

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.*

CDC looking for tick culture

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 journals¹⁵⁻¹⁷ and have been presented at the last three international conferences on Lyme Borreliosis (Stockholm, 1990;¹⁸ Arlington, VA 1992;¹⁹ Bologna, Italy 1994²⁰). 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 moille 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*.²³ 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 argu-

ments 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 *Aspila 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.²⁴

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	3.278	1.394	1.022	0.81 (equiv)	41	25, 28, 30, 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,²⁵ but positive by other published criteria.²⁶⁻²⁷ 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 data²⁸ 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."²⁹

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

miss 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 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 Britain³⁰ and also in the northeastern U.S.³¹ 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 (ELISA) 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	1991 FLA	1992 FLA	1993 FLA	IgM Western blot	IgG Western blot
				+=>1.0 (3SD)	+=>1.0 (3SD)	+=>1.0 (3SD)	+=>1.0 (3SD)		
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	15,25,37,41,45,60,68,75	
18	1360	119	16	0.38	0.396	0.257	0.29	41	29,41,48,57,62,75
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	0.578	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	143a	19	0.398	0.274	0.214	0.23	62,63,64	21,34,37,41,60,62
4	1386	143b	38	0.626	0.358	0.307	0.19	39,63,64,73,83	15,34,41,60,62
5	1388	105a	4	0.686	0.538	0.295	0.21		28,41,62
5	1389	105b	32	0.809	0.528	0.283	0.27	41	29,41,62
14	1397	137a	31	1.846	0.192	0.488	0.39	41,66	34,41,62,66,75,83
14	1398	117b	92	1.816	0.623	0.519	0.31	37,39,41,58,66	15,21,34,41,62,78,83
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	16,25,28,34,37,41,56,62
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	41,78,83
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	0.785	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	0.577	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	0.23	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.²¹ The syphilis spirochete has still not been cultivated in a cell free media.²²

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.¹ 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,²³ as we have other authors,²⁴ these were specifically excluded in this Missouri study. We know that atypical *B. burgdorferi* exist in Missouri²⁵ and that Zoller et al published that strain dependent differences of Western blot band intensities exist.²⁶ Additionally, there are numerous publications which have included faint bands. Engstrom et al. showed that most less intense bands should be counted.²⁷ 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,²⁷ 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.²⁸

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.^{27,28}

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

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			Ma Controls*		
	#	21 pts.	%	#	320 pts.	%
20	2	9.5		2	6.6	<.001
31	3	14		7	2.2	<.001
36	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.

by the CDC were compared by us to the Ma study controls.²⁸ (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.²⁹ 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.

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 > .90.²⁴ We believe this needs to be explained. All 11 patients had a negative rapid plasma reagins (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 chaffeensis*. 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 patients²⁹ 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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Additionally, the CDC indicated the presence of an 83kDa, 39kDa, 21kDa, or 18kDa band was also highly specific for Lyme arthritis, even when faint.²³ 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 Bunkis 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.²⁷ 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 re-

searched. 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.²⁸ 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.²⁹ 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.^{30,31} The CDC paper dismisses the positive IFA and PCR results of Feir et al³² by stating that *B. burgdorferi* failed to be amplified using a second primer pair.¹ 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

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 borrellosis. 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.³³ Missouri EM patients often have histological and serological results that are clearly abnormal and are consistent with a borrellosis.

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 2/2 months, and another with arthritis at 4/2 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.¹⁹ 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 borrellosis, 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.⁴ 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 borrellosis - whether it is caused by *B. burgdorferi*, an atypical *B. burgdorferi*, or some other infectious agent or spirochete. Further research is needed.

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