

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

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**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. Concurrence 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.

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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  $42.8 \pm 5.7$  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  $\geq 1:64$  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 chi<sup>2</sup> 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).

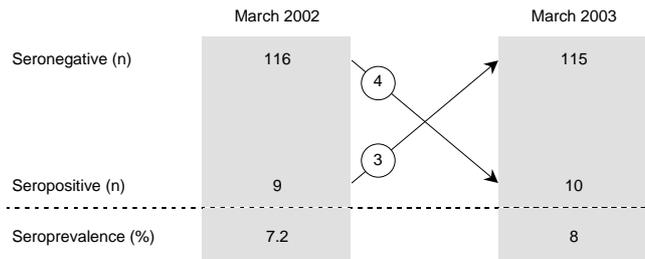
## RESULTS

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.

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

<i>A. phagocytophilum</i>	<i>Borrelia burgdorferi</i> IgG			Total
	positive	borderline	negative	
positive	4 (3.2%)	0	5 (4%)	9
negative	23	6	87	116
Total	27	6	92	125



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

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

	Seroconversion		Reconversion	
	Negative	Positive	Positive	Negative
<i>Anaplasma phagocytophilum</i> IgG (at entry)				
<i>Anaplasma phagocytophilum</i> IgG (at the end)				
Σ N = 125	n = 4	n = 3	n = 6	n = 112
Arthralgia, n (%)	1 (25)	0	1 (16.7)	2 (7.2)
Erythema migrans, n (%)	0	0	1 (16.7)	5 (4.5)
Fever, n (%)	0	0	1 (16.7)	4 (3.6)
Headache, n (%)	0	1 (33)	1 (16.7)	6 (5.4)
Malaise, n (%)	0	0	1 (16.7)	2 (1.8)

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 peak antibody titers, the high titers -  $\geq 640$  - 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. Wittesjö *et al.* in Sweden revealed an even higher anti-*A. phagocytophilum* seroprevalence among Aspö Island residents, reaching 28% [17].

We have observed the 3% seroconversion and 2.4% reconversion 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 reconversion 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 joint 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 seroconversions 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].

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