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FIRST CASE OF HUMAN GRANULOCYTIC ANAPLASMOSIS FROM SLOVAKIA

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Abstract: In order to evaluate the potential risks of human granulocytic anaplasmosis in Slovakia, blood and serum samples of hunters and foresters from the northern parth of Slovakia were tested. We present the first case of HGA from Slovakia confirmed by nested PCR amplification of the *16S* rRNA gene fragment of *A. phagocytophilum*.

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INTRODUCTION

Human granulocytic anaplasmosis (HGA) is a tick-borne emerging disease caused by the obligate intracellular bacterium *Anaplasma phagocytophilum* transmitted by the hard tick *Ixodes ricinus*. In 2002 for the first time, *A. phagocytophilum* was detected in *I. ricinus* ticks from southwestern Slovakia [8]. Subsequently, the pathogen has been isolated from spleens of *Apodemus flavicollis* and *Clethrionomys glareolus* from the same part of the country [9]. The presence of *A. phagocytophilum* has been confirmed also in red deer (14.1%), roe deer (18.2%) [11] and European brown bear (24.3%) in central Slovakia [12]. Recently, a relatively high proportion of the population with serum antibodies against *A. phagocytophilum* has been detected in Slovakia [5], but the acute infection has not been described yet. Therefore, to evaluate the risk of HGA in Slovakia, we examined the risk groups of population – foresters and hunters. The presented case of granulocytic anaplasmosis can contribute for better defining the biological and public health significance of HGA in Europe.

CASE DESCRIPTION

On 30 January 2009, a 54-year-old man originating from the northern part of Slovakia was admitted to the Department of Infectious Diseases, Military Hospital, Ružomberok to take part in the study investigating tick-borne diseases in forestry rangers and hunters from this region. Information about risk factors such as residence, outdoor leisure activities, potential tick exposure, nonspecific clinical symptoms (fever, headache, myalgia, arthralgia) were collected via a questionnaire. At admission, the physical examination did not show any signs of disease; the patient complained of long-term arthralgias, myalgias and acute diarrhoea. He did not notice an elevated body temperature. In personal communication, the patient recalled the attachment of several ticks on his chest in August and September 2008, and skin lession at the site of the ticks bites. In November 2008, he succumbed to the overcame disease, suffering from flu-like symptoms with intense arthralgias, headaches, tiredness, restlessess, swelling and fever of about 37°C. The patient is a hunter, and had close contact with numerous game

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animals (deer, red deer, wild boars) over the past months. Nevertheless, he had not been outside this region during previous years.

At admission, laboratory tests revealed a peripheral blood leukocyte count of 6.2 × 10^9/l (normal range 3.5–10.8 × 10^9/l), and a platelet count of 290 × 10^9/l (normal range 130–400 × 10^9/l). The haemoglobin level, erythrocyte sedimentation rate and the level of liver enzymes were also within normal limits. The patient showed mild hyperglycemia and hypocholesterolemia. At presentation, his body temperature was 36.9°C.

A commercial extraction kit was used to isolate DNA from the patient's whole blood sample (NucleoSpin Blood Kit, Machery-Nagel, Germany). The presence of *Borrelia burgdorferi sensu lato* spirochetes was tested by PCR targeting flagellin gene [4]. For detection of *A. phagocytophilum*, a nested PCR amplification of *16S* rRNA gene fragment [6] was performed. Nuclease-free water was added instead of DNA as a negative control, and sequenced *A. phagocytophilum* DNA, isolated from an infected deer blood sample, was used as a positive control for PCR analysis. DNA amplicon was purified using PCR Clean-Up Kit (Sigma-Aldrich, Germany) and sequenced at the Department of Molecular Biology (Faculty of Natural Sciences Comenius University, Bratislava, Slovakia). Sequences were compared to the GenBank entries by Blast N2.2.13. All procedures, DNA isolation, PCR and electrophoresis were performed in separate rooms using different pipettes, racks, wearing separate laboratory coats, disposable gloves, and disposable sterile filter tips in each laboratory to prevent carry-over contamination and to avoid false positive results. Giemsa stained blood smear was performed. The serum sample was tested with an IFA for the presence of antibodies against *A. phagocytophilum* (Focus Technologies) with 1:20 (IgM) and 1:64 (IgG) dilution, as recommended by the manufacturer. The detection of anti-*Borrelia* anitibodies was performed using enzyme-linked immunosorbent assay (ELISA) [10].

The presence of *A. phagocytophilum* in the whole blood sample was confirmed based on PCR amplification of a 546-bp long fragment of 16S rRNA. Obtained sequence (GenBank accession No. GQ179652) share 99% similarity to *A. phagocytophilum* B3F isolate isolated from the blood of moufflon (*Ovis musimon*) in the Czech Republic, (EU839851) as well as *A. phagocytophilum* c3a isolate from the blood of red deer (*Cervus elaphus*) in the Czech Republic (EU839849), and showed 98% similarity with *A. phagocytophilum* HZ (CP000235). The IFA test for *A. phagocytophilum* specific IgM and IgG antibodies was negative. The presence of borreliae was not detected by PCR. In the first blood-taking serologic test for *Borrelia* specific IgM antibodies was borderline, while result for IgG antibodies remained negative. After three months, serological results were estimated as borderline for IgM and negative for IgG antibodies against *B. burgdorferi s.l.* again. Examination of blood smear revealed the identification of morulae in granulocytes.

DISCUSSION

According to a declaration of the WHO (2008), confirmation of HGA requires *A. phagocytophilum* isolation from blood, and/or identification of morulae in granulocytes, and/or positive PCR results with subsequent sequencing of the amplicons to demonstrate specific anaplasmal DNA. Seroconversion, or at least a fourfold increase in antibody titers to *A. phagocytophilum*, was also used as criteria for confirmed HGA [1].

Our case meets the criteria of a confirmed case of human granulocytic anaplasmosis based on positive PCR results with subsequent sequencing of the amplicons and identification of morulae in granulocytes. PCR used in the study was determined as assay with the highest analytical sensitivity and specificity [7]. As in a reported case of HGA from Sicily [2], our patient demonstrated HGA without detectable antibodies against the pathogen for up to three months after detection of the infection. Accordingly, HGA should be considered in patients with erythema migrans and atypical changes for lyme borreliosis, such as fever, even without laboratory abnormalities, especially in hunters, forestry rangers or blood donors.

The clinical manifestations of HGE include fever, headaches, myalgia, arthralgia, diarrhoea, and in half of the patients laboratory abnormalities like leucocytopenia or thrombocytopenia occur. The disease can vary from subclinical or mild to severe, even fatal [3]. In spite of fulfillment of criteria for confirmed HGA, our patient had a relatively mild illness with uneventful recovery. However, further studies are required to evaluate the risk of human granulocytic anaplasmosis for people in Slovakia.

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