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

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**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 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 other 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
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-labelled 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. Semiquantitative 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).

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

| Gender | Age | TBE (CFT) | Primary diagnosis |
|--------|-----|-----------|------------------|
| Female | 54  | -         | Z 94.9 Transplanted organ and tissue status, unspecified |
| Female | 44  | -         | R 50.9 Fever, unspecified |
| Female | 12  | -         | R 59.9 Enlarged lymph nodes, unspecified |
| Female | 21  | -         | G 35 Multiple sclerosis |
| Male   | 41  | -         | R 50.9 Fever, unspecified |
| Male   | 16  | -         | R 50.9 Fever, unspecified |
| Male   | 38  | -         | G 82 Paraplegia and tetraplegia |
| Male   | 66  | -         | G 96.9 Disorder of central nervous system, unspecified |
| Female | 65  | -         | G 163.9 Cerebral infarction, unspecified |
| Male   | 30  | -         | R 50.9 Fever, unspecified |
| Male   | 71  | -         | B 02.9 Herpes zoster without complication |
| Male   | 19  | -         | G 31.9 Degenerative diseases of nervous system, unspecified |
| Male   | 19  | -         | M 60.9 Myositis, unspecified |
| Female | 24  | -         | G 62.9 Polyneuropathy, unspecified |
| Female | 66  | -         | G 96.9 Disorder of central nervous system, unspecified |
| Male   | 17  | -         | G 30 Alzheimer's disease |
| Male   | 4   | -         | M 54.9 Dorsalgia, unspecified |
| Male   | 22  | -         | J 03.9 Acute tonsillitis, unspecified |
| Male   | 5   | -         | G 51.9 Facial nerve disorders, unspecified |
| Male   | 56  | -         | R 50.9 Fever, unspecified |
| Female | 36  | -         | R 50.9 Fever, unspecified |
| Male   | 3   | -         | G 51.9 Facial nerve disorders, unspecified |

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

| Gender | Age | TBE (CFT) | Primary diagnosis |
|--------|-----|-----------|------------------|
| Female | 54  | -         | Z 94.9 Transplanted organ and tissue status, unspecified |
| Female | 44  | -         | R 50.9 Fever, unspecified |
| Female | 12  | -         | R 59.9 Enlarged lymph nodes, unspecified |
| Female | 21  | -         | G 35 Multiple sclerosis |
| Male   | 41  | -         | R 50.9 Fever, unspecified |
| Male   | 16  | -         | R 50.9 Fever, unspecified |
| Male   | 38  | -         | G 82 Paraplegia and tetraplegia |
| Male   | 66  | -         | G 96.9 Disorder of central nervous system, unspecified |
| Female | 65  | -         | G 163.9 Cerebral infarction, unspecified |
| Male   | 30  | -         | R 50.9 Fever, unspecified |
| Male   | 71  | -         | B 02.9 Herpes zoster without complication |
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| Male   | 19  | -         | M 60.9 Myositis, unspecified |
| Female | 24  | -         | G 62.9 Polyneuropathy, unspecified |
| Female | 66  | -         | G 96.9 Disorder of central nervous system, unspecified |
| Male   | 17  | -         | G 30 Alzheimer's disease |
| Male   | 4   | -         | M 54.9 Dorsalgia, unspecified |
| Male   | 22  | -         | J 03.9 Acute tonsillitis, unspecified |
| Male   | 5   | -         | G 51.9 Facial nerve disorders, unspecified |
| Male   | 56  | -         | R 50.9 Fever, unspecified |
| Female | 36  | -         | R 50.9 Fever, unspecified |
| Male   | 3   | -         | G 51.9 Facial nerve disorders, unspecified |

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

| Gender | Patients with suspected TBE No. (%) | Control group No. (%) | Relative risk (95% CI) | p-value |
|--------|------------------------------------|-----------------------|------------------------|---------|
| Males  | 13 (20)                            | 1 (5.6)               | 3.60 (0.5 – 25.7)      | 0.20    |
| Females| 9 (18.8)                           | 9 (18)                | 1.04 (0.5 – 2.4)       | 0.92    |
| Σ      | 22 (19.5)                          | 10 (14.7)             | 1.32 (0.6 – 2.6)       | 0.42    |

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 (voles, mice), but also insectivores and carnivores. Hosts that support virus circulation indirectly by enabling tick reproduction are different species of wild and domestic mammals (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 groups 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.

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REFERENCES

1. Chen S, Dumler JS, Bakken JS, Walker AR. Identification of a granulocytotropic Ehrlichia species as the etiological agent of human disease. J Clin Microbiol. 1994; 32: 589–595.
2. Dumler JS, Barbet AF, Bekker CPJ, Dasch GA, Palmer GH, Ray SC, Rickisya H, Runarwiga FR. Reorganization of genera in the families Rickettsiidae and Anaplasmataceae in order Rickettsiales: unification of some species of Ehrlichia with Anaplasma, Cowdria with Ehrlichia and Ehrlichia with Neorickettsia, descriptions of six new species combinations and designation of Ehrlichia equi and “HGE agent” as subjective synonyms of Ehrlichia phagocytophilum. Int J Syst Evol Microbiol. 2001; 51: 2145–2165.
3. Brouqui P, Dumler JS, Lienhard R, Brossard M, Raoult D. Human granulocytic ehrlichiosis in Europe. Lancet 1995; 346: 782–783.
4. Petrovec M, Lotrič-Furlan S, Zupanc TA, Strle F, Brouqui P, Roux V, Dumler JS. Human disease in Europe caused by a granulocytic Ehrlichia species. J. Clin. Microbiol. 1997; 35: 1556–1559.
5. Blanco IR, Oteo JA. Human granulocytic ehrlichiosis in Europe. Clin Microbiol Infect. 2002; 8: 763–772.
6. Hulinska D, Votypana J, Pich J, Vlcek E, Valesova M, Bojar M, Hulinsky V, Smetana K. Molecular and microscopic evidence of Ehrlichia spp. and Borrelia burgdorferi sensu lato in patients, animals and ticks in the Czech Republic. New Microbiol. 2002; 25: 437–448.
7. Sabatini A, Cammarata E, Frangione C, De Nardo S, Bissi A, Ciupian I, Tempera G, Del Piano M. Multicentric study of seroprevalence of Borrelia burgdorferi and Anaplasma phagocytophilum in high-risk groups in regions of central and southern Italy. Int J Immunopathol Pharmacol. 2004; 17: 219–225.
8. Grzeszczuk A. Anaplasma phagocytophilum in Ixodes ricinus ticks and human granulocytic anaplasmosis seroprevalence among forestry rangers in Białystok region. Adv Med Sci. 2006; 51: 283–286.
9. Ehrhard S, Koebel CH, Goehringer F, Socolovschi C, Jaulhac B, Raoult D, Brouqui P. Emergence of human granulocytic anaplasmosis in France.Ticks Ticks-borne Dis. 2012; 3: 402–404.
10. Chmielewska-Badora J, Zwołinski J, Clasak E, Wójcik-Fatla A, Buczek A, Durbiewicz J. Prevalence of Anaplasma phagocytophilum in Ixodes ricinus ticks determined by polymerase chain reaction with two pairs of primers detecting 16S rRNA and aκA genes. Ann Agric Environ Med. 2007; 14: 281–285.
11. Wójcik-Fatla A, Szymierska J, Wdowiak L, Buczek A, Durbiewicz J. Coincidence of three pathogens (Borrelia burgdorferi sensu lato, Anaplasma phagocytophilum and Babesia microti) in Ixodes ricinus ticks in the Lublin macroregion. Ann Agric Environ Med. 2009; 16: 151–158.
12. Nováková M, Vichova B, Malajtňova V, Lesňáková A, Pochybová M, Petko B. First case of human granulocytic anaplasmosis from Slovakia. Ann Agric Environ Med. 2010; 17: 129–133.
13. Carlon JA, Fikrig E. Invasion and survival strategies of Anaplasma phagocytophilum. Cell Microbiol. 2003; 5: 743–754.
14. Dumler JS, Madigan JE, Pusterla N, Bakken JS. Ehrlichioses in humans: epidemiology, clinical presentation, diagnosis, and treatment. Clin. Infect. Dis. 2007; 45: suppl I, 45–51.
15. Kruty E, Reahacek J, Vryvova V, Gurycova D. Infection of ticks with Borrelia burgdorferi and Francisella tularensis in Slovakia. Bratisl Lek Listy. 1990; 91: 251–256.
16. Kožuch O, Labuda M, Lysy J, Weismann P, Kreiner J. Longitudinal study of natural foci of Central European encephalitis virus in West Slovakia. Acta Virol. 1990; 34: 537–544.
17. Kožuch O, Labuda M, Lysy J, Weismann P, Krippel E. Longitudinal study of natural foci of Central European encephalitis virus in West Slovakia. Acta Virol. 1990; 34: 537–544.
18. Suss J. Tick-borne encephalitis 2010: Epidemiology, risk areas, and prevention in six Central and Eastern European countries: report from a meeting of experts convened to discuss TBE in their region. Vaccine 2011; 29: 4556–4564.