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Review Article

THE EMERGENCE OF LYME DISEASE IN A CHANGING ENVIRONMENT IN NORTH AMERICA AND CENTRAL EUROPE*

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ABSTRACT

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Lyme disease has recently begun to emerge as a significant threat to human health, both in Europe and the United States. Late sequellae, resembling those of neurosyphilis and multiple sclerosis, may occur many years after initial infection. Spontaneous abortion accompanies arthritis, carditis and neuritis as burdensome short-term sequellae. Thousands of new infections are recognized each year on each side of the Atlantic, although reporting may be incomplete. The disease was described in Europe nearly a century ago and named erythema chronicum migrans, but its etiology has only recently been defined. The name "Lyme disease" was coined to describe a particularly intense American focus of disease, but the term has gained wide acceptance on both continents. The identity of the American and European etiological agents involved has yet to be determined.

In America, a deer-associated, often bird-transported tick transmits this mouse-reservoired spirochete. The European situation seems more complex because the vector tick feeds on a greater variety of vertebrates. The reservoir hosts of the spirochete have yet to be determined. The role of *Ixodes ricinus* and possible other vectors in perpetuating transmission of the European infection remains to be defined. Whether *I. ricinus* as well as *I. dammini* merely serve as a bridge to the human population or are important for the maintenance of the feral cycle remains to be seen.

The capacity of a tick to maintain transmission of Lyme disease spirochetes depends upon a complex set of properties, including competence as a host for the spirochete, a pattern of feeding that focuses on a particular reservoir favored by a pattern of tick activity, during each transmission season, in which nymphs feed before larvae. Transmission would be favored by an environment, such as that of islands, in which the variety of potential reservoir hosts is restricted. Hosts, for example reptiles, that might fail to support growth of the spirochete would serve to dilute effective transmission in nature.

*The present review lays stress on selected papers focusing on the epidemiological aspects and the experimental approach to the newly emerged disease rather than reviewing the complex literature concerning Lyme disease.

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Similarly, the capacity of a vertebrate to maintain the infection requires long-term support of the spirochete in a tissue site accessible to vector ticks, tolerance of repeated feeding by vector ticks and a pattern of host activity that exposes the host to numerous bites.

The intensity of infection depends upon a continuous pattern of transmission in which each generation is infected anew. The rare event in which the vector inherits infection would serve mainly to transport the spirochete to a new site, most effectively by migrating birds.

Due to the dispersed nature of Lyme disease and its recent emergence as an important hazard to health, measures for prophylaxis have only recently been devised. Lyme disease can be treated with antibiotics. But the effectiveness of such therapy depends upon correct and prompt diagnosis; delayed treatment is less effective, presumably because the spirochete becomes sequestered in immune-privileged sites.

INTRODUCTION

Infectious disease in highly developed regions of the world has assumed a new prominence in clinical experience. Among other infections, thousands of cases of Lyme disease, AIDS and Legionnaire's disease have come to burden human health in parts of North America and Europe. Each affects a different segment of the population: Lyme disease particularly threatens those who visit, work or reside in the most scenic and desirable parts of affected regions.

Lyme disease was recognized as a clinical entity soon after the turn of the century, when the Swedish physician, Afzelius, coined the term "*erythema chronicum migrans*" to describe a rare, seemingly innocuous rash of unknown etiology (Afzelius, 1921). The presently accepted term for this condition was coined later to describe an epidemic occurring in the Connecticut (U.S.A.) village of Old Lyme in which some 51 people, mostly children, suffered an atypical arthritis during the mid 1970s (Steere et al., 1977). This outbreak of "Lyme arthritis" was soon found to involve a broad spectrum of symptoms, including the peculiar annular rash experienced by European patients. "Lyme disease" then came to replace the narrower designation for this increasingly common disease both in North America and Europe. Environmental change seems to fuel its developing incidence.

ETIOLOGY

Diagnosis of Lyme disease was hampered, until recently, by the subjectivity imposed by the absence of an identifiable etiological agent. Thus the discovery, in 1982, of its spirochetal nature set the stage for a surge of publications on many facets of the biology of this agent. This seminal discovery was registered by Willy Burgdorfer and his associates while studying ticks collected from an island site in New York that was notably affected by various tick-borne diseases (Burgdorfer et al., 1982). The agent was grown in culture, and antisera soon became available for testing against the sera of clinically diagnosed

patients. Numerous investigators later confirmed that clinical Lyme disease correlated with seropositivity. Similarly infected ticks were discovered in Europe as well as in other parts of the United States (Burgdorfer et al., 1983). The spirochete that Burgdorfer discoverd came to be named *Borrelia burgdorferi* in his honor.

CLINICAL COURSE

The pathognomonic rash appears initially as a dark central punctation that becomes surrounded by a bright red margin. Gradually, the margin expands outwards as a transient, migrating ring. Satellite lesions may appear and disappear. A flu-like illness generally accompanies the rash. Weeks or months later a syndrome may develop that includes chronic meningoneuritis, myocarditis and recurrent intermittent oligoarthritis. Heart-block may be life-threatening. Spontaneous abortions have been attributed to Lyme disease. Arthritic attacks may recur, sometimes becoming chronic and involving erosion of cartilage and bone. Years later, a third phase of this disease may ensue. Demyelenization of nerves, in the course of this chronic development, simulates syphilis and results in the psychiatric and neurological signs characteristic of lues. These late developments may include dementia and masquerade as multiple sclerosis. Spirochetes have been discovered in a variety of tissues, including blood, synoveal fluids, skin and heart, as well as other organs (Steere, 1985).

DIAGNOSIS AND TREATMENT

Clinical diagnosis is based on a characteristic presentation of symptoms, and the peculiar ring-like rash serves as a uniquely useful sign, when present. This *erythema migrans* (EM) is present in about 3/4 of diagnosed patients. A history of tick-bites is generally misleading in the northeastern United States, because the nymphal ticks mainly transmitting infection are so small that they generally escape notice. The apparently irrelevant and much larger dog tick, *Dermacentor variabilis*, frequently attacks people and is more commonly reported to physicians. Serological procedures, involving polyclonal as well as monoclonal reagents, and employing immunofluorescence and ELISA technology, now provide objective bases for clinical diagnosis. Serological conversion provides definitive diagnosis. The spirochetal etiological agent can rarely be demonstrated in blood or in biopsied tissue. Because treatment is most effective when begun early in the course of infection, prompt and convenient diagnostic criteria are essential.

Lyme disease has come to eclipse other tick-borne human infections. Rocky Mountain spotted fever in the United States and tick-borne encephalitis in Europe remain prominent as candidate agents to be considered in differential

diagnosis of tick-transmitted infections. Because the rickettsial and the viral infection are both more life-threatening, they require special attention. However, the spirochetal disease assumes an increasingly important role in the clinic because of its incidence and the burdensome nature of its chronic features.

B. burgdorferi is sensitive to antibiotics, particularly tetracycline, or penicillin in the case of children. Oral treatment, using moderate doses of antibiotic, generally clears infection and aborts development of humoral antibody. About 10% of the patients fail to become spirochete-free. Treatment of chronic Lyme disease is more difficult, presumably because the agent has become sequestered in immune-privileged sites. Parenteral administration of massive quantities of penicillin generally is recommended. As in the case of syphilis, successful treatment may release large quantities of antigen, resulting in allergic Jarisch–Herxheimer reactions due to formation of immune-complexes (Steere, 1985).

ROLE OF *IXODES* TICKS AS VECTORS OF LYME DISEASE

Correlative, epidemiological evidence initially implicated *Ixodes* ticks as vectors of Lyme disease in North America (Spielman et al., 1985). Outbreaks of infection were known only where *Ixodes dammini* was abundant, and the seasonal incidence of human disease coincided with the season of greatest nymphal abundance. This reasoning was strengthened by experimental evidence implicating nymphal *I. dammini* in another zoonotic disease, caused by *Babesia microti*. Comparable evidence for Lyme disease transmission could not precede demonstration of the etiologic agent, and such did not occur until 1983. In fact, tick-derived material from a field site provided the basis for this initial discovery. Soon thereafter, similar spirochetes were demonstrated in *I. ricinus*, the sheep tick that is so abundant in Europe (Burgdorfer et al., 1983). *I. pacificus* serves as the vector in western North America. Although other ticks may play some role in transmission, *Ixodes* ticks appear to be crucial where human disease occurs.

Laboratory evidence that *I. dammini* is competent as a vector of Lyme disease was derived from experimental induction of the characteristic erythematous rash in rabbits by using vector ticks. Indeed, antispirochete antibodies were detected in the serum of patients suffering from Lyme disease, and spirochetes were isolated from their tissues. Recent studies demonstrate that virtually all *I. dammini* become infected after feeding on spirochete-infected hosts and that infection can readily be transmitted, even via the bite of a single tick. Where transmission is intense, as many as half of wild-caught nymphs carry spirochetes, both in the United States and in Europe.

Intense human infection by the agent of Lyme disease is generally distributed along coastal portions of the northeastern United States and the northern Great Plains, particularly affecting portions of the states of Massachusetts,

Rhode Island, Connecticut, New York, New Jersey, Delaware, Wisconsin and Minnesota. In "high risk" sites, as many as 10% of residents may become infected each year. Elsewhere in the United States, human disease is much less episodic. Thus, occasional cases occur in virtually all parts of the country and in Canada. Episodic transmission is limited to sites in which *I. dammini* is abundant. A segment of the Pacific coast, near San Francisco, provides the sole exception to this generality. There, *I. pacificus* serves as a vector. Scattered cases of Lyme disease are associated with the lone star tick, *Amblyomma americanum*, or the southern deer tick, *I. scapularis*, which may occasionally transmit human infection. In Europe, the focality of Lyme disease remains to be defined.

ENVIRONMENTAL CORRELATES FOR HUMAN DISEASE

Its recent recognition in North America and emergence as a threat to human health in Europe suggests that present environmental trends may influence the intensity of transmission of Lyme disease. Alternatively, this increasing incidence may represent an artifact due to increased physician awareness and improved diagnostic procedures. It is true that clinical diagnoses are now more effective than in the recent past, but relevant features of the environment have also been subject to dramatic change during the past few decades.

In North America, *I. dammini* was recognized as a distinct species as late as 1979 (Spielman et al., 1979). The oldest known collections of this tick date back to the early 1920s, when a group of French and American scientists collaborated on a biological control effort aiming to reduce the abundance of ticks on Naushon Island. This sparsely populated and privately owned island, located near Cape Cod in Massachusetts, had been maintained in a relatively pristine state since colonial times. Interestingly, the island archives document visits for the purpose of illegal hunting by Hessian mercenary troops, employed by the English during the American War of Independence. Later, this island served as a rare hunting resource in the region, being visited for that purpose by American presidents, including Ulysses S. Grant and Theodore Roosevelt. Elsewhere, deer, *Odocoileus virginianus*, had become virtually extinct. In the course of the biological control effort, specimens of *I. dammini* were collected and preserved. They were misidentified as *I. scapularis*, and similar specimens were collected there throughout the following decades. *I. scapularis* is endemic to the southeastern portion of the country. The remote Naushon site remained as an isolated, but mistaken, northern outpost in its range. Since the early 1970s, *I. dammini* has been collected from a much larger region, where earlier it must have been exceedingly rare (Spielman et al., 1985).

The changing pattern of abundance of deer in the United States parallels that of Lyme disease. Before 1600, the native Indian inhabitants of the U.S.A. had maintained a stable relationship with their neighboring fauna (Cronon,

1983). A park-like landscape, conducive to the development of deer, was formed by deliberate burning. The Indians began to overexploit their available resources when durable goods were introduced from Europe. Furs, hides and meat provided their most accessible vehicle of exchange. The resulting decline in deer numbers accelerated the need for cloth, thereby intensifying the exploitation of the remaining animals as a means of exchange. By the middle 1600s virtually all large animals from southern New England and the middle Atlantic states had been destroyed. Agricultural efforts by the colonists then maintained the region in a deforested condition until the early 1900s, when agriculture was displaced to the West. Simultaneously, efforts to eradicate Texas cattle fever included a nationwide program of deer eradication. Deer did not begin to reestablish themselves in the North-East until the 1930s, and it was not until the 1950s that the presence of deer became commonplace.

The concept is implied that *I. dammini* was once widely distributed in the northeastern United States, but that it virtually disappeared as a result of deforestation and disappearance of white-tailed deer; the tick survived only in the few isolated sites where these deer remained. Such refugia may have included Naushon, Gardiner and Shelter Islands along the northeastern coast, as well as Long Point in Ontario (Canada) and perhaps another site in northeastern Wisconsin. During the past 20 years or so, the increasing abundance of deer seems to have permitted this tick to begin to regain its original North American distribution.

EXPERIMENTAL EVIDENCE OF THE ROLE OF DEER

In order to verify by experiment the correlative evidence relating the abundance of deer ticks to that of white-tailed deer, the deer on a tick-infested island in Massachusetts were removed, virtually to extinction. The experiment took place recently on Great Island, a small island off the coast of Massachussetts connected to the mainland by a narrow causeway. A marked decrease in the abundance of larval *I. dammini* promptly followed, but nymphs seem to have persisted beyond the expected time decline, two years later. The two-year cycle of the tick imposes such a delay because nymphs derive from adults that engorge a year and a half earlier. Several factors seem to have buffered the response to this deer-removal experiment. Those deer removed late in the winter, after many adult ticks had already engorged, would have permitted development of numerous larval and nymphal ticks. Of course, hosts other than deer may sustain the adult stage of the tick, and foxes seem to serve as effective reservoir hosts. But neither alternative would explain why nymphs seem to decline in numbers less rapidly than do larvae. Even if removal of deer were promptly followed by decreasing abundance of nymphal *I. dammini*, various considerations would seem to render deer destruction impractical as a measure

generally intended to protect human health (Wilson et al., 1984; Wilson et al., in prep., a).

SEASONAL FACTORS AFFECTING TRANSMISSION

The seasonal pattern of activity of American *I. dammini* has been well-described (Spielman et al., 1985). Blood serves as the sole nutrient, and each of the three trophic stages feed only once. Larvae are most abundant from July through September, and virtually disappear during the rest of the year. Remarkably, maximum abundance of the next developmental instar, the nymph, occurs earlier in the year, during May and June (Wilson and Spielman, 1985). This can be explained by the two-year cycle of the tick. Adult *I. dammini* quest for hosts mainly during late fall and early winter, but feeding resumes during spells of warm weather and continues until early spring. Ticks of this species mate while feeding; engorged females then detach and fall to the ground. Males remain on their hosts, where they may continue sexual activity (Wilson and Spielman, 1985).

In Central Europe, the seasonal activity of the various stages of *I. ricinus* is less coherent than in the case of American *I. dammini.* All stages of the tick occur simultaneously throughout the season of activity, beginning in March/April and as a rule falling virtually to nil before the end of November (Apitzsch, 1967; Bauch, 1971, 1972, 1973; Aeschlimann, 1972; Mass, 1975; Walter and Liebisch, 1980). There are several papers indicating that nymphal and adult activity of *I. ricinus* occurs about 3–4 weeks before that of larvae (Loew et al., 1964; Aeschlimann, 1972; Gilot et al., 1975; Walter and Liebisch, 1980). In most locations, the tick's feeding activity was found to be minimal in midsummer, thereby describing a bimodal pattern: one peak during April through June and the other during late August through September. However, environmental factors may modify the seasonal pattern of activity, e.g. Bauch (1972) found that a warm and dry microclimate in summer favors a bimodal seasonal activity of Central European *I. ricinus* while a cool and moist one does not. All stages of *I. ricinus* are able to survive the winter; eggs and engorged stages, however, seem to be more successful in surviving. Like *I. dammini*, each generation spans two or three years and perhaps even more (Bauch, 1971; Walter and Liebisch, 1980).

The seasonal questing activities of Central European *I. ricinus* and Northeast American *I. dammini* are not congruent and this may have important epidemiological implications. Assuming that larval and nymphal ticks serve to maintain spirochetal infection in some rodent reservoir, transmission would be most efficient if nymphs, already infected the year before, feed in each transmission season before the larvae. Such a pattern in the sequence of seasonal tick activities would not only promote prevalence of infection in the vector population but also increase risk of infection in man. Of course, the well-delim-

ited season of transmission to man of spirochetes borne by nymphs of *I. dammini* derives from this seasonal activity pattern. Since the seasonality of the Central European vector is different, it could render *I. ricinus* less effective in perpetuating infection than *I. dammini*. However, recent studies in Ireland by Gray (1984, 1985), on larval and nymphal activity of the sheep tick *I. ricinus*, state findings which reveal similarities to those reported from immature stages of *I. dammini* in the U.S.A. In Gray's study, larval activity was almost unimodal, with a relatively small peak in spring and almost all activity taking place in midsummer (July–August). While nymphal activity was highest in spring (March–May) and in autumn (October–November), nymphs were always more frequent in spring than in autumn (Gray, 1984).

ROLE OF DEER IN THE EPIZOOTIOLOGY OF LYME DISEASE

The host associations of American *I. dammini* differ from those of European *I. ricinus*. Adult *I. dammini* specialize in a narrow range of hosts, adult *I. ricinus* are more indiscriminate. Both ticks feed abundantly on deer and fox, *Vulpes vulpes*, but adult *I. ricinus* attack lagomorphs. Rabbits, *Sylvilagus floridanus*, are not suitable as hosts for *I. dammini*. Adults have never been found on such hosts in nature, and experimental evidence demonstrates that lagomorphs rapidly become solidly immune to attack. In contrast to *I. dammini*, which is found solely where deer are abundant, *I. ricinus* is said to occur in the absence of deer. Sheep or cattle can serve as suitable hosts, but animals such as lagomorphs cannot be excluded. While deer may not be necessary as hosts in Europe, their presence may promote transmission of Lyme disease on both continents (Milne, 1947; Rosicky and Cerny, 1954; Negrobov et al., 1965; Walter and Liebisch, 1980; Wilson et al., 1985).

More objective evidence describes the relationship between population density of white-tailed deer and the abundance of *I. dammini* (Wilson et al., 1985). The abundance of larvae was estimated on 13 islands in coastal Massachusetts. White-footed mice (*Peromyscus leucopus*) were most intensively infested by larvae on islands most commonly frequented by deer. The abundance of nymphal *I. dammini* did not correlate with that of deer, apparently because birds transported larvae between islands. This finding reflected the outcome of the previously described deer-removal experiment. Adult *Ixodes* ticks, which are less abundant than larvae or nymphs, accumulated where the deer were removed. Contact with deer, therefore, seems to serve as the crucial bottleneck in the cycle of the tick. Thus, in North America if not in Central Europe, deer play an indirect but dominant role in the epizootiology of human Lyme disease. It is possible that their abundance in Central Europe correlates with that of the vector as it has been shown for North America.

ROLE OF RODENTS IN THE EPIZOOTIOLOGY OF LYME DISEASE

To the extent that vector ticks acquire the Lyme disease spirochete in the course of feeding, any diversion from suitable reservoir hosts would dilute the force of transmission (Levine et al., 1985). Larval and nymphal *I. ricinus* feed on a broad range of hosts, such as mice, birds and lizards, but large mammals, including people, serve as host as well. Where human infections are clustered in North America, immature *I. dammini* mainly parasitize white-footed mice, but also other small rodents and even birds. Relative abundance of vertebrates greatly influences their utilization as hosts by ticks, and white-footed mice are by far the most abundant vertebrates in these sites. Lizards are absent. This relationship between a particular mammal and the vector tick, occurring where risk of Lyme disease is greatest, suggests that white-footed mice serve as the most effective reservoirs of infection.

In spite of this relationship with mice, density of deer seems to limit the abundance of *I. dammini*, and this profoundly affects the circumstances of transmission. If the abundance of this tick is independent of that of mice, the density of ticks on mice would vary inversely with that of mice! Intensity of transmission depends on the number of vectors on each reservoir host and not on absolute abundance of the vector. Intuitive reasoning might focus control efforts on any animal that is implicated as an important reservoir for a pathogen, but with deleterious effects upon human health. Not only would reduced abundance of mice focus additional bites upon each surviving mouse, but questing nymphs might accumulate in such a manner that human residents of the region would be subject to additional bites. Destruction of mice may, in fact, cause increased transmission of Lyme disease, particularly affecting human hosts. These considerations may not apply to *I. ricinus* due to its apparent lack of host-specificity.

VECTORIAL CAPACITY AND VECTOR COMPETENCE

The role of a vector in the maintenance of a zoonosis, a disease transmissable from an animal reservoir to man, can be described in terms of vector competence and vectorial capacity (Spielman and Rossignol, 1984). The term vector competence concerns the relationship between vector and pathogen, including any factor that applies to uptake of the pathogen, its development and ultimate delivery to an animal serving as reservoir for that pathogen. Horizontal competence applies when infection is not inherited by the vector, as when nymphs transmit infection acquired by the preceeding larval stage. Vertical competence applies to inherited infection, as within transovarial (congenital) transmission. Vectorial capacity includes vector competence as well as any behavioral or environmental factors that may influence dissemination of the pathogen by the vector. Previous sections deal with various components of vectorial capac-

ity, mainly vector abundance and narrowness of host range; the following discussion focuses on vector competence of *I. dammini* and its European relative *I. ricinus*.

VERTICAL VERSUS HORIZONTAL TRANSMISSION

Transovarial transmission of *B. burgdorferi* by *I. dammini* has been demonstrated, but such vertical transmission seems to be rare (Levine et al., 1985). To determine whether larval *I. dammini* had acquired spirochetal infection through transovarial transmission, larvae collected in endemic sites were fed on noninfected hamsters. After molting, resulting nymphs were examined for spirochetes. Surprisingly, in a site in which about 80% of adults harbored spirochetes fewer than 1% of these nymphs were infected. This suggests that vertical transmission of *B. burgdorferi* contributes little to natural nidality in the northeastern United States. Laboratory observations confirm that *I. dammini* lacks vertical vector competence for the agent of Lyme disease. European *I. ricinus*, on the other hand, may more frequently inherit infection (Burgdorfer et al., 1983). Thus, most of the laboratory-reared progeny of two adults, collected in Switzerland, proved to be infected. However, these original females differed from most field-derived ticks in that they harbored numerous spirochetes disseminated throughout their bodies. Spirochetal infection was present only in about 30% of *I. ricinus* collected in that site. Infrequent vertical transmission appears to contribute little to perpetuation of spirochetal infection or to risk of human disease. Establishment of a focus would be greatly facilitated through importation of the agent by birds. An infected larva might be transported to a remote site while feeding on a migrating bird or other vagile vertebrate. Even if that transport host were incompetent as a reservoir of infection, the resulting nymph would infect its next host and might establish a new focus of infection.

ROUTE OF INFECTION

Until recently, the course of development of the Lyme disease spirochete in its vector remained obscure. The pathogen was first isolated from the gut of *I. dammini*, a finding confirmed so many times that other sites of infection seemed unlikely. Disseminated infection was rarely noted. Hemolymph-borne infection was noted in only 4 of 71 unengorged female *I. ricinus* and 2 of another 39 that had survived oviposition. Each of these ticks exhibited spirochetes in the contents of their guts. This apparently infrequent dissemination suggested that the salivary glands could not commonly be infected. Thus, regurgitation or defecation seemed likely routes for transmission, or perhaps release in the course of grooming (Burgdorfer et al., 1983).

As ixodid ticks salivate copiously while engorging, saliva could effectively

serve as a vehicle for spirochetes or another of the numerous infectious pathogens transmitted by these ticks (Ribeiro et al., 1985). Spirochetes could not infect the salivary glands, however, without traversing the hemolymph. Spirochetes do disseminate during a relatively brief span of time in the course of attachment; in nymphal *I. dammini* dissemination follows at about 2 days after attachment and in adults at about 4 days. Salivary infection coincides with onset of rapid engorgement. Spirochetes were detected in saliva soon thereafter. Infection becomes generalized in about half of the ticks with a midgut infection, and half of these produced saliva contaminated with spirochetes. This suggests that transmission may be prevented by prompt removal of attached ticks. Because adult ticks are larger than nymphs and take longer to transmit, they are generally removed from their human hosts before transmission has occurred.

ANTIHEMOSTATIC ROLE OF SALIVA

The pharmacological activity of the saliva of ticks may profoundly affect vector competence. Like other hematophagous organisms, ticks must overcome the hemostatic mechanism of their hosts in order to obtain blood and maintain its flow (Ribeiro et al., 1985). Aggregation of platelets, which serves as a crucial obstacle confronting a feeding tick, depends mainly on release of adenosine diphosphate (ADP, released by damaged cells), exposure of collagen fibrils (as a result of vessel injuries), thrombin (activated by the coagulation cascade) and platelet-aggregation factors (released by leukocytes). Thromboxane (released by activated platelets), ADP and serotonin are further potential stimuli for vessel constriction and platelet aggregation. Thus, thrombogenesis, including platelet aggregation and fibrin-induced coagulation, threatens feeding success.

Because ixodid ticks take so long to feed, they must evade their host's immune response. Inflammation, with its attendant edema and hemostasis, would otherwise inhibit feeding. The saliva of *I. dammini* contains a prostaglandin (PGE2) and a kininase, in addition to a set of directly antihemostatic components. The diversity of inflammatory response expressed by different hosts and the narrow host range of certain ticks reflects Trager's seminal discovery of host-specific immune evasion in ticks. Salivary diversity may contribute to such specificity. *I. dammini* salivates an extraordinary pharmacological cocktail including antihemostatic, antiinflammatory and immunsuppressive components. Like bloodsucking bugs, tsetse flies and mosquitoes, tick saliva prevents platelet aggregation induced by ADP, collagen or platelet-aggregation factors. Another component serves as an anticoagulant by inhibiting thrombin formation. Interestingly, the apyrase component of saliva degrades one nucleotide that stimulates platelet aggregation (ADP) as well as another that is said to stimulate feeding (ATP). This paradox extends to hematophagous bugs,

mosquitoes and tsetse flies, in which an evolutionary convergence centers around the common problem of overcoming hemostasis. Since inflammation and platelet aggregation are induced redundantly, other yet undescribed components surely lie hidden in the saliva of ticks.

ANTIINFLAMMATORY ROLE OF SALIVA

Because ATP stimulates mast cell degranulation and aggregation of neutrophils, salivary apyrase would serve an antiinflammatory as well as an antihemostatic role in the feeding of ticks (Ribeiro et al., 1985). By inhibiting inflammation, this enzyme would indirectly counteract hemostasis due to reduced release of thromboxane, platelet-aggregation factors and vasoactive amines. Apyrase converts ATP to AMP, which is pharmacologically inactive or inhibitory to purinergic P2 receptors. In addition, PGE2 is antiinflammatory, inhibiting degranulation of mast cells, activation of macrophages, neutrophils and T cells. As a result, interleukin-2 production is inhibited, at least in vitro. Prostaglandin also prevents release of lysosomal enzymes from polymorphonuclear cells. T cells serve as primary stimuli for the cascade of cellular events leading to antibody production, thereby providing yet another role for the prostaglandin content of saliva. The saliva of *I. dammini* is an unusually rich source of PGE2, perhaps containing enough of this material to generally suppress the ability of certain hosts to mount a humoral response against this tick. In this manner, the site of tick attachment is modified by the tick such that pathogens placed there may be privileged against rejection. Lyme disease spirochetes would be protected in such an environment while accomplishing the transition between such diverse hosts as a tick and a mammal. This many-faceted process of immune evasion remains to be explored in all of its fascinating complexity. Horizontal transmission of the spirochete requires that individual vertebrate hosts permit repeated feeding by *Ixodes* ticks and they fail to reject the pathogen. The antiinflammatory role of saliva contributes to these crucial components of vector competence.

ANALGESIC EFFECTS OF SALIVA

The bite of a tick must not be painful; pain would provoke grooming by a host which might lead to the tick's destruction. In this context, salivary PGE2 constitutes of a paradox, because it induces release of bradykinin from serum (Ribeiro et al., 1985). Bradykinin, in turn, powerfully potentiates pain induced by pressure, as would result from the edema-promoting effects of PGE2. In this manner certain components of saliva produce a spectrum of effects that may hinder as well as help feeding. The advantageous effects of PGE2, for example, include increased flow of blood due to erythema, inhibition of mast cell degranulation and immune suppression, thereby preventing production of

antibodies against salivary antigens. On the other hand, this same salivary component potentiates pain produced by bradykinin and the production of edema. However, the saliva negates its own counterproductive effects through the action of a kininase that degrades bradykinin. In this manner ticks regulate, to their advantage, the hemostatic and inflammatory responses of their hosts through the action of an integrated complex of salivary components.

Diverse inflammatory and immune mechanisms characterize the many kinds of mammals that could serve as hosts for *Ixodes* ticks and reservoirs of Lyme disease. It may be that a high degree of specificity permits these ticks to evade their host's immune responses. The remarkable pharmacological arsenal of components in the saliva of *I. dammini* would thereby contribute to the transmission of the Lyme disease spirochetes as well as other tick-borne pathogens.

Among the various and disparate functions of the ticks' salivary glands are those regulating body water balance and ionic equilibrium. In their non-parasitic phase, water-deficient ticks use saliva to compensate for water loss. They secrete a hygroscopic solution, evidently produced by the type I alveoli, onto the rostrum. This secretion takes up water vapour from subsaturated atmospheres when the relative humidity exceeds a certain critical value. The water-enriched secretion is subsequently imbibed by the mouth and transferred via the pharyngeal pump into the midgut (Knülle and Rudolph, 1983). In their parasitic phase and in the course of feeding, ticks secrete their surplus water and most of the excess NaCl, acquired with the blood meal, into the host. This is accomplished by certain cells of the alveoli II and III which are not prominent in unfed ticks (Kaufman, 1983). In fact, the salivary glands of ticks are involved in osmoregulation and function as excretory organs.

RESERVOIR HOSTS OF THE SPIROCHETE

The capacity of the white-footed mouse to serve as a reservoir of the Lyme disease spirochete in North America is suggested by the frequency with which larval and nymphal *I. dammini* feed on this rodent. In addition, *P. leucopus* serves as reservoir for *Babesia microti*, another *I. dammini*-transmitted pathogen; reservoir capacity for one pathogen implies similar capacity for any other pathogen transmitted by the same vector, assuming similar reservoir competence (Spielman et al., 1985).

This inferential evidence implicating mice as reservoirs for *B. burgdorferi* was supplemented by the results of a recent field study wherein evidence of spirochetal infection was sought in *I. dammini* collected by a variety of techniques (Levine et al., 1985). White-footed mice were each infested by about four nymphs, and about half of these contained spirochetes. Since nymphs feed for 3 or 4 days, these mice must have been exposed to infection almost daily. In fact, spirochetal infection appeared to be universal. Spirochetal infection was far more frequent in ticks derived from larvae or nymphs found on these

mice than in others collected by any other means. Thus, spirochetal infection in the vector correlates with frequency of contact with the white-footed mouse.

Such ground-associated birds as thrush may harbor numerous immature *Ixodes* ticks, particularly the larval stage of *I. dammini*, which quests mainly during August and September when bird migration is in full force. At this time, tick-infested birds become most abundant in sites lying in migratory routes leading away from locations where mice are infested. Earlier in the summer, bird infestation coincides with that of mice. Spirochetes similar to *B. burgdorferi* have been isolated from birds, but the role of birds as reservoir for this agent have not yet been quantified (Anderson et al., 1986). In any case, certain birds clearly serve as transport hosts for *I. dammini* ticks, particularly in a southward direction. The mode of transport of the spirochete is problematic (Wilson et al., in prep., b). Birds are also well-known as hosts for larval and nymphal *I. ricinus*.

The reported presence of spirochetes in the blood of white-tailed deer that were killed in road accidents suggests that deer may serve as reservoir for *B. burgdorferi* (Bosler et al., 1984). Although serological tests confirm that these mammals may be exposed to spirochetal antigen, the role of deer as reservoirs of the spirochete remains to be confirmed. Particularly relevant would be an effort to determine whether larval *Ixodes* ticks can acquire infection from deer, thereby demonstrating reservoir competence. Immature *I. dammini* do feed on deer, but this would occur less frequently than on rodents, which invariably are far more abundant. Deer would be relevant as sources of infection for adult *Ixodes* only if vertical infection contributed to the force of transmission. Larval *I. dammini* are rarely infected in nature, as discussed. In the absence of more definitive information, the role of deer in the epidemiology of Lyme disease would seem to be limited to that of a crucial food source for the adult stage of the vector tick, *I. dammini.*

COMPETENCE OF MICE AS HOSTS FOR THE SPIROCHETE

A competent reservoir host for the Lyme disease spirochete must readily be infected and should permit the agent to proliferate in a site accessible to the *Ixodes* vector. Rabbits, for example, appear to support spirochetal infection and to become infectious for *I. dammini.* However, this finding has not been expressed in quantitative terms. Only a few larval *I. dammini* became infected with spirochetes after they had been allowed to engorge on a rabbit infected with spirochetes in the laboratory. In contrast, virtually all ticks that had engorged on spirochete-infected white-footed mice became infected (Donahue et al., 1986). Moreover, even a single nymph is generally sufficient to transmit the pathogen. The prepatent period seldom exceeds a week, at which time half of the attached larvae became infected. Infectivity peaks after about three weeks and then gradually declines. This demonstrates that white-footed mice rapidly

become highly infectious for ticks and remain so for the duration of a transmission season. In the laboratory the golden hamster, *Mesocricetus auratus*, proved to be as susceptible to spirochetal infection as is the white-footed mouse.

In addition to its competence as a host for the Lyme disease spirochete, a competent reservoir host must permit repeated feeding by the tick. Rabbits become immune to the bites of *I. dammini* after one or two exposures, and hamsters behave similarly. White-footed mice, on the other hand, never come to reject this tick. A host like the golden hamster would not contribute to the maintenance of transmission, since vector ticks generally were unable to continue development after repeated engorgement on the hamster. Vector-specific immune tolerance of the white-footed mouse is certainly another essential parameter of the host specificity of *I. dammini.*

The significance of a vertebrate species as reservoir for a zoonosis depends upon the proportion of vectors acquiring infection from this particular host. Serological evidence alone, or evidence of occasional infection does not demonstrate reservoir capacity. In the northeastern United States, at least, the white-footed mouse appears to serve as the cricial reservoir host mainly responsible for perpetuating Lyme disease as well as babesiosis (Spielman et al., 1985).

AMERICAN-EUROPEAN COMPARISONS

In the northeastern part of the United States, the major features of Lyme disease have begun to emerge. *I. dammini* serves as vector, both for maintaining infection in nature and as the "bridge" transmitting the spirochete to human hosts. The white-footed mouse, *P. leucopus*, is the main reservoir host. Voles, *Microtus pennsylvanicus*, seem less important. The availability of deer regulates reproduction by adult *I. dammini* and hence the abundance of the vector.

In Central Europe, mice of the genus *Apodemus* occupy an ecological niche similar to that of the white-footed mouse in the United States. Although their competence as reservoir hosts for *B. burgdorferi* has not been tested, they are suspect as the primary reservoir hosts. *Clethrionomys* voles remain as candidate hosts. Other possibilities cannot be excluded.

I. ricinus, however, is definitely implicated at least as a bridge vector, transmitting the Lyme disease spirochete to man. Other ticks restricted to mice, such as *I. trianguliceps*, may play a role in maintaining zoonotic transmission, as suggested by its role as a vector of *B. microti.* Like *I. dammini*, adult *I. ricinus* feed on deer, but cattle and sheep promote their abundance as well (Milne, 1947). In any case, the rising incidence of diagnosed Lyme disease in Europe parallels that in the United States and both coincide with a recent dramatic increase in the numbers of deer. The American deer herd began to recover from its centuries-long eclipse in the 1930s. A corresponding event in Central Europe occurred 30 years later. It is well known that in the 1940s the deer herd had

been largely destroyed in the aftermath of the second World War, when the disrupted economy forced the residents of the region to exploit all available animals for food. At that time the region was also partly deforested, owing to a shortage of other sources of heating fuel during the series of exceptionally cold postwar winters. Thereafter, rapid economic improvement, accompanied by a growing environmental consciousness, stimulated people to encourage the growth of forests and the return of deer. In Europe, as in North America, deer abundance in densely populated regions has now reached unprecedented levels. This apposition of people, rodents and deer may be the root cause of the present zoonosis.

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