Lyme and/or Lyme-like Disease in Missouri

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Missouri patients who fulfill the strict CDC surveillance definition for Lyme disease have been reported in significant numbers since 1989, although there are no viable Missouri human cultures of *Borrelia burgdorferi*. The Missouri erythema migrans rashes are indistinguishable from those in other areas, and the clinical syndrome appears similar to Lyme disease nationally. The authors suspect atypical *B. burgdorferi*, and/or other *Borrelia* spirochetes of causing this clinical borreliosis syndrome.

Missouri patients who fulfill the strict CDC surveillance definition for Lyme disease have been reported in significant numbers since 1989. The etiology has been unclear and confusing. This enigma was previously addressed in this journal in 1992.[1]

The clinical syndrome is better defined, but the exact etiologies are unproven. We participated with the Centers for Disease Control and Prevention (CDC) on an epidemiologic and diagnostic study of 45 Missouri patients with physician diagnosed erythema migrans (EM) (considered a diagnostic marker for Lyme disease). This study will be published in the August issue of the *Journal of Infectious Diseases*.[2] The CDC conclusion was that the etiology remains idiopathic, but that evidence implicating *Borrelia burgdorferi* is absent. The study design, exclusion of available information, decisions excluding data relevant to the objective evaluation of the problem, and arbitrary authorship were such that we, as the state epidemiologist who initiated the study and the primary clinician who supplied over half of the study patients, took the significant step of declining authorship. We believe that additional information should have been made available.

Visually, Missouri EM rashes are indistinguishable from those associated with Lyme disease elsewhere. Many authors consider erythema migrans pathognomonic for Lyme disease.[3-14] Photographs of Missouri EM rashes have been published in peer reviewed journals[15-17] and have been presented at the last three international conferences on Lyme Borreliosis (Stockholm, 1990;[18] Arlington, VA 1992;[19] Bologna, Italy 1994[20]). Missouri case data show an EM incidence with a summer peak, and rash location on the body, histology, treatment response, incubation time, tick exposure, age, gender, multiple lesions, associated signs and symptoms, and sequelae are all similar to Lyme disease reported nationally. Thus, clinically, the Missouri physician cannot distinguish this rash and syndrome from Lyme disease diagnosed elsewhere. If Lyme disease is a clinical diagnosis, as the world's literature and the CDC state,[21] then this is clinical Lyme disease.

Atypical isolates of *B. burgdorferi* have been cultured from *Ixodes dentatus* ticks in Southeast Missouri.[22] Live motile spirochetes, similar to *Borrelia*, have been visualized in lone star ticks (*Amblyomma americanum*) from the homesites of Missouri EM patients. Further intrigue is added by the growing evidence that some *A. americanum* (lone star ticks) are infected with a very different and as-yet - unidentified spirochete that may be a completely new species of *Borrelia*.[2] One then ponders the question whether this syndrome is caused by a new species of *Borrelia* that is not *B. burgdorferi*; is it, or is it not, Lyme disease if it cannot be distinguished clinically? There are arguments on both sides, depending on whether one makes the diagnosis microbiologically or clinically. For example, the putative etiologic agent for cat-scratch disease has changed from *Afipia felis* to *Bartonella henselae*, and yet the clinical diagnosis remains the same. To use a *Borrelia* analogy, there are numerous *Borrelia* species that cause relapsing fever, both tick borne and louse borne, and yet the diagnosis of "relapsing fever" remained. The issue of a possible different *Borrelia* bacteria in lone star ticks is significant. What is different about Missouri EM patients is that a minority accurately describe the lone star tick as being associated with their clinical Lyme disease. This has also been reported in other states, but the extent to which this may occur is unknown.[23]

Case Report

Case illustration (CDC #6, ID #111): This case illustrates why a borreliosis is suspected in Missouri. A 41-year-old white man presented on July 5, 1991 with an EM rash (Fig 1) which was painless, non-pruritic and with documented expansion. There was no history of recent tick exposure outside Missouri.

Laboratory findings: A biopsy was taken and he was enrolled in a national study. The EKG on July 5, 1991 was normal. An EM rash biopsy photo (Fig 3) shows histology consistent with erythema migrans, and (Fig 2) shows a modified Dieterle stain of the EM biopsy with a dermal spirochete visualized by Dr. Paul Duray of Harvard.

On July 12, 1991 his electrocardiogram showed atrial fibrillation which resolved spontaneously. All subsequent

Table 1 - Testing by CDC						
CDC Case #6	1991 WCS +>1.0 (3SD)	1991 FLA +>1.0 (3SD)	1992 FLA +>1.0 (3SD)	1993 FLA +>1.0 (3SD)	IgM Western Blot	IgG Western Blot
Day 8	2.278	1.394	1.022	0.81 (equiv)	41	**25, 28,** 36,41, 57, 60, 62
Day 36	2.260	1.354	1.025	0.82 (equiv)	**66**	**15, 25, 28,** 34, **41,** 56, 62

EKG's with over a three year follow up have been normal. Blood Pressure = 112/78; Cholesterol = 165; Glucose = 102; T4 = 7.6; serum ferritin < 200, normal weight, no personal or family history of heart disease and normal echocardiogram. His only medication was amoxicillin 500 mg TID.

Other tests include a positive Lyme ELISA by Dr. R.J. Johnson, of the University of Minnesota, negative RA, negative ANA, negative RPR, and a positive biopsy by Dr. Paul Duray of Harvard (now at the NIH).

Discussion

Eight ELISAs were done by the CDC and none was negative (Table 1). The Western blots were indeed negative by Dressler's criteria,[24] but positive by other published criteria.[25-27] We disagree with the CDC and do not believe this case constitutes "absence of evidence" of serological reactivity to *B. burgdorferi* or a related spirochete. Furthermore, not only were positive CDC FLA ELISA results omitted from the JID manuscript,[2] diagnostically significant less intense Western blot bands by the CDC's own research data[28] were ignored. Knowing that atypical *B. burgdorferi* or "related spirochetes" might very likely produce a response such as this, we disagree with the CDC conclusion of "no evidence." We also know that "most patients with early disease have a good IgG response, and some reinfected patients may have only an IgG response."[25]

Considerable information consisting of test results and information, histology results, and complications were all excluded in the CDC analysis. Very few pathologists have published on the histopathology of Lyme disease. Four of these published experts have reviewed Missouri EM cases and all four (Dr. Duray, Harvard; Dr. DeKoning, Netherlands; Dr. S. Granter, Harvard; and Dr. A. MacDonald of Beaumont, TX) agree that both histology consistent with Lyme erythema migrans and spirochetes which appear similar to *Borrelia* are present in Missouri rash biopsies.

The incubation time of the Missouri EM rashes is important because of the etiological implications. On a questionnaire of 20 Missouri patients in the CDC study, the incubation time differed from that contemporaneously recorded in the patient's charts (3 days vs. 5.5 days).[2] Based on that, one might improperly conclude that the chart data were inaccurate. However, the chart data were entered before the CDC study was even begun. Additionally, the differences were due to discrepancies in only four patients where there were long delays of up to a year between the rash and the questionnaire. The chart data were also verifiable by third parties. Furthermore, 10 of these 20 CDC study patients were a subset of 25 consecutive Missouri erythema migrans patients that were included in a national study. They were documented exhaustively long before the CDC study began. The median incubation time for that larger series of 25 patients was seven days, exactly what one would expect. In 1993, the Missouri Department of Health collected data on 30 other consecutive erythema migrans patients from all over the state and the median incubation time was 6 days. The published incubation times usually vary from 2-30 days with a median of 6 or 7 days. Another series of 14 EM rashes following witnessed lone star tick bites (including three of the CDC study patients) had a median incubation time of 7 days.[20] Although the short 3 day time on the CDC questionnaire might support a suggestion for hypersensitivity reaction or other noninfectious cause of these rashes, we believe that data to be inferior, incomplete, and contradicted. Also, the mean duration of the EM rashes following known tick bites in the CDC study was 14 days, arguing against a hypersensitivity reaction as does the median duration of rash expansion of seven days reported by 31 CDC study cases.

There was no correlation between documented Missouri rash size and reported duration. However, with the known impact of treatment on EM rashes and the considerable variation in antibiotic therapy the lack of correlation is not surprising.

We also disagreed with the arbitrary exclusion of available histopathology evaluations of biopsies on EM study patients. These biopsies clearly exclude such entities as granuloma annulare, etc. Obviously, there are probably some instances where a hypersensitivity reaction could be mistaken for an erythema migrans, but that is a completely inadequate explanation for the phenomenon that is being reported in Missouri. Many have had tick bites all their lives and have never had a similar rash. Also many have been followed for several years since their erythema migrans rashes, have had further tick bites, and have had no further rashes. The vast majority of these clinically diagnosed erythema migrans simply cannot be explained as hypersensitivity reactions or toxic phenomenon, based on clinical data, histopathological examinations, clinical history, and follow up.

The absence of a viable human *B. burgdorferi* culture in Missouri is significant. However, absence of proof is not proof of absence and with an organism such as this where increasing heterogeneity is being found, we do not know for sure that atypical variants do not have some atypical growth requirements. This phenomenon exists in other areas and has been reported in Great Britain[29] and also in the northeastern U.S.[30] Also, at least one uncultivable (at least by present methods) organism that may be a *Borrelia* probably exists in lone star ticks.[2] We caution against using the criteria of failure to grow in a highly defined specific medium such as BSK-II as proof of nonexistence. Even

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Table 2.

Results of tests for antibodies to *Borrelia burgdorferi* in serum from Missouri patients with suspected erythema migrans (all patients of Dr. Masters) by enzyme immunoassay (EIA) using whole-cell sonicated (WCS) antigens and flagellar (FLA) antigens and immunoblots performed by the CDC. Bold print indicates strong bands.

CDC Case #	Samp. #	Pt ID#	Days After Rash	1991 WCS +=>1.0 (3SD)	1991 FLA +=>1.0 (3SD)	1992 FLA +=>1.0 (3SD)	1993 FLA	IgM Western blot	IgG Western blot
17	1355	125	37	0.38	0.575	0.192	0.16	39,66.75	ND
11	1358	136a	27	0.552	0.443	0.375	0.23		35, 41, 52, 60, 75
11	1359	136b	100	0.677	0.689	0.204	0.18		15, 25, **37, 41**, 45, 60, 62, 75
18	1360	119	16	0.38	0.396	0.257	0.29	41	**29, 41, 45, 57,** 62,**75**
1	1362	116a	3	1.432	0.519	0.591	0.55	41	**28, 37, 39, 41, 45, 58,** 66
1	1363	116b	34	1.690	0.434	0.678	0.51	ND	**15, 39, 41, 47, 56, 58**
1	1364	116c	97	1.425	0.721	0.638	0.54	**66**,75	**15, 39, 41, 47, 56, 58**
3	1371	121a	5	0.811	0.434	0.731	0.15		25, **41** ,45, **57, 62**
3	1372	121b	46	1.116	0.387	0.700	0.21	31, 45, 58	**15,** 25, 29, **41,** 45, **57, 62**
12	1374	137a	22	0.953	0.575	0.449	0.73	**41**	25, **34, 41, 56, 66,** 75
12	1375	137b	119	0.833	0.415	0.344	0.54		15, **42**
19	1380	138	54	0.507	0.179	0.272	0.18	**41, 60, 66, 75**	**41, 62, 68**
13	1382	103a	30	1.118	0.472	0.378	0.37	**41**	30, **41**
13	1383	103b	58	1.082	0.557	0.344	0.41	66, 75	15, **29, 41**
4	1385	145a	19	0.398	0.274	0.214	0.23	**62, 63, 64**	**21, 34**, 37, 41, **60**, 62
4	1386	145b	38	0.626	0.358	0.307	0.19	39, **63, 64,** 75, 83	15, **34**, 41, 60, 62
5	1388	105a	4	0.686	0.538	0.295	0.21		**29, 41**, 62
5	1389	105b	32	0.809	0.528	0.283	0.27	41	29, **41**, 62
14	1397	117a	31	1.846	0.792	0.488	0.39	**41, 66**	**34, 41**, 62, 66, **75**, 83
14	1398	117b	92	1.836	0.623	0.519	0.31	**37**, 39, 41, **58, 66**	15, **21, 34**, 41, **62, 75,** 83
2	1404	118a	3	2.694	0.772	0.234	0.22	41	18, 29, 37, **41, 45**, 57, **66, 75**
2	1405	118b	35	1.931	0.668	0.301	0.28	41	18, 37, **41, 45**, 57, **66, 75**
2	1406	118c	185	0.900	0.435	0.193	0.25	34	**15,** 37, **41,** 45, 57, **66, 75**, 83
6	1407	111a	8	2.278	1.394	1.022	0.81	41	**25, 28, 36, 41,** 57, 60, 62
6	1408	111b	36	2.260	1.354	1.025	0.82	**66**	**15, 25, 28,** 34, 37, **41,** 56, 62
20	1410	131	99	0.364	0.292	0.481	0.13	25, 37, 66	**29, 41**, 62, 83
7	1411	132a	3	0.318	0.340	0.280	0.3	41, **75**	15, 18, **41**, 75, 83
7	1412	132b	31	0.440	0.321	0.295	0.21	**79**	**41,75,**83
8	1415	140a	3	0.835	0.406	0.314	0.35	**25**, 41, 83	**15, 28**, 29, 36, 39, 41, **62**
8	1416	140b	94	1.096	0.415	0.197	0.18	**25**, 37, 60, 66, 83	**15, 28, 29, 37**, 41, 60, **62**
15	1418	141a	1	1.207	0.783	0.386	0.23	**41**	28, **30, 35, 41**, 45, 62
15	1419	141b	24	0.587	0.425	0.380	ND	**41**	**30, 35**, 36, **41, 45**, 62
15	1420	141c	176	0.716	0.443	0.324	0.27	41, 58, 60, 66, 83	**15, 29, 30, 41, 62**
21	1424	133	94	1.606	1.087	0.693	0.64	83	**41**
9	1425	113a	3	0.452	0.106	0.130	0.13	**41**	**15**, 25, **34**, 41, 57
9	1426	113b	33	0.782	0.821	0.546	0.29	41	15, 25, 28, **34, 41**, 62
16	1428	120a	25	1.338	0.377	0.507	0.79	**25, 31, 39, 41**	15, 25, **41**, 55, 62, 75
16	1429	120b	97	0.700	0.481	0.423	0.32	**15**, 25, 31, 37, 39, 41	28, 41, 62, 66, 75, 83
10	1431	114a	8	2.188	0.358	0.394	0.39	**41**	**15, 41, 60**
10	1432	114b	39	2.636	0.472	0.208	0.25	28, **37**, 39, **41**	15, 25, **41**, 60
22	1433	127	76	0.552	0.679	0.261	0.10	ND	ND

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Dr. Kelly, when originally cultivating relapsing fever spirochetes, had to use different media for different species.[31] The syphilis spirochete has still not been cultivated in a cell free media.[32]

The CDC concluded that in the Missouri study of 22 patients tested there was no serologic evidence of a *B. burgdorferi* or related spirochetal infection.[2] All 22 were patients of Dr. Masters and the results are presented in Table 2. We disagreed with the CDC's negative conclusion and present here data that was excluded from the CDC manuscript. There were 45 patients in the CDC study and 40 of these patients had no less than 57 different positive Lyme serologies performed by 7 different laboratories.

Even though the CDC itself has researched and presented on the diagnostic utility of using faint or less intense Western blot bands for diagnosing Lyme disease,[28] as have other authors,[25] these were specifically excluded in this Missouri study. We know that atypical *B. burgdorferi* exist in Missouri[22] and that Zoller et al published that strain dependent differences of Western blot band intensities exist.[33] Additionally, there are numerous publications which have included faint bands. Engstrom et al. showed that most less intense bands should be counted.[25] Obviously, band intensity is not only a subjective interpretation by the laboratory, it can also be a function of technique and storage. The Missouri Western blots were performed by the CDC on stored sera that had gone through multiple freeze/thaw cycles. Also, in the Engstrom paper,[25] they could not match a number of their protein bands with those described by Dressler and thus there is published concern about the arbitrary use of such a rigid Western blot interpretation. We also have questions about the arbitrary decisions on IgM band specificity, as well as exclusion of OspA (31kDa) and OspB (34kDa) from the diagnostic criteria, which is contrary to all previously published literature.[24]

The gravamen of the CDC analysis is their assumption that their FLA ELISA is accurate in its negative results and their WCS ELISA is wrong in its positive results in detecting *B. burgdorferi* or a related spirochete. If one were biased toward a negative conclusion as we believe the CDC authors were, then the sensitivity of relatively comparable tests would take precedence. The CDC admits the ELISA test with Whole Cell Sonicate (WCS) is more sensitive.[2,32,35]

We believe that the differences should be examined and compared not only with the positive, but also with the negative control population. Of the 22 Missouri EM patients tested serologically by the CDC, 11 of the 22 had discordant results by having positive WCS ELISAs, using a three standard deviation cutoff for positive. The CDC did not mention that 38 non-Lyme Missouri controls were also tested by the WCS ELISA method and that only one of the 38 tested positive, as compared to 50% of the Missouri EM rash patients. The odds of this result occurring by chance exceed 25,000,000 to one. Missouri controls were not tested with the CDC FLA ELISA. Furthermore, if one takes the 21 Missouri patients who had both IgM and IgG Western blots and compares them to published studies in which faint bands were included, there are some interesting results. Using the same criteria, the Western blots on these 21 Missouri patients performed by the CDC were compared by us to the Ma study controls.[36] (Table 3) Of the major published *Borrelia* associated Western blot bands such as 20kDa, 31kDa, 34kDa, 39kDa, 66kDa, and 83kDa, the p values in the Missouri EM patients were significantly higher than the Ma study controls ($p < .002$) in every instance. Fawcet et al published a series of 200 non-Lyme controls in 1992 and of their Western blots, only one patient of 200 controls had four IgG bands.[26] As shown in Table 2, the vast majority of these Missouri EM patients had four or more IgG bands. These Missouri EM patients have Lyme serologies meaningfully different from published negative control populations.

Table 3. IgM & IgG Lyme Disease Western blot Bands

A comparison of 21 Missouri EM patients' Borrelia associated bands when tested by the CDC with published normal non-Lyme controls.

kDa	Missouri EM #	21 pts. %	Ma Controls* #	320 pts. %	p
20	2	9.5	2	0.6	< .001
31	3	14	7	2.2	< .002
34	6	28	8	2.5	< .001
39	7	33	4	1.3	< .001
41	21	100	138	43	< .001
66	11	52	42	13	< .001
83	8	38	4	1.3	< .001

* Ma et al. Serodiagnosis of Lyme borreliosis by Western Immunoblot: Reactivity of Various Significant Antibodies against *Borrelia burgdorferi*. *Journal of Clinical Microbiology*, February 1992, p. 370-6.

Analysis of the 11 CDC study patients with positive WCS ELISAs and negative or equivocal flagellar (FLA) ELISAs is also significant. The CDC had previously stated their WCS and FLA ELISAs were highly correlated with an $r > .90$.[34] We believe this needs to be explained. All 11 patients had a negative rapid plasma reagin (RPR), rheumatoid arthritis (RA), and antinuclear antibody (ANA) to help rule out cross-reactivity. All tested negative for antibodies to *Francisella tularensis*, *Rickettsia typhi*, *Rickettsia rickettsii*, arboviruses, and *Ehrlichia chafeensis*. One out of 10 patients tested positive to *Q fever (Coxiella burnetii)*. Two patients also tested positive to a CDC 1991 Lyme FLA ELISA. As mentioned, when compared with published controls patients[36] the p value for Western blot *Borrelia* associated bands (20kDa, 31kDa, 34kDa, 39kDa, and 83kDa) was .002 or less. Nine of these 11 patients had four or more bands on at least one Western blot performed by the CDC and eight of the eleven had five or more bands. The CDC itself has presented research that indicates that the presence of five IgG bands, even faint, have a high correlation with Lyme disease.[28] Eight of these 11 patients were also part of a national prospective study. Lyme serologies were done by Dr. R.J. Johnson of the University of Minnesota and 2 Standard Deviations (SD) was considered borderline and 3 SD positive. All eight (100%) of this subgroup had one or more Lyme ELISAs at least two SD from normal with many having strongly positive tests.

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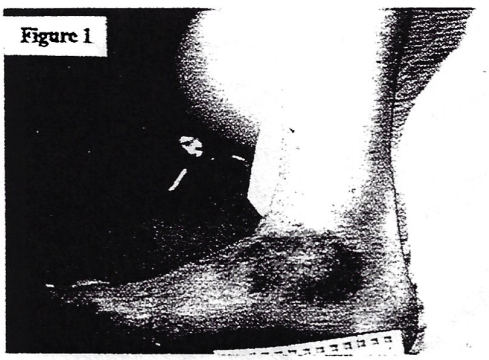
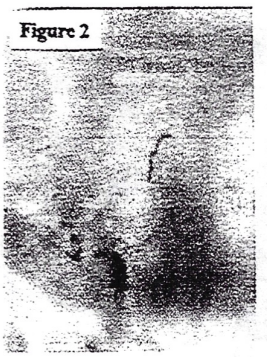


Figure 1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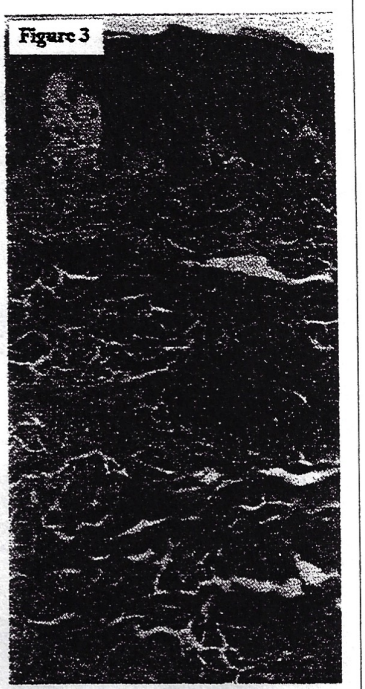
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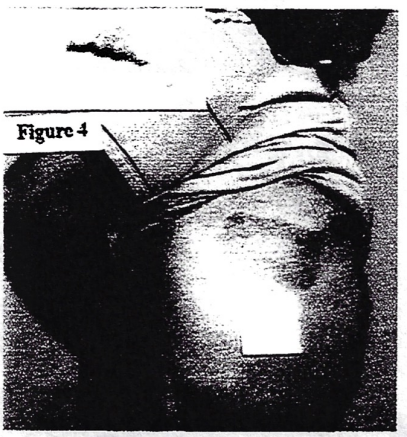


Figure 4

Left: J.S. Tick Bite,
Bollinger Co., MO.
Personally removed tick.
Rash started one week after bite.
Pictures taken 14 days after the tick bite.
Positive U. of CT Western blot.
Neg RPR; Neg RA; Neg ANA.
Positive pathology by three different pathologists:

1. Dr. P. Duray, Harvard
2. Dr. J. DeKoning, Netherlands
3. Dr. P. Cordes, Cape Girardeau, MO

Non-pruritic painless expanding rash.
Tick bites before and after this have NOT resulted in rashes.

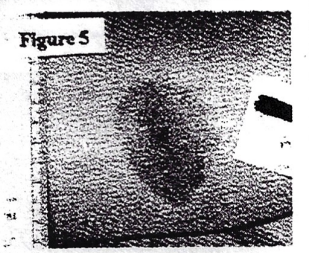


Figure 5

Above: Rash following tick bite in a 10-year old boy who had never traveled outside of Missouri. Western blot analysis initially showed no bands but 3 months later revealed IgG bands (29, 31, 41, and 66kDa). Results of rheumatoid factor and rapid plasma reagin tests were negative.


Figure 7

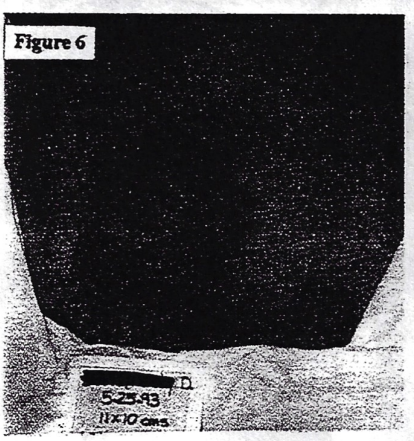


Figure 6

Above: M.D.
- Witnessed adult female lone star tick bite on back on 5/12/93
- Rash first noticed on 5/21/93 (9 days incubation time)
- Rash enlarged to 10x11 cm on 5/25/93
- Rash non-pruritic and painless
- Many previous tick bites, has never had one do this
- No tick exposure out of MO
- No allergies whatsoever
- Initial Lyme serologies all negative
- Neg RA; Neg RPR
- Lyme Western blot (Mardex) on 6/28/93: IgM 41kDa, IgG 39kDa, 41kDa, 58kDa, 60kDa
- Three *Borrelia burgdorferi* isolates from *Ixodes dentatus* obtained from her farm
- See histopathology on right

Biopsy of M.D. Rash
above: The epidermis and keratin layer are intact, with a lymphoplasmacellular perivascular infiltrate in the papillary dermis. These features characterize the histopathology of erythema migrans. H&E stain.
Magnification x400.
Photo courtesy of P. Duray, Harvard.


Figure 8

Above: H5332 monoclonal antibody stain of an adult female lone star tick midgut.
Bollinger County, MO.
Photo provided by Dorothy Feir, Ph.D.
Saint Louis University.

Examples of Missouri Erythema Migrans in CDC Case Study

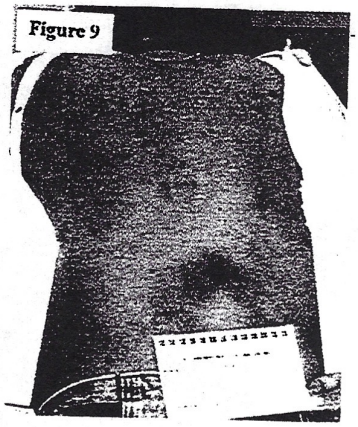
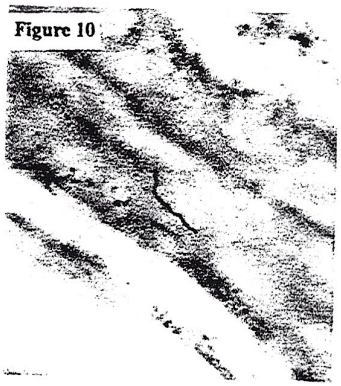


Figure 9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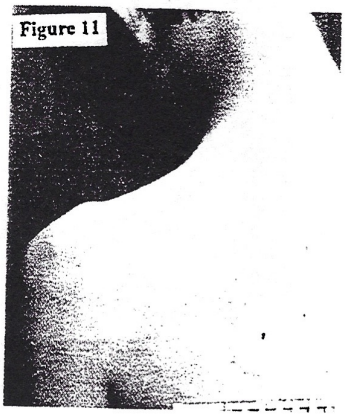
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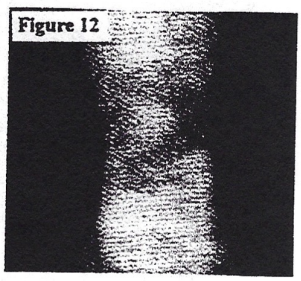
Figure 12

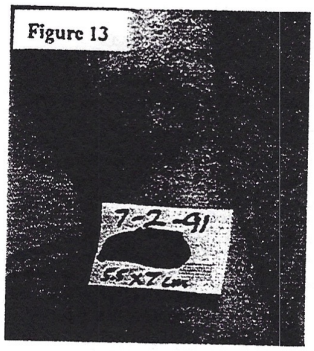

Figure 13

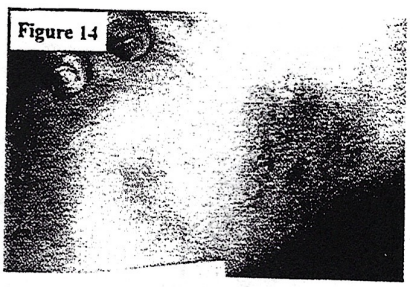
Figure 14

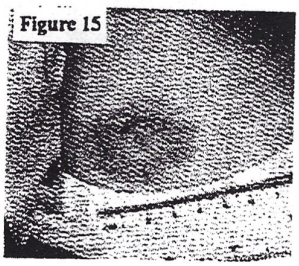
Figure 15

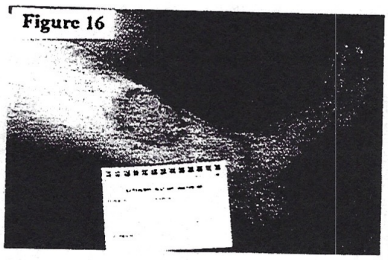
Figure 16

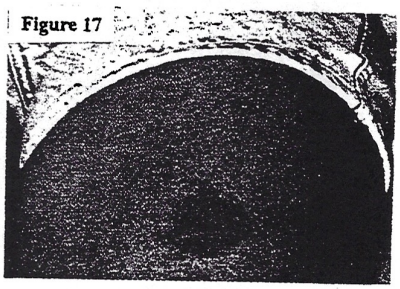
Figure 17

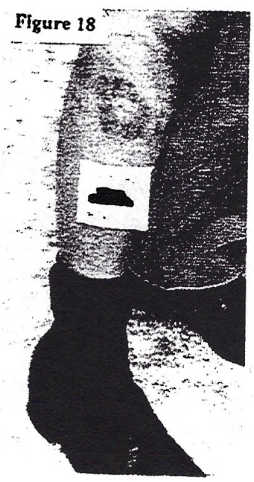
Figure 18


Figure 19


Figure 20


Figure 21

Continued from page 349

Additionally, the CDC indicated the presence of an 83kDa, 39kDa, 21kDa, or 18kDa band was also highly specific for Lyme arthritis, even when faint.[28] As one can see from Table 2 many of these bands were present in the Missouri EM patients tested by the CDC. Engstrom et al showed that low intensity bands can have diagnostic utility and need not be excluded.[25] Bunikis et al showed that variable reactivity against different Lyme disease *Borrelia* spp. exists and concluded that differences in antigen compositions among *Borrelia* spp. may result in variable immune responses.[37] Knowing that different *Borrelia* spp. may exist and that atypical *B. burgdorferi* definitely exist in Missouri,[22] we believe that all of the available data should be evaluated before negative conclusions are made and published.

We know from Oliver and Kollar's work that *B. burgdorferi* are in Missouri *I. dentatus* ticks that preferentially feed on rabbits (but also occasionally on humans).[22] Bridge vectors should be researched. In Ryder et al's tick transmission study using geographically unmatched spirochetes in *Amblyomma americanum*, they showed a transstadial transmission rate of 1:60 (1.7%) that left open the question of whether lone star ticks could be involved in occasional human cases.[38] One cottontail rabbit in Southeast Missouri at the farm of an EM patient was examined and had *I. dentatus* ticks on it as well as over 1000 lone star larvae.[39] Even at a 1.7% rate, that single rabbit could theoretically be the source of infection for 17 lone star nymphs. Other researchers have seen spirochetes appearing similar to *Borrelia* that stained variably with H5332 monoclonal antibodies for *B. burgdorferi* in this area.[29,40-42] The CDC paper dismisses the positive IFA and PCR results of Feir et al[32] by stating that *B. burgdorferi* failed to be amplified using a second primer pair.[2] Three ticks were tested using the two primers and only one failed to amplify *B. burgdorferi* DNA with the second primer. The ability to amplify *B. burgdorferi* DNA using one set of primers while failing using a different set of primers is not uncommon and can possibly be attributed to strain variation. For example, Oliver et al (1994) found in one *B. burgdorferi* isolate from Missouri, that FLA DNA was amplified while OspA DNA was not using a second primer pair.[22] In the Feir et al study, PCR positivity was significantly associated with IFA positivity, and there were no PCR positive tests from IFA negative ticks from areas believed to be free of Lyme disease or from laboratory reared ticks.[32]

Figures on pages 350 and 351

Figure 1 Case Illustration. CDC Case #6, I.D.#111. MO EM rash, right foot, transient atrial fibrillation. **Figure 2** Modified Dieterle stain of EM biopsy in figure 1 showing a dermal spirochete. Photo courtesy of Dr. P. Duray, M.D., Harvard. **Figure 3** Histology of EM biopsy, case in figure 1 & 2, showing a dense lymphoplasmacellular infiltrate consistent with erythema migrans (Photo courtesy of P. Duray, M.D., Harvard). **Figure 4** Missouri EM, Bollinger County; (insert: Steiner-Steiner silver stain showing a dermal spirochete in his EM biopsy). **Figure 5** "Target" lesion characteristic of EM, Scott County. **Figure 6** MO EM after lone star tick bite. **Figure 7** Histology of EM in figure 6. Photo courtesy of P. Duray, M.D., Harvard. Also, on modified Dieterle stain of this biopsy, a dermal spirochete was visualized. **Figure 8** Lone star tick midgut smear showing spirochetes. **Figure 9** DS, CDC Case #21, ID#133 - positive CDC FLA ELISA in 1991, and positive IgM and IgG ELISA by Dr. R.J. Johnson. **Figure 10** Modified Dieterle stain showing a dermal spirochete in CDC case #21, figure 9. Photo courtesy of P. Duray, M.D., Harvard. **Figure 11** CDC Case #1, ID #116 (also seroconverted to Q fever) and seroconverted to Lyme with IgG ELISA by Dr. R.J. Johnson. **Figure 12** K.K. CDC study case. Enlarging rash noticed three weeks after tick bite while turkey hunting in Phelps County. PCR was positive for Lyme disease; **Figure 13** PH CDC Case#5, ID #105. **Figure 14** FN CDC Case #7, ID #132. **Figure 15** BW CDC Case #22, ID#127. **Figure 16** LH CDC Case #4, ID#145. **Figure 17** CP CDC Case #8. ID#140, CDC WCS ELISA seroconversion. **Figure 18** LA CDC Case #18, ID#119. **Figure 19** BT CDC Case #9, ID#113. **Figure 20** CC CDC Case #3, ID#121 (seroconverted on WCS ELISA). **Figure 21** LL CDC Case #2, ID#118 (Western blot seroconversion at North American Laboratories). Also developed a migratory oligoarticular arthritis. **Figures 5, 12, and 15** reprinted with permission of *Postgraduate Medicine*. **Figure 21** reprinted with permission of *Journal of Spirochetal and Tick-borne Diseases*.

Conclusion

In summary, we have presented evidence documenting Missouri patients that have clinical presentations that meet diagnostic and surveillance criteria for Lyme disease and that cannot be easily explained in the absence of a borreliosis. Previously, some believed that because Missouri did not have the *Ixodes dammini* deer tick, Lyme disease was not possible. We now know that *I. dammini* is not a valid separate tick species but is the same as *I. scapularis*, which is common in Missouri.[43] Missouri EM patients often have histological and serological results that are clearly abnormal and are consistent with a borreliosis.

We also believe that this illness can have marked sequelae. In the CDC study of 45 patients treated early,[2] there were still two cases of associated arthritis with visibly swollen joints and one carditis. Also, there were at least two cases of marked non-specific symptoms such as fatigue, impaired cognition, myalgias, etc. Three of these patients, including the patient with carditis at three weeks, one with arthritis at 2 1/2 months, and another with arthritis at 4 1/2 months were not reported by the CDC because of the study design.

We previously published on all 672 Missouri cases reported from 1989-1992 that met the CDC Lyme surveillance definition and compared the signs and symptoms to Lyme disease reported elsewhere. We concluded that Lyme disease reported in Missouri was similar in terms of signs and symptoms to Lyme disease reported nationally.[15] We agree with the CDC that physicians should maintain a high index

of suspicion, not only for this syndrome, but for other Missouri tick borne illnesses such as rickettsiosis, tularemia, babesiosis, and ehrlichiosis. We also believe that there are insufficient available data to exclude clinical borreliosis, possibly caused by more than one variety of spirochete. At this time there is no alternative plausible diagnosis and we encourage physicians to comply with the Missouri law requiring reporting of Lyme disease.[44] Even though the exact etiology or etiologies of this clinical syndrome remain unproven, patients who meet the clinical criteria for Lyme disease should be reported. The Missouri Department of Health recognizes the etiological controversy and in order to further understand this illness and resolve the issue of Lyme vs. Lyme-like disease, data are needed and patients need to be evaluated. We have both identified and unidentified spirochetes in Missouri ticks that are biting our patients who then become ill with signs and symptoms that are extremely difficult to explain in the absence of a borreliosis - whether it is caused by *B. burgdorferi*, an atypical *B. burgdorferi*, or some other infectious agent or spirochete. Further research is needed.

References

1. Donnell HD. The enigma of Lyme disease in Missouri. *MoMed* 1992; 89(10):714-6.
2. Campbell GL, Paul WS, Schriefer ME et al. Epidemiologic and diagnostic studies of patients with suspected early Lyme disease, Missouri, 1990-1993. *Journal of Infectious Diseases* August 1995 (In press)
3. Malane MS, Grant-Kels JM, Feder HM, Luger SW. Diagnosis of Lyme disease based on dermatologic manifestations. *Ann Intern Med.* 1991;114:490-8.
4. Rahn DW, Malawista SE. Lyme disease: Recommendations for diagnosis and treatment. *Ann of Intern Med.* 1991;114(6):472-81.
5. Massarotti EM, Luger SW, Rahn DW et al. Treatment of early Lyme disease. *Amer J of Med.* 1992;92:396-403.
6. Corpuz M, Hilton E, Lardis P, et al. Problems in the use of serologic tests for the diagnosis of Lyme disease. *Arch Intern Med.* 1991;151:1837-40.
7. Cooper JD, Schoen RT. Epidemiology, clinical features, anddiagnosis of Lyme disease. *Curr Opin in Rheuma* 1992;4:520-8.
8. Manian FA. The latest strategies for diagnosing and treating Lyme disease. *Modern Medicine* 1992;60:72-81.
9. Lyme disease still baffling. *CAP Today* 1990;4(10).
10. Diagnosis and treatment of Lyme disease. *Clinical Courier* 1991;9(5).
11. Lightfoot RW, Luft BJ, Rahn DW, et al. Empiric parenteral antibiotic treatment of patients with fibromyalgia and fatigue and a positive serologic result for Lyme disease. *Ann of Intern Med* 1993;119(6):503-9.
12. Kazakoff MA, Sinusas K, Macchia Christine. Liver function test abnormalities in early Lyme disease. *Arch Fam Med* 1993;2:409-13.
13. Goldings EA, Jericho J. Lyme disease. Clinics in Rheumatic Diseases 1986;12(2):343-67.
14. Treatment of Lyme disease. *The Medical Letter* 1989;31:57-9.
15. Masters EJ, Donnell HD, Fobbs M. Missouri Lyme disease: 1989 through 1992. *J SpirochetalTick-borne Dis* 1994;1:12-7.
16. Masters EJ. Erythema migrans. Rash as key to early diagnosis of Lyme disease. *Postgrad Med* 1993;94:133-4,137-42.
17. Masters EJ, King LE. Differentiating Loxoscelism from Lyme Disease. Emer Med. 1994;26(10):46-9.
18. Masters EJ, Feir D, Reppell C. Lyme borreliosis in Missouri: significant new area, new significant vector. [abstractW/TH-P-10] In programs and abstracts from the 4th International Conference on Lyme Borreliosis (Stockholm, Sweden), 1990.
19. Masters EJ, Rawlings J, Fobbs M, Donnell HD. Epidemiology of erythema migrans in Missouri [abstract 351]. In programs and abstracts from the 5th International Conference on Lyme Borreliosis (Arlington,VA), 1992.
20. Masters EJ, Duray P, Granter S, Cordes P. Annular Rashes Following Definite Lone StarTick Bites (P029T). In: Program and abstracts of the 6th International Conference on Lyme Borreliosis, Bologna, Italy, 1994.
21. CDC Surveillance Summary: Volume 4, #3, June 1993.
22. Oliver J, Kollars T, Chandler, Masters E, et al. UnusualTick Isolates from Southeast MissouriAssociated Geographically andTemporally with Human Lyme Disease [abstract 0002M]. In programs and abstracts from the 6th International Conference on Lyme Borreliosis (Bologna, Italy), 1994.
23. SchulzeTL, Bowen GS, Bosler EM et al. *Amblyomma americanum*: a potential vector of Lyme disease in New Jersey. *Science* 224:601-603.
24. Dressler F, Whalen JA, Reinhardt BN, Steere AC. Western blotting in the serodiagnosis of Lyme disease. *J Infect Dis* 1993;167:392-400.
25. Engstrom SM, Shoop E, Johnson RC. Immunoblot interpretation criteria for serodiagnosis of early Lyme disease. *J Clin Microbiol* 1995;33:419-27.
26. Fawcet et al. Frequency and specificity of antibodies that crossreact with Borrelia burgdorferi antigens. *J of Rheumatology* 1992;19(4):582-7.
27. Mitchell PD, Reed KD, Aspeslet TL et al. Comparison of four immunoserologic assays for detection of antibodies to *Borrelia burgdorferi* in patients with culture-positive erythema migrans. *Journal of Clin Micro* 1994;32(8):1958-62.
28. Weinstein A, Kowal K, Bailey RE, Craven R. Western blot band intensity in Lyme disease. [P109T]. In: Program and abstracts of the 6th International Conference on Lyme Borreliosis (Bologna, Italy), 1994.
29. Curtin SM, Maggs AF, Pennington TH. Lyme borreliosis. the *Lancet* 1994;344:188-9.
30. Brunet LR, Spielman A, Telford III SR. Heterogeneity of *Borrelia burgdorferi* within guts of individual field-collected adult *Ixodes dammini* ticks. [P073W] In: Programs and abstracts from the 6th International Conference on Lyme Borreliosis (Bologna, Italy) 1994.
31. Kelly RT. Cultivation and physiology of relapsing fever Borreliae. *The Biology of Parasitic Spirochetes*. RC Johnson, Editor: Academic Pres N.Y. 1976.
32. Feir D, Santanello CR, et al. Evidence supporting thepresence of Borrelia burgdorferi in Missouri. *Am J Trop Med Hyg* 1994;51(4):475-82.
33. Zoller et al. Validity of Western immunoblot band patterns in the serodiagnosis of Lyme borreliosis. *J Clin Micro* 1991;174-82.
34. Quan TJ, Craven RB, Mayer LW, et al. Sensitivity and specificity of whole-cell sonicate and flagella ELISA for *Borrelia burgdorferi* antibody [abstract 73]. In: Program and abstracts of the 5th International Conference on Lyme Borreliosis (Arlington, VA), Rockville, MD: Federation of American Societies for Experimental Biology, 1992.
35. Quan TJ, Robbins KE, Craven RB, et al. Precision of whole-cell sonicate and flagella ELISA for Borrelia burgdorferi antibody [abstract 74]. In: Program and abstracts of the 5th International Conference on Lyme Borreliosis (Arlington, VA), Rockville, MD: Federation of American Societies for Experimental Biology, 1992.
36. Ma, et al. Serodiagnosis of Lyme borreliosis by Western immunoblot: reactivity of various significant antibodies against *Borrelia burgdorferi*. *J Clin Micro* 1992:370-6.
37. Bunikis J, Olden B, Westman G, Bergstrom S. Variable serum immunoglobulin responses against different Borrelia burgdorferi sensu lato species in a population at risk for and patients with Lyme disease. *J of Clin Microb* 1995;33(6):1473-8.
38. Ryder JW, Pinger RR, Glancy T. Inability of *Ixodes cookei* and *Amblyomma americanum* nymphs (Acari:Ixodidae) to transmit *Borrelia burgdorferi*. *J Med Entomol* 1992;29:525-530.
39. Dr. Tom Kollars, Georgia Southern University, Statesboro, GA, Personal comm.
40. Dr. Lawrence W. Hencke, Department of Biological Sciences, Arkansas State University, State University, AR, Personal comm.
41. Kardatzke JT, Neidhardt K, Dzuban DP, Sanchez JL, Azad AF. Cluster of tick-borne infections at Fort Chaffee, Arkansas: rickettsias and Borrelia burgdorferi in ixodid ticks. *J Med Entomol* 1992;26:669-72.
42. Kocan AA, Mukolwe SW, Murphy GL, et al. Isolation of *Borrelia burgdorferi* (Spirochaetales: Spirochaetaceae) from *Ixodes scapularis* and *Dermacentor albopictus* ticks (Acari:Ixodidae) in Oklahoma. *J Med Entomol* 1992;29:630-633.
43. Oliver JH, Owsley MR, Hutcheson HJ et al. Conspecificity of the ticks Ixodes scapularis and I. dammini (Acari:Ixodidae). *J Med Entomol* 1993;30(1):54-63.
44. State of Missouri. Code of State Rules 1995.19 CSR 20-20.020 Reporting Communicable, Environmental and Occupational Diseases.

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