Anaplasma phagocytophilum in *Ixodes ricinus* ticks and human granulocytic anaplasmosis seroprevalence among forestry rangers in Białystok region

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Abstract

Purpose: Human granulocytic anaplasmosis, former ehrlichiosis, is a tick-borne zoonosis of increasing recognition.

The aim of the study was: 1) to assess the prevalence of *Anaplasma phagocytophilum* infection in *Ixodes ricinus* ticks collected in recreational forests in Białystok vicinity, the capital of podlaskie voivodship; 2) to evaluate the prevalence of IgG and IgM antibodies to *A. phagocytophilum* among forestry rangers from the same region.

Results: Of the 372 ticks examined, 54 (14.5%) yield the positive PCR reaction. The highest prevalence was detected in females, up to 27.8% (37/133), almost one third lower in males – 9.2% (13/142), followed by nymphs – 4.1% (4/97). Human seropositivity study revealed IgG antibodies against *A. phagocytophilum* in 9 out of 231 individuals (3.9%). No IgM antibodies were found. Sixty-seven individuals 67/231 (29%) reported erythema migrans. IgM anti-*Borrelia burgdorferi* antibodies were detected in 32 out of 121 (26.4%) persons tested, IgG – in 43 out of 231 (18.6%).

Conclusions: The data obtained show relatively low *A. phagocytophilum* seroreactivity among professionally exposed to tick group of forestry workers despite high *A. phagocytophilum* infection level in the competent vector – *I. ricinus* ticks.

Key words: *Anaplasma phagocytophilum*, *Ixodes ricinus*, ticks, antibodies, human granulocytic anaplasmosis, forestry rangers, Białystok region, Poland.

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Introduction

Anaplasma phagocytophilum is a unique bacterium infecting and multiplying successfully in granulocytes of broad range of hosts, including domestic and wild animals – canids, horses, sheep, cattle, European bisons and rodents, as well as humans. Infection may be subclinical or manifesting as a non specific febrile disease, called granulocytic anaplasmosis, tick fever or pasture fever in sheep [1-3]. *A. phagocytophilum* is transmitted by ticks from *Ixodes persulcatus* complex, which in Europe is mainly *I. ricinus* [1,4]. Ticks and tick-borne diseases are endemic in north-eastern Poland. This region has also the highest incidence of tick-borne encephalitis in Poland – 7.8/100 000 vs 0.46/100 000 for the whole country in 2005 and Lyme borreliosis (63.1 vs 11.5) [5]. Ecological and socio-economical changes in our region lead to increased ticks abundance and therefore augmented exposure of human population to tick transmitted pathogens.

Forestry workers constitute the professional group greatly exposed to ticks. The aim of the study was: 1) to assess the prevalence of *A. phagocytophilum* infection in *I. ricinus* ticks collected in recreational forests in Białystok vicinity, the capital of podlaskie voivodship; 2) to evaluate the prevalence of IgG and IgM antibodies to *A. phagocytophilum* among forestry rangers from the same region.

Material and methods

Ticks

Host seeking ticks were collected by flagging lower vegetation in different forested areas in Białystok vicinity – Pietrasze, Strzelnica, Dzikie and in Knyszyn Primeval Forest – Bobrowa, Korytne, Supraśl-Pólko, Królowy Most and in Biebrza National Reserve, *Tab. 1*. Collected ticks were individually evaluated prior to DNA extraction by a qualified entomologist with regard to species and gender according to Siuda [6]. The ticks were killed in hot water, placed in separate vials (adult) or pooled by

Table 1. Anaplasma phagocytophilum **infection rate in host seeking** *Ixodes ricinus* **ticks collected in different forested areas in north-eastern Poland**

Collection site	Year	No positive *I. ricinus*/No tested (% positive)				
		Female	Male	Total adults	Nymphs	Total
Białystok – Pietrasze	2003	6/36 (16.6)	4/49 (8.2)	10/85 (11.8)	-	10/85 (11.8)
Białystok – Strzelnica	2001	1/3 nc	0/4	1/7 nc	-	1/7 nc
Supraśl – Pólko	2006	8/28 (28.5)	5/24 (20.8)	13/52 (25.0)	1/31 (3.2)	14/83 (16.8)
Królowy Most	2006	12/32 (40.0)*	3/27(11.1)*	15/59 (25.4)#	1/23 (4.3)#	16/82 (19.5)
Bobrowa	2001	2/7 nc	0/4 nc	2/11 (18.2)	0/2 nc	2/13 (15.4)
Korytne	2001	5/21 (23.8)	1/28 (3.6)	6/49 (12.2)	2/24 (8.3)	8/73 (11.0)
Dzikie	2001	3/6 nc	0/5 nc	3/11 (27.3)	0/11	3/22 (13.6)
Biebrzański PN	2002	-	0/1 nc	0/1 nc	0/6 nc	0/7 nc
Total		37/133 (27.8)*	13/142 (9.2)*	50/275 (18.1)#	4/97 (4.1)#	54/372 (14.5)

nc – not calculated, No < 10; * – difference statistically significant between females and males; # – difference statistically significant between adults and nymphs

2-5 (nymphs) and fixed in 70% ethanol for further investigation by PCR for the presence of *A. phagocytophilum*.

Human study population

Study participants were recruited from the following forest inspectorates localized in Białystok vicinity: Dojlidy, Supraśl, Czarna Białostocka, Waliły in July, August 2004 or 2005. Serum samples were collected from 231 forestry workers, 40 females and 191 males, aged 49 ± 12 years. The questionnaire regarding age, length and character of employment in the forest, Lyme borreliosis and tick-borne encephalitis history, ticks exposure and actual complains such as fever, arthralgia, mialgia was filled by a physician.

DNA extraction

DNA was extracted by the ammonium hydroxide lysis (NH_4OH) according to Rijpkema et al. [7]. Lysates were stored in -20°C until examination.

Polymerase Chain Reaction

PCR was performed according to Pancholi et al. [8]. The primers EHR 521 (5'-TGT AGG CGG TTC GGT AAG TTA AAG-3') and EHR 747 (5'-GCA CTC ATC GTT TAC AGC GTG-3') were used to amplify the 16S rRNA (rrs) gene fragment specific for *A. phagocytophilum*. The tick lysates from positive reactions obtained in our previous investigation [9] served as a positive control and the double distilled water as a negative control in each PCR run. All PCR reactions were carried out in Perkin Elmer GeneAmp PCR System 9700 thermal cyclers. Amplification products were analyzed after electrophoresis in a 2% agarose gel stained with ethidium bromide. DNA bands of 247 base pairs (bp) were considered positive results. All positive samples were confirmed with nested PCR reaction amplifying rrs gene according to Massung [10].

Serological tests

Human granulocytic anaplasmosis (HGA) Anti-*A. phagocytophilum* IgM and IgG antibodies were detected by indirect immunofluorescence technique applying commercial kits with HL60 cells infected with the human isolate of *A. phagocytophilum* (Focus technologies HGE IFA IgG /IgM Test Kit, USA). The serum screening dilution was 1:64 according to the manufacturer and results ≥1:64 were considered positive.

Lyme borreliosis In order to examine the anti-*Borrelia burgdorferi* serological response Borrelia recombinant IgG and/ /or IgM kits (Biomedica, Austria) applying MiniBoss were used, according to the producer.

Statistics

Statistical analysis was performed with Pearson's χ^2 test and $p \leq 0.05$ was considered significant.

Results

Ticks

Of the 372 ticks examined, 54 (14.5%) yield the positive PCR reaction. The highest prevalence was detected in females, up to 27.8% (37/133), almost one third lower in males – 9.2% (13/142), followed by nymphs – 4.1% (4/97). The overall infection rate varied depending on the collection site; among adults from 27.3% in Dzikie to 11.8% in Białystok-Pietrasze; differences in nymphs were less pronounced – from 0% to 8.3%.

Human seropositivity

IgG antibodies against *A. phagocytophilum* were detected in 9 out of 231 individuals (3.9%), *Tab. 2. A. phagocytophilum* antibodies were detected in employee from 3 forest inspectorates – Supraśl 2/36 (5.5%), Dojlidy 3/39 (7.7%) and Czarna Białostocka 4/72 (5.6%). No seropositive individuals were found neither in Żednia 0/30 nor in Waliły 0/45 inspectorates. Such a low prevalence of specific antibodies might not allow finding any significant differences depending on sex, age, length and character of forestry employment – field or office work, Lyme borreliosis serological status and history (erythema migrans), and actually reported complains (fever, arthralgia, mialgia). Surprisingly, no significant differences were detected between individuals reporting tick bites in the last year – 6/147 (2.6%) and those denying it – 3/63 (1.3%). Thus regression analysis was

***Table 2*. Prevalence of IgG against *A. phagocytophilum* and IgG/IgM against *B. burgdorferi* among forestry workers in north-eastern Poland**

Anti-*Anaplasma phagocytophilum* IgG		*Borrelia burgdorferi*							
		Erythema migrans (No=231)		IgM (No=121)			IgG (No=231)		
		+	-	+	-	+/-	+	-	+/-
Positive	9	2	7	0	4	0	0	8	1
Negative	222	65	157	32	79	6	43	170	9
Total	231	67	164	32	83	6	43	178	10

not possible. IgM antibodies against *A. phagocytophilum* were not detected in any person evaluated.

Lyme borreliosis

Sixty-seven individuals 67/231 (29%) reported erythema migrans in their medical history. Twelve foresters had erythema migrans in the year of examination, but only 3 out of 10 persons tested had IgM antibodies to *B. burgdorferi*. IgM anti-*Borrelia burgdorferi* antibodies were detected in 32 out of 121 (26.4%) persons tested, IgG – in 43 out of 231 (18.6%), however, the results were not confirmed by Western Blot tests, *Tab. 2*.

Discussion

Our results show pretty high *A. phagocytophilum* infection rate in *I. ricinus* ticks collected in north-eastern Poland, reaching 18.1% average in adults and even 40% among females in certain locations (Królowy Most). The frequency of infection significantly raises from nymphal to adult stage of ticks. This observation points to the role of little rodents and small, and intermediate mammals, harboring *I. ricinus* larvae, in *A. phagocytophilum* circulation in nature since this bacterium is not transovarially transmitted [1,4]. *A. phagocytophilum* infection rate demonstrated in our study is similar to that found in mid-eastern Poland [11]. Slovac research conducted in suburban forest near Košice revealed 12.5% adult ticks infected with *A. phagocytophilum* [12]. Several other Polish studies showed the *A. phagocytophilum* infection rates ranging from 0% in Szczecin province (Głębokie Public Bath and Landscape Park in Ińsko) [13], 13.1% in Lublin province [11], 14% in Tricity Forest on the Baltic coast [14] and to 16% in Białowieża Primeval Forest [15].

Despite the high *A. phagocytophilum* infection rate in *I. ricinus* ticks, a very low presence of antibodies (3.9%) was revealed in the present study. Beginning the investigation among forestry workers and choosing the summer months, which followed the highest tick activity, higher prevalence and eventually acute granulocytic anaplasmosis cases detection was expected. Other Polish studies in forestry rangers demonstrated higher levels of seropositivity from 17.7% (20/113) to 20.0% (13/63) in mid-eastern Poland and 9.6% (46/478) in northern and north-eastern Poland [11,16,17]. Analogically to our results, very low *A. phagocytophilum* seropositivity – 1.5% was detected in English farmers [18], nevertheless south-western German data from Baden-Württemberg forestry workers show seroprevalence ranging from 5% to 16% in various counties [19]. Northern Italy investigation showed 8.8% (16/181) sera positive by IFA, although the authors considered only one of them – 0.6%, truly positive since confirmed by Western blot [20]. However, application of Western blot is not required for granulocytic anaplasmosis diagnosis according to ESCMID Study Group [21]. All three above studies demonstrated anti-*B. burgdorferi* seropositivity surpassing those of *A. phagocytophilum* from 3 to 20 times [11, 19,20], however, the anti-*B. burgdorferi* assays results should be interpreted with caution since they where not followed by Western blot, what is required for Lyme borreliosis diagnostics [21]. The reasons of such low levels of *A. phagocytophilum* seropositivity are unclear. One of the factors may be very variable year to year tick infection rate observed in our region during 4 years period [22]. Another factor, postulated by Massung and co-workers in USA, is presence of *A. phagocytophilum* variants non-pathogenic for humans [23], however, the latter hypothesis require closer characterization of strains postulated.

The results obtained show relatively high *A. phagocytophilum* infection rate in *I. ricinus* ticks and very low seropositivity among forestry rangers, a professional group highly exposed to ticks.

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