



High seroprevalence for indigenous spotted fever group rickettsiae in forestry workers from the federal state of Brandenburg, Eastern Germany



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ABSTRACT

In the last decade six *Rickettsia* species, including *Rickettsia slovaca* have been characterized in Germany. All of these species could be linked to distinct clinical syndromes in humans. However, due to lack of seroepidemiological data an estimation of the prevalence and the public health impact of rickettsial infections in Germany is difficult. The aim of the present study was to determine the seroprevalence of spotted fever group (SFG) rickettsiae in a population with an elevated exposure risk to ticks. For that purpose, 559 sera of forestry workers in the federal state of Brandenburg, Eastern Germany, were screened for SFG-rickettsiae reactive IgG antibodies. Positive sera were subsequently titrated by microimmunofluorescence assay against *R. helvetica*, *R. raoultii*, *R. felis*, "*R. monacensis*" and *R. slovaca*. The total average IgG seroprevalence rate against SFG rickettsiae of 27.5% was found to be represented by 9.7% *R. helvetica*, 5% *R. raoultii*, 2.7% *R. felis*, 0.5% "*R. monacensis*" and 0.5% *R. slovaca*. The remaining 9.1% positive test results were of non-differentiable origin. IgG seroprevalences ranged from 11% to 55% in the different forestry districts. Older and male participants had a significantly higher probability for seropositivity and higher anti-rickettsia antibody titer level. In addition, the number of recent as well as the recalled lifetime tick bites was significantly associated with seropositivity and higher titers against SFG rickettsiae. In conclusion, we found an unexpected high total seroprevalence against SFG rickettsiae in forestry workers and serological evidence confirming the occurrence of *R. raoultii*, *R. felis*, "*R. monacensis*" and *R. helvetica* in the federal State of Brandenburg.

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1. Introduction

Rickettsiae are small Gram-negative, obligate intracellular coccobacilli. They belong to the genus *Rickettsia* within the family Rickettsiaceae in the order Rickettsiales. Rickettsiae are classified

into four groups: typhus group (TG), spotted fever group (SFG), *Rickettsia bellii* group, and *Rickettsia canadensis* group (Merhej and Raoult, 2011), of which the SFG rickettsiae include the major number of known *Rickettsia* spp. (Dobler and Wölfel, 2009). To date more than twenty different species have been identified worldwide and many more are in the status of species Candidatus (Eremeeva, 2012; Merhej and Raoult, 2011). In Germany, six *Rickettsia* species with different pathogenicity have been identified, so far (Fig. 1A). All of them belong to the SFG and have been mainly detected in fleas (*R. felis*) or in ticks.

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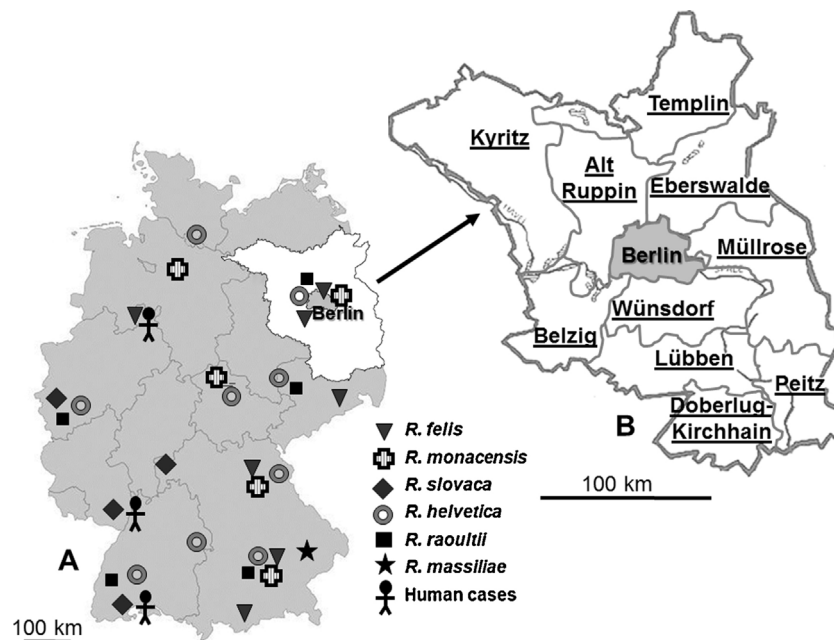


Fig. 1. Current knowledge of the distribution of *Rickettsia* spp. in Germany, identified by molecular biological analysis of ticks and rodents and description of human cases including serological analysis of spotted fever group rickettsiae and typhus group rickettsiae (A) (Dautel et al., 2006; Dobec et al., 2009; Dobler et al., 2009; Dobler and Wölfel, 2009; Franke et al., 2010; Gilles et al., 2008; Hartelt et al., 2004; Hildebrandt et al., 2011; Pichon et al., 2006; Pluta et al., 2010, 2009; Rieg et al., 2011; Schorn et al., 2011; Schreiber et al., 2014; Silaghi et al., 2008, 2011; Simser et al., 2002; Wölfel et al., 2006) and locations of the investigated forestry districts in the federal State of Brandenburg (B).

R. slovaca (Raoult et al., 1997, 2002) and *R. raoultii* are known as causative agents of tick-borne lymphadenopathy (TIBOLA) whereby *R. slovaca* is being considered the more pathogenic (Parola et al., 2009). Recently, two cases of *R. slovaca*-induced TIBOLA have been described in Baden-Wuerttemberg and Rhineland Palatinate, Germany (Pluta et al., 2009; Rieg et al., 2011). In 2000, flea-borne spotted fever, which is caused by *R. felis* (Raoult et al., 2001), was confirmed in two patients from North Rhine-Westphalia, Germany (Richter et al., 2002). *R. helvetica* is associated with a non-specific fever without erythema (“uneruptive fever”) as well as with meningitis (Fournier et al., 2000; Nilsson et al., 2010) whereas “*R. monacensis*” (Jado et al., 2007) and *R. massiliae* may cause classical spotted fever. However, no clinical cases due to rickettsiae other than *R. slovaca* or *R. felis* have been reported in Germany, yet.

In Germany, only limited data on the prevalence of rickettsiae in vector ticks and fleas or reservoir hosts such as cats, dogs and rodents are available (Schex et al., 2011). For *R. slovaca* and “*R. monacensis*” a few studies indicate a low infection rate of the vector ticks (Dobler and Wölfel, 2009; Franke et al., 2010; Pluta et al., 2009). *R. massiliae* was detected only once in a single location in Germany (Dobler and Wölfel, 2009). Therefore, its presence as an indigenous rickettsia species in Germany needs to be confirmed. *R. helvetica*, *R. raoultii*, and *R. slovaca* are mainly detected in their distinct tick vectors *Ixodes ricinus*, *Dermacentor reticulatus*, and *Dermacentor marginatus*, respectively. So far, the occurrence of *R. raoultii*, “*R. monacensis*”, *R. helvetica* in ticks and *R. felis* in fleas was reported in the region of the investigated forest districts (Fig. 1). *I. ricinus* is still the most frequent tick species in Germany, but increasing populations of *Dermacentor* spp. have been recorded in several federal states including Brandenburg, since 2005. In Brandenburg, *Dermacentor* ticks occur in regions in the east, northeast and south of Berlin. The infection rate of *Dermacentor* spp. with *R. raoultii* is reported to be up to 80% (average 50%) in certain locations in the area northeast of Berlin (Dautel et al., 2006; Graser et al., 2009; Talaska, 2009). Until now, no prevalence data are available

for *R. massiliae* and *R. slovaca* in the federal State of Brandenburg, Eastern Germany.

Rickettsial diseases can be diagnosed by cultural, molecular and serological methods. Strong cross-reactivities in immunofluorescence assay (IFA) or enzyme-linked immunosorbent assay (ELISA) occur within the SFG and TG rickettsiae, respectively, whereas cross-reactions between the different groups are low (Anacker et al., 1987). Therefore, screening with an IFA for IgG antibodies against a single SFG rickettsia (in this study *R. conorii*) in a low sample dilution can be used to detect SFG antibody positive samples. Subsequently, a differentiation can be achieved by comparative IFA antibody titration against the different SFG rickettsial species (Brouqui et al., 2004; Jansen et al., 2008).

The aim of the present study was to investigate the seroprevalence against SFG rickettsiae in a high-risk population in Eastern Germany and to gain information about presumable risk factors for acquiring an infection with SFG rickettsiae.

2. Material and methods

2.1. Collection of samples

A total of 559 serum samples from male ($n=495$; 89%) and female ($n=64$; 11%) forestry workers, employed in ten different forestry districts throughout the federal state of Brandenburg (Fig. 1B, Table 1), had been collected between May to June 2008 and stored as previously described (Mertens et al., 2011). Informed consent was obtained from all study participants prior to examination.

To identify demographic and occupational factors (e.g. gender, age), information on tick bite exposure and frequency of work in the forest, all participants completed a self-administered questionnaire that contained closed-ended questions. The study subjects were categorized into six different age groups (1 = 20–35 years; 2 = 36–40y; 3 = 41–45y; 4 = 46–50y; 5 = 51–55y; 6 = 56–65y). The tick bite rate of the participants was classified in four categories

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