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Lyme Disease—A Tick-Borne Spirochetosis?

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Lyme Disease—A Tick-Borne Spirochetosis?

Abstract. *A treponema-like spirochete was detected in and isolated from adult* Ixodes dammini, *the incriminated tick vector of Lyme disease. Causally related to the spirochetes may be long-lasting cutaneous lesions that appeared on New Zealand White rabbits 10 to 12 weeks after infected ticks fed on them. Samples of serum from patients with Lyme disease were shown by indirect immunofluorescence to contain antibodies to this agent. It is suggested that the newly discovered spirochete is involved in the etiology of Lyme disease.*

Lyme disease is an epidemic inflammatory disorder that usually begins with a skin lesion called erythema chronicum migrans (ECM). Weeks to months later the lesion may be followed by neurologic or cardiac abnormalities, migratory polyarthritis, intermittent attacks of oligoarticular arthritis, or chronic arthritis in the knees (*1*).

Although in the United States cases of ECM were first reported from Wisconsin (*2*) and southeastern Connecticut (*3*), Lyme disease as a new form of inflammatory arthritis was first recognized in 1975 in Lyme, Connecticut (*4*). It has since been reported from other northeastern, midwestern, and western states (*5*).

Epidemiologic evidence suggests that Lyme disease is caused by an infectious agent transmitted by ticks of the genus *Ixodes*. In the Northeast and Midwest *Ixodes dammini* and, in the West, *I. pacificus* have been incriminated as potential vectors (*6, 7*). Until recently, all attempts to isolate the causative agent either from ticks or from patients were unsuccessful.

Recently we isolated from *I. dammini* a spirochete that binds immunoglobulins of patients convalescing from Lyme disease. We also recorded the development of lesions resembling ECM in New Zealand White rabbits on which ticks harboring this spirochete had fed.

Adult *I. dammini* were collected in late September and early October 1981 by flagging lower vegetation on Shelter Island, New York—a known endemic focus of Lyme disease (*8*). Of 126 such ticks that were dissected, 77 (61 percent; 65 males and 12 females) contained spirochetes. The spirochetes were distributed mainly in the midgut but were occasionally also seen in the hindgut and rectal ampule. No other tissues, including the salivary glands, contained spirochetes. The organisms stained moderately well with Giemsa (Fig. 1); in wet preparations examined by dark-field mi-

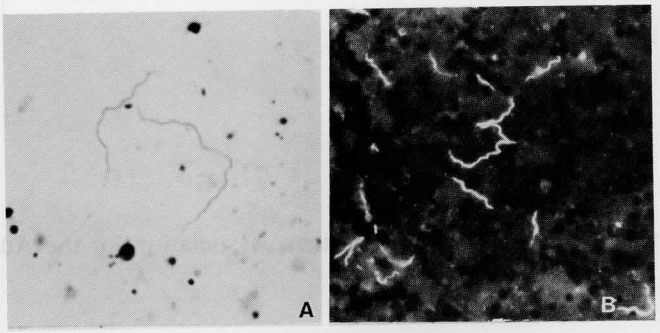

Fig. 1. *Ixodes dammini* spirochetes in midgut tissues of its tick vector. (A) Giemsa staining (×1200). (B) Serum of patient J.G. examined by indirect immunofluorescence (×570).

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Table 1. Serologic evaluation (indirect immunofluorescence) of serum from persons with Lyme disease.

Patient*	Disease contracted	Serum collected	Serum dilution end point
B.B.	May 1978	September 1978	1:1280
B.Br.	July 1980	July 1980	1:240
E.D.	July 1980	July 1980	1:80
C.G.	June 1979	March 1980	1:640
J.G.	June 1979	March 1980	1:1280
L.H.	June 1980	September 1980	1:640
J.S.	July 1979	January 1982	1:640
A.S.	July 1977	March 1980	1:80
C.T.	June 1979	March 1980	1:320
Controls: Four samples from New York and ten from Montana			≦1:20

*Diagnosed by E.G. except for J.S., whose serum was submitted to the New York State Health Department. All patients contracted the disease while visiting Shelter Island, New York.

croscopy they moved sluggishly and rotated slowly. The degree of infection varied; some ticks contained only a few spirochetes, others contained large numbers often to the extent that clumps of spirochetes were present throughout the midgut.

Electron microscopy (*9*) of midgut diverticula revealed spirochetes closely associated with the microvillar brush border of the gut epithelium (Fig. 2). Fine structural features of the organism were similar to those reported for *Treponema* species (*10*). Irregularly coiled, the spirochetes range from 10 to 30 μm in length and from 0.18 to 0.25 μm in diameter. The ends appear tapered with four to eight filaments inserted subterminally at each end. Insertion points of the filaments are in a row paralleling the cell's long axis. Cross sections of the cells show six to eight filaments interspersed between the outer membrane and the cytoplasmic membrane in the asymmetric region of the section profile (Fig. 2).

The *I. dammini* spirochete was isolated by inoculating 0.1 ml of a suspension prepared from midgut tissues of four infected ticks into 8.5 ml of modified Kelly's medium (*11*). After 5 days of incubation at 35°C, all the culture tubes contained spirochetes that could be regularly subcultured and maintained at 35°C.

When about 300 *I. dammini* were allowed to feed on eight New Zealand White rabbits (*12*), they appeared to have no immediately adverse effects. Blood smears examined daily for 14 days after placement of the ticks were negative for spirochetes. However, 10 to 12 weeks after the ticks had engorged, up to 15 small (2 to 3 mm in diameter) macules and papules appeared in the skin of the back and lateral trunk of each rabbit. Within 3 to 5 days, these lesions had enlarged (up to 5 cm in diameter) to slightly elevated annular or oval lesions with bright red to reddish-violet margins. Similar lesions on the abdomen, the site of tick attachment, were recorded on only one of the eight rabbits. All lesions persisted for at least 8 weeks.

Sections of biopsy specimens were stained with hematoxylin and eosin. These sections showed that the skin lesions consisted of a thickened, slightly hyperkeratotic epidermis with the dermis showing dense mononuclear cell infiltration and edema of the superficial layer. Limited attempts to isolate spirochetes from suspensions of biopsied skin lesions in Kelly's medium were negative.

Even though microscopic examination of repleted *I. dammini* showed that at least two ticks harboring spirochetes had fed on each rabbit, we are not certain whether the described skin reaction on the rabbits is causally related to the spirochetes or is due to other factors associated with the ticks' feeding process.

When tested by an indirect immunofluorescence method (*13*), antibodies to the spirochetes in titers of ≧ 1:1280 were present in the serum of all rabbits on which ticks had fed 30 and 60 days earlier. The serum of rabbits that had not been exposed to ticks did not react at dilutions of > 1:20.

That the *I. dammini* spirochete is antigenically related to the etiologic agent of Lyme disease was suggested by the positive reactions we obtained when we examined serum samples from nine patients with clinically diagnosed Lyme disease by means of indirect immunofluorescence (Fig. 1) (*14*). Antibody titers ranging from 1:80 to 1:1280 were recorded for persons who had Lyme disease currently or as many as 32 months previously (Table 1). In contrast, serum samples from four people from New York and ten from Montana with no history of the disease did not react with the spirochete in titers higher than 1:20.

Our observations suggest that the

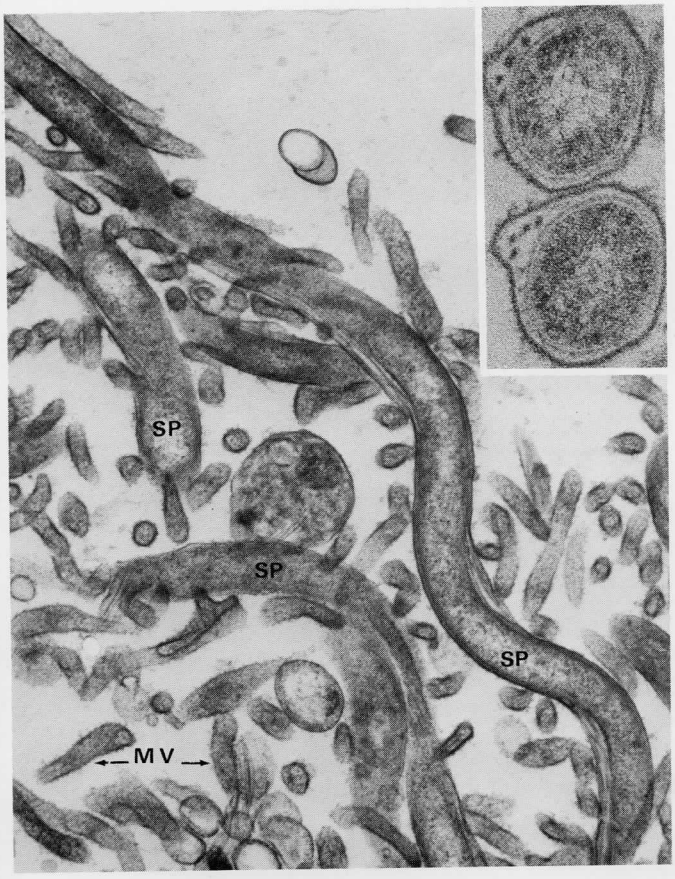

Fig. 2. Electron micrograph of *I. dammini* spirochetes (*SP*) associated with microvillar brush border (*MV*) of the tick's midgut (×55,440). Inset shows cross section of spirochetes (×122,100).

treponema-like organism isolated from *I. dammini* may be involved in the etiology of Lyme disease. It is interesting that organisms presenting the morphological characteristics of spirochetes were said to be associated with ECM in Europe as early as 1948 (*15*). Although this was never confirmed, a recent study (*16*) showing that resolution of lesions and concurrent symptoms occurs faster in patients treated with penicillin suggests a penicillin-susceptible bacterium as an etiologic agent of Lyme disease.

Our results establish the susceptibility of the domestic rabbit to the *I. dammini* spirochete and demonstrate the possible value of the indirect immunofluorescence test as a diagnostic tool for Lyme disease. They also suggest the need for additional investigations not only into the epidemiology and ecology of Lyme disease and related disorders, such as ECM of Europe (*17*), but also into the relations between the spirochete and its vector *I. dammini*.

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8. The ticks were first examined by the hemolymph test [W. Burgdorfer, *Am. J. Trop. Med. Hyg.* **19**, 1010 (1970)]. Subsequently they were dissected for the preparation of multiple smears from gut, malpighian tubules, salivary glands, central ganglion, and testes or ovary. Smears were stained according to the Giménez method [D. F. Giménez, *Stain Technol.* **39**, 135 (1964)] or with Giemsa. Once spirochetes were detected, wet preparations of tissues were examined also under dark field.
9. For electron microscopy, diverticula of midgut were removed by dissection and were processed according to S. F. Hayes and W. Burgdorfer [*J. Bacteriol.* **137**, 605 (1979)].
10. K. Hovind-Hougen, *Acta Pathol. Microbiol. Scand. Sect. B Suppl. No. 225* (1976).
11. Kelly's medium [R. Kelly, *Science* **173**, 443 (1971)] modified by addition of CMRL medium 1066 (Gibco No. 330-1540) and Yeastolate (Difco) for final concentrations of 5 and 0.2 percent, respectively (H. G. Stoenner, in preparation).
12. Fifteen to twenty *I. dammini* females and equal numbers of males for mating (males may ingest small amounts of blood) were placed on each of eight rabbits. The ticks were contained in metal capsules attached by adhesive tape to the shaved abdomen of each rabbit.
13. In accordance with the data of R. N. Philip, E. A. Casper, R. A. Ormsbee, M. G. Peacock, and W. Burgdorfer [*J. Clin. Microbiol.* **3**, 51 (1976)] midgut smears of infected ticks or cultured spirochetes were used as antigen. Fluorescein isothiocyanate–conjugated goat antibody to rabbit immunoglobulin (Chappel Laboratories) was used at a 1:50 dilution in phosphate-buffered saline with 1 percent bovine serum albumin.
14. Fluorescein isothiocyanate–conjugated goat antibody to human immunoglobulin (BBL, Cockeysville, Md.) was used at 1:100 dilution in phosphate-buffered saline with 1 percent bovine serum albumin.
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17. Since submission of this manuscript, microscopic examination by one of us (W.B.) of midgut smears from *Ixodes pacificus* from Oregon and of *I. ricinus* from Switzerland also revealed, in some instances, the presence of spirochetes.
18. We thank the Nature Conservancy Incorporation for permission to collect ticks in their Shelter Island Preserve. We also thank E. Bosler, S. Guirgis, D. Massey, and J. Coleman for their assistance in collecting ticks. Special thanks also to W. H. Hadlow, Epidemiology Branch, Rocky Mountain Laboratories, for the histologic characterization of the rabbit lesions.

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