Clinical and molecular features of one case of human infection with *Anaplasma phagocytophilum* from Podlaskie Province in eastern Poland

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**Abstract**

The article focuses on the clinical and laboratory diagnosis of human granulocytic anaplasmosis (HGA) caused by *Anaplasma phagocytophilum* infection in one of 28 patients (3.6%; n=1/28 tested samples) with early Lyme borreliosis. The clinical and laboratory results of a 42-year-old patient fulfilled criteria of confirm anaplasmosis and suggest an acute stage of illness. The described case provides strong presumptive evidence that infection in this patient was acquired with a pathogenic strain of *A. phagocytophilum* through a tick bite. A positive DNA with PCR for *A. phagocytophilum* infection was sequenced and analyzed phylogenetically. Physicians should consider the possibility of anaplasmosis in patients with early Lyme borreliosis, and *A. phagocytophilum* should be considered as a differential diagnosis in all patients from an endemic region of potential high risk factors for tick-borne diseases.

**Key words**

*Anaplasma phagocytophilum*, anaplasmosis, coinfection, Lyme borreliosis, clinical and molecular diagnosis

**INTRODUCTION**

*Anaplasma phagocytophilum* is a gram-negative, obligate intracellular bacterium that has tropism for neutrophils and causes a tick-borne rickettsial disease, known as human granulocytic anaplasmosis (HGA), formerly human granulocytic ehrlichiosis [1]. HGA is an emerging zoonosis worldwide and recently recognized as an important and frequent tick-borne disease in USA and in many parts of Europe and Asia. The clinical symptoms of HGA range from asymptomatic seroconversion to mild, severe, or fatal disease [1]. In the north-east of Poland, an endemic region for tickborne encephalitis, Lyme borreliosis (LB), babesiosis, the epidemiology of HGA, especially in the inhabitants of rural areas with increased possibility of contact with the vector *Ixodes ricinus*, is still poorly recognized. The first three cases of acute human HGA in this part of Poland were identified by an indirect immunofluorescence assay and confirmed using PCR [2]. Although in Poland the seroprevalence of HGA in ‘high risk’ groups, mostly forestry workers, has been assessed in a few prospective studies [3, 4, 5]. Also, in a *Borrelia*- seropositive person from southeastern Poland, co-infection was shown with *A. phagocytophilum* and *Babesia* spp. [6]. However, in some countries of Europe, from relatively high seroprevalence of HGA (from 32% in Cyprus to 8.8% in Italy) to low (1.3 % in the Netherlands) the cases were revealed mostly in forestry workers [7, 8, 9]. In Europe, populations of *I. ricinus*, the main vector of *A. phagocytophilum* from the zoonotic reservoir, are infected to a variable extent, approximately from 4% – 67% [10]. Quite recently, the presence of a strain or genetic variants of *A. phagocytophilum* pathogenic for humans and animal zoonotic reservoirs has been revealed in *I. ricinus* ticks and roe deer populations in from Poland [11]. To-date, a few cases of infection with *A. phagocytophilum* through blood transfusion have been reported [12], as well as sporadic cases of nosocomial and perinatal transmission [13, 14].

The presented study investigates the clinical, molecular and phylogenetic features of *A. phagocytophilum* for inpatients and outpatients at the Department of Infectious Diseases and Neuroinfections at the Medical University of Białystok, with early LB and due to clinical findings suspected for HGA.

**MATERIALS AND METHOD**

Retrospectively, in 2011, blood samples were taken from 28 patients – 16 males and 12 females, mean age 45.2 years (ranges 18–75) during outpatient hospitalizations for tick-borne diseases (TBDs) at the Department of Infectious Diseases and Neuroinfections at the Medical University in Białystok, and examined for the presence of *A. phagocytophilum* specific DNA in co-infection with LB. The diagnosis of early LB was based on erythema migrans and other clinical judgments. Two ml of whole blood were collected into 0.001M EDTA and stored at -20°C until a retrospective analysis could be performed.

Molecular detection of *A. phagocytophilum*. DNA isolation and PCR analysis. The genomic DNA was extracted from
Phylogenetic characterization of A. phagocytophilum.
DNA sequence alignments and phylogenetic analysis were conducted using MEGA version 5.0. After testing the data for the best substitution model, phylogenetic trees were obtained using Maximum Likelihood as the tree construction method and Tamura-Nei model parameter algorithm as a distance method. For comparison, sequences of A. phagocytophilum strains obtained from GenBank (www.ncbi.nlm.nih.gov) were implemented in the sequence alignment. The stability of inferred phylogenies was assessed by bootstrap analysis of 1,000 randomly generated sample trees.

Nucleotide sequence accession numbers. New nucleotide sequences were deposited in GenBank with Accession No. KF11754 for 16S rRNA gene, and Accession No. KF015601 for groESL (a heat-shock protein) gene.

RESULTS
Case clinical history. Among 28 patients with early LB, one was infected with A. phagocytophilum. The patient, aged 42, was admitted to hospital because of malaise, general muscles pain and weakness 10 days after removing the tick from the right leg: bruising and erythema with a diameter of about 10 cm appeared at the site. Erythematous changes in the right lower leg with swollen, ankle was stated. The patient was limping because of the pain in the limb. Erythema is likely to respond with suspected LB. The married patient does not work because he is a pensioner, takes antidepressants (lerivon, anafranil), and smokes 1–2 packs of cigarettes a day.

Results of laboratory tests performed on admission: SR 2/6, ALAT 68 U/l, AspAT 74 U/ml, cholesterol 216 mg/dl, blood pressure 140/100, ECG: regular sinus rhythm 77/min. Morphology: WBC: 5.84, RBC: 4.72, Hgb15.6 g/dl, Hct 46.5%, MCV 98.5 fl, MCH 33.1pg, MCHC 33.5 g/dl, plt 170. Antibodies against LB were negative in both classes (IgM 3 BBU/ml, IgG 1 BBU/ml (Biomedica, Germany), ALAT 24 U/ml, AspAT-22 U/ml, cholesterol 235 mg/dl, CRP 1.8. The serological result for anaplasmosis in the IFA was negative with IgG antibodies titer less than 64.

Molecular feature and phylogenetic analysis of A. phagocytophilum. A. phagocytophilum DNA was detected in one blood sample (3.6%, n=1/28 tested samples) (Fig. 1). The 540 bp fragment of the 16S rRNA gene and the 1200 bp fragment of the groESL heat shock operon were further analyzed for one isolate. Sequencing analysis of the PCR products using both sets of primers showed 100% homology with A. phagocytophilum strains pathogenic for humans (16S rRNA:AF189153; A. phagocytophilum: AF033101) and animals (16S rRNA:EU839852, AF507941; groESL: EU381150, AF482760). Scrutiny of the phylogenetic tree, based on the partial groESL operon sequences, confirmed that the presented human isolate clustered with A. phagocytophilum pathogenic for human and domestic animals in Europe and in North America (Fig. 2). Thus, this variant is considered to represent zoonotic genotypes.

DISCUSSION
The causative agent of HGA known as A. phagocytophilum (previously described as A. phagocytophila) belongs to the order Rickettsiales, family Anaplasmataceae [10]. In Europe, HGA remains a rare disease, compared with the USA, and in infected persons who are symptomatic, illness onset occurs 5–21 days after a tick bite. Typical symptoms include...
with age [21]. Also, there is experimental data on the mice model that high blood cholesterol levels resulting from an interaction between dietary and genetic factors, facilitate *A. phagocytophilum* infection and up-regulate a proinflammatory chemokine and its receptor, which may contribute to HGA pathogenesis [22].

The described case provides strong presumptive evidence that infection in this patient was acquired with a pathogenic strain/variant of *A. phagocytophilum* through a tick bite, as proved by molecular and phylogenetic analyses. *A. phagocytophilum* strains have been characterized using a variety of genetic markers, including the genes encoding the small-subunit rRNA (16S rRNA), a heat-shock protein (groESL), the major surface proteins (msp) and the AnkA protein (ankA). Although the 16S rRNA and groESL genes demonstrate a minor degree of variation in their nucleotide sequences, several genetic variants have been detected in sequences derived from different mammalian hosts, ticks, and geographical regions [23]. In the current case, amplification of partial sequences from the 16S rRNA and groESL genes, DNA sequencing and phylogenetic analysis provided reliable evidence of anaplasmosis due to the human strain of *A. phagocytophilum*. It is well known that the zoonotic pathogen of *A. phagocytophilum* has a high degree of biological and clinical diversity; some of strains/variants are pathogenic in both humans and animals, while others only for animals [11, 24].

In the forested areas of eastern Poland, infections of *A. phagocytophilum* transmitted by the *I. ricinus* were previously reported in foresters with an approximate prevalence of 10% [2, 3, 25]. Patients who develop post-ticks bite non-specific fever, accompanied by acute thrombocytopenia and high level of cholesterol, should be evaluated by clinical examination and routine laboratory testing to determine if the illness is potentially a *A. phagocytophilum* infection, especially in co-infection with LB.

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REFERENCES


