Occurrence of antibodies to *Anaplasma phagocytophilum* in patients with suspected tick-borne encephalitis

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**Abstract**

**Introduction and objectives.** Human granulocytic anaplasmosis (HGA) is an emerging tick-borne infectious disease caused by *Anaplasma phagocytophilum*. In Europe, the first serological evidence of HGA was described in 1995 in Switzerland, and the first clinical case was confirmed in 1997 in Slovenia. Since then, many European countries, including Slovakia, have reported the occurrence of HGA. The aim of this study was to examine the occurrence of IgG antibodies against *A. phagocytophilum* in blood sera of humans with suspected tick-borne encephalitis.

**Material and methods.** 181 people were examined for the presence of anti-*A. phagocytophilum* IgG antibodies; 113 were patients with suspected TBE (65 males, 48 females), and 68 from the control group (18 males, 50 females). Respondents were aged 2–80 years (mean age: 31.39; STD: 17.1). Anti-*A. phagocytophilum* IgG antibodies were detected by the IFA IgG test. Relative risk (RR) and their 95% confidence intervals (95% CI) were estimated for the occurrence of IgG *A. phagocytophilum* antibodies.

**Results.** Of the total number of 181 people examined, 32 (17.7%) showed positive for IgG antibodies against *A. phagocytophilum*, 22 of whom were patients with suspected TBE (19.5%) and 10 people from control group (14.7%). The RR of occurrence of IgG *A. phagocytophilum* was 1.3-times higher in the patients with suspected TBE than in the control group.

**Conclusion.** None of the examined patients with suspected TBE had the disease confirmed. However, as shown by the results, the relative risk of occurrence of anaplasmosis is higher in people examined for some another vector-borne disease (in this case TBE). Therefore, the performance of screening examinations in patients suspected of having any tick-borne disease is very important.

**Key words**

*Anaplasma phagocytophilum*, human granulocytic anaplasmosis, *Ixodes ricinus*, tick-borne encephalitis, Slovakia

**INTRODUCTION**

Human granulocytic anaplasmosis (HGA) is an emerging tick-borne infectious disease caused by *Anaplasma phagocytophilum* (formerly *Ehrlichia phagocytophila*) [1]. This gram-negative obligate intracellular pathogen was first identified in humans in 1990, when a patient from Wisconsin in the USA died of an acute febrile illness 2 weeks after a tick bite. The pathogen was isolated in 1994 by polymerase chain reaction (PCR) and its taxonomic name was changed in 2001 to the current form of *A. phagocytophilum* [1, 2].

In Europe, the first serological evidence of HGA was described in 1995 in Switzerland [3], and first clinical case was confirmed in 1997 in Slovenia [4]. Since then, many European countries have reported the occurrence of HGA [5, 6, 7, 8, 9, 10, 11]. In Slovakia, the first case of HGA was confirmed in 1997 in Slovenia [4]. Since then, many European countries, including Slovakia, have reported the occurrence of HGA. The aim of this work was to study the occurrence of IgG antibodies in the blood sera of humans with suspected TBE, and to compare the obtained results with prevalence in the control group.

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headache, malaise, generalized myalgia and arthralgia. Other symptoms, such as nausea, abdominal pain, diarrhea, cough and meningitis, are less commonly reported [14].

Anaplasmosis is usually transmitted to human and animals through the bite of an infected tick. In Europe, including Slovakia, the main vector of *A. phagocytophilum* is the common tick, *Ixodes ricinus*, which can also transmit other pathogens, such as the tick-borne encephalitis (TBE) virus, the bacteria *Borrelia burgdorferi*, and the spotted-fever group of bacteria *Rickettsia* [15, 16]. Of these, tick-borne encephalitis is currently the most important disease transmitted by *Ixodes ricinus* ticks in central Europe. The disease typically takes a biphasic course. The initial symptoms are non-specific, and symptoms may include fever, malaise, anorexia, muscle pain, headache and nausea or vomiting, symptoms similar to HGA. The second phase of the disease occurs in 20% – 30% of patients and involves the central nervous system, with symptoms of meningitis, encephalitis or meningoencephalitis.
MATERIALS AND METHODS

A total of 181 human serum samples were examined for the presence of IgG antibodies against *A. Phagocytophilum*. 113 patients (65 males, 48 females) were selected on the basis of showing clinical symptoms during a differential diagnosis and examined for tick-borne encephalitis. For comparison, 68 healthy individuals (18 males, 50 females) who denied having any contact with ticks and who showed no clinical signs of vector-borne diseases were also examined. The examined patients were between 2–80 years of age (mean age: 31.39, STD: 17.1).

Blood from patients suspected of having tick-borne encephalitis was taken by neurologists and infectologists and sent to a virology laboratory, where the samples were examined for TBE using a complement fixation test. Residual sera were subsequently delivered to our institute, where they were stored at -20°C until tested in the laboratory.

Anti-*A. phagocytophilum* IgG antibodies were detected using an Indirect Immunofluorescence Antibody (IFA) IgG test (Focus Diagnostics, California, USA). The IFA assay is a two stage 'sandwich' procedure: in the first stage, the patient’s serum is diluted in PBS and placed on a slide in contact with the substrate, and incubated. Following incubation, the slide is washed in PBS to remove unbound serum antibodies. In the second stage, each antigen well is overlaid with fluorescein-labelled antibody to human IgG. The slide is incubated, allowing the antigen antibody complexes to react with the fluorescein-labeled anti-human IgG. After the slide has been washed, dried, and mounted, it is examined using fluorescence microscopy.

Positive reactions appear as an apple-green fluorescence of the morulae. Semi-quantitative endpoint titers are obtained by testing serial dilutions of positive specimens. The serum screening dilution was 1:64, according to the test producer.

Statistical analysis. Basic descriptive statistics were used to analyse the obtained results. Relative risks (RR) and their 95% confidence intervals (95% CI) were estimated for the occurrence of IgG *A. phagocytophilum* antibodies. The contributions of gender and risk group on the prevalence *A. phagocytophilum* antibodies were assessed using a logistic regression model. Statistical significance was defined as p value <0.05.

RESULTS

In the case of a positive immunological reaction to the presence of antibodies against *A. phagocytophilum*, the apple-green fluorescence of the morulae was detected. Patients whose sera reacted at the titre 1:64 and higher were considered to be positive.

Of the total number of 181 people included in the study, 32 (17.7%) showed positivity for IgG antibodies against *A. phagocytophilum*. 22 of them were patients with suspected TBE (19.5%) and 10 were from control group (14.7%). The highest positivity was detected in males and females with suspected TBE (20% resp. 18.8%), while positivity in females from the control group was 18% and in men from control group 5.6% (Tab. 1).

None of the 22 anti-*A. phagocytophilum* IgG antibodies positive patients with suspected TBE had the disease confirmed (Tab. 2).

Upon comparing the relative risk of occurrence of IgG *A. phagocytophilum* antibodies in the group of patients with suspected TBE and the control group, it was found that the risk of infection was almost 1.3-times higher in patients with suspected TBE than in the control group. This risk was 3.6-times higher for males with suspected TBE, compared with males in the control group, and upon comparing the group of females with suspected TBE with those from control group, the relative risk for both groups was approximately the same. Therefore, no significant difference was observed between positive cases in the groups of people with suspected TBE and the control group (Tab. 3).

### Table 1. Prevalence of IgG *A. phagocytophilum* antibodies

<table>
<thead>
<tr>
<th>Gender</th>
<th>Age (CFT)</th>
<th>TBE</th>
<th>Primary diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>32</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>49</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Table 2. Epidemiological data of patients with suspected TBE with positive anti-*A. phagocytophilum* IgG antibodies

<table>
<thead>
<tr>
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<td>49</td>
<td></td>
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</tr>
</tbody>
</table>

### Table 3. Significant difference between positive cases of patients with suspected TBE and control group in relation to gender

<table>
<thead>
<tr>
<th>Gender</th>
<th>Suspected TBE</th>
<th>Control group</th>
<th>Relative risk (95% CI)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>32 (19.5)</td>
<td>10 (14.7)</td>
<td>3.2 (0.6 – 2.6)</td>
<td>0.42</td>
</tr>
<tr>
<td>Female</td>
<td>18 (18.8)</td>
<td>9 (18)</td>
<td>1.04 (0.5 – 2.4)</td>
<td>0.92</td>
</tr>
</tbody>
</table>

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DISCUSSION

Tick-borne diseases are the most common vector-borne diseases in Europe. Lyme borreliosis, tick-borne encephalitis, Crimean-Congo haemorrhagic fever and rickettsiosis are endemic in certain regions of Europe. Lyme borreliosis and tick-borne encephalitis are of primary importance in public health, but the overall burden of these tick-borne diseases in Europe remains unclear [17].

TBE is endemic across much of Central and Eastern Europe. The reported incidence of the disease is increasing, with numbers estimated to be as high as 8,755 cases per year [18]. In Slovakia, around 60–80 cases are reported annually. The main vectors of the TBE virus in Europe are ticks of the family *Ixodidae*, mainly *Ixodes ricinus* (Central, Northern and Eastern Europe) and *Ixodes persulcatus* (parts of the Baltic States, Finland, Russia, Siberia). Competent reservoir hosts are mainly small rodents (foxes, bats, hares, deer, wild boar, sheep, cattle, goats, and dogs). Humans are incidental and dead-end hosts. In addition to being bitten by an infected tick, in endemic areas humans can also acquire TBE infection by consuming infected raw dairy products [19, 20].

The same ticks that transmit TBE in Slovakia can also transmit other pathogens, including *Anaplasma phagocytophilum*. Therefore, simultaneous infection with multiple organisms is possible.

The real infection rate of HGA in Europe is still difficult to establish. Seroprevalence rates range from zero to up to 28.0% [21]. No official epidemiological data on the prevalence of this infection in the human population are available in Slovakia. Only a few studies have been published relating to anaplasmosis, with results of prevalence ranging from 7% – 25% [22, 23]. The total prevalence of *A. phagocytophilum* antibodies in the presented sample (17.7%) corresponds with the findings of these studies. Despite a higher number of positive cases in the group of patients with suspected TBE, no significant difference in the occurrence between the study and control groups was found. The most probable reason seems to be the disproportion in the gender ratio in the control group (three times more females than males), which affected the overall value of the prevalence of *A. phagocytophilum* antibodies in the control group.

In the presented study, the highest positivity was detected in group of patients with suspected TBE. There are several studies that confirm the possible co-infection of multiple vector-borne pathogens. In Europe, these combinations also include infections by *A. phagocytophilum* and the TBE virus, but generally the frequency of simultaneous diseases is usually low. In the current study, none of the examined patients with suspected TBE had the disease confirmed. However, as shown by the results, the relative risk of occurrence of anaplasmosis is higher in people who are examined for some other vector-borne disease (in this case TBE). Therefore, the performing of screening examinations in patients suspected of having any tick-borne diseases is very important, especially in the case of negative results, not only for TBE and Lyme borreliosis, but also for anaplasmosis.

Acknowledgments

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REFERENCES