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Inadequacy of IgM Antibody Tests for Diagnosis of Rocky Mountain Spotted Fever

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Abstract. Among 13 suspected Rocky Mountain spotted fever (RMSF) cases identified through an enhanced surveillance program in Tennessee, antibodies to *Rickettsia rickettsii* were detected in 10 (77%) patients using a standard indirect immunofluorescent antibody (IFA) assay. Immunoglobulin M (IgM) antibodies were observed for 6 of 13 patients (46%) without a corresponding development of IgG, and for 3 of 10 patients (30%) at least 1 year post-onset. However, recent infection with a spotted fever group rickettsiae could not be confirmed for any patient, based on a lack of rising antibody titers in properly timed acute and convalescent serologic specimens, and negative findings by polymerase chain reaction testing. Case definitions used in national surveillance programs lack specificity and may capture cases that do not represent current rickettsial infections. Use of IgM antibodies should be reconsidered as a basis for diagnosis and public health reporting of RMSF and other spotted fever group rickettsiae in the United States.

INTRODUCTION

Rocky Mountain spotted fever (RMSF) is an acute tick-borne infection caused by the bacterium *Rickettsia rickettsii*. A member of the spotted fever group of rickettsiae (SFGR), *R. rickettsii* is transmitted by a variety of tick vectors in the United States, including *Dermacentor variabilis* (the American dog tick), *Dermacentor andersoni* (the American wood tick), and *Rhipicephalus sanguineus* (the brown dog tick).[1] Other SFGR present in the United States that are known to be pathogenic to humans include *Rickettsia parkeri*, *Rickettsia phillipi*, *Rickettsia massiliae*, and possibly *Rickettsia montanensis* and *Rickettsia amblyommii*.[2–6] However, these known and suspected human pathogens have been linked to primarily mild systemic signs, and to date, only *R. rickettsii* has been shown to result in serious human illness, including fatal infection.

The incidence of reported RMSF and other SFGR has increased nationally over the past decade.[1] In Tennessee, a region long considered endemic for RMSF, only 87 cases were reported during 2001, but this number rose to 696 in 2012. Among the 696 cases reported during 2012, 22% were among residents of the West Tennessee health region, even though these counties only account for 9% of the population in Tennessee (Dunn J, Tennessee Department of Health Services, unpublished data). In addition to increased reports of illness, spatial clusters of severe outcomes in RMSF cases residing in the West Tennessee health region were recently identified in a national study, suggesting this as an area where enhanced surveillance might be used to identify more cases and improve patient outcomes, including preventing deaths.[7]

Despite the recent dramatic increase in incidence, the number of SFGR cases meeting a confirmed case definition declined both nationally and in Tennessee during the corresponding time period.[1] Over half of the Tennessee cases in 2001 met a confirmed national surveillance case definition, versus < 1% of Tennessee SFGR cases in 2012 (Dunn J, Tennessee Department of Health Services, unpublished data). This difference likely reflects changes in diagnostic testing patterns among healthcare providers. Further complicating matters is the fact that the national surveillance case definition for SFGR requires presence of fever, but an increasing body of evidence suggests that some SFGR infections, including RMSF, may not include fever.[5]

To better understand the epidemiology of RMSF, especially factors related to case ascertainment and severe outcomes, a study was designed to closely follow suspected RMSF patients identified by providers in West Tennessee during 2010–2012.

METHODS

The study was conducted under approval by Centers for Disease Control and Prevention (CDC's) Human Subjects Review Board, protocol no. 5754, and the Tennessee Department of Health Institutional Review Board. Physicians practicing in Carroll, Decatur, Henderson, and Henry counties were offered Continuing Medical Education on the diagnosis, management, and treatment of RMSF; attendance at the training and participation in the study were voluntary. Participating providers were offered the option of using CDC's Rickettsial Reference Diagnostic Laboratory for free testing of suspected RMSF patient specimens including whole blood, serum, and skin biopsies, and were asked to inform the patients of the opportunity to participate in the study. Patients whose initial samples were tested at CDC were contacted by regional or state health department personnel and invited to participate in additional evaluation and testing, free of charge. Participation was voluntary; patients consenting to participate were nominally compensated with a $25 gift card for each additional visit involving travel and additional specimen collection.

Suspected RMSF cases included patients with a fever (≥ 100.4°F or 38°C), for whom no other clear alternative diagnosis was present and who had at least one of the following: 1) a history of a tick bite in the 2 weeks before illness onset; 2) non-pruritic skin manifestations (petechial rash, maculopapular rash, or eschar); or, 3) two or more of the following: headache, myalgia, nausea, vomiting, abdominal pain. Based on the judgment of some providers, three afebrile patients with

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other clinically consistent signs (as described in criteria no.3) were included in the study. Enrolled patients participated in the following visits and evaluations:

- Visit 1: Visit during acute illness, examination conducted by primary provider. Occurred 0–2 weeks after the onset of fever or other symptoms. Serum for indirect immunofluorescent antibody (IFA) testing (immunoglobulin M [IgM] and IgG) and whole blood for polymerase chain reaction assay (PCR) were collected.
- Visit 2: Interview and specimen collection conducted at local health department, occurred 2–4 weeks after onset. Serum for IFA (IgM and IgG) was collected.
- Visit 3: Interview and specimen collection conducted at local health department, occurred 4–8 weeks after onset. Serum for IFA (IgM and IgG) was collected.
- Visit 4: Interview and specimen collection conducted at local health department, occurred ~1 year after onset. Serum for IFA (IgM and IgG) was collected.

The IgG and IgM IFA testing was conducted using *R. rickettsii* antigen according to previously described methods.[8] Ehrlichiosis IgG IFA was conducted using *Ehrlichia chaffeensis* antigen by the same method. The PCR was conducted as previously described, using a Pan-*Rickettsia* real-time PCR technique.[9] A "confirmed RMSF or SFGR" case was defined according to the current National Surveillance Case definition as a febrile patient with suspected RMSF, and either a positive PCR result indicating infection with a *Rickettsia* species or evidence of at least a 4-fold change (rise or fall) in IgG antibody titers to *R. rickettsii* antigen.[10] With respect to the timing of specimens collected during this study, antibodies were generally expected to rise during Visits 1–3, and fall between Visits 3–4.

Suspected RMSF cases that did not meet a confirmed case definition were further grouped as "probable SFGR" according to the following three criteria.

- *First Visit case classification* (using information available to providers as a result of Visit 1, which represents the most common provider–patient scenario). Probable cases = anti-*R. rickettsii* IgM or IgG antibody titer ≥ 1 : 64 present during Visit 1. Afebrile cases were permitted per the provider's discretion and clinical suspicion.
- *National Surveillance case classification* (using case definition criteria established by the Council of State and Territorial Epidemiologists). Probable case = anti-*R. rickettsii* IgM or IgG antibody titer ≥ 1 : 64 during at least one of Visits 1–3. Case must be febrile and have at least one other case defining symptom (headache, myalgia, rash/eschar, anemia, thrombocytopenia, or elevated hepatic transaminases), according to the national case definition. (NNDSS)
- *Modified Study case classification:* Probable case = anti-*R. rickettsii* IgM AND IgG antibody response ≥ 1 : 64 during at least one of Visits 1–3. Afebrile cases were permitted per the provider's discretion and clinical suspicion.

RESULTS

Participating providers identified 13 suspected RMSF cases from this four-county region in West Tennessee during September 2010–December 2011 (Table 1). Mean age of the patients was 50.5 years (range = [23 – 84], median = 43 years). A wide range of clinical symptoms was observed (Table 2). All 13 cases were treated with doxycycline during their acute illness.

All suspected RMSF patients were screened for IgG antibodies to *E. chaffeensis* using convalescent sera collected during Visit 3; all tested negative for antibodies to this agent. Whole blood samples collected during Visit 1 for each of these 13 suspected patients were all negative for *Rickettsia* species by PCR. No skin biopsies were collected. Antibodies (IgM and/or IgG) to *R. rickettsii* antigen were observed in at least one collected serum sample from 10 of 13 (77%) patients. However, recent infection with a SFGR could not be confirmed for any patient, based on the observed serologic results. Only one patient (Patient no. 1) showed a 4-fold change in IgG antibody titer between Visit 1 and Visit 3; this patient lacked an IgM titer, and the IgG titer decreased when an increase would have been more likely expected, given the timing of the specimens. In addition, this patient was afebrile, therefore not meeting the criteria for the confirmed case definition. No other patients showed a significant change in IgG antibodies between collected serum specimens.

The IgM antibodies to *R. rickettsii* antigen were commonly observed in this patient cohort, occurring in 9 of 13 (69%) of suspected RMSF patients. However, the pattern of reactivity

TABLE 1

Suspected Rocky Mountain spotted fever patients and indirect immunofluorescent antibody (IFA) diagnostic test results. Titers < 1 : 64 were considered negative*

	Visit 1 (0–2 weeks)		Visit 2 (2–4 weeks)		Visit 3 (4–8 weeks)		Visit 4 (1 yr)	
Patient	IgM	IgG	IgM	IgG	IgM	IgG	IgM	IgG
1	Negative	1:4096	Negative	1:2048	Negative	1:1024	Negative	1:512
2	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
3	1:128	Negative	1:256	Negative	1:128	Negative	1:128	Negative
4	1:128	Negative	1:128	Negative	n/a	n/a	n/a	n/a
5	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
6	1:256	1:256	1:512	1:256	n/a	n/a	1:256	1:128
7	Negative	Negative	Negative	Negative	Negative	Negative	1:64	Negative
8	Negative	Negative	n/a	n/a	1:64	Negative	Negative	Negative
9	Negative	1:128	Negative	1:128	Negative	1:128	Negative	1:128
10	Negative	Negative	Negative	Negative	1:64	Negative	Negative	Negative
11	1:64	Negative	1:64	1:64	1:64	Negative	n/a	Negative
12	1:128	Negative	1:256	Negative	1:128	Negative	Negative	Negative
13	1:64	1:128	Negative	1:64	n/a	n/a	n/a	n/a

*n/a = not applicable (did not complete this part of the study visit or sample unable to be tested).

TABLE 2
Probable Spotted fever group Rickettsiae (SFGR) case classification of suspected Rocky Mountain spotted fever (RMSF) patients

Patient	Fever present	Clinical presentation	Confirmed RMSF or SFGR case	Probable SFGR case classification		
				First presentation	National surveillance	Modified study
1	No	Headache, myalgia, vomiting. (Additional: chills, sweats, cough, arthralgia, dizziness, fatigue, stiff neck, diarrhea, dark colored urine)	No	√		
2	Yes	Fever, headache, myalgia. (Additional: sweats, arthralgia, dizziness, abdominal pain, fatigue)	No			
3	Yes	Fever, headache, myalgia. (Additional: chills, cough, fatigue, dark colored urine, jaundice)	No	√	√	
4	Yes	Fever, headache, myalgia, vomiting, rash. (Additional: sweats, chills, cough, arthralgia, dizziness, fatigue, dark colored urine)	No	√	√	
5	Yes	Fever, headache, myalgia, rash. (Additional: sweats, chills, arthralgia, dizziness, abdominal pain, fatigue, stiff neck, diarrhea)	No			
6	Yes	Fever, headache, myalgia, rash. (Additional: sweats, chills, cough, arthralgia, dizziness, abdominal pain, fatigue, stiff neck)	No	√	√	√
7	Yes	Fever, headache, vomiting. (Additional: sweats, chills, arthralgia, dizziness, fatigue, stiff neck)	No		√	
8	No	Sweats, dizziness	No			
9	No	Headache, myalgia, rash. (Additional: Arthralgia, dizziness, fatigue)	No	√		
10	Yes	Fever, rash. (Additional: sweats, chills, cough, dizziness, fatigue)	No		√	
11	Yes	Fever, headache, myalgia, rash. (Additional: sweats, chills, cough, arthralgia, stiff neck, diarrhea)	No	√	√	√
12	Yes	Fever, headache, myalgia, vomiting. (Additional: chills, cough, dizziness, fatigue, dark colored urine)	No	√	√	
13	Yes	Fever, myalgia. (Additional: sweats, chills, arthralgia, abdominal pain, fatigue, stiff neck, diarrhea	No	√	√	√

noted among patients was not that expected with a recent acute SFGR infection. When present, IgM titers were often high during Visit 1 and did not rise within the first few weeks of infection. Notably, IgM was seen in 6 of 13 patients (46%) without a corresponding development of IgG antibodies. IgM antibodies were also detected in 3 of 10 patients (30%) who submitted specimens for serologic testing 1 year after onset, including one patient (Patient no. 7) for whom the only positive antibody test was this IgM positive at 1 year. In contrast, IgG antibodies were observed in 5 of 13 (38%) of patients; only 3 of 13 (23%) patients had both IgM and IgG antibodies observed.

The 13 suspected RMSF cases in this study were classified as "probable SFGR," by three different methods (Table 2). According to the First Visit classification, eight patients in this series would have been diagnosed as probable RMSF. Eight patients (including two who did not meet a First Visit case classification) met the National Surveillance case classification currently in use in U.S. SFGR surveillance programs, and would have been reported and counted by state and federal authorities. The Modified Study case classification, which only included patients exhibiting reactivity to both IgM and IgG, classified three patients as probable SFGR. Although it is interesting to see the varied numbers according to each method, these probable case classifications do not change the fact that *none* of the patients in this cohort were shown to have confirmed RMSF or other SFGR, based on a thorough analysis of clinically appropriate, optimally timed acute and convalescent sera and application of gold-standard techniques.

DISCUSSION

Although the number of patients studied was small, our results indicate that immunologic reactions resulting in false positive IgM findings occur for RMSF and the SFGR in the United States, impacting clinical diagnostic interpretation and public health reporting. The use of IgM as a diagnostic indicator using microimmunofluorescence techniques like IFA has been previously called into question for *R. conorii,* a related SFGR and the causative agent of Mediterranean spotted fever in the eastern hemisphere. Patients suffering from *R. conorii* infection have been shown to develop IgM antibodies that cross-react to a variety of bacterial antigens, including *Legionella*.[11] Anti-*R. conorii* IgM is frequently directed against nonspecific lipopolysaccharides, and has been shown to be subjected to a high rate of false positivity, based on Western blotting techniques.[12] The bacterial organism *Coxiella burnetii*, which causes Q fever in humans, is known to elicit a general non-specific immunological arousal in a significant number of patients, including antibodies to *Rickettsia* species, and it is possible that an immunological background of other bacterial infections may influence the prevalence of SFGR IgM-positive results in both sick and healthy patient populations.[13]

This study is subject to several important limitations. Our results relied on either a positive PCR or changing antibody titers as the gold standard for diagnosis of confirmed RMSF and SFGR. The Pan-*Rickettsia* PCR assay used in this study has the advantage of detecting multiple SFGR species, in addition to *R. rickettsii*.[9] Clinical sensitivity and specificity testing of the PCR assay shows reliable and reproducible detection at eight organisms per reaction and the unlikely occurrence of false positive results (Kato C, CDC, unpublished data). Although it performs well in seriously ill (i.e., critical or fatal patients) because of adequate numbers of *R. rickettsii* organism present in blood samples, its sensitivity for the detection of infection in mild to moderately ill patients has not been well evaluated. For mild or moderately ill patients, a 4-fold rise in serum antibodies provides the best evidence for current infection.

Delay or prevention of antibody development has been previously observed in patients infected with mildly pathogenic SFGR, such as *R. africae*, although this finding has not

been reported in patients infected with more highly pathogenic SFGR, such as *R. conorii*.[14] In the case of *R. africae*, early treatment with doxycycline is suspected to prevent a robust antibody response. Because the SFGR that circulate in Tennessee may include less pathogenic strains such as *R. montanensis* and *R. parkeri*, in addition to the more virulent *R. rickettsii*, the species responsible for patient presentation in this study are unknown. Therefore, the pattern of antibody development in our patient's infections may have been influenced by heightened physician awareness for suspected RMSF, and early and empiric doxycycline administration.

We only evaluated patient specimens using *R. rickettsii* antigen, given that this is the standard in use in commercial laboratories. However, it is possible that more varied serologic responses may be observed if other antigens were used, particularly in light of a recent finding from Georgia suggesting an afebrile presentation in a patient showing a higher immunologic response to *R. montanensis* antigen than to *R. rickettsii*.[5] Finally, because participation was voluntary for both healthcare providers and patients, the suspected RMSF cases identified in this study are unlikely to represent all SFGR that occurred in this region during the study period, and should not be used to presume estimates of incidence.

Based on our findings, current national SFGR case definitions that permit a probable case classification using IgM alone may not capture true rickettsial infections. Recent trends in national surveillance data suggest that physicians are increasingly relying on single serum samples to evaluate suspected RMSF, and that few are taking steps to acquire convalescent specimens to confirm infections.[1] This diagnostic approach could negatively impact physician perceptions of the severity and seriousness of RMSF (thus inadvertently delaying antibiotic treatment), and also distorts the national surveillance data critical for our understanding of the spatial, temporal, and epidemiologic risk factors for RMSF. The design of effective public health interventions is dependent on good surveillance data, and current testing algorithms and surveillance practices may be hampering their development.

Based on our results, more studies should be conducted to better evaluate the use of IgM antibody testing for rickettsial infections, and to develop appropriate algorithms for determination of probable cases. Requiring the presence of both IgM and IgG antibodies to designate probable cases may help improve specificity. The current national case definition for SFGR permits the designation of "probable" SFGR on the basis of IgM seropositivity alone and does not exclude patients lacking evidence of changing antibody titers in paired, properly timed specimens. Until more data are available, reliance on IgM antibodies alone should be reconsidered as a reasonable basis for diagnosis and surveillance of RMSF and other SFGR.

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