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OCCURRENCE OF IGG ANTIBODIES TO *ANAPLASMA PHAGOCYTOPHILUM* IN HUMANS SUSPECTED OF LYME BORRELIOSIS IN EASTERN SLOVAKIA

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Abstract: Human granulocytic anaplasmosis (HGA) and Lyme borreliosis (LB) are tick-borne and emerging infectious diseases caused by the *Anaplasma phagocytophilum* and *Borrelia burgdorferi* species. In Europe, including Slovakia, the principal vector of both pathogens is the common tick – *Ixodes ricinus*, in which double infections with these pathogens have been reported. The aim of our study was evidence of IgG antibodies against *A. phagocytophilum* in blood sera of humans with suspects LB from several Clinics of University Hospitals, and the evaluation of the possibility of *B. burgdorferi* and *A. phagocytophilum* co-infection in examined patients. The serological method ELISA was used to detect IgM and IgG antibodies against *B. burgdorferi*. Anti-*A. phagocytophilum* IgG antibodies were analyzed by the *A. phagocytophilum* Indirect Immunofluorescence Antibody (IFA) IgG test. A total of 214 human samples (91 men, 123 women) were obtained from patients living in Košice town and in villages around Košice (Eastern Slovakia). IgG antibodies against *A. phagocytophilum* were detected in 15 cases (6 men, 9 women), which represented 7.0% positivity. Two cases of the co-infection *B. burgdorferi* with *A. phagocytophilum*, which equals 0.93% of the total number, were found.

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INTRODUCTION

Human granulocytic anaplasmosis (HGA) is tick-borne zoonosis caused by *Anaplasma phagocytophilum*. *A. phagocytophilum* (formerly *Ehrlichia phagocytophila*, *E. equi* and human granulocytic ehrlichiosis – HGE agent) belongs to the *Anaplasmataceae* family [10]. It has affinity to granulocytic cells where the bacteria replicates within cytoplasmic vacuoles to form microcolonies (morulae, Latin for "mulberry") that do not fuse with lysosomes [4].

The first case of human infection by *A. phagocytophilum* was found in the United States in 1994 [5]. Since then, the number of patients has increased in the United States [11]. In Europe, the first human cases of this disease were described in 1997, in Slovenia [22], and serological and PCR analyses suggest that *A. phagocytophilum* is distributed throughout Europe and in some parts of the Middle East and Asia [2, 3, 6, 15, 16, 28].

HGA is febrile systematic illness and the severity of this disease ranges from asymptomatic seroconversion to death. Infection is often characterized by fever, severe headache, malaise, myalgia, leucopenia, thrombocytopenia, and elevated hepatic transaminases. The illness is rarely fatal, but death may occur as a result of opportunistic infections, often with catalase-positive organisms [11].

The principal vector of *A. phagocytophilum* in Europe is tick *Ixodes ricinus*. This tick is known as vector of several microorganisms, such as *Borrelia burgdorferi*, tick-borne encephalitis (TBE) virus, *Coxiella burneti*, spotted fever group rickettsiae [17, 19, 23].

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In Slovakia, TBE and Lyme borreliosis (LB) are the most familiar tick-borne diseases. In common with the vector of these diseases the double infections with both LB and HGA pathogens have been reported [9]. There is the assumption that co-infection may also occur in humans.

Therefore, the aim of our study was evidence of IgG antibodies against *A. phagocytophilum* in blood sera of humans suspected of LB, and evaluation of the possibility of *B. burgdorferi* and *A. phagocytophilum* co-infection in the examined patients.

MATERIAL AND METHODS

A total 214 human serum samples (91 men and 123 women) from several clinics of the University Hospital (Clinic of Orthopaedics – 71 samples, Clinic of Neurology – 46 samples, Clinic of Dermatovenerology – 34 samples, other clinics – 63 samples) with suspected Lyme borreliosis were analyzed for the presence of antibodies against *A. phagocytophilum*. All sera from patients were obtained before treatment. Analyzed sera were stored at -20°C until use in the serological test.

The groups of examined people were selected by age as follows: in the age group 0–19 years there were 9 patients, in the age group 20–29 years – 32 patients, in the age group 30–39 years – 38 patients, in the age group 40–49 years – 46 patients, in the age group 50–59 years – 47 patients, in the age group 60–69 years – 21 patients, and 21 patients were older than 70 years. All 214 examined people were living in Eastern Slovakia (124 in Košice town and 90 in villages around Košice town).

For the presence of IgM and IgG antibodies against *B. burgdorferi* the sera were tested at the Institute of Medical and Clinical Microbiology of the P. J. Šafárik University, Faculty of Medicine in Košice with ELISA test kit (f. Biomedica) according to manufacturer's instructions. IgG and IgM concentrations were estimated in BBU/ml by quantitative measurements. People whose BBU/ml was more than 11 were considered positive.

Anti-*A. phagocytophilum* IgG antibodies were detected by the Focus Diagnostics Indirect Immunofluorescence Antibody (IFA) IgG test, which is intended for the detection of human serum IgG class antibodies to *A. phagocytophilum*, as an aid in the diagnosis of HGA. Blood sera were processed and results interpreted according to the test producer. The people whose blood sera reacted at the titer 1:64 and higher were considered positive.

RESULTS

In a positive case, the apple-green fluorescence of the morulae was detected.

IgG antibodies against *A. phagocytophilum* were detected in 15 (7.0%) out of the total number of 214 examined sera. Six positive samples coming from the Clinic of Orthopaedics, 4 from the Clinic of Neurology, 2 from the Clinic of Dermatovenerology and 3 from others clinics (Fig. 1).

Of 15 patients positive diagnosed with *A. phagocytophilum* IgG antibodies there were 6 men and 9 women with various primary diagnosis (Tab. 1).

With regard to the age of the patients, IgG antibodies against *A. phagocytophilum* were found in 5 (15.6%) persons aged 20–29, 2 (5.3%) aged 30–39, one (2.2%) in the 40–49 age group, 5 (10.6%), aged 50–59, one (4.8%) in the age group 60–69, and one (4.8%) in the group older than 70 years (Tab. 1).

Table 1. Presence of IgM and IgG *B. burgdorferi* antibodies in patients positive for *A. phagocytophilum*.

Gender	Age	Place of residence	*B. burgdorferi*		Primary diagnosis
			IgM	IgG	
Male	34	town	neg.	neg.	M 54.4 – Lumbago with sciatica
Male	59	village	neg.	neg.	M 53.1 – Cervicobrachial syndrome
Female	24	village	neg.	neg.	M 25.5 – Pain in joint
Female	52	town	+++	neg.	M 13.0 – Polyarthritis unspecified
Female	52	village	neg.	neg.	S 22.0 – Fracture of thoracic vertebra
Female	73	town	neg.	+	M 13.0 – Polyarthritis unspecified
Male	35	village	neg.	neg.	G 44.8 – Other specified headache syndrome
Male	57	town	neg.	neg.	G 96.9 – Disorders of CNS, unspecified
Female	47	town	neg.	neg.	G 50.0 – Trigeminal neuralgia
Female	64	town	±	neg.	I 63.9 – Cerebral infarction unspecified
Female	22	village	neg.	neg.	L 52.0 – Erythema nodosum
Female	25	village	neg.	neg.	L 94.0 – Localized scleroderma
Male	28	town	neg.	neg.	B 99.0 – Other and unspecified infectious diseases
Male	28	town	neg.	neg.	D 75.0 – Familial erythrocytosis
Female	59	town	neg.	neg.	H 06.0 – Disorders of lacrimal system in diseases classified elsewhere

'neg.' < 9 BBU/ml; '±' 9–11 BBU/ml; '+' 11–20 BBU/ml; '+++' >30 BBU/ml

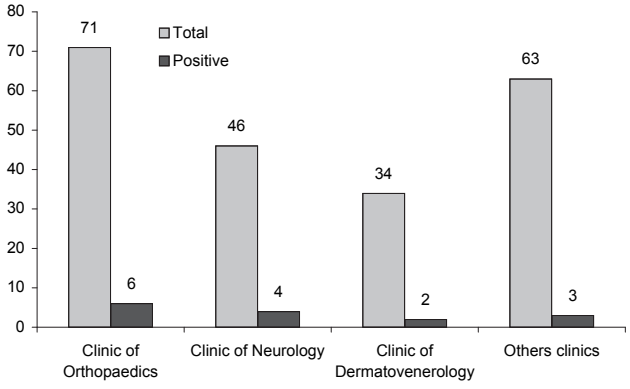

Figure 1. Prevalence of IgG against *A. phagocytophilum* among patients suspected of Lyme borreliosis.

With regard to the place of residence, anti-IgG *A. phagocytophilum* antibodies were confirmed in 9 (7.3%) of 124 humans living in Košice town, and 6 (6.6%) from 90 people living in villages (Tab. 1).

Positive anti-*B. burgdorferi* antibodies were found in 20.6% of people (44 positive), of which 20 were men and 24 were women.

Of the total number of 214 human sera examined, only 2 cases (2 women) were detected who had coinfection of *B. burgdorferi* with *A. phagocytophilum*, which represented 0.93% (Tab. 1).

DISCUSSION

Infections caused by *A. phagocytophilum* pathogen have been described in many European countries. In Slovakia, HGA is a less well known tick-borne disease and data on their prevalence and morbidity are absent. Only few studies have been published relating to anaplasmosis. In 2008, Kocianova *et al.* [18] examined 76 human sera from patients with LB and one person with a history of recent tick bite and clinical symptoms indicating LB. All the people came from an area of central Slovakia endemic for LB. IgG antibodies against *A. phagocytophilum* were detected in 25% of patients.

In central Europe, both pathogens – *A. phagocytophilum* and *B. burgdorferi* – are transmitted by the tick *I. ricinus* [12]. Acute HGA with clinical signs is rarely documented [21], the patients often showing only an immune response to *A. phagocytophilum*. The most commonly used technique for HGA diagnosis is IFA, which should include both IgM and IgG specific antibody screens for maximal certainty. In the absence of treatment, detectable IgM levels generally rise 3–5 days post-infection, or 24 hours after the initial onset of fever, falling again to undetectable levels in about 30–60 days. IgG levels often are detectable about 7–10 days post-infection, peaking at 14–21 days and persisting for approximately a year.

Seroprevalence rates of *A. phagocytophilum* in humans in Europe range from zero or very low to up to 28.0% [25]. Prevalence of IgG antibodies to *A. phagocytophilum* among forestry rangers from the Białystok region (north-eastern Poland) was 3.9% [14], from Lublin province (eastern Poland) – 23.0% [28]. Other Polish studies in forestry rangers demonstrated seropositivity from 17.7% –20.0% in mid-eastern Poland and 9.6% in northern and north-eastern Poland [8, 24, 27]. 1.5% seropositivity of *A. phagocytophilum* has been detected in English farmers [26]. *A. phagocytophilum* has been studied in blood donors in Macedonia (North Greece) revealing a 7.3% prevalence of antibodies to *A. phagocytophilum* [1]. In Crete (Greece), seroprevalence of *A. phagocytophilum* among blood donors was 21.4% [7]. In the Czech Republic IgG antibodies against *A. phagocytophilum* were detected in 7.9% of analyzed sera [20].

In our study we examined 214 people from Eastern Slovakia with suspected borreliosis for the presence of antibodies against *A. phagocytophilum*. The total seropositivity was 7.0%. During the examination in relation to the age categories, the highest positivity was observed in the age group of 20–29 years. With regard to place of residence, significant difference was not detected in outcome between people urban dwellers and rural dwellers (7.3% *vs.* 6.6%). Single infection of *B. burgdorferi* was detected in 20.6%. Co-infection *A. phagocytophilum* with *B. burgdorferi* was confirmed only in 2 women from Košice town. Our results correspond with the results of a study performed by Derdakova *et al.* [9]. They examined *I. ricinus* ticks collected from a suburban park in Košice town where LB is highly endemic for the presence of *A. phagocytophilum* and *B. burgdorferi*. 8.3% of the tested ticks carried single infection of *A. phagocytophilum*, 38.3% were infected with *B. burgdorferi*, and in 5% of tested ticks, a double infection of both pathogens was detected.

These results, together with results obtained from our study, indicate the importance of performing screening examinations of patients with suspected LB, especially in the case of negative results. Clinical signs of both diseases are very similar, and studies from Slovakia acknowledge that pathogen *A. phagocytophilum* circulate in ticks *I. ricinus*, which are the principal vectors of disease.

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