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Spotted fever group rickettsiae transmitted by *Dermacentor* ticks and determinants of their spread in Europe

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Abstract

Rickettsiae from the spotted fever group, i.e. the etiological agents of tick-borne lymphadenopathy/*Dermacentor*-borne necrotic erythema and lymphadenopathy (TIBOLA /DEBONEL) syndrome, are associated with ticks, including *Dermacentor marginatus* and *Dermacentor reticulatus*. The expansion of these ticks into new areas increases the risk of infection of their hosts with tick-borne pathogens. The study summarises the importance of 2 species from the genus *Dermacentor*, i.e. *D. marginatus* and *D. reticulatus*, in the spread of spotted fever group rickettsiae in various regions of Europe. The study also focuses on the determinants of the presence of vectors and transmission of rickettsiae, as well as the effects of human infections with these pathogens. The climate changes observed nowadays affect vectors and increase the incidence and spread of tick-borne diseases worldwide. Due to the existing risk of exposure to an increasing number of people, knowledge about the course of these serious diseases and their etiological factors should be disseminated among healthcare professionals as well as in society. There is a great challenge for epidemiological services to provide access to medical and veterinary facilities in order to diagnose and treat rickettsioses. Therefore, the development of a strategy for tick control and the popularisation of knowledge concerning prophylaxis of tick-borne diseases is indispensable.

Key words

Rickettsia; spotted fever group rickettsiae; TIBOLA/DEBONEL; tick-borne diseases; *Dermacentor reticulatus*; *Dermacentor marginatus*

INTRODUCTION

Tick-borne rickettsioses are caused by various bacterial species from the genera *Rickettsia*, *Ehrlichia*, *Neoehrlichia*, and *Anaplasma* [1, 2, 3, 4]. The greatest medical importance in this bacterial group is ascribed to spotted fever group rickettsiae (SFGR) (genus *Rickettsia*). The association of different rickettsia species with their hosts is determined by many factors, mainly the pathogen traits, the vector competence of tick species, and the presence of zoonotic reservoirs indispensable for the persistence of pathogens in a specific environment. The presented study analyses the role of *Dermacentor* ticks in the transmission of SFG rickettsiae in Europe, especially in its central and south parts, and the effects of infection with these pathogens in humans. The increase is emphasised in the risk of attacks by *Dermacentor reticulatus* and *Dermacentor marginatus* ticks on humans and animals related to climate and weather change, and human activity affecting the landscape and the structure of plants and animals, the latter of which are potential hosts of various developmental stages of these ticks.

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Spread of spotted fever group rickettsiae. SFGR are rod-shaped bacteria with a size of 0.3–0.5 × 0.8–2 μm with trilaminar cell walls surrounded by a thick electron-lucent layer (halo zone) without flagella. As reported by Socolovschi et al. [5], as many as 16 out of the 25 *Rickettsia* species identified to-date may infect humans, and 13 species have been detected in the inhabitants of Europe. Species with confirmed pathogenicity and remarkable epidemiological importance include *Rickettsia conorii* subsp. *conorii* (agent of Mediterranean spotted fever, MSF), *Rickettsia conorii subsp. israelensis* (Israeli spotted fever, ISF), *Rickettsia conorii subsp. caspia, Rickettsia conorii subsp. indica* (Indian tick typhus), *Rickettsia massiliae*, *Rickettsia monacensis* (MSF-like illness), *R. sibirica subsp. mongolitimonae* (lymphangitis-associated rickettsiosis, LAR), *Rickettsia slovaca* and *Rickettsia raoultii* (SENLAT- scalp eschars and neck lymphadenopathy, formerly referred to as TIBOLA- tickborne lymphadenopathy or DEBONEL- *Dermacentor*-borne necrotic erythema and lymphadenopathy), *Rickettsia aeschlimannii* (symptoms similar to those of MSF), *Rickettsia helvetica*, and *R. sibirica subsp. sibirica* [6, 7, 8, 9, 10, 11]. In central Europe, 5 *Rickettsia* species have been detected in ticks: *R. slovaca*, *R. raoultii*, *R. helvetica*, *R. monacensis*, and *Candidatus Rickettsia mendelii* [12, 13]. With the exception of *R. mendelii*, all these species have been identified in *D. marginatus* and *D. reticulatus* (Fig. 1).

Figure 1. *Dermacentor reticulatus*: (a) female, (b) male

SFGR have been detected in ticks from the genus *Dermacentor* across Europe, from the UK [14], Denmark [15], The Netherlands [16], and the Baltic countries [17] in the north, through countries in the western [8, 18, 19], central [20–30], and eastern parts [8, 31–34] and Spain [35], Portugal [36–38], Italy [39–43], Greece [44], and Croatia [45] in the south. In Poland, the presence of SFG rickettsiae in *D. reticulatus* has been documented in many regions of the country [46–53].

Ticks are infected with rickettsiae while feeding on a host with rickettsiosis. Within the tick population, rickettsiae are disseminated via transovarial [5, 33, 54, 55], transstadial [5, 33] and, probably less frequently, transspermal [56] transmission. The possibility of transmission of SFGR *R. massiliae* and *R. rickettsii* during the co-feeding of ixodid ticks has also been suggested [5].

In natural conditions, rickettsiae may be transferred in another way. The phenomenon of oral-anal contact between two *D. reticulatus* and *Ixodes ricinus* ticks, described recently for the first time, may indicate an alternative route of transmission of some pathogenic and non-pathogenic microorganisms from one population of sympatric tick species to another [57]. The scale of this type of interspecific tick contacts in natural conditions and their epidemiological importance are as yet unknown and require further investigations. The possibility of transmission of rickettsiae during the feeding of hungry or not fully engorged specimens on engorged ticks from the same species, also needs elucidation. Such research is even more advisable as hyperparasitism has been described in various ticks, including those with great epidemiological importance [58]. It has been experimentally shown that some pathogens, such as *Dipetalonema viteae, Borrelia crocidurae* and *Borrelia burgdorferi* s.l., can be transmitted during parasitization of some ticks by others [59–61].

As in the case of other tick-borne pathogens, the prevalence of rickettsiae in ticks differs between regions and between different years in the regions. In the northern regions, *D. reticulatus* occurrence varies, with the prevalence of *R. raoultii* in adult ticks varying from 0% – 36.9% (mean 4.9%) in different parts of Lithuania and Latvia [17]. In Belarus, as many as 41.9% of *D. reticulatus* specimens collected from animals and 44.5% of these ticks found on vegetation were infected with *Rickettsia* spp., with the dominance of *R. raoultii* (35.5% and 22.6% in specimens collected from animals and vegetation, respectively) [34]. Higher SFGR infection rates in *Dermacentor* ticks have been reported from many localities in central and southern Europe.

In recreational areas in Białowieża (Podlaskie Province, north-eastern Poland), *R. raoultii* DNA was detected in 56.7% of *D. reticulatus* specimens collected from vegetation [47]. Later, Stańczak et al. [53] identified rickettsial DNA in 41% of adult *D. reticulatus* in this province. In Lublin Province (eastern Poland), the SFGR prevalence in this species during the study period was 53.0% [49]. *Dermacentor reticulatus* ticks from Łęczyńsko-Włodawskie Lakeland (Lublin Province) have been most frequently diagnosed with *R. raoultii* infection (43.8%) [52]. In the endemic regions of north-eastern (Warmińsko-Mazurskie Province) and central Poland (Mazowieckie Province), *Rickettsia* spp. were identified in 44.1% of *D. reticulatus* [50]. A higher percentage of SFGR (60%) was detected in adult stages of the species collected from dogs and cats in the Wrocław agglomeration [51].

Infection with SFG rickettsiae has been detected in 85.15% and 97.0% of *D. marginatus* ticks in southern and northern Spain, respectively [62, 63]. In Slovakia during 2004 – 2010, the infection rate ranged from 25.49% – 36.17% depending on the tick collection. The prevalence of *R. raoultii* was 8.1 – 8.6% and 22.3 – 27% in *D. marginatus* and *D. reticulatus*, respectively. In turn, the prevalence of *R. slovaca* was higher in *D. marginatus* (20.6 – 24.3%) than in *D. reticulatus* (1.7 – 3.4%) [26]. In hunting and recreational forest areas in southern Hungary, *R. raoultii* infection has been confirmed in 58% of examined *D. reticulatus* specimens [30]. A high prevalence of *Rickettsia* spp. (70.5%) in questing *D. reticulatus* and only 11.4% cases of *I. ricinus* have been reported from Saxony (Germany) [64]. As in the case of *D. reticulatus*, the infection rate in *D. marginatus* varied widely from 0.7% – 13.3% in Germany [24, 25], 32.1% in Italy [40] and 41.5% in Portugal [37].

Particularly noteworthy is the fact that SGFR are transferred into new areas together with the spread of *Dermacentor* ticks into urban and suburban areas. In a Kiev city park (Ukraine), 10.1% of *D. reticulatus* ticks were infected by *R. raoultii* [65]. As many as 36% of ticks collected in a park in Rome (Italy), including 14% of *D. marginatus*, 26% of *Rhipicephalus turanicus*, and 70% of *I. ricinus*, exhibited the presence of rickettsial DNA. *D. marginatus* were also found to be infected by *R. monacensis* [43]. SFGR may co-infect *D. reticulatus* ticks with other pathogens, i.e. TBE virus, *Anaplasma phagocytophilum*, *B. burgdorferi*, *Babesia* spp., or *Toxoplasma gondii* [52]. Ticks can also be infected by 2 SFG *Rickettsia* species. In Croatia, the coexistence of *R. helvetica* and *R. slovaca* was noted in 1% of *D. reticulatus* ticks [45]. Co-infections of SFGR (*R. slovaca* and *R. helvetica*) with *Borrelia lusitaniae* were also found in ticks, including *D. marginatus* (23.3%), collected in a Safari Park in Alentejo (Portugal) [37].

The circulation of *Rickettsia* spp. in nature proceeds with the participation of vectors – ticks and hosts of their juvenile and adult stages [66–70]. Larvae and nymphs of *D. reticulatus* and *D. marginatus* ticks parasitize small rodents and insectivores, while adult stages feed on medium-sized and large animals. *R. raoultii* has also been detected in blood collected 24 h after *D. reticulatus* was placed in feeding units [71]. In Slovakia, 11% of small mammals are infected by SFG rickettsiae [70]. The dominant species is *R. helvetica*, which has been detected most frequently in the striped field mouse

(*Apodemus agrarius*), the yellow-necked mouse (*Apodemus flavicollis*), the bank vole (*Myodes glareolus*), and the common vole (*Microtus arvalis*) in natural and suburban habitats. In turn, *R. felis* was identified in *A. flavicollis* captured in a suburban habitat. *R. slovaca* was detected in one *A. flavicollis* captured in a natural habitat. The highest species diversity of rickettsiae was observed in *A. flavicollis*, and the highest prevalence of the bacteria was recorded in *M. glareolus* [66–69]. The prevalence of *Rickettsia* spp. in the investigated small mammals (*A. agrarius, A. flavicollis, M. arvalis, M. agrestis, Mustela nivalis, M. glareolus, Sorex araneus,* and *Talpa europaea*) from Germany (Saxony) was 25.3% [64].

The strong relationship between rickettsiae and host species is supported by the 7-fold higher prevalence of *Rickettsia* spp. in *M. glareolus* infested with *D. reticulatus* than that in non-infested animals. In south-western Poland, DNA of SFG rickettsiae was detected in tissues of 17.6% of wild-living rodents – *A. agrarius, A. flavicollis*, and *M. glareolus* [72]. Some of the hosts of *D. reticulatus* and *D. marginatus* adult stages, i.e. wild and domestic large ungulates, carnivores, horses, or wild boars, can also be reservoirs of rickettsiae in nature [40, 51, 73, 74].

Analyses of mammalian biological material for the presence of the DNA of SFG rickettsiae and/or antibodies against pathogens have confirmed the widespread distribution and high prevalence of rickettsiae in *Dermacentor* spp. hosts in the European occurrence range of these ticks [51, 74–76]. For instance, high seroprevalence of *R. slovaca* in Spain was recorded in domestic ruminants – 65% in bullfighting cattle, 21% in goats, and 16% in sheep [77].

Ticks infected by SFG rickettsiae can invade humans and feed on the new host, which poses a high risk of transmission of the pathogens during blood ingestion. Cases of tick-borne lymphadenopathy (TIBOLA) caused by *R. slovaca* have been reported in patients who had contact with horses [78]. Humans can be infected not only by a feeding *Dermacentor* female tick, but also by a foraging male specimen [78, 79].

Impact of environmental factors on *Dermacentor* ticks and transmission of SFG rickettsiae. Climate change leading to global warming alters tick fauna in various habitats and the structure of animals, i.e. potential hosts of various tick development stages and animal reservoirs of pathogens. Examples of species with high dynamics of expansion in the last 10 years include *D. reticulatus* [80–83] and *D. marginatus* [84–86]. These ticks migrate with their hosts via ecological routes along rivers. The transfer of *D. reticulatus* ticks with hosts is the quickest route of extension of the occurrence range of this species. *D. reticulatus* females and males alone are able to move at a distance of 66.35 ± 100 cm and 54.85 ± 45 cm over 7 weeks, respectively [87]. Studies conducted on other tick species suggest that pathogens may influence the physical activity of ticks. It has been demonstrated that *Rickettsia* increase and *Arsenophonus* limit the locomotor activity of larvae of 3 tick species, i.e. *Amblyomma americanum, Dermacentor variabilis*, and *Ixodes scapularis*, which are pathogen vectors in eastern North America [88]. The presence of *Rickettsia* bacteria in *D. variabilis* ticks affect their behaviour [89].

Twenty years ago in Poland, *D. reticulatus* ticks were found only in the eastern and north-eastern parts of the country [90–92]. Currently, tick localities are reported from many other places in the eastern [92, 93], central and north-eastern [94–97] western [98–100], and in south-western [101] provinces. In Europe, a similar trend towards expansion of the occurrence range is observed in other species with great epidemiological importance [102].

Due to the climate and weather changes, adult *D. reticulatus* stages in central Europe can attack hosts even during winter [103]. Active specimens were found in January in the habitat of this species in eastern Poland, which is usually a diapause period for these ticks. Similarly, *D. reticulatus* activity in winter has been reported from western Poland [104]. Besides the expansion of the occurrence range of *Dermacentor* ticks in Europe, their population size is also increasing. Over 10 years, the number of *D. reticulatus* adults in a meadow ecosystem used for grazing cattle in eastern Poland has increased two- and three-fold in spring and autumn, respectively [105]. The dynamics of the seasonal activity of this species with 2 peaks, in spring and autumn, and a period of decline in the activity in July persists in central Europe. However, the beginning of spring activity and the end of autumn activity shift in time together with the climate change [106]. Depending on the temperature, ticks can attack hosts already at the end of the calendar winter and in early spring. Favourable weather conditions contribute to the prolongation of the autumn activity of *D. reticulatus*. The risk of pathogen infection of hosts is increased at the peak of tick diurnal activity. In eastern Poland, adult *D. reticulatus* stages exhibit the highest activity from 12.00 midday – 16.25 in spring and from 10.00 a.m. – 18.25 in autumn, with a peak at 14.00 – 14.25 in both these periods [107].

The behaviour of ticks during the parasitic phase can be modified when 2 species coexist on the same host. During *D. reticulatus* and *D. marginatus* co-feeding on the same host, 2 inter-specific systems with different physiological features are formed, which may influence the transmission of tick-borne pathogens. In the inter-specific group, *D. marginatus* females attach to the host for a longer time, and although their feeding time is shorter than in a mono-specific group, feeding efficiency does not decrease [108]. The current study demonstrates that the co-feeding of *I. ricinus* and *D. reticulatus* ticks on the same host influences their non-parasitic stage of the life cycle by increasing significantly the egg amount and hatching success in *I. ricinus* feeding in inter-specific groups [109]. Additionally, the dynamics of tick feeding on hosts changes with the intensity of invasion [110] and tick gender structure [111].

Human infection with SFG rickettsiae. Human infections with SFG rickettsiae have been reported from various European countries, in particular from those located in the southern and central parts of the continent. As many as 8 species of different spotted fever group (SFG) rickettsiae, i.e. *R. slovaca*, *Rickettsia* sp. IRS3/IRS4, *R. massiliae*/Bar29, *R. aeschlimannii*, *Rickettsia* sp. RpA4/DnS14, *R. helvetica*, *Rickettsia* sp. DmS1, and *R. conorii*, were identified in 10 species of ticks infesting humans in north-western Spain [35]. *R. slovaca, R. raoultii*, and *R. roji* transmitted by *D. marginatus* and/or *D. reticulatus* are the agents of TIBOLA (tickborne lymphadenopathy) or DEBONEL (*Dermacentor*-borne necrotic erythema and lymphadenopathy) syndromes, which are also referred to as SENLAT, and reflects their clinical symptoms. The first case of *R. slovaca* infection following a tick bite was described in 1980. The patient was diagnosed with acute meningoencephalitis, febrile relapses, and prolonged persistence of neurasthenic disorders [112]. The next case of *R. slovaca* infection in humans was documented in 1997 in a patient in France [113]. Human infection with *R. slovaca* was also diagnosed in, e.g. Slovakia, Bulgaria, Hungary, Germany, Italy, Spain and Portugal [9, 38, 39, 114–118]. Cases of human *R. raoultii* infections have also been reported from several other countries, e.g. France, Slovakia and Poland [116, 118, 119]. In Poland, *Rickettsia* spp. were detected for the first time in a patient with TIBOLA/DEBONEL symptoms in Warsaw [120].

SFG rickettsiae introduced with tick saliva during feeding enter human blood. Via interaction between the adhesive protein (OmpB), which is an abundant surface protein in *Rickettsia*, and Ku70 receptors in host cells, rickettsiae penetrate the vascular endothelial cells and are divided in the cell cytosol. Since rickettsiae penetrate other cells without passing through the intracellular space, they avoid the immune response. This promotes the spread of the infection in the organism. Most of the clinical symptoms caused by SFG rickettsiae are attributed to disseminated endothelial infection. *Rickettsia* spp. and other intracellular microorganisms have been identified in pathological aortic valves in humans [121]. Rickettsial infection results in increased microvascular permeability, generalised vascular inflammation, oedema, and release of vasoactive mediators, which exert an effect on coagulation and promote the release of pro-inflammatory cytokines.

The presence of antibodies against tick-borne SFG rickettsiae was detected in 36.0% of inhabitants of areas with a high risk of tick attacks in eastern Poland. In groups exposed to contact with ticks occupationally, antibodies against tick-borne SFG rickettsiae were confirmed in 50.7% of forestry workers and 21.3% of agriculture workers [122]. In Catalonia (north-eastern Spain), the seroprevalence of *R. slovaca* in the general population was reported to range from 3.7% – 5.5% [123], whereas in a group exposed to tick bites seroprevalence was as high as 16.9% [124].

Tick-borne rickettsioses (TBR) in humans may be asymptomatic [125], but usually with moderate symptoms [3, 9, 125]. However, deaths of patients have also been reported [9, 38].

The length of the TIBOLA/DEBONEL incubation time varies from 1 – 15 days (typically between 5 – 10 days). The most common symptoms of *R. slovaca* infection include asthenia, headache, painful adenopathies, painful scalp eschar, and oedema of lymph nodes close to the tick bite. Low fever, and rarely rash, as well as face oedema may develop in patients. Symptoms of SENLAT syndrome may also include inoculation eschar, cervical lymphadenopathy, high fever, malaise, and headaches [6, 38, 115–117, 126]. Alopecia around the eschar persisting for a long time (several months), even after antibiotic therapy, has been reported [116]. Clinical symptoms of *R. raoulti*-induced TIBOLA syndrome are similar to those of *R. slovaca* infection, except for the absence of alopecia.

There are known cases of an emerging syndrome of scalp eschar and neck lymphadenopathy after a tick bite [127]. SFGRs can cause chronic symptoms in patients. Nillson et al. [128] reported the occurrence of *R. helvetica* in a patient with chronic perimyocarditis and suggested that the pathogen may play an important role in its etiology.

Due to the colonisation of new areas by *D. marginatus* and *D. reticulatus*, which are the main SFGR vectors in Europe, the risk of human and animal infections (including companion animals such as dogs and cats) with these rickettsiae and other tick-borne pathogens is increasing. The challenge for medical and epidemiological services is therefore to provide access to medical and veterinary facilities in order to diagnose and treat rickettsioses. Development of a strategy for tick control and popularisation of prophylaxis of tick-borne diseases are also indispensable.

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