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Review

Rickettsiae and rickettsial diseases in Croatia: Implications for travel medicine

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Summary *Aim:* To review the current state of knowledge concerning rickettsiae and rickettsioses in Croatia and to discuss their implications for travellers.
Methods: The PubMed database was searched from 1991 to 2015 by combining the words "rickettsia," "rickettsiosis", "travellers" and "Croatia".
Results: Since 1969, Croatia appears to be free of epidemic typhus (ET) caused by *Rickettsia prowazekii* and the last case of Brill-Zinsser disease was recorded in 2008.

Mediterranean spotted fever (MSF) caused by *Rickettsia conorii* is the most frequent human rickettsial infection in Croatia, followed by murine typhus caused by *Rickettsia typhi*. Human cases of MSF and murine typhus have been predominantly observed along the eastern Adriatic coast from Zadar to Dubrovnik and between Zadar and Split, respectively.

Rickettsia akari, etiologic agent of rickettsialpox, was isolated from blood of a patient diagnosed with MSF in Zadar, but no cases of rickettsialpox were reported.

Several species of pathogenic (*Rickettsia slovaca*, *Rickettsia aeschlimannii*, *Ricketsia helvetica*, and *Ricketsia raoultii*) and species of undetermined pathogenicity (*Ricketsia hoogstraalii* sp. nov.) rickettsiae were identified in ticks collected in different ecological regions of Croatia.

A search of the literature revealed no evidence of rickettsial infection in travellers visiting Croatia. Three imported cases of *Rickettsia africae* were observed in travellers returning from South Africa.
Conclusion: Rickettsiae and rickettsial diseases continue to be present in Croatia. As they can be acquired while travelling, physicians should consider rickettsial infection in the differential diagnosis of patients returning from Croatia and presenting with febrile illness.

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1. Introduction

Rickettsiae are gram-negative, obligate intracellular bacteria belonging to the family *Rickettsiaceae*, order *Rickettsiales*. Members of the genus *Rickettsia* may be classified into spotted fever group (SFG) rickettsiae, typhus group (TG) rickettsiae, the *Rickettsia bellii* group, and the *Rickettsia canadensis* group [1,2]. The largest group comprises the spotted fever group rickettsiae (SFG) and consists of a multitude of pathogenic and non-pathogenic species, transmitted mainly by ticks [1,3—6]. Rickettsial infections are prevalent throughout the world and may cause serious diseases in humans [1—6]. Rickettsioses are increasingly being recognized among international travellers [7,8]. Besides that, rickettsiae are also recognized as potential threat for humans during the current refugee crisis in Europe [9].

Since 1988, more than 450 cases of travel-associated rickettsioses have been reported in the literature [10,11].

The diseases known as rickettsioses have been recorded throughout the littoral of Croatia, including Istria and Hrvatsko Primorje [12]. The Croatian coastal area between Zadar and Dubrovnik is an endemic area for *Rickettsia conorii* and *Rickettsia typhi* [[13]; Fig. 1].

As shown in Table 1, over 13 million travellers visited Croatia in 2014. Out of them, more than 11 million people visited Adriatic Croatia and over 5 million spent vacation in Dalmatia, which is known to be endemic for rickettsiae [13,14]. The surface of this area is 12,951 km^2 and it is populated with 856,758 inhabitants [15,16].

The purpose of this article is to review the current state of knowledge on rickettsiae and rickettsioses and the frequency of rickettsial diseases among travellers to/from Croatia.

2. Methods

The sources of data used for this review were the PubMed database, published abstracts and articles reporting original data on rickettsiae and rickettsioses in Croatia, as well as data from the National Institute of Public Health mandatory infectious disease reports [17]. We searched the PubMed database from 1991 to 2015 by combining the words "rickettsia," "rickettsiosis", "travellers" and "Croatia". Other data were obtained from the National and University Library in Zagreb, Croatia. The articles reporting original data on rickettsia and rickettsioses in Croatia were selected for further evaluation.

3. Results

A total of 36 references were revealed in the PubMed database. Of those, 18 publications, concerning infections of human and reservoirs, were selected for the current review.

In Croatia, rickettsioses are notifiable diseases, and the diagnosis of rickettsioses usually depends on clinical—epidemiological evidence supported by serologic confirmation. For diagnostic purposes, prior to 1988, complement fixation test was used. After 1988, the indirect immunofluorescence antibody assay (IFA) has been performed to determine the titres of IgM and IgG antibodies in patients' sera. In the 1990s, a few seroepidemiological studies were conducted with the aim to collect data related to the presence of rickettsiae as well as to determine their importance for public health in Croatia. As ticks are the most important vectors and reservoirs of SFGR, including human rickettsial pathogens, molecular methods were used to investigate, identify and characterize SFG rickettsiae in ticks collected in ecologically different areas of Croatia.

4. Typhus group (TG) rickettsiae

4.1. Epidemic typhus and Brill-Zinsser disease

During World War II (WW II), outbreaks of louse-borne epidemic typhus (ET) due to *Rickettsia prowazekii* transmitted by body louse (*Pediculus humanus corporis*) were common on the territory of former Yugoslavia, especially in mountainous areas of Bosnia and Herzegovina [18]. Epidemic typhus had great impact on the public health in the region. Between 1945 and 1969, a total of 2794 human cases of ET were officially reported in Croatia [17]. Improvement of socioeconomic conditions after WW II influenced a marked decrease of ET. In the decade after WWII, from 1945 to 1954, 2750 cases were recorded with an annual incidence between 7 and 2169. Most of the cases occurred sporadically. According to official notifications, the last case of ET was reported in 1969 [12,17,19].

According to data from the Croatian Institute of Public Health a total of 174 cases of Brill-Zinsser disease, recrudescent form of louse-borne epidemic typhus, were recorded in Croatia from 1957 to 2013. In the period from 1995 to 2013, only 3 cases were reported and the last case was recorded in 2008. Most of the patients gave a history of epidemic typhus during the period from 1941 to 1953, and more than half of them originated from Bosnia and Herzegovina. The mean age of these patients was 55.4 years (range 37—81) [12,19].

4.2. Murine or endemic typhus

Murine or endemic typhus is an infectious disease caused by *R. typhi*. In Croatia the diseases was recognized on the island of Solta in 1942, where nine proven cases of murine typhus were recorded [12,13]. Since then, sporadic cases have been observed nearly every year. Between 1992 and 2003, 12 cases of murine typhus were reported [12]. These cases originated and occurred on the Croatian littoral and islands between Zadar and Split. Rats (which are the main host of *R. typhi*) are abundant in these littoral areas. Climatic and ecological conditions are also favourable for maintaining rat fleas (*Xenopsylla cheopis*) which may act both as reservoirs and as vectors of *R. typhi*. In the Zadar- and Split-Dalmatia County, murine typhus cases were distributed in practically all age groups, and cases occurred during the entire year [12,13,20]. The signs and symptoms of patients included fever (100%), headache (75.0%), rash (70.8%), malaise (53.2%), and arthralgia/myalgia (43.6%). Rash was distributed over the trunk and limbs, sparing the face, palms and soles [13]. Asymptomatic infections with *R. typhi* are more common than clinical disease [3,4]. Seroepidemiologic studies have shown the Zadar area to be highly endemic for *R. typhi* [20]. Antibody prevalence in

Figure 1 Map of Croatia showing the geographic distribution of *R. conorii* (♦) and *R. typhi* (▲) infections.

humans living along the coast and on the islands around Zadar was between 23.0% and 66.0% [20]. However, overt manifestations of the disease or the need for hospital treatment are not as common as might be expected from the high exposure of the area population to *R. typhi*. Most infections probably are asymptomatic, or cases go undiagnosed and spontaneously resolve. In the area of Split, 21.4% of the examined healthy persons had antibodies to *R. typhi* [21]. Positive serology was more common in those who reported contact with domestic animals. The prevalence of antibodies according to sex does not differ significantly, indicating the degree of exposure for men and women in the area was very similar. On the most southern Adriatic island of Mljet antibodies to *R. typhi* have been observed in the sera of 8.2% of healthy residents examined [13,21].

5. Spotted fever group rickettsiae (SFGR)

5.1. Mediterranean spotted fever (MSF)

R. conorii is the etiologic agent of Mediterranean spotted fever (MSF), a disease widely distributed in Mediterranean countries, including the south of Croatia. The first clinical description of spotted fever disease in Croatia was reported by Tartaglia in 1935 in the city of Split. The first well documented, serologically confirmed cases of MSF in Croatia were detected among inhabitants and tourists in Split and its adjacent suburban—semirural area in 1982 [12,13,22]. Since then, there has been a growing interest in the study of MSF in Croatia. Human cases of MSF have been

Table 1 Statistical data for different NUTS levels in Croatia, Adriatic Croatia and Dalmatian part of Croatia.

Administrative unit	Area (km^2)	Population (according to 2011 census)	Population density (people per km^2)	Number of tourists (2014)
1	2	3	4	5
Croatia (NUTS I)	56,594	4,284,889	75.7	13,128,416
Adriatic Croatia (NUTS II)	24,705	1,411,935	57.8	11,494,216
Dalmatia	12,951	856,758	66.2	5,492,269
Zadar County (NUTS III)	3646	170,017	44.5	1,186,908
Šibenik-Knin County (NUTS III)	2984	109,375	37.7	746,177
Split-Dalmatia County (NUTS III)	4540	454,798	102.5	2,212,903
Dubrovnik-Neretva County (NUTS III)	1781	122,568	69.0	1,346,281

NUTS = Nomenclature of Units for Territorial Statistics.
Sources. Column 2 — Magaš, D. Geography of Croatia. University of Zadar, Zadar, Croatia, 2015. Columns 3 and 4 — Croatian Bureau of Statistics. Census of population, households and dwellings 2011. Zagreb, Croatia, 2016. Column 5 — Croatian Bureau of Statistics. Tourism 2014, Statistical report No. 1539. Zagreb, Croatia, 2015.

detected mostly in the southern part of Croatia from Zadar to Dubrovnik. Sporadic cases were also observed in northern parts of the Croatian littoral (Istria, Hrvatsko Primorje) [12,13,22]. The first clinical isolate of *R. conorii* (strain Malish) was obtained from the blood of a male patient living in area of Split [23]. In addition, *R. conorii* was isolated not only from the main tick vector *Rhipicephalus sanguineus*, but also from *Rh. bursa* and *Dermacentor marginatus* [24].

Similar results were found in serological studies from Split and Zadar area. Males were more frequently diagnosed as having MSF than females. All age groups were affected, with a peak in the 0—10 years age group (23.8%). Cases arise more frequently from people living in suburban—semirural settlements than in those from urban areas [13,25]. During the 12-year period of observation, 75 patients with MSF were reported in Croatia, 66 of them in the littoral [12]. Annual incidence of MSF ranged from 1 to 17 cases per year. Most cases occurred from July through September, with a peak incidence in August. Typical cases presented with high fever, headache and maculopapular rash of the palms and soles. Eschar, at the site of the tick bite, was found in 63.5% and 23.3% of patients from Split and Zadar, respectively [13,25,26]. Although MSF has been considered to be a mild disease in Croatia, a case with malignant form manifested as meningoencephalitis and one with severe respiratory distress syndrome have been reported [26]. In Croatia, the only reported fatal case of MSF was a 58-year-old male from Zadar [26].

Serologic evidence of circulation of *R. conorii* among residents was obtained in all the surveyed localities in southern Croatia. In the region of Split, a prevalence (43.7%) of antibodies to *R. conorii* was observed, which was similar to that recorded in the region of Zadar (41.6%), but higher than 14.7% described on the island of Mljet [27]. Further, antibodies to *R. conorii* have been found in sera of dogs, cattle, goats and sheep in all the surveyed localities [28,29].

5.2. Rickettsialpox

Rickettsialpox is a disease caused by *Rickettsia akari*, which is maintained in mice through the mite bites [3,4]. In 1991, *R. akari* was isolated from blood of a patient clinically diagnosed with MSF in Zadar [30]. Results from a subsequent seroepidemiological investigation showed that 24.8% persons living in the area of Zadar and adjacent islands had antibodies to *R. akari* [30]. Because cross-reactivity is known to occur between SFG rickettsiae, such a high seroprevalence raises the question of whether rickettsiae other than *R. akari* are involved in asymptomatic human infection in the region.

5.3. Rickettsiae identified in ticks in Croatia

Ticks are the main vectors of SFGR [1,3,4,8]. During the past two decades, several rickettsiae have been characterised by molecular techniques in ticks collected in various parts of Croatia. In the area of Split the presence of three SFG rickettsiae with yet uncertain pathogenicity for humans in Croatia were detected. Specific sequences of *Rickettsia slovaca*, *Rickettsia aeschlimannii* and *Rickettsia rhipicephali* were detected in *D. marginatus*, *Hyalomma marginatum* and *Rh. turanicus* ticks, respectively [31,32]. *Ricketsia hoogstraalii* sp. nov. strain Croatica as a novel SFGR with an unknown pathogenicity was found in *Haemaphysalis sulcata* ticks collected from sheep and goats in southern Croatia in 2006 [33]. *Dermacentor reticulatus* ticks collected in north-western Croatia were infected with *Ricketsia helvetica* (10%), *R. slovaca* (2%) or co-infected with both species (1%) [34].

5.4. Rickettsial diseases in travellers

According to the GeoSentinel Surveillance Network, rickettsial diseases were reported in 280 international travellers, out of whom 82.5% had spotted fever (SFG) rickettsiosis during 1996—2008 [8].

Reports on human cases of rickettsial diseases acquired in Croatia in travellers were found neither in this multicenter analysis nor in the PubMed search.

Sporadic cases of ricketsiosses in domestic travellers were recorded in continental Croatia and could be related to person's travel to the littoral [12,17,26].

Three imported cases of *Rickettsia africae* in travellers returning from South Africa to Croatia, confirmed by PCR and sequencing, were described recently [35].

6. Discussion

Rickettsial pathogens are maintained in natural cycles involving vertebrate hosts and arthropods. They are transmitted by arthropod vectors (ticks, mites, lice, fleas) and are found in the regions abundant with specific hosts and vectors [1—5,36]. It has been suggested that climatic changes and global warming are influencing arthropods and arthropod-borne pathogens such as rickettsiae. As a result of globalization, changes in geographical distribution of known rickettsial species, as well as emergence of imported novel rickettsioses could occur in different geographic areas and climatic regions [5,6,36].

Croatia is a well known as an endemic region for rickettsial diseases. Contrary to the period after World War II, nowadays epidemic typhus represents no public health problem in Croatia. However, the risk of infection with *R. prowazekii* is still present as imported epidemic typhus or as recurrence as a Brill-Zinsser disease [36]. Unlike with most other rickettsioses, humans are primary reservoir of epidemic typhus [36—39]. Because this clinical form is bacteraemic, it can initiate an outbreak of ET when body louse (*P. humanus corporis*) infestation is prevalent in a persons living under poor socioeconomic and sanitary conditions (homeless population, refugees, migrants) [40,41]. Hence, the current refugee crisis rickettsioses might have implication for migrants and refugees from Middle East and Asia, using Croatia as a migratory route to the European Union (EU) [9,41]. The disease is still considered a major threat by public-health authorities, despite the efficacy of antibiotics, because poor sanitary conditions are favourable to body louse proliferation [37—39]. Finally, *R. prowazekii* is a potential category B bioterrorism agent, because it is stable in dried louse faeces and can be transmitted through aerosols [38,39].

SFG rickettsioses are the most commonly diagnosed rickettsial diseases among international travellers [7,8]. MSF was the most prevalent rickettsiosis in Croatia, followed by murine typhus [12]. The number of reported cases of MSF was low in comparison to the high prevalence of specific antibodies to *R. conorii* (a prevalence of 43.7%, 41.6% and 14.7% in the region of Split, Zadar, and on the most southern Adriatic island of Mljet, respectively) [27]. Even bigger discrepancy was observed between the low number of reported cases of murine typhus and high prevalence of antibodies to *R. typhi*. Seroepidemiologic studies have shown the Zadar area to be highly endemic for *R. typhi*. Antibody prevalence in humans living along the coast and on the islands around Zadar was between 23.0% and 66.0% [20]. In the area of Split, 21.4% of the examined healthy persons had antibodies to *R. typhi* [21]. There is a strong discrepancy between the frequency of infection and the incidence of subclinical and manifest clinical disease. Most infections probably are asymptomatic, or cases go undiagnosed and spontaneously resolve. Because a typical clinical presentation of MT is not regularly present, the disease is probably often underdiagnosed due to lack of awareness. As a result of the broad and persistent presence of peridomestic rat reservoirs and their fleas, and due to the fact that the faeces of infected fleas can contaminate broken skin, respiratory tract or conjunctivae, we should be aware of the possibility of epidemic occurrence of MT in the littoral region of Croatia.

After *R. akari* was isolated from the blood of a patient from Zadar, no further cases of rickettsialpox were reported in this area. It may be attributable to misdiagnosing with other exanthematous diseases, as has happened with the patient from whose blood *R. akari* was isolated [30].

Application of molecular techniques has resulted in the discovery of several species of pathogenic (*R. slovaca*, *R. aeschlimannii*, *R. helvetica*, and *Ricketsia raoultii*) and species of undetermined pathogenicity (*R. hoogstraalii* sp. nov.) rickettsiae in ticks collected in different ecological regions of Croatia [30—34]. Although *R. slovaca*, *R. helvetica*, and *R. aeschlimannii* are proven causative agents of human diseases, so far it is not known whether they cause disease in Croatia [1,5,36]. Hence, febrile diseases caused by these rickettsie should be included in the differential diagnosis of febrile illnesses occurring after a tick bite in Croatia.

Although it has been reported in several European countries [1,3,4,36], the prevalence of *Rickettsia felis* infections has not been studied in Croatia. *R. felis* causes flea-borne spotted fever, since it is transmitted by cat fleas.

Increased international travel has greatly increased the chances of more rickettsiae to be introduced to previously non-endemic northern regions [42]. These "imported" rickettsioses could be a cause of concern especially for inexperienced physicians.

Since 1988, when the first comprehensive series of travel-associated rickettsioses was published, >450 travel-associated cases have been published worldwide. Of 24,920 returned travellers seen at a GeoSentinel clinic from March 1997 through March 2006, 28% cited fever as a main reason for seeking care. Rickettsioses, as a specific infections causing systemic febrile illness were diagnosed in 2% of such patients and 20% of them were hospitalized [43]. In one other study, among 99,355 travellers who sought medical care at a GeoSentinel site during June 1996—December 2008, 13,763 had fever, and 211 (1.5%) of them were diagnosed with rickettsial disease [8]. This increase is likely attributable to improvements in diagnostic awareness and the availability of microbiological tests, as well as to increases in travel to areas of endemicity and in risk behaviour [11].

The report of three imported cases of *R. africae* in travellers returning from South Africa to Croatia is consistent with the fact that African tick bite fever is currently the most commonly encountered rickettsiosis in travel medicine [10,11]. No other case of ricketsioses imported to Croatia has been found in the literature search so far.

As Croatia is well-known endemic region for different rickettsioses, infections among visitors may appear and can be introduced to previously non-endemic countries through frequent travel. This is particularly interesting, because most infections occur in the autochtonous population from July to September, on the southern coast between Zadar and Dubrovnik, which is the main tourist destination.

Surprisingly, in spite of the high prevalence of antibodies to different species of rickettsia in humans and animals, ricketssiae detected in ticks and the confirmed clinical rickettsioses in the autochthonous population, reports on rickettsial infection in millions of travellers visiting Croatia every year are missing.

This can be explained because a typical clinical picture of rickettsioses is not regularly present and the diseases are probably often underdiagnosed due to lack of awareness. Physicians should be aware of the epidemiology of rickettsial infections in the tourist destinations and knowledge of specific geographical and seasonal distribution could be helpful in diagnosing illness.

Another possible reason for underreporting of rickettsial diseases in travellers may be influenced by several difficulties and pitfalls in laboratory diagnosis of rickettsial infections. Although a specific microbiological diagnosis should always be attempted and the definitive diagnosis of rickettsial infection requires laboratory confirmation, this approach is associated with several obstacles [7,8,44]. Serology, which is the most commonly used method for laboratory diagnosis of rickettsial infections, seldom yields diagnostic results on serum collected at the time of first presentation. IFA assay for detection of IgG antibodies and using paired acute and convalescence sera is currently considered to be the reference standard for serologic diagnosis of rickettsial infections [45,46]. However, IFA assay is insensitive during the first week of rickettsial infection, which is the period during which most patients seek medical attention and when the majority of specimens are collected for evaluation [47]. IFA assay is highly sensitive at detecting antibodies 2—3 weeks after illness onset, although patients infected with *Rickettsia africae* might not show seroconversion until 4 weeks after illness onset [48—50]. The majority of commercial reference laboratories, that conduct testing for rickettsial pathogens, test for IgG antibodies. Some laboratories also perform IFA assays and other serologic testing for IgM antibodies. However, false positive IgM antibodies are frequently detected in patients in whom no other supportive evidence of a recent rickettsiosis exists [50,51]. Further, very early therapy with a tetracycline-class drug can sometimes diminish or delay the development of antibodies to rickettsiae [52,53]. In addition, due to extensive cross-reactions, IFA is usually unable to determine the causative agent to the species level [11,44]. To circumvent this problem, serum samples may be shipped to a reference laboratory for Western blotting in conjunction with cross-adsorption assays [54].

The methods that are capable of identifying the infectious aetiology (PCR and isolation) are not widely available [7]. Isolation of causative rickettsiae is performed in only a few laboratories and requires biosafety level 3 conditions [7,8,44].

Therefore, although laboratory confirmation is important, a clinical diagnosis and empiric treatment with doxycycline are the standard practical approach [7]. In Croatia, a clinical entity known as rickettsioses accounted for the majority (68.1%) of rickettsial disease cases during the observation period between 1992 and 2003, indicating that the exact rickettsial species has not been definitely differentiated [12].

Because of all the above mentioned reasons, precise data on the incidence of rickettsioses among international travellers are unfortunately not available [11].

7. Conclusion

In conclusion, coastal and insular Croatia is endemic for several rickettsiae. This finding is of particular importance in light of the increasing popularity of the Croatian littoral as a holiday destination. Travellers within Croatia might be exposed to different arthropod vectors (ticks, fleas, mites) during travel, which can result in illness after they return home. Moreover, tourists increasingly take their own pets (dogs, cats) with them when they go on a trip. Pets could bring infected vectors to travellers home.

Because a typical clinical picture of rickettsioses is not regularly present, the diseases are probably often underdiagnosed due to lack of awareness. Physicians should be aware of the epidemiology of rickettsial infections in their local area and knowledge of seasonal distribution could be helpful in diagnosing illness.

Given a history of residence in or travel to an endemic area, exposure to ticks, fleas or mites and onset in spring, summer or fall in cases with compatible clinical signs, infection with a rickettsia must be suspected. Physicians should consider rickettsial infection in the differential diagnosis of patients (travellers) returning from Croatia and presenting with febrile illness. It is important to recognize the variety of clinical manifestations of the infection, use specific laboratory diagnostic assays, and begin with proper antimicrobial therapy. Early initiation of treatment decreases the risk of severe forms and mortality of the diseases caused by rickettsiae.

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Conflict of interest

None.

References

[1] Parola P, Paddock CD, Socolovschi C, Labruna MB, Mediannikov O, Kernif T, et al. Update on tick-borne rickettsioses around the world: a geographic approach. Clin Microbiol Rev 2013;26:657—702. http://dx.doi.org/10.1128/CMR.00032-13.

[2] Merhej V, Raoult D. Rickettsial evolution in the light of comparative genomics. Biol Rev Camb Philos Soc 2011;86:379—405. http://dx.doi.org/10.1111/j.1469-185X.2010.00151.

[3] Walker DH, Valbuena GA, Olano JP. Pathogenic mechanisms of diseases caused by Rickettsia. Ann N Y Acad Sci 2003;990:1—11.

[4] Raoult D, Roux V. Rickettsioses as paradigms of new or emerging infectious diseases. Clin Microbiol Rev 1997;10:694—719.

[5] Parola P, Socolovschi C, Jeanjean L, Bitam I, Fournier PE, Sotto A, et al. Warmer weather linked to tick attack and emergence of severe rickettsioses. PLoS Negl Trop Dis 2008;2:

e338. http://dx.doi.org/10.1371/journal.pntd.0000338 [Epub 2008 Nov 18].
[6] Parola P, Rovery C, Rolain JM, Brouqui P, Davoust B, Raoult D. *Rickettsia slovaca* and *R. raoultii* in tick-borne rickettsioses. Emerg Infect Dis 2009;15:1105—8.
[7] Walker DH. Rickettsial diseases in travellers. Travel Med Infect Dis 2003;1:35—40.
[8] Jensenius M, Davis X, von Sonnenburg F, Schwartz E, Keystone JS, Leder K, et al. Multicenter GeoSentinel analysis of rickettsial diseases in international travellers, 1996—2008. Emerg Infect Dis 2009;15:1791—8. http://dx.doi.org/10.3201/eid1511. 090677.
[9] Catchpole M, Coulombier D. Refugee crisis demands European Union-wide surveillance! Euro Surveill 2015;20. http://dx.doi.org/10.2807/1560-7917.ES.2015.20.45.30063.
[10] McDonald JC, MacLean JD, McDade JE. Imported rickettsial disease: clinical and epidemiological features. Am J Med 1988; 85:799—805.
[11] Jensenius M, Fournier PE, Raoult D. Rickettsioses and the international traveler. Clin Infect Dis 2004;39:1493—9 [Epub 2004 Oct 22 2004].
[12] Mulic R, Mihajlovic N, Ropac D, Zoric I, Alambasic K. Epidemiological characteristics of rickettsial diseases in Croatia. Mil Med 2006;171:64—8.
[13] Punda-Polić V, Luksić B, Capkun V. Epidemiological features of Mediterranean spotted fever, murine typhus, and Q fever in Split-Dalmatia County (Croatia), 1982—2002. Epidemiol Infect 2008;136:972—9. Epub 2007 Sep. 13.
[14] Croatian Bureau of Statistics. Tourism 2014, statistical report no. 1539. 2015. Zagreb, Croatia.
[15] Magaš D. Geography of Croatia. Zadar, Croatia: University of Zadar; 2015.
[16] Croatian Bureau of Statistics. Census of population, households and dwellings 2011. 2016. Zagreb, Croatia.
[17] Infectious diseases in Croatia. In: Baklaic Z, Deckovic-Vukres V, Kuzman M, editors. Croatian health service yearbook 2008. Zagreb, Croatia: Croatian National Institute of Public Health; 2009. p. 193—210.
[18] Punda-Polić V, Leko-Grbić J, Radulović S. Prevalence of antibodies to rickettsiae in the north-western part of Bosnia and Herzegovina. Eur J Epidemiol 1995;11:697—9.
[19] Turčinov D, Kuzman I, Puljiz I. Brill-Zinsser disease still occurs in Croatia: a retrospective analysis of 25 hospitalized patients [in Croatian]. Liječ Vjesn 2002;124:293—6.
[20] Dobec M, Hrabar A. Murine typhus on the northern Dalmatian islands, Yugoslavia. J Hyg Epidemiol Microbiol Immunol 1990; 34:175—81.
[21] Punda-Polić V, Sardelic S, Bradaric N, Klismanic-Nuber Z. Prevalence of antibodies to *Rickettsia typhi* in southern Croatia. In: Raoult D, Brouqui P, editors. Rickettsiae and rickettsial diseases at the turn of the third millennium. Amsterdam: Elsevier; 1999. pp.343—6.
[22] Radulovic S, Walker DH, Weiss K, Dzelalija B, Morovic M. Prevalence of antibodies to spotted fever group rickettsiae along the eastern coast of the Adriatic sea. J Clin Microbiol 1993;31:2225—7.
[23] Sardelić S, Fournier PE, Punda-Polic V, Bradaric N, Grgic D, Ivic I, et al. First isolation of *Rickettsia conorii* from human blood in Croatia. Croat Med J 2003;44:630—4.
[24] Radulovic S, Feng HM, Crocquet-Valdes P, Morovic M, Dzelalija B, Walker DH. Antigen-capture enzyme immunoassay: a comparison with other methods for the detection of spotted fever group rickettsiae in ticks. Am J Trop Med Hyg 1994;50:359—64.
[25] Punda-Polić V, Klismanic Z, Capkun V, Bradaric N. Demographic and epidemiologic features of Mediterranean spotted fever cases in the region of Split, Croatia. Ann N Y Acad Sci 2003;990:143—8.
[26] Dželalija B, Punda-Polić V, Medić A, Mraović B, Šimurina T. A case of Mediterranean spotted fever associated with severe respiratory distress syndrome. Microbes Infect 2015;17: 870—3. http://dx.doi.org/10.1016/j.micinf.2015.08.012 [Epub 2015 Sep. 3].
[27] Punda-Polic V, Klišmanic Z, Capkun V. Prevalence of antibodies to spotted fever group rickettsiae in the region of Split (southern Croatia). Eur J Epidemiol 2003;19:451—5.
[28] Punda-Polic V, Bradaric N, Klismanic-Nuber Z, Mrljak V, Giljanovic M. Antibodies to spotted fever group rickettsiae in dogs in Croatia. Eur J Epidemiol 1995;11:389—92.
[29] Punda-Polic V, Poljak S, Bubic A, Bradaric N, Klismanic-Nuber Z. Antibodies to spotted fever group rickettsiae and *Coxiella burnetii* among domestic animals in southern Croatia. Acta Microbiol Immunol Hung 1995;42:339—44.
[30] Radulovic S, Feng HM, Morovic M, Djelalija B, Popov V, Crocquet-Valdes P, et al. Isolation of *Rickettsia akari* from a patient in a region where Mediterranean spotted fever is endemic. Clin Infect Dis 1996;22:216—20.
[31] Punda-Polic V, Petrovec M, Trilar T, Duh D, Bradaric N, Klismanic Z, et al. Detection and identification of spotted fever group rickettsiae in ticks collected in southern Croatia. Exp Appl Acarol 2002;28:169—76.
[32] Duh D, Petrovec M, Trilar T, Punda-Polic V, Bradaric N, Klismanic Z, et al. A follow-up study on newly recognized Spotted Fever Group Rickettsiae in ticks collected in southern Croatia. Ann N Y Acad Sci 2003;990:149—51.
[33] Duh D, Punda-Polic V, Avsic-Zupanc T, Bouyer D, Walker DH, Popov VL, et al. *Rickettsia hoogstraaalii* sp. nov., isolated from hard and soft-bodied ticks. Int J Syst Evol Microbiol 2010; 60:977—84.
[34] Dobec M, Golubic D, Punda-Polic V, Kaeppeli F, Sievers M. *Ricketsia helvetica* in *Dermacentor reticulatus* ticks. Emerg Infect Dis 2009;15:98—100.
[35] Svoboda P, Lukas D. Cases of imported Rickettsia africae infection among travellers to South Africa. In: Abstract book. 1st Croatian congress on travel, tropical, migration medicine & HIV, Dubrovnik, Croatia; 2015 October 1—4; 2015. p. 39 [p. 11].
[36] Blanco JR, Oteo JA. Rickettsiosis in Europe. Ann N Y Acad Sci 2006;1078:26—33.
[37] Badiaga S, Brouqui P. Human louse-transmitted infectious diseases. Clin Microbiol Infect 2012;18:332—7. http://dx.doi.org/10.1111/j.1469-0691.2012.03778.
[38] Diaz JH. Environmental risk factors for epidemic typhus in the United States: wintertime is typhus time. J La State Med Soc 2012;164:16—20.
[39] Bechah Y, Capo C, Mege JL, Raoult D. Epidemic typhus. Lancet Infect Dis 2008;8:417—26.
[40] Badiaga S, Brouqui P, Raoult D. Autochthonous epidemic typhus associated with *Bartonella quintana* bacteremia in a homeless person. Am J Trop Med Hyg 2005;72:638—9.
[41] Antinori S, Mediannikov O, Corbellino M, Grande R, Parravicini C, Bestetti G, et al. Louse-borne relapsing fever (*Borrelia recurrentis*) in a Somali refugee arriving in Italy: a re-emerging infection in Europe? PLoS Negl Trop Dis 2016; 10(5):e0004522. http://dx.doi.org/10.1371/journal.pntd.0004522 [eCollection 2016].
[42] Pietzsch ME, Hansford KM, Cull B, Jahfari S, Sprong H, Medlock JM. Detection of *Dermacentor marginatus* and a possible *Rickettsia slovaca* case in the United Kingdom — the risk of the visiting traveller. Travel Med Infect Dis 2015;13(2): 200—1. http://dx.doi.org/10.1016/j.tmaid. 2015.01.002 [Epub 2015 Feb 10].
[43] Wilson ME, Weld LH, Boggild A, Keystone JS, Kain KC, von Sonnenburg F, et al. Fever in returned travelers: results from the GeoSentinel surveillance network. Clin Infect Dis 2007;44: 1560—8 [Epub 2007 May 7].

[44] Biggs HM, Behravesh CB, Bradley KK, Dahlgren FS, Drexler NA, Dumler S, et al. Diagnosis and management of tickborne rickettsial diseases: Rocky Mountain spotted fever and other spotted fever group rickettsioses, ehrlichioses, and anaplasmosis — United States. MMWR Recomm Rep 2016;65(RR-2): 1—44. http://dx.doi.org/10.15585/mmwr.rr6502a1.

[45] Walker DH, Bouyer DH. Rickettsia and orientia. In: Jorgensen JH, Pfaller MA, Carroll KC, Funke G, Landry ML, Richter SS, et al., editors. Manual of clinical microbiology. 11th ed. Washington, DC: American Society of Microbiology Press; 2015. p. 1122—34 [chapter 64].

[46] Reller ME, Dumler JS. Ehrlichia, Anaplasma, and related intracellular bacteria. In: Jorgensen JH, Pfaller MA, Carroll KC, Funke G, Landry ML, Richter SS, et al., editors. Manual of clinical microbiology. 11th ed. Washington, DC: American Society of Microbiology Press; 2015. p. 1135—49 [chapter 65].

[47] Walker DH, Dumler JS. Rickettsiae: spotted fever and typhus group. In: Lennette EH, Lennette DA, Lennette ET, editors. Diagnostic procedures for viral, rickettsial, and chlamydial infections. 7th ed. Washington, DC: American Public Health Association; 1995. p. 575—81 [chapter 40].

[48] Clements ML, Dumler JS, Fiset P, Wisseman CL, Snyder MJ, Levine MM. Serodiagnosis of Rocky Mountain spotted fever: comparison of IgM and IgG enzyme-linked immunosorbent assays and indirect fluorescent antibody test. J Infect Dis 1983;148:876—80.

[49] Newhouse VF, Shepard CC, Redus MD, Tzianabos T, McDade JE. A comparison of the complement fixation, indirect fluorescent antibody, and microagglutination tests for the serological diagnosis of rickettsial diseases. Am J Trop Med Hyg 1979;28: 387—95.

[50] 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.

[51] McQuiston JH, Wiedeman C, Singleton J, Carpenter LR, McElroy K, Mosites E, et al. Inadequacy of IgM antibody tests for diagnosis of Rocky Mountain spotted fever. Am J Trop Med Hyg 2014;91:767—70.

[52] Davis JP, Wilfert CM, Sexton DJ, Burgdorfer W, Casper EA, Philip RN. Serologic comparison of *R. rickettsii* isolated from patients in North Carolina to *R. rickettsii* isolated from patients in Montana. In: Burgdorfer W, Anacker RL, editors. Rickettsiae and rickettsial diseases. New York, NY: Academic Press, Inc.; 1981. p. 139—47.

[53] Philip RN, Casper EA, MacCormack JN, Sexton D, Thomas LA, Anacker RL, et al. A comparison of serologic methods for diagnosis of Rocky Mountain spotted fever. Am J Epidemiol 1977;105:56—67.

[54] La Scola B, Rydkina L, Ndihokubwayo JB, Vene S, Raoult D. Serological differentiation of murine typhus and epidemic typhus using cross-adsorption and Western blotting. Clin Diagn Lab Immunol 2000;7:612—6.