suspected of also having LD or HA require an additional antimicrobial (e.g., doxycycline) (131).

STRATEGIES FOR PREVENTING COINFECTIONS FROM *IXODES* TICKS

Prevention of LD and other diseases transmitted by *Ixodes* ticks has posed a substantial public health challenge. The majority of prevention programs stress personal protection measures such as tick habitat avoidance (including information on when and where people are at risk), protective clothing, repellents, and frequent tick checks to locate and remove ticks before they can transmit disease. Although these efforts are useful, completely avoiding tick habitats, maintaining regular repellent use, wearing protective clothing in warm weather, or locating attached ticks (especially smaller nymphs) often can be impractical or impossible (157). Research demonstrating the efficacy of personal protection measures is lacking, and tick-borne disease case numbers continue to increase in parts of the world where such diseases are endemic (79).

While not routinely recommended, a single-dose prophylactic antibiotic treatment (e.g., doxycycline) of known tick bites to prevent potential LD is often practiced in the United States. At present, tick bite antibiotic prophylaxis is the only available

preventive measure proven effective in preventing LD (132). However, doxycycline should be taken only by individuals who have had a recognized *Ixodes* tick bite within the previous 72 h, and where there is reasonable information to suggest that the tick remained attached long enough (24 to 48 h) to potentially transmit *B. burgdorferi* if it was infected. Medical providers should remain mindful of the facts that the majority of LD patients do not recall having had a tick bite and the probability of LD transmission from any given tick bite is usually low (176). In addition, this practice has not been evaluated in Europe or Asia for *B. burgdorferi* sensu lato species or for other tick-borne diseases.

An LD vaccine was made available to the public in 1999 but was withdrawn from the market in 2002 after a decline in sales caused by concerns about the vaccine's safety and usefulness. Because of the difficulty of preventing tick exposure, the majority of tick-borne disease prevention programs stress early recognition and treatment of tick-borne disease cases.

The management of ticks in the environment for the prevention of LD and other tick-borne diseases has been reviewed at length (69, 79). Tick control measures (e.g., acaricides applied to tick habitats or hosts) are available; however, they can be costly, and often landowners are reluctant to use pesticides on their property. Habitat modification (e.g., burning tick habitat, leaf litter and brush removal, or landscaping to create dry barriers) can be part of prevention efforts, but these efforts are labor-intensive and require routine maintenance. Given the increasing numbers of tick-borne disease cases despite multiple prevention measures, human coinfections with tick-borne pathogens will likely continue to be a public health problem into the foreseeable future.

RESEARCH NEEDS

Our knowledge of individual tick-borne zoonoses has increased dramatically during the past 2 decades, but relatively little progress has been made in our understanding of coinfections. Funding agencies should focus on research that can be applied to the recognition, treatment, or prevention of tick-borne coinfections among humans. Additional prospective studies are needed in North America and Europe to assess the immunologic and clinical effects of the most common coinfections, including Lyme borreliosis with either HA or babesiosis. The sensitivity, specificity, and predictive value of laboratory markers of coinfection should be evaluated to facilitate prompt clinical detection.

In addition, clarification is needed on the geographic distribution of *B. burgdorferi* sensu lato genogroup, *B. microti/divergens*, and *A. phagocytophilum* for all areas of the world, including nontemperate climates. Limited or no information exists on the incidence or effects of coinfection with other tick-borne pathogens (e.g., TBE and Powassan flaviviruses, spotted fever group rickettsiae, *Fransicella tularensis*, and *Bartonella* spp.). Prospective cohort studies involving healthy persons with frequent exposure to tick habitats are needed to assess the absolute risk for coinfection. This risk might vary in time and space because of increasing human exposure, climate and habitat change, and the expanding geographic distribution of *Ixodes* ticks. Finally, research is needed to understand the role of genetic heterogeneity among coinfecting pathogens. In particular, further efforts are needed to assess competition between

variant clones of *A. phagocytophilum* and the impact of these clones on disease transmission and severity.

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