Case report

Rocky Mountain spotted fever in Panama: a cluster description

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Abstract

Rocky Mountain spotted fever (RMSF) is a tick-borne infection caused by *Rickettsia rickettsii*. We report a cluster of fatal cases of RMSF in 2007 in Panama, involving a pregnant woman and two children from the same family. The woman presented with a fever followed by respiratory distress, maculopapular rash, and an eschar at the site from which a tick had been removed. She died four days after disease onset. This is the second published report of an eschar in a patient confirmed by PCR to be infected with *R. rickettsii.* One month later, the children presented within days of one another with fever and rash and died three and four days after disease onset. The diagnosis was confirmed by immunohistochemistry, PCR and sequencing of the genes of *R. rickettsii* in tissues obtained at autopsy.

Key words: *Rickettsia rickettsii*; cluster analysis; Panama; Rocky Mountain spotted fever; pregnancy; Central America

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Introduction

Rocky Mountain spotted fever (RMSF) is a systemic tick-borne infection caused by *Rickettsia rickettsii,* a Gram-negative, obligate intracellular bacterium with two immunodominant cell surface proteins, protein A (OmpA) and protein B (OmpB). Serologic diagnosis is based on the identification of serum antibodies reactive to these proteins [1]. However, serology is neither sensitive nor informative during the acute phase of the illness [2], and diagnostic reagents are not widely available in Panama. Furthermore, due to cross-reactivity among *Rickettsia* species, serology is non-specific. Molecular diagnosis by amplification and sequencing of several rickettsial genes such as *omp*A, *omp*B and the citrate synthase (*glt*A*)* genes, enable species-specific identification of rickettsiae [3,4]. Unfortunately, PCR testing is performed only in specialized laboratories and is only moderately sensitive, primarily due to a very small number of rickettsiae present in human blood, even during the most severe stage of infection [5].

The early diagnosis of RMSF therefore depends heavily on clinical suspicion. Patients with fever, headache, malaise, myalgias, and rash, however, can have a variety of infections and excluding these can result in significant diagnostic delays. Untreated, RMSF has a case fatality as high as 30% [6] and timely treatment with doxycycline decreases mortality and morbidity. Tetracyclines are the drugs of choice, but during pregnancy chloramphenicol may be considered [7].

In Panama, the first cases of RMSF were reported by Rodaniche in 1950 [8] and in 1953, the tick *Amblyomma cajennense* was identified as the main vector of *R. rickettsii* in the region [9].

Subsequently there were no case reports until 2004, when a child was diagnosed with the disease [10]. In this manuscript we report a family cluster of three fatal cases that included a pregnant woman and two children in 2007.

Case reports

Case 1

In October 2007, a pregnant 22-year old female at 23 weeks' gestation presented to a medical center in Panama City, with a one-day history of fever, headache, myalgias, retro-orbital pain, and cough. She was prescribed amoxicillin and sent home. At the time of her visit to the center, a tick was removed from the internal face of her upper left leg. Two days later (three days after onset of symptoms), she was admitted to the intensive care unit in respiratory distress. An eschar (Figure 1A) was noted at the site of tick removal and she had a generalized maculopapular rash over her chest and extremities (Figure 1B). The patient was intubated and two hours later developed a bloody tracheal exudate. Tests revealed severe thrombocytopenia (32 x 10^3 platelets/mm^3); leukocytosis (12.4 x 10^3 cells/mm^3) with 97% neutrophils; hypokalemia (3.1 g/dl); hypocalcemia (6.3 mg/dl); hypoalbuminemia (2.2 g/dl); hyperbilirubinemia (8.1 mg/dl); alanine aminotranferase (AST) 112 U/l; and lactate dehydrogenase (LDH) 1517 U/l. Serologic testing was performed for *R. rickettsii*, *Leptospira*, *Treponema pallidum*, and HIV at the Hospital Santo Tomás. Molecular tests were performed for influenza, dengue and *Borrelia*. All serologic and molecular tests were negative. Bacterial blood cultures were also negative. The patient died one day after hospitalization, four days after disease onset. Autopsy findings included myocarditis, pulmonary edema, centrilobular renal necrosis, active splenic vasculitis, hepatomegaly with centrilobular hepatic ischemia and necrosis, moderate cerebral edema, and a normal fetus of adequate weight for gestational age. Formalin-fixed, paraffin-embedded spleen tissue, obtained from the mother at autopsy, was sent to ICGES and subsequently to the United States Centers for Disease Control and Prevention (CDC) for confirmatory testing. No tissue samples were taken from the fetus.

Case 2

At the end of November 2007, the niece of Case 1, a 5-year-old girl, presented with fever, nausea, and abdominal pain of three days duration. She was treated with diclofenac but due to worsening abdominal pain was taken to a pediatric hospital with suspected bacteremia. Physical examination revealed an awake, alert, but tearful girl with mild dehydration and abdominal tenderness in the right lower quadrant. She was treated with ampicillin and intravenous fluids. Several hours later, she developed petechiae on the chest, extremities and palate. Four hours later, she became disoriented with generalized petechiae, cyanosis and weakened pulse. Laboratory examination showed thrombocytopenia (15.0 x 10^3 platelets/mm^3), and leukocytosis (10.4 x 10^3 cells/mm^3) with 49% blasts and toxic granulations in the neutrophils. The patient developed a generalized tonic-clonic seizure and died, four days after disease onset. Fresh, unfixed liver and spleen samples were sent to ICGES for diagnostic evaluation.

Case 3

The three-year-old sister of Case 2 presented at the end of November with a two-day history of fever, rash and one episode of syncope. She was taken to the emergency room and pronounced dead on arrival. She died the same day as her sister, three days after disease onset. Fresh, unfixed liver and spleen samples were also sent to ICGES.

Diagnostic studies

All three cases were diagnosed as RMSF based on PCR amplification of a 401bp *glt*A fragment of *Rickettsia* from the liver and spleen samples of each patient using primers CS-78F and CS-323R and sequencing [11]. The *glt*A sequences obtained from each patient by ICGES were identical to each other (NCBI accession numbers JF739385 - JF739387) and had 100% sequence identity to the homologous fragment of the *glt*A from *R. rickettsii*. A 532-bp *omp*A fragment was also amplified from spleen and liver samples from the adult female patient using primers Rr190.70 and Rr190.602 [12] at the CDC. The restriction fragment profile of the *omp*A fragments obtained using enzymes *Pst*I and *Rsa*I was identical to that of *R. rickettsii* (Figure 1C) and the nucleotide sequences of the amplicons were identical to the homologous fragment of *R. rickettsii* (NCBI accession number JF912515). Abundant spotted fever group rickettsial antigens were identified in the spleen of the Case 1 patient (Figure 1D) by using an immunohistochemical staining technique at CDC [2]. Serologic evaluation for the presence of IgG and IgM class antibodies against *R. rickettsii* using indirect immunofluorescent assays (IFA; Focus Technologies,

Figure 1. Anatomical, molecular, and histological analyses of an adult case of Rocky Mountain spotted fever presenting in Panama

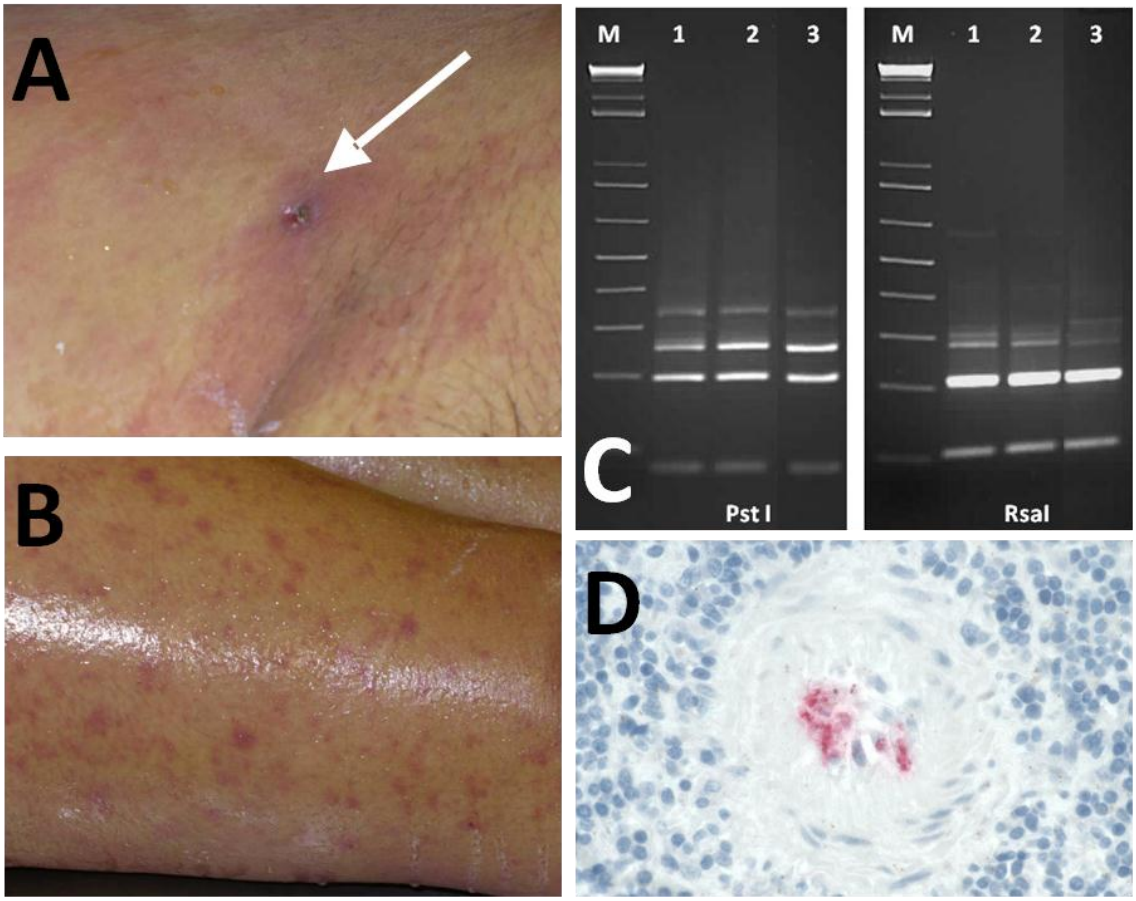

Figure 1: A) Site of the tick bite in Case 1 (left leg). Infected eschar indicated by arrow; **B)** Skin of Case 1 (post-mortem) demonstrating macular erythematous lesions on moderately jaundiced skin; **C)** Restriction fragment length polymorphism analysis of *omp*A fragments amplified from spleen and liver of patient #1. Amplified DNA was treated for 2 hr at 37°C with *Pst*I and *Rsa*I restriction endonucleases, digested specimens were loaded in the following order: lane M, 1 Kb Plus DNA ladder (InVitrogen Life Technologies); lane 1, DNA from spleen of Case 1; lane 2, DNA from liver of Case 1; lane 3, positive control DNA from *R. rickettsii*, and separated in 3% agarose gel for 1 hr; **D)** Immunohistochemical staining of spotted fever group rickettsial antigens (red) in vascular endothelial cells in the spleen of Case 1 (polyclonal anti-*R. rickettsii* antibody with immunoalkaline phosphatase and naphthol-fast red with hematoxylin counterstain, original magnification ×158).

Cypress, USA) was negative for the adult female patient at four days after disease onset. Serologic tests (EIA) for *Leptospira*, hantavirus, and dengue were also negative. Blood cultures of the children were negative for *Neisseria meningitidis*.

Discussion

In 2007, the Gorgas Memorial Institute of Health Studies (ICGES) and the CDC used molecular tools to confirm RMSF as the cause of death in three Panamanian patients. The fatal outcome of these cases was related to rapid disease progression, lack of appropriate antibiotic therapy, and the infrequency with which rickettsioses present in Panama. In Case 1, diagnosis was further complicated because more common conditions associated with pregnancy, such as HELPP syndrome, can have a similar presentation to RMSF [7].

One interesting aspect of this report is the family clustering. Case 1 was the aunt-in-law of the two young sisters and all lived in the same house, which had many openings to the external environment, domestic dogs, and a semi-rural setting. Attempts to collect ectoparasites from the dogs and house after the patients' deaths were unsuccessful as an intensive pest-eradication regime using insecticide had been used by the family. Although the parents of the two children could not recall tick bites on their daughters, there was 100% identity among the *glt*A sequences of *R. rickettsia* detected in the tissue samples from the three patients, and the deaths occurred within a month of each other. Family clusters of RMSF cases have been previously reported in many other

countries, and result when patients share environmental exposures [6].

Another important aspect of this report is the presence of an eschar in Case 1. Eschars appear to be more common in less severe rickettsioses, such as *Rickettsia parkeri* rickettsiosis [13-15]; rickettsialpox, caused by *Rickettsia akari*; 364D rickettsiosis, [15]; and *Rickettsia massiliae* rickettsiosis [16] [17]. By contrast, the occurrence of an eschar on a patient with RMSF is described rarely: the first and second cases, both fatal and diagnosed by IFA, occurred in North Carolina and Tennessee in 1981 [18]; and the third case, which represents the first molecular confirmation of an eschar-associated rickettsioses caused by *R. rickettsii*, was reported in North Carolina in 2011 [19].

The first cases of RMSF in Panama were reported in 1950 [8], and after more than 50 years of no known occurrence of RMSF, six cases were confirmed between 2004 and 2007. Two as yet unpublished cases occurred in 2006 in the community of Macano in the Province of Coclé (personal communication, Panama Ministry of Health). Cyclic fluctuations in the incidence of RMSF have been reported in the United States, with resurgences in the US and Latin America also noted during the last decade [20-23]. None of the recent Panamanian cases had travelled outside Panama, suggesting that *R. rickettsii* and its vectors are still present in the country. In Panama, the diagnosis of RMSF is performed only in specialized laboratories, contributing to under-diagnosis and reporting of the disease.

The six documented cases of RMSF in the last decade in Panama have had a case fatality rate of 100%. Given the scarcity of reports of RMSF in the region, there is a low level of awareness of the disease amongst physicians. Also, the similar presenting signs of RMSF to far more common endemic infections, such as dengue, leads to low clinical suspicion of the disease. The detection of an increasing number of fatal cases of RMSF suggests that clinicians should ask about tick bites in patients presenting with febrile illnesses in Panama and in other countries of Central America and consider early empiric therapy with doxycycline or chloramphenicol as appropriate. In suspected RMSF cases with compatible clinical manifestation, tick bite is not always evident or recollected and should not dissuade physicians from doxycycline therapy. Also, treatment should not be delayed until laboratory test results are available.

Increased awareness of RMSF and referral of suspected cases for testing is necessary to improve surveillance of the disease in Panama, particularly as glucose-6-phosphate dehydrogenase deficiency, a genetic condition which accelerates the progression of fulminant RMSF [24], is commonly reported in Panama [25]. It is also extremely important to promote public awareness about the potential health consequences of tick bites, as one of the most effective measures against RMSF is close inspection of the head, body and clothes after exposure to wooded and rural areas and careful removal of any attached ticks with tweezers [2].

Disclaimer

The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention.

References

1. Dantas-Torres F (2007) Rocky Mountain spotted fever. Lancet Infect Dis 7: 724-732.
2. Paddock CD, Greer PW, Ferebee TL, Singleton J, Jr., McKechnie DB, Treadwell TA, Krebs JW, Clarke MJ, Holman RC, Olson JG, Childs JE, Zaki SR (1999) Hidden mortality attributable to Rocky Mountain spotted fever: immunohistochemical detection of fatal, serologically unconfirmed disease. J Infect Dis 179: 1469-1476.
3. Chapman AS, Bakken JS, Folk SM, Paddock CD, Bloch KC, Krusell A, Sexton DJ, Buckingham SC, Marshall GS, Storch GA, Dasch GA, McQuiston JH, Swerdlow DL, Dumler SJ, Nicholson WL, Walker DH, Eremeeva ME, Ohl CA (2006) 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 55: 1-27.
4. Fenollar F, Raoult D (2004) Molecular genetic methods for the diagnosis of fastidious microorganisms. APMIS 112: 785-807.
5. Kaplowitz LG, Lange JV, Fischer JJ, Walker DH (1983) Correlation of rickettsial titers, circulating endotoxin, and clinical features in Rocky Mountain spotted fever. Arch Intern Med 143: 1149-1151.
6. Levy C, Burnside J, Tso T, Englender S, Auslander M, Billings S, Bradley K, Bos J, Burnsed L, Brown J, Mahoney D, K Chamberlain, Porter M, Duncan C, Johnson B, Ethelbah R, K Robinson, Wessel M, Savoia S, Garcia C, Dickson J, Kvamme D, Yost D, Traeger M, Krebs J, Paddock C, Shieh W, Guarner J, Zaki S, Swerdlow D, McQuiston J, Nicholson W, Demma L, Burnside J, Tso T, Englender S, Auslander M, Billings S, Bradley K, Bos J, Burnsed L, Brown J, Mahoney D, K Chamberlain, Porter M, Duncan C, Johnson B, Ethelbah R, K Robinson, Wessel M, Savoia S, Garcia C, Dickson J, Kvamme D, Yost D, Traeger M, Krebs J, Paddock C, Shieh W, Guarner J, Zaki S, Swerdlow D, McQuiston J, Nicholson W, Demma L (2004) Fatal cases of Rocky Mountain spotted fever in family clusters--three states, 2003. MMWR Morb Mortal Wkly Rep 53: 407-410.

7. Stallings SP (2001) Rocky Mountain spotted fever and pregnancy: a case report and review of the literature. Obstetrical and gynecological survey 56: 37-42.
8. De Rodaniche EC, Rodaniche A (1950) Spotted fever in Panama; isolation of the etiologic agent from a fatal case. Am J Trop Med Hyg 30: 511-517.
9. De Rodaniche EC (1953) Natural infection of the tick, Amblyomma cajennense, with Rickettsia rickettsii in Panama. Am J Trop Med Hyg 2: 696-699.
10. Estripeaut D, Aramburu MG, Saez-Llorens X, Thompson HA, Dasch GA, Paddock CD, Zaki S, Eremeeva ME (2007) Rocky Mountain spotted fever, Panama. Emerg Infect Dis 13: 1763-1765.
11. Labruna MB, Whitworth T, Horta MC, Bouyer DH, McBride JW, Pinter A, Popov V, Gennari SM, Walker DH (2004) Rickettsia species infecting Amblyomma cooperi ticks from an area in the state of Sao Paulo, Brazil, where Brazilian spotted fever is endemic. J Clin Microbiol 42: 90-98.
12. Regnery RL, Spruill CL, Plikaytis BD (1991) Genotypic identification of rickettsiae and estimation of intraspecies sequence divergence for portions of two rickettsial genes. J Bacteriol 173: 1576-1589.
13. Romer Y, Seijo AC, Crudo F, Nicholson WL, Varela-Stokes A, Ryan-Lash R, Paddock CD (2011) Rickettsia parkeri Rickettsiosis, Argentina. Emerg Infect Dis 17.
14. Conti-Diaz IA, Moraes-Filho J, Pacheco RC, Labruna MB (2009) Serological evidence of Rickettsia parkeri as the etiological agent of rickettsiosis in Uruguay. Rev Inst Med Trop Sao Paulo 51: 337-339.
15. Cragun WC, Bartlett BL, Ellis MW, Hoover AZ, Tyring SK, Mendoza N, Vento TJ, Nicholson WL, Eremeeva ME, Olano JP, Rapini RP, Paddock CD (2010) The expanding spectrum of eschar-associated rickettsioses in the United States. Arch Dermatol 146: 641-648.
16. Garcia-Garcia JC, Portillo A, Nunez MJ, Santibanez S, Castro B, Oteo JA (2010) A patient from Argentina infected with Rickettsia massiliae. Am J Trop Med Hyg 82: 691-692.
17. Vitale G, Mansuelo S, Rolain JM, Raoult D (2006) Rickettsia massiliae human isolation. Emerg Infect Dis 12: 174-175.
18. Walker DH, Gay RM, Valdes-Dapena M (1981) The occurrence of eschars in Rocky Mountain spotted fever. J Am Acad Dermatol 4: 571-576.
19. Breitschwerdt EB, Hegarty BC, Maggi RG, Lantos PM, Aslett DM, Bradley JM (2011) *Rickettsia rickettsii* transmission by a Lone Star Tick, North Carolina. Emerg Infect Dis 17: 873-875.
20. Galvao MA, Dumler JS, Mafra CL, Calic SB, Chamone CB, Cesarino Filho G, Olano JP, Walker DH (2003) Fatal spotted fever rickettsiosis, Minas Gerais, Brazil. Emerg Infect Dis 9: 1402-1405.
21. Hidalgo M, Miranda J, Heredia D, Zambrano P, Vesga JF, Lizarazo D, Mattar S, Valbuena G (2011) Outbreak of Rocky Mountain spotted fever in Cordoba, Colombia. Mem Inst Oswaldo Cruz 106: 117-118.
22. Openshaw JJ, Swerdlow DL, Krebs JW, Holman RC, Mandel E, Harvey A, Haberling D, Massung RF, McQuiston JH (2010) Rocky Mountain spotted fever in the United States, 2000-2007: interpreting contemporary increases in incidence. Am J Trop Med Hyg 83: 174-182.
23. Paddock CD, Fernandez S, Echenique GA, Sumner JW, Reeves WK, Zaki SR, Remondegui CE (2008) Rocky Mountain spotted fever in Argentina. Am J Trop Med Hyg 78: 687-692.
24. Walker DH, Hawkins HK, Hudson P (1983) Fulminant Rocky Mountain spotted fever. Its pathologic characteristics associated with glucose-6-phosphate dehydrogenase deficiency. Arch Pathol Lab Med 107: 121-125.
25. de Gurrola GC, Arauz JJ, Duran E, Aguilar-Medina M, Ramos-Payan R, Garcia-Magallanes N, Pacheco GV, Meraz EA (2008) Kernicterus by glucose-6-phosphate dehydrogenase deficiency: a case report and review of the literature. J Med Case Reports 2: 146.

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