

Serologic evidence of *Anaplasma phagocytophilum* infections in patients with a history of tick bite in central Slovakia

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Serologische Evidenz von *Anaplasma phagocytophilum*-Infektionen bei Patienten mit Zeckenstichanamnese aus der Zentral Slowakei

Zusammenfassung. Ziel der Studie war es, das Risiko einer Anaplasmen-Infektion in bekannten Endemie-Gebieten der Lyme-Borreliose in der Zentral-Slowakei zu erfassen. Die Zirkulation von *Anaplasma phagocytophilum* in Zecken und Wildtieren wurde schon seit Jahren in Naturherden dieses Gebietes beobachtet. Aus dieser Region wurden Serumproben von Patienten gewonnen, die nach einem Zeckenstich klinische Symptome der Lyme-Borreliose entwickelt hatten. Insgesamt wurden Seren von 76 Patienten mittels eines kommerziellen Immunofluoreszenz Assays zum Nachweis von IgG Antikörpern gegen *A. phagocytophilum* getestet; 19 (25%) waren positiv. Eine Infektion mit *A. phagocytophilum* wurde bei einem Kind (4%), 12 Erwachsenen (39%) im Alter zwischen 22 und 56 Jahren und bei sechs Personen (32%) über 56 Jahre serologisch bestätigt. Ein statistisch signifikanter Unterschied in der Seroprävalenz ($P < 0,01$) wurde zwischen Kindern und Erwachsenen ermittelt. IgG-Antikörper gegen *A. phagocytophilum* wurden bei sieben Patienten mit klinischer Lyme-Borreliose, bei sieben Personen mit Antikörpern gegen *Borrelia burgdorferi* sensu lato und bei fünf Borrelien-seronegativen Personen ermittelt. Die häufigsten Symptome bei den *A. phagocytophilum* seropositiven Patienten waren Kopfschmerzen, Gelenks- und Muskelschmerzen, Fieber, Ausschlag, und Lymphknotenschwellung. Diese Patienten stammten aus den Gemeinden Vtáčnik (5/19), Štiavnické vrchy (1/19), Kremnické vrchy (10/19) und Žiarska kotlina (3/19). Die Ergebnisse zeigen, dass ein Risiko für Infektionen mit *A. phagocytophilum* in den Naturherden der Zentral-Slowakei besteht, sodass die humane Anaplasrose in die Differentialdiagnose mit einbezogen werden sollte, insbesondere dann, wenn eine akut fieberhafte Erkrankung nach Zeckenstich auftritt.

Summary. The aim of this study was to determine the risks of human anaplasmosis in an area of central Slovakia endemic for Lyme borreliosis. The circulation of *Anaplasma phagocytophilum* in ticks and wild animals has been observed in natural foci in this area for several years. Samples of human sera from patients with Lyme borreliosis and persons with a history of recent tick bite and clinical symptoms indicating Lyme borreliosis were collected in central Slovakia.

A total of 76 human sera were analyzed using an indirect HGE IgG immunofluorescent assay kit. IgG antibodies against *A. phagocytophilum* were found in 19 (25%) sera (15 female, 4 male patients). *A. phagocytophilum* infection was serologically confirmed in one (3.8%) child, 12 (38.7%) persons aged 22–56 and six (31.6%) persons older than 56. A statistically significant difference in seroprevalence ($P < 0.01$) was observed between children (3.8%, 1/26) and adults (36%, 18/50). Antibodies against *A. phagocytophilum* were detected in seven patients with clinically diagnosed Lyme borreliosis and in another seven individuals with assessed anti-borrelia antibodies. IgG antibodies against *A. phagocytophilum* were detected in five persons seronegative for borrelia. The most frequent clinical symptoms in patients with positive *A. phagocytophilum* serology were cephalgia, arthralgia, myalgia, fever, exanthema, neurological symptoms and lymphadenopathy. Positive sera were obtained from patients living in villages and towns in the orographic entities Vtáčnik (5/19), Štiavnické vrchy (1/19), Kremnické vrchy (10/19) and Žiarska kotlina (3/19). Our results demonstrate the risk of acquiring *A. phagocytophilum* infection in natural foci in central Slovakia. Human anaplasmosis should be considered in the differential diagnosis, especially in cases of acute febrile illness with tick-bite history.

Key words: *Anaplasma phagocytophilum*, seroprevalence, tick bite, Lyme borreliosis.

Introduction

Tick-borne diseases are known or considered to occur in Slovakia because of the abundance of *Ixodes ricinus*, the most prevalent exophilic tick species in central Europe [1, 2]. This tick is a known vector of several microorganisms such as tick-borne encephalitis (TBE) virus, *B. burgdorferi* s.l., ehrlichiae, spotted fever group (SFG) rickettsiae and *Coxiella burnetii* [3–5]. TBE and Lyme borreliosis (LB) are the most familiar tick-borne diseases in Slovakia. The first described outbreak of TBE occurred in Rožňava in 1951 [6] and the disease has been monitored since 1952. The average morbidity in the period 1952–2006 was 1.47 per 100 000 inhabitants. LB has been monitored since 1987; the average morbidity in the period 1987–2006 was 10.38 per 100 000 inhabitants (published in the epidemiological information system of the Slovak Republic, www.epis.sk). Human anaplasmosis and rickettsioses are caused by *A. phagocytophilum* and rickettsiae, respectively. These illnesses are less well known tick-borne diseases in Slovakia and data on their prevalence and morbidity are absent.

Human anaplasmosis (previously named human granulocytic ehrlichiosis) is caused by *Anaplasma phagocytophilum*, a gram-negative obligate intracellular bacterium that primarily infects granulocytes [7]. In Europe, it is transmitted by *I. ricinus*. The disease was described in the USA in 1994 [8]. The first European case was reported in Slovenia in 1997 [9]. In the following years, evidence of human infections caused by *A. phagocytophilum* and also serological evidence were reported from several European countries [10]. Nevertheless, symptomatic infection in Europe appears to be rare and only 66 cases have been reported, despite a median seroprevalence rate of 6.2% [11]. Clinical presentations of human anaplasmosis are nonspecific and usually consist of fever, chills, headache, fatigue, malaise, nausea, vomiting, diarrhea, cough, nonspecific rash, myalgia and/or arthralgia. The disease is often accompanied by leucopenia, thrombocytopenia, anemia, and increased activity of hepatic enzymes. Systemic complications may involve the respiratory tract (sore throat, cough, pulmonary infiltrates, acute respiratory distress syndrome), the digestive system (nausea, vomiting, abdominal pain, gastrointestinal bleeding) or hepatitis. Other possible complications include meningitis, pericarditis, renal failure and disseminated intravascular coagulation. The acute stage of infection with *A. phagocytophilum* is indicated by the presence of morulae in the granulocytes, a positive polymerase chain reaction from whole blood and/or isolation of *A. phagocytophilum* from blood. An indirect immunofluorescence assay (IFA) is used for diagnosis. Serological tests are commonly used but are often negative during the initial phase of the disease [12, 13].

The occurrence of bacteria belonging to the former *Ehrlichia phagocytophila* group in Slovakian ticks was first described in 2002 [14]. *A. phagocytophilum* was detected in questing *I. ricinus* ticks and in the spleens of wild animals from central Slovakia [15–17].

The aim of this study was to determine the seroprevalence of *A. phagocytophilum* in patients with a history of tick bite, undiagnosed febrile illness and suspected or diagnosed LB in an endemic area of central Slovakia.

Methods

Serum samples and data on patients' age, sex, presence of antibodies to *Borrelia burgdorferi* s.l. (tested by Euroimmun Anti-Borrelia IgG and IgM ELISA kit), tick bite and disease symptoms were obtained in cooperation with sentinel physicians and medical laboratory workers. In the period 2002–2005 a total of 76 sera were collected from patients who had history of tick bite and symptoms resembling LB and resided in areas where a higher incidence of the disease was reported. The patients worked in various occupations; 6.58% were forest and agricultural workers.

Sera were analyzed using an HGE IgG IFA kit (Focus Technologies, USA). Material was processed and results interpreted according to the recommendations of the manufacturer. Seroprevalence was stratified by sex, age and geographic area. The chi-squared test was used to compare rates of seroprevalence.

Results

Sera were collected from 76 patients: 25 men and 51 women. Twenty-six patients were children aged 6–19 years, 31 were persons aged 20–56, and 19 were older than 56 years. IgG antibodies against *A. phagocytophilum* (AP IgG) were detected in 19 (25%) patients: 15 (29.4%) females and in 4 (16%) males (Table 1). With regard to the age of the patients, *A. phagocytophilum* infection was serologically confirmed in one (3.8%) child, in 12 (38.7%) persons aged 22–56 and six (31.6%) persons older than 56. A statistically significant difference in seroprevalence was observed between children (3.8%, 1/26) and adults (36%, 18/50) ($P < 0.01$). AP IgG was detected in 14 patients who were seropositive to *B. burgdorferi* s.l. and LB was confirmed in seven of these patients. AP IgG was detected in five persons seronegative for borrelia.

Patients with positive *A. phagocytophilum* serology suffered from arthralgia/myalgia (57.9%), fever (26.3%), lymphadenopathy (10.5%), exanthema (26.3%) and neurological symptoms (21%). Some of these patients exhibited interesting symptoms. One of these persons, an anemic patient who had been followed for a long time in the hematology department, had complained of aggravation of strong arthralgias and intense fatigue; however, LB serology was negative. Another patient, also with negative LB serology, suffered from arthralgias, myalgia, atypical exanthema, an affected respiratory system and lymphadenopathy. A female patient with multiple sclerosis and low antibodies against *B. burgdorferi* s.l. was admitted to a neurologic unit after a tick bite because of sudden aggravation of her health condition with strong headache and impairment of motility. Two forest workers with diagnosed LB suffered from erythema migrans, fever, arthralgia, headache and tiredness.

During the period of the study, sera positive for *A. phagocytophilum* were obtained from patients living in villages and towns in the mountains of Vtáčnik (5/19), Štiavnické vrchy (1/19) and Kremnické vrchy (10/19), and in the basin of Žiarska kotlina (3/19).

Discussion

In Slovakia, human anaplasmosis is a less well known disease but is growing in importance. Data on the occurrence and morbidity of this emerging tick-borne zoonosis

Table 1. Presence of antibodies against *B. burgdorferi* s.l. and confirmed Lyme borreliosis in patients positive for *A. phagocytophilum* in IFA

Ap-positive patients	Localities	Ap IgG only	Ap IgG+ Bb s.l. IgM+ IgG	Ap IgG+ LB confirmed
female	Nová Baňa	+	–	–
female	Malá Lehota	–	+	+
female	Malá Lehota	–	+	+
female	Žiar nad Hronom	–	+	+
female	Žiar nad Hronom	–	+	–
male	Žiar nad Hronom	–	+	+
female	Kremnica	–	+	+
female	Kremnica	–	+	+
female	Kremnica	–	+	–
female	Kremnica	–	+	–
female	Kremnica	–	+	+
female	Kremnica	–	+	–
female	Kremnica	–	+	–
male	Kremnica	–	+	–
male	Kremnica	+	–	–
male	Kremnica	–	+	–
female	Hodruša Hámre	+	–	–
female	Horná Ves	+	–	–
female	Vel'ká Lehota	+	–	–
<i>Total: 19</i>		<i>5</i>	<i>14</i>	<i>7</i>

Ap *Anaplasma phagocytophilum*; Bb *Borrelia burgdorferi* sensu lato; LB Lyme borreliosis.

are absent and the clinical and laboratory follow-up are not yet established in practice.

Human infections with *A. phagocytophilum* and antibodies reactive to granulocytic ehrlichiae have been described in several countries of Europe [10, 18, 19]. In western Austria, 9% of sera from Tyrolean blood donors tested by IFA showed the presence of antibodies to *A. phagocytophilum* [20]. In Poland, a seroprevalence of 19.8% was found in foresters and 5.4% in blood donors from the Lublin region [21]. In Spain, *A. phagocytophilum* antibodies were detected in 1.4% of tested people in La Rioja region, where ticks were found to be positive for this bacterium [22]. In Italy, antibodies reactive with *A. phagocytophilum* were found in 5.7% of workers exposed to tick bites and in 0.9% of the control group [23]. In the Czech Republic IgG antibodies against *A. phagocytophilum* were detected in 7.9% of sera analyzed [24].

Seroprevalence rates of *A. phagocytophilum* in humans in Europe range from zero or very low to up to 28%. The proportion of seropositive persons increases with age and is higher in persons exposed to ticks [19]. All the sera in our study were obtained from persons who frequently reported tick bites, and a seroprevalence of 25% was determined. Antibodies were detected in 3.8% of children and 36% of adults from five locations. Symptoms such as arthralgia, myalgia, fever and headache in patients with positive HGA serology were similar to those described in patients with anaplasmosis in a prospective study in Slovenia [13].

Moss and Dumler [25] described simultaneous early Lyme disease and human anaplasmosis in a child in the USA. European studies have also shown that *A. phagocytophilum* infections might occur in individuals with LB. In study in northern Italy, *A. phagocytophilum* was serologically detected in five of 79 patients tested for tick-borne diseases. Three of these patients were coinfecting with *B. burgdorferi* s. l. [26]. In Poland, *A. phagocytophilum* DNA was amplified from the blood of patients with neuroborreliosis, arthralgia and disseminated LB [27]. In the Lublin region of Poland, 4.5% of 334 sera from forest workers were positive to both *A. phagocytophilum* and *B. burgdorferi* s. l. [21]. Antibodies against both *A. phagocytophilum* and *B. burgdorferi* s. l. were detected in 1.4% of Tyrolean blood donors [20] and in 2.8% of male soldiers aged 18–29 in Germany [28]. Antibodies against *A. phagocytophilum* and *B. burgdorferi* s. l. were identified in 3.8% of tested patients in the Czech Republic [24]. In our study, antibodies against *B. burgdorferi* s.l. had been detected in 14 of 19 patients with AP IgG. LB was diagnosed in seven of these persons.

A. phagocytophilum seems to be widely distributed in natural foci in Europe, though its prevalence in *I. ricinus* varies in different regions [10]. The median infection prevalence in *I. ricinus* is 3% [11]. *A. phagocytophilum* has been found in 4.4% of adult *I. ricinus* collected in suburban, agricultural and recreational areas of central Slovakia [15], therefore inhabitants of these regions, especially persons who are often bitten by ticks and people

such as forest workers professionally exposed to ticks may be at risk of acquiring this tick-transmitted illness.

This is the first report confirming that inhabitants and visitors in central Slovakia are exposed to *A. phagocytophilum* infection. Human anaplasmosis should therefore be considered in patients with unexplained febrile illness or nonspecific symptoms following a tick bite, even though antibodies against *B. burgdorferi* s.l. were identified or LB was diagnosed.

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