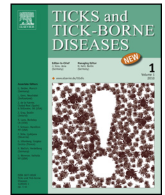
Ticks and Tick-borne Diseases xxx (2015) xxx–xxx

Contents lists available at ScienceDirect

Ticks and Tick-borne Diseases

journal homepage: www.elsevier.com/locate/ttbdis



Original article

Molecular examinations of *Babesia microti* in rodents and rodent-attached ticks from urban and sylvatic habitats in Germany

Anna Obiegala [a,b,*], Martin Pfeffer [b], Kurt Pfister [a], Carolin Karnath [b], Cornelia Silaghi [a,c]

[a] Comparative Tropical Medicine and Parasitology, Ludwig-Maximilians-Universität München, Leopoldstraße 5, 80802 Munich, Germany
[b] Institute of Animal Hygiene and Veterinary Public Health, University of Leipzig, An den Tierkliniken 1, 04103 Leipzig, Germany
[c] Institute of Parasitology, National Reference Center for Vector Entomology, Vetsuisse Faculty, University of Zürich, Winterthurerstrasse 266a, 8057 Zurich, Switzerland

ARTICLE INFO

Article history:
Received 7 January 2015
Received in revised form 2 March 2015
Accepted 3 March 2015
Available online xxx

Keywords:
Tick-borne pathogen
Babesia microti
Ixodes ricinus
Ixodes trianguliceps
Rodent

ABSTRACT

Small mammals serve as reservoir hosts for tick-borne pathogens, especially for those which are not transmitted transovarially in ticks – such as *Babesia microti*. Molecular investigations on the prevalence of *B. microti* in wild small mammals and on attached ticks from differently structured areas may provide information on the circulation of *B. microti* in different ecological niches. In 2012 and 2013, 622 rodents (396 *Myodes glareolus*, 178 *Apodemus flavicollis*, 36 *Apodemus sylvaticus*, 4 *Apodemus agrarius*, 7 *Microtus arvalis*, 1 *Microtus agrestis*) were captured from three differently structured habitats (urban, sylvatic, recultivated) in Germany. Attached ticks were collected from 449 small mammals (3250 *Ixodes ricinus*, 7 *Ixodes trianguliceps*, 133 *Dermacentor reticulatus*). A representative selection of a maximum of 5 ticks per developmental stage and species per 30 rodents of each species, location and year resulting in 965 ticks was further investigated. DNA was extracted from tick, blood and spleen samples, and tested by PCR for the partial *18S rRNA* gene of *B. microti* with subsequent sequencing. The prevalence was significantly higher in rodents from the sylvatic site (4.6%) than in rodents captured at both other sites (–0.6%) ($\chi^2 = 11.95$; $p = 0.00125$). Body and spleen weight of infected *M. glareolus* from the sylvatic site were significantly higher compared to those from non-infected individuals from that site ($p = 0.00288$ and $p = 0.00017$, respectively). *Babesia microti* DNA was detected in 3 out of 965 attached ticks (0.3%; 95%CI: 0–1) from all sites, but they derived exclusively from rodents captured at the sylvatic site. At the same site, *I. ricinus* nymphs (7.7%; 95%CI: 1–25.3) were significantly more often infected than *I. ricinus* larvae (0%; 95%CI: 0–1.3)($\chi^2 = 26.72$; $p < 0.0001$). The majority of positive rodents was also found at that site. *I. trianguliceps* occurred exclusively and the majority of *M. glareolus* at that site. Thus, it may be assumed that the circulation of *B. microti* is more efficient where this tick species and voles exist sympatrically than in areas with a predominant occurrence of *Apodemus* species.

© 2015 Elsevier GmbH. All rights reserved.

Introduction

Small mammals serve as hosts for ixodid ticks. Microtine voles have been discussed as reservoir hosts for the intraerythrocytic protozoan *Babesia microti* (phylum Apicomplexa, order Piroplasmida) (Gray et al., 2002). It was first described in a vole near Lisbon in 1910 as *Smithia microtia* (*Microtus incertus*, now *Microtus arvalis* subspecies *incertus*, the common vole (Walter, 1981). *Babesia microti* has since been detected in small mammals, humans and hard ticks from Europe and the USA (Kogut et al., 2005; Hildebrandt et al., 2007; Silaghi et al., 2012; Hersh et al., 2012). Human babesiosis caused by *B. microti* displays subclinical to fatal malaria-like symptoms. It primarily occurs in the USA (Vannier and Krause, 2012). In Europe however, severe cases of human babesiosis have mainly been caused by *Babesia divergens*-like parasites in immunocompromised patients, but cases have also been reported in immunocompetent patients (Hildebrandt et al., 2013; Mathis et al., 2006; Martinot et al., 2011). Transplacental transmission and transmission via tick bites have been described before (Vannier and Krause, 2009). However the first and so far only autochthonous human case caused by *B. microti* in Europe was transmitted by

* Corresponding author at: Institute of Animal Hygiene and Veterinary Public Health, University of Leipzig, An den Tierkliniken 1, D-04103 Leipzig, Germany. Tel.: +49 341 97 38 166; fax: +49 341 97 38 198.
E-mail addresses: Anna.Obiegala@vetmed.uni-leipzig.de (A. Obiegala), Pfeffer@vetmed.uni-leipzig.de (M. Pfeffer), Kurt.Pfister@tropa.vetmed.uni-muenchen.de (K. Pfister), Karnath@vetmed.uni-leipzig.de (C. Karnath), Cornelia.Silaghi@uzh.ch (C. Silaghi).

http://dx.doi.org/10.1016/j.ttbdis.2015.03.005
1877-959X/© 2015 Elsevier GmbH. All rights reserved.

blood transfusion (Hildebrandt et al., 2007). While transovarial transmission in ticks was confirmed for *B. divergens* and other *Babesia* species, experimentations have failed to demonstrate transovarial transmission for *B. microti* . Therefore mammalian hosts are essential for its maintenance in natural transmission cycles (Gray et al., 2002). As information on the enzootic life cycle of *B. microti* is still incomplete, the objectives of this study were to detect and compare *B. microti* prevalences in wild rodents and attached ticks as well as to assess their role in the circulation of this protozoan at ecologically different habitats in Germany.

Material and methods

Collection of wildlife samples

In 2012 and 2013 small mammals were captured at three differently structured sites in Germany. The urban site "R" (7.4 ha, 49°00′55.72″ N, 12°05′08.89″ E) is a small park near the city center of Regensburg. It is surrounded by walls and high traffic roads. The park is highly frequented by visitors. Large wild animals are absent. Sylvatic site "T" (641 ha, 48°06′36.42″ N, 10°34′33.40″ E), located near Tussenhausen in Bavaria, is a large forest area (641 ha). This mixed forest is dominated by beeches, oaks and spruces. Different wild animal species are present and the anthropogenic influence is low. Forestry is not cultivated extensively. Therefore, there is little to no interaction between wild and domestic animals and humans (Forstdirektion Oberbayern). The recultivated site "S" is subdivided in three locations (51°15′32.2″ N, 12°21′02.5″ E, 51°17′01.3″ N, 12°21′00.6″ E, 51°26′97.2″ N, 12°32′25.6″ E), formerly described as site E-G, which surround an artificially created lake "Lake Cospuden", near Leipzig, Saxony (Silaghi et al., 2012). The lake belongs to a recultivated area, which was created out of a former brown coal mining area. Today, the surroundings of this lake are of public health interest as it is the largest recreational area near Leipzig (http://www.leipzigerneuseenland.de/). Bushes and trees less than 20 years old characterize this site. Nevertheless a high diversity of large and small wild mammals is present. Details of the investigated sites have been described previously (Silaghi et al., 2012; Obiegala et al., 2014; Overzier et al., 2013). Small mammals were trapped using Sherman© live animal traps (H. B. Sherman Traps, Inc., Tallahassee, Fla. U.S.A.) (official permit Site R1: 55.1-8646.4-140, Site T: 55.1-8646-2/30, Site S: AZ 36.11-36.45.12/4/12-001). Traps, baited with apple slices, were placed for at least two consecutive nights per month and site and were checked twice a day. Collected animals were anaesthetized with CO_2 and then euthanized by cervical dislocation. Blood was taken by heart puncture. Animals were sexed, weighed and necropsy was performed to obtain spleen samples. All small mammals were identified by the use of taxonomic keys (Stresemann, 1989). Further, a conventional PCR targeting the *cytochrome b* gene (Parson et al., 2000) yielding an amplicon of 354 base pairs was performed to verify morphological identification for a randomly selected number of 69 individuals as described (Obiegala et al., 2014).

DNA extraction and PCR analysis

DNA was extracted from ticks, blood and spleen samples from small mammals with the Maxwell® 16 LEV blood DNA kit (Promega GmbH, Mannheim, Germany) and the corresponding Maxwell® 16 System (Promega GmbH) as described (Obiegala et al., 2014). PCR amplification of an *18S rRNA Babesia* gene fragment (411–452 bp) was performed using a previously published PCR protocol (Casati et al., 2006) with modifications as described (Schorn et al., 2011). Amplicons were detected by agarose gel electrophoresis and purified with the Qiaquick PCR purification kit (Qiagen, Hilden, Germany) for sequencing at Eurofins MWG Operon, Ebersberg, Germany. Sequencing was performed with forward and reverse primers used for PCR amplification. Sequences were analyzed with the Chromas Lite program (Technelysium Pty Ltd., South Brisbane, Australia) and aligned to available sequences in the GenBank with BLASTn (National Center for Biotechnology Information, Bethesda MD, USA) (Schorn et al., 2011).

Statistical analysis

Graph Pad Software (Graph Pad Software, Inc., San Diego, CA, USA) was used for the calculation of confidence intervals (CI 95%) of prevalence rates with the Clopper and Pearson method. Pearson's Chi-squared test with a type I error α of 0.05 and Fisher's exact test for the comparison of sample sizes <5 were used to compare *B. microti* prevalences. Comparisons of multiple values were adjusted with the Bonferroni correction. To compare body and spleen weight of infected and non-infected *M. glareolus* at the sylvatic site the one-tailed Wilcoxon–Mann–Whitney-Test with $\alpha = 2.5$ was used. Results with p values <0.05 were considered to be positive.

Results

Collection of wildlife samples

Altogether 622 rodents of six species (4 *Apodemus agrarius*, 36 *A. sylvaticus*, 178 *A. flavicollis*, 7 *Microtus arvalis*, 1 *Mi. agrestis*, 396 *M. glareolus*) were collected. From these animals 443 blood and 585 spleen samples were available for further examination (Table 1). Altogether 3391 ticks of three species (7 *Ixodes trianguliceps*, 3250 *I. ricinus*, 133 *Dermacentor reticulatus*) were collected from 449 of the 622 small mammals. Altogether 92.1% of all collected ticks were larvae. Nymphs were exclusively collected from *M. glareolus* and *A. flavicollis*. Tick infestation rates ranged from 1 to 112 ticks with a mean infestation of 8 ticks and a median infestation of 16 ticks per small mammal. For *M. glareolus* the mean infestation rate was 4 ticks and the median infestation was 6 ticks. The mean infestation on *A. flavicollis* was 10 ticks per mouse and the median infestation was 23 per mouse. For *A. sylvaticus* the mean infestation rate was 4 ticks and the median infestation was 9. *I. trianguliceps* ticks were found on *M. glareolus* and *A. flavicollis* exclusively in the sylvatic site T. A total of 98.5% of all *D. reticulatus* ticks were collected from *M. glareolus* and only 1.5% from *A. flavicollis*. All *D. reticulatus* were found at the recultivated site S. According to the availability of different rodent and tick species, 5 ticks per tick stage and species of 30 individuals of each rodent species were selected per year and site. This resulted in 965 ticks from 186 rodents for further analysis (Table 2).

Babesia *spp. detection and sequence analysis*

In total 13 out of 622 (2.1%; 95%CI: 1.2–3.6) small mammals were positive for *B. microti*. Of those, 11 rodents were from site T (4.6%; 95%CI: 2.5–8.2), two from site S (0.6%; 95%CI: 0–2.2) and none from site R1 (Table 1). The prevalence for *B. microti* was significantly higher at site T than at site R1 and site S ($\chi^2 = 11.95$; $p = 0.00125$). At site T, *M. glareolus* (7.2%; 95%CI: 3.8–12.9) were significantly more often infected than *A. flavicollis* (1%; 95%CI: 0–6) ($\chi^2 = 5.03$; $p = 0.025$). Body and spleen weight was significantly higher in infected ($n_1 = 10$) than in non-infected *M. glareolus* ($n_2 = 229$) from site T ($U = 976$; $p = 0.00288$; respectively for the spleen weight: $U = 1084.5$; $p < 0.001$) (Fig. 1). The ratio between body and spleen weight was 242:1 for non-infected and 48:1 for infected *M. glareolus*. The infestation rate of ticks on infected rodents ranged from 0 to 29. Altogether, 3 out of 965 (0.3%; 95%CI: 0–1) attached ticks were positive for *B. microti* (Table 2). These

Please cite this article in press as: Obiegala, A., et al., Molecular examinations of *Babesia microti* in rodents and rodent-attached ticks from urban and sylvatic habitats in Germany. Ticks Tick-borne Dis. (2015), http://dx.doi.org/10.1016/j.ttbdis.2015.03.005

Download English Version:

https://daneshyari.com/en/article/5807298

Download Persian Version:

https://daneshyari.com/article/5807298

Daneshyari.com