Zoonosis Update

In 1976, public health officials investigated a cluster of suspected juvenile rheumatoid arthritis cases that occurred among residents of Lyme, Connecticut, and neighboring communities.[1] More than 50 residents were evaluated for recurrent, usually short-lived (1 to 2 weeks' duration) attacks of swelling and pain in a few large joints. Clinical, laboratory, and epidemiologic evidence failed to substantiate an immune-mediated pathogenesis. An arthropod-transmitted bacterium was suspected as the etiologic agent, as many patients also had an expanding, red, annular rash that resembled erythema chronicum migrans (a lesion identified in Europe in the early 20th century that was associated with tick bites and was responsive to penicillin).[2,3] An infectious cause for the disease was confirmed when spirochetal bacteria isolated from *Ixodes dammini* (now considered *I scapularis*) ticks[4]

bacterial proteins) and prevention (identification of potential vaccine candidates) of Lyme borreliosis.

Ecology and Transmission

Borrelia burgdorferi is maintained in nature in a cycle that involves hard ticks of the *Ixodes* genus as vectors and small mammals or birds as reservoir hosts. *Ixodes* spp are 3-host ticks that attach to a host and take a blood meal at each life stage (larva, nymph, and adult), then drop off the host to molt in the environment.[25] Larval and nymphal stages of *Ixodes* spp are found in moist, protected areas, such as under leaf litter in humid hardwood forests. The principal hosts of immature *Ixodes* ticks are small rodents, lizards, and ground-feeding birds. The immature ticks typically require 2 to 4 days of attachment to the host to complete a blood meal. At the adult stage, an *Ixodes* tick climbs to the tips of grasses, where it waits (or quests) for a large mammal host to brush against it. Adult ticks feed typically for 5 to 7 days. The abundance and activity of each life stage differ by season and are dependent on weather, sunlight, and host availability.[26,27]

In the northeastern and upper midwestern United States, *I scapularis* (commonly known as the deer tick or black-legged tick) is the principal vector of *B burgdorferi*. Larval and nymphal *I scapularis* acquire *B burgdorferi* primarily from infected white-footed mice (*Peromyscus leucopus*).[5,28,29] *Borrelia burgdorferi* is transmitted trans-stadially from larva to nymph and from nymph to adult. *Ixodes scapularis* that become infected as larvae or nymphs can subsequently transmit the agent as nymphal and adult ticks when they feed in the summer and fall, respectively.[30] Transovarial transmission (adult female to egg) is rare and inefficient.[25,31,32] White-tailed deer (*Odocoileus virginianus*) are the preferred hosts of adult *I scapularis;* however, because they are poor reservoirs for *B burgdorferi*,[33] they serve chiefly to maintain the population of ticks and not that of *B burgdorferi*. Furthermore, birds may introduce infected

Connecticut. Between 1995 and 2000, reported incidence of Lyme borreliosis exceeded 10 cases/100,000 person-years in 7 states (Connecticut, Delaware, Maryland, New Jersey, New York, Pennsylvania, and Rhode Island). Each year, > 90% of reported cases occur among residents of the northeastern and upper-central states. In these same areas, serologic evidence of exposure to *B burgdorferi* has been observed in a high proportion of dogs (in some instances > 50%).[60,61] In contrast, seroprevalence estimates among clinically normal dogs in the southern and western United States have been uniformly low (≤ 3.5%) and generally within the margin of error of false-positives for the assays used.[62-64]

Risk of infection with *B burgdorferi* is correlated with the opportunity of being bitten by an infected tick and dependent on the density of vector ticks in an endemic area, the proportion of ticks infected, and the duration and nature of the susceptible host's activities in that area. Most cases of Lyme borreliosis are believed to be acquired from the bites of nymphal ticks, which are most abundant in the late spring and early summer. In 1 study,[65] > 50% of humans reported to have Lyme borreliosis in an endemic county of New York experienced onset of illness in June or July. Residence in or near areas of relatively undisturbed and dense vegetation poses the greatest risk.[65] Outdoor recreational activities in similar vegetated areas can also increase chance of infection.[66] Persons whose occupation places them in wooded areas (eg, forestry or wildlife workers) may occasionally be exposed to infected ticks.[67] Dogs and horses that have ongoing access to densely vegetated areas near their home (peridomestic exposure) or occasional recreation-

swelling among cattle in an endemic region.[99,100] Attempts to experimentally infect cattle with *B burgdorferi* suggest they have a low susceptibility.[101]

Diagnosis

Diagnosis of Lyme borreliosis can be made on the basis of history of exposure to *Ixodes* ticks in an endemic area, compatible clinical signs, laboratory evidence of infection, consideration and exclusion of other diseases, and, possibly, response to antimicrobial treatment.[83,102] Laboratory results alone are not prima facie evidence of infection but must be interpreted with regard to the pretest probability of the disease existing in the patient.[103,104] Establishing the prior probability of Lyme borreliosis is particularly important for species such as dogs that lack a pathognomonic sign of infection like the erythema migrans rash in humans.

Given the fastidious growth requirements of *B burgdorferi*, attempts to culture spirochetes from blood or other tissues are difficult and most often unrewarding. Thus, most commercially available clinical laboratory tests rely on detection of antibodies in serum. Serologic assays for IgM, IgG, or combined immunoglobulin against *B burgdorferi* are available through most commercial laboratories. The sensitivity of serologic assays is directly dependent on the kinetics of the immunologic response following infection. In humans, serum concentration of IgM against *B burgdorferi* increases within 2 to 3 weeks of infection, peaks around 3 to 6 weeks, and then gradually decreases.[105] Changes in serum IgG concentration lag that of IgM; IgG concentration begins to increase 4 to 6 weeks after infection, peaks at 6 to 8 weeks or later, and remains high for months to years.[6] In most or all dogs, IgG seroconversion occurs prior to onset of clinical signs, usually within 4 to 6 weeks after exposure.[81,102]

Enzyme immunoassays (EIAs) and immunofluorescent assays (IFAs) are the most commonly available serologic tests; however, despite their high sensitivity, these tests generally have poor specificity.[104] Unstandardized and variable procedures for manufacture and validation of commercial test kits further reduce the reliability of these assays.[106,107] In a laboratory proficiency study[108] in which seroimmunologic tests for 14 different pathogens were evaluated, assays for *B burgdorferi* antibody had the poorest correlation between reference and nonreference laboratories. To improve test reliability for human patients, the CDC currently recommends a 2-step serodiagnostic strategy: an initial EIA or IFA, with specimens that yield positive or equivocal results for *B burgdorferi* further tested by western immunoblotting for IgM or IgG antibody, whichever is appropriate for the patient's stage of illness.[109]

In dogs, the immunoblot band pattern does not merely enhance test reliability but provides indispensable information to differentiate serologic responses induced by natural infection with *B burgdorferi* from those produced by vaccination.[77] Dogs that are vaccinated react most strongly to spirochetal proteins in the 31- to 34-kd rauri

reduce the chance of recrudescent Lyme borreliosis. Following experimental inoculation of dogs with *B burgdorferi*, spirochetes were repeatedly cultured from skin biopsy specimens prior to treatment; after 30 days of azithromycin (25 mg/kg [11.4 mg/lb], PO, q 24 h), ceftriaxone (25 mg/kg, IV, q 24 h), or doxycycline (10 mg/kg [4.5 mg/lb], PO, q 12 h), examination of biopsy specimens from multiple tissues failed to yield any viable spirochetes.[84] In a similar study[132] of experimentally infected dogs, administration of immunosuppresive dosages of corticosteroids led to a recurrence of severe lameness in 2 of 2 dogs that had not received antimicrobial treatment but in none of 12 that received antimicrobial treatment.

Treatment is rarely indicated for dogs with serologic evidence of *B burgdorferi* exposure in the absence of clinical disease. As stated previously, although the number of seropositive dogs in an endemic area can be high, the proportion of these that will develop clinical signs is low. Serologic status determined at a singular point in time is not predictive of future illness; a study[78] of dogs without signs of illness in an endemic area of Connecticut showed that the incidence of signs of Lyme borreliosis over a 20-month observation period did not differ between dogs that were initially seropositive and those that were seronegative. A course of antimicrobials prescribed solely on the basis of arbitrarily timed serologic findings is unlikely to reduce morbidity or to be effective in preventing reexposure in an endemic area.

Prevention and Control

The foundation for preventing Lyme borreliosis in domestic animals and humans is the reduction of the risk of tick bites at the environmental or individual level. Avoiding tick bites prevents not only Lyme borreliosis but also other tick-borne diseases, such as ehrlichiosis and babesiosis, in regions where these pathogens are present. A knowledge of the ecologic requirements for the tick-borne diseases that are present in a given area is critical toward selection and implementation of the most effective integrated prevention strategies.[133] In areas where Lyme borreliosis is a peridomestic risk, tick density may be managed locally by targeting animal hosts or by modifying the environment to decrease the availability of tick habitat. Products that kill or repel ticks can reduce the likelihood that ticks will attach to pets. Induced immunity through vaccination may provide additional protection in some highly endemic areas.

Some of the most effective approaches to environmental control of ticks target the reservoir animals that sustain *I scapularis* populations. The use of permethrin-treated cotton balls as nesting material decreased the load of immature *I scapularis* on white-footed mice.[134] Although the number of ticks that infested rodents appeared to decrease with this method over a 3-year period, the number of questing ticks did not differ between treated and untreated sites.[135] The concept of targeting small mammals for tick control was developed commercially as rodent bait boxes that contain fipronil.[b] The success of this targeted approach depends on limiting access of alternative tick hosts to treated areas; therefore, large-scale implementation may be impractical. Bait boxes have not proven as effective in the reduction of tick numbers in the western United States, probably because *I pacificus* feeds on a wider range of vertebrate hosts, compared with *I scapularis*.[136] White-tailed deer have also been effectively targeted in attempts to control adult *I scapularis*. Feeding stations were designed whereby deer that rub against 4 amitraz-impregnated posts transfer acaracide onto their heads and necks (regions of the body where *I scapularis* ticks most frequently attach on deer).[137] In areas where these feeding stations were deployed, the number of adult *I scapularis* observed on deer carcasses was less than that observed on carcasses in areas without the feeding stations.

Environmental approaches to tick control (eg, pesticide application and landscape modification) are designed to decrease suitable habitat for ticks. Acaracides can be a useful adjunctive treatment on limited spatial scales, but applications must be targeted to specific areas and timed appropriately in order to maximize control and minimize excess pesticide residue in the environment.[138,139] Fencing that excludes deer (ie, > 2 m in height) can be constructed around small areas such as a residential property to decrease the number of adult *Ixodes* ticks deposited and thus reduce the number of tick progeny in the environment.[140] A swath of mulch or other inert material placed between wooded areas and lawns can provide an effective impediment to tick movement into areas where they are likely to encounter people and pets.

Control of ticks on dogs is facilitated by the availability of collars impregnated with permethrin or amitraz and topical solutions containing fipronil,[c] permethrin,[d] or selamectin.[e] The myriad of recently developed ectoparasiticides and their control efficacy have been reviewed.[141] Amitraz-impregnated collars appear to be more effective at interrupting the tick life cycle and longer acting than topical applications of fipronil.[142] The appropriate use of amitraz-impregnated collars on dogs can provide effective tick control and thereby prevent infection with *B burdorferi*.[143] Amitraz is also available as a spray or dip for tick control on domestic livestock; its use is contraindicated in horses, pregnant or nursing bitches, and cats. Selamectin is effective in control of brown dog ticks (*Rhipicephalus sanguineus*) and American dog ticks (*Dermacentor variabilis*) on dogs and is safe to use on cats. However, in a study[144] in Europe, topical application of permethrin was more effective at repelling European *Ixodes* spp, compared with topical application of selamectin. Topical administration of permethrin products is contraindicated for cats.[141]

Ideally, owners should examine their pets after visiting tick-infested areas. Although a thorough inspection may not reveal all ticks, prompt removal of those that are found can prevent most tick-borne diseases because there is often a lag period between the initiation of feedi

parts as close to the skin as possible and pulling gently, firmly, and perpendicularly away from the skin. A variety of commercial products[f-h] are available that can be effective in removal of nymphal and adult ticks when used properly.[146] Crushing the tick during the removal procedure does not appear to increase likelihood of transmission of *Borrelia* spirochetes.[145] After the tick's removal, the bite site should be washed with an antiseptic compound. The efficacy of antimicrobial prophylaxis for dogs after tick bites is unknown. When considering a prophylactic course of antimicrobials for a tick bite, veterinarians should carefully weigh the risk of tick-borne disease versus the risk of an adverse drug reaction.

Two whole-cell *B burgdorferi* bacterin vaccines are available for canids.[i,j] An initial efficacy study[147] by 1 of the manufacturers indicated that the vaccine protected laboratory dogs against the development of lameness following syringe-delivered challenge with several different strains of *B burgdorferi*. Results of a large-scale field study[61] indicated that the vaccine was safe and effective at preventing development of Lyme borreliosis in many breeds of dogs. A survey[148] of dogs from a single practice in a Lyme borreliosis-endemic area showed a higher prevalence of seroreactivity to the *B burgdorferi* C6 antigen among unvaccinated dogs (64%), compared with the prevalence among dogs that had received annual vaccination with the whole-cell bacterin (5%). Results of a study[149] involving a model of Lyme borreliosis in hamsters indicated that immunity that develops subsequent to administration of the bacterin is specific to *B burgdorferi* infections (ie, not protective against *B afzelii* or *B garinii*) and short-lived (< 1 year). Findings of a similar study[150] suggested that immune-mediated arthritis was associated with the *B burgdorferi* bacterin; however, it remains uncertain whether there is an association between immune-mediated arthritis in dogs and use of this vaccine.

A recombinant subunit vaccine that contains the highly immunogenic Osp A is the next generation of vaccines available for prevention of Lyme borreliosis in dogs.[k] Because Osp A is expressed by *B burgdorferi* primarily in the gut of ticks prior to feeding, immunity against Osp A is thought to function through complement-mediated lysis of *B burgdorferi* in the tick's gut soon after the tick begins its blood meal.[151] In a study[152] involving use of the subunit vaccine in dogs, protection against *B burgdorferi* transmission by naturally infected *I scapularis* was demonstrated. A recombinant *B burgdorferi* vaccine for horses, also based on the Osp

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