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Original article

Rickettsioses in Denmark: A retrospective survey of clinical features and travel history

Lukas Frans Ocias[a,b,c,*], Bo Bødker Jensen[d,e], Steen Villumsen[f], Anne-Mette Lebech[g], Sigurdur Skarphedinsson[d], Ram Benny Dessau[b,c], Karen Angeliki Krogfelt[a,c]

[a] Department of Bacteria, Parasites and Fungi, Statens Serum Institut, Artillerivej 5, 2300 København S, Denmark
[b] Department of Clinical Microbiology, Slagelse Hospital, Ingemannsvej 46, 4200 Slagelse, Denmark
[c] ScandTick Innovation Study Group
[d] Clinical Center for Emerging and Vector-Borne Infections, Odense University Hospital, Søndre Blvd. 29, 5000 Odense, Denmark
[e] Institute for Regional Health Research, Center of Southern Jutland, University of Southern Denmark, Winsløwparken 19, 3, 5000 Odense, Denmark
[f] Department of Infectious Diseases, Rigshospitalet, Blegdamsvej 9, 2100 København Ø, Denmark
[g] Department of Infectious Diseases, Hvidovre University Hospital, Kettegård Alle 30, 2650 Hvidovre, Denmark

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ABSTRACT

Rickettsia spp. can be found across the globe and cause disease of varying clinical severity, ranging from life-threatening infections with widespread vasculitis to milder, more localized presentations. Vector and, to some degree, reservoir are hematophagous arthropods, with most species harboured by ticks. In Denmark, rickettsiae are known as a cause of imported travel-related infections, but are also found endemically in ticks across the country. Data are, however, lacking on the geographical origin and clinical features of diagnosed cases.

In this study, we have examined the travel history and clinical features of two groups of patients; 1) hospital-patients diagnosed with rickettsioses in the years 2010–2015 and 2) patients from primary health care (PHC) centers in Denmark having demonstrated anti-rickettsia antibodies in the years 2012–2015. The patients were identified using the Danish National Patient Registry (DNPR) and through the serological database at the State Serum Institute, where the laboratory diagnosis of rickettsioses is currently centralized. Data were collected for 86 hospital patients and 26 PHC center patients by reviewing hospital medical records and performing telephone interviews with PHC centers.

Of the hospital patients, 91% (78/86) had a history of international travel 14 days prior to symptom start, with most having imported their infection from southern Africa, South Africa in particular (65%), and presenting with a clinical picture most compatible with African tick-bite fever caused by *R. africae*. Only two patients presented with a CRP > 100 mg/L and no mortalities were reported. At the PHC centers, most patients presented with mild flu-like symptoms and had an unknown (50%) or no history (19%) of international travel, raising the possibility of endemic rickettsioses. In view of our findings, rickettsioses do not appear to constitute a major public health problem in Denmark, with most cases being imported infections and potential endemic cases presenting as mild infections.

1. Introduction

The *Rickettsia* genus encompasses small, Gram-negative, aerobic, obligate intracellular, pleomorphic bacteria that can be transmitted to humans via hematophagous arthropods such as ticks, fleas, mites and lice. Twenty-six different species have so far been identified worldwide, of which at least fifteen are potentially pathogenic to humans (Parola et al., 2013). They can be serotypically divided into the spotted fever group (SFG) and the typhus group (TG) of which the SFG is the most frequently encountered. The pathophysiology is characterized by invasion and replication in vascular endothelial cells causing varying degrees of vasculitis in small to medium-sized blood vessels and resulting in symptoms such as fever, rash, headache, myalgia, arthralgia and sometimes eschar ("tache noir") (Raoult and Roux, 1997). Clinical severity is associated with the underlying species ranging from potentially fatal disease such as Rocky Mountain spotted fever caused by

Abbreviations: SFG, spotted fever group; TG, typhus group; PCR, polymerase chain reaction; PHC, primary health care; DNPR, Danish National Patient Registry; DNA, deoxyribonucleic acid; ICD-10, International Classification of Diseases, Tenth Revision; SSI, State Serum Institute; IFA, indirect immunofluorescence assay

* Corresponding author at: Statens Serum Institut, Artillerivej 5, 2300 København S, Denmark.
E-mail address: luoc@ssi.dk (L.F. Ocias).

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Rickettsia rickettsii to the more benign African tick-bite fever caused by *Rickettsia africae*.

SFG rickettsiae are an important cause of imported travel-related infections, but can also be found endemically in the Scandinavian countries (Askling et al., 2009; Jensenius et al., 2003; Kantsø et al., 2010; Nilsson et al., 1999a; O'Brien et al., 2001; Quarsten et al., 2015; Skarphédinsson et al., 2007; Stensvold et al., 2015; Svendsen et al., 2009; Wölfel, 2017). In Denmark, *Rickettsia helvetica* and *Rickettsia monacensis* have been detected by PCR in *Ixodes ricinus* ticks (Kantsø et al., 2010; Skarphédinsson et al., 2007; Stensvold et al., 2015; Svendsen et al., 2009; Wölfel, 2017). Of these, *R. helvetica* is the most prevalent SFG rickettsia and has been found in ticks all over the country with one study by Svendsen et al. showing a prevalence of 13%, making it one of the most common tick-borne organisms (Kantsø et al., 2010; Skarphédinsson et al., 2007; Stensvold et al., 2015; Svendsen et al., 2009). Despite this fact, data are lacking on the occurrence and clinical features of both endemic and imported rickettsioses in Denmark.

In this study, we have reviewed the clinical features and travel history of two groups of patients; 1) in-hospital patients diagnosed with rickettsial disease and 2) primary health care (PHC) center patients exhibiting anti-rickettsia antibodies. The aim was to elucidate the clinical spectrum of infection in Danish patients afflicted by rickettsioses and to examine the geographical origin of imported cases in these two cohorts.

2. Materials and methods

2.1. Data sources for study cohorts

Personal identification numbers of hospital patients were extracted from the Danish National Patient Registry (DNPR). The DNPR, established in 1977, records data on all patients discharged from non-psychiatric hospitals. Since 1995 it has also included data on all visits to outpatient departments and emergency clinics in Denmark (Lynge et al., 2011). Diagnoses are coded by the attending physician according to the International Classification of Diseases, Tenth Revision [ICD-10]. From this registry we extracted personal identification numbers of patients appointed a clinical diagnoses of rickettsiosis.

Personal identification numbers of PHC center patients were extracted from the serological database at the State Serum Institute (SSI), where the laboratory diagnosis of rickettsioses is currently centralized, thus giving us access to serological data from across the country.

2.2. In-hospital patient cohort

The DNPR was searched for 15 ICD-10 codes relating to the diagnosis of rickettsiosis (Table A1 in Supplementary material), in the years 2010–2015. The search yielded 430 national records. Three university hospitals were selected for data collection; Rigshospitalet in Copenhagen, Hvidovre University Hospital in Hvidovre, a suburb to Copenhagen, and Odense University Hospital in the city of Odense on the island of Funen. These hospitals were chosen as they house major departments of infectious diseases, diagnosing and treating a wide variety of infectious diseases, both endemic and imported. In the years 2010–2015, a total of 120 patients had been appointed ICD-10 codes of rickettsial disease at these hospitals, representing 28% of the national total; 37 (31%) at Rigshospitalet, 16 (13%) at Hvidovre University Hospital and 67 (56%) at Odense University Hospital. These patients had their medical records reviewed by LFO and BBJ and were screened for eligibility. Thirty-two patients were excluded as they were given another diagnosis in their electronic medical records, despite being appointed an ICD-10 code indicating rickettsioses. Another two patients were excluded as their medical records were unavailable. In the end, a total of 86 hospital patients were found to be eligible (Fig. 1). The clinical manifestations and travel history of these patients were used in the final analysis. A cut-off of 14 days prior to symptom start was chosen for the travel history as it corresponds to the maximum incubation period of most rickettsial diseases.

2.3. PHC center patient cohort

The SSI serological database was searched for sera submitted from PHC centers and tested for anti-rickettsia antibodies in the years 2012–2015. A total of 293 samples were identified, of which 94 (32%) exhibited anti-rickettsia antibodies. Seventy-nine individual patients could be identified, after duplicate samples had been removed. The PHC centers having requested the serological analysis were contacted using telephone interviews and inquired about the patient's clinical manifestations and any mention of travel history in the corresponding medical records. In case no travel history was mentioned in the medical records, it was noted as "unknown". To mitigate recall bias, we started with the PHC centers that had most recently requested the analysis and then continued in a consecutive manner until 2012. Interviews were conducted by a medical student and followed a strict questionnaire (Fig. B1 in Supplementary material). In the event that data could not be directly provided over the telephone, they were faxed over to SSI. Forty-nine patients were excluded due to the PHC centers failing to provide relevant data and four patients were excluded due to stationary or diminishing IgM without IgG seroconversion on convalescent sera. In the end, data were provided for 26 patients from 25 PHC centers (Fig. 2). Fourteen of these patients had also been tested for antibodies against *Borrelia burgdorferi* sensu lato, *Anaplasma phagocytophilum*, *Bartonella* spp. and *Francisella tularensis*. Antibodies against one of these pathogens were detected in four patients. However, as three of them had high IgG titer values against SFG rickettsiae (1:512, 1:1024, 1:1024), prior exposure to rickettsiae was still very likely. The fourth patient was asymptomatic and exhibited IgM antibodies against SFG rickettsia (1:128) and IgG antibodies against *Anaplasma phagocytophilum*. The antibody titers of the included patients are presented in Table 1.

2.4. Laboratory diagnosis of rickettsiosis

2.4.1. Serology

Serological analysis at SSI is performed using a commercially available indirect immunofluorescense assay (IFA) produced by Focus Diagnostics (IF0100G, *Rickettsia* IFA IgG; Focus Diagnostics, Inc., Cypress, CA, USA). The assay exploits the cross-reactivity of antibodies within the SFG rickettsiae and TG rickettsiae and uses antigens from *R. rickettsii* (RR) and *Rickettsia typhi* (RT) to discriminate between these two groups. The assay has been used for several years at SSI and has been shown to detect antibodies against both *R. africae* (Johansen and Thybo, 2011) and *R. helvetica* (unpublished data). A titer of 1:64 or above is considered positive for IgM. For IgG, the same applies for a titer of 1:512 or above. Both the IgG and IgM cut-off values are based on Danish healthy blood donors to achieve a specificity of 95%. This has resulted in a slight increase of the manufacturer's cut-off value for IgG (Kantsø et al., 2009). The IFA IgG kit has been evaluated for cross-reactivity with other bacteria using a panel of 80 sera from patients with antibodies against organisms that might cross-react with rickettsiae including *Salmonella* spp., *Campylobacter jejuni*, *Helicobacter pylori*, *Coxiella burnetii*, *Mycoplasma pneumonia*, *Chlamydophila pneumoniae*, *Bartonella henselae* and *Borrelia burgdorferi* sensu lato. Cross-reactivity was reduced considerably using the modified cut-off levels with none of the examined samples displaying reactivity at an IgG titer of 1:512 or above. Furthermore, neither the IFA IgM nor the IgG kits have during the years of routine use and using the current cut-off levels displayed any significant cross-reactivity with other tick-borne pathogens such as *Anaplasma phagocytophilum*, *Borrelia burgdorferi* sensu lato, *Bartonella* spp. and *Francisella tularensis*. To further prevent cross-reactivity, all samples underwent rheumafactor adsorption, prior to IgM determination.

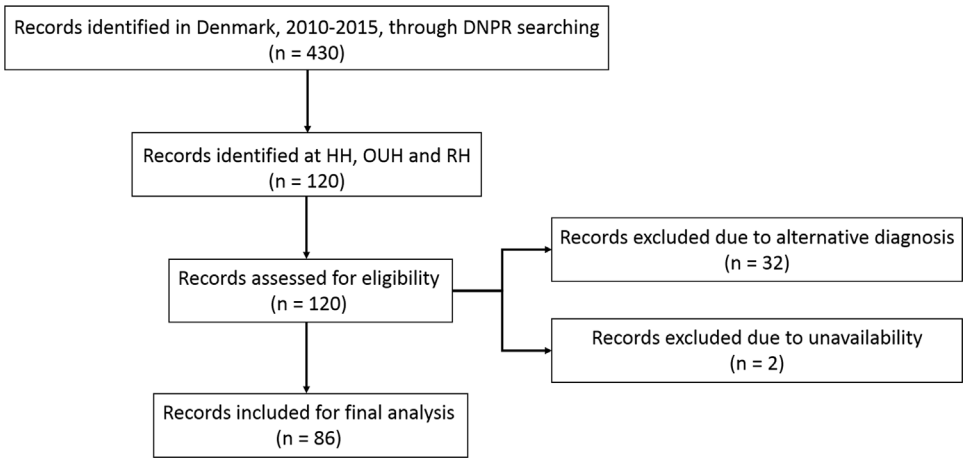


Fig. 1. Inclusion process for the hospital cohort. HH: Hvidovre Hospital. OUH: Odense University Hospital. RH: Rigshospitalet.

2.4.2. PCR

A real-time PCR with genus-specific primers (CS-F and CS-R) was used to amplify a 74-bp fragment of the *R. rickettsii* citrate synthase (gltA) gene as previously described (Stenos et al., 2005).

3. Results and discussion

We found that rickettsioses do not appear to pose a major public health problem in Denmark, with most of the hospital patients presenting with mild infections caused by SFG rickettsiae. Only two of the hospital patients presented with a considerable inflammatory response as reflected in an elevated CRP over 100 mg/L. Treatment of choice was doxycycline in 90% (77/86) of the hospital cases, with the remaining cases receiving no treatment at all (8/86) or azithromycin (in a pregnant woman). No fatalities were reported and all hospital patients recovered from their infections. Of the 79 PHC patients exhibiting anti-rickettsia antibodies in the years 2012–2015, clinical data were obtained for 26 (33%), none of whom presented with severe or acute life-threatening symptoms.

Considering a Danish population of 5,500,000, an incidence of 1.3/100,000 per year can be estimated for the hospital cases, most of which are imported. For the PHC centers, an incidence of 0.8/100,000 per year can be estimated, assuming that the number of patients with rickettsiosis and no detectable antibody reactivity at the time of diagnosis, is the same size as those who are seropositive. Combined, these two estimates give an estimated national incidence of around 2.0/100,000 per year (Appendix C in Supplementary material). The travel history and clinical manifestations of both cohorts are presented in Figs. 3 and 4 and Tables 2 and 3.

3.1. Hospital

3.1.1. Imported rickettsial infections

Almost all (91%) of the hospital patients had a travel history outside

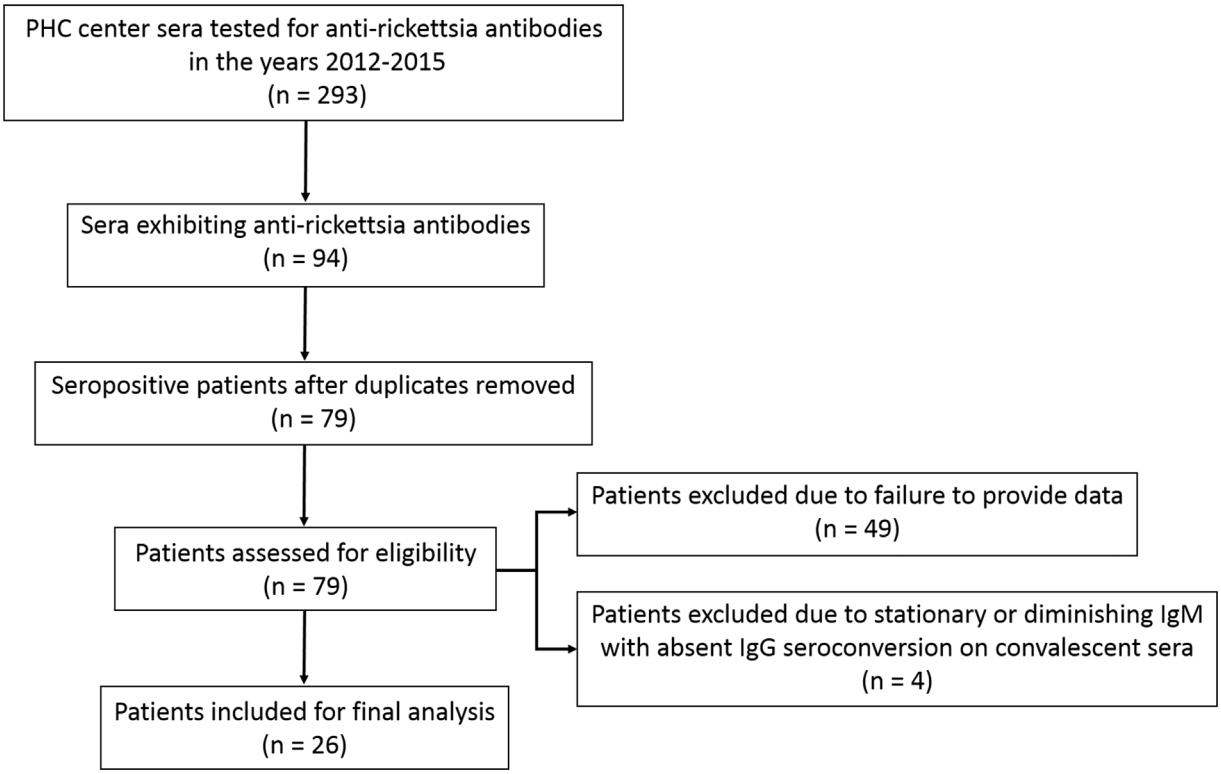


Fig. 2. Inclusion process for the PHC center cohort.

Table 1
Antibody titers against *Rickettsia* spp. in the hospital (a) and PHC center (b) cohorts. Serology was requested for 54 (63%) in-hospital patients. Of these, 31 displayed initial antibody reactivity against *Rickettsia* spp. Nineteen of the hospital patients displayed solely RR IgM, five had RR IgG and the remaining had some constellation of two or more types of antibodies. Of the 26 PHC center patients, 17 patients displayed solely RR IgM, one displayed RR IgG alone and eight had some constellation of two or more types of antibodies. The cut-offs used were 1:64 for IgM and 1:512 for IgG. All sera were analyzed at SSI. RR: *R. rickettsii*. RT: *R. typhi*.

	1:64	1:128	1:256	1:512	1:1024	1:2048	1:4096	1:8000	1:16000	1:32000
a.										
RR IgM	5	10	3	3	1	1	0	0	1	1
RR IgG	–	–	–	2	2	0	2	1	0	0
RT IgM	0	1	0	0	0	0	0	0	1	0
RT IgG	–	–	–	3	0	0	1	0	0	0
b.										
RR IgM	4	11	4	1	2	1	0	0	0	0
RR IgG	–	–	–	1	2	3	1	1	0	0
RT IgM	1	0	1	0	0	0	0	0	0	0
RT IgG	–	–	–	1	1	0	0	0	0	0

of Denmark with the incidence slightly peaking in late winter and early spring (January through April). Cases were, however, reported throughout the whole year (Table 4). Fever/chills and eschar(s) were the most common clinical manifestations seen in 84% (72/86) and 57% (49/86) of the patients respectively. The diagnosis was most often made on clinical grounds alone (24/86) in patients with characteristic clinical manifestations, such as eschar(s), and a relevant travel history. *Rickettsia* DNA was detected using PCR in eleven patients, with all samples coming from swabs or skin biopsies except for one blood specimen. Of the 15 patients who also had convalescent sera submitted for analysis, only five demonstrated IgG seroconversion or a greater than two-fold dilution rise in titer.

A key finding was that a significant majority of the hospital patients had a travel history to southern Africa, South Africa in particular (65%), and a clinical picture compatible with African tick-bite fever caused by *R. africae*. Despite other SFG rickettsiae such as *Rickettsia conorii*, *Rickettsia aeschlimannii* and *Rickettsia mongolitimonae* being present in this region, 16% (14/86) of the patients had multiple eschars, a feature characteristic of African tick-bite fever, and 33% (28/86) had visited Krueger National Park for safari or game hunting, a known risk factor for this type of travel-related rickettsiosis (Parola, 2006; Pretorius and Birtles, 2004, 2002). All except two of the patients presenting with multiple eschars had visited South Africa with the remaining having visited Zimbabwe and Kenya, both endemic for *R. africae*. None of these patients presented with severe infection indicative of the more virulent Mediterranean spotted fever caused by *R. conorii* (Pretorius et al., 2004; Raoult et al., 1986). Only one patient in the hospital cohort was diagnosed with TG rickettsiosis (murine typhus), following a visit to Tunisia, but underwent spontaneous remission without treatment. A similar distribution of rickettsioses has been reported in several single-center and multi-center studies, with African tick-bite fever being the most common rickettsial infection in returned travelers (Freedman et al., 2006; Jelinek and Löscher, 2001; Jensenius et al., 2003, 2002)

3.1.2. Endemic rickettsial infections

Despite eight of the hospital patients denying any recent travel history in their medical records, thereby raising the possibility of an endemic rickettsiosis, only one of them demonstrated convincing laboratory evidence of infection with a greater than two-fold dilution rise in titer and IgG seroconversion in the convalescent sera. This low number of possible endemic rickettsioses in the hospital cohort is in line with several other studies, reporting asymptomatic seroconversion or mild, self-limiting disease during infection with *R. helvetica*, likely perceived as too mild for hospital referral by many primary care physicians (Fournier et al., 2004, 2000; Lindblom et al., 2016; Phongmany

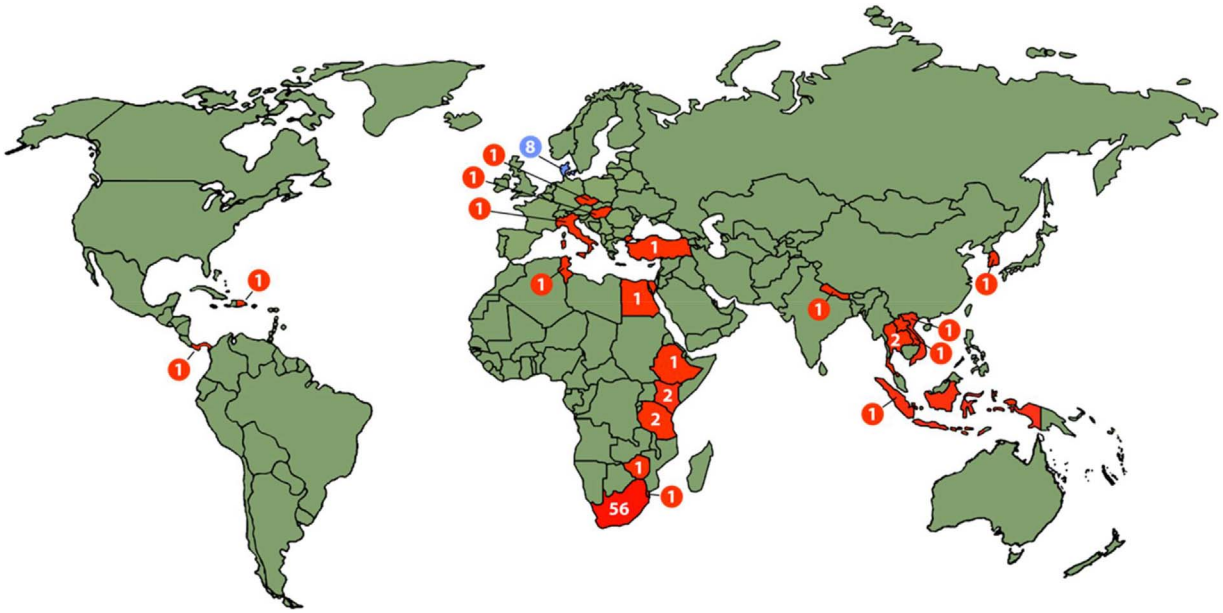


Fig. 3. Travel history of hospital patients (n = 86) 14 days prior to symptom start. Most patients (65%) had visited South Africa. Eight patients denied international travel. Other destinations included: Kenya, Tanzania, Zimbabwe, Ethiopia, Swaziland, Indonesia, Vietnam, Egypt, Tunisia, Nepal, Turkey, Panama, Czech Republic, Italy, Hungary, South Korea, Laos, Thailand, and The Dominican Republic.

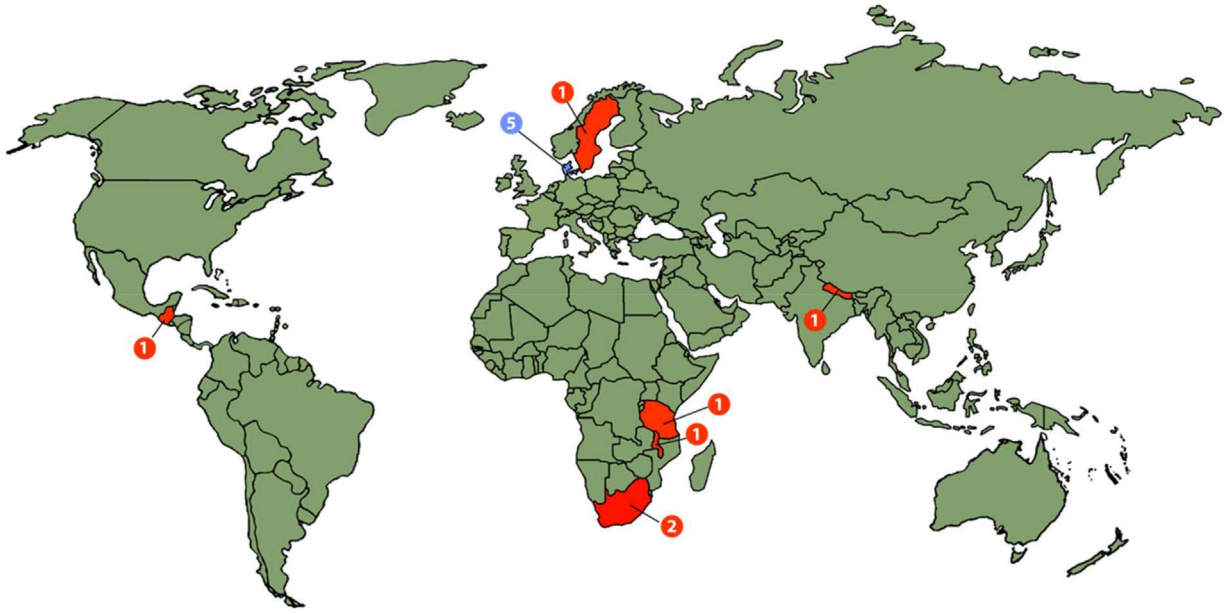


Fig. 4. Travel history of PHC center patients (n = 26). Half of all patients had an unknown travel history as it was not commented upon in the medical records. For the rest of the patients, most (five) had stayed in Denmark. Four patients had visited sub-Saharan Africa (South Africa, Tanzania and Malawi). Other destinations included: Guatemala, Nepal and Sweden. One patient had visited an unspecified country in Africa.

et al., 2006). Serious infections with *R. helvetica* have been described by a Swedish research group (Nilsson, 2009; Nilsson et al., 2011, 2010, 1999b). However, such reports are scarce and have, so far, not described cases in other geographical locations, outside of Sweden, where *R. helvetica* is also endemic in ticks.

3.2. PHC centers

For the PHC centers, only eight patients (31%) exhibiting antirickettsia antibodies had international travel mentioned in their medical records. Data on travel history for most patients (50%) was absent and five patients (19%) denied recent international travel in their medical records, again raising the possibility of endemic rickettsiosis. This is, however, impossible to establish with certainty as only five of the PHC patients had convalescent sera examined for antibodies of which only one, after a trip to Africa, demonstrated a greater than two-fold dilution rise in titer.

All PHC cohort patients presented with relatively mild disease, with the most frequently reported clinical indication for requesting serological analysis being a witnessed tick or insect bite (38%) followed by fever/chills (23%) and headache (19%). One of the patients was asymptomatic, but was worried about having been infected with a tick-borne pathogen following a tick bite and wanted to be tested to ease his mind. Another one was asymptomatic, apart from the presence of an erythema migrans lesion. Use of serology in such patients, diminishes the positive predictive value and should be avoided. Five patients

Table 2
The most common clinical manifestations of patients diagnosed with rickettsiosis at hospitals.

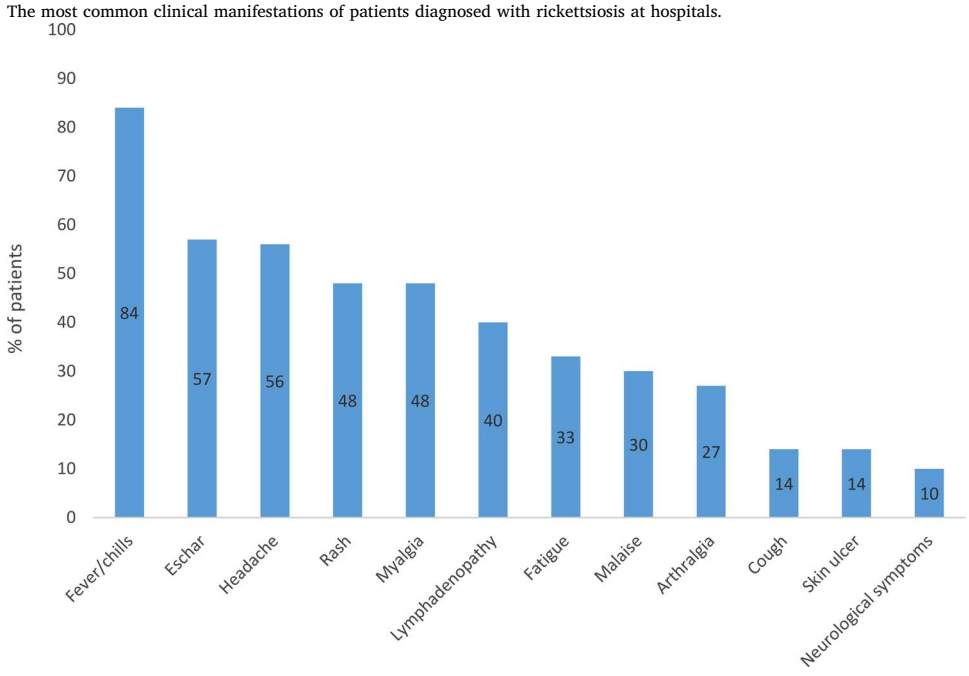


Table 3
Clinical indications for requesting *Rickettsia* serology at PHC centers in Denmark.

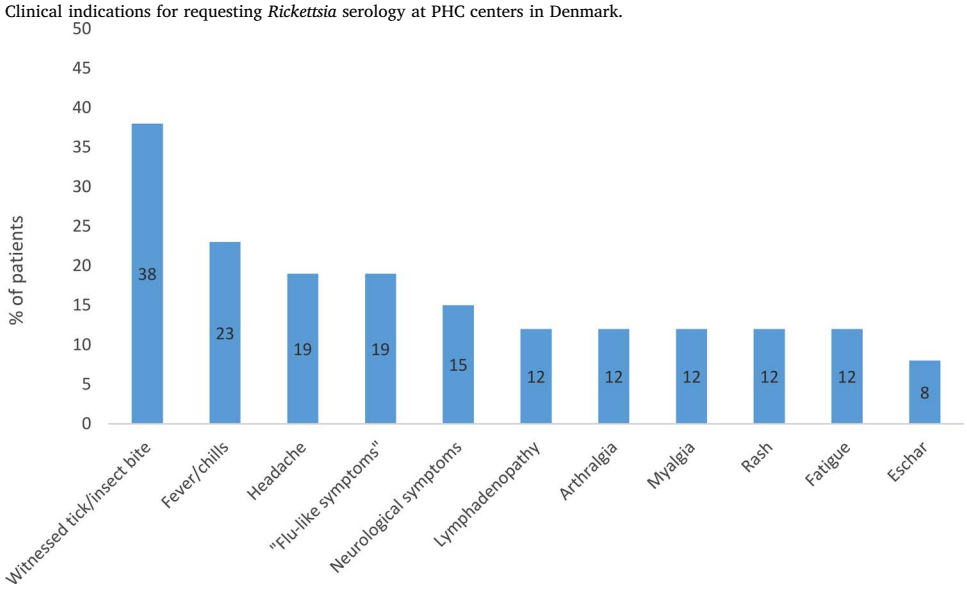
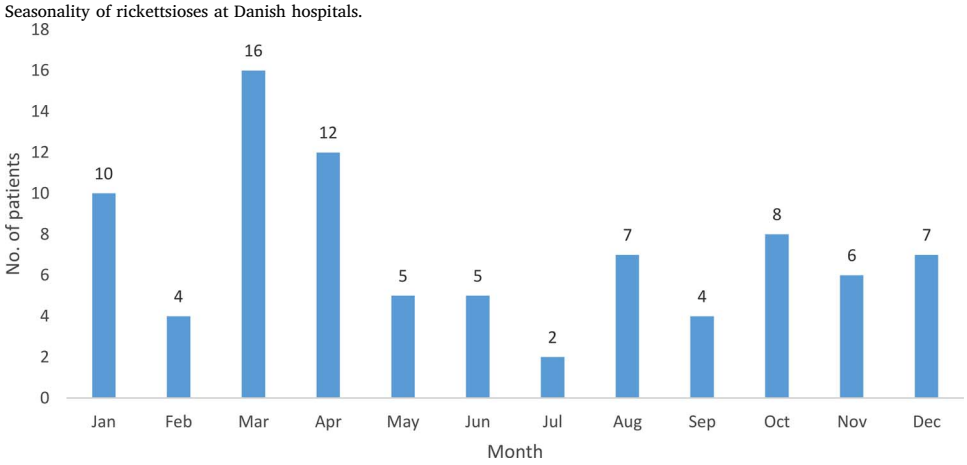


Table 4
Seasonality of rickettsioses at Danish hospitals.



(19%) presented with “flu-like symptoms”, an amalgamate of several symptoms, of which the precise composition is subjective which is why we have chosen to have this complex as a separate entity. Whether symptoms such as fever/chills, headache or myalgia should be put under this term could be discussed. Only two patients at the PHC centers, both of whom had recently visited South Africa, were tested due to the presence of an eschar. However, an unspecified skin ulcer was present in one other patient, with no recorded history of international travel, and could potentially represent a true eschar.

3.3. Strengths and limitations

This is the first overview of the clinical manifestations and travel history of patients with rickettsioses in Denmark, with most prior papers consisting of case reports and a short epidemiological report from 2012 (Johansen and Thybo, 2011; Kibsgaard et al., 2012a, 2012b; Madsen et al., 2004; Nielsen et al., 2004). Contrary to previous papers, we have included both hospital and PHC center patients, painting a more complete picture of the burden of rickettsial infections. There are, however, several weaknesses in this study, which are important to address.

First of all, the analyzed data constitute only a small percentage of Danish PHC patients suspected of rickettsioses as these patients had all been chosen based on a positive serology and clinical data were only provided for 33%. Basing the population solely on patients demonstrating anti-rickettsia antibodies also carries the risk of underestimating the number of patients afflicted by rickettsioses with a weak or delayed antibody response as well as patients diagnosed on clinical grounds alone or using solely PCR (Fournier et al., 2002). However, as only six PHC patients demonstrated a positive PCR analysis in the years 2012–2015, three of whom had visited South Africa, this latter concern does not seem to be a major problem.

Furthermore, it is important to remember that, especially IgM antibodies, have a tendency to cross-react and the possibility that some of the patients could potentially have been infected with some other organism cannot be ruled out. Two-hundred and seventy-nine sera with detectable anti-rickettsia antibodies were, in the years 2008–2015, also tested for *Borrelia burgdorferi* sensu lato, *Anaplasma phagocytophilum*, *Bartonella* spp. and *Francisella tularensis*. Of these, 72 (26%) displayed antibodies against one other tick-borne organism and 10 (4%) exhibited antibody reactivity against two other bacteria (unpublished data). Whether this was due to cross-reactivity, prior exposure or co-infection is not known.

Lastly, only five of the PHC center patients were followed-up with convalescent samples making the diagnosis less certain in many cases, especially in patients presenting with nonspecific symptoms. This is especially problematic when attempting to diagnose infections caused by the endemic *R. helvetica* which often lack a rash or the characteristic

eschar (Fournier et al., 2004; Phongmany et al., 2006). However, given the high specificity of the serological assay used (95% for both IgM and IgG), prior exposure to *Rickettsia* spp. is still probable in these patients.

4. Conclusion

Rickettsioses in Denmark most often present as mild infections. Among hospital patients, imported infections are the most common, with South Africa in particular dominating as the destination of choice for travelers with rickettsiosis returning to Denmark, most of whom have presumably been infected with African tick-bite fever caused by *R. africae*. The diagnosis is most often made on clinical grounds alone with a majority of patients presenting with a characteristic eschar and a relevant travel history to South Africa in the winter and early spring months. Only one in-hospital patient presenting with mild symptoms had convincing evidence of having acquired the infection in Scandinavia

Among patients who tested positive for anti-rickettsia antibodies at PHC centers, 19% had no history of international travel and 50% had no mention of travel history in the medical records. These patients most often presented with mild, nonspecific flu-like symptoms and only rarely presented with cutaneous manifestations such as rash or eschar.

Ethics statement

The study was approved by the Regional Medical Ethics Committee (approval No. 53005) and the Danish Data Protection Agency (approval No. 15/09765).

Conflicts of interest

All authors report no conflicts of interest.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.ttbdis.2018.01.016.

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