Clinical Infectious Diseases

MAJOR ARTICLE

Searching and Finding the Hidden Treasure: A Retrospective Analysis of Rickettsial Disease Among Dutch International Travelers

Sophia G. de Vries,[1] Louise E. van Eekeren,[1] Hans van der Linden,[2] Benjamin J. Visser,[1] Martin P. Grobusch,[1] Jiri F. P. Wagenaar,[1,2] Marga G. A. Goris,[2] and Abraham Goorhuis[1]

[1]Center of Tropical Medicine and Travel Medicine, Department of Infectious Diseases, Division of Internal Medicine, Amsterdam University Medical Center, Academic Medical Center, University of Amsterdam, Amsterdam, The Netherlands, and [2]Leptospirosis Reference Center, Department of Medical Microbiology, Amsterdam University Medical Center, Academic Medical Center, University of Amsterdam, Amsterdam, The Netherlands

(See the Editorial Commentary by Raoult on pages 1179–80.)

Background. Rickettsial disease (RD) is a prevalent and underestimated cause of febrile illness worldwide, especially in the absence of an inoculation eschar. We attempted to quantify this underestimation at our clinic, by investigating past cases of febrile illness in travelers who had tested negative for leptospirosis, a disease that can initially present similarly to non-eschar RD, and which we routinely consider when other important causes of unspecified febrile illness have tested negative.

Methods. We performed a retrospective analysis in febrile returned travelers from Asia, Africa, or the Americas between 2010 and 2017, who had tested negative for leptospirosis. Serologic immunofluorescence assays were performed for *Orientia tsutsugamushi* (scrub typhus), typhus group, and spotted fever group RD. We performed a medical records review of all patients who tested positive. In case of a fitting medical history, cases were deemed either confirmed (based on convalescent serology) or suspected (based on single serology).

Results. Among 97 patients, convalescent serology was available in 16 (16.5%) patients, and a single serology in 81 (83.5%) patients. RD was the likely diagnosis in 8 of 16 (50.0%) patients with convalescent serology, and in 8 of 81 (9.9%) with single serology. Of the 16 confirmed/suspected cases, 11 (69%) had been missed and 7 (44%) had not received adequate empiric antibiotic therapy.

Conclusions. This study shows that non-eschar RD is an important and poorly recognized cause of illness in travelers, even in a specialized travel clinic. A lower threshold to test and treat for RD is warranted in returning travelers with febrile illness.

Keywords. epidemiology; Netherlands; rickettsioses; serology; travelers.

Rickettsial diseases (RD) are zoonotic infections, transmitted to humans by predominantly arthropod vectors [1], although leeches and mosquitoes have also been described as vectors [2, 3]. The disease may be mild to life-threatening [4], especially when treatment is delayed [5, 6]. Substantial morbidity is reported worldwide in autochthonous populations, as well as in travelers [7–15]. RD generally presents as an indifferent acute febrile illness, with nonspecific accompanying symptoms such as nausea, vomiting, lymphadenopathy, headache, skin rash, and, sometimes, an inoculation eschar. The prevalence of the latter varies widely per specific RD [16]: from 0% in patients with murine typhus (caused by *Rickettsia typhi*), to 30%–90% in patients with African tick bite fever (caused by *Rickettsia africae*) [11]. Clinically, the symptomatology of RD is often similar to other acute febrile illnesses such as malaria, dengue fever, and leptospirosis [17], especially if an eschar is absent at presentation.

The disease is caused by intracellular bacteria of the Rickettsiaceae family, ordered into 2 genera: *Orientia* (consisting of *Orientia tsutsugamushi*, causing scrub typhus) and *Rickettsia* [18]. The *Rickettsia* genus is divided in 4 biogroups: (1) the spotted fever group (SFG), which, among others, includes *Rickettsia conorii* (causing Mediterranean spotted fever [MSF]), *R. africae* (causing African tick bite fever), and *Rickettsia rickettsii* (causing Rocky Mountain spotted fever); (2) the typhus group (TG), which comprises *R. typhi* and *Rickettsia prowazekii*, causing endemic and epidemic typhus, respectively; (3) a translational group, including *Rickettsia felis*, *Rickettsia australis*, and *Rickettsia akari*; and (4) a nonpathogenic group [18, 19]. Rickettsial organisms have been identified on all continents except Antarctica [20]. *Rickettsia typhi* and *R. felis* and are distributed globally; SFG

Received 11 October 2019; editorial decision 27 December 2019; accepted 28 January 2020; published online January 30, 2020.

Correspondence: A. Goorhuis, Department of Infectious Diseases Amsterdam UMC, AMC University of Amsterdam, Meibergdreef 9, 1105 AZ, Amsterdam, The Netherlands (a.goorhuis@amsterdamumc.nl).

Clinical Infectious Diseases® 2021;72(7):1171–8

DOI: 10.1093/cid/ciaa091

RD has been reported on all continents; and scrub typhus (cause by *O. tsutsugamushi*) is traditionally prevalent in the tropical Pacific triangle, but there are recent reports from South America and sub-Saharan Africa [20].

Currently, the cornerstone of diagnosis is still the indirect detection of *Rickettsia*-specific antibodies in patient sera by serologic methods, such as immunofluorescence or Western blotting. Because antibodies are detected at a later stage after infection, typically 15 days or more [21–23], these methods have limited clinical impact in the acute stage of disease, when most initial diagnostic testing is done [5]. Additionally, there is cross-reactivity between species [24]. For a specific diagnosis in the acute phase of illness, molecular detection methods are preferred [25–28], but these are not widely available. Also, reported diagnostic accuracy of the different tests varies considerably, also based on the specimen type (eg, whole blood, serum), and reference tests are suboptimal, with differences in applied techniques and targets [29].

Because of the unspecific clinical presentation of RD and difficulties in laboratory diagnostics in the early phase of disease, patients may be undiagnosed or misdiagnosed. In a previous study based on reported literature, we estimated that the diagnosis of RD was missed in 66.5% of patients with scrub typhus, and in 57.9% of patients with MSF in autochthonous populations [16]. However, these percentages applied to patients who presented with or without an inoculation eschar. Among patients in whom an inoculation eschar was absent, RD was missed in 87.0% of patients with scrub typhus and 81.6% of patients with MSF.

In travelers, this proportion could even be higher due a low index of suspicion by physicians in areas that are not endemic for the disease. This underestimation is of growing concern, given the expansion of international travel to endemic regions such as Asia and Africa, resulting in increased numbers of imported infections such as RD [30].

We hypothesize that in the absence of an inoculation eschar, the diagnosis of RD is missed in a substantial proportion of returned travelers presenting with acute febrile illness. Our hospital houses the Dutch Leptospirosis Reference Center (NRL), which means that testing for leptospirosis can be easily performed upon clinical suspicion. The disease is usually considered when diagnostic routine testing for other important causes of unspecified febrile illness turns out negative (ie, malaria, typhoid fever, dengue, chikungunya, and Zika virus infection), even in the absence of evident exposure to fresh water, as this is often difficult to ascertain in retrospect. Therefore, and because leptospirosis and RD can have clinical similarities at initial presentation, we hypothesized that missed diagnoses of RD would likely be found among patients who had presented with unspecified febrile illness and who had tested negative for leptospirosis. Finding these missed diagnoses would provide us a rough indication of the underdiagnosis of non-eschar RD at our travel clinic. In this study, we retrospectively assessed sera of a group of leptospirosis-negative returned travelers for the presence of antibodies to SFG and TG rickettsioses and *O. tsutsugamushi*.

METHODS

This retrospective cohort study was performed as a collaboration of the NRL and the Center for Tropical and Travel Medicine, both part of the Amsterdam University Medical Center (UMC).

We selected samples from adult (aged ≥ 18 years) travelers, in whom leptospirosis had been clinically suspected but had tested negative. All had presented at the Center of Tropical Medicine and Travel Medicine of the Amsterdam UMC between January 2010 and July 2017, and had recently returned from Africa, the Americas, or Asia, and had an available stored serum sample.

Laboratory Diagnostics

Diagnostic tests were performed in December 2015 and June 2017 at the NRL. Serum samples had been stored at −20°C. If available, convalescent samples were tested. All samples were tested with several immunofluorescence assays (IFAs). Two different kits were used:

1. The *Rickettsia* Screen IFA Antibody Kit, immunoglobulin G (IgG) and immunoglobulin M (IgM) (Fuller Laboratories, Fullerton, California), using *R. conorii and R. typhi* substrate antigens. A positive result was defined as a titer ≥ 1:128 (IgG) or ≥ 1:64 (IgM), a ≥ 4-fold titer rise between acute and convalescent samples, or seroconversion.
2. *Orientia tsutsugamushi* IFA Antibody Kit, IgG and IgM (Fuller Laboratories), using the Boryong, Gilliam, Karp, and Kato antigen strains of *O. tsutsugamushi*. A positive result was defined as a titer ≥ 1:128 (IgG) or ≥ 1:64 (IgM), a ≥ 4-fold titer-rise between acute and convalescent samples, or seroconversion.

Cutoff titers were determined based on the low prevalence of RD in the research population, as the occurrence of autochthonous infections in the Netherlands is rare [31]. The IFAs were performed by 2 trained individuals (S. G. d. V. and H. v. d. L.). In case of positivity or doubt, both interpreted all sample results independently. For a subset of samples, further dilutions were prepared once the sample was positive.

Medical Records Review

The medical records of all patients who tested positive for RD were reviewed. Epidemiological and clinical data were extracted, including travel history, reason for travel, tick exposure during travel, whether or not the differential diagnosis had included RD, whether or not the patient had initially been tested for RD, the final clinical diagnosis, whether or not the patient had received treatment with antirickettsial drugs,

and the follow-up. Countries of exposure were grouped. Tetracyclines, macrolides, and fluoroquinolones were considered as effective treatments for RD. Finally, all clinical data of patients with positive laboratory tests were reviewed by 2 clinicians (S. G. d. V. and A. G.), to assess whether RD was indeed the most likely diagnosis.

Case Definitions

A "laboratory-confirmed case" was defined as a ≥4-fold titer increase, or seroconversion in convalescent samples. A "laboratory-suspected case" was defined as an IFA-positive single serum sample, with the earlier mentioned cutoff titers. A "definitive-confirmed case" was defined as a laboratory-confirmed case in combination with a compatible clinical course and no other likely or confirmed diagnosis. A "definitive-suspected case" was defined as a laboratory-suspected case in combination with a compatible clinical course and no other likely or confirmed diagnosis.

Laboratory- and definitive-confirmed and suspected cases were categorized in 4 groups: SFG rickettsiosis, TG RD, indeterminate RD (either SFG or TG, but IFA could not differentiate between the 2), and scrub typhus.

Data Analysis

Data were anonymized, organized, and analyzed using Microsoft Excel software (Microsoft Corporation, 2010). Data were de-identified and not attributable to individual patients. For numerical variables with a normal distribution, including age and laboratory values, mean and standard deviation was calculated. For numerical variables with a nonnormal distribution, including variables about the disease course, median and interquartile range were calculated.

RESULTS

Figure 1 provides the study flow and main results. In short, 97 patients met the inclusion criteria, of whom 16 (16.5%) had

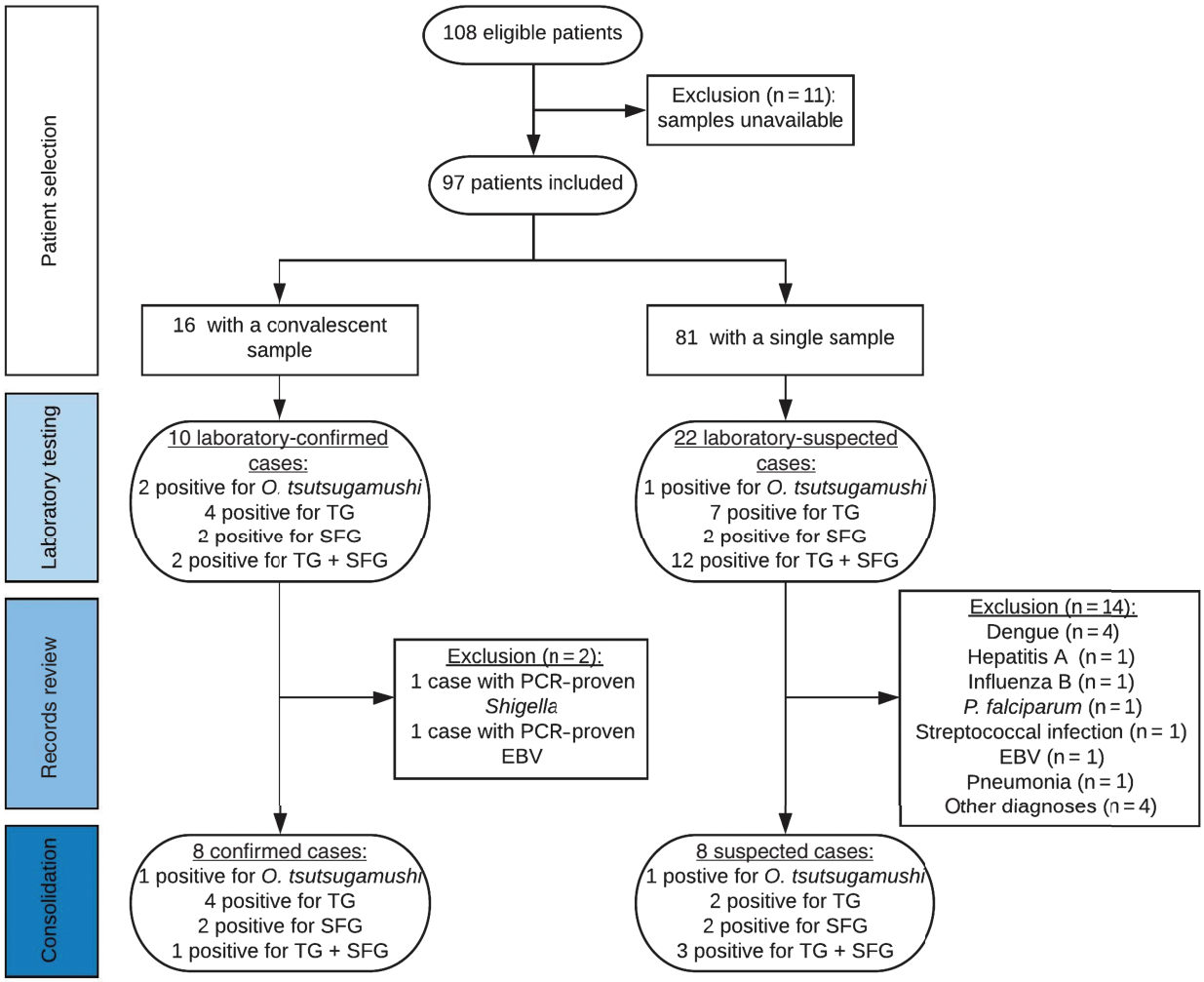

Figure 1. Flow diagram of the study and main results. Abbreviations: EBV, Epstein-Barr virus; PCR, polymerase chain reaction; SFG, spotted fever group; TG, typhus group.

a convalescent sample available and 81 (83.5%) only a single sample. In total, 32 (33%) patients tested IFA positive: 10 of 16 (62.5%) of patients with a convalescent sample (laboratory-confirmed cases), and 22 of 81 (27.2%) of patients with a single sample (laboratory-suspected cases).

Medical Records Consolidation

Of the 32 patients who were IFA positive (10 laboratory-confirmed and 22 laboratory-suspected cases), medical data were extracted. After medical records review, 2 of 10 laboratory-confirmed cases were excluded, resulting in 8 of 16 (50%) definitive-confirmed cases among patients with a convalescent sample, which is 8 of 97 (8.2%) definitive-confirmed cases in the whole cohort. The 2 excluded cases comprised immunocompetent patients: 1 with polymerase chain reaction (PCR)–proven shigellosis, and 1 with PCR-proven Epstein-Barr virus infection.

Of the 22 laboratory-suspected cases, 14 were excluded, resulting in 8 of 81 (9.9%) definitive-suspected cases among patients with a single sample, which is 8 of 97 (8.2%) definitive-suspected cases in the whole cohort. The 14 excluded patients comprised 4 with a dengue infection (2 PCR-confirmed, 2 with positive IgM and dubious IgG); 1 with acute hepatitis A virus (HAV) infection (anti-HAV IgM positive); 1 with PCR-proven influenza B infection; 1 with blood smear–positive *Plasmodium falciparum* malaria; 1 with a streptococcal infection complicated by glomerulonephritis; 1 with a recent (IgM positive) Epstein-Barr virus infection; 1 with lobar pneumonia; 1 with bacterial cellulitis of the leg; 1 with an autoimmune-mediated encephalitis; 1 with a cerebral and retinal vasculitis (although the latter could have been due to RD); and 1 with relapsing fevers.

In total, we thus identified 16 of 97 (16.5%) patients with either definitive-confirmed RD (8 patients) or definitive-suspected RD (8 patients).

Demographics and Laboratory Findings

Demographic characteristics are depicted in Table 1. Of the 16 definitive-confirmed/suspected cases, 2 were IFA-positive for *O. tsutsugamushi*, 6 for TG RD, and 4 for SFG RD; in 4 cases, reactivity was indeterminate TG/SFG (Figure 1). Details of the laboratory findings can be found in Table 2.

Clinical Findings

Table 3 summarizes general clinical characteristics and laboratory findings of the 16 definitive-confirmed/suspected patients. Table 2 provides a detailed overview of clinical and diagnostic information of all definitive-confirmed/suspected cases. A total of 5 patients (31.3%) had initially been diagnosed with RD by the treating clinician, 4 of them based on diagnostics performed at the reference laboratory. Of the 16 definitive-confirmed/suspected patients, 9 (56.3%) had received adequate antibiotic treatment. The course of illness of the 8 who had not received treatment was not well documented.

DISCUSSION

In this study, we provided a rough estimate of the extent of missed diagnoses of RD among ill returning travelers, by investigating a cohort of patients who had tested negative for leptospirosis, a disease that can initially present similar to RD, and which we routinely consider when other important causes of unspecified febrile illness have tested negative. Among 97 patients, we identified 16 (16.5%) patients with definitive-confirmed or suspected RD, based on both laboratory and clinical criteria. Of these 16 patients, 5 (31.3%) had actually been correctly diagnosed by the treating physician, whereas 11 (68.7%) had been missed. Only 9 (56.3%) patients had received adequate empirical antibiotic treatment.

Table 1. Demographic Characteristics

Demographic Data	All (N = 97)	Definitive-Confirmed and Definitive-Suspected Cases (n = 16)
Male sex	52 (53.6)	11 (68.8)
Age, y, mean ± SD (range)	37.5 ± 14.5 (8.5–70.6)	44.8 ± 14.0 (24.0–68.2)
Region of travel		
Southeast Asia	58 (59.8) (Asia all regions)	9 (56.3)
Sub-Saharan Africa	23 (23.7) (Africa all regions)	3 (18.8)
Latin America/Caribbean	16 (16.5) (Americas)	3 (18.8)
Northern Africa	…	1 (6.3)
Rickettsial disease included in differential diagnosis	NA	9 (56.0)
Initially diagnosed with rickettsiosis	NA	4 (25.0)
Day postonset of disease at collection of positive rickettsiosis sample, mean ± SD (range)	NA	17.3 ± 7.6 (1–36)
Hospital admission	NA	5 (33.3)
Deaths	NA	0 (0)

Data are presented as no. (%) unless otherwise indicated.

Abbreviations: NA, not applicable; SD, standard deviation.

Table 2. Clinical and Laboratory Details of Definitive-Confirmed and Definitive-Suspected Cases

Patient No.	Sex, Age (y)	Destination	Main Symptoms	Initial Diagnosis	Antibiotics Administered?	Convalescent Sample?	Day of Sample Collection*	IFA Positive for:	Laboratory Findings
Definitive-Confirmed cases									
81	Male, 33	Malaysia and Borneo	Fever, headache, arthralgia, myalgia, rash	Arbovirus or nematode infection	Yes (doxycycline)	Yes	6 + 27	*Orientia tsutsugamushi*	Day 6: IgM^-, IgG^- Day 27: IgM 1:512, IgG 1:128
79	Male, 35	Thailand	Fever, chills, headache, arthralgia, myalgia, rash, nausea, vomiting, diarrhea, abdominal pain, elevated CRP	Leptospirosis	Yes (ceftriaxone)	Yes	4 + 18	TG	Day 4: IgM^-, IgG^- Day 18: IgM^+, IgG^-
27	Female, 61	Indonesia	Fever, chills, arthralgia, myalgia, cough, dyspnea, nausea, diarrhea, rectal blood loss, anorexia, elevated CRP	TG rickettsial disease	Yes (amoxicillin, ceftriaxone, and doxycycline)	Yes	10 + 20	TG	Day 10: IgM 1:64, IgG^- Day 20: IgM 1:64, IgG 1:128
32	Female, 58	Congo	Fever, chills, headache, myalgia, cough, throat pain, conjunctival suffusion, improvement after treatment with doxycycline for 2 d	Rickettsial disease or flulike illness	Yes (doxycycline)	Yes	9 + 20	TG	Day 9: IgM^-, IgG^- Day 20: IgM 1:64, IgG 1:128
44	Female, 29	Uganda	Presentation after hospital admission for malaria. Headache, arthralgia, myalgia, abdominal pain, cough, dyspnea, icterus, splenomegaly (Hb 4.7 mmol/L [or 7.57 g/dL]), elevated liver enzymes and bilirubin	Hemolytic anemia after malaria	Yes (ciprofloxacin)	Yes	18 + 85	TG	Day 18: IgM 1:64, IgG^- Day 85: IgM 1:64, IgG 1:128
92	Male, 62	South Africa	Fever, chills headache, arthralgia, myalgia	Rickettsial disease	Yes (doxycycline)	Yes	6 + 72	SFG	Day 6: SFG and TG IgM 1:64 Day 72: SFG IgG 1:128
63	Male, 68	Morocco	Fever, chills, nausea, petechiae	SFG rickettsial disease	No	Yes	19 + 39	SFG	Day 19: IgM 1:64, IgG^- Day 39: IgM 1:64, IgG 1:128
23	Male, 29	Indonesia	Fever, myalgia, headache, itchy rash	Viral infection	Yes (doxycycline)	Yes	4 + 18	Mixed TG/SFG	Day 4: IgM^- Day 18: IgM 1:512 TG/SFG
Definitive-suspected cases									
25	Female, 47	Thailand	Fever, nausea, vomiting, diarrhea	Leptospirosis	Yes (ceftriaxone, gentamicin)	No	1	SFG	IgM 1:512
66	Male, 40	Suriname	Fever, chills, arthralgia, myalgia, rash, red eyes, lymphadenopathy, elevated CRP	Self-limiting arboviral infection	No	No	5	SFG	IgM 1:64
51	Female, 60	Thailand	Fever, cough	Viral infection (not specified)	No	No	16	Mixed TG/SFG	IgM 1:64 TG/SFG
69	Male, 24	French Guyana	Headache, myalgia, chills, anorexia, rash	Dermatomycosis	No	No	36	Mixed TG/SFG	IgM 1:64, IgG 1:128 TG/SFG
88	Male, 30	Puerto Rico	Fever, headache, arthralgia, dyspnea, nausea, rash	Viral infection (not specified)	No	No	14	Mixed TG/SFG	IgM 1:64 TG/SFG
11	Male, 42	Thailand	Headache, myalgia, rash, lymphadenopathy, aminotransferase elevation	CMV	No	No	22	*O. tsutsugamushi*	IgM 1:256
4	Male, 48	Indonesia	Headache, myalgia, sore throat	TG rickettsial disease	Yes (doxycycline)	No	24	TG	IgM 1:256
26	Male, 51	Thailand and Cambodia	Fever, chills, headache, arthralgia, abdominal pain, elevated CRP	Viral infection (not specified)	No	No	2 (but 19 d after return)	TG	IgM 1:256

*The numbers represent the timing of serologic sampling in days after onset of symptoms.
Abbreviations: CMV, cytomegalovirus; CRP, C-reactive protein; Hb, hemoglobin; IFA, immunofluorescence assays; IgG, immunoglobulin G; IgM, immunoglobulin M; SFG, spotted fever group; TG, typhus group.

Table 3. Symptoms and Clinical Laboratory Findings of Definitive-Confirmed and Definitive-Suspected Cases

Finding	All (n = 16), No. (%)
Symptoms and signs	
Fever	13 (81.3)
Headache	12 (75.0)
Myalgia	10 (62.5)
Arthralgia	9 (56.3)
Chills	9 (56.3)
Gastrointestinal symptoms[a] (≥ 1)	8 (50.0)
Respiratory symptoms[b] (≥ 1)	8 (50.0)
Skin rash	5 (31.3)
Lymphadenopathy	2 (12.5)
Symptoms of bleeding[c] (≥ 1)	1 (6.3)
Urogenital symptoms[d] (≥ 1)	1 (6.3)
Eschar	0 (0.0)
Laboratory abnormalities[e]	
Elevated CRP (> 5 mg/L)	5/12 (41.7)
Elevated ALT (SGPT) (> 45 U/L)	5/16 (31.3)
Elevated AST (SGOT) (> 40 U/L)	4/13 (30.8)
Leukocytosis (> 10.5×10^9/L)	4/16 (25.0)
Elevated bilirubin (> 17 μmol/L)	2/11 (18.2)
Low platelet count (< 150×10^9/L)	2/14 (14.3)
Elevated creatinine (> 110 μmol/L)	2/16 (12.5)
Low hemoglobin (male: < 8.5 mmol/L; female: < 7.5 mmol/L)	1/16 (6.3)
Leukocytopenia (< 4.5×10^9/L)	1/16 (6.3)
Hypokalemia (< 3.5 mmol/L)	0/7 (0.0)

All symptoms and laboratory findings were recorded at the day of presentation to the clinic. Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; CRP, C-reactive protein; SGOT, serum glutamic-oxaloacetic transaminase; SGPT, serum glutamic pyruvic transaminase.

[a]Gastrointestinal symptoms include nausea, vomiting, diarrhea, and abdominal pain.

[b]Respiratory symptoms include cough, sore throat, hemoptysis, and dyspnea.

[c]Symptoms of bleeding include hematemesis, melena, and rectal bleeding.

[d]Urogenital symptoms include dysuria, hematuria, and oliguria.

[e]Denominators vary as not all clinical symptoms were available for all patients.

Interestingly, the highest proportion (9/16 [50%]) of RD was found in the group of patients who twice tested negative for leptospirosis in convalescent samples, as opposed to 10% (8/81) in the group of patients who were only tested once. Obviously, this was driven by the desire of the clinician to establish a diagnosis in a patient in whom pathology was highly suspected. To turn this around: If a patient had tested negative for leptospirosis in single sample testing, there was a 10% chance that RD was the missed underlying cause, which increased to 50% in case of a negative convalescent test, ordered by the treating physician for clinical reasons.

There are no other clinical studies that have tried to estimate the underdiagnosis of RD in travelers, only the recently published finding from our group among autochthonous populations, that in the absence of an inoculation eschar, 82%–87% of RD cases were missed [16]. In our setting of a specialized academic travel clinic, where clinicians are familiar with RD, we also missed almost 70% of non-eschar RD. Therefore, one can assume that the underdiagnosis in general clinics is much higher.

The currently existing body of evidence on RD in travelers mainly comprises a multitude of case reports and case series, of which an overview can be found in a review by Delord and colleagues [14]. Additionally, a few cohort studies have been published [9, 10, 32–38]. However, in these studies, patients were retrospectively identified based on the diagnosis made by the treating physician, which makes underestimation very likely, precluding the possibility to estimate underdiagnosis [9, 10, 32, 33]. Five studies used prospective methods [34–38] but investigated diagnosed infections, or only RD presenting with an inoculation eschar, precluding the possibility to assess underdiagnosis of non-eschar RD.

The results presented here should be interpreted with caution, as there are several limitations. First, all patients had presented to a specialized travel clinic in an academic medical center, with a lower-than-average threshold of suspicion for RD.

Second, the group of patients in our study is not representative for the overall group of travelers with fever. Because we were interested in underdiagnosis of RD, and studied a specific subset of patients who had tested negative for leptospirosis, we "missed" the typical presentations of RD who had presented with an eschar. These patients are readily diagnosed at our clinic based on the clinical presentation, precluding the need for further diagnostic testing for leptospirosis or other diseases. The fact that the diagnostic process for leptospirosis had been initiated typically implies that more common causes of fever had already been excluded (eg, malaria, dengue, chikungunya, Zika virus infection, common bacterial infections). Thus, we studied a selected group of patients with a higher a priori likelihood of less common illnesses, such as non-eschar RD. For this study however, this was intentional, because we expected to find missed cases of non-eschar RD in this population. Obviously, an important criterion to test for leptospirosis is exposure to fresh water, which means that we missed additional cases of non-eschar RD among patients who were never tested for leptospirosis because they were not exposed to fresh water. It is possible that this population was tested for RD more frequently.

Third, important limitations apply to the laboratory methods. The diagnostic process for RD is changing rapidly [29]. Whereas many reference laboratories are still working with IFA or the microimmunofluorescence assay as reference standards [20], molecular detection methods are gaining popularity [29], as they can diagnose the illness in its early stage. Because of restrictions in the type and quality of samples available for this study, we only used serology-based methods. It is known that there are many limitations to IFA in general: (1) poor sensitivity in the acute phase of illness (and thus limited diagnostic value of single samples); (2) high variation and lack of consensus in cutoff limits; (3) interreader heterogeneity; and (4) cross-reactivity of IgM with other species and antibody persistence beyond the acute phase of illness [20, 23, 29, 39]. All of these limitations apply to this study. For the majority of patients, only a single

sample was available. Therefore, dynamics in antibody titers could not be assessed, resulting in unconfirmed or even missed diagnoses of RD. Also, due to material constraints, not all samples underwent further diluting; presented dilutions could have been higher for some samples. Almost certainly, some positive IgM titers were based on cross-reactivity, or on previous infections. Although the latter is less likely in the Dutch population, coinfections with tick-borne Rickettsiae have been described in the Netherlands [40]. Remarkably, we observed cross-reactivity between SFG and TG groups in a considerable number of samples. It is possible that this has been caused by *R. felis* infections, a rickettsial illness that has been on the rise globally in the past years [41].

Finally, the retrospective nature of this study itself introduced limitations. For example, the clinical information was extracted from patient files and was often incomplete. Also, though not expected [42], long-term freezing could have affected the quality of the serum samples.

The most important message from this study is that even in a specialized travel clinic where clinicians are familiar with the diagnosis of RD, this diagnosis is still missed in a substantial proportion of patients, especially when an inoculation eschar is absent. In retrospect, in our study, 68.7% of the confirmed/suspected RD cases had been missed and 43.7% did not receive adequate (empirical) antibiotic therapy. Although no deaths occurred in this small group of patients, the hospitalization rate was high (33.3%), which emphasizes the importance of timely recognition and treatment of this disease. In a nonspecialized clinical setting, the proportion of missed diagnoses of RD will probably be higher, as we also estimated earlier [16].

There is a dire need for properly conducted prospective studies among febrile travelers, to reach a credible estimation of the burden of this disease as an imported cause of febrile illness. A lower threshold to test for RD by clinicians is justified, and RD should be included in the testing algorithm of febrile illnesses.

Notes

Author contributions. Concept: S. G. d. V., H. v. d. L., J. F. P. W., M. G. A. G., M. P. G., and A. G. Laboratory analysis: S. G. d. V., H. v. d. L., and V. J. Chart review: L. E. v. E. and S. G. d. V. Data analysis: S. G. d. V. and L. E. v. E. First draft manuscript: S. G. d. V., L. E. v. E., and B. J. V. Revision of the manuscript: All authors.

Financial support. This work was internally funded by the Amsterdam University Medical Center.

Potential conflicts of interest. The authors: No reported conflicts of interest. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest.

References

1. Parola P, Davoust B, Raoult D. Tick- and flea-borne rickettsial emerging zoonoses. Vet Res **2005**; 36:469–92.
2. Dieme C, Bechah Y, Socolovschi C, et al. Transmission potential of *Rickettsia felis* infection by *Anopheles gambiae* mosquitoes. Proc Natl Acad Sci U S A **2015**; 112:8088–93.
3. Slesak G, Inthalath S, Dittrich S, Paris DH, Newton PN. Leeches as further potential vectors for rickettsial infections. Proc Natl Acad Sci U S A **2015**; 112:E6593–4.
4. Botelho-Nevers E, Raoult D. Host, pathogen and treatment-related prognostic factors in rickettsioses. Eur J Clin Microbiol Infect Dis **2011**; 30:1139–50.
5. La Scola B, Raoult D. Laboratory diagnosis of rickettsioses: current approaches to diagnosis of old and new rickettsial diseases. J Clin Microbiol **1997**; 35:2715–27.
6. Botelho-Nevers E, Socolovschi C, Raoult D, Parola P. Treatment of *Rickettsia* spp. infections: a review. Expert Rev Anti Infect Ther **2012**; 10:1425–37.
7. Taylor AJ, Paris DH, Newton PN. A systematic review of mortality from untreated scrub typhus (*Orientia tsutsugamushi*). PLoS Negl Trop Dis **2015**; 9:e0003971.
8. Botelho-Nevers E, Rovery C, Richet H, Raoult D. Analysis of risk factors for malignant Mediterranean spotted fever indicates that fluoroquinolone treatment has a deleterious effect. J Antimicrob Chemother **2011**; 66:1821–30.
9. Jensenius M, Davis X, von Sonnenburg F, et al. Multicenter GeoSentinel analysis of rickettsial diseases in international travelers, 1996–2008. Emerg Infect Dis **2009**; 15:1791–8.
10. Jensenius M, Han PV, Schlagenhauf P, et al. Acute and potentially life-threatening tropical diseases in Western travelers—a GeoSentinel multicenter study, 1996–2011. Am J Trop Med Hyg **2013**; 88:397–404.
11. Parola P, Paddock CD, Socolovschi C, et al. Update on tick-borne rickettsioses around the world: a geographic approach. Clin Microbiol Rev **2013**; 26:657–702.
12. Fang LQ, Liu K, Li XL, et al. Emerging tick-borne infections in mainland China: an increasing public health threat. Lancet Infect Dis **2015**; 15:1467–79.
13. Eldin C, Parola P. Update on tick-borne bacterial diseases in travelers. Curr Infect Dis Rep **2018**; 20:17.
14. Delord M, Socolovschi C, Parola P. Rickettsioses and Q fever in travelers (2004–2013). Travel Med Infect Dis **2014**; 12:443–58.
15. Schlagenhauf P, Weld L, Goorhuis A, et al. Travel-associated infection presenting in Europe (2008–12): an analysis of EuroTravNet longitudinal, surveillance data, and evaluation of the effect of the pre-travel consultation. Lancet Infect Dis **2015**; 15: 55–64.
16. van Eekeren LE, de Vries SG, Wagenaar JFP, Spijker R, Grobusch MP, Goorhuis A. Under-diagnosis of rickettsial disease in clinical practice: a systematic review. Travel Med Infect Dis **2018**; 26:7–15.
17. Raeber PA, Winteler S, Paget J. Fever in the returned traveller: remember rickettsial diseases. Lancet **1994**; 344:331.
18. Merhej V, Raoult D. Rickettsial evolution in the light of comparative genomics. Biol Rev Camb Philos Soc **2011**; 86:379–405.
19. Murray GG, Weinert LA, Rhule EL, Welch JJ. The phylogeny of *Rickettsia* using different evolutionary signatures: how tree-like is bacterial evolution? Syst Biol **2016**; 65:265–79.
20. Abdad MY, Abou Abdallah R, Fournier PE, Stenos J, Vasoo S. A concise review of the epidemiology and diagnostics of rickettsioses: *Rickettsia* and *Orientia* spp. J Clin Microbiol **2018**; 56. doi:10.1128/JCM.01728-17.
21. Dumler JS, Taylor JP, Walker DH. Clinical and laboratory features of murine typhus in south Texas, 1980 through 1987. JAMA **1991**; 266:1365–70.
22. Fournier PE, Jensenius M, Laferl H, Vene S, Raoult D. Kinetics of antibody responses in *Rickettsia africae* and *Rickettsia conorii* infections. Clin Diagn Lab Immunol **2002**; 9:324–8.
23. Blacksell SD, Bryant NJ, Paris DH, Doust JA, Sakoda Y, Day NP. Scrub typhus serologic testing with the indirect immunofluorescence method as a diagnostic gold standard: a lack of consensus leads to a lot of confusion. Clin Infect Dis **2007**; 44:391–401.
24. Raoult D, Dasch GA. Immunoblot cross-reactions among *Rickettsia*, *Proteus* spp. and *Legionella* spp. in patients with Mediterranean spotted fever. FEMS Immunol Med Microbiol **1995**; 11:13–8.
25. Giulieri S, Jaton K, Cometta A, Trellu LT, Greub G. Development of a duplex real-time PCR for the detection of *Rickettsia* spp. and typhus group rickettsia in clinical samples. FEMS Immunol Med Microbiol **2012**; 64:92–7.
26. Papp S, Rauch J, Kuehl S, Richardt U, Keller C, Osterloh A. Comparative evaluation of two *Rickettsia typhi*-specific quantitative real-time PCRs for research and diagnostic purposes. Med Microbiol Immunol **2017**; 206:41–51.
27. Paris DH, Blacksell SD, Stenos J, et al. Real-time multiplex PCR assay for detection and differentiation of rickettsiae and orientiae. Trans R Soc Trop Med Hyg **2008**; 102:186–93.
28. Watthanaworawit W, Turner P, Turner C, et al. A prospective evaluation of real-time PCR assays for the detection of *Orientia tsutsugamushi* and *Rickettsia* spp. for early diagnosis of rickettsial infections during the acute phase of undifferentiated febrile illness. Am J Trop Med Hyg **2013**; 89:308–10.
29. Paris DH, Dumler JS. State of the art of diagnosis of rickettsial diseases: the use of blood specimens for diagnosis of scrub typhus, spotted fever group rickettsiosis, and murine typhus. Curr Opin Infect Dis **2016**; 29:433–9.
30. Jensenius M, Fournier PE, Raoult D. Rickettsioses and the international traveler. Clin Infect Dis **2004**; 39:1493–9.

31. Rijksinstituut voor Volksgezondheid en Milieu. *Rickettsia* spp. Available at: https://www.rivm.nl/wilde-knaagdieren-en-zo-nosen/ziekteverwekkers/rickettsia-spp-rickettsiose. Accessed 6 May 2019.
32. Herbinger KH, Hanus I, Felbinger TW, et al. Elevated values of clinically relevant transferases induced by imported infectious diseases: a controlled cross-sectional study of 14 559 diseased German travelers returning from the tropics and subtropics. Am J Trop Med Hyg **2016**; 95:481–7.
33. Herbinger KH, Hanus I, Schunk M, et al. Elevated values of C-reactive protein induced by imported infectious diseases: a controlled cross-sectional study of 11 079 diseased German travelers returning from the tropics and subtropics. Am J Trop Med Hyg **2016**; 95:938–44.
34. Parola P, Soula G, Gazin P, Foucault C, Delmont J, Brouqui P. Fever in travelers returning from tropical areas: prospective observational study of 613 cases hospitalised in Marseilles, France, 1999–2003. Travel Med Infect Dis **2006**; 4:61–70.
35. Jensenius M, Hoel T, Raoult D, et al. Seroepidemiology of *Rickettsia africae* infection in Norwegian travellers to rural Africa. Scand J Infect Dis **2002**; 34:93–6.
36. Jensenius M, Fournier PE, Vene S, et al. African tick bite fever in travelers to rural sub-Equatorial Africa. Clin Infect Dis **2003**; 36:1411–7.
37. Morand A, Angelakis E, Ben Chaabane M, Parola P, Raoult D, Gautret P. Seek and find! PCR analyses of skin infections in West-European travelers returning from abroad with an eschar. Travel Med Infect Dis **2018**; 26:32–6.
38. Beltrame A, Angheben A, Casolari S, et al. Imported rickettsioses in Italy. Travel Med Infect Dis **2012**; 10:201–4.
39. Phetsouvanh R, Thojaikong T, Phoumin P, et al. Inter- and intra-operator variability in the reading of indirect immunofluorescence assays for the serological diagnosis of scrub typhus and murine typhus. Am J Trop Med Hyg **2013**; 88:932–6.
40. Koetsveld J, Tijsse-Klasen E, Herremans T, Hovius JW, Sprong H. Serological and molecular evidence for spotted fever group *Rickettsia* and *Borrelia burgdorferi* sensu lato co-infections in the Netherlands. Ticks Tick Borne Dis **2016**; 7:371–7.
41. Angelakis E, Mediannikov O, Parola P, Raoult D. *Rickettsia felis*: the complex journey of an emergent human pathogen. Trends Parasitol **2016**; 32:554–64.
42. Dard C, Bailly S, Drouet T, Fricker-Hidalgo H, Brenier-Pinchart MP, Pelloux H. Long-term sera storage does not significantly modify the interpretation of toxoplasmosis serologies. J Microbiol Methods **2017**; 134:38–45.