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>
<thead>
<tr>
<th>Gender</th>
<th>Age</th>
<th>Place of residence</th>
<th>B. burgdorferi</th>
<th>Primary diagnosis</th>
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<tbody>
<tr>
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<td>IgM</td>
<td>IgG</td>
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<tr>
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<tr>
<td>Female</td>
<td>59</td>
<td>town</td>
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</tr>
</tbody>
</table>

‘neg.’ < 9 BBU/ml; ‘±’ 9–11 BBU/ml; ‘+’ 11–20 BBU/ml; ‘+++’ >30 BBU/ml
Antibodies were found in. The most commonly used tech among blood do
2000, in Europe range from zero or very low to up to 28.0%
sisting for approximately a year. 7–10 days post-infection, peaking at 14–21 days and per
in about 30–60 days. IgG levels often are detectable about
initial onset of fever, falling again to undetectable levels
tainty. In the absence of treatment, detectable IgM levels
IgM and IgG specific antibody screens for maximal cer
nique for HGA diagnosis is IFA, which should include both
[21], the patients often showing only an immune response
[12]. Acute HGA with clinical signs is rarely documented
in China where Lyme disease is endemic.

With regard to the place of residence, anti-IgG A. phago-
cytoplphilum antibodies were confirmed in 9 (7.3%) of 124
humans living in Košice town, and 6 (6.6%) from 90 peo-
ple 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 represent-
ed 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 stud-
ies have been published relating to anaplasmosis. In 2008,
Kocianova et al. [18] examined 76 human sera from pa-
tients 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 tech-
nique for HGA diagnosis is IFA, which should include both
IgM and IgG specific antibody screens for maximal cer-
tainty. 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 per-
sisting 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),
erseroprevalence of A. phagocytophilum among blood do-
nors was 21.4% [7]. In the Czech Republic IgG antibod-
ies against A. phagocytophilum were detected in 7.9% of
analyzed sera [20].

In our study we examined 214 people from Eastern Slo-
vakia with suspected borreliosis for the presence of anti-
bodies against A. phagocytophilum. The total seropos-
itivity 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 re-
results correspond with the results of a study performed by
Derdakova et al. [9]. They examined I. ricinus ticks col-
lected 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 ex-
aminations 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**

This study was supported by VEGA MŠ SR No. 1/0412/09, MZ
SR 2006/31-SAV-02 and KEGA MŠ SR No. 3/6155/08.

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