INCIDENCE AND PREVALENCE OF INFECTION WITH 
ANAPLASMA PHAGOCYTOPHILUM. PROSPECTIVE STUDY IN HEALTHY 
INDIVIDUALS EXPOSED TO TICKS

Anna Grzeszczuk, Beata Puzanowska, Henryka Mięgoć, Danuta Prokopowicz

Department of Infectious Diseases, Medical University of Białystok, Białystok, Poland


Abstract: The seroprevalence of human granulocytic anaplasmosis (former human granulocytic ehrlichiosis, HGE) has been documented in several studies, but little data exists on incidence rates in healthy individuals. In a prospective study, we tested 125 healthy adults (mean age 43 years) - workers of the Białowieża Primeval Forest National Park, north-eastern Poland - for Anaplasma phagocytophilum IgG antibodies using an indirect immunofluorescence antibody assay, and for Borrelia burgdorferi IgG with ELISA in a 12-month interval. The data concerning clinical symptoms consistent with human granulocytic anaplasmosis were collected using a standardized questionnaire. Of these 125 subjects, 9 were anti-A. phagocytophilum positive at the study entry. Four participants (3.2%) seroconverted from IgG negative to positive during the observation period. Three subjects (2.4%) converted from initially anti-A. phagocytophilum positive to negative. Specific IgG antibodies against Borrelia burgdorferi were detected in 27 (21.6%) individuals. Concordance of Borrelia burgdorferi and Anaplasma phagocytophilum was observed in 3.2%, whereas 4% were Anaplasma phagocytophilum IgG positive and Borrelia burgdorferi IgG negative (not significant). Clinical symptoms associated with human granulocytic anaplasmosis were not present in seroconverting individuals. The obtained results confirm the occurrence of Anaplasma phagocytophilum infection in north-eastern Poland with asymptomatic clinical course.

Address for correspondence: Anna Grzeszczuk, MD, Department of Infectious Diseases, Medical University of Białystok, ul. Żurawia 14, 15-540 Białystok, Poland.
E-mail: oliwa@amb.edu.pl

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Granulocytic anaplasmosis (former human granulocytic ehrlichiosis) - Anaplasma (A.) phagocytophilum infection - was for years a concern of veterinary medicine [8]. The first human patients were described in the US in 1994 [5]. Through 2002, a review of the CDC Morbidity and Mortality Weekly Reports documented 1,423 cases of human granulocytic anaplasmosis through US national surveillance. While in Europe only sporadic cases have been documented so far [4].

Recently, the first Polish cases were detected in north-eastern Poland [16] and the Białowieża Primeval Forest was described as a natural focus of A. phagocytophilum infection, with Ixodes ricinus ticks identified as a vector, and forestry employment as a risk factor of seropositivity [10].

The seroprevalence of human granulocytic anaplasmosis has been documented in several studies but little data exists on incidence rates [4, 11, 17, 18]. The aim of this study was to prospectively analyse the prevalence and incidence of A. phagocytophilum infection in a healthy population exposed to ticks. Clinical symptoms associated with A. phagocytophilum infection were also documented in this population.
MATERIALS AND METHODS

The serum sample pairs from 125 healthy Białowieża Primeval Forest workers (102 males and 23 females) taken in March 2002 (study entry) and March 2003 (end of the study) were examined. The mean age was 42.8 ± 5.7 years. All individuals were bitten by ticks and the reported number of bites ranged 1–30.

Samples were assayed for IgG against *A. phagocytophilum* and *Borrelia (B.)* burgdorferi. To evaluate the anti-*A. phagocytophilum* IgG status the indirect immunofluorescence antibody assay (HGE IFA IgG Test Kit, MRL Diagnostics, USA) was employed. This uses HL60 cells infected with the human isolate of *A. phagocytophilum* for detection and semi-quantification of specific antibodies. The serum screening dilution was 1:64, according to the instruction of the manufacturer. Titers ≥ 1:64 were considered positive. In order to examine the anti-*Borrelia burgdorferi* serological response, *Borrelia* recombinant IgG kit (Biomedica, Austria) was used, according to the producer’s instructions.

The participants were asked about clinical symptoms during the observation period, using a standardized questionnaire.

The chi² test was used for statistical analyses and Odds ratio and confidence intervals were calculated applying Statistica 5.0 PL software.

The study was accepted by the local ethical committee (R-T-003/164 /2003).

RESULTS

At the study entry, 9 of 125 (7.2%) individuals tested positive for *A. phagocytophilum* IgG. At follow-up, almost the same number reacted positive - 10/125 (8%). However, during the observation period of 12 months, 4 individuals (3.2%) seroconverted from initially negative to anti-*A. phagocytophilum* IgG positive. Simultaneously, we observed almost the same number of subjects - 3 of 125 (2.4%), who reconverted from positive at the study entry to negative after 12 months (Tab. 1, Fig. 1).

Clinical symptoms associated with *A. phagocytophilum* seropositivity were rare in all individuals (Tab. 2), including those seropositive and those in whom seroconversion was detected during the study observation.

Concerning the *B. burgdorferi* status, specific IgG antibodies were detected in 27 individuals (21.6%). Borderline results were found in a further 6 persons (4.8%), while 92 study subjects were negative (Tab. 1). The *A. phagocytophilum* and *B. burgdorferi* co-infection was detected in 4 individuals (3.2%) at the study entry. Individuals with positive Lyme borreliosis serology were not more likely to have anti-*A. phagocytophilum* antibodies than the seronegative ones (p > 0.05; OR = 3.03; CI = 0.71-12.18).

DISCUSSION

Specific tests to confirm the *A. phagocytophilum* diagnosis include microscopic detection of morulae in granulocytes, culture of bacteria, and polymerase chain reaction during acute phase of infection. Of these methods, culture appears to have the greatest sensitivity in the acute phase prior to antimicrobial treatment [2]. However, since *A. phagocytophilum* is an obligate intracellular microorganism, it restricts application of this method to centres well-equipped and experienced in

Table 1. Prevalence of IgG antibodies to *Anaplasma phagocytophilum* and *Borrelia burgdorferi* at the study entry.

<table>
<thead>
<tr>
<th></th>
<th>Borrelia burgdorferi IgG</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>A. phagocytophilum</em></td>
<td>positive</td>
<td>borderline</td>
</tr>
<tr>
<td>positive</td>
<td>4 (3.2%)</td>
<td>0</td>
</tr>
<tr>
<td>negative</td>
<td>23</td>
<td>6</td>
</tr>
<tr>
<td>Total</td>
<td>27</td>
<td>6</td>
</tr>
</tbody>
</table>

Figure 1. Dynamics of seroprevalence of anti-*Anaplasma phagocytophilum* IgG antibodies in 125 Białowieża Primeval Forest National Park workers.

Table 2. Symptoms reported during the study period among 125 Białowieża Primeval Forest National Park workers.

<table>
<thead>
<tr>
<th></th>
<th>Seroconversion</th>
<th>Reconversion</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Anaplasma phagocytophilum IgG (at entry)</strong></td>
<td><strong>Negative</strong></td>
<td><strong>Positive</strong></td>
</tr>
<tr>
<td><strong>Anaplasma phagocytophilum IgG (at the end)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n = 125</td>
<td>n = 4</td>
<td>n = 3</td>
</tr>
<tr>
<td>Arthralgia, n (%)</td>
<td>1 (25)</td>
<td>0</td>
</tr>
<tr>
<td>Erythema migrans, n (%)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Fever, n (%)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Headache, n (%)</td>
<td>0</td>
<td>1 (33)</td>
</tr>
<tr>
<td>Malaise, n (%)</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>
cellular culture. The series of serological studies in individuals with culture confirmed human granulocytic anaplasmosis revealed seroconversion in most cases [1, 13]. However, the titers of both IgG and IgM antibodies varied significantly between individuals tested [1, 13]. In some individuals, who had very high pick antibody titers, the high titers - ≥ 640 - suggestive of the recent infection, were still observed 6 months and 1 year after treatment of A. phagocytophilum infection [1].

The number of individuals seropositive for A. phagocytophilum IgG in our study (7.2% and 8%) is similar to the one obtained in earlier studies from the same region [9, 10] and is lower than those observed in other European studies. In south-western Germany (Saarlouis) Woessner et al. [18] detected 15% seropositivity among young soldiers, with a 6.4% annual incidence rate. Wittejsjö et al. in Sweden revealed an even higher anti-A. phagocytophilum seroprevalence among Åspö Island residents, reaching 28% [17].

We have observed the 3% seroconversion and 2.4% seroconversion rates in Białowieża Primeval Forest National Park workers, highly exposed to ticks, while in Sweden the seroconversion rate was as high as 11% over a single tick season [17].

The similar seroconversion and recrudescence rates found in our observation may reflect a steady state of infection, since the specific antibodies are not life-lasting but persist for months, at the longest for a few years after inoculation [1, 3].

The clinical symptoms consistent with acute human granulocytic anaplasmosis, such as fever, malaise, headache, muscle and join pain following tick bite were not reported by individuals with seroconversion. It is an interesting observation in accordance with the general phenomenon described in Europe. Despite similar seroprevalence in North America and in the ‘old world’, the number of acute cases of human granulocytic ehrlichiosis diagnosed in the former is about several hundred times higher. However, the asymptomatic serovaccinations were described in the highly endemic area of New York State [11].

Detected A. phagocytophilum seropositivity is significantly lower than the B. burgdorferi seropositivity among the same individuals, which is 21.6% (27/125). In other Polish studies, the seropositivity rate among forestry workers reaches as high as 60% [12]. Simultaneous infection of Ixodes ricinus ticks with A. phagocytophilum, Borrelia burgdorferi s.l. [6, 7, 14, 15] may cause co-infections in humans with a potentially more severe course.

In conclusion, our study reveals further evidence of Anaplasma phagocytophilum infection in north-eastern Poland, along with asymptomatic seroconversion, which may partially explain the very low number of acute cases of human granulocytic anaplasmosis confirmed in our country [14, 16].

REFERENCES


