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Assessing the risk of human granulocytic anaplasmosis and lyme borreliosis after a tick bite in Bavaria, Germany

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ABSTRACT

To date, only isolated incidences of human granulocytic anaplasmosis (HGA) have been reported in Europe. However, entomological studies in Bavaria, Germany showed a prevalence of *Anaplasma phagocytophilum* of between 2 and 9.5% in the tick vector *Ixodes ricinus*. In this study we assessed the risk of pathogenic *A. phagocytophilum* infection after a tick bite in Bavaria. The seroprevalence of anti-*Borrelia burgdorferi* sensu lato (s.l.) antibodies was investigated as an indicator of past exposure, seroconversion as actual exposure of participants to ticks.

Patients with a tick bite in the preceding four weeks were recruited by participating doctors. Questionnaires on demographics, tick exposure and clinical signs were completed by patients and doctors, respectively. Two blood samples, taken at an interval of two weeks, were tested for antibodies against *A. phagocytophilum* and *B. burgdorferi* s.l.

One of 107 recruited patients showed serological evidence of an acute infection of *A. phagocytophilum* but had no clinical signs. Four out of six patients with serological evidence of an acute *B. burgdorferi* s.l. infection, presented with erythema migrans. A seroprevalence of 7.5% for *A. phagocytophilum* and 13.1% for *B. burgdorferi* s.l. was detected.

The comparatively high seroprevalence of *B. burdorferi* s.l. and *A. phagocytophilum* antibodies indicate frequent past exposure of participants to ticks. The finding of one acute infection of *A. phagocytophilum* in the absence of clinical signs, supports entomological evidence that the strains of *A. phagocytophilum* predominantly present in the Bavarian tick population may cause transient infections but are of low pathogenicity in humans.

1. Introduction

Human granulocytic anaplasmosis (HGA) was first described in the United States in the 1990s (Bakken et al., 1994; Chen et al., 1994; Dumler et al., 2005). It is caused by *Anaplasma phagocytophilum*, an obligate intracellular bacterium. Initially, the causative pathogen was grouped in the genus *Ehrlichia* and named *E. phagocytophila* or Human Granulocytic Ehrlichiosis (HGE) agent. The pathogen was later reclassified as the species *A. phagocytophilum* (Dumler et al., 2001), together with the veterinary pathogens previously known as *E. equi* and *Cytoecetes phagocytophila* (Dumler et al., 2005). Despite being classified as a single species, phylogenetic analysis has identified different genetic variants of *A. phagocytophilum* associated with predilection for different host species (Scharf et al., 2011; Huhn et al., 2014). Only certain variants have been isolated from human HGA cases and appear to be associated with pathogenicity in immunocompetent humans. The most frequent manifestations of HGA, reported in 70–95% of clinical cases, are pyrexia, malaise, myalgia and headaches, with a minority of cases presenting with arthalgia, involvement of the gastrointestinal or respiratory tract, the liver or the central nervous system. On rare occasions the infection is fatal. Laboratory findings include thrombocytopenia, leucopenia, elevated C-reactive protein, and elevated liver enzymes (Aguero-Rosenfeld et al., 1996; Bakken et al., 1996; Walker and Dumler, 1996).

HGA is endemic in the Midwest and the East Coast of the US. Reported cases rose continuously and have now possibly reached a plateau at around 2500 cases a year reported in 2011, 2012 and 2013 (CDC, 2012, 2013, 2014, 2015; Thomas et al., 2009).

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The geographical distribution of *A. phagocytophilum* in the US corresponds to the focal distribution of *B. burgdorferi*, with which it shares the tick vector species *Ixodes (I.) scapularis* and *I. pacificus*. Despite comparatively low absolute case numbers of HGA across the US, the incidence of up to 58/100,000 population reported in some endemic areas in the US is of similar magnitude to the reported incidence of Lyme disease in these areas (Bakken et al., 1996; Ijdo et al., 2000, http://www.cdc.gov/lyme/stats/chartstables/casesbyyear.html). In Europe, *A. phagocytophilum* is transmitted to humans by *I. ricinus*. The first serological evidence of a human infection with *A. phagocytophilum* was reported in the mid-1990s in Switzerland (Brouqui et al., 1995). The first confirmed clinical HGA case was described in Slovenia in 1997, with a moderately severe but self-limiting illness including fever, headache, nausea, vomiting, malaise, intense myalgia, and arthralgia (Petrovec et al., 1997). Despite an *A. phagocytophilum* prevalence of 0.25–24.4% in the tick vector across Europe (Cinco et al., 1997; Alberdi et al., 1998), only sporadic clinical cases of HGA have been reported in Europe (Blanco and Oteo, 2002).

In Germany no autochthonous human clinical infections with *A. phagocytophilum* have been reported to date. However, a range of studies showed a seroprevalence between 1.0% and 4.4% for blood donors and control populations without a specific history of tick bites (Fingerle et al., 1997; Hunfeld and Brade, 1999; Bätzing-Feigenbaum et al., 2000; Randolph, 2002; Kowalski et al., 2006). A seroprevalence ranging from 14.0 to 19.5% was reported for forestry workers (Fingerle et al., 1997; Bätzing-Feigenbaum et al., 2000). In individuals with acute Lyme disease or serological evidence of past exposure to *B. burgdorferi* sensu lato (s.l.), an *A. phagocytophilum* seroprevalence of 4.5–13.1% was detected (Fingerle et al., 1997; Hunfeld and Brade, 1999; Kowalski et al., 2006). In comparison, the seroprevalence for *B. burgdorferi* s.l. in Germany ranges from 13.4% to 34.6% in people with high occupational risk of exposure to ticks (Oehme et al., 2002; Reimer et al., 2002; Rieger et al., 2002) and from 1.8 to 12.0% in people with leisure time exposure or no known exposure to ticks (Nübling et al., 2002; Rieger et al., 2002; Wilking et al., 2015).

Since *A. phagocytophilum* is transmitted by the same vector (*I. ricinus*) as *B. burgdorferi* s.l. in Europe, transmission to humans is presumed to be governed by similar risk factors. *A. phagocytophilum* prevalence of 1.6–9.5% was detected in *I. ricinus* in Germany, with a higher prevalence in adult ticks than in nymphs (Fingerle et al., 1999; Silaghi et al., 2008; Schorn et al., 2011a,b). A prevalence of *B. burgdorferi* s.l. of up to 36.2% (178/492) was detected in ticks in Bavaria (Fingerle et al., 1999). In contrast to *B. burgdorferi* s.l., which is endemic across Bavaria, the presence of *A. phagocytophilum* in vector ticks appears to be focal, with significant differences in prevalence in the tick vector reported between localities (Fingerle et al., 1999) and types of habitats (Silaghi et al., 2008). Higher infection rates were observed in ticks from a variety of inner city parks than in ticks from woodland habitats (Silaghi et al., 2008) suggesting separate sylvatic and urban *A. phagocytophilum* transmission cycles. Nevertheless, the influence of the host community structure on *A. phagocytophilum* transmission cycles still remains unclear. Partial sequencing of the 16SrRNA gene identified 6 different *A. phagocytophilum* variants of *I. ricinus* from a range of Bavarian inner city parks. Two out of 45 infections showed 100% sequence similarity of the 16SrRNA target with the original human infective HGA agent. However, the variant of *A. phagocytophilum* infection most frequently detected in ticks in Bavaria (*Ehrlichia* sp. Frankonia 2) (Silaghi et al., 2008; Schorn et al., 2011a) has, to date, only been isolated from a single clinical HGA case from Slovenia (Scharf et al., 2011) but has been found more frequently in dogs and cats (Silaghi et al., 2008; Scharf et al., 2011). Based on the available data this variant is suspected to be of low pathogenicity in humans. No clinical infections of *A. phagocytophilum* in humans have been recorded in Bavaria to date. Potential reasons include episodes of HGA not being recorded due to lack of awareness or misdiagnosis. An alternative reason could be that little or no transmission of *A. phagocytophilum* to humans takes place in Bavaria. A closed transmission cycle between ticks and vertebrate hosts such as dogs or rodents could result in maintenance of the observed prevalence of *A. phagocytophilum* in ticks with few or no spill-over infections in humans. It is also conceivable that transmission of *A. phagocytophilum* to humans does take place on a regular basis, but that the variant of *A. phagocytophilum* present in the tick vector in Bavaria is specific to other host species, causing only transient infections without relevant clinical signs in humans. No studies assessing the risk of acute *A. phagocytophilum* infections in humans have previously been carried out in Bavaria. The primary aim of this study was to investigate whether there is a risk of infection with *A. phagocytophilum* after tick bite in Bavaria. The risk of *B. burgdorferi* s.l. infection after a tick bite was investigated in parallel as an indicator of the level of past exposure to tick-borne pathogens experienced by participants. As a secondary aim, the activity of the participants at the time of tick exposure as well as regular leisure or occupational activities were investigated as potential risk factors for contracting a tick-borne infection.

2. Methods

2.1. Study design and sample size

A cross-sectional study was designed. The target population were residents of Bavaria over 18 years of age, experiencing a tick bite in Bavaria in the preceding 4 weeks. As determined according to Cannon and Roe (1982), a sample size of 100 participants was required for proof of presence of acute *A. phagocytophilum* infections after tick bite in Bavaria. This sample size would permit the detection of at least one acute infection of *A. phagocytophilum*, with a probability of over 85%, assuming an acute infection in 2% of people after a tick bite (Cannon and Roe, 1982).

All general practice (GP) and internal medicine surgeries, who were registered for the syndromic or virological influenza sentinel surveillance in Bavaria in June 2010 ($n = 134$), were asked to participate in the study. Participating surgeries recruited patients over 18 years of age with recent tick exposure. Recent tick exposure was defined as patients who presented to their doctor with a tick bite or reported a history of a tick bite in the 4 weeks preceding the time point of the first sample.

2.2. Biological samples

After written consent was obtained, venous blood samples were collected from participating patients on two occasions, with an interval of two weeks between sampling time points. Submission of a third blood sample was requested if interpretations of the serological results from the first two time points were ambiguous.

2.3. Survey

For each of the sampling time points, doctors were asked to fill in a short structured questionnaire recording any clinical signs, the history of chronic diseases and any prescription of antibiotics. After giving consent to participate in the study, patients were asked to fill in a structured questionnaire with questions on demographics including place of residence, geographical location and activity at the time of tick exposure (if remembered) as well as regular leisure activities and potential occupational risk factors for tick exposure.