

Bacterial Zoonoses Branch
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Infectious Diseases
National Center for Infectious Diseases
Centers for Disease Control

Volume 3 -: No. 3 Date: September 1992

RESULTS OF CDC ATTEMPTS TO ISOLATE B. burgdorferi FROM SUSPECTED ERYTHEMA MIGRANS LESIONS, UNITED STATES, 1990-1992

Culture of *Borrelia burgdorferi* from patients is currently the only unequivocal means of confirming a diagnosis of Lyme disease. Positive cultures from patients have been reported from Connecticut [1], New York [2, 3], and Wisconsin [4]; in addition, CDC has isolated the organisms from patients in California and Maryland (unpublished data). These states all have high incidence rates of Lyme disease and well-documented, established enzootic cycles of *B. burgdorferi* transmission. Although isolation of *B. burgdorferi* has also been reported from patients in Texas [5], an established enzootic cycle in that state has not been documented.

The highest culture yields of *B. burgdorferi* from clinical samples (ca. 70%) have been obtained from punch biopsies of erythema migrans (EM) lesions [1-3], the characteristic dermatologic sign of acute Lyme disease. Additionally, *B. burgdorferi* has been cultured from cerebrospinal fluid (CSF), blood, joint fluid, and cardiac muscle, but yields have been low or attempts few [5-7].

Beginning in 1990, CDC has collaborated with clinicians to culture *B. burgdorferi* from patients with clinically suspected Lyme disease. Emphasis has been on states where enzootic transmission of *B. burgdorferi* has not been proven, or in which cases of Lyme disease have been diagnosed clinically, but never confirmed by culture. Specialized culture medium (BSK) has been shipped to interested clinicians, and samples have been obtained and placed in the culture medium, and then returned to the Diagnostic and Reference Section, Bacterial Zoonoses Branch, CDC, Fort Collins, Colorado for incubation and examination. Cultures are examined periodically for 2-3 months before considered to be negative.

As of August 10, 1992 a total of 72 punch biopsy samples from suspected EM lesions from 69 patients of 42 clinicians have been submitted for culture (Tables 1 and 2). None of 60 samples was culture-positive from states where the isolation of *B. burgdorferi* has not been reported from humans, animals or ticks (Arkansas, Florida, Kansas, Missouri, North Carolina, and Tennessee), or where low enzooticity has been detected, but no isolates from humans have been documented (Virginia). In contrast, 5 of 12 (42%) samples were culture-positive from states where the endemicity of *B. burgdorferi* is proven, and where substantial areas of enzooticity have been documented (California, Maryland, New Jersey, New York, and Wisconsin). This difference is statistically significant (0/60 vs. 5/12, Fisher's exact test, 2-tailed p < 0.001).

CDC will continue these efforts to culture biopsies of EM lesions, particularly in states where the isolation of B. burgdorferi has not been documented, but where cases of Lyme disease are being diagnosed clinically. Ongoing problems with false positive results from serologic tests underscore the importance of establishing the presence of Lyme disease in these states by isolating B. burgdorferi from suspected EM lesions.

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Table 1. Results of CDC attempts to culture *Borrelia burgdorferi* from suspected erythema migrans lesions, 1990-1992, in states where isolation of *B. burgdorferi* has not been reported*, or where limited areas of enzooticity have been documented—.

State	No. clinicians	No. Patients	No. skin biopsies cultured	No. cultures positive
Arkansas	1	2	2	0
Florida	2	2	4	0
Kansas	1	4	4	0
Missouri	14	25	28	0
North Carolina	3	6	6	0
Tennessee	2	6	5	0
Virginia	8	12	11	0
Total	31	57	60	0

^{*} Arkansas, Florida, Kansas, Missouri, North Carolina, Tennessee

+ Virginia

Table 2. Results of culture of *Borrelia burgdorferi* from suspected erythema migrans lesions, 1990-1992, in states where isolation of *B. burgdorferi* has previously been reported from humans, animals or ticks, and where substantial areas of enzooticity of *B. burgdorferi* have been documented.

State	No. clinicians	No. patients	No. skin biopsies cultured	No. cultures positive
California	6	7	7	3
Maryland	2	2	2	1
New Jersey	1	1	1	0
New York	1	1	1	0
Wisconsin	1	1	1	1
Total	11	12	12	5

NEW SPECIES OF LYME BORRELIA DESCRIBED

Researchers at the Pasteur Institute in Paris have described a new species named *Borrelia garinii* associated with Lyme borreliosis (1). They also report on a number of isolates in Group VS461, named after the type strain, that preliminary evidence suggests will be included in a 3rd Lyme borreliosis-associated species of *Borrelia*. *Borrelia burgdorferi* sensu stricto have been isolated from Europe and the United States. *Borrelia garinii* and Group VS461 isolates are from Asia and Europe only; none has been described from the United States. DNA homology and ribotyping were used to delineate these new genospecies. DNA relatedness is summarized below:

Percent homology when probed with (N=strains) *

Strains of	B31 DNA	20047 DNA	VS461 DNA
B. burgdorferi s.s.	80-100 (12)	49-67 (4)	48-50 (2)
B. garinii	51-55 (4)	75-100 (13)	53-74 (9)
Group VS461	51 (1)	54-73 (7)	87-100 (7)

^{*}limited ranges may be due to small number of strains tested; B31 and 20047 are the type strains for *Borrelia burgdorferi* sensu stricto and *Borrelia garinii*.

Although these findings make the diagnosis, epidemiology, immunology, pathogenesis, etc. of Lyme borreliosis more complicated, they may help explain some of the quandaries about Lyme disease. For example, it is thought that Lyme arthritis is less common in patients in Europe than the United States (4). We can speculate that this may be because *B. garinii* and/or Group VS461 strains have less arthritogenic potential. Western blotting patterns of patient sera from Europe and the U. S. have different reactivities (2).

Comparisons of the ospA genes of strain B31 [which is Borrelia burgdorferi sensu stricto] and strain Ip90 [which is Borrelia garinii] show their nucleotide sequences are 86% identical and predicted amino acid sequences are 79% identical (3). The greater similarity of ospA genes compared to total DNA may be because the osp genes are located on the 49 kbp linear plasmid which could have been recently introduced into Borrelia.

Although all 3 genospecies are detected by current diagnostic tests, there is no simple assay at this time to differentiate *Borrelia burgdorferi* sensu stricto from *Borrelia garinii* or Group VS461. Ribotyping or DNA hybridization are the only methods which currently can be used to speciate Lyme disease spirochetes.

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COMPARISON OF REPORTED CASES OF LYME DISEASE FROM 1990 TO 1991 BY STATE/ TERRITORY AND REGION

Final data from 1991 have been received from nearly all states and territories (Table 3). Directions of change listed are not expected to differ when final minor data adjustments are made. Reporting of Lyme disease cases increased in all regions except the Pacific states where a 28% decrease was registered in 1991 compared to 1990. For the entire United States, reported cases increased 19% in 1991.

KNOWN DISTRIBUTION OF Ixodes dammini AND Ixodes pacificus IN THE UNITED STATES

In previous editions of LDSS, we have requested the assistance of our readers in a project to collect data on the distribution of known vectors of Lyme disease in the nation. These contributions along with surveys by public health officials and investigators and a review of the published literature have been collated to provide the accompanying map (Figure 1) and tabular data by counties (Table 4). In the Northeast and upper Midwest, 18 states appear to have established populations of *I. dammini* ticks; and an additional 3 states have reported identifying them as present, but have not yet shown that they are established. *I. pacificus* ticks appear to be established in 5 western states and have been reported in 2 others.

While these data are valuable, they should be interpreted cautiously. Most areas not listed as infested in this compilation have not yet been evaluated. Two other considerations are that 1) although an entire county is listed as infested, the infestation is often quite focal, and 2) the data only refer to the presence of tick populations—they do not distinguish infected from uninfected populations. Some areas listed as harboring these vector ticks may be remote and present a small risk of disease transmission to humans. In many counties, *Ixodes* spp. tick populations may be very focal and may or may not be infected.

Ixodes scapularis, another member of the Ixodes ricinus complex, is found throughout the southern United States. It is a competent vector of B. burgdorferi in the laboratory, but has a low rate of B. burgdorferi infection in nature and does not frequently bite humans. Further studies are needed in the southern United States to map the

distribution of *B. burgdorferi* in ticks and vertebrate hosts, and to confirm cases of suspected human infection with *B. burgdorferi* by isolating the organism from patient specimens.

REPORTING OF LYME DISEASE CASES IN 1991 AND 1992 BY NETSS

The numbers of Lyme disease cases reported through National Electronic Telecommunication Surveillance System (NETSS) in the period January through September 3, 1992 are shown in Figure 2. Of the total 4,999 cases reported through Week 36, 3,999 (80%) were reported from the mid-Atlantic and New England regions. The negative data shown in Week 30 correct an erroneous transmission of data from one state in a previous reporting period. Figure 3 shows the numbers of cases reported through NETSS during 1991 for comparison. The total number for 1991 (N=9,469) is still provisional and is expected to be revised slightly in the next few weeks. This number already differs from the final surveillance total which is being published in the 1991 MMWR Annual Summary. The deadline for submitting final data for that publication has passed although a few states are still receiving data requiring modifications of their final 1991 numbers.

ERRATA

In the previous issue of LDSS (1992;3(2):2-4), two points require clarification. The statement that *Borrelia burgdorferi* grows best in "anaerobic conditions" should read in "microaerophilic conditions". That article also contains some apparently conflicting recommendations on optimal growth temperatures (32-33 degrees C and 33-34 degrees C). *B. burgdorferi* grows well throughout the range from 32-35 degrees C and shows marked decrease in growth at 38 degrees C (Barbour AG. *Yale J Biol Med* 1984;57:521-25, 1984).

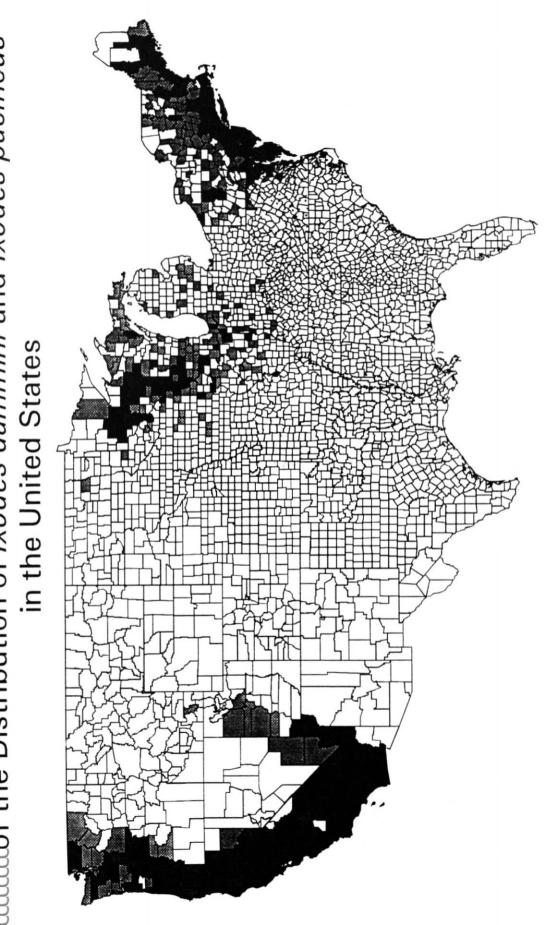
Lyme Disease Surveillance Summary (LDSS) is edited by Drs. Robert Craven and David Dennis. If you have information to contribute or wish to receive a LDSS, please contact them at:

CDC/DVBID Lyme Disease Surveillance Summary P.O. Box 2087 Fort Collins, CO 80522

Table 3. Totals of reported cases of Lyme disease by state/territory and region, 1990-1991.

	REPORTED CASES			
STATE/TERRITORY OR REGION	1990	1991	% AND/OR DIRECTION OF CHANGE	
MAINE	9	15		
NEW HAMPSHIRE	4	38	٠	
VERMONT	11	7		
MASSACHUSETTS	117	265		
RHODE ISLAND	101	142		
CONNECTICUT	704	1192		
NEW ENGLAND REGION	946	1,659	+75.4%	
NEW YORK *	3244	3944		
NEW JERSEY	1074	915		
PENNSYLVANIA	553	718		
MID. ATLANTIC REGION	4,871	5,577	+14 5%	
ОНЮ	36	112		
INDIANA	15	16		
ILLINOIS	30	51		
MICHIGAN	134	46		
WISCONSIN	337	424		
E.N. CENTRAL REGION	562	649	+17.6%	
MINNESOTA	70	84		
IOWA	16	22		
MISSOURI	205	207		
NORTH DAKOTA	3	2		
SOUTH DAKOTA	2	1		
NEBRASKA	0	25		
KANSAS	14	- 22		
W N. CENTRAL REGION	310	363	+17.1%	
DELAWARE	54	73		
MARYLAND	238	282		
oc	5	5	EVEN	
VIRGINIA	129	151		
WEST VIRGINIA	11	43		
NORTH CAROLINA	87	73		
SOUTH CAROLINA	7	10		
GEORGIA	161	25		
FLORIDA	7	35	1 .	

	REPORTED CASES			
STATE/TERRITORY OR REGION	1990	1991	% AND/OR DIRECTION OF CHANGE	
S. ATLANTIC REGION	699	697	-0.3%	
KENTUCKY	18	44		
TENNESSEE	28	35		
ALABAMA	33	13		
MISSISSIPPI	7	8		
E.S. CENTRAL REGION	96	100	+16.3%	
ARKANSAS	22	31		
LOUISIANA	3	6		
OKLAHOMA	13	29		
TEXAS	44	57		
W.S. CENTRAL REGION	82	123	+50.0%	
MONTANA	0	0	EVEN	
IDAHO	1	2		
WYOMING	5	11		
COLORADO	0	1		
NEW MEXICO	0	3		
ARIZONA	0	1		
UTAH	1	2		
NEVADA	2	5		
MOUNTAIN REGION	9	25	+177.8%	
WASHINGTON	30	7		
OREGON +	11	5		
CALIFORNIA +	345	265		
ALASKA	0	0	EVEN	
HAWAII	2	0		
PACIFIC REGION	388	277	-28.6%	
GUAM	0	0	EVEN	
PUERTO RICO	0	0	EVEN	
VIRGIN ISLANDS	0	0	EVEN	
AMERICAN SAMOA	0	0	EVEN	
C.N.M.I	0	0	EVEN	
TERRITORIES	0	0	EVEN	
UNITED STATES TOTAL	7943	9469	+19.2%	



CALIFORNIA:

alameda

amador

calaveras

del norte

el dorado

humboldt

imperial

fresno

glenn

invo

kern

lake

lassen

los angeles

madera

mariposa

merced

napa

nevada

orange

placer

plumas

riverside

sacramento

s.bernadino

san benito

san diego

s.francisco

s.l.obispo

san mateo

santa clara

santa cruz

shasta

sierra

siskivou

sononma

stanislaus

solano

sutter

tehema

trinity

tulare

santa barbara

san joaquin

monterey

mendocino

marin

contra costa

butte

colusa

toulumne

ventura

CONN:

fairfield

hartford

litchfield

middlesex

new haven

new london

DELAWARE:

tolland

windam

kent

sussex

IDAHO:

bannock

boone

brown

carroll

du page

edgar

fayette

grundy

iroquois

io daviess

kankakee

henry

knox

lee

la salle

lawrence

mchenry

macoupin

mclean

menard

mercer

monroe

ogle

peoria

putnam

rock island

sangamon

piatt

coles

ILLINOIS:

cumberland

new castle

volo

vuba

will

allen

cass

dubois

jasper

la porte

madison

marshall

morgan

newton

parke

pike

porter

starke

wabash

warren

IOWA:

clayton

deleware

dubuque

dallas

floyd

linn

polk

iackson

iohnson

marshall

muscatine

winnebago

winneshiek

MAINE:

franklin

hancock

kennebec

knox

lincoln

oxford

penobscot

androscoggin

cumberland

allamakee

cerro gordo

washington

vigo

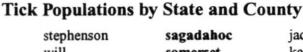
st joseph

lake

fountain

winnebago

INDIANA:



vork

allegeny

baltimore

calvert

cecil

caroline

charles

dorchester

frederick

garrett

harford

howard

montgomery

queen annes

p. georges

st marys

somerset

washington

wicomioo

worcester

MASS:

barnstable

berkshire

bristol

dukes

essex

franklin

hampden

middlesex

nantucket

plymouth

worcester

baraga

berrien

clinton

delta

chippewa

dickinson

genesee

gogebic

iosco

MICHIGAN:

hampshire

talbot

kent

anne arundel

sagadahoc somerset waldo washington

iackson

kent lappeer leelanau

livingston

mackinac

MARYLAND:

manistee marquette menominee oakland

oceana

aitkin

anoka

carlton

chisago

dakota

douglas

houston

kanabec

mille lacs

morrison

olmstead

ramsey

st louis

winona

washington

N. DAKOTA:

N.HAMPSHIRE

grand forks

belknap

cheshire

grafton

hillsborough

merrimack

rockingham

NEW JERSEY:

strafford

sullivan

atlantic

bergen

carroll

coos

scott

pine

isanti

crown wing

carver

ontonagon

schoolcraft

MINNESOTA:

erie franklin fulton iuniata

lackawanna lancaster lebanon lehigh luzerne

lycoming mckean mercer monroe

montgomery n.hampten philadelphia potter sullivan warren

westmorland york

wayne

R.ISLAND:

bristol kent newport providence washington

UTAH:

beaver iuab millard tooele utah

washington

VERMONT:

addison bennington caledonia lamoille windham windsor

VIRGINIA: accomack caroline p.william

vork

W.VIRGINIA: iefferson

WASHINGTON

chelan clallam clark cowlitz gravsharbor island **jefferson** king kitsap klickitat lewis mason okanogon pacific

s.juan skagit skamania snohomish thurston wahkiakum whatcom

yakima

pierce

WISCONSIN:

adams ashland barron buffalo burnett chippewa clark columbia crawford dane eau claire grant iowa jackson iefferson

la crosse lafayette lincoln manitowoc marathon marinette milwaukee monroe oneida

outagamie polk portage price racine richland rock rusk st croix sauk sawver taylor trempealeau vernon walworth washburn waukesha winnebago

> NOTE: Counties that are in **bold** type are counties considered to have established populations, on the basis of surveys of public health officials and investigators and a review of the published literature. Counties in normal type have reported ticks but do not meet criteria for established populations.

Figure 2.

Reported Lyme Disease Cases by Week of Report, U.S., 1992

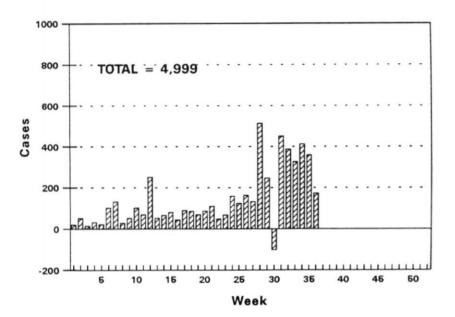


Figure 3.
Reported Cases of Lyme Disease by Week of Report, U.S., 1991

