Ticks and Biting Insects Infected with the Etiologic Agent of Lyme Disease, *Borrelia burgdorferi*

LOUIS A. MAGNARELLI* AND JOHN F. ANDERSON

Department of Entomology, The Connecticut Agricultural Experiment Station, P.O. Box 1106, New Haven, Connecticut 06504

Members of 18 species of ticks, mosquitoes, horse flies, and deer flies were collected in southeastern Connecticut and tested by indirect fluorescent-antibody staining methods for *Borrelia burgdorferi*, the etiologic agent of Lyme disease. An infection rate of 36.2% (116 tested), recorded for immature *Ixodes dammini*, exceeded positivity values for all other arthropod species. Prevalence of infection for hematophagous insects ranged from 2.9% of 105 *Hybomitra lasiophthalma* to 14.3% of seven *Hybomitra epistates*. Infected *I. dammini* larvae and nymphs coexisted with infected *Dermacentor variabilis* (American dog tick) immatures on white-footed mice (*Peromyscus leucopus*), but unlike *I. dammini*, none of the 55 adult American dog ticks collected from vegetation harbored *B. burgdorferi*. Groups of 113 field-collected mosquitoes of *Aedes canadensis* and 43 *Aedes stimulans* were placed in cages with uninfected Syrian hamsters. Of these, 11 females of both species contained *B. burgdorferi* and had fed fully or partially from the hamsters. No spirochetes were isolated from the hamsters, but antibodies were produced in one test animal.

The causative agent of Lyme disease, *Borrelia burgdorferi*, has been detected in or isolated from ticks (2, 5, 9–11, 21), human tissues (8, 32), and the blood and organs of wildlife (1–3, 5, 19). Although *Ixodes dammini* is the chief vector of *B. burgdorferi* in the northeastern United States, Wisconsin, and Minnesota, other arthropods, such as mosquitoes, horse flies, and deer flies, have also been found harboring this bacterium (22). Evidence of transmission by hematophagous insects, however, is limited to infrequent associations between bites and the development of erythema migrans (13, 15, 22), a unique skin lesion that marks the early stage of Lyme disease (31, 32).

Members of several species of arthropods harbor *B. burgdorferi* during the summer, but little is known about the prevalence of infection in different sites and years. Moreover, it is unclear whether naturally infected mosquitoes, horse flies, and deer flies retain spirochetes in their digestive tracts, complete gonotrophic cycles, and efficiently transmit *B. burgdorferi* to vertebrate hosts. The objectives of this study were to compare prevalences of infection for ticks and biting insects in two rural communities in Connecticut where human infections with Lyme disease have been documented, to determine reproductive life histories for spirochete-infected blood-seeking mosquitoes, horse flies, and deer flies, to determine whether *B. burgdorferi* can survive in experimentally infected horse flies, and to attempt to isolate *B. burgdorferi* from Syrian hamsters fed upon by naturally infected mosquitoes in the laboratory.

MATERIALS AND METHODS

Study sites and sampling. Ticks, mosquitoes, deer flies, and horse flies were collected during 1986 and 1987 in Salem and Norwich, communities in southeastern Connecticut where Lyme disease is endemic (23, 33). During spring and summer, immatures of *I. dammini* and *Dermacentor variabilis* (American dog tick) were removed from *Peromyscus leucopus* (white-footed mouse) captured in or near forests, while adult ticks of both species were obtained during spring

* Corresponding author.

or summer by flagging vegetation along trails near woodlands. Blood-seeking mosquitoes and deer flies were captured in an insect net during the summer (time of collection, 1000 to 1500 h) as they approached the investigator. Mosquitoes were removed by an aspirator and separated from deer flies before being transported to the laboratory. Horse flies were collected during June and July by erecting dry-ice-baited canopy traps (28). All insects were kept on crushed ice or in styrofoam containers in the field and while in transit.

Dissection and identification of *B. burgdorferi*. Midgut tissues were dissected from ticks and smeared onto glass microscope slides as described previously (5, 9). Heads were removed from mosquitoes and placed on other microscope slides. Internal contents were expelled and smeared by applying pressure to cover slips placed over the heads. Anterior digestive tracts, including the salivary glands and proventriculus, were dissected from field-collected horse flies and deer flies (tabanids) and from experimentally infected females of a salt marsh horse fly, *Tabanus nigrovittatus*. These tissues were smeared onto slides in the same manner as the tick and mosquito tissues. After drying at 37°C, all preparations were fixed in acetone for 10 min and overlaid with murine monoclonal antibody (H5332) diluted 1:4 or 1:8 in phosphate-buffered saline (PBS) solutions. This antiserum was directed to outer surface protein A, a polypeptide of approximately 31 kilodaltons (6, 7) that is common to all North American isolates of *B. burgdorferi*. The specificity of this monoclonal antibody, dilutions of reagents, application of fluorescein isothiocyanate-labeled goat anti-mouse immunoglobulin G (IgG) (1:40), and other procedures used in indirect fluorescent-antibody (IFA) staining have been reported (5–7, 21, 22).

Reproductive life histories. Ovaries were dissected from mosquitoes and tabanids to determine the number of completed gonotrophic cycles. Tissues were teased apart in Ringer physiological saline solution, and ovarioles were examined microscopically to determine whether dilatations or relics (yellow bodies) had formed in follicular tubes. The presence of these structures is evidence of egg development

and oviposition (parity) and can be used to determine the number of ovarian cycles completed. Dissection procedures, terminology for ovarian structures, and interpretations of parity are those of Detinova (12). In general, females of most biting insects ingest blood from vertebrate hosts, mature their eggs, and deposit them in or near aquatic or semiaquatic habitats. Multiple blood feedings and ovarian cycles can occur (12, 20, 34).

Feeding trials and isolation attempts. Blood-seeking mosquitoes of *Aedes canadensis* and *Aedes stimulans* were collected from woodlands in Salem and Norwich. Groups of females, separated by species, were placed into screened cages with anesthetized, uninfected Syrian hamsters in the laboratory. These mammals can be used to isolate *B. burgdorferi* (17). All test animals were initially immobilized with Penthrane (Abbott Laboratories, North Chicago, Ill.) and subsequently injected with ketamine hydrochloride (Vetalar; Parke-Davis, Morris Plains, N.J.). Hamsters were shaved along the head and back to allow mosquitoes to feed readily. After 1 h, the blood-engorged mosquitoes were separated from the unfed individuals, dissected, and screened for spirochetes by IFA staining methods. Two additional hamsters were each inoculated intraperitoneally with 150 μl of Barbour-Stoenner-Kelly (BSK) medium containing living *B. burgdorferi* (CT strain 22956), a 10-day-old culture of a primary isolate recovered from a kidney of *P. leucopus* captured in Armonk, N.Y. The number of spirochetes in the culture medium was about 4.0 × 10⁶/ml. For negative controls, four normal hamsters (not exposed to mosquitoes) were kept in separate cages adjacent to those of the hamsters fed upon by mosquitoes or challenged by inoculation.

Kidneys and spleens were aseptically removed, triturated, and inoculated into BSK medium as described previously (1, 3, 17). Cultures were held at 31°C for 4 to 7 weeks, and samples of medium were examined by dark-field microscopy to detect living spirochetes. In addition, blood samples were collected from hamsters, and sera were stored at −60°C until IFA analyses for antibodies to *B. burgdorferi* could be performed.

Laboratory experiments were conducted to determine whether *B. burgdorferi* survives in females of *T. nigrovittatus*. Blood-seeking horse flies were collected in canopy traps during July 1985 and August 1986 in a salt marsh in Milford, Conn. After being transferred to sugar-free cages in the laboratory, the insects were held overnight with distilled water at 21 ± 3°C. On the next day, they were placed on membrane feeding devices (26) for 20 to 30 min. The food source was kept in a glass reservoir and consisted of 2.0 ml of fresh, citrated beef blood mixed with equal volumes of 7-day-old BSK medium containing living *B. burgdorferi* (CT strain 2591) or PBS (negative control). Following feeding trials, females were dissected at hourly or daily intervals, and duplicate preparations of head and digestive tract tissues were examined for spirochetes by dark-field microscopy and IFA staining methods.

Serologic testing. Hamster serum samples were screened for total immunoglobulins to *B. burgdorferi* by IFA staining procedures (5, 21). Polyvalent fluorescein isothiocyanate-labeled goat anti-hamster IgG (Kirkegaard & Perry Laboratories, Gaithersburg, Md.) was diluted 1:30 in PBS solution. Sera from inoculated hamsters, obtained during earlier experiments, served as positive controls; *B. burgdorferi* infections were confirmed by reisolating the spirochetes from kidney or spleen tissues. Uninfected (normal) hamster sera were also included as controls. In preliminary analyses of nine normal serum samples, there was no nonspecific reac-

TABLE 1. Total arthropods collected and number of specimens infected with *B. burgdorferi* in Salem and Norwich, Conn., during 1986 and 1987

Arthropod species	Salem		Norwich	
	No. of specimens collected	No. (%) infected[a]	No. of specimens collected	No. (%) infected[a]
Ticks[b]				
I. dammini	116	42 (36.2)	2	0
D. variabilis	52	10 (19.2)	14	0
Mosquitoes				
A. canadensis	113	11 (9.7)	10	0
A. cinereus	19	0	5	0
A. stimulans	21	2 (9.5)	23	1 (4.4)
A. triseriatus	18	2 (11.1)	36	0
Deer flies				
C. callidus	106	9 (8.5)	57	6 (10.5)
C. cincticornis	20	0	9	0
C. geminatus	49	0	0	—[c]
C. macquarti	36	3 (8.3)	2	0
C. univittatus	144	13 (9.0)	1	0
C. vittatus	101	3 (3.0)	10	0
Horse flies				
H. epistates	7	1 (14.3)	1	0
H. hinei	51	2 (3.9)	17	0
H. lasiophthalma	101	8 (7.9)	105	3 (2.9)
H. sodalis	38	0	0	—
T. lineola	15	0	0	—
T. quinquevittatus	12	0	2	0

[a] Determined by IFA staining with murine monoclonal antibody H5332.
[b] Larvae and nymphs removed from white-footed mice.
[c] —, Not determined.

tivity at dilutions of ≥1:16. Therefore, reactions of test sera at or above a 1:16 dilution were considered positive. All serum samples were retested to determine reproducibility.

Statistical analyses. When sample sizes were adequate (*n* > 30), variances were computed and tested for homogeneity by an *F* test (30). Statistical differences in sample means were then determined by an appropriate Student's *t* test. All analyses were conducted at the *P* < 0.01 level of significance.

RESULTS

Ticks, mosquitoes, and tabanids of 12 species harbored *B. burgdorferi*. The percentage of infected *I. dammini* immatures (36.2%) removed from white-footed mice in Salem was significantly greater than those of all other arthropods studied (Table 1). The second highest rate (19.2%) was recorded for immatures of *D. variabilis*. Spirochetes in midgut preparations of *I. dammini* usually exceeded an average of 50 per 40× microscopic field. Tissues of naturally infected horse flies and deer flies normally contained ca. 10 to 50 spirochetes per field, while after considerable searching, preparations of head tissues from mosquitoes rarely exceeded 15 spirochetes per field. When present, *B. burgdorferi* was found most readily in tissues from *I. dammini*. In Norwich, females of *A. stimulans*, *Chrysops callidus*, and *H. lasiophthalma* also contained *B. burgdorferi*; the prevalence of infection was 10.5% or less.

Examinations of ovarian tissues from 1,062 blood-seeking mosquitoes and tabanids revealed that the majority had completed at least one gonotrophic cycle and, therefore, had taken at least one blood meal before depositing eggs (oviposition) and starting the second ovarian cycle. For uninfected insects, the numbers of parous specimens (i.e., with evi-

TABLE 2. Parity of biting insects with or without *B. burgdorferi* in Salem and Norwich, Conn. during 1986 and 1987

Arthropod species	Total dissected	No. of infected females[a]		No. of uninfected females	
		Nulliparous	Parous	Nulliparous	Parous
A. canadensis	121		11	35	75
A. cinereus	20			9	11
A. stimulans	43		2	2	39
A. triseriatus	38		2	22	14
C. callidus	162		15	102	45
C. cincticornis	28			13	15
C. geminatus	46			12	34
C. macquarti	37		3	21	13
C. univittatus	123		13	40	70
C. vittatus	100	1	1	58	40
H. epistates	8		1	2	5
H. hinei	68		1	32	35
H. lasiophthalma	201		11	66	124
H. sodalis	38			36	2
T. lineola	15			8	7
T. quinquevittatus	14			8	6
Total	1,062	1	60	466	535

[a] Not all infected mosquitoes were dissected to determine reproductive life history. Nulliparous, No evidence of egg maturation and oviposition; parous, at least one ovarian cycle completed.

dence of oviposition) were higher (52.2 to 95.1%) than nulliparous individuals (i.e., no evidence of oviposition) in 9 of 16 species studied (Table 2). Nearly all of the 61 infected insects had completed at least one gonotrophic cycle. One female of *Chrysops vittatus* harbored *B. burgdorferi* but had not yet oviposited.

During 1986 and 1987, 133 *P. leucopus* were captured in Salem and Norwich and examined for ticks. Infected larvae (*n* = 22) and nymphs (*n* = 20) of *I. dammini* and *D. variabilis* (seven larvae and three nymphs) were removed from 30 white-footed mice during June and July in Salem. An additional 34 mice from Salem carried either or both ticks, but no spirochetes were detected in these ectoparasites. The remaining eight mice had no ticks attached at the time of capture. The numbers of infected *I. dammini* larvae (30.2% of 73) and nymphs (46.5% of 43) in Salem exceeded those of *D. variabilis* (21.9% of 32 larvae and 15% of 20 nymphs). Although the prevalence of infected *I. dammini* nymphs during 1986 (45.8%) was nearly equal to that recorded in 1987 (47.4%), the fourfold difference in numbers of larvae harboring *B. burgdorferi* (10 and 44.2%) was statistically significant. In Norwich, 61 white-footed mice were captured, and of these, 4 carried uninfected *I. dammini* (two larvae) and *D. variabilis* (13 larvae and 1 nymph).

Adults of *I. dammini* and *D. variabilis*, collected in Salem during the spring or summer of 1986 and 1987, were screened by IFA methods for *B. burgdorferi*. Of the 61 males and 83 females of *I. dammini* tested, 19 (31.2%) and 41 (49.4%), respectively, were found to be carrying the Lyme disease spirochete. Application of IFA staining procedures to midgut tissues from 26 males and 29 females of *D. variabilis*, obtained in the same habitat as *I. dammini*, revealed no spirochetes.

To determine whether mosquitoes could transmit *B. burgdorferi*, field-caught blood-seeking females of *A. canadensis* and *A. stimulans* were allowed to feed on uninfected hamsters in the laboratory. Five groups of 113 *A. canadensis* females were each placed with five separate hamsters (16 to 35 mosquitoes per group), while two groups of 43 *A. stimulans* females (15 and 28 per group) were placed with two

other hamsters. Of these, 71 *A. canadensis* and 30 *A. stimulans* ingested partial or complete blood meals. *B. burgdorferi* was detected in the head tissues of nine and two blood-fed specimens, respectively. At least one infected mosquito had fed partially or completely from each of the exposed hamsters. No isolations were made from the spleens or kidneys of seven hamsters fed upon by mosquitoes or from four others held as negative controls. However, *B. burgdorferi* was recovered from the two hamsters that had been inoculated with BSK medium containing these spirochetes. In addition, one of five hamsters fed upon by *A. canadensis* had antibodies to *B. burgdorferi* at a titer of 1:32. An infected mosquito ingested blood from this hamster, and the antibody titer was reproducible. The remaining hamsters exposed to mosquitoes or held as negative controls had no antibodies. Those inoculated with spirochetes had antibody titers of 1:64 or 1:256.

Fifty-seven females of *T. nigrovittatus* ingested infected blood from membrane feeding devices. Of these, 28 had living spirochetes in their heads (including anterior digestive tract tissues). Duplicate preparations were positive by IFA staining. Although 26 infected females were examined within 24 h after feeding, two females harbored living spirochetes for 2 to 3 days after ingesting infected blood. In IFA tests of 15 females that ingested uninfected blood in the laboratory and of 66 host-seeking *T. nigrovittatus* collected in Milford, no spirochetes were detected.

DISCUSSION

Members of several species of arthropods harbor *B. burgdorferi*, but the prevalence of infection was highly variable. In *I. dammini*, the chief vector of *B. burgdorferi* in Connecticut (4, 5, 21, 32), the proportion of infected ticks differed from 11 to 54%, depending on the site, season, and sampling method. At Shelter Island, N.Y., an infection rate of 61% has been reported (9). The presence of *B. burgdorferi* in biting insects also varied. During 1985, 14 species of hematophagous insects were found to be carrying this bacterium in Norwich, Conn. (22); rates were as high as 21% for *C. callidus*. In the present study, females of three species from Norwich contained *B. burgdorferi*, and the prevalence of infection for *C. callidus* was 50% lower.

Horse flies and deer flies can disperse readily from breeding areas (16, 35). The infected females collected in Norwich may have acquired *B. burgdorferi* elsewhere. This study site does not appear to be an important focus for Lyme disease, because the numbers of *I. dammini* on mice were very low and *B. burgdorferi* was not found in these ticks. In addition, we did not collect *I. dammini* adults while flagging vegetation during the spring and fall. Although the sources of infection for mosquitoes and tabanids are unknown, the number of infected specimens may vary with changes in population densitites of large mammals such as white-tailed deer (*Odocoileus virginianus*), horses, or cattle. Antibodies to *B. burgdorferi* have been detected in deer (21, 24) and horses (27; L. A. Magnarelli, J. F. Anderson, E. Shaw, J. E. Post, and F. C. Palka, Am. J. Vet. Res., in press), indicating that these animals were exposed to the Lyme disease spirochete or to another closely related *Borrelia* organism. However, isolation and identification of *B. burgdorferi* are needed to confirm that the large mammals are spirochetemic and serve as reservoirs of infection. In comparison, white-footed mice are abundant in forests and known to be competent reservoirs for *B. burgdorferi* (2, 3, 14, 18). With relatively small home ranges, and as important hosts for *I.*

dammini, these rodents serve to maintain *B. burgdorferi* infections in foci and to infect ticks during the warmer months. Therefore, the presence of infected *I. dammini* is convincing evidence that the sampling area is a focus for Lyme disease.

Infected *I. dammini* and *D. variabilis* coexisted on white-footed mice. This reinforces the epidemiological significance of this rodent in Lyme borreliosis. Since transovarial transmission of *B. burgdorferi* is low in *I. dammini* (25, 29), larvae mainly acquire these spirochetes by feeding on infected hosts. Based on lower percentages of infected *D. variabilis* nymphs and the absence of *B. burgdorferi* in questing adults of this species, transstadial transmission in *D. variabilis* is probably inefficient. In addition, there are no convincing reports indicating an association between American dog tick bites and the development of erythema migrans in humans. Therefore, adults of this species do not appear to be vectors of *B. burgdorferi*.

The occurrence of infected ticks and biting insects in Salem indicates that *B. burgdorferi* is widely distributed among hematophagous arthropod populations. As in East Haddam and Lyme, two communities that border Salem where Lyme disease is also endemic (23, 33), *I. dammini* is abundant. Verification of *B. burgdorferi* in arthropods from Salem confirms earlier clinical reports on human infections in this community. Since birds carry infected *I. dammini* larvae and nymphs (4), the range of this tick may continue to expand, and Lyme disease may become endemic in other communities in southcentral and southeastern Connecticut.

Ovarian examinations of biting insects revealed that nearly all of the infected, blood-seeking mosquitoes and tabanids had completed at least one ovarian cycle. Unlike ticks, mosquitoes and tabanids can ingest multiple blood meals from different mammals during a gonotrophic cycle (20, 34) and in the process may acquire *B. burgdorferi* from one or more infected hosts. Maximal periods of survival for *B. burgdorferi* in naturally infected biting insects are unknown, but in experimentally infected mosquitoes, *B. burgdorferi* lived less than 6 days in the insect's digestive system (26). This, coupled with the relatively low number of spirochetes found in head tissues of field-caught females and no isolates of *B. burgdorferi* from hamsters fed upon by infected mosquitoes, indicates that these insects may not be suitable hosts for this bacterium. The low-level immune response in a hamster fed upon by an infected female of *A. canadensis* may have been directed against dead or weakened *B. burgdorferi*. Although anecdotal, there are records of deer fly bites and the subsequent development of erythema migrans in persons who had Lyme disease (22). In addition, *B. burgdorferi* survives for brief periods in *T. nigrovittatus*. Further studies are needed to confirm that deer flies or horse flies can mechanically transmit *B. burgdorferi*.

ACKNOWLEDGMENTS

LITERATURE CITED

1. **Anderson, J. F., P. H. Duray, and L. A. Magnarelli.** 1987. Prevalence of *Borrelia burgdorferi* in white-footed mice and *Ixodes dammini* at Fort McCoy, Wis. J. Clin. Microbiol. **25:** 1495–1497.
2. **Anderson, J. F., R. C. Johnson, L. A. Magnarelli, and F. W.** Hyde. 1985. Identification of endemic foci of Lyme disease: isolation of *Borrelia burgdorferi* from feral rodents and ticks (*Dermacentor variabilis*). J. Clin. Microbiol. **22:**36–38.
3. **Anderson, J. F., R. C. Johnson, L. A. Magnarelli, F. W. Hyde, and J. E. Myers.** 1987. Prevalence of *Borrelia burgdorferi* and *Babesia microti* in mice on islands inhabited by white-tailed deer. Appl. Environ. Microbiol. **53:**892–894.
4. **Anderson, J. F., and L. A. Magnarelli.** 1984. Avian and mammalian hosts for spirochete-infected ticks and insects in a Lyme disease focus in Connecticut. Yale J. Biol. Med. **57:**627–641.
5. **Anderson, J. F., L. A. Magnarelli, W. Burgdorfer, and A. G. Barbour.** 1983. Spirochetes in *Ixodes dammini* and mammals from Connecticut. Am. J. Trop. Med. Hyg. **32:**818–824.
6. **Barbour, A. G., R. A. Heiland, and T. R. Howe.** 1985. Heterogeneity of major proteins in Lyme disease borreliae: a molecular analysis of North American and European isolates. J. Infect. Dis. **152:**478–484.
7. **Barbour, A. G., S. L. Tessier, and W. J. Todd.** 1983. Lyme disease spirochetes and ixodid tick spriochetes share a common surface antigenic determinant defined by a monoclonal antibody. Infect. Immun. **41:**795–804.
8. **Benach, J. L., E. M. Bosler, J. P. Hanrahan, J. L. Coleman, G. S. Habicht, T. F. Bast, D. J. Cameron, J. L. Ziegler, A. G. Barbour, W. Burgdorfer, R. Edelman, and R. A. Kaslow.** 1983. Spirochetes isolated from the blood of two patients with Lyme disease. N. Engl. J. Med. **308:**740–742.
9. **Burgdorfer, W., A. G. Barbour, S. F. Hayes, J. L. Benach, E. Grunwaldt, and J. P. Davis.** 1982. Lyme disease—a tick-borne spirochetosis? Science **216:**1317–1319.
10. **Burgdorfer, W., A. G. Barbour, S. F. Hayes, O. Peter, and A. Aeschlimann.** 1983. Erythema chronicum migrans—a tick-borne spirochetosis. Acta Trop. **40:**79–83.
11. **Burgdorfer, W., R. S. Lane, A. G. Barbour, R. A. Gresbrink, and J. R. Anderson.** 1985. The western black-legged tick, *Ixodes pacificus*: a vector of *Borrelia burgdorferi*. Am. J. Trop. Med. Hyg. **34:**925–930.
12. **Detinova, T. S.** 1962. Age-grouping methods in Diptera of medical importance. World Health Organization, Geneva.
13. **Doby, J. M., J. F. Anderson, A. Couatarmanac'h, L. A. Magnarelli, and A. Martin.** 1986. Lyme disease in Canada with possible transmission by an insect. Zentralbl. Bacteriol. Mikrobiol. Hyg. Ser. A **263:**488–490.
14. **Donahue, J. G., J. Piesman, and A. Spielman.** 1987. Reservoir competence of white-footed mice for Lyme disease spirochetes. Am. J. Trop. Med. Hyg. **36:**92–96.
15. **Hard, S.** 1966. Erythema chronicum migrans (Afzelii) associated with mosquito bite. Acta Dermatol. Venereol. (Stockholm) **46:**473–476.
16. **Hocking, B.** 1953. The intrinsic range and speed of flight of insects. Trans. R. Entomol. Soc. London **104:**223–345.
17. **Johnson, R. C., N. Marek, and C. Kodner.** 1984. Infection of Syrian hamsters with Lyme disease spirochetes. J. Clin. Microbiol. **20:**1099–1101.
18. **Levine, J. F., M. L. Wilson, and A. Spielman.** 1985. Mice as reservoirs of the Lyme disease spirochete. Am. J. Trop. Med. Hyg. **34:**355–360.
19. **Loken, K. I., C. Wu, R. C. Johnson, and R. F. Bey.** 1985. Isolation of the Lyme disease spirochete from mammals in Minnesota. Proc. Soc. Exp. Biol. Med. **179:**300–302.
20. **Magnarelli, L. A., and J. F. Anderson.** 1980. Feeding behavior of Tabanidae (Diptera) on cattle and serologic analyses of partial blood meals. Environ. Entomol. **9:**664–667.
21. **Magnarelli, L. A., J. F. Anderson, C. S. Apperson, D. Fish, R. C. Johnson, and W. A. Chappell.** 1986. Spirochetes in ticks and antibodies to *Borrelia burgdorferi* in white-tailed deer from Connecticut, New York State, and North Carolina. J. Wildlife Dis. **22:**178–188.
22. **Magnarelli, L. A., J. F. Anderson, and A. G. Barbour.** 1986. The etiologic agent of Lyme disease in deer flies, horse flies, and mosquitoes. J. Infect. Dis. **154:**355–358.
23. **Magnarelli, L. A., J. F. Anderson, and W. A. Chappell.** 1984. Geographic distribution of humans, raccoons, and white-footed mice with antibodies to Lyme disease spirochetes in Connecti-

cut. Yale J. Biol. Med. **57**:619–626.

24. **Magnarelli, L. A., J. F. Anderson, and W. A. Chappell.** 1984. Antibodies to spirochetes in white-tailed deer and prevalence of infected ticks from foci of Lyme disease in Connecticut. J. Wildlife Dis. **20**:21–26.

25. **Magnarelli, L. A., J. F. Anderson, and D. Fish.** 1987. Transovarial transmission of *Borrelia burgdorferi* in *Ixodes dammini* (Acari: Ixodidae). J. Infect. Dis. **156**:234–236.

26. **Magnarelli, L. A., J. E. Freier, and J. F. Anderson.** 1987. Experimental infections of mosquitoes with *Borrelia burgdorferi*, the etiologic agent of Lyme disease. J. Infect. Dis. **156**:694–695.

27. **Marcus, L. C., M. M. Patterson, R. E. Gilfillan, and P. H. Urband.** 1985. Antibodies to *Borrelia burgdorferi* in New England horses: serologic survey. Am. J. Vet. Res. **46**:2570–2571.

28. **Pechuman, L. L.** 1981. The horse flies and deer flies of New York (Diptera, Tabanidae). Search (Ithaca) **18**:1–68.

29. **Piesman, J., J. G. Donahue, T. N. Mather, and A. Spielman.** 1986. Transovarially acquired Lyme disease spirochetes (*Borrelia burgdorferi*) in field-collected larval *Ixodes dammini*

(Acari: Ixodidae). J. Med. Entomol. **23**:291.

30. **Snedecor, G. W., and W. G. Cochran.** 1967. Statistical methods. Iowa State University, Ames.

31. **Steere, A. C., N. H. Bartenhagen, J. E. Craft, G. J. Hutchinson, J. H. Newman, D. W. Rahn, L. H. Sigal, P. N. Spieler, K. S. Stenn, and S. E. Malawista.** 1983. The early clinical manifestations of Lyme disease. Ann. Intern. Med. **99**:76–82.

32. **Steere, A. C., R. L. Grodzicki, A. N. Kornblatt, J. E. Craft, A. C. Barbour, W. Burgdorfer, G. P. Schmid, E. Johnson, and S. E. Malawista.** 1983. The spirochetal etiology of Lyme disease. N. Engl. J. Med. **308**:733–740.

33. **Steere, A. C., and S. E. Malawista.** 1979. Cases of Lyme disease in the United States: locations correlated with distribution of *Ixodes dammini*. Ann. Intern. Med. **91**:730–733.

34. **Tempelis, C. H.** 1975. Host-feeding patterns of mosquitoes, with a review of advances in analysis of blood meals. J. Med. Entomol. **11**:635–653.

35. **Thornhill, A. R., and K. L. Hays.** 1972. Dispersal and flight activities of some species of *Tabanus* (Diptera: Tabanidae). Environ. Entomol. **1**:602–606.