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

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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* are distributed globally; SFG
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 to 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
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 | 173 ± 76 (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.
| 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 (ceftriaxone, 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 flu-like 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, ceterus, splenomegaly (Hb 4.7 mmol/L [or 7.57 g/dL]), elevated liver enzymes and bilirubin | Hemolytic anemia after malaria | Yes (k-pifloxicacin) | 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, gentamycin) | 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 | Dermatomyositis | 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.
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 (e.g., 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 \emph{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, to reach a credible estimation of the absolute 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

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References

1. Parola P, Davoust B, Raoult D. Tick- and flea-borne rickettsial emerging zoonoses. Vet Res 2005; 36:469–92.
2. Deme C, Bechah Y, Socolovschi C, et al. Transmission potential of \emph{Rickettsia felis} infection by \emph{Anopheles gambiae} mosquitoes. Proc Natl Acad Sci U S A 2015; 112:8088–93.
3. Sleasak G, Inhalaal S, Ditttrich 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, ParaP. Treatment of \emph{Rickettsia} spp. infections: a review. Expert Rev Anti Infect Ther 2012; 10:425–37.
7. Taylor AJ, Paris DH, Newton PN. A systematic review of mortality from untreated scrub typhus (\emph{Orientia tsutsugamochi}). PLoS Negl Trop Dis 2015; 9:ea003971.
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, Schlangenhaus 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; 12:443–55.
15. Schlangenhaus 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 Ekeren LE, de Vries SG, Wagenaar JFP, Spikker 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. Raebber 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 \emph{Rickettsia} using different evolutionary signatures: how tree-like is bacterial evolution? Syst Biol 2016; 65:265–79.
20. Abdel M, Abou Abdallah R, Fournier PE, Stenos J, Vasoo S. A concise review of the epidemiology and diagnostics of rickettsioses: \emph{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, Jensensius M, Laderl H, Vene S, Raoult D. Kinetics of antibody responses in \emph{Rickettsia afericas} and \emph{Rickettsia conorii} infections. Clin Diagn Lab Immunol 2002; 9:324–8.
23. Blacksell SD, Bryant NJ, Paris DH, Douat 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 \emph{Rickettsia}, \emph{Proteus} spp. and \emph{Legionella} spp. in patients with Mediterranean spotted fever. FEMS Immunol Med Microbiol 1995; 11:13–8.
25. Giuliani S, Jaton K, Cometta A, Trellu LT, Greub G. Development of a duplex real-time PCR for the detection of \emph{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 \emph{Rickettsia typhi}-specific quantitative real-time PCRs for research and diagnostic purposes. Med Immunol Immunol Med 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. Watthanaowarat W, Turner P, Turner C, et al. A prospective evaluation of real-time PCR assays for the detection of \emph{Orientia tsutsugamochi} and \emph{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. Herberger 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. Herberger 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, Thojjakong 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, Tijssse-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, Droiset T, Fricker-Hidalgo H, Brenner-Pinchart MP, Pelloix H. Long-term sera storage does not significantly modify the interpretation of toxoplasmosis serologies. J Microbiol Methods 2017; 134:38–45.