PATIENTS AND METHODS

Seventeen patients who presented to a primary care clinic in Cape Girardeau, Mo, from May 6, 1990, to September 15, 1993, with rashes similar, if not identical, to erythema migrans and documented Lone Star tick (*Amblyomma americanum*) bites were studied. For inclusion in this study, positive identification of the arthropod as a Lone Star tick required confirmation of the distinctive white dot on the back of the adult female. Witnessed nymph tick bites were excluded unless the tick was saved for definitive identification. Some patients have been subjects of other studies. Five patients (patients 3, 5, 6, 8, and 9) were enrolled in a double-blind, randomized controlled trial comparing azithromycin with amoxicillin in the treatment of erythema migrans.[16] Remarkably, these patients were not distinguishable in this study from patients in accepted Lyme disease endemic areas. Six patients (patients 1, 3, 4, 5, 7, and 9) were in a Centers for Disease Control and Prevention (CDC) retrospective study of Missouri patients with suspected early Lyme disease.[4] Fourteen of the rashes had biopsy specimens of the peripheral margins available for study. All biopsy specimens were formalin fixed and paraffin embedded by standard techniques. Sections were also stained using a modified Dieterle silver stain to identify spirochetes. This stain was performed as previously described.[17]

Table 1. Clinical Characteristics of Study Patients*

Patient No./ Sex/ Age, y	Date	Tick†	Bite Location	Incubation Time, d	Rash Diameter, cm	Central Clearing	Other Symptoms‡	Multiple Lesions	EM Pathology§	Positive Spirochetes‖
1/F/47	5-6-90	F	Back	15	5.2	–	+	–	+	–
2/F/43	5-23-90	F	Abdomen	7	6.0	+	–	–	+	–
3/M/33	7-8-90	F	Back	7	7.5	+	–	–	+	+
4/M/29	7-18-90	F	Groin	5	5.2	–	–	–	+	+
5/F/30	7-21-90	N	Back	3	7.0	+	–	–	+	+
6/F/62	4-18-91	F	Back	2	5.5	+	+	+	+	+
7/M/12	4-27-91	F	Back	14	9.0	+	–	–	ND	ND
8/M/25	6-5-91	M	Back	10	9.0	+	–	–	ND	ND
9/M/27	6-29-91	F	Back	2	15.0	+	+	–	+	ND
10/M/37	7-10-92	M	Back	6	10.0	+	–	–	+	–
11/M/69	4-26-93	F	Groin	4	12.0	+	–	–	+	+
12/F/14	5-12-93	F	Back	9	11.0	+	–	–	+	–
13/F/27	5-18-93	N	Abdomen	14	5.5	+	+	–	+	+
14/F/43	5-20-93	N	Abdomen	13	9.5	+	+	–	+	–
15/M/38	5-31-93	M	Leg	9	2.5	–	–	–	+	ND
16/M/48	7-12-93	F	Thorax	5	8.5	+	–	–	ND	ND
17/F/34	9-15-93	N	Back	11	5.1	+	–	–		

*Plus sign indicates positive; minus sign, negative; and ND, not done.
†N indicates nymph; M, adult male; and F, adult female.
‡Flulike illness.
§Histopathological findings consistent with erythema migrans (EM).
‖Positive silver stain.

RESULTS

CLINICAL FINDINGS

The clinical data are summarized in **Table 1**. Seventeen patients, 9 male and 8 female, were studied. Their ages ranged from 12 to 69 years (median, 33 years).

All rashes were measured and photographed. They had a median incubation time of 7 days, with a range from 2 to 15 days. The relationships among the tick sizes and sex, incubation times, and rash diameters are illustrated in **Figure 1**. The nymph tick bites were associated with rash incubation times ranging from 3 to 14 days; 3 of 4 had incubation times of 11 days or longer. Rash location included the back (10 cases), anterior thorax or abdomen (4 cases), groin (2 cases), and leg (1 case). The median rash diameter was 7.5 cm, with the largest being 15 cm (range, 2.5-15 cm). All rashes were similar to, and often indistinguishable from, erythema migrans in patients from areas with endemic Lyme disease. Examples of rashes are shown in **Figure 2** through **Figure 5**. Fourteen of the 17 rashes had central clearing. Sixteen of these rashes were solitary erythemas. Patient 6 had multiple lesions. This is consistent with the experience at the study site clinic in which approximately 15% of cases show multiple lesions. None was significantly pruritic or painful. Five patients (patients 1, 6, 9, 13, and 14) had mild associated flulike constitutional symptoms. The rashes occurred from April 18 to September 5 (median, May 31) during the years 1990 to 1993. There was no correlation between tick stage or sex and rash diameter or incubation time. Nymph-associated rash incubation time ranged from 3 to 14 days and adult female–associated rash incubation time ranged from 2 to 15 days. Most patients have been followed up since, and although many have had additional tick bites, none has developed an erythema migrans–like rash.

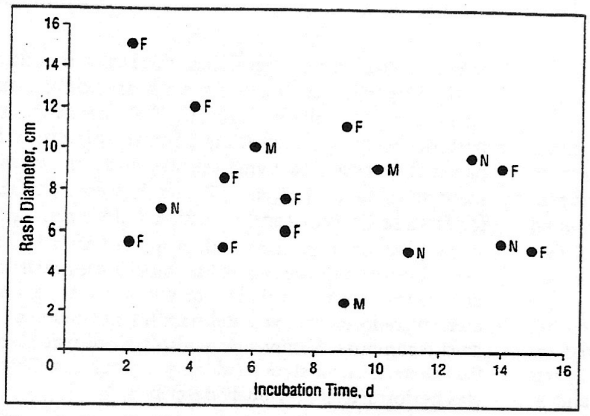

Figure 1. *Lone Star tick sex and age relative to rash size and incubation time. F indicates adult female; M, adult male; and N, nymph.*

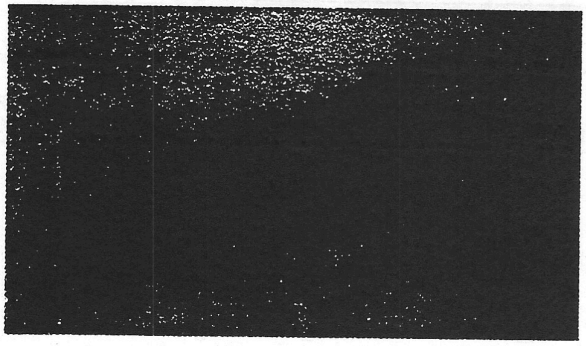

Figure 2. *Patient 6. Rash on back following an adult female Lone Star tick bite. The tick was saved and was placed on her near the rash at the 2-o'clock position for this photograph. Note the distinctive white dot on the tick's back. There were 4 smaller associated lesions. Histopathological findings were consistent with erythema migrans and apparent dermal spirochetes were seen with silver stains. She had many serology results consistent with a borreliosis.*

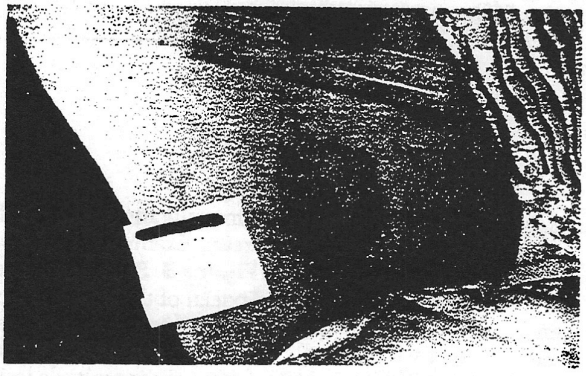

Figure 3. *Patient 14. Rash (9.5 cm in diameter) with a 13-day incubation time following a Lone Star nymph bite. Histopathological findings were consistent with erythema migrans and apparent dermal spirochetes were seen with silver stains.*

LYME DISEASE SEROLOGY

Ten of the 17 case patients had Lyme serology results inconsistent with test-negative non-Lyme (uninfected) controls. (Control subjects were randomly selected from consenting office patients and emergency department patients having blood drawn for other purposes and volunteers with

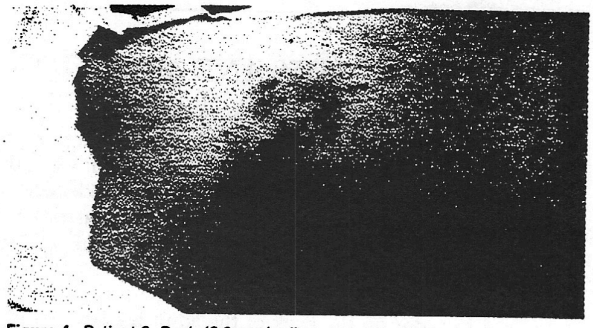

Figure 4. *Patient 2. Rash (6.0 cm in diameter) with a 7-day incubation time following an adult female Lone Star tick bite.*

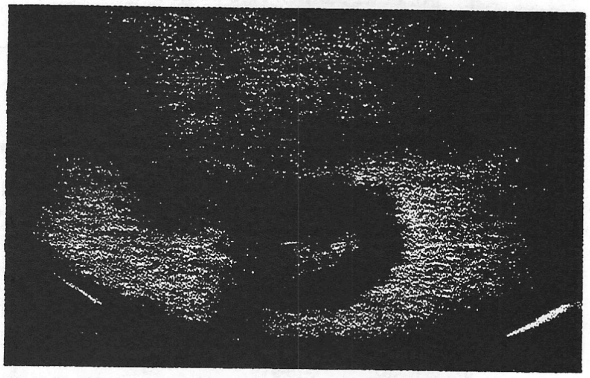

Figure 5. *Patient 10. Annular rash (10 cm in diameter) with a 6-day incubation time following an adult male Lone Star tick bite. The tick midgut stained negative with H5332 immunofluorescent assay at 1:100 dilution.*

no history compatible with a borreliosis.) Eight Lyme serology results from the 17 case patients had results suggestive of a borreliosis. Frozen serum samples were available on 3 patients and underwent multiple serologic evaluations. Extensive testing for other diseases and causes of possible cross-reactivity were negative with 1 exception: patient 7 tested positive for *Coxiella burnetii* (cause of Q fever). All tested patients had negative test results for rheumatoid factor, antinuclear antibody, and syphilis serology. Patients 3, 5, and 7 did not have Western immunoblots. Patient 8 had multiple negative enzyme-linked immunoabsorbent assays (ELISAs). Patient 16 did not return after his initial visit when the biopsy was done and was unavailable for follow-up. Patient 9 seroconverted in the treatment study.[16] A CDC whole-cell sonicated ELISA on patient 9 was also strongly positive at 3.176 (positive >1.0). Results of serologic evaluations in patients 5, 6, and 7 are presented in **Table 2**. Additionally, 13 months after the tick bite, patient 6 (Figure 2) had a Western immunoblot with IgM of 59 and IgG of 20, 34, 38, 39, 41, 50, 60, 63, and 75 kd. Patient 6 was also the only patient in this series with multiple lesions (5) and associated constitutional symptoms. She also had the most positive results of Lyme tests, including a positive biopsy result. Results of Lyme Western blots are presented in **Table 3**. These data show the unusually high frequency of 4 or more IgG Western blot bands, *B burgdorferi*–associated bands, and positive ELISAs, all of which are inconsistent with published data on non-Lyme controls.[18-21] Six patients (patients 3, 5, 6, 7, 8, and 10) were enrolled in a national erythema migrans

Table 2. Lyme Serology Results*

Patient No.	CDC Case No.	No. of Days After Rash	1991 WCS + ≥1.0 (3 SD)	1991 FLA + ≥1.0 (3 SD)	1992 FLA + ≥1.0 (3 SD)	1993 FLA	IgM Western Blot	IgG Western Blot
5	21	94	1.606	1.087	0.693	0.64	83	**41**
6	14	31	1.846	0.792	0.488	0.39	**41, 66**	**34, 41,** 62, 66, **75,** 83
	14	92	1.836	0.623	0.519	0.31	**37,** 39, **41, 58, 66**	15, **21, 34,** 41, **62, 75,** 83
7	1	3	1.432	0.519	0.591	0.55	41	**28, 37, 39, 41, 45, 58,** 66
	1	34	1.690	0.434	0.678	0.51	ND	**15, 39, 41, 47, 56, 58**
	1	97	1.425	0.721	0.638	0.54	**66,** 75	**15, 39, 41, 47, 56, 58**

**Results of tests for antibodies to* Borrelia burgdorferi *in serum from Missouri patients with suspected erythema migrans by enzyme immunoassay using whole-cell sonicated (WCS) antigens and flagellar (FLA) antigens and immunoblots performed by the Centers for Disease Control and Prevention (CDC). Boldface indicates strong bands.*

Table 3. Lyme Western Blot Examples

Patient No.	No. of Days After Rash	IgM Western Blot	IgG Western Blot
1	24	66	38, 41, 50
2	68	None	None
3	180	59	None
4	101	None	31, 34, 38
10	180	41, 66	None
11	39	None	None
12	47	41	39, 41, 58, 60
13	29	35, 41	41, 62
14	18	31, 34, 38, 60, 66	41, 45, 66
15	57	25, 36, 41, 63	18, 23, 29, 30, 31, 41, 63, 70, 75, 100
17	135	None	20, 30, 41, 75

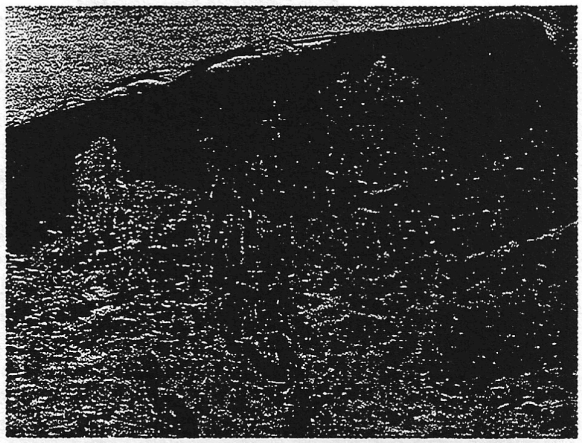

Figure 6. *Patient 11. Scanning magnification of peripheral rash biopsy specimen shows superficial and deep perivascular mononuclear cell infiltrates (hematoxylin-eosin).*

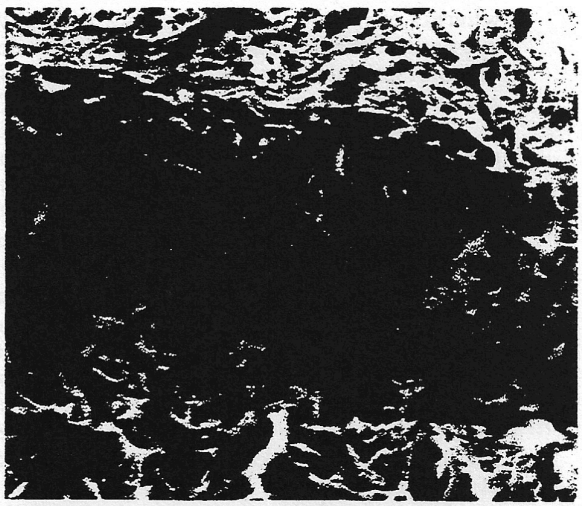

Figure 7. *Patient 12. Histopathological biopsy findings of Lone Star tick–associated erythema migrans from the peripheral rash margin shows a perivascular cuff of lymphocytes lined by plump reactive endothelial cells. These findings, while not specific, are typical of erythema migrans (hematoxylin-eosin, ×400).*

treatment study, but patient 3 dropped out of the study. Test results of patient 8 were all negative, whereas the other 4 patients had 1 or more positive Lyme ELISAs.[16]

PATHOLOGICAL FINDINGS

Biopsies were performed on all 17 rashes at the peripheral margin and cultured in BSK II medium with negative results. All 14 rashes with biopsy specimens for histopathological evaluation revealed findings consistent with erythema migrans (**Figure 6** and **Figure 7**). Six of 11 biopsy specimens examined with the modified Dieterle method[17] showed silver-positive structures consistent with dermal spirochetes (**Figure 8**).

EPIDEMIOLOGY

In a separate tick survey, Lone Star ticks containing *Borrelia*-appearing spirochetes variably reactive to H5332 were found in the counties of 16 case patients.[22] One patient was bitten in a county in Southern Illinois where ticks have not yet been examined. Ticks (2 nymphs and 3 adult males) from 5 study patients were examined with midgut smears and stained negative to H5332 immunofluorescent assay at 1:100 dilution.

TREATMENT

All 17 patients were treated early and aggressively with oral antibiotics. The most common regimens were amoxicillin or doxycycline for 20 or more days. No sequelae or symptoms indicative of treatment failure were found in this small group.

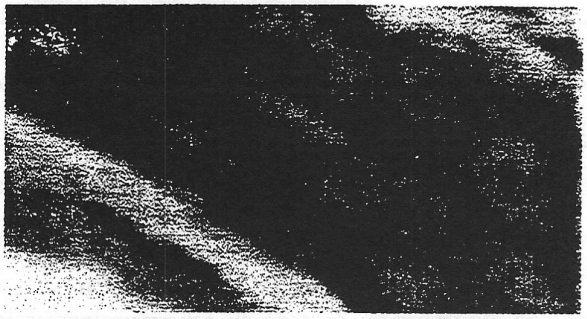

Figure 8. *Patient 5. Modified Dieterle silver stain of peripheral biopsy specimen of rash showing an apparent dermal spirochete. Reprinted with the permission of* Missouri Medicine.[26]

COMMENT

Our study shows that Lone Star ticks are associated with rashes similar to, or even indistinguishable from, erythema migrans rashes associated with Lyme disease in CDC-accepted endemic areas. Photographs of Missouri physician-diagnosed erythema migrans rashes have been published.[23-26] A few points regarding the rashes we evaluated deserve mentioning. It is not surprising that most of the rashes in this study were on the back since 10 of the 17 ticks were adults: this location would allow the larger adult tick to go unnoticed for a longer time and better transmit possible pathogens or antigens. Positive identification of the distinctive white dot on the back of the adult female tick was required for inclusion in this study; however, nymphal and adult male forms had to be saved for definitive identification. This study has an obvious selection bias for patients bitten by adult female forms. Therefore, it is not surprising that only 4 of 17 study cases presented here involved nymphal ticks, whereas our experience in dealing with physician-diagnosed erythema migrans during the past decade in Missouri indicates that the majority of these rashes are associated with nymphal ticks.

The summer peak incidence, histological findings, treatment response, rash diameter, incubation time, patient age and sex, frequency of multiple lesions, and signs and symptoms were similar to that associated with Lyme disease reported nationally. Notably different was the inability to culture spirochetes in BSK II media. We did not necessarily expect good culture results with a medium designed for spirochetes from other *Ixodes* ticks. If the rashes we encountered are indeed associated with borrelial infection, the BSK II media may not be satisfactory for isolation of potential spirochetes associated with the Lone Star tick. It has been shown that that BSK II culture media can select for specific genotypes of *B burgdorferi*.[27]

Lyme serology testing argues against a B31 *B burgdorferi* cause. However, the serology results are also inconsistent with a test-negative, uninfected control population.[18-21,26] We know that different strain variants can have different test results[28,29] and that *B burgdorferi sensu lato* in Europe can test negative with culture-proven disseminated disease.[30] With more *B burgdorferi sensu lato* being cultured in the South (eg, the farm of patient 12),[2] this possibility needs to be explored. In 3 patients in our study there was a dramatic and unexplained difference in ELISA testing of Missouri patients using whole-cell sonicated antigens and flagellar antigens. This was observed in a serologic study by the CDC.[4] Previously, the CDC whole-cell sonicated and flagellar ELISAs were highly concordant, but not in Missouri patients. The whole-cell sonicated ELISA tested positive in approximately 45% of Missouri patients with erythema migrans, but tested negative in 37 (96%) of 38 Missouri control subjects, whereas the flagellar ELISA was almost always negative. The discordant results were such that the odds of this occurring by chance were 1 in 25 million. These results are consistent with the possibility of a related *Borrelia* that frequently cross-reacts with the whole-cell sonicated ELISA, but rarely with flagellar ELISA.[4,18,26] Similarly, Missouri Western blot results are usually negative by the strict criteria of Dressler et al[31] adopted by the CDC where neither outer surface protein A (31 kd) nor outer surface protein B (34 kd) are counted.[31] The results are also inconsistent with test-negative non-Lyme controls.[18-21,26]

The treatment of patients with erythema migrans and erythema migrans–like rashes outside CDC-accepted endemic areas of Lyme disease is controversial. We believe, given the likelihood of a borrelial etiology, that these rashes should be treated with antibiotics as would an erythema migrans rash in an accepted endemic area. This is also the view of others.[3,4] No sequelae or symptoms indicative of treatment failure were found in this small group, which is similar to observations of others.[32] Nationally, the erythema migrans treatment failure rate has been variously reported at between 5% and 10%.[23] Until a borrelial cause is either proven or refuted for these cases, proper treatment and follow-up will be controversial.

In conclusion, we have presented evidence that rashes visibly similar or indistinguishable from other *Ixodes* tick–vectored Lyme erythema migrans can be associated with Lone Star tick bites. Collateral evidence suggests the possibility that *Borrelia* play a pathogenic role in these patients. If proven, the clinical and epidemiological implications of a Lone Star–vectored borreliosis are great, especially in view of the prevalence of the tick in the South and south central United States, as well as evidence that it is becoming more widespread. For example, the increase in prevalence of the Lone Star tick from 2 New York counties in the 1970s to 46 of the 62 New York counties today has been documented.[33] Also, there is the possibility in areas where there are both *Ixodes scapularis* (*Ixodes dammini*)[34] and Lone Star (*Amblyomma americanum*) ticks that frequently bite humans (eg, New Jersey), that a Lone Star–vectored borreliosis could result in physician-diagnosed or -suspected Lyme disease that could often be seronegative. Clearly, the pathogenic role of *Borrelia* in these patients needs further investigation.

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