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James E. Childs
Centers for Disease Control and Prevention, Atlanta, james.childs@yale.edu

Patricia W. Greer

Tara L. Ferebee

Joseph Singleton Jr.

Don B. McKechnie

See next page for additional authors

Authors

James E. Childs, Patricia W. Greer, Tara L. Ferebee, Joseph Singleton Jr., Don B. McKechnie, Tracee A. Treadwell, John W. Krebs, Matthew J. Clarke, Robert C. Holman, James G. Olson, Christopher D. Paddock, and Sherif R. Zaki

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Viral and Rickettsial Zoonoses Branch, Infectious Disease Pathology Activity, and Office of the Director, Division of Viral and Rickettsial Diseases, National Center for Infectious Diseases, Centers for Disease Control and Prevention, Atlanta, Georgia

Rocky Mountain spotted fever (RMSF) is the most severe tickborne infection in the United States and is a nationally notifiable disease. Since 1981, the annual case-fatality ratio for RMSF has been determined from laboratory-confirmed cases reported to the Centers for Disease Control and Prevention (CDC). Herein, a description is given of patients with fatal, serologically unconfirmed RMSF for whom a diagnosis of RMSF was established by immunohistochemical (IHC) staining of tissues obtained at autopsy. During 1996–1997, acute-phase serum and tissue samples from patients with fatal disease compatible with RMSF were tested at the CDC. As determined by indirect immunofluorescence assay, no patient serum demonstrated IgG or IgM antibodies reactive with *Rickettsia rickettsii* at a diagnostic titer (i.e., ≥64); however, IHC staining confirmed diagnosis of RMSF in all patients. Polymerase chain reaction validated the IHC findings for 2 patients for whom appropriate samples were available for testing. These findings suggest that dependence on serologic assays and limited use of IHC staining for confirmation of fatal RMSF results in underestimates of mortality and of case-fatality ratios for this disease.

The notoriety of Rocky Mountain spotted fever (RMSF) was established a century ago when physicians in the northwestern United States first reported an acute febrile disease of devastating severity [1, 2]; during 1885–1912, the mean annual case-fatality ratio (CFR) for RMSF in the Bitterroot Valley of western Montana exceeded 65% [3]. The profound lethality of RMSF continued through the preantibiotic era, and CFRs of 18.4–28.1 occurred in the United States during 1930–1939 [4, 5]. Mortality attributable to RMSF plummeted following the discovery of effective antirickettsial therapies in the late 1940s, and CFRs remained relatively constant at 5%–10% for the next 30 years [6–8]. Since 1981, yearly CFRs have averaged 3.4% overall and have dropped to as low as 1.2%. [9] (Centers for Disease Control and Prevention [CDC], unpublished data). The numbers of reported RMSF fatalities also declined over this period, from a mean of 13 confirmed deaths per year for 1981–1992 [9] to a mean of 7 confirmed deaths per year for 1993–1996 (CDC, unpublished data). The reason(s) for the apparent decline in total reported RMSF fatalities is not known, although these changes may be linked to increasing physician awareness and earlier diagnosis of tickborne diseases and perhaps to concurrent decreases in reporting of RMSF.

Annual reporting of the number of cases of RMSF by state health departments has been done since 1920 [10]. Currently, cases of RMSF are reported to the CDC via two national surveillance systems. The first system was established in 1970 and consists of standardized case report forms completed by private physicians and local or state health department personnel. Information submitted through case report forms is used by the CDC to periodically compile clinical, epidemiologic, and laboratory summaries of RMSF [7–11]. For surveillance summaries assembled from case report–form data, cases of RMSF are classified as confirmed, probable, or unconfirmed according to guidelines established by the Council of State and Territorial Epidemiologists [12]. Case classifications are made on the basis of results of confirmatory laboratory tests, which are interpreted in the context of an illness clinically compatible with RMSF.

The second method of national surveillance for RMSF, the National Electronic Telecommunications System for Surveillance (NETSS), was initiated in 1984 to evaluate trends in the regional incidence of RMSF. NETSS data are accumulated at the CDC by weekly telephone reports filed by state health departments and are summarized annually in the Morbidity and Mortality Weekly Report. NETSS provides no information on laboratory test results, clinical course, or patient outcome. Consequently, total mortality and CFR determinations for RMSF are derived solely from laboratory-confirmed cases reported to the CDC through case report forms [9].

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Reprints or correspondence: Dr. Christopher D. Paddock, Viral and Rickettsial Zoonoses Branch, Mailstop G-13, Centers for Disease Control and Prevention, 1600 Clifton Rd., N.E., Atlanta, GA 30333.

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Starting in 1981, the CDC required the use of specific assays, including indirect immunofluorescence (IFA), indirect hemagglutination, complement fixation, and latex agglutination in the serologic confirmation of all reported cases of RMSF [8, 13]. Serologic assays comprise the most widely available and frequently used methods for confirming cases of RMSF; however, these tests are often nonconfirmatory during the first 7–10 days of illness [13, 14]. In this report, we describe 9 patients with fatal RMSF for whom diagnoses were confirmed by immunohistochemical (IHC) staining of tissues for spotted fever group rickettsiae. Confirmatory serologic criteria were absent for all patients, underscoring the hidden mortality attributable to this disease and the value of histopathology and immunopathology in evaluating patients with suspected fatal RMSF.

Materials and Methods

Patient samples. Formalin-fixed, paraffin-embedded tissues from 16 patients with suspected fatal RMSF were submitted to the CDC from May 1996 to December 1997 for laboratory confirmation. For 12 of these patients, acute-phase serum samples were also available for testing. Unfixed tissues or whole blood collected at autopsy were available for testing for 5 cases. Routine hematoxylin-eosin–stained tissue sections were examined for all cases. Clinical summaries, laboratory reports, and autopsy reports were reviewed when available.

Immunohistochemistry. Tissues obtained at autopsy were tested for spotted fever group rickettsiae by use of a modified immunoalkaline phosphatase technique [15]. In brief, 3-μm sections were deparaffinized in xylene, rehydrated in a graded alcohol series, and rinsed in distilled water. Tissue sections were digested in 0.1 mg/mL proteinase K (Boehringer Mannheim, Indianapolis) for 15 min and blocked with 20% normal swine serum in Tris-saline-Triton (NSS/TST). Slides were incubated in a 1/500 dilution of polyclonal rabbit anti–*Rickettsia rickettsii* antiserum (complement fixation titer of 1024, prepared as previously described [16]) for 60 min. The sections were washed three times in NSS/TST, incubated in biotinylated swine anti-mouse and anti-rabbit antibody (Dako, Carpenteria, CA) for 15 min, washed in NSS/TST, and incubated with alkaline phosphatase–conjugated streptavidin for 15 min. The slides were rinsed, incubated in naphthol phosphate–fast red chromogen reagent for 15 min, counterstained in Mayer's hematoxylin for 6 min, and mounted with aqueous mounting medium.

The primary hyperimmune antiserum used in this assay demonstrates reactivity to multiple spotted fever group rickettsiae, including *Rickettsia akari, Rickettsia conorii, and R. rickettsii* [16]. The specificity of the anti–spotted fever group rickettsial antibody was confirmed in IHC experiments by demonstrating the absence of reactivity with normal patient tissues and with multiple tissues infected with nonrelevant bacterial pathogens, including *Coxiella burnetii, Francisella tularensis, Ehrlichia chaffeensis, Ehrlichia equi, Legionella pneumophila* type I, *Neisseria meningitidis* group C, *Rickettsia typhi, Treponema pallidum,* and *Yersinia pestis.* Similarly, nonimmune rabbit serum and several primary hyperimmune antisera to nonrelevant bacteria (as listed above) did not react with patient tissues that were positive as determined by IHC testing for spotted fever group rickettsiae.

The IHC staining technique for spotted fever group rickettsiae was validated by use of autopsy-derived tissues from 7 patients with fatal RMSF in whom the diagnosis was confirmed by serologic testing (median IFA titer, 256; range, 64–1024), including 2 patients additionally tested by use of polymerase chain reaction (PCR) to confirm the 17-kDa rickettsial surface protein gene.

IFA. Patient serum was diluted from 1/8 to 1/256 and evaluated for antibodies reactive with spotted fever group rickettsiae by use of IFA [17]. IgG and IgM antibodies were detected by use of fluorescein isothiocyanate–conjugated goat anti–human IgG and IgM antibodies, respectively (Kirkegaard & Perry, Gaithersburg, MD). All IgM determinations were made after absorption of IgG, using the Quik-Sep IgM Protein G affinity method (Isolab, Akron, OH). End-point titers were recorded as the reciprocal of the highest dilution exhibiting specific rickettsial fluorescence. Patients with a reciprocal IFA titer ≥64 were considered seropositive [12].

Nucleic acid amplification and sequencing. Extraction kits (QIAGEN, Chatsworth, CA) were used to obtain DNA from 200 μL of EDTA-anticoagulated whole blood and from 0.2 × 0.2 × 0.2 cm fragments of fresh tissues obtained at autopsy. Extracts were evaluated by use of a nested PCR assay designed to amplify a 246-bp segment of the 17-kDa rickettsial surface protein gene from whole blood and unfixed tissues [18] (DB McKechnie, unpublished data). This assay has not been optimized to evaluate formalin-fixed tissues, and no fixed tissue samples were evaluated. In brief, 10 μL of extracted DNA was amplified in a 100-μL primary reaction, using 0.5 μ*M* each of primers R17.126 and R17.507. One μL of the primary reaction mix was amplified in a 100-μL nested reaction, using 0.2 μ*M* each of primers TZ15 and TZ16 [19]. Primary reactions (40 cycles) and nested reactions (30 cycles) were performed in a thermal cycler (GeneAmp Systems 9600; Perkin-Elmer, Foster City, CA), with a profile consisting of denaturation at 95°C for 2 min, followed by denaturation at 94°C for 30 s, annealing at 55°C for 30 s, extension at 72°C for 1 min, and an additional extension for the last cycle at 72°C for 5 min. PCR products were sequenced by use of the dideoxy terminator system on an automated sequencer (ABI Prism 377; Applied Biosystems, Foster City, CA) and analyzed with computer software (Wisconsin Sequence Analysis Package; Genetics Computer Group, Madison).

Review of RMSF case report forms. RMSF case report forms submitted by state health departments to the CDC for years 1981–1996 were reviewed to assess the magnitude of total confirmed cases and confirmed fatalities in national reporting of RMSF. Confirmed cases met one or more of the Council of State and Territorial Epidemiologists criteria for RMSF [12]: (1) a ≥4-fold change in titer of antibody reactive with *R. rickettsii* by IFA, latex agglutination, complement fixation, or indirect hemagglutination tests; (2) the detection of *R. rickettsii* DNA by PCR; (3) the demonstration of *R. rickettsii* in biopsy or autopsy tissues by specific immunostains, including immunofluorescence, immunoperoxidase, and immunoalkaline phosphatase stains; and (4) the isolation of *R. rickettsii* from a clinical specimen. In addition, patients with a single titer ≥64 by IFA or ≥16 by complement fixation were categorized as confirmed cases for the purpose of surveillance [9]. Total numbers of fatal cases confirmed by serology, immunopathology, PCR, or isolation were compared to determine the relative

frequencies of each test category used to establish the diagnosis of RMSF during this 16-year interval. The median number of days from onset of disease to death was determined for all laboratory-confirmed cases of RMSF reported in during 1981–1996.

Results

Results of confirmatory tests for RMSF. RMSF was confirmed in 12 of 16 deceased patients on the basis of IHC testing alone or in combination with serology or PCR. None of the 4 patients for whom IHC results were negative had a positive serology or PCR result. All 12 laboratory-confirmed cases had unequivocal IHC evidence of rickettsial antigen in tissues. Three of the 12 IHC-confirmed RMSF fatalities additionally demonstrated serum antibody reactive with *R. rickettsii* at titers ≥64 and are not included in further discussion. Nine patients had positive IHC results with either a negative or unavailable serologic result (table 1). Acute-phase serum samples were available for 7 of 9 patients evaluated in the series. For 2 (patients 5 and 6), RMSF was not considered before autopsy; serum for these patients was not collected and therefore was unavailable for testing. Serum specimens were obtained a median of 5 days (range, 2–8) following onset of illness. None of the 7 samples demonstrated specific IgG or IgM antibodies reactive to *R. rickettsii* at a dilution ≥1/64. None of these patients met Council of State and Territorial Epidemiologists or CDC surveillance criteria for serologically confirmed RMSF [9, 12] on the basis of the best recognized and most widely available confirmatory assay.

Patient characteristics. Patients for whom IHC testing demonstrated spotted fever group rickettsiae in tissues but who lacked confirmatory serology for RMSF originated from eight states (Alabama, Georgia, Kansas, Kentucky, Idaho, Mississippi, North Carolina, and West Virginia). There were 4 males and 5 females, who ranged in age from 2 months to 65 years (table 1).

All patients presented with an acute febrile illness during April–October. Myalgias and vomiting were reported in 6 and 7 of these patients, respectively. A macular or petechial rash (or both) was reported for 6 patients, and headache was reported for 3. The triad of fever-rash-headache was identified for 3 patients. A history of tick bite preceding the illness was described for 5 patients. The triad of fever–rash–tick bite was identified for 3 patients. Thrombocytopenia (range, 14,000–72,000/μL) was reported in 8 patients. One or more electrolyte abnormalities were described for 7 patients, including hyponatremia (serum sodium ≤134 mEq/L), hypocalcemia (serum calcium ≤8.6 mEq/L), and hypokalemia (serum potassium ≤3.1 mEq/L) in 4, 4, and 3 patients, respectively. Aspartate aminotransferase levels were elevated (≥136 IU/L) in 4 patients. A median of 7 days (range, 5–10) elapsed from onset of symptoms to death. Fulminant RMSF (i.e., death occurring in the first 5 days of illness [20]) was reported for 2 patients. RMSF was considered in the premortem differential diagnosis in 3 patients, and 4 patients received antimicrobial therapy active against *R. rickettsii* (doxycycline for 3, chloramphenicol for 1) before their deaths.

Immunohistochemistry. A minimum of 4 tissues from each patient were evaluated by IHC staining. Tissues (no. of patients) included liver (9), myocardium (8), spleen (8), kidney (8), lung (7), adrenal gland (5), pancreas (5), cerebral cortex (5), cerebellum (4), skin (4), stomach (4), colon (4), bone marrow (3), lymph node (3), small intestine (2), trachea (2), skeletal muscle (2), thymus (2), and thyroid (2). The following tissue types were represented as single samples from patients in the series: coronary artery, aorta, hippocampus, medulla, pons, pineal gland, choroid plexus, ovary, tongue, and appendix. A bone marrow biopsy obtained 1 day before death was available for 1 patient (patient 7).

Rickettsial antigens reactive with the anti-*R. rickettsia* antibody stain were visualized in multiple tissues from each patient (table 1). Staining was most apparent within and associated

Table 1. Patient characteristics and confirmatory laboratory findings in persons with fatal, serologically unconfirmed Rocky Mountain spotted fever.

Patient	Age, sex	No. of days from onset of illness to death	No. of days from onset of illness to serum sample	Reciprocal IFA titer		PCR	IHC
				IgG	IgM		
1	28 years, F	6	5	<8	<8	NA	+
2	19 years, M	10	8	32	32	NA	+
3	29 years, F	5	4	<8	<8	NA	+
4	65 years, F	7	7	32	16	+	+
5	58 years, F	9	NA	NA	NA	NA	+
6	49 years, M	7	NA	NA	NA	+	+
7	35 years, M	5	5	<8	16	NA	+
8	3 years, F	7	7	<8	<8	NA	+
9	2 months, M	6	2	<8	<8	NA	+

NOTE. IFA = indirect immunofluorescence assay; PCR = polymerase chain reaction amplification of rickettsial 17-kDa surface protein gene sequence from whole blood or unfixed patient tissues collected at autopsy; IHC = immunoalkaline phosphatase stain for spotted fever group rickettsiae, including *Rickettsia rickettsii,* in formalin-fixed, paraffin-embedded tissues obtained at autopsy; NA = serum, whole blood, or unfixed tissue samples unavailable for testing.

with vascular endothelium of capillaries, arterioles, venules, arteries, and veins (figure 1A–D). Rickettsial antigens and discrete coccobacilli were primarily located within the cytoplasm and occasionally in nuclei of infected endothelial cells (figure 1A–C); less frequently, they were located within fixed and circulating mononuclear phagocytic cells, including bone marrow histiocytes (figure 1D), Kupffer's cells, pulmonary alveolar macrophages, and monocytes. Aggregates of fragmented, granular, or coalesced rickettsial antigens were occasionally seen in foci of intense staining (figure 1B). Abundant extracellular rickettsial antigen was distributed throughout the splenic red pulp in 5 of 8 patients for whom spleen was examined by IHC staining.

Although observations were generally limited to evaluation of a single section representing a particular organ, tissues stained with hematoxylin and eosin frequently revealed lymphohistiocytic infiltrates around and involving small vessels, which subsequently demonstrated abundant rickettsial antigen by IHC staining. Inflammatory lesions identified by routine staining included hepatic portal triaditis (9/9 patients), focal interstitial pneumonitis (7/7 patients), meningitis (5/7 patients), interstitial nephritis (4/8 patients), focal myocarditis (4/8 patients), and focal vasculitis in the dermis (4/4 patients).

Rickettsiae were also identified in minimally inflamed or noninflamed vessels, and intensely inflamed foci occasionally failed to demonstrate stainable bacteria. Rickettsial antigens were characteristically distributed as discontinuous foci in vessels in each of the major organs examined. Rickettsiae were identified in or around small vessels in sections of skin from each patient for whom this tissue was available; however, the organisms were often sparse and distributed sporadically in the tissue. Other frequently observed lesions included pulmonary intra-alveolar hemorrhage (7/7 patients), erythrophagocytosis by Kupffer's cells (6/9 patients), and microscopic cortical necroses in the adrenal glands (3/5 patients). Multifocal arteriolar microinfarcts or glial nodules involving the cerebral cortex and brainstem were seen in 3 of 7 patients in this series.

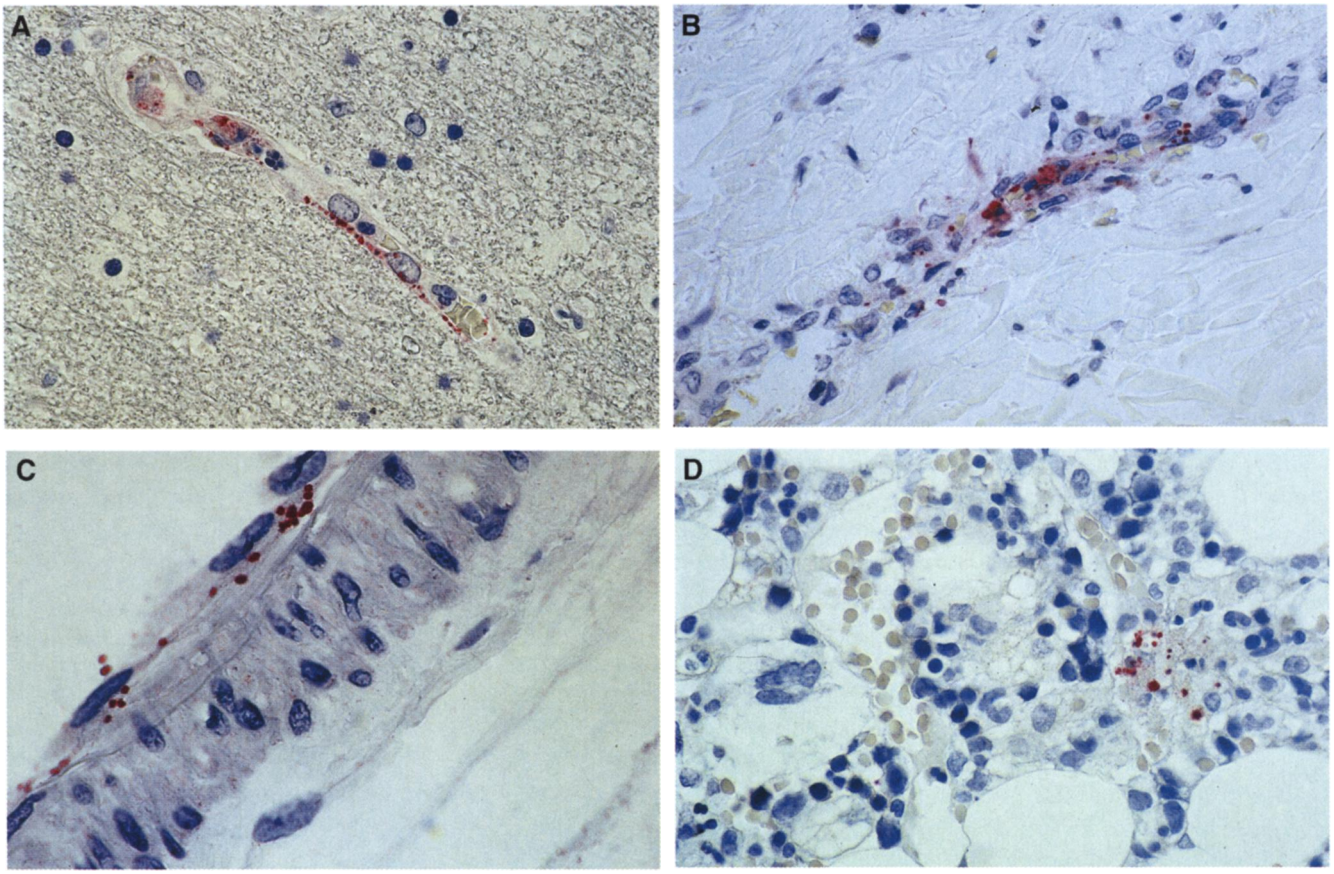

Figure 1. Immunohistochemical localization of spotted fever group rickettsial antigens in tissues of patients with fatal Rocky Mountain spotted fever. Immunoalkaline phosphatase stain with naphthol phosphate–fast red substrate and hematoxylin counterstain. *A,* Intracytoplasmic and intranuclear spotted fever group rickettsiae in infected endothelial cells in white matter of cerebral cortex (patient 2); original magnification, ×158. *B, Rickettsia rickettsii* within damaged endothelium of dermal capillary (patient 4); original magnification, ×158. *C,* Coccobacillary forms of *R. rickettsii* in cytoplasms of infected endothelial cells in a leptomeningeal vein (patient 6); original magnification, ×250. *D,* Rickettsiae in cytoplasms of bone marrow histiocytes (patient 7); original magnification, ×158.

Nucleic acid amplification from patient blood and tissues. Rickettsial 17-kDa gene sequences were amplified from blood or fresh tissues of all 4 of the 12 IHC assay–positive patients for whom appropriate samples were obtained. These included 2 of the 9 serologically unconfirmed patients. For each case with unfixed tissues available for evaluation, PCR analysis validated the IHC results. PCR products of the expected size were amplified from peripheral blood, spleen, and liver of patient 4 and from skeletal muscle and myocardium of patient 6. Sequence analysis of 208 bp of the amplicons was used to verify 17-kDa rickettsial surface protein gene sequences; they demonstrated 100% homology to the corresponding sequence of *R. rickettsii* (GenBank accession no. M16486).

RMSF fatalities reported in the United States, 1981–1996. Case report forms that were submitted to the CDC for 1981–1996 were reviewed: 5388 (57.9%) of 9312 forms revealed laboratory-confirmed cases of RMSF (209–602 cases/year), including 182 laboratory-confirmed fatalities (4–24 confirmed fatalities/year). The mean annual CFR for this 16-year interval was 3.4% (range, 1.2%–5.3%). Of the total confirmed RMSF fatalities, 109 (59.8%) were diagnosed by serology, 67 (36.8%) by immunopathology, and 15 (8.2 %) by PCR or isolation of *R. rickettsii* (table 2). Ninety-two (33.6%) of 274 deaths reported to the CDC during this period were unconfirmed by laboratory testing. For 159 laboratory-confirmed deaths during 1981–1996 for which dates of onset and dates of death were reported, the median time to death was 8 days; 107 (67%) of these deaths occurred in the first 9 days of illness. For laboratory-confirmed deaths occurring during 1981–1984 (65 patients) and during 1993–1996 (20 patients), the median time to death was also 8 days.

Table 2. Laboratory-confirmed cases of fatal Rocky Mountain spotted fever reported to the Centers for Disease Control and Prevention (CDC), 1981–1996.

Year	Total no. of laboratory-confirmed cases	Case-fatality ratio (%)[a]	Laboratory criteria for confirmation of fatal cases[b]		
			Serology[c]	Immuno-pathology[d]	Other[e]
1981	369	13/341 (3.8)	5	7	1
1982	399	20/379 (5.3)	13	7	1
1983	602	24/553 (4.3)	14	10	4
1984	398	19/361 (5.3)	14	5	1
1985	334	16/320 (5.0)	9	6	2
1986	346	11/325 (3.4)	3	7	1
1987	257	5/241 (2.1)	3	2	0
1988	362	15/348 (4.3)	14	2	0
1989	299	4/274 (1.5)	2	2	0
1990	300	13/263 (4.9)	4	8	1
1991	289	8/254 (3.1)	3	4	2
1992	263	8/227 (3.5)	6	2	0
1993	209	9/192 (4.7)	6	3	0
1994	270	8/204 (3.9)	6	0	2
1995	325	5/294 (1.7)	5	0	0
1996	366	4/348 (1.2)	2	2	0

NOTE. Data were determined from RMSF case report forms submitted by state health departments to the CDC.

[a] Case-fatality ratios determined from confirmed cases for which patient outcome (i.e., fatal or nonfatal) is specified; 8.6% of case report data are incomplete and do not designate patient outcome.

[b] Laboratory criteria defined by Council of State and Territorial Epidemiologists [12]. Total no. of laboratory confirmations may exceed total no. of confirmed fatalities when multiple assays are used to establish diagnosis.

[c] Includes indirect immunofluorescence, latex agglutination, complement fixation, and indirect hemagglutination assays for detecting antibodies reactive with *Rickettsia rickettsii.*

[d] Includes immunofluorescence, immunoperoxidase, and immunoalkaline phosphatase stains for detecting spotted fever group rickettsiae in tissues.

[e] Includes polymerase chain reaction assay or isolation of *R. rickettsii* from patient blood or tissues.

Discussion

The epidemiologic and clinical characteristics of the 9 patients described in this report were strongly compatible with RMSF. All patients resided in areas where *R. rickettsii* was endemic, became ill in mid-spring to early fall, and presented with an acute febrile syndrome characterized by myalgias, vomiting, and/or rash. The IFA, which was used to detect antirickettsial antibody, determined that none of the 9 patients examined in this series fulfilled CDC surveillance criteria for laboratory-confirmed RMSF. Had IFA been the only test attempted, none of these 9 fatalities would be included in yearly CFR estimates for RMSF. We detected 12 fatal cases that were identified by IHC staining in a 14-month interval from 1996 through 1997. By comparison, immunopathology was used to confirm only 9 of 38 RMSF deaths reported to the CDC during 1991–1995 (table 2). These data suggest that IHC staining is underrecognized and underutilized as a diagnostic tool and that many, if not most, deaths caused by *R. rickettsii* in the United States are missed, unconfirmed, or unreported.

Detection of antibody to spotted fever group rickettsiae remains the best recognized and most utilized laboratory method to confirm RMSF. The IFA test is generally considered the reference standard in RMSF serology [13] and is the test currently used by the CDC and most state public health laboratories. Most patients with RMSF lack diagnostic IFA titers (i.e., $\geq$64) until the second week of disease, an observation supported by studies of patients with strong clinical and epidemiologic evidence of RMSF [21, 22], culture-confirmed *R. rickettsii* infection [23, 24], and $\geq$4-fold changes in antibody titer over the course of their illness [25]. IFA titers $\geq$16 may appear within 5–6 days after onset of symptoms [14]; however, <15% of patients demonstrate IgG or IgM titers $\geq$64 in the first 7 days of illness [21]. About 40%–50% of patients lack a diagnostic titer 7–9 days after onset of illness [13, 14], and as many as 25% of all patients with RMSF lack a diagnostic titer by the end of the second week of disease [13, 21].

The kinetics of the serologic response to infection with *R. rickettsii* is particularly important in view of the temporal course of fatal RMSF. Treatises on this disease from the 1930s and 1940s reported the majority of deaths to occur in the second and third weeks of illness [20, 26]. Patients in our series died of RMSF a median of 7 days following onset of illness, which

is in agreement with many contemporary case series, in which at least half of all deaths occur within the first 7–9 days of the disease [10, 27–29]. In the present study, deaths reported during 1981–1996 occurred a median of 8 days after onset of symptoms. These findings are consistent with some of the earliest descriptions of the clinical course of disease: 43 (49%) of 88 spotted fever deaths reported by Wilson and Chowning in 1904 died within the first 8 days of illness, and 65 (68%) of these patients died 6–10 days after onset of symptoms [2].

In this context, it could be reasonably expected that >50% of all patients with fatal RMSF lack diagnostic antibody at the time of death. In a 1978 study of 44 deaths attributable to RMSF, <25% of cases demonstrated confirmatory serologic evidence of infection with *R. rickettsii* [6]. Reporting of RMSF primarily on the basis of antibody-detection diagnostics will underestimate the absolute number of RMSF deaths, although the effect on the CFR is less clearly defined. The CFR depends both on the appropriate identification of fatal cases and on a positive test for a case to enter the denominator. Because many cases are confirmed for surveillance reports on the basis of one positive laboratory test (most often a serologic test) in the presence of clinically compatible disease, patients who survive their infection but are tested early in the disease course may be missed. Perseverance by physicians to obtain convalescent-phase serum will certainly assist in accurately defining the total number of cases among persons who survive the disease; however, this is not an option in fatal cases. Unless methods other than serology are attempted in patients with fatal RMSF, the number of deaths attributable to this disease will be underestimated. In the past 16 years, however, confirmatory methods other than serology (i.e., immunopathology, PCR, or isolation) have been used to diagnose <40% of the RMSF fatalities reported to the CDC (table 2).

In this case series, lesions were regularly observed in sections of kidney, heart, lung, liver, and skin by use of routine hematoxylin and eosin staining, and rickettsiae were consistently identified in these anatomic locations by use of IHC staining. Although no single lesion is pathognomonic for RMSF [30], a number of histopathologic findings are recognized and may suggest subsequent IHC evaluation for rickettsiae. Suggestive pathology observed in this study and documented in previous series includes multifocal lymphohistiocytic infiltrates in the interstitium of the kidneys [27, 31, 32], heart [33, 34], and lung [35, 36] and in the hepatic portal triads [37, 38]. Adrenal cortical necroses were identified in several patients in this series and characteristically contained abundant rickettsial antigens. Multifocal necroses may be observed in the adrenal cortices of persons dying of RMSF [39–41], and adrenal glands of these patients often contain rickettsiae [36, 38]. Skin represents the most accessible organ for histopathologic and IHC evaluation of RMSF; however, rickettsial antigens are often distributed sporadically in this tissue, and even relatively large segments of skin obtained at autopsy from patients with petechial or ecchymotic lesions may demonstrate broad areas with no demonstrable rickettsiae [39, 42, data herein].

Lesions involving the central nervous system, including multifocal glial nodules and arteriolar microinfarcts, are among the most suggestive histopathologic features of RMSF [30]. However, these findings are seldom observed in patients who die before day 11 of illness [43]. Glial nodules and arteriolar microinfarcts were observed together in a patient in this series who died on day 10 after onset; however, these lesions would probably be absent in most patients with serologically unconfirmed disease. In several of the patients in this series, typical mononuclear inflammatory lesions were minimal, yet IHC staining revealed numerous organisms. This was particularly true in the patients who died early in the course of disease, which is in agreement with previous descriptions of the pathology in patients with fulminant RMSF [41].

With remarkably few exceptions, pathologists have had difficulty demonstrating rickettsiae in patient tissues by use of conventional histochemical methods. Even tissues stained with classical stains for *Rickettsia* species (e.g., Giemsa, Macchiavellos, and Pinkerton's stains) are characteristically difficult to interpret and may produce misleading results [30]. Immunologic staining techniques for detecting *R. rickettsii* revolutionized the detection of these organisms in patient tissues and have been used in diagnostic capacities since 1976. Previous studies have emphasized the clinical utility of immunostaining of skin biopsy specimens to guide patient care in suspected cases of RMSF [44–49] or as a method to investigate the pathophysiology of RMSF by evaluating the anatomic distribution of rickettsial antigens in specific organs, including kidney [31, 32, 42], heart [33], lung [35, 36], skin [47–50], liver [37, 38], stomach, small intestine, colon, and pancreas [51]. These stains additionally represent powerful tools for use in confirming fatal spotted fever infection [39, 52, 53]. In this context, IHC staining for *R. rickettsii* may uncover otherwise undiagnosed RMSF fatalities, particularly when conventional samples (e.g., acute-phase serum and whole blood) may not be available. As an example, IHC staining has been used to diagnose a fatal rickettsial infection from archived tissues nearly a century old [53].

Abundant, intensely staining rickettsial antigens were identified in multiple tissues from all patients in this series, including tissues from all 4 patients receiving specific antirickettsial therapy. Antimicrobial therapy may diminish or even abrogate the detection of rickettsiae by IHC staining. Morphologic disruption and scarcity of rickettsiae have been observed in skin biopsy specimens after 24–48 h of tetracycline or chloramphenicol therapy [45], and positive immunostaining has not been previously described in biopsy or autopsy tissues from patients receiving >48 h of therapy [30–32, 42, 48]. In this study, discrete coccobacilli were visualized in sections from multiple organs in patients receiving ≥1 days of antirickettsial therapy, including 1 patient who received doxycycline for 72 h (figure 1A). Autopsy-derived tissues may be less susceptible to antibiotic-in-

duced false-negative reactivity because of increased numbers of rickettsiae and larger areas of tissue available for evaluation. The duration of therapy necessary to abolish immunologic detection of *R. rickettsii* in patient tissues may ultimately vary by tissue type, bacterial load, or sensitivity of the assay. However, the inability of immunostaining to detect rickettsial antigens in autopsy tissues after 5–7 days of antirickettsial therapy has been documented consistently [30–32, 35].

Epidemiologic and clinical clues should be fundamental in guiding requests for IHC testing. Reasonable contexts for use of this assay include any unexplained, fatal, febrile illness characterized by (1) occurrence of disease between early spring and late summer, particularly in recognized *R. rickettsii*–endemic regions (observed in 8/9 patients in this series), (2) a clinical course that includes thrombocytopenia in combination with a macular or petechial rash, elevated hepatic transaminase levels, or electrolyte abnormalities (observed in 7/9 patients in this series), or (3) a history of tick bite preceding the illness (observed in 5/9 patients in this series). Although lymphohistiocytic infiltrates involving small vessels are often identified in ≥1 tissues, the histopathology of fatal RMSF may be subtle or represented as nonspecific findings, such as hepatic erythrophagocytosis, adrenal necroses, or pulmonary intra-alveolar hemorrhage. The diagnostic dilemma posed by fulminant RMSF may be even greater [30]. In these settings, clinical and epidemiologic clues gleaned from the patient history and hospital course assume considerable importance for the pathologist and clinician.

An accurate count of mortality attributable to RMSF remains elusive. The CDC determines the number of deaths and the CFR for RMSF from data provided by case report forms, and underreporting of fatal cases may contribute to falsely low mortality estimates. Although RMSF is a nationally notifiable disease, many cases are missed or unreported. Prospective active surveillance for RMSF in regions where the disease is hyperendemic suggests that as many as 50% of all cases (including confirmed but unreported deaths due to RMSF) are missed by passive surveillance mechanisms (e.g., NETSS and case report forms) [54].

Phenomena independent of reporting error may also contribute to recent declines in CFRs. The requirement of rigorous laboratory criteria has understandably reduced the number of confirmed fatalities attributable to RMSF; one-third of all deaths reported during 1981–1996 were unconfirmed by laboratory assays. The effect of more stringent confirmatory criteria on the CFR is less clear, as 42% of the 9312 RMSF "cases" reported to the CDC through case report forms during 1981–1996 were classified as probable or unconfirmed. During this same interval, expanding physician awareness of *Borrelia* and *Ehrlichia* infections has resulted in broader use of antirickettsial therapies, particularly the use of doxycycline in febrile patients with a history of tick bite. Prompt administration of effective antimicrobials in misdiagnosed yet appropriately treated cases of RMSF may indirectly and fortuitously lower deaths and possibly CFRs associated with this disease.

Regardless of real and artifactual influences on RMSF death statistics, confirmation of fatal disease remains fundamental in compiling accurate mortality data. We believe that this process is greatly augmented by immunopathology. Immunostaining for spotted fever group rickettsiae is offered by the CDC and a few state health departments and university-based hospital and commercial laboratories in the United States. Increased awareness and availability of these assays will enhance efforts to better define mortality attributable to RMSF. Additionally, by diagnosing otherwise unconfirmed or unexplained deaths, immunopathology can help identify foci of endemicity for *R. rickettsii* and promote awareness, prevention, and early recognition of this severe and potentially lethal disease.

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