Rickettsia 364D: A Newly Recognized Cause of Eschar-Associated Illness in California

Marc R. Shapiro,[1] Curtis L. Fritz,[2] Karen Tait,[3] Christopher D. Paddock,[5] William L. Nicholson,[5] Kyle F. Abramowicz,[5] Sandor E. Karpathy,[5] Gregory A. Dasch,[5] John W. Sumner,[5] Patricia V. Adem,[5] Jamesina J. Scott,[4] Kerry A. Padgett,[2] Sherif R. Zaki,[5] and Marina E. Eremeeva[5]

[1]St Helena Hospital Clearlake, Clearlake, [2]Division of Communicable Disease Control, California Department of Public Health, Sacramento, and [3]Lake County Health Services Department and [4]Lake County Vector Control District, Lakeport, California; and [5]Division of Viral and Rickettsial Diseases, Centers for Disease Control and Prevention, Atlanta, Georgia

Background. Four spotted fever group rickettsiae (SFGR) are known to infect humans in the United States. A member of the SFGR designated 364D and detected in *Dermacentor occidentalis* ticks has not previously been identified as a human pathogen.

Methods. An 80-year-old man from a rural northern California community presented with an eschar on his forearm. A skin punch biopsy of the lesion was evaluated by immunohistochemistry and molecular analysis. Serum specimens obtained from the patient and 3 other area residents with similar illnesses were tested by immunofluorescence and Western immunoblot for antibodies to SFGR. Ticks were collected near the patient's residence and tested for SFGR.

Results. Abundant intracellular rickettsiae and fragmented rickettsial antigens were observed in the mononuclear inflammatory infiltrates of the biopsy. Nucleotide sequences of DNA fragments amplified from the biopsy were identical to those of 364D. Convalescent sera from all four patients exhibited high immunoglobulin G titers to *Rickettsia rickettsii, Rickettsia rhipicephali,* and 364D antigens. Three adult *D. occidentalis* were positive for 364D, *R. rhipicephali,* and an unidentified *Rickettsia* species.

Conclusions. This is the first confirmation of human disease associated with the SFGR 364D, which was likely transmitted by *D. occidentalis*. Although the patients described here presented with a single cutaneous eschar as the principal manifestation, the full spectrum of illness associated with 364D has yet to be determined. Possible infection with 364D or other SFGR should be confirmed through molecular techniques in patients who present with "spotless" Rocky Mountain spotted fever or have serum antibodies to *R. rickettsii* with group-specific assays.

Four species of spotted fever group rickettsiae (SFGR)—*Rickettsia rickettsii, Rickettsia parkeri, Rickettsia felis,* and *Rickettsia akari*—are well-documented causes of infections in humans in the United States [1–4]. Rocky Mountain spotted fever (RMSF), which is caused by *R. rickettsii,* is most frequently reported in the Mississippi River Valley and southern Atlantic states but is rarely reported west of the Rocky Mountains [5,

6]. The first human case of RMSF in California was identified in 1903, with 238 cases reported between 1903 and 1973 (California Department of Public Health, unpublished data). However, although these cases were diagnosed principally on clinically compatible symptoms (ie, fever, headache, and rash), since 1981, only 1–2 cases have been reported each year that meet both the clinical and laboratory criteria (ie, a ≥4-fold change in specific antibody titer to *R. rickettsii*) for a confirmed case of RMSF [5, 7, 8], as specified in the national surveillance case definition (http://www.cdc.gov/ncphi/disss/nndss/casedef/case_definitions.htm).

Each of the classically recognized vectors of RMSF—namely *Dermacentor variabilis* and *Dermacentor andersoni*—and a newly recognized US vector (*Rhipicephalus sanguineus* [2]) occur in California. Historically, a large proportion of cases of RMSF in California were reported from the northeastern Modoc Plateau area where *D.*

andersoni occurs. However, since 1950, an increasing proportion of cases have been reported outside the Modoc Plateau in areas of the state where *D. andersoni* is absent, suggesting that other ticks might be responsible for occasional transmission of the disease [9]. Both *D. variabilis* and *Dermacentor occidentalis* (the Pacific Coast tick) (Figure 1) are abundant in the coastal ranges from spring to early summer, coincident with many recent California RMSF cases [9, 10]. However, in California to date, *R. rickettsii* has not been detected in *D. variabilis* but only in *R. sanguineus* and *D. occidentalis* [11–13]. In addition to uncertainty about which tick species are the principal vectors for RMSF in California, because *R. rickettsii* has never to our knowledge been isolated from an autochthonously exposed California patient, other species of SFGR may cause illnesses that resemble and are misdiagnosed as RMSF [14–16].

Several investigators have speculated that 364D, a SFGR closely related to *R. rickettsii* and commonly found in *D. occidentalis,* might infect humans in California [11, 13, 16–19]. *Dermacentor occidentalis* rarely harbors *R. rickettsii* in California; a recent study detected *R. rickettsii* in only 0.3% of *D. occidentalis* in southern California [13]. In contrast, 364D has been detected in up to 11% of *D. occidentalis* from 8 California counties where *D. andersoni* is absent [13, 19]. Because the clinical features and commercial serodiagnostic assays for RMSF lack specificity within the SFGR, an unknown proportion of these cases may represent infection with other species of SFGR. Herein, we report 1 laboratory-confirmed case and 3 similar possible cases of infection with 364D from a rural region of northern California.

CASE REPORT

An 80-year-old male resident (patient 1) of Lake County, California, presented to the emergency department of a local hospital in July 2008 with progressive swelling and erythema of his left forearm. Although not disclosed at the time, the patient later recalled having a small black spot at the site 3 days earlier that he initially attributed to a possible insect bite; whether this represented a nascent eschar or the arthropod itself is unknown. He had not travelled outside Lake County during the 10 weeks preceding his illness. The patient had no contact with wild or domestic animals.

Physical examination revealed a 2×3-cm vesicular patch with surrounding erythema on the proximal forearm. The patient reported left axillary pain and tenderness, but was afebrile and without other symptoms. Trimethoprim-sulfamethoxazole (160/800 mg every 12 h) was prescribed for suspected bacterial infection and the patient was released.

The patient presented to his internist three days later at which time the forearm lesion consisted of a 0.4-cm ulcer with raised erythematous margins, surrounded by a 2.0–2.5-cm zone of induration, maceration, and vesiculation (Figure 2A). Generalized erythema extended approximately to the wrist and elbow. Because a rickettsial etiology was considered at this time, antimicrobial therapy was changed to doxycycline (100 mg every 12 h), and an acute-phase serum sample was collected for serological testing. The specimen was negative (titer, <1:64) for immunoglobulin (Ig) G antibodies to *R. rickettsii* by immunofluorescence assay (IFA) conducted at a commercial reference laboratory.

Seven days later, the lesion had developed into a 0.8-cm ulcer beneath an unroofed, irregular 1.5-cm eschar, surrounded by a few smaller satellite ulcers. The ulcer resembled cutaneous lesions observed in 3 other patients (patients 2–4) from the same community (Figure 1B and Table 1) identified previously by one of us (M.S.) between 2002 and 2008. These patients were diagnosed with a SFGR infection based on compatible

Figure 1. Adult female *(left)* and adult male *(right)* Pacific Coast tick *(Dermacentor occidentalis).* Photographs appear courtesy of James Gathany (Centers for Disease Control and Prevention).
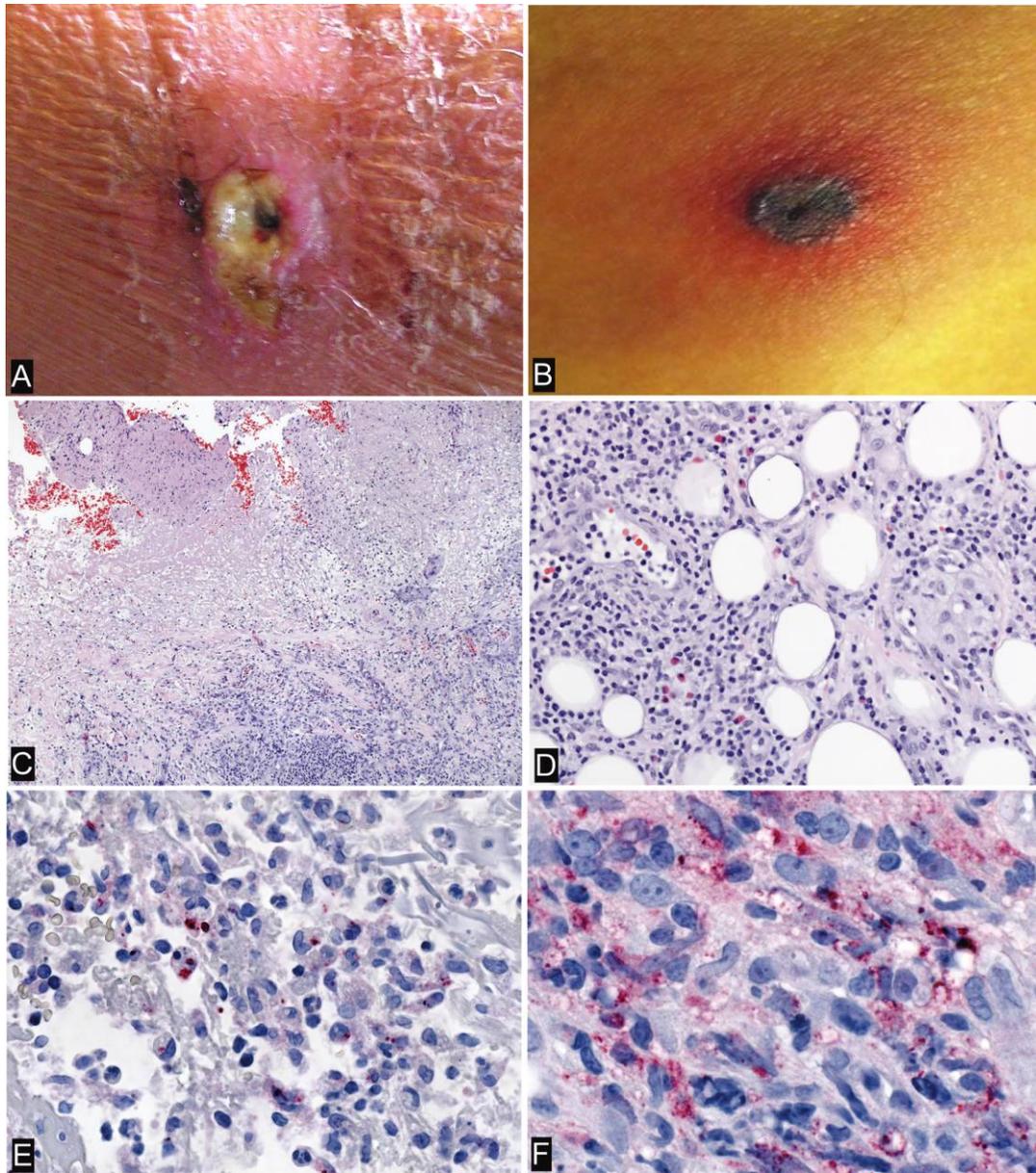
Figure 2. Appearance of ulcer following unroofing of eschar in patient 1 *(A)* and ulcer with intact eschar in patient 2 *(B),* and histopathology and immunohistochemical localization of spotted fever group rickettsiae in the eschar of patient 1. *C,* Extensively ulcerated epidermis with abundant fibrin, hemorrhage, and necrotic debris, representing the superficial aspect of the eschar (original magnification, ×12.5). *D,* Mixed inflammatory cell infiltrates comprising lymphocytes, macrophages, and eosinophils that involve the deep dermis and extend into the subcutaneous adipose tissue (original magnification, ×50). *E,* Intracellular rickettsiae *(red)* in dermal inflammatory cells (original magnification, ×100). *F,* Abundant intracellular and extracellular rickettsial antigens *(red)* in a focus of mononuclear inflammatory cells in the mid-dermis (original magnification, ×158). Hematoxylin and eosin stains are shown in panels *C* and *D,* and immunoalkaline phosphatase stains with a polyclonal anti–*Rickettsia rickettsii* antibody are shown in panels *E* and *F.*

clinical presentations, positive responses to treatment with doxycycline, and positive results reported for *R. rickettsii* serologic tests conducted at a commercial laboratory. A 0.3-cm punch biopsy specimen of the skin and eschar was obtained from patient 1, fixed in 10% buffered formalin, and sent to the Centers for Disease Control and Prevention (Atlanta, GA) for laboratory evaluation of eschar-associated infectious agents, including *Bacillus anthracis* and SFGR. One week after completing a 14-day course of doxycycline, the skin lesion had completely resolved. The patient remained afebrile and reported no systemic symptoms throughout the course of his illness. A convalescent-phase serum sample collected 27 days after onset and evaluated at the same commercial laboratory was positive (titer, 1:512) for IgG antibodies to *R. rickettsii*.

Table 1. Clinical Characteristics of 4 Patients with Cutaneous Eschar and Suspected 364D Rickettsiosis in Lake County, California, 2002–2008

Characteristic	Patient 1	Patient 2	Patient 3	Patient 4
Age, years	80	59	60	53
Sex	Male	Male	Female	Male
Time of onset	July 2008	July 2008	July 2002	September 2003
Location of eschar	Forearm	Hip	Forearm	Shoulder
Lymphadenitis and/or lymphadenopathy	Yes	No	Yes	No
Fever	No	No	Yes	Yes
Rash	No	No	No	No
Headache	No	Yes	Yes	Yes
Fatigue	No	No	Yes	Yes
Nausea, vomiting, and/or diarrhea	No	No	No	No
Photophobia and/or stiff neck	No	No	No	No
Myalgia and/or arthralgia	No	No	Yes	No
Leukocyte count, cells $\times$ 10^9/L	5.6	NA	8.2	3.1
Platelet count, platelets $\times$ 10^9/L	193	NA	155	237
Aspartate aminotransferase level, U/L	NA	NA	42	NA
Alanine aminotransferase level, U/L	NA	NA	57	NA
Initial diagnosis	Rickettsiosis, anthrax	Rickettsiosis	Rickettsiosis, anthrax, tularemia	Rickettsiosis
Antimicrobial treatment (duration, days)	Trimethoprim-sulfamethoxazole (3), doxycycline (14)	Doxycycline (14)	Doxycycline (1), ciprofloxacin (14, 7), doxycycline (14)	Doxycycline (14)
Hospitalization	No	No	No	No
Outcome	Resolution	Resolution	Resolution	Resolution

NOTE. NA, not available.

METHODS

Serological testing. Antibodies to SFGR antigens were measured by IFA and by western blotting (WB). *R. rickettsii* strain Sheila Smith, *R. rhipicephali* strain 3–7-6♀, and 364D were cultivated in Vero E6 cells and prepared as antigens for IFA and WB as previously described [3, 20]. Serum samples were screened by IFA at consecutive 2-fold serum dilutions starting at 1:32 to end-point using heavy chain specific conjugates. IgG and IgM titers ≥1:64 were considered to be positive; seroconversion was defined as a ≥4-fold change in titer between 2 serum specimens collected at least 2 weeks apart. For WB, purified antigens were separated through 10% SDS polyacrylamide gel and blots were prepared using 0.45-μm nitrocellulose membranes as described [20]. All patient serum samples were tested by immunoblot at a final dilution of 1:100.

Histopathologic and immunohistochemical tests. The fixed tissue sample was processed for paraffin embedding. Sections (3 μm) were stained with hematoxylin and eosin, Grocott's methenamine silver, Warthin-Starry, and Gram stains and by an immunoalkaline phosphatase technique [3] using a polyclonal anti–*R. rickettsii* antibody diluted at 1:500, 2 monoclonal anti–*B. anthracis* antibodies (1:200 and 1:1000), and a polyclonal anti–*Sporothrix schenckii* antibody (1:500).

Molecular analyses. DNA was extracted from a 10-μm section of the formalin-fixed, paraffin-embedded skin biopsy specimen. Polymerase chain reaction (PCR) detection and molecular identification of rickettsiae were performed by amplification of the fragments of the 17 kDa protein antigen and outer membrane protein (OmpA) genes using nested or semi-nested assays [4, 15]. RR0155-*rpm*B and RR1240-*tlc*5 intergenic regions were amplified with nested primers [21]. Specific information on the molecular assays and results are provided in Table 2.

Collection and analysis of ticks. Ticks were collected near the residences of patients 1 and 2 in August 2008 using flannel flags and carbon dioxide traps. Ticks were identified to species using standard taxonomic keys and maintained at 4°C until processed for DNA for SYBR Green PCR testing and PCR and sequencing of *omp*A amplicons (Table 2) [13].

RESULTS

Serological tests. The convalescent-phase specimen from patient 1 exhibited high IgG titers to *R. rickettsii*, *R. rhipicephali*, and 364D antigens, ranging from 1:1024 to 1:2048. Similar reactivity patterns were detected for serum samples collected and tested from patients 2–4 (Tables 1 and 3). Earlier serum specimens were available for only 2 of the presumptive cases; seroconversion to all 3 antigens was demonstrated for patient

Table 2. Oligonucleotide Primers and Accession Numbers for Corresponding Sequences Determined for a Confirmed Case of 364D Rickettsiosis

Target, primer identification	Primer sequence 5′→3′	Application	NCBI GenBank accession number for sequence obtained from patient specimen	Reference
17 kDa protein gene				
R17–122	CAGAGTGCTATGAACAAACAAGG	Primary PCR	…	[4]
R17–500	CTTGCCATTGCCCATCAGGTTG	…	…	…
TZ15	TTCTCAATTCGGTAAGGGC	Nested PCR	FJ666086	[4]
TZ16	ATATTGACCAGTGCTATTTC	…	…	…
Outer membrane protein A gene (ompA)				
RR190–70	ATGGCGAATATTTCTCCAAAA	Primary PCR	…	[13]
RR190–701	GTTCCGTTAATGGCAGCATCT	…	…	…
RR190–602[a]	AGTGCAGCATTCGCTCCCCCT	Semi-nested PCR	FJ666087	[13]
RR190–547[b]	CCTGCCGATAATTATACAGGTTTA	…	…	[13]
190-FN1	AAGCAATACAACAAGGTC	Nested PCR	…	[4]
190-RN1	TGACAGTTATTATACCTC	…	…	…
RR0155-rpmB intergenic region				
RR0155-PF	GGATTTCATTTAAAGAGCGATTAGG	Primary PCR	…	Present study
RR0155-PR	GAAAAGATAGGCACTGATCACATTC	…	…	…
RR0155-FN	TTTCTAGCAGCGGTTGTTTTATCC	Nested PCR	FJ666088	[21]
RR0155-RN	TTAGCCCATGTTGACAGGTTTACT	…	…	…
RR1240-tlc5 intergenic region				
RR1137-PF	CTTTCTATGTCATTCCTGACTTCC	Primary PCR	…	Present study
RR1137-PR	CAAAACATTAAGGTACTCATCGG	…	…	…
RR1240-FN	CGGGATAACGCCGAGTAATA	Nested PCR	FJ666089	[21]
RR1240-RN	ATGCCGCTCTGAATTTGTTT	…	…	…

NOTE. NCBI, National Center for Biotechnology Information; PCR, polymerase chain reaction.
[a] Used with RR190–70 in seminested PCR assay after primary amplification with RR190–70/RR190–701.
[b] Used with RR190–701 in SYBR Green PCR assay.

3. Patient 4 exhibited seroconversion when tested using *R. rhipicephali* antigen, but only 2-fold titer increases were seen for both *R. rickettsii* and 364D.

Antibodies to SFGR were also characterized by WB (Figure 3). A convalescent-phase serum sample from patient 1 exhibited strong reactivity with the high molecular weight protein antigens, OmpA and OmpB. The reactivity was strongest with antigens from *R. rhipicephali,* followed by *R. rickettsii* and 364D. Similar patterns were observed for serum samples from patients 2–4. Serum samples from patients 1 and 3 exhibited reactivity to rickettsial lipopolysaccharide; the serum sample from patient 2 also reacted to the GroEL 60 kDa heat-shock protein antigen, although both intensities were much weaker compared to reactivity with the high–molecular weight protein antigens.

Histopathologic and immunohistochemical tests. The biopsy specimen from patient 1 showed prominent necrosis of the epidermis and superficial dermis, with fragmented and intact inflammatory cells comprising lymphocytes, macrophages, and neutrophils admixed sparsely with the necrotic tissue (Figure 2C). The most intense inflammatory infiltrates, consisting of lymphocytes and macrophages, were distributed diffusely throughout the full thickness of the dermis and extended into the subcutaneous fat (Figure 2D). Unusual components of this lesion included numerous eosinophils in some areas of dermatitis and panniculitis (Figure 2D) and the absence of conspicuous vasculitis. No bacteria or fungi were detected by using

Table 3. Immunofluorescence Assay Results Demonstrating Antibody Reactivity of Serum Specimens from Confirmed and Presumptive Cases of 364D Rickettsiosis

Patient	No. of days after onset	IgG (IgM) IFA titer to antigen[a]		
		Rickettsia rickettsii	*Rickettsia rhipicephali*	364D
1	27	1:2048	1:2048	1:1024
2	14	1:512	1:256	1:256
3	15	<1:32 (1:32)	<1:32 (<1:32)	<1:32 (<1:32)
	29	1:1024	1:1024	1:256
4	30	1:256 (1:256)	1:64 (1:128)	1:128 (1:256)
	45	1:512	1:256	1:256

NOTE. IFA, immunofluorescence assay; IgG, immunoglobulin G; IgM, immunoglobulin M.
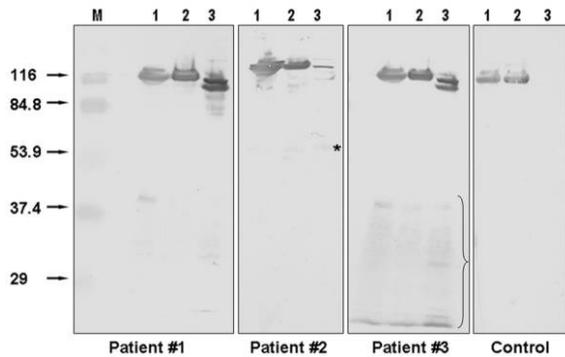[a] Titers ≥1:64 are considered positive for this study.

Figure 3. Western blotting reaction of serum samples obtained from persons with confirmed and presumptive cases of 364D rickettsiosis with spotted fever group rickettsial antigens. Reactions of immunoglobulin G (at 1:100 dilution) antibodies are shown with rickettsial whole cell antigens solubilized at room temperature, including 364D *(lane 1)*, *Rickettsia rickettsii (lane 2)*, and *Rickettsia rhipicephali (lane 3)*. Reactivity of the rickettsial lipopolysaccharide is shown with brackets, reactivity of the GroEL is marked with an asterisk (*). Arrows at the left indicate positions of the protein molecular weight markers *(lane M)* with the following sizes, from the top to the bottom: 116 kDa, 84.8 kDa, 53.9 kDa, 37.4 kDa, and 29.0 kDa. Serum from a patient suffering from Rocky Mountain spotted fever was used as the positive control.

Grocott's methenamine silver, Warthin-Starry, or Gram stains or with the IHC stains for *B. anthracis* or *S. schenkii.* The IHC stain for SFGR demonstrated abundant intracellular rickettsiae and fragmented rickettsial antigens in the mononuclear inflammatory infiltrates (Figure 2*E* and 2*F*).

Molecular analyses. A 208-bp fragment of the 17 kDa *Rickettsia* antigen gene was amplified from DNA extracted from a formalin-fixed skin sample. Two *omp*A fragments (70–602 nucleotides and 101–678 nucleotides) were also amplified; their sequences were identical to that of the homologous fragment of 364D (GenBank accession no. EU109181), which differs from *R. rickettsii* in 3 nucleotide positions. The 361-bp and 292-bp fragments of the RR0155-*rpm*B and RR1240-*tlc*5 intergenic regions were identical to the homologous fragments from the type strain of 364D, and differed by 13 and 7 nucleotides, respectively, from the homologous regions of *R. rickettsii* Sheila Smith (Table 2) [21].

Analysis of ticks. Fourteen *D. variabilis*, 4 *Haemaphysalis leporispalustris*, 19 *D. occidentalis*, and 149 *Ornithodoros coriaceus* were tested. Three adult *D. occidentalis* were positive for SFGR: 364D (1 male), *R. rhipicephali* (1 male), and an unidentified *Rickettsia* (1 female). Identification to species of *Rickettsia* could not be completed for the third tick due to the low amount of rickettsial DNA detected. All positive ticks were collected within 1 km of patient 1's residence; the single adult *D. occidentalis* collected on his property was negative. Rickettsial DNA was not detected in any other tick species.

DISCUSSION

The patient described here presented with a cutaneous lesion that progressed to an ulcer as the chief clinical abnormality. The differential diagnosis for isolated ulcers and eschars encompasses numerous noninfectious and infectious etiologies, including anthrax, tularemia, lymphogranuloma venereum, orf, and cat scratch disease. Although the classic dermatologic manifestation of infection with *R. rickettsii* is a diffuse maculopapular or petechial rash that involves the palms and soles, atypical cutaneous manifestations including "spotless" fever and eschars have also been occasionally reported in patients with serum antibodies to *R. rickettsii* [22–24]. Other SFGR, including *R. conorii* and *R. aeschlimannii,* have been reported to cause illnesses in which an eschar was the sole clinical manifestation [25, 26]. More than 10% of patients seen at a university medical center in an endemic area who met national surveillance criteria for probable or confirmed RMSF had atypical or no cutaneous manifestations [27]. Similarly, California cases reported as RMSF frequently lack the classic triad of fever, macular rash, and history of tick bite. Diagnoses in most of these cases are based principally on commercial laboratory reports of serum antibodies to *R. rickettsii*. However, as standard serologic assays do not differentiate infections caused by SFGR, an unknown proportion of such cases in California and elsewhere may represent infection with other SFGR. In the present study, all 4 patients had elevated convalescent-phase serum antibody titers to *R. rickettsii, R. rhipicephali,* and 364D; of the 2 patients for whom paired serum samples were available, patient 3 seroconverted to all 3 agents and patient 4 seroconverted to *R. rhipicephali* and had 2-fold rises in titer to *R. rickettsii* and 364D. None of the 4 patients had IFA or WB results that permitted specific serological identification of the etiology of the spotted fever group rickettsiosis they experienced. These results illustrate the profound cross-reactivity among SFGR antigens that diminishes the specificity of conventional serologic assays and underscore the need for culture or molecular-based techniques to accurately identify the etiologic agent responsible for a SFG rickettsiosis [1, 3, 4, 6, 20, 28]. This level of diagnostic specificity will help to define the clinical spectrum of infection with other SFGR, including 364D, and may explain "atypical RMSF" in areas where *R. rickettsii* is not prevalent or not the sole SFGR present. A biopsy of the eschar was instrumental for correctly identifying the pathogen in the case described here and should be considered for patients who present with similar illnesses [3, 4, 6, 22–24, 27]. Prolonged persistence of the SFGR in areas of poorly perfused tissue at the margin between viable and necrotic tissue in gangrenous extremities and eschars has been previously described [29, 30], and a similar process was perhaps observed in patient 1 who received extended antibiotic treatment prior to the biopsy procedure.

Three other patients (patients 2–4) with cutaneous lesions

were investigated retrospectively. Each reported headache 2–14 days before noticing the skin lesion, and 2 reported fever. Two patients recovered after a 2-week course of doxycycline. The remaining patient initially started receiving doxycycline, but the regimen was changed to ciprofloxacin (500 mg twice per day for 2 weeks) for suspected cutaneous anthrax, then subsequently completed 2 weeks of doxycycline after anthrax was excluded and an erythematous macule persisted. Each had elevated serum IgG to SFGR, including *R. rickettsii*, *R. rhipicephali*, and 364D, but the lack of molecular analysis of the skin lesions precluded a definitive identification of the true etiologic agent. Indeed, prior infection with any SFGR may produce cross-reactive serum antibodies that not only confound interpretation of results of serologic assays but may provide some level of protective immunity that reduces the severity of clinical disease following infection with other SFGR, as has been previously reported in dogs experimentally infected with *R. rhipicephali* [31].

Approximately 15 of the 20 SFGR species known worldwide are recognized as pathogenic to humans. In 1966, a SFGR named 364D was first identified in *D. occidentalis* ticks in Ventura County, California [11, 17–19]. Isolates of 364D evaluated to date are serologically and biologically homogeneous, cause mild illness in guinea pigs and voles, and are cytopathic for Vero cells and kill chicken embryos [19]. Recent molecular testing has identified unique differences between 364D and isolates of *R. rickettsii* in 18 of 28 intergenic regions [21] and comparisons of the complete genome sequences of 364D with those of *R. rickettsii* Sheila Smith and Iowa further support species status for 364D (authors' unpublished data).

Among California ticks, 364D has been identified only in *D. occidentalis*, a species that occasionally bites humans [32] and is common throughout much of California [10]. In northern California, adult *D. occidentalis* are most active March through May [33], whereas nymphal *D. occidentalis* are more commonly encountered June through August [34]. One study [13] estimated that 7.8% of *D. occidentalis* in southern California were infected with 364D, compared with <1% with *R. rickettsii*. 364D and *R. rhipicephali* were detected in *D. occidentalis* ticks in Lake County first in 1980 [19] and again in the present study. Pending experimental transmission studies to verify its vector competence, the available evidence indicates that *D. occidentalis* is the likely vector for 364D in California.

The 4 patients described here were from a single practice area and had similar, mild illnesses; further investigation is required to elucidate the clinical spectrum of infection with this newly recognized pathogen, the range of its distribution, and its contribution to human morbidity. Because 364D shares many antigens with other SFGR that preclude serological differentiation, molecular identification or isolation of rickettsiae from patient tissues is required for a definitive etiologic diagnosis of this and any other spotted fever group rickettsiosis.

References

1. Schriefer ME, Sacci JB Jr, Dumler JS, Bullen MG, Azad AF. Identification of a novel rickettsial infection in a patient diagnosed with murine typhus. J Clin Microbiol **1994**; 32:949–954.
2. Demma LJ, Traeger MS, Nicholson WL, et al. Rocky Mountain spotted fever from an unexpected tick vector in Arizona. N Engl J Med **2005**; 353:587–594.
3. Paddock CD, Koss T, Eremeeva ME, Dasch GA, Zaki SR, Sumner JW. Isolation of *Rickettsia akari* from eschars of patients with rickettsialpox. Am J Trop Med Hyg **2006**; 75:732–738.
4. Paddock CD, Finley RW, Wright CS, et al. *Rickettsia parkeri* rickettsiosis and its clinical distinction from Rocky Mountain spotted fever. Clin Infect Dis **2008**; 47:1188–1196.
5. Dalton MJ, Clarke MJ, Holman RC, et al. National surveillance for Rocky Mountain spotted fever, 1981–1992: epidemiologic summary and evaluation of risk factors for fatal outcome. Am J Trop Med Hyg **1995**; 52:405–413.
6. Chapman AS, Bakken JS, Folk SM, et al. Diagnosis and management of tickborne rickettsial diseases: Rocky Mountain spotted fever, ehrlichioses, and anaplasmosis—United States: a practical guide for physicians and other health-care and public health professionals. MMWR Recomm Rep **2006**; 55:1–27.
7. California Department of Health Services. Communicable diseases in California, 1994–1998. July **2001**. Available at http://www.cdph.ca.gov/data/statistics/Pages/CDdata.aspx. Accessed 6 June 2009.
8. California Department of Health Services. Communicable diseases in California, 1999–2000. December **2003**. Available at: http://www.cdph.ca.gov/data/statistics/Pages/CDdata.aspx. Accessed 6 June 2009.
9. Rotramel GL, Schwan TG, Doty RE. Distribution of suspected tick vectors and reported cases of Rocky Mountain spotted fever in California. Am J Epidemiol **1976**; 104:287–293.
10. Furman DP, Loomis EC. The ticks of California (Acari: Ixodida). University of California Publications, Bulletin of the California Insect Survey, Vol. 25. Berkeley: University of California Press, **1984**.
11. Philip RN, Lane RS, Casper EA. Serotypes of tick-borne spotted fever group rickettsiae from western California. Am J Trop Med Hyg **1981**; 30:722–727.
12. Wikswo ME, Hu R, Metzger ME, Eremeeva ME. Detection of *Rickettsia rickettsii* and *Bartonella henselae* in *Rhipicephalus sanguineus* ticks from California. J Med Entomol **2007**; 44:158–162.
13. Wikswo ME, Hu R, Dasch GA, et al. Detection and identification of spotted fever group rickettsiae in *Dermacentor* species from southern California. J Med Entomol **2008**; 45:509–516.
14. Mediannikov O, Paddock CD, Parola P. Other rickettsiae of possible or undetermined pathogenicity. In: Raoult D, Parola P, eds. Rickettsial diseases. New York: Informa Healthcare, **2007**:163–177.

15. Eremeeva ME, Bosserman EA, Demma LJ, Zambrano ML, Blau DM, Dasch GA. Isolation and identification of *Rickettsia massiliae* from *Rhipicephalus sanguineus* ticks collected in Arizona. Appl Environ Microbiol **2006**; 72:5569–5577.

16. Paddock CD. *Rickettsia parkeri* as a paradigm for multiple causes of tick-borne spotted fever in the western hemisphere. Ann N Y Acad Sci **2005**; 1063:315–326.

17. Cory J, Yunker CE, Howarth JA, et al. Isolation of spotted fever group and *Wolbachia*-like agents from field-collected materials by means of plaque formation in mammalian and mosquito cells. Acta Virol **1975**; 19:443–445.

18. Philip RN, Casper EA, Burgdorfer W, Gerloff RK, Hughes LE, Bell EJ. Serologic typing of rickettsiae of the spotted fever group by microimmunofluorescence. J Immunol **1978**; 121:1961–1968.

19. Lane RS, Philip RN, Casper EA. Ecology of tick-borne agents in California. II. Further observations on rickettsiae. In: Burgdorfer W, Anacker RL, eds. Rickettsiae and rickettsial diseases. New York: Academic Press, **1981**:575–584.

20. Raoult D, Dasch GA. Line blot and western blot immunoassays for diagnosis of Mediterranean spotted fever. J Clin Microbiol **1989**; 27: 2073–2079.

21. Karpathy SE, Dasch GA, Eremeeva ME. Molecular typing of isolates of *Rickettsia rickettsii* by use of DNA sequencing of variable intergenic regions. J Clin Microbiol **2007**; 45:2545–2553.

22. Helmick CG, Bernard KW, D'Angelo LJ. Rocky Mountain spotted fever: clinical, laboratory, and epidemiological features of 262 cases. J Infect Dis **1984**; 150:480–488.

23. Walker DH, Gay RM, Valdes-Dapena M. The occurrence of eschars in Rocky Mountain spotted fever. J Am Acad Dermatol **1981**; 4:571–576.

24. Ramsey PG, Press OW. Successful treatment of Rocky Mountain 'spotless' fever. West J Med **1984**; 140:94–96.

25. Mansueto S, Tringali G, Di LR, Maniscalco M, Montenegro MR, Walker DH. Demonstration of spotted fever group rickettsiae in the tache noire of a healthy person in Sicily. Am J Trop Med Hyg **1984**; 33:479–482.

26. Pretorius AM, Birtles RJ. *Rickettsia aeschlimannii:* a new pathogenic spotted fever group rickettsia, South Africa. Emerg Infect Dis **2002**; 8: 874.

27. Sexton DJ, Corey GR. Rocky Mountain "spotless" and "almost spotless" fever: a wolf in sheep's clothing. Clin Infect Dis **1992**; 15:439–448.

28. Dasch GA, Eremeeva ME. Laboratory diagnosis of Rocky Mountain spotted fever and other rickettsioses in the Americas: contemporary approaches and prospects for future improvements. In: Proceedings of the XIV Colegio Brasileiro de Parasitologia Veterinaria and 2 Simposio Latino-Americano de Ricquetsioses (Ribeiro Preto, Brazil). **2006**:124–131.

29. Hove MG, Walker DH. Persistence of rickettsiae in the partially viable gangrenous margins of amputated extremities 5 to 7 weeks after onset of Rocky Mountain spotted fever. Arch Pathol Lab Med **1995**; 119: 429–431.

30. Walker DH, Gear JH. Correlation of the distribution of *Rickettsia conorii*, microscopic lesions, and clinical features in South African tick bite fever. Am J Trop Med Hyg **1985**; 34:361–371.

31. Burgdorfer W, Brinton LP, Krynski WL, Philip RN. *Rickettsia rhipicephali,* a new spotted fever group rickettsia from the brown dog tick, *Rhipicephalus sanguineus.* In: Rickettsiae and rickettsial diseases: proceedings of the 2nd International Symposium on Rickettsia and Rickettsial Diseases. Bratislava: VEDA Publishing House of the Slovak Academy of Sciences, **1978**:307–16.

32. Merten HA, Durden LA. A state-by-state survey of ticks recorded from humans in the United States. J Vector Ecol **2000**; 25:102–113.

33. Lane RS. Risk of human exposure to vector ticks (Acari: Ixodidae) in a heavily used recreational area in northern California. Am J Trop Med Hyg **1996**; 55:165–173.

34. Casher L, Lane R, Barrett R, Eisen L. Relative importance of lizards and mammals as hosts for ixodid ticks in northern California. Exp Appl Acarol **2002**; 26:127–143.