The Expanding Spectrum of Eschar-Associated Rickettsioses in the United States

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Background: Until recently, Rickettsia rickettsii was the only substantiated cause of tick-borne spotted fever group (SFG) rickettsiosis in humans in the United States. Rickettsia parkeri, originally thought to be nonpathogenic in humans, was recently proved to be another cause of tick-borne SFG rickettsiosis.

Observations: We report 3 cases of SFG rickettsiosis and discuss the epidemiology, clinical presentation, histopathologic features, and laboratory findings that support confirmed or probable diagnoses of R. parkeri infection and describe the expanding list of eschar-associated SFG rickettsioses recognized in US patients.

Conclusions: The SFG rickettsioses share many clinical manifestations and extensive antigenic cross-reactivity that may hamper specific confirmation of the causative agent.

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Report of Cases

Case 1

In August 2008, a 55-year-old US service-man presented to a hospital in San Antonio, Texas, with a 5-day history of fatigue, chills, fever, and headache accompanied by an irregular, nonpruritic, morbilliform rash on his trunk that had erupted the morning of presentation. Approximately 7 days before the rash developed, the patient discovered a red papule on the dorsal aspect of his left foot that he assumed was a fire ant bite that had occurred while he was playing golf in central South Carolina. He traveled from South Carolina to Texas 1 day before he arrived at the hospital and did not recall any antecedent tick, mite, or mouse exposure. The foot lesion slowly evolved over several days until it developed a central scab with surrounding erythema.

Physical examination revealed a mildly uncomfortable patient with obvious rigors. He had a temperature of 38.4°C and a heart rate of 65/min and was normotensive. The results of the rest of his examination were normal. Dermatologic exami-
nation of the dorsal aspect of his left foot revealed an erythematous and indurated plaque with a central eschar (Figure 1A). There were approximately 20 red macules and papules, several containing a central small pustule (Figure 1B), scattered on his trunk and the proximal aspect of his extremities. His scalp, face, palms, soles, conjunctiva, and mucous membranes were clear. There was 1 tender left inguinal lymph node. The results of laboratory studies, including a complete blood cell count, a basic chemistry panel, and liver-associated enzyme levels, were normal except for a C-reactive protein level of 34 mg/L (reference range, 0.08 to 4.90 mg/L) (to convert to nanomoles per liter, multiply by 9.524).

The patient was admitted to the hospital, and a 4-mm punch biopsy specimen was obtained from a pustule on the right upper back area. Oral doxycycline therapy was begun at a dosage of 100 mg twice daily for a presumed diagnosis of SFG rickettsiosis. The morning after admission, the patient had defervesced and was feeling im-

Figure 1. Patient 1. A, An erythematous and indurated plaque with a central eschar on the dorsal aspect of the left foot. B, A characteristic red papule with a central pustule on the left inner thigh area. C, Superficial papillary dermal edema with periadnexal and perivascular mixed infiltrate (hematoxylin-eosin, original magnification ×10). D, A vascular lumen filled with neutrophils and without evidence of vasculitis (hematoxylin-eosin, original magnification ×60). E, Immunohistochemical localization of antigens of spotted fever group rickettsiae (red) in the dermal perivascular infiltrate using an immunalkaline phosphatase stain with a polyclonal anti–Rickettsia rickettsii antibody diluted 1/500 (naphthol-fast red and hematoxylin counterstain, original magnification ×158).
proved, and his exanthem had already begun to resolve. He was discharged on a 14-day course of doxycycline.

Sections of the skin biopsy specimen demonstrated superficial papillary dermal edema with periadnexal and perivascular lymphocytes, macrophages, and abundant interstitial neutrophils. Several areas showed vascular lumina filled with neutrophils, but no distinct vasculitis was identified. Exocytosis of neutrophils into the epidermis was also evident (Figure 1C and D). Immunohistochemical (IHC) testing for SFG rickettsiae using a polyclonal anti-\( R. \) rickettsii antibody was performed on the formalin-fixed, paraffin-embedded skin biopsy specimen as previously described.\(^4,9\) Immunohistochemical staining of the biopsy specimen demonstrated SFG antigens and intact rickettsiae in foci of the perivascular infiltrate (Figure 1E). Acute- and convalescent-phase serum specimens were tested for IgG antibodies that are reactive with \( R. \) rickettsii, \( R. \) akari, and \( R. \) parkeri using indirect immunofluorescence assays. All of the acute-phase serologic test results were negative. The convalescent-phase specimen, obtained 18 days later, revealed antibody titers of 8192 and 16384 for \( R. \) rickettsii, \( R. \) akari, and \( R. \) parkeri, respectively, and 4096 for \( R. \) parkeri. DNA was extracted from a 10-µm-thick, formalin-fixed, paraffin-embedded section of the skin biopsy tissue and tested using a direct polymerase chain reaction (PCR) assay that targeted a segment of the 17-kDa antigen gene and nested and seminested PCR assays that targeted a segment of the SFG rickettsial OmpA gene, as described previously.\(^4,10\) No amplicons of the expected sizes were obtained.

CASE 2

In August 2008, a 38-year-old man from Houston, Texas, presented to a clinic with what he described as a nonhealing lesion on his left shoulder. He compared the lesion to a boil that swelled, drained pus, and quickly ulcerated, subsequently forming a scab. Two days later, he experienced the rapid onset of fever, headache, myalgias, arthralgias, and fatigue. He was examined by his primary care provider, who initiated trimethoprim-sulfamethoxazole therapy for a presumptive boil. However, his symptoms progressed and within 12 hours he developed a nonpruritic, erythematous macular rash on his trunk that spread to his extremities. Approximately 1 week before the onset of these symptoms, physical examination revealed a crusted, 0.8 × 0.8-cm eschar on the upper area of his left leg (Figure 3A) and multiple erythematous macules and papules on his arms, legs, and palms (Figure 3C and D). Serum samples and skin biopsy specimens were obtained from the eschar and a macule. The patient began doxycycline therapy (100 mg twice daily), and his constitutional symptoms resolved by the second day of treatment, followed by the resolution of his rash a few days later. Sections of the eschar biopsy specimen showed extensive ulceration with a necrotic and hemorrhagic crust. Hemorrhage, necrosis, and mixed inflammatory cell infiltrates that included abundant neutrophils and macrophages extended into the superficial to middle dermis directly subjacent to the necrotic crust (Figure 3B). Immunohistochemical staining of the eschar biopsy specimen revealed sparse SFG antigens in the inflammatory infiltrates. The macule biopsy specimen showed an edematous dermis and sparse perivascular lymphocytes without vasculitis (Figure 3E). No SFG were identified in the macule biopsy specimen on IHC staining.

A 532–base pair segment of ompA was amplified from DNA extracted from a section of the formalin-fixed, paraffin-embedded biopsy specimen. This amplicon was sequenced and showed 99% similarity with the corresponding ompA segment of \( R. \) parkeri. Paired serum samples collected from the patient at the time of his initial presentation and 15 days later showed IgG antibody titers of 128 and 256 for \( R. \) rickettsii antigens and 512 and 4096 for \( R. \) parkeri antigens when tested with indirect immunofluorescence assays.

CASE 3

In July 2009, a 62-year-old man from Shelbyville, Texas, presented with a 1-week history of symptomatic red “spots” on his arms and legs, associated with fever and lethargy. He recalled a “chigger bite” on his leg a few days before the onset of these symptoms. Physical examination revealed a crusted, 0.8 × 0.8-cm eschar on the upper area of his left leg (Figure 2A). Acute- and convalescent-phase serum specimens were tested for IgG antibodies that are reactive with \( R. \) rickettsii, \( R. \) akari, and \( R. \) parkeri using indirect immunofluorescence assays. All of the acute-phase serologic test results were negative. The convalescent-phase specimen, obtained 15 days later showed IgG antibody titers of 128 and 256 to \( R. \) rickettsii and \( R. \) typhi, respectively, and 4096 for \( R. \) parkeri. DNA was extracted from formalin-fixed, paraffin-embedded tissue sections as described previously and tested with PCR assays that targeted segments of the 17-kDa antigen gene and the OmpA gene; however, no amplicons of the expected sizes were obtained.

Inflammatory infiltrates (Figure 2D). The serum specimen showed no significant titer to \( R. \) rickettsii or \( R. \) typhi. DNA was extracted from formalin-fixed, paraffin-embedded tissue sections as described previously and tested with PCR assays that targeted segments of the 17-kDa antigen gene and the OmpA gene; however, no amplicons of the expected sizes were obtained.

COMMENT

Herein, we describe 3 patients from the southern United States who presented to clinicians with eschar-associated illnesses during summer months. One patient (case 3) was conclusively diagnosed as having \( R. \) parkeri rickettsiosis. Based on clinical, epidemiologic, and laboratory criteria, the other 2 patients were presumptively diagnosed as having \( R. \) parkeri rickettsiosis, but the diagnoses were not confirmed by PCR assays. Rickettsiae are transferred to humans by various hematophagous arthropods, including lice, fleas, ticks, and mites.\(^1\) For years, arthropod-induced, eschar-associated rickettsioses in humans in the United States
were attributed only to infections caused by *Rickettsia* rickettsii\(^1\) and *R. akari*, the cause of rickettsialpox.\(^2\) More recently, it has become apparent that a broader collection of *Rickettsia* species indigenous to the Western Hemisphere cause eschar- and rash-associated illnesses in US patients.

There are many similar clinical characteristics among the various imported and domestic eschar-associated rickettsioses encountered by clinicians in the United States (Table 1). Notably, *R. parkeri* rickettsiosis, cat flea–associated rickettsiosis, African tick bite fever caused by *Rickettsia africae*, Mediterranean spotted fever caused by *Rickettsia conorii*, and rickettsialpox share findings of fever, myalgia, malaise, headache, and a maculopapular or vesicular eruption. Cat flea–associated rickettsiosis is a newly recognized infection with a cosmopolitan distribution; however, only a few confirmed cases of this emerging rickettsiosis have been documented.\(^3\) *Rickettsia africae* is not endemic to the United States; however, African tick bite fever is increasingly described among travelers returning from southern Africa and the West Indies, where the pathogen and tick...
vectors exist.\textsuperscript{14} \textit{Rickettsia conorii} is also occasionally imported into the United States by travelers.\textsuperscript{15} \textit{Rickettsia massiliae}, which is an emerging pathogen in the Mediterranean region, causes an eschar and a maculopapular rash and is transmitted by \textit{Rhipicephalus} species ticks\textsuperscript{7}; however, although no confirmed cases have been identified in the United States, \textit{R massiliae} has recently been identified in brown dog ticks in some western states.\textsuperscript{8}

Rickettsialpox also presents as a febrile, eschar-associated illness, but this SFG rickettsiosis, which has been described almost exclusively in large urban areas of the northeastern United States, is associated with the bite of the house mouse mite (\textit{Liponyssoides sanguineus}).\textsuperscript{3} Recently, a reported case of rickettsialpox from rural North Carolina\textsuperscript{12} that occurred within the recognized geographic range of \textit{Amblyomma maculatum} demonstrated clinical features that were closely compatible with \textit{R parkeri} rickettsiosis.\textsuperscript{3} Although the findings of serologic assays suggested an infection with \textit{R akari}, it is also possible that this case represented infection with \textit{R parkeri}. The most recently identified cause of eschar-associated rickettsiosis in the United States is \textit{Rickettsia} species 364D, which is now associated with at least 1 confirmed and several probable cases of disease in California.\textsuperscript{6} \textit{Rickettsia parkeri} has been convincingly linked to disease in humans in at least 9 US states,\textsuperscript{4} and the Gulf Coast tick (\textit{A maculatum}) is recognized as the principal vector.\textsuperscript{3} The classic range of \textit{A maculatum} in the United States reaches across the middle Atlantic and southeastern states that border the Atlantic Ocean or the Gulf of Mexico. \textit{Rickettsia parkeri} has been detected in \textit{A maculatum} collected in many of these states.\textsuperscript{3} Interestingly, patient 2 most likely acquired his SFG rickettsiosis at a location less than 50 miles from an area of coastal Texas where
adult *A. maculatum* ticks were collected in 1937 to obtain the first isolate of *R. parkeri*. To our knowledge, patient 3 represents the first confirmed case of *R. parkeri* rickettsiosis originating in Texas.

At least 16 other confirmed or probable cases of *R. parkeri* rickettsiosis have been described in the United States; each confirmed case was defined by isolation of *R. parkeri* DNA from a clinical specimen, and probable cases were defined as clinically and epidemiologically compatible illnesses, with at least 1 supporting serologic or IHC test result using group-specific assays for SFGR. Other cases of relatively mild eschar-associated SFGR rickettsiosis that occurred within the range of *A. maculatum* but were originally described as Rocky Mountain spotted fever or rickettsialpox might also have represented infections with *R. parkeri*.12,17

Most cases of *R. parkeri* rickettsiosis occur between July and early September. Symptoms usually begin 2 to 10 days after the tick bite and include a “sore” or “pimple” at the site of the tick bite as well as generalized symptoms of fever, fatigue, myalgia, headache, and a generalized rash. A low- to moderate-grade fever is present. Almost all patients report an eschar that typically precedes the onset of fever by 0 to 4 days. Nearly all develop a maculopapular, vesiculopapular, or papulopustular exanthem 0.5 to 4 days after the onset of fever. In general, the patients describe their myalgias and headaches as mild. A few report tender lymphadenopathy. In general, only about one-third of patients are ill enough to require hospitalization. Patients typically defervesce within 12 to 48 hours of the initiation of doxycycline therapy.3

Generally, a biopsy specimen of the eschar is preferred for microscopic, IHC, and PCR studies of suspected SFG rickettsioses (Table 2). The eschar is the site of initial rickettsial proliferation and an excellent source from which rickettsiae can be isolated. Eschar biopsy specimens can also provide important diagnostic information for patients with other eschar-associated diseases, including cutaneous anthrax, tularemia, nontuberculous mycobacterioses, mycoses, leishmaniasis, and poxvirus infections.

Microscopically, the eruptions of the rickettsial diseases are very similar. The biopsy specimens from the lesions in our 3 cases showed the characteristic perivascular and periadnexal mixed inflammatory infiltrate with lymphocytes, macrophages, and neutrophils.3,19 Most IHC tests use polyclonal anti–*R. rickettsii* antibodies that will react with many different species of SFGR, including *R. rickettsii*, *R. akari*, *R. africae*, *R. conorii*, *R. helvetica*, *R. parkeri* species 364D, and *R. rickettsii*, making the results of IHC studies insufficient for identifying the specific infecting agent.3,5,6,18 Although IHC staining of the biopsy specimens from each of our patients showed evidence of infection with SFGR, specific identification of the infecting pathogen was not possible in 2 of the 3 cases because IHC stains are only SFGR specific (Table 2). The acute and convalescent serologic tests are also only SFGR specific and have the same limitations as IHC staining. For example, a convalescent serum specimen from patient 1 demonstrated robust antibody titers against all 3 SFGR antigens tested.

Table 2. Eschar-Associated Rickettsioses (Imported and Domestic) Encountered by Clinicians in the United States (US)

<table>
<thead>
<tr>
<th>Disease</th>
<th>Pathogen</th>
<th>Vector</th>
<th>Recognized Distribution of Disease in the US</th>
<th>Distinguishing Cutaneous Features</th>
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<tbody>
<tr>
<td><em>Rickettsia parkeri</em></td>
<td><em>R. parkeri</em></td>
<td>Gulf coast tick (<em>Amblyomma maculatum</em>)</td>
<td>Southeastern US</td>
<td>Eschar in &gt;90% of cases, occasionally multiple; maculopapular rash, vesicular or pustular in &gt;40% of cases</td>
</tr>
<tr>
<td>Rickettsialpox</td>
<td><em>Rickettsia akari</em></td>
<td>House mouse mite (<em>Liponyssoides sanguineus</em>)</td>
<td>Large urban centers in eastern US</td>
<td>Eschar in &gt;70% of cases, rarely multiple; papular rash almost always associated with vesicles or pustules</td>
</tr>
<tr>
<td>364D rickettsiosis</td>
<td><em>Rickettsia</em> species 364D</td>
<td>Pacific Coast tick (<em>Dermacentor occidentalis</em>)</td>
<td>Northern California</td>
<td>Eschar uniformly present, often with regional lymphadenopathy; rash absent in each of the few identified cases</td>
</tr>
<tr>
<td>Cat flea–associated</td>
<td><em>Rickettsia felis</em></td>
<td>Multiple fleas, including cat flea (<em>Ctenocephalides felis</em>) and rat flea (<em>Xenopsylla cheopis</em>)</td>
<td>Vector and pathogen broadly distributed throughout the US; disease infrequently diagnosed</td>
<td>Eschar infrequent; maculopapular rash in most patients</td>
</tr>
<tr>
<td>rickettsiosis</td>
<td><em>Rickettsia</em> species 364D</td>
<td>Pacific Coast tick (<em>Dermacentor occidentalis</em>)</td>
<td>Vector and pathogen broadly distributed throughout the US; disease infrequently diagnosed</td>
<td>Eschar infrequent; maculopapular rash in most patients</td>
</tr>
<tr>
<td>African tick bite fever</td>
<td><em>Rickettsia africæ</em></td>
<td>Bont ticks (<em>Amblyomma hebraeum</em>, <em>Amblyomma variegatum</em>)</td>
<td>Pathogen and vectors not endemic to US; disease of travelers to sub-Saharan Africa and West Indies</td>
<td>Eschar in &gt;90% of cases, multiple infection in &gt;50% of cases; maculopapular rash, vesicular in &gt;40% of cases</td>
</tr>
<tr>
<td>Mediterranean spotted</td>
<td><em>Rickettsia</em> conorii</td>
<td>Brown dog tick (<em>Rhipicephalus sanguineus</em>)</td>
<td>Pathogen not endemic to US; disease of travelers to the Mediterranean region and northern Africa</td>
<td>Eschar usually singular; maculopapular rash not associated with vesicles or pustules</td>
</tr>
<tr>
<td>rickettsiosis</td>
<td><em>R. massiliae</em></td>
<td>Rhipicephalus species ticks</td>
<td>Pathogen and vector are endemic to US; disease currently not diagnosed in the US</td>
<td>Eschar occasionally multiple; maculopapular rash not associated with vesicles or pustules</td>
</tr>
</tbody>
</table>
infecting *Rickettsia* species in some circumstances; however, and as seen in this study, the sensitivity of PCR assay can be diminished when skin biopsy specimens are processed in formalin for routine histopathologic evaluation.3 If a species-specific diagnosis is desired in a case involving a suspected eschar-associated rickettsiosis, fresh skin biopsy specimens of the eschar and rash lesions should be collected and placed on sterile saline-moistened gauze pads and shipped chilled (not frozen) to an appropriate reference laboratory for PCR or culture-based assays.3,18 These cases highlight the challenges of confirming the diagnosis of eschar-associated rickettsioses and the importance of collecting appropriate clinical samples in an apt time frame to identify the expanding list of pathogens that may cause similar illnesses in the United States.

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**Author Contributions:** Drs Cragun, Ellis, Tyring, and Paddock had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. **Study concept and design:** Cragun, Ellis, and Tyring. **Acquisition of data:** Cragun, Bartlett, Ellis, Hoover, Tyring, Mendoza, Vento, Nicholson, Eremeeva, Rapini, and Paddock. **Analysis and interpretation of data:** Cragun, Ellis, Tyring, Vento, Nicholson, Eremeeva, Olano, and Paddock. **Drafting of the manuscript:** Cragun, Bartlett, Ellis, Mendoza, Vento, Eremeeva, and Paddock. **Critical revision of the manuscript for important intellectual content:** Cragun, Bartlett, Ellis, Hoover, Tyring, Vento, Nicholson, Eremeeva, Olano, and Paddock. **Administrative, technical, or material support:** Cragun, Hoover, Tyring, Mendoza, Vento, Nicholson, Eremeeva, Olano, and Paddock. **Study supervision:** Cragun, Ellis, Hoover, and Paddock. **Laboratory testing:** Nicholson, Eremeeva, Olano, and Paddock.

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### Table 2. Diagnostic Tests for Confirmation of Eschar-Associated Spotted Fever Group Rickettsioses

<table>
<thead>
<tr>
<th>Assay</th>
<th>Optimal Specimen</th>
<th>Advantages</th>
<th>Limitations</th>
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<tr>
<td>IFA assay</td>
<td>Approximately 1 mL of serum or plasma, shipped at −20°C or 4°C</td>
<td>Commercial IFA assays widely available; results often more rapid relative to other diagnostic assays</td>
<td>Specimens obtained during the first 7-10 d of illness are often negative, paired samples (eg, to include a second sample collected ≥4 wk after acute illness) may be needed to confirm infection; IFA results indicate only infection with SFGR, and are not species specific</td>
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<tr>
<td>IHC testingb</td>
<td>0.4-cm punch biopsy specimen from central aspect of eschar, fixed in 10% formalin, or embedded in paraffin, shipped at room temperature</td>
<td>Demonstrates SFGR in context with the histopathologic features to confirm active infection; can be used to make a retrospective diagnosis from paraffin-embedded tissue months to years after active infection; results generally available more quickly than PCR or culture</td>
<td>Assay limited to specialized infectious disease laboratories, including CDCb; IHC results indicate only infection with SFGR, and are not species specific</td>
</tr>
<tr>
<td>PCR assayb</td>
<td>0.4-cm punch biopsy specimen from central aspect of eschar, preferably shipped fresh, in sterile saline-moistened gauze at 4°C or −20°C; can also be attempted from paraffin-embedded tissue</td>
<td>Provides species-specific identity of <em>Rickettsia</em> species responsible for infection</td>
<td>Assay limited to specialized infectious disease laboratories, including CDCb; results may take several weeks</td>
</tr>
<tr>
<td>Cell culture isolationb</td>
<td>0.4-cm punch biopsy specimen from central aspect of eschar, shipped fresh, in sterile saline-moistened gauze at 4°C</td>
<td>Criterion standard of diagnosis; provides species-specific identity of <em>Rickettsia</em> species responsible for infection</td>
<td>Assay limited to specialized infectious disease laboratories, including CDCb; results may take several weeks</td>
</tr>
</tbody>
</table>

Abbreviations: CDC, Centers for Disease Control and Prevention; IFA, immunofluorescence antibody; IHC, immunohistochemical; PCR, polymerase chain reaction; SFGR, spotted fever group rickettsiae.

a Infectious Diseases Pathology Branch and Rickettsial Zoonoses Branch (see author affiliations for contact information).

b Skin biopsy specimen is ideally collected before the initiation of appropriate antibiotic therapy.
Additional Contributions: David Walker, MD, University of Texas Medical Branch, Galveston, assisted in IHC testing in case 2, and John Sumner, BS, Centers for Disease Control and Prevention, Atlanta, Georgia, performed the molecular testing of the skin biopsy specimens in cases 1 and 2.

REFERENCES