



Diagnostic Testing for Rocky Mountain Spotted Fever: Unraveling the Uncertainty

Introduction

Georgia is endemic for Rocky Mountain spotted fever (RMSF), a tickborne disease of varying presentation and severity. Often, non-specific clinical presentation and lack of known tick bite make diagnosis difficult. Patients initially present with a non-specific flu-like illness of 3 or 4 days duration, with no classical diagnostic signs and symptoms. Beyond the challenge of recognizing a case based on clinical manifestation and history of tick exposure, there is confusion about which diagnostic tests to order and how to interpret the results. Not all available tests are appropriate for confirmation of RMSF, and timing of tests should be considered in their interpretations.

Clinical Presentation

Rocky Mountain spotted fever is characterized by fever (usually over 102°F), headache (sometimes severe), and a maculopapular or petechial rash that begins at the extremities (characteristically involving the palms and soles) and spreads to the trunk. Malaise, myalgias, nausea, vomiting, abdominal pain, and conjunctivitis are also common. However, the classic diagnostic triad of fever, rash, and history of tick exposure is present in only a very small proportion of patients during the first three days of illness, when patients are likely to first seek medical care (1). Additionally, only 88% of patients eventually develop a rash, so absence of rash should not rule out the diagnosis of RMSF (1). Rash may be difficult to observe with darker skin tones. Severe complications include disseminated intravascular coagulation, adult respiratory distress syndrome, skin necrosis, renal impairment, hypotension, altered mental status, myocarditis, seizures, coma, and even death. Differential diagnoses include gastroenteritis, measles, scarlet fever, ehrlichiosis, Lyme disease, leptospirosis, meningococcemia, Epstein-Barr virus infection, cytomegalovirus infection, toxic shock syndrome, and bacterial sepsis. In the absence of proper and timely therapy, the RMSF mortality rate exceeds 20% and death can occur within 8 to 15 days of onset (2).

Treatment

Doxycycline is the treatment of choice for all patients with RMSF, including young children. Chloramphenicol is appropriate for treatment of RMSF when tetracyclines are contraindicated (as in pregnancy), but studies have shown that patients treated with chloramphenicol are more likely to die from RMSF than those treated with a tetracycline (3). An infectious disease consult may be necessary, as chloramphenicol is unavailable in oral form in the United States and can cause severe adverse effects. Treatment

should continue for 7 to 10 days, or at least 3 days after fever subsides. Empiric therapy is indicated for any patient suspected of having RMSF, but prophylactic treatment after a tick bite before symptoms develop is not recommended (4). Treatment should never be delayed while awaiting development of a rash or laboratory results; delay in treatment has been associated with severe and fatal cases (4).

Laboratory Testing

Testing for RMSF is not indicated in the absence of a compatible clinical syndrome. A presumptive diagnosis can be made and treatment given based on clinical and epidemiologic findings without waiting for laboratory results. A confirmed diagnosis cannot be made without supporting laboratory results. Cases diagnosed without laboratory findings do not meet the national surveillance case definition and are not included in public health statistics.

The most commonly used serologic method, and the gold standard for diagnosing RMSF, is the IgG indirect fluorescence antibody (IFA) test on paired sera taken 3 weeks apart. The first serum specimen should be collected upon first presentation to a healthcare provider (during the first week of illness). This may be tested immediately, but the first specimen is often negative and thus uninformative. Alternatively, this specimen should be appropriately stored (refrigerated at 4°C or frozen in a non-frost-free freezer) until 3 weeks later when the second serum specimen is drawn. Both specimens can be sent together to the laboratory for IgG testing by IFA. It is sometimes necessary to send a third sample taken 6 weeks after disease onset to demonstrate seroconversion. A single negative acute specimen does not rule out RMSF, but is often misinterpreted as such, and in these cases a second specimen is never drawn. Sending both specimens together eliminates this propensity and the two sera can be assayed at the same time, eliminating interassay variation that can provide misleading results. Another note is that convalescent specimens drawn several weeks or months following recovery are not useful, thus every attempt must be made to collect them in a timely manner.

Other widely available tests include IgM antibody testing by IFA or enzyme-linked immunosorbent assay (ELISA) for IgM or IgG. We do not recommend IgM testing for rickettsial diseases, especially in the absence of IgG testing, as false positives and false negatives are common. Unlike viral testing, the IgM test

does not provide earlier detection of antibodies to rickettsiae as IgM and IgG antibodies develop around the same time and can both be negative in the first week of illness. In addition, IgM antibodies can remain elevated for many weeks or months, so detection of IgM antibodies cannot be used to signify etiology of an acute illness. In recent years, use of an ELISA test for IgG or IgM has become more commonly employed by large commercial laboratories. The ELISA tests currently available are qualitative tests, and although results are reported as a number, they can only be interpreted as “positive”, “equivocal”, or “negative”. Therefore, convalescent testing is unable to show a 4-fold change as required for confirmation of RMSF. Furthermore, the ELISA tests have not been adequately validated on a sufficient number of samples. Once they have been validated, they may still only be appropriate for use as a screening tool as the results are qualitative, not quantitative. Then those screened positive by ELISA should be tested by IFA to determine a relative level of anti-rickettsial antibodies.

For patients with rash who have not begun antibiotic therapy, acute diagnosis is achievable by use of polymerase chain reaction (PCR) assay of a skin punch biopsy from the rash site. Immunohistochemistry (IHC) may also be used to demonstrate the organisms within the endothelium of affected tissues. If the patient does not have a rash, these tests are not useful. PCR and IHC are also confirmatory on autopsied tissues of the lung,

Key Points about Laboratory Testing

- Order IgG IFA testing. IgM tests provide presumptive, never confirmatory, evidence of infection and should be interpreted as such.
- Serologic testing **MUST** occur among paired sera, 3 weeks apart. This has been the gold standard for many years and works well.
- The best test to diagnose an acutely ill RMSF patient at the time of presentation is PCR of a rash biopsy. Paired serologic tests should accompany any adjunct testing.

liver, spleen, kidney, brain, whole blood, or skin from patients who have died of presumptive RMSF. PCR and IHC are not widely available, but can be arranged by contacting GDPH at 404-657-2588.

How to Report a Case of RMSF

The national surveillance case definition has undergone revision in recent years, and will likely be modified to address laboratory diagnostic issues again in the near future. The surveillance case definition is a tool used by Public Health to capture actual cases of disease while excluding as many non-cases as possible. This case definition should not be used to diagnose individual patients. RMSF is reportable to Public Health within 7 days of diagnosis. To report a case electronically, log on to Georgia’s State Electronic Notifiable Disease Surveillance System (SENDSS) at <http://sendss.state.ga.us>. Alternatively, complete a Notifiable Disease Report Form (form 3095) and mail to your County Health Department, District Health Office, or State Health Office. When reporting cases of RMSF, be sure to include clinical signs and symptoms in addition to laboratory results, as clinical compatibility is a requirement of the surveillance case definition.

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Important Things You Did Not Know About RMSF

- Very few RMSF cases (3%) have fever, rash, AND history of tick bite upon first presentation to a healthcare provider.
- Many RMSF cases never develop a rash, so absence of rash does NOT rule out RMSF.
- Empiric antibiotic therapy is the rule for anyone suspected of having RMSF. Never wait for lab results!
- Prophylactic antibiotic therapy is NOT recommended after a tick bite in the absence of tickborne disease symptoms.
- Doxycycline is the antibiotic treatment of choice, even in young children.
- Pharyngitis is a sign of possible RMSF; the patient has the rash in his/her throat. Doctors have misdiagnosed this as strep throat and people have died as a result.

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Recommended Reading

1. CDC. Diagnosis and management of tick-borne 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* 2006;55(No.RR-4): 1-29. <http://www.cdc.gov/mmwr/preview/mmwrhtml/rr5504a1.htm>
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*The findings and conclusions are those of the authors and do not necessarily represent the views of the Centers for Disease Control and Prevention.

Tick Surveillance Notes: *Rickettsia rickettsii* in *Rhipicephalus sanguineus* Ticks from Gordon County

Rickettsia rickettsii, the agent of Rocky Mountain spotted fever (RMSF), is most commonly found in *Dermacentor* spp. ticks, specifically *Dermacentor variabilis* (the American dog tick) in Georgia. Although recognized as an efficient experimental vector, *Rhipicephalus sanguineus* (the brown dog tick) was not implicated as a vector of human disease in the United States until 2004 (1). This short communication is the first report of a *R. rickettsii*-infected *Rb. sanguineus* tick in Georgia.

In May 2005, tick dragging was conducted by the Georgia Division of Public Health in cooperation with the Northwest Georgia Health District and the Gordon County Health Department in response to a complaint of large numbers of ticks in a Gordon County homeowner's yard. The ticks were identified as *Rb. sanguineus* (the brown dog tick) and submitted to the Rickettsial Zoonoses Branch, Division of Viral and Rickettsial Diseases, Centers for Disease Control and Prevention for testing. Of eight adult ticks collected and preserved in isopropanol, one female and two male *Rb. sanguineus* tested positive for spotted fever group rickettsial DNA using a quantitative SYBR-Green PCR assay (2). One of the males was found to be infected with *R. rickettsii* by amplification and

restriction analysis of a 602-bp fragment of the rOmpA species specific rickettsial gene. The identity of the spotted fever group rickettsiae in the female and the other male tick was not determined due to a low copy number of rickettsial DNA in those ticks.

The importance of *Rb. sanguineus* ticks in the maintenance and transmission cycles of *R. rickettsii* in Georgia is unknown. In general, *R. rickettsii* prevalence in the expected vector, *D. variabilis*, is <1%. However, in Gordon County, prevalence in *Rb. sanguineus* was 12.5%, which is quite high. Gordon County experienced an unusually high number of RMSF cases in 2005 (n=7), which can be at least partially attributed to a fatal RMSF case in a Gordon County child that year resulting in increased awareness and testing. Whether *Rb. sanguineus* ticks played a part in this increase is unknown. *Rhipicephalus sanguineus* ticks are characterized by their tendency to create large infestations in areas frequented by dogs. If you suspect RMSF in a dog owner who describes a large tick infestation in their yard, please call the Georgia Division of Public Health at 404-657-2588.

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Notice: End of Tick Attach Study

The Tick Attach Study was conducted by the Georgia Division of Public Health (GDPH), the University of Georgia, and the Georgia Poison Center between April 2005 and December 2006. Moving forward, GDPH cannot accept ticks for testing except in rare cases (call 404-657-2588 for more information). However, we can identify ticks. Instructions for submitting a tick for identification can be found on our website at <http://www.health.state.ga.us/epi/vbd/tick.asp>. There is a short form (PDF) that should be completed and sent with the tick. Thanks to all who contributed ticks to the Tick Attach Study. Look for study results in an upcoming Georgia Epidemiology Report.



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Reported Cases of Selected Notifiable Diseases in Georgia, Profile* for January 2007

Selected Notifiable Diseases	Total Reported for January 2007	Previous 3 Months Total Ending in January			Previous 12 Months Total Ending in January		
	2007	2005	2006	2007	2005	2006	2007
Campylobacteriosis	37	122	98	107	586	592	578
<i>Chlamydia trachomatis</i>	3930	7946	9087	9877	34356	34181	39096
Cryptosporidiosis	16	21	47	51	172	166	284
<i>E. coli</i> O157:H7	1	7	1	3	25	28	45
Giardiasis	37	212	162	124	911	723	676
Gonorrhea	1540	3936	4351	4433	16026	16259	19281
<i>Haemophilus influenzae</i> (invasive)	13	42	32	39	125	107	121
Hepatitis A (acute)	10	41	12	16	287	116	58
Hepatitis B (acute)	10	113	35	34	444	177	186
Legionellosis	2	7	9	12	45	38	39
Lyme Disease	0	0	1	1	10	7	7
Meningococcal Disease (invasive)	3	7	5	8	16	14	21
Mumps	0	0	1	0	2	2	4
Pertussis	3	15	8	7	34	47	29
Rubella	0	0	0	0	1	0	0
Salmonellosis	113	334	341	376	1933	1953	1906
Shigellosis	90	129	193	437	630	701	1422
Syphilis - Primary	2	34	30	15	122	131	105
Syphilis - Secondary	7	137	117	52	498	526	399
Syphilis - Early Latent	6	66	97	37	373	415	300
Syphilis - Other**	17	219	227	108	887	983	833
Syphilis - Congenital	0	1	2	1	6	4	7
Tuberculosis	27	133	113	108	531	494	510

* The cumulative numbers in the above table reflect the date the disease was first diagnosed rather than the date the report was received at the state office, and therefore are subject to change over time due to late reporting. The 3 month delay in the disease profile for a given month is designed to minimize any changes that may occur. This method of summarizing data is expected to provide a better overall measure of disease trends and patterns in Georgia.

** Other syphilis includes latent (unknown duration), late latent, late with symptomatic manifestations, and neurosyphilis.

AIDS Profile Update
HIV/AIDS UPDATE (by Date of Report)

Report Period	Disease Classification	Total Cases Reported*			Percent Female	Risk Group Distribution						Race Distribution			
		<13yrs	>=13yrs	Total		MSM	IDU	MSM&IDU	HS	Unknown	Perinatal	White	Black	Hispanic	Other
Latest 12 Months**:	HIV, non-AIDS	17	2,924	2,941	45	31	8	2	16	42	0	21	74	4	1
3/06-2/07	AIDS	10	2,090	2,100	28	33	8	2	13	44	0	22	72	6	<1
Five Years Ago:	HIV, non-AIDS	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3/02-2/03	AIDS	2	1,583	1,585	26	39	9	3	16	32	-	20	74	5	1
Cumulative:	HIV, non-AIDS	243	10,079	10,322	34	30	8	3	12	47	2	22	74	3	1
07/81-2/07	AIDS	274	36,366	36,640	20	44	15	5	14	21	0	31	66	3	<1

Yrs - Age at diagnosis in years **MSM - Men having sex with men** **IDU - Injection drug users** **HS - Heterosexual**

* Case totals are accumulated by date of report to the Epidemiology Section ** Due to a change in the surveillance system, case counts may be artificially low during this time period

***HIV, non-AIDS was not collected until 12/31/2003