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Serologic Evaluation of Patients from Missouri with Erythema Migrans-Like Skin Lesions with the C_6 Lyme Test

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Southern tick-associated rash illness (STARI), also known as Masters disease, affects people predominantly in the Southeast and South Central United States. These patients exhibit skin lesions that resemble erythema migrans (EM), the characteristic skin lesion in early Lyme disease. The etiology of STARI remains unknown, and no serologic test is available to aid in its diagnosis. The C_6 Lyme enzyme-linked immunosorbent assay was used to evaluate coded serum specimens from patients with STARI at two laboratory sites. The specimens tested at one site consisted of acute- and convalescent-phase samples that were obtained from nine STARI patients from Missouri and from one patient with documented *Borrelia lonestari* infection who acquired this infection in either North Carolina or Maryland. All of these samples were C_6 negative. Seventy acute- or convalescent-phase specimens from 63 STARI patients from Missouri were C_6 tested at the second site. All but one of these STARI specimens were also negative. In contrast, of nine acute- and nine convalescent-phase serum specimens obtained from culture-confirmed Lyme disease patients with EM from New York state, seven were C_6 positive at the acute stage, and eight were positive at convalescence. The C_6 test is negative in patients with STARI, providing further evidence that *B. burgdorferi* is not the etiologic agent of this disease.

In the United States, the majority of cases of Lyme borreliosis are reported from the Northeast, Mid-Atlantic, Midwest, and Far West regions of the country. The skin lesion known as erythema migrans (EM) is the disease's most common clinical sign; the spirochete *Borrelia burgdorferi*, the etiologic agent of Lyme disease, is often recovered from EM skin biopsy cultures.

EM-like skin lesions may also develop in patients from the Southeast and South Central United States, often preceded by the bites of *Amblyomma americanum* ticks (14). Skin biopsy cultures from such lesions have not yielded *B. burgdorferi* (21), and, moreover, the tick *A. americanum* has been shown in the laboratory to be an incompetent vector for this spirochete (5, 19). Therefore, *B. burgdorferi* is not thought to be the cause of the EM-like lesions in patients from the Southeast and South Central United States (14, 21). This condition is referred to either as southern tick-associated rash illness (STARI) or as Masters disease.

Clinically, Masters disease also differs from Lyme borreliosis, despite the fact that patients may present in both cases with, in addition to the EM-like sign, symptoms such as joint pain, fatigue, fever, chills, and headache. In a comparative prospective clinical evaluation of patients from Missouri and New York presenting with EM, the lesions in the Missouri cases were significantly smaller in size, more circular in shape, and more likely to have central clearing than those from patients in New York (22). In addition, Missouri case patients were less likely to be symptomatic or to have multiple skin lesions than were New York case patients, and they recovered more rapidly after antibiotic treatment (22). Thus, there are clear distinctions between the clinical presentations of Lyme and STARI patients (22).

The etiology of STARI has not been elucidated. In a single reported case, the EM-like lesion was caused by *Borrelia lonestari*, a spirochete known to infect *A. americanum* (3). However, in a recent microbiological evaluation of Missouri patients with EM, *B. lonestari* was not detected by PCR in any of 31 skin biopsy specimens collected from 30 Missouri patients (21). Thus, the etiology of STARI is unknown.

There is no serologic test available to aid in the diagnosis of STARI. Enzyme-linked immunosorbent assays (ELISAs) with *B. burgdorferi* whole-cell extracts as antigens have been used with Missouri EM patients, but with some exceptions, the overall outcome of such testing has been negative (14, 21). Detection of antibody to C_6, a peptide that reproduces the sequence of the sixth invariable region (IR_6) within the central domain of the VlsE protein of *B. burgdorferi*, is used currently for the serologic diagnosis of Lyme disease in humans (The C_6 Lyme disease ELISA [Immunetics]) (1, 4, 9, 10, 12, 13, 15–18) and in canines (Canine SNAP 3Dx test [Heartwork Ag, E.C.A., Lyme Ab]; IDEXX [Lyme quantitative C_6 antibody test]) (2, 6–8, 11). In view of the clinical similarity of STARI and EM and the likelihood that the *B. burgdorferi* antigen extracts used in whole-cell Lyme ELISA lacked VlsE—the linear plasmid lp28-1, which encodes VlsE, is lost after as few as five culture passages (20)—we reasoned that anti-C_6 antibodies should be evaluated in the sera of patients from Missouri with EM-like lesions.

The C_6 Lyme ELISA (Immunetics, Cambridge, MA) was used to evaluate coded serum specimens from patients with STARI. The test was used according to the manufacturer's instructions, and evaluations were conducted independently at

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TABLE 1. C_6 serology of STARI and Lyme disease patients

Disease	Source[a]	Test site	No. of patients		
			Total	C_6 positive	C_6 negative
STARI	MO	FDI	9	0	9
	MO	TNPRC	63	1	62
	MD or NC	FDI	1	0	1
Lyme disease	NY	FDI	9	8	1

[a] MO, Missouri; MD, Maryland; NC, North Carolina; NY, New York.

two separate laboratory sites. The specimens tested at Focus Diagnostics, Inc. (FDI) consisted of acute- and convalescent-phase specimens from nine STARI patients from Missouri and from one patient who had a proven *B. lonestari* infection acquired in either North Carolina or Maryland. Seventy acute- or convalescent-phase specimens from 63 STARI patients from Missouri were tested at the Tulane National Primate Research Center (TNPRC).

All of the samples tested at FDI were C_6 negative. All but one of the STARI specimens tested at TNPRC were also negative by this test. In contrast, of nine acute-phase and nine convalescent-phase serum specimens obtained from culture-confirmed Lyme disease patients with EM from New York State and tested at FDI, seven were C_6 positive at the acute stage, and eight were positive at convalescence. Our results, which are summarized in Table 1, show that the C_6 test is negative in patients with STARI and provide further evidence that *B. burgdorferi* is not the etiologic agent of this disease.

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