Rickettsia sibirica mongolitimonae Infection, France, 2010–2014

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To further characterize human infections caused by *Rickettsia sibirica mongolitimonae*, we tested skin biopsy and swab samples and analyzed clinical, epidemiologic, and diagnostic characteristics of patients with a rickettsiosis. The most common (38%) indigenous species was *R. sibirica mongolitimonae*. Significantly more cases of *R. sibirica mongolitimonae* infection occurred during spring and summer.

Tickborne rickettsioses are zoonoses caused by spotted fever group (SFG) *Rickettsia* spp. (*1*). The first human infection with *R. sibirica mongolitimonae* was reported in France in 1996 (*2*). This patient had rope-like lymphangitis from the eschar to the draining lymph node, and *R. sibirica mongolitimonae* infection was thus named lymphangitis-associated rickettsiosis (*3*,*4*). Since then, other cases with or without rope-like lymphangitis have been described (*5*). Several SFG rickettsioses that have been considered nonpathogenic for decades are now associated with human infections, making these diseases useful as a paradigm for understanding emerging and reemerging infections (*6*). To further characterize human infections caused by *R. sibirica mongolitimonae*, we tested skin biopsy and swab samples and analyzed the clinical, epidemiologic, and diagnostic characteristics of patients with a rickettsiosis.

The Study

During 2010–2014, we tested skin biopsy (*7*) and cutaneous swab samples from rickettsiosis inpatients and outpatients throughout France. These samples were received frozen or in transport media; when possible, serum samples were also collected and sent at room temperature. For patients with positive *Rickettsia* results, epidemiologic and clinical data were collected.

We extracted total genomic DNA from samples by using a QIAamp tissue kit (QIAGEN, Hilden, Germany). We screened samples for *Rickettsia* spp. by using a quantitative PCR assay selective for a 109-bp fragment of a hypothetical protein (*8*). For positive samples, PCR amplification and sequencing selective for the *glt*A and *omp*A genes were performed (*8*). Samples were cultured in human embryonic lung fibroblasts (*9*). All serum samples were tested by immunofluorescence assay for SFG rickettsial antigens and typhus group rickettsiae (*10*). Student *t* or χ^2 tests were performed by using Epi Info version 6.0 software (Centers for Disease Control and Prevention, Atlanta, GA, USA). Means were compared by using analysis of variance or the Kruskal-Wallis test, on the basis of results of the Bartlett test for inequality of population variances. Proportions were compared by using the Mantel-Haenszel χ^2 or Fisher exact tests when the expected value of a cell was <0.05. *R. sibirica mongolitimonae* seasonality was assessed by using the autocorrelation module of PASW software version 17.02 (http://www.spss.com.hk/statistics/). $p < 0.05$ was considered significant.

We classified patients as definitively having a rickettsiosis if direct evidence of rickettsial infection was found on culture or molecular assays. Of 465 patients examined, 91 (20%) were infected with *Rickettsia* spp., most commonly *R. africae* (n = 36, 40%), followed by *R. conorii* (n = 21, 23%), *R. sibirica mongolitimonae* (n = 20, 22%), and *R. slovaca* (n = 14, 15%). Two cases of *R. sibirica mongolitimonae* infection in France have been reported (*11*,*12*).

For patients infected with *R. sibirica mongolitimonae*, median age ± SD (interquartile range) was 43 ± 21 (2–70) years, and most (12, 60%) were male (online Technical Appendix Table 1, http://wwwnc.cdc.gov/EID/article/22/5/14-1989-Techapp1.pdf). The most common *Rickettsia* species in France was *R. sibirica mongolitimonae.* Only 1 patient mentioned recent travel to Spain; all others denied recent travel. Five patients mentioned recent outdoor activities, 8 mentioned frequent contact with dogs, and 1 mentioned contact with horses. A tick bite or tick handling was reported by 6 patients. An autocorrelation analysis revealed significant seasonality for *R. sibirica mongolitimonae* cases (p<0.001). Significantly more cases occurred during spring (April–June) (11 cases, 55%; p = 0.006), followed by summer (July–September) (8 cases, 40%, p = 0.01). One case occurred in October and none in winter.

The symptoms at disease onset included fever for all patients (duration 4–14 days), myalgia (n = 11, 55%), and headache (n = 3, 15%). Generalized maculopapular rash and an inoculation eschar developed in all patients. One patient had 3 eschars (buttocks, right hand, breast). A rope-like lymphangitis from the eschar to the draining lymph node was detected in 7 (35%) patients. One patient was admitted to an intensive care unit. For all 5 patients for whom an initial laboratory examination was available, increased liver enzymes (alanine aminotransferase, aspartate aminotransferase) and thrombocytopenia were found; 2 patients had hypoproteinemia. Oral doxycycline (7–14 days) was given to 19 patients; pristinamycin (7 days) was given to 1 patient. All outcomes were successful.

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Table. Epidemiologic and clinical characteristics of the main spotted fever group rickettsioses identified at the Unité de Recherche sur les Maladies Infectieuses et Tropicales Émergentes, Marseilles, France, 2010–2014*

Characteristic	*Rickettsia africae*	*R. conorii*	*R. slovaca*	*R. sibirica mongolitimonae*
No. cases	36	21	14	20
Geographic location	Zimbabwe and South Africa	Algeria, France, Morocco, Portugal, South Africa	France	France, Spain
Median age ± SD (IQR), y	58 ± 12 (31–80)	53 ± 18 (10–80)	36 ± 23 (6–65)	43 ± 21 (2–70)
Female sex	14 (39)	7 (33)	9 (64)	8 (40)
Recent travel	36	17	0	1
Clinical signs				
Fever	35 (97)	21 (100)	5 (36)	20 (100)
Rash	24 (67)	20 (95)	3 (21)	19 (95)
Enlarged lymph nodes	15 (42)	3 (14)	14 (100)	12 (60)
Lymphadenopathy location				
Cervical	1 (3)	3 (14)	14 (100)	5 (25)
Inguinal	14 (39)	0	0	3 (15)
Axillary	0	0	0	4 (20)
Eschar	36 (100)	18 (86)	14 (100)	20 (100)
Multiple eschars	13 (36)	0	0	3 (15)
Eschar location				
Scalp	0	2 (10)	14 (100)	0
Lower limbs	32 (89)	3 (14)	0	7 (33)
Upper limbs	2 (6)	2 (10)	0	4 (20)
Trunk	2 (6)	5 (24)	0	3 (15)
Neck	0	0	0	4 (20)
Lymphangitis	0	0	0	7 (35)
Treatment (duration, d)				
Doxycycline	34 (1–20)	21 (7–21)	12 (1–7)	19 (7–14)
Amoxicillin	2 (7)	None	None	None
Pristinamycin	None	1 (7)	None	1 (7)
Azithromycin	None	None	2 (4)	None

*Values are no. (%) patients unless otherwise indicated. IQR, interquartile range.

An eschar swab sample was available for 13 patients (*13*), and a skin biopsy sample was available for 10; all samples were positive for *R. sibirica mongolitimonae*. An acute-phase serum sample was also available for 13 patients; results of serologic testing were positive for only 2 (15%). A convalescent-phase serum sample was available from 5 patients; results were positive for 4 (80%). A skin biopsy sample was also positive for *R. sibirica mongolitimonae* by culture.

Statistical comparison of the 4 rickettsioses (Table) showed that a recent travel history was more common among patients with *R. africae* infection ($p<0.001$). *R. slovaca* infection was associated with absence of fever or rash ($p<0.001$ for each). Multiple eschars were associated with *R. africae* infection ($p<0.001$). An eschar on the neck was a characteristic of infection with *R. sibirica mongolitimonae* ($p = 0.002$); on the scalp, *R. slovaca* ($p<0.001$); on the trunk, *R. conorii* ($p = 0.05$); and on the lower limbs, *R. africae* ($p<0.001$). For patients with rope-like lymphangitis, the probability of *R. sibirica mongolitimonae* infection was 100% ($p<0.001$). Cervical lymphadenitis was associated with *R. slovaca* ($p<0.001$), inguinal lymphadenitis with *R. africae* ($p<0.001$), and axillary lymphadenitis with *R. sibirica mongolitimonae* infection ($p = 0.01$).

Conclusions

R. sibirica mongolitimonae is considered a rare pathogen; only 30 cases of infection with this organism have been reported in Europe and Africa (online Technical Appendix Table 2), of which 11 patients had lymphangitis, 27 inoculation eschars, and 18 a rash. In agreement with previous authors, we found that the most common signs of *R. sibirica mongolitimonae* infection were fever and rash. The addition of rope-like lymphangitis cases to those in the literature revealed that 17 (35%) of patients with *R. sibirica mongolitimonae* infection had this manifestation. Ramos et al. proposed that the term lymphangitis-associated rickettsiosis may be unwarranted for *R. sibirica mongolitimonae* infection because it is not found in all patients infected with this organism and because other rickettsioses produce lymphangitis (*14*). However, only *R. sibirica mongolitimonae* infection is associated with rope-like lymphangitis extending from the eschar to the draining lymph node; to our knowledge, only 1 case of mild, local, but not rope-like lymphangitis in a patient with *R. africae* infection has been described (*15*). In accordance with previous reports from France and Spain (*3*,*14*), we found that *R. sibirica mongolitimonae* infection was seasonal and that most cases occurred in the spring and summer.

Our strategy for diagnosing *Rickettsia* spp. infection on the basis of skin biopsy and cutaneous swab samples modified our knowledge of the epidemiology of SFG rickettsioses in France. We provide evidence that *R. sibirica mongolitimonae* infection is a frequent rickettsiosis, probably more frequent than *R. conorii* infection, which for decades has been considered the most common *Rickettsia* species in France.

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Expanding Distribution of Lethal Amphibian Fungus *Batrachochytrium salamandrivorans* in Europe

Technical Appendix

Technical Appendix Table 1. Field sites where *Bsal* was detected, sampled species, numbers of *Bsal*-positive and total sampled specimens*

Site no., location, and amphibian collected	No. *Bsal*-positive/total tested (year)	Observed prevalence (Bayesian 95% credible intervals)	Remarks
The Netherlands			
1, Bunderbos, deciduous forest			
Fire salamander	3/3 (2010)	1.00 (0.42–1.00)	Past mass deaths; 99.9% population decline (1997–2014)
	1/1 (2011)	1.00	
	1/1 (2012)	1.00	
	0/3 (2014)	0 (0–0.61)	
	2/14 (2015)	0.14 (0.04–0.40	
	0/1 (2016)	0	
Alpine newt	1/1 (2013)	1.00	Possibly declining (monitoring started in 2013)†
	1/39 (2014)	0.03 (0.01–0.13)	
	1/10 (2015)	0.10 (0.02–0.43)	
	0/6 (2016)	0 (0–0.43)	
2, Putberg, deciduous forest			
Smooth newt	0/2 (2014)	0 (0–0.70)	Possibly declining†
Alpine newt	0/10 (2014)	0 (0–0.31)	Possibly declining†
	1/1‡ (2014)	1.00	
	1/1‡ (2015)	1.00	
3, Meerssen, garden pond			
Fire salamander	0/1 (2015)	0	No evidence of decline§
Smooth newt	4/43 (2015)	0.09 (0.04–0.21)	No evidence of decline§
Alpine newt	0/9 (2015)	0 (0–0.30)	No evidence of decline§
4, Wormdal, clusters of natural ponds in nature conservation area¶			
Smooth newt	1/22 (2015)	0.05 (0.01–0.21)	87% decline(2000–2013)†#
Alpine newt	0/12 (2015)	0 (0–0.26)	96% decline (2000–2013)†#
5, Pepinusbeekdal, extensive agriculture			
Smooth newt	1/2‡ (2014)	0.50 (0.09–0.88)	No evidence of decline†
6, Berg en Dal, garden pond			
Alpine newt	12/12‡ (2015)	1.00 (0.74–1.00)	Yearly mass deaths; species still present§
7, Vijlenerbosch, deciduous forest			
Alpine newt	0/1 (2013)	0	No evidence of decline§
	0/30 (2014)	0 (0–0.11)	
	1/18 (2015)	0.05 (0.02–0.24)	
Smooth newt	0/8 (2014)	0 (0–0.31)	No evidence of decline§
	0/11 (2015)	0 (0–0.26)	
Palmate newt	0/1 (2014)	0	No evidence of decline§
	0/9 (2015)	0 (0–0.30)	
Belgium			
8, Eupen, deciduous forest			
Fire salamander	1/2 (2013)	0.50 (0.09–0.88)	Deaths, probably fire salamanders severely declining, no monitoring trend available
9, Robertville, deciduous forest			
Fire salamander	16/30 (2014)	0.53 (0.36–0.69)	Deaths, severe decline, monitoring ongoing
10, Liège, deciduous forest			

Site no., location, and amphibian collected	No. *Bsal*-positive/total tested (year)	Observed prevalence (Bayesian 95% credible intervals)	Remarks
Fire salamander	5/5 (2014)	1.00 (0.55–.00)	Deaths
11, Duffel, garden pond			
Alpine newt	2/30‡ (2015)	0.07 (0.02–0.22)	2 dead in fyke; no evidence of decline
Smooth newt	0/16 (2015)	0 (0–0.20)	No deaths; no evidence of decline
Germany			
12, Weisse Wehe, deciduous forest			
Fire salamander	4/11‡ (2015)	0.36 (0.15–0.65)	No evidence of decline†
13, Solchbachtal, mixed forest			
Fire salamander	0/2 (2014)	0 (0–0.70)	Decreased newts and salamanders§
	1/51 (2015)	0.02 (0.01–0.10)	
Palmate newt	0/19(2014)	0 (0–0.18)	Decreased newts and salamanders§
Alpine newt	0/5(2014)	0 (0–0.44)	Decreased newts and salamanders§
14, Belgenbachtal, mixed forest			
Fire salamander	21/22‡ (2015)	0.96 (0.79–0.99)	Remarkable deaths (16 dead), noted only since Nov 2015†

**Bsal, Batrachochytrium salamandrivorans.* Data provide an overview of novel information and previously published data. Site numbers correspond to those on map (Figure).
†Population monitored.
‡Includes individual(s) found dead by chance.
§Anecdotal reports.
¶At this site, crested newts and smooth newts decreased with similar percentages over the same period (−96%; −94%, respectively).
#http://www.ravon.nl/EID_SI_Spitzen_et_al_2016.

Technical Appendix Table 2. Field sites studied where *Bsal* was not detected, number of sampled species and specimens*

Site no., location, and amphibian collected	Number of specimens tested (year)	Observed prevalence (Bayesian 95% credible intervals)	Remarks
Belgium			
15, Nerenbos, deciduous forest			
Fire salamander	30 (2015)	0 (0–0.11)	No evidence of decline†
16, Heilig Geestgoed, deciduous forest			
Fire salamander	30 (2015)	0 (0–0.11)	No evidence of decline†
17, Kasteel van Horst, deciduous forest			
Fire salamander	30 (2015)	0 (0–0.11)	No evidence of decline†
18, Smetledebos, deciduous forest			
Fire salamander	30 (2015)	0 (0–0.11)	No evidence of decline†
19, Kluisbos, deciduous forest			
Fire salamander	30 (2015)	0 (0–0.11)	No evidence of decline†
20, Hallerbos, deciduous forest			
Fire salamander	30 (2015)	0 (0–0.11)	No evidence of decline†
21, Buggenhoutbos, deciduous forest			
Fire salamander	30 (2015)	0 (0–0.11)	No evidence of decline†
22, Raspaillebos, deciduous forest			
Fire salamander	30 (2015)	0 (0–0.11)	No evidence of decline†
23, Haeyesbos, deciduous forest			
Fire salamander	30 (2015)	0 (0–0.11)	No evidence of decline†
24, t Burreken, deciduous forest			
Fire salamander	30 (2015)	0 (0–0.11)	No evidence of decline†
Germany			
25, Lamersiefen, deciduous forest			
Fire salamander	17 (2014)	0 (0–0.19)	No evidence of decline†
	32 (2015)	0 (0–0.11)	
26, Fischbach, deciduous forest			
Fire salamander	36 (2014)	0 (0–0.09)	No evidence of decline; 3 dead-found specimens Tested negative for *Bsal* via histology (2014)†
	51 (2015)	0 (0–0.07)	
Alpine newt	1 (2015)	0	Live-studied specimen by chance; no evidence of decline

Site no., location, and amphibian collected	Number of specimens tested (year)	Observed prevalence (Bayesian 95% credible intervals)	Remarks
Palmate newt	1 (2015)	0	Live-studied specimen by chance; no evidence of decline
27, Kallerbach, deciduous forest			
Fire salamander	24(2015)	0 (0–0.15)	No evidence of decline†
28, Rosbach, deciduous forest			
Fire salamander	47 (2015)	0 (0–0.07)	No evidence of decline†
29, Zweifallshammer, deciduous forest			
Fire salamander	41 (2015)	0 (0–0.08)	No evidence of decline†
30, Peterbach, mixed forest			
Palmate newt	12 (2014)	0 (0–0.26)	No evidence of decline
Alpine newt	4 (2014)	0 (0–0.52)	No evidence of decline
31, Haftenbach, deciduous forest			
Fire salamander	46 (2015)	0 (0–0.08)	No evidence of decline†
32, Sauerbach, deciduous forest			
Fire salamander	22 (2015)	0 (0–0.15)	No evidence of decline†
Alpine newt	1 (2015)	0,00	No evidence of decline
33, Härtgessief, deciduous forest			
Fire salamander	15 (2014)	0 (0–0.19)	Strong evidence of decline†
34, Kottenforst, deciduous forest			
Fire salamander	51 (2015)	(0–0.07)	No evidence of decline
35, Großkampenberg, mixed forest			
Alpine newt	4 (2015)	0 (0–0.52)	No evidence of decline
Palmate newt	1 (2015)	0	No evidence of decline
36, Lützkampen -mixed forest			
Alpine newt	8 (2015)	0 (0–0.31)	No evidence of decline
37, Ferschweiler- mixed forest			
Alpine newt	2 (2015)	0 (0–0.70)	No evidence of decline
Palmate newt	8 (2015)	0 (0–0.31)	No evidence of decline
38, Ernzen, mixed forest			
Fire salamander	4 (2015)	0 (0–0.52)	No evidence of decline†
The Netherlands			
39, Moerveld surroundings (A), Bunderbos vicinity			
Alpine newt	13 (2015)	0 (0–0.22)	No evidence of decline‡
40, Moerveld surroundings (B), Bunderbos vicinity			
Alpine newt	34 (2015)	0 (0–0.11)	No evidence of decline‡
41, Snijdersbergweg 21, garden pond			
Alpine newt	60 (2015)	0 (0–0.06)	No evidence of decline‡
42, Mevr van der Meijstraat 12, garden pond			
Alpine newt	19 (2015)	0 (0–0.18)	No evidence of decline‡
43, Mevr van der Meijstraat 20, garden pond			
Alpine newt	17 (2015)	0 (0–0.19)	No evidence of decline‡
44, Snijdersbergweg 20, 2 garden ponds			
Alpine newt	30 (2015)	0 (0–0.11)	No evidence of decline‡
45, Snijdersbergweg 23b, garden pond			
Alpine newt	15 (2015)	0 (0–0.19)	No evidence of decline‡
46, Broekhoven, garden pond			
Fire salamander	2 (2015)	0 (0–0.70)	No evidence of decline‡
47, Meerssen, deciduous forest			
Fire salamander	57 (2013)	0 (0–0.06)	No deaths; no evidence of decline†
	43 (2014)	0 (0–0.08)	
	29 (2015)	0 (0–0.11)	
	2 (2016)	0 (0–0.70)	
48, Carisberg, deciduous forest			
Alpine newt	8 (2014)	0 (0–0.31)	No information available
Palmate newt	23 (2014)	0 (0–0.14)	No information available
Smooth newt	2 (2014)	0 (0–0.70)	No information available
Additional far-out sites (Germany)			
N.S., Solling, deciduous forest			
Fire salamander	23 (2015)	0 (0–0.14)	No evidence of decline‡
N.S., Ilsenburg, deciduous forest			
Fire salamander	8 (2015)	0 (0–0.31)	No evidence of decline‡
N.S., Lelm, deciduous forest			
Alpine newt	57 (2015)	0 (0–0.06)	No evidence of decline‡
Palmate newt	6 (2015)	0 (0–0.43)	No evidence of decline‡

Site no., location, and amphibian collected	Number of specimens tested (year)	Observed prevalence (Bayesian 95% credible intervals)	Remarks
Smooth newt	16 (2015)	0 (0–0.20)	No evidence of decline‡
Crested newt	29 (2015)	0 (0–0.11)	No evidence of decline‡
N.S., Kleiwiesen, exposed ponds surrounded by deciduous forest			
Alpine newt	27 (2015)	0 (0–0.13)	No evidence of decline‡
Smooth newt	117 (2015)	0 (0–0.03)	No evidence of decline‡
Crested newt	27 (2015)	0 (0–0.13)	No evidence of decline‡
N.S., Waldecker Schlossgrund, deciduous forest			
Fire salamander	22 (2015)	0 (0–0.15)	No evidence of decline‡
N.S., Closewitz, exposed ponds surrounded by deciduous forest			
Crested newt	23 (2015)	0 (0–0.14)	No evidence of decline‡
Additional far-out site (the Netherlands)			
N.S., Veluwe, deciduous forest			
Italian crested newt	0 (2015)	0 (0–0.11)	No evidence of decline‡

**Bsal, Batrachochytrium salamandrivorans*; N.S., not shown on map (Figure). Data provide an overview of novel information and previously published data. Site numbers correspond to those on map (Figure).
†Population monitored.
‡Anecdotal report.