LYME disease SURVEILLANCE SUMMARY

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Infectious Diseases
National Center for Infectious Diseases
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DISTRIBUTION BY COUNTY OF *IXODES DAMMINI* IN THE UNITED STATES, 1991

CDC sent data collection forms and made telephone calls to epidemiologists, entomologists, and laboratory directors at state and county health departments, federal agencies, and universities throughout the United States. Data requested included the occurrence of known and putative tick vectors of Lyme disease by county, numbers and stages of specimens collected, and rates of infection with *Borrelia burgdorferi*. Presented here are counties in which at least one specimen of *Ixodes dammini* has been identified at any time, regardless of infection with *B. burgdorferi* (Figure 1).

The distribution of *I. dammini* in the eastern United States has expanded slowly during the 1980's and 1990's. Although the heaviest concentrations of *I. dammini* ticks still occur along the eastern seaboard and the Minnesota-Wisconsin border area, established populations of *I. dammini* have been newly described in upstate New York, western Pennsylvania, the upper peninsula of Michigan, and in northwestern Illinois. The geographic spread and temporal increase of Lyme disease in New York was recently reviewed by White, DJ et al. (JAMA 266:1230, 1991). It must be stressed that the appearance of occasional adult *I. dammini* in a new location is not necessarily evidence of an established population. In order to prove that a tick population is well established, all 3 life stages of the tick should be collected and collections made over several years (Piesman, J. Can J Infect Dis 2:55, 1991).

Only a few counties in the southeastern United States (VA and NC) were reported as positive for *I. dammini*. Obviously, the difficulties in distinguishing *I. dammini* from *I. scapularis* make any attempt to map the distribution of *I. dammini* in the southeastern United States difficult. Intensive studies on the biological and genetic relatedness of these 2 tick species are being conducted at present. The results of such studies will add much to our understanding of the Lyme disease spirochete transmission risk in the southern United States.

PROGRESS UPDATE: ACADEMIC REFERENCE CENTER (ARC) STUDY OF LYME DISEASE SEROLOGIC TESTS.

In a previous issue of LDSS, we described a multicenter cooperative study to evaluate precision of Lyme disease serologic tests employed by CDC and five academic institutions active in Lyme disease research. Progress in two areas has led to modification of the original study design after consultation among all participants.

The collection in the past year of considerable numbers of culture-confirmed serum specimens has made it possible to evaluate accuracy as well as precision in the multicenter comparison. Thus, the serum panel to be tested has been increased from the original 200 to 600 specimens. This panel is divided into three groups: specimens from Lyme disease cases, specimens from non-case controls, and diagnostic specimens with serologically "equivocal" results. The latter group is composed of sera from patients living in states not considered endemic for Lyme disease, but who were suspected of having Lyme disease by their physicians. Many specimens in this latter group yielded serologic test results with the CDC WCS ELISA which were close to the cut-off level (either positive or negative), and provided an opportunity to evaluate cut-off levels employed by the various centers. Non-case control serum specimens were obtained from healthy blood donors from non-endemic areas. Each group of 600 specimens contains replicates for the measurement of precision. The testing of specimens is in progress.

CULTURE OF ERYTHEMA MIGRANS LESIONS AS A DIAGNOSTIC TEST IN LYME DISEASE.

Bacteriologic diagnosis of Lyme disease by culturing clinical specimens for *B. burgdorferi* has long been considered as unrewarding, a definitive serologic test has not been identified, and the protean clinical manifestations of Lyme disease make it difficult to accurately classify sera for use in serologic test development.

This dilemma was discussed during the First National Conference on Lyme Disease Testing in Dearborn, MI hosted by the Association of State and Territorial Public Health Laboratory Directors (ASTPHLD), the FDA and CDC. One of the concerns raised at the Conference was the extent to which deficiencies in the BSK medium being employed were responsible for the poor isolation rates of *B. burgdorferi* from blood and other body tissues. Since that meeting, CDC has supported research on the culture of aspirates and biopsies of erythema migrans (EM) lesions and blood of patients and has funded a study to monitor the development of spirochetemia in a primate model.

CDC supported research, as well as recently published data from Burger et al. (J Clin Microbiol 30:359-361, 1992), demonstrate that *B. burgdorferi* organisms can be recovered from EM lesions in most untreated patients.

The technique for obtaining skin biopsies is minimally invasive and has been well tolerated and accepted by patients. We encourage its use. Requirements for culture are good quality BSK II medium and an incubator which can be maintained at a temperature range of 33 to 34 degrees C. Neither requirement should impose difficulties for most diagnostic microbiology laboratories. We welcome *B. burgdorferi* isolates and serum specimens from patients with culture positive EM lesions for inclusion in our National Lyme Disease Serologic Reference Collection. These reference materials are being distributed, upon request, for test development and standardization. If you have material to contribute, please call the editors at (303) 221-6453.

INSTRUCTIONS FOR CULTURE OF *BORRELIA* FROM HUMAN TISSUES, INCLUDING THE STORAGE AND USE OF CULTURE MEDIUM

Culture of *Borrelia burgdorferi* from clinical specimens is currently the only unequivocal way to diagnose Lyme disease. This organism can readily be isolated from biopsies or aspirates of EM lesions, with yields in the 50-80% range. Yields may typically be lower from other tissues or fluids, but relatively few studies have been reported. Tissues or fluids for culture can include skin (by biopsy or needle aspirate), fetal tissues, placenta, whole blood, serum, cerebrospinal fluid (CSF), synovial fluid, urine or other tissues or fluids. Instructions for obtaining and handling specimens are as follows:

A. STORAGE

- Frozen BSK II medium in individual tubes containing 4.5 or 7.0 ml should be used.
- Keep media frozen at or below -20°C. Medium will keep at least 6 months at these temperatures.
- DISCARD tubes of medium that have turned dark red at the air interface when thawed (a light red or pink color at the air interface is normal and these tubes are satisfactory for use).

B. INOCULATION

- This spirochete grows best in microaerophilic to anaerobic conditions. Media containing specimen should have minimal air-fluid interface.
- Thaw tube(s) of medium and, if time allows, bring to room temperature.
- As soon as possible after sample is collected, inoculate 4.5 ml tube of medium with 0.5 ml whole blood, serum, CSF, synovial fluid, urine, or other fluids (e.g., skin aspirate). Tubes containing 7.0 ml of medium can be inoculated with up to 1.0 ml of fluids. Biopsy material should be placed whole into the tube of medium.
- Incubate inoculated tube(s) for a minimum of 6 weeks at 32-33° C before discarding as negative. Examine cultures by dark-field micros copy at 10 day intervals or when turbidity or acid production indicates growth. Spirochetal cultures may be sent to State Health Laboratories or by prior arrangement to CDC for a confirmation of identification by current methodologies.

C. BIOPSY AND NEEDLE ASPIRATION OF SKIN LESIONS

- Skin biopsies typically are done with a standard 2-4 mm diameter punch instrument using standard sterile technique. Generally, the biopsy site is anesthetized (1 cc of a 1% lidocaine plus 1:100,000 epinephrine solution) and then disinfected (iodine tincture followed by isopropyl alcohol). Using a gentle twisting action, the punch instrument is used to cut the skin to a depth of 3-7 mm. The punch instrument is then removed. The skin punch is grasped with fine-tipped forceps, pulled gently away from the body, and snipped at the base with iris scissors. Hemostasis is achieved in the usual fashion; generally, pressure alone is adequate. The biopsy specimen(s) can be divided with a scalpel if more than one test is planned. Biopsy samples are inoculated directly into thawed culture medium.

- A 2-needle, 2-person aspiration technique (''cutaneous lavage'') was developed in 1990-1991 by Gary Wormser, Gilda Forseter and colleagues at New York Medical College, based on a similar method described by Piesman *et al.* (*J Infect Dis* 163:895-897, 1991) for use in rabbits. After local anesthesia and disinfection (as above), a 25 ga. 5/8" needle connected to a 3 cc syringe containing 2 cc of non-bacteriostatic normal saline is introduced intradermally and enough saline is injected to create a visible wheal at least 1 cm in diameter. While this first needle remains in place, a second operator introduces a 20 ga. 1½'' needle attached to a 3 cc syringe at a slanting angle into the wheal at a site opposite to that of the first needle. The second needle and syringe are then removed and used to collect fluid exuding from the second needle track. The rate and amount of fluid obtained from the second needle site depends on the rate and amount of additional saline injected into the wheal through the first needle and syringe. Aspiration fluid is inoculated directly into thawed culture medium. Themethod seems to be less sensitive then culture of skin biopsy material.

- A good location for isolation of spirochetes from EM lesions appears to be about 4mm inside the perimeter (Berger *et al.*, *J Clin Microbiol* 30:359-361, 1992).

REPORTING OF LYME DISEASE CASES IN 1992 BY NETTS

The numbers of Lyme disease cases reported through NETSS in the period January through May 9, 1992 are shown in Figure 2. Of the total 1,351 cases reported through Week 19, 1,122 (83%) were reported from the mid-Atlantic and New England regions.

Lyme Disease Surveillance Summary (LDSS) is edited by Drs. Robert Craven and David Dennis. If you have information to contribute or wish to receive a LDSS, please contact them at:

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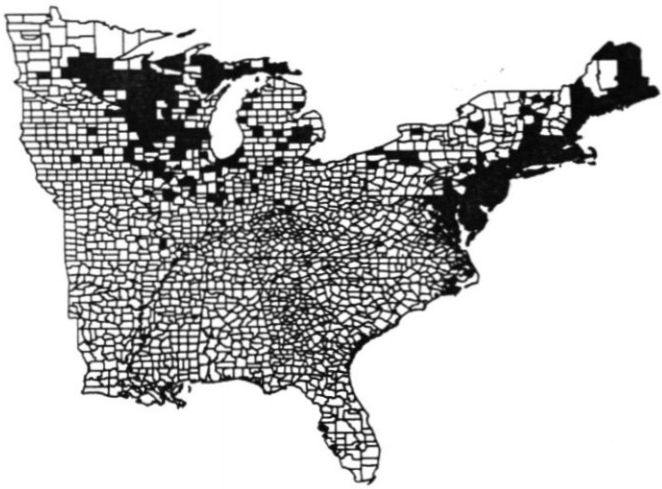
Figure 1.

Distribution of *Ixodes dammini* in the U.S. by County, 1991



CDC\NCID\DVBID

□ Not reported
■ Ixodes dammini reported

Figure 2.

REPORTED LYME DISEASE CASES, U.S., 1992