Geographic Distribution of Humans, Raccoons, and White-Footed Mice with Antibodies to Lyme Disease Spirochetes in Connecticut

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An indirect immunofluorescence test was used during 1982–1983 to identify antibodies to Lyme disease spirochetes in humans, white-footed mice, and raccoons. Serologic tests detected IgM or total Ig antibodies in serum samples from 67 persons. Onset of illness, as marked by erythema chronicum migrans (ECM), occurred mainly during July and August. The majority of the persons with Lyme disease lived in south central and southeastern Connecticut. Analyses also verified prior spirochetal infections in 29 of 323 (9 percent) white-footed mice and in three of 34 (9 percent) raccoons captured at sites with or without evidence of human infections. Results indicate potential for Lyme disease at numerous localities in Connecticut.

Lyme disease is endemic at several locations in south central and southeastern Connecticut. Based on clinical records, human infections may have originated in at least 33 communities [1]. The suspected tick vector, *Ixodes dammini*, is present at many of these sites [2] and may transmit spirochetes, the etiologic agent of this disease [3,4], to humans and other animals [4,5–9].

It is often difficult to determine accurately sources of human infection from case histories. The characteristic skin lesion, erythema chronicum migrans (ECM), may appear within three weeks after a tick bite [1,10], and, consequently, persons may not recall tick bites. When ticks are found, many people are unable to determine precisely the time or place of exposure. Therefore, in addition to clinical records, supportive data are needed to assess the risk for Lyme disease in various communities. Serologic analyses of wildlife provide important information on the distribution of zoonotic diseases. The purpose of this study was to expand on earlier investigations [1,8,9] by indicating residences of persons who recently had Lyme disease and by identifying areas where white-footed mice (*Peromyscus leucopus*) and raccoons (*Procyon lotor*) have had spirochete infections.

MATERIALS AND METHODS

Sera of persons thought to have Lyme disease were typically drawn 7–21 days after onset of ECM or arthritis. Specimens were forwarded along with case histories
to the Connecticut Agricultural Experiment Station by the Virology Laboratories of the Connecticut State Health Department. Additional clinical data were obtained from the attending physicians if sera reacted positively in serologic tests. In addition to age, sex, and place of residence, the following information was recorded: history of tick bites, date of onset of illness, presence of ECM, and development of other manifestations such as arthritic, cardiac, and neurologic disorders. Because travel histories were unknown, residences of patients were separated into two categories—probable areas for Lyme disease (defined by multiple cases or by evidence of spirochetes in ticks or mammals) and possible sites for infection (single cases in areas not previously reported).

White-footed mice and raccoons were captured as described earlier [11] from late spring to late fall during 1976–1982. Following anesthetization, blood was drawn from the heart, and sera were stored at −60°C. Mammals were returned to their respective habitats.

Human sera were obtained during July 1982–October 1983 and were screened for antibodies to Lyme disease spirochetes by indirect immunofluorescent antibody (IFA) tests. Sources of reagents and procedures for isolation and maintenance of spirochetes, antigen preparations, and for analyses of human and wildlife sera have been reported [5,8,12]. In initial tests, we used a polyvalent conjugate, fluorescein isothiocyanate-labeled goat anti-human total immunoglobulins (Ig), to detect antibodies in patients. Subsequently, all serum samples were retested with fluorescein-tagged goat anti-human IgM immunoglobulins. Conjugated rabbit antisera to raccoon and white-footed mouse globulins (Ig) were produced in hyperimmunized animals at the Centers for Disease Control in Atlanta, Georgia. Confirmation of Lyme disease in humans was based on a history of ECM with one or more of the following criteria: a fourfold rise in IgM or total Ig titers or single titers ≥ 1:64. Without documentation of ECM, serologic reactions at or above these dilutions were considered presumptive evidence of Lyme disease. Titers of ≥ 1:64 and ≥ 1:32 were significant for raccoons and white-footed mice, respectively. All tests included antigen and positive and negative serum controls.

RESULTS

We compiled records for 67 persons who had significant levels of IgM or total Ig antibodies to spirochetes. Clinical histories for 18 males (5–58 years) and 16 females (2–73 years) with ECM (Table 1) indicated that onset of illness, as marked by expanding skin lesions, was most prevalent during July and August (n = 27); 13 people recalled tick bites. Headaches, fever, fatigue, myalgias, or malaise accompanied ECM. Arthralgias and/or arthritis developed weeks or months after ECM in 18 individuals. Four of these also had neurologic complications (two each with facial palsy and meningitis). Serologic analyses revealed elevated Ig antibody titers (1:128–1:8,192) in samples from all 34 persons and identified IgM antibodies (1:128–1:2,048) in 14 of these. Seroconversions (negative to positive) were recorded for two of seven paired serum samples.

The residences of 34 persons who had ECM are located mainly in south central and southeastern Connecticut (Fig. 1). Of the 25 communities identified, the following probably had multiple cases: Old Lyme (three), East Haddam (three), Colchester (three), Middletown (two), Lyme (two), and Portland (two). Single cases were reported from coastal areas (Old Saybrook, New London, Madison, Stonington, and Darien) and inland sites (Amston, East Lyme, New Britain, Moodus, Un-
TABLE 1
Seasonal Occurrence of Human Cases of Lyme Disease Based on the Presence of ECM and Serologic Evidence, July 1982–October 1983

| Time of ECM Appearance | No. of Cases | % of Total | History of Tick Bite | Arthralgias | Arthritis | Cardiac Disorders* | Neurologic Disorders* |
|------------------------|--------------|------------|----------------------|-------------|-----------|-------------------|----------------------|
| 1982                   |              |            |                      |             |           |                   |                      |
| July–Aug.              | 12           | 35         | 6                    | 10          | 5         |                   | 3                    |
| Sept.–Oct.             | 3            | 9          | 2                    | 2           | 1         |                   |                      |
| Nov.–Dec.              | 0            | 0          |                      |             |           |                   |                      |
| 1983                   |              |            |                      |             |           |                   |                      |
| Jan.–April             | 0            | 0          |                      |             |           |                   |                      |
| May–June               | 4            | 12         | 2                    | 2           | 2         |                   |                      |
| July–Aug.              | 15           | 44         | 5                    | 4           | 2         | 1                 | 1                    |
| Sept.–Oct.             | 0            | 0          |                      |             |           |                   |                      |
| Total                  | 34           | 13         | 18                   | 8           | 1         | 1                 | 4                    |

*Myocarditis noted without accompanying arthritic or neurologic disorders
*Meningitis (two), facial palsy (two)

casville, Chester, Stafford Springs, Lisbon, Hartford, Higganum, South Norwalk, Bristol, Glastonbury, and Salem).

Clinical data were available for an additional 19 males (4–61 years) and 14 females (7–71 years) who may have also had Lyme disease. Although ECM was not noted, serologic tests detected Ig antibodies to spirochetes in sera collected throughout the year (Table 2). Similar to the previous group, the number of cases reported was relatively high (n = 15) during July and August. The majority (n = 24) sought medical attention after developing arthralgias, and of these, eight reported tick bites. Serologic tests also identified IgM antibodies in sera from 13 patients (n = 8 during June–August). Like those who had ECM, these individuals all had total Ig and IgM antibodies at titers of $1:128-1:16,384$ and $1:128-1:2,048$, respectively. Analyses of paired samples revealed seroconversions for four of 12 persons.

FIG. 1. Distribution of residences of persons who had ECM and antibodies to spirochetes, 1982–1983.
### TABLE 2
Seasonal Occurrence of Presumptive Human Cases of Lyme Disease Based on Serologic Evidence, July 1982-October 1983

| Time of Medical Examination | No. of Cases | % of Total | History of Tick Bite | Arthralgias | Arthritis | Neurologic Disorders* |
|-----------------------------|--------------|------------|----------------------|-------------|-----------|-----------------------|
| 1982                        |              |            |                      |             |           |                       |
| July-Aug.                   | 4            | 12         | 1                    | 2           | 1         |                       |
| Sept.-Oct.                  | 1            | 3          |                      | 1           |           |                       |
| Nov.-Dec.                   | 5            | 15         | 5                    | 5           | 2         |                       |
| 1983                        |              |            |                      |             |           |                       |
| Jan.-April                  | 4            | 12         | 1                    | 4           | 3         |                       |
| May-June                    | 4            | 12         | 1                    | 2           | 1         |                       |
| July-Aug.                   | 11           | 33         | 3                    | 8           | 3         |                       |
| Sept.-Oct.                  | 4            | 12         | 2                    | 2           | 2         | 1                     |
| Total                       | 33           | 12         | 8                    | 24          | 14        | 4                     |

*Encephalitis (one), facial palsy (three)

Residences of the 33 persons with antibodies to spirochetes but without records of ECM are distributed principally throughout south central and southeastern Connecticut (Fig. 2). There was evidence of Lyme disease for individuals living in Amston (n = 5), Norwich (four), Marlborough (two), Hadlyme (two), and Essex (two). Additional cases were noted for Moodus, Lyme, Portland, Old Lyme, Groton, Colchester, East Lyme, East Haddam, Glastonbury, Salem, Manchester, Haddam, Waterford, Noank, Killingworth, North Franklin, Newington, and Lebanon.

Immunofluorescence tests verified prior spirochetal infections in 29 of 323 white-footed mice (Table 3) and in three of 34 raccoons (Table 4). Positive raccoons were captured during July and August at three sites where no human cases have been reported. Similarly, serologic tests revealed antibodies in white-footed mice from three additional locations, two of which had evidence of spirochete infections in humans. These areas were scattered throughout southern Connecticut (Fig. 3).
TABLE 3
Sera of White-Footed Mice Analyzed for Antibodies to Spirochetes in Connecticut

| Sites          | No. of Samples at Reciprocal Titration Endpoints |
|----------------|-----------------------------------------------|
|                | 32-64 | 128-256 | 512-1,024 |
| Branford       | 52    | 2 (4)    | 1         |
| Colchester     | 13    | 0        | 1         |
| Danielson      | 6     | 0        | 1         |
| East Lyme      | 75    | 7 (9)    | 2         |
| New Fairfield  | 10    | 0        | 5         |
| Stonington     | 124   | 20 (16)  | 1         |
| Thompson       | 12    | 0        | 19        |
| Woodbridge     | 23    | 0        |           |
| Voluntown      | 8     | 0        | 25        |
| Total          | 323   | 29 (9)   | 25        |

TABLE 4
Sera of Raccoons Analyzed for Antibodies to Spirochetes in Connecticut

| Sites         | Number of Samples at Reciprocal Titration Endpoints |
|---------------|----------------------------------------------------|
|               | 64-128 | 256-512 |
| Cheshire      | 3      | 0       |
| East Haven    | 3      | 1       |
| Guilford      | 14     | 0       |
| Hamden        | 4      | 0       |
| Hebron        | 4      | 0       |
| North Branford| 1      | 1       |
| North Haven   | 1      | 0       |
| West Hartford | 2      | 0       |
| Woodbridge    | 2      | 1       |
| Total         | 34     | 3       |

FIG. 3. Distribution of raccoons and white-footed mice with antibodies to spirochetes, 1976-1982.
DISCUSSION

Human cases of Lyme disease were clustered chiefly in south central and southeastern Connecticut. If these people were bitten by infected ticks near their homes, spirochetes may have been acquired in 38 communities, 17 of which have been previously reported [1]. Recent records for some sites such as Darien, Amston, Middletown, Portland, and South Norwalk probably reflect a greater awareness of Lyme disease among physicians and health officials. Although it appears from this study that Lyme disease may also be spreading in the state, we recognize that some people may have traveled to endemic areas and were bitten by infected ticks elsewhere. Even though the precise places of origins for these human infections are unknown, our results agree with those of Steere and Malawista [1] and show potential for this disease at several locations.

Lyme disease occurs in both coastal and inland areas where the vector, I. dammini, is found and where deer populations are increasing [2]. Microscopic examinations of midgut tissues from immature or adult I. dammini have revealed spirochetes at East Haddam, Haddam, East Lyme, Lyme, Guilford, and Voluntown [4,5,9]. Therefore, there are numerous foci where persons may encounter infected ticks. Documentation of Lyme disease or ECM in different states and continents [1,4], where I. dammini or related ticks occur, indicates a distribution over broad geographic regions. If I. dammini populations increase, this tick may disperse and extend the range of this disease.

Based on the timing of ECM, an important clinical marker for Lyme disease [1,13], persons were infected primarily during the summer. This confirms earlier studies [14,15] and suggests a relationship between the prevalence of new human cases and relatively high populations of I. dammini nymphs [2,16]. Although 21 persons reported tick bites in the present study, specimens were not available for identification. Therefore, it is unclear whether these people had encountered I. dammini or Dermacentor variabilis, another common tick found throughout the state [2]. Nonetheless, with increased exposure to ticks during warmer months, there is greater potential for Lyme disease.

Unlike D. variabilis, I. dammini larvae and nymphs will feed on humans [17]. The juvenile stages of the latter species may harbor spirochetes [4,5], and because these ticks are small, they are likely to be overlooked even after they engorge blood. This may account, in part, for the relatively low number of persons with histories of tick bites and reinforces the importance of checking for ticks after visiting infested habitats.

Comparisons of our two case populations indicate similar distributions of where spirochete infections for Lyme disease may have been acquired. These results also show the usefulness of serologic tests even when knowledge of ECM is lacking. The detection of IgM or total Ig antibodies, combined with clinical and geographic information, may provide important clues as to the cause of certain arthritic, cardiac, or neurologic disorders. As indicated earlier [4], significant rises in IgM and IgG titers can be demonstrated if blood samples are drawn at appropriate times in the course of illness. In addition to time of sampling, the relatively low number of seroconversions noted in our study might be due to differences in immune responses or, as noted by Steere et al. [4], to the effects of antibiotics.

We detected antibodies to spirochetes in white-footed mice and raccoons from six widely separated coastal and inland sites. This adds to previous work [8] which identified antibodies in both mammals at East Haddam and Lyme and, collectively, pro-
vides supportive evidence for Lyme disease in areas where humans may have been infected. Of the 329 white-footed mouse and 78 raccoon sera collected from these sites during 1978–1982, 10 percent and 23 percent, respectively, contained antibodies [8]. Similar studies of white-tailed deer at these and other locations in south central and southeastern Connecticut [9] revealed positivity rates of 29–39 percent. Because spirochetes have also been isolated from the blood of these hosts [5,7], we believe that these mammals may serve as reservoirs for this agent.

Since several hosts are commonly parasitized by *I. dammini* [2,7–9,16], sampling wildlife populations may prove useful in the identification and monitoring of endemic areas. The present and earlier findings [5,8,9] show a close correlation among the distributions of infected ticks, mammals with antibodies to spirochetes, and residences of persons who had Lyme disease. However, some communities, such as Colchester and Guilford, appear to have had human cases [1], but there is no evidence of spirochete infections in raccoons or white-footed mice. This could be due to insufficient sampling. Thus, surveillance programs should include tests for spirochetes in ticks and serologic analyses of mammalian sera from scattered sites within towns. Studies should also be conducted over several months to determine if there are temporal differences in vector-pathogen-host interrelationships.

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**REFERENCES**

1. Steere AC, Malawista SE: Cases of Lyme disease in the United States: Locations correlated with distribution of *Ixodes dammini*. Ann Intern Med 91:730–733, 1979
2. Anderson JF, Magnarelli LA: Vertebrate host relationships and distribution of ixodid ticks (Acari: Ixodidae) in Connecticut, USA. J Med Entomol 17:314–323, 1980
3. Burgdorfer W, Barbour AG, Hayes SF, et al: Lyme disease—a tick-borne spirochetosis? Science 216:1317–1319, 1982
4. Steere AC, Grodzicki RL, Kornblatt AN, et al: The spirochetal etiology of Lyme disease. New Eng J Med 308:733–740, 1983
5. Anderson JF, Magnarelli LA, Burgdorfer W, et al: Spirochetes in *Ixodes dammini* and mammals from Connecticut. Am J Trop Med Hyg 32:818–824, 1983
6. Benach JI, Bosler EM, Hanrahan JP, et al: Spirochetes isolated from the blood of two patients with Lyme disease. New Eng J Med 308:740–742, 1983
7. Bosler EM, Coleman JL, Benach JL, et al: Natural distribution of the *Ixodes dammini* spirochete. Science 220:321–322, 1983
8. Magnarelli LA, Anderson JF, Burgdorfer W, et al: Parasitism by *Ixodes dammini* (Acari: Ixodidae) and antibodies to spirochetes in mammals at Lyme disease foci in Connecticut, USA. J Med Entomol 21:52–57, 1984
9. Magnarelli LA, Anderson JF, Chappell WA: Antibodies to spirochetes in white-tailed deer and prevalence of infected ticks from foci of Lyme disease in Connecticut. J Wildl Dis 20:21–26, 1984
10. Steere AC, Broderick TF, Malawista SE: Erythema chronicum migrans and Lyme arthritis: Epidemiologic evidence for a tick vector. Am J Epidemiol 108:312–321, 1978
11. Magnarelli LA, Anderson JF, Burgdorfer W: Rocky Mountain spotted fever in Connecticut: Human cases, spotted-fever group rickettsiae in ticks, and antibodies in mammals. Am J Epidemiol 110:148–155, 1979
12. Magnarelli LA, Meegan JM, Anderson JF, et al: Comparison of an indirect fluorescent antibody test with an enzyme-linked immunosorbent assay for the serologic analyses of Lyme disease. J Clin Microbiol, in press
13. Steere AC, Malawista SE, Hardin JA, et al: Erythema chronicum migrans and Lyme arthritis: The expanding clinical spectrum. Ann Intern Med 86:685-698, 1977
14. Steere AC, Malawista SE, Snydman DR, et al: A cluster of arthritis in children and adults in Lyme, Connecticut (abstract). Arthritis Rheum 19:824, 1976
15. Steere AC, Malawista SE, Snydman DR, et al: Lyme arthritis: An epidemic of oligoarticular arthritis in children and adults in three Connecticut communities. Arthritis Rheum 20:7-17, 1977
16. Carey AB, Krinsky WL, Main AJ: *Ixodes dammini* (Acari: Ixodidae) and associated ixodid ticks in south-central Connecticut, USA. J Med Entomol 17:89-99, 1980
17. Wallis RC, Brown SE, Kloter KO, et al: Erythema chronicum migrans and Lyme arthritis: Field study of ticks. Am J Epidemiol 108:322-327, 1978