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 systemic 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].
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*.

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

'neg.' < 9 BBU/ml; ‘±’ 9–11 BBU/ml; ‘+’ 11–20 BBU/ml; ‘+++’ >30 BBU/ml
Occurrence of IgG antibodies to Anaplasma phagocytophilum in humans suspected of Lyme borreliosis in eastern Slovakia

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 Bialyštok region (northeastern 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 Dědakova 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.

Acknowledgement

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REFERENCES