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SHORT COMMUNICATION

A case report of *Rickettsia*-like infection in a human patient from Slovakia

Dana Zubriková [1] · Ivana Heglasová [1,2] · Daniela Antolová [1] · Lucia Blaňarová [1] · Bronislava Víchová [1]

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Abstract

Herein we report a case of a 29-year-old man, suffering from a disease of unknown etiology and indicated a history of a bite by the unknown flying insect to both ear lobes in his anamnesis. The patient developed several nonspecific symptoms, such as fever, tension headache, and bilateral swelling of cervical lymph nodes (lymphadenitis). Moreover, he described perspiration, shivering, chills, epistaxis, several collapses, myalgia, and bone pains. Successive medical examinations of the patient confirmed several diagnoses. In addition, a blood sample was examined for the presence of tick-borne pathogens. PCR assay targeting gltA gene fragment of *Rickettsia* spp. brought a positive result. DNA of *Rickettsia*–like organism was confirmed by PCR and further sequencing. The rickettsial infection was successfully treated with doxycycline with a significant improvement of his health. This study brings a report of the molecular evidence of *Rickettsia*-like infection in a human patient from Slovakia, who suffered from several health problems accompanied by nonspecific febrile clinical symptoms. Although the patient did not report the tick bite before the development of clinical symptoms, he reported that he was bitten by an unknown flying insect. Thus, the possibility of rickettsial infection should be considered in similar nonspecific febrile illnesses when the bite of a flying insect is reported in personal anamnesis.

Keywords Nonspecific febrile illness · Lymphadenitis · *Rickettsia* · Human infection · Insect · Slovakia

Introduction

Rickettsiae are obligate intracellular Gram-negative bacteria from the order Rickettsiales with a worldwide distribution. Many species are responsible for serious diseases in humans and other mammals (Raoult and Roux 1997; Parola et al. 2013). However, the pathogenicity of rickettsiae varies widely between species (Abdad et al. 2011).

Bacteria are transmitted via the bites of arthropod vectors such as ticks, mites, fleas, and lice or through infectious fluids (such as feces) inoculated into the skin (Raoult and Roux 1997; Weinert et al. 2009; Miťková et al. 2015). Recent studies have shown that the genus *Rickettsia* could be divided into four different phylogenetic groups: the spotted fever group (consisting of *R. helvetica*, *R. slovaca*, *R. raoultii* and/or *R. monacensis*), the typhus group (*R. typhi*, *R. prowazekii*), ancestral group (*R. bellii* and *R. canadensis*) and transitional group (*R. akari* and *R. felis*) (Murray et al. 2016). Nowadays, novel *Rickettsia* species of undetermined pathogenicity continue to be detected from arthropod vectors around the world (Parola et al. 2013).

In Slovakia, several pathogenic species of rickettsiae have so far been confirmed (*R. slovaca*, *R. raoultii*, *R. monacensis* strains IRS3 and IRS4, *R. helvetica*, *R. africae* and *R. felis*) in *Ixodes ricinus* (Linnaeus, 1758) and *Dermacentor* spp. ticks (Boldiš et al. 2008; Sekeyová et al. 1998, 2012a, b, 2013; Heglasová et al. 2018; Špitalská et al. 2008, 2015). In Slovakia, *R. africae*, usually transmitted by *Amblyomma*

✉ Bronislava Víchová
vichova@saske.sk

Dana Zubriková
cernanska@saske.sk

Ivana Heglasová
heglasova@saske.sk

Daniela Antolová
antolova@saske.sk

Lucia Blaňarová
blanarova@saske.sk

[1] Institute of Parasitology of the Slovak Academy of Sciences, Hlinkova 3, 040 01 Košice, Slovak Republic

[2] Department of Zoology, Faculty of Natural Sciences, Comenius University, Mlynská dolina B- 1, 842 15 Bratislava, Slovakia

spp., *Hyalomma* spp., and/or *Rhipicephalus* spp. ticks, was identified in *Ceratophyllus garei* Rothschild, 1902 flea collected from reed warblers (*Acrocephalus scirpaceus* Hermann, 1804) migrated from Africa (Sekeyová et al. 2012c). Recently the bacterial microbiome of field-collected *Dermacentor marginatus* (Sulzer, 1776) and *D. reticulatus* (Fabricius, 1794) ticks from Slovakia was analyzed and *Rickettsia* spp. showed high abundance in both tick species (Zhang et al. 2019). In humans, *R. helvetica*, *R. slovaca*, *R. raoultii* and *R. conorii* have been identified by serological examination and/or molecular methods (Řeháček et al. 1975; Kováčová et al. 2006; Sekeyová et al. 2012a, b).

In this study, a rare case of human rickettsiosis caused by a *Rickettsia*-like organism is presented.

Case presentation

A 29-year-old man indicated in his anamnesis, that he was bitten to both ear lobes by an unknown flying insect during outdoor activities in December 2014. At the time of the reported insect bites, he was working outside near the sewage treatment plant near Spišska Nová Ves town (Eastern Slovakia). The patient did not report any travel or tick bite history within the period when his health problems started. However, he lives in a village house with his family and breeds a dog, a parrot, and a hamster. He admitted that in the past, he was removing ticks from the dog as well as from himself.

After indicated bites, both his ear lobes started to suppurate, and approximately one week after he developed symptoms such as fever, severe tense headache, and bilateral swelling of cervical lymph nodes (lymphadenitis), where his cervical lymph nodes reached 5 cm in diameter. He suffered from weakness and weight loss and described also repeated epistaxis, perspiration, shivering, chills, myalgia, and bone pains.

In February 2015, the patient was hospitalized for one week at the Department of Neurology since he suffered from severe headaches and pre-collapse states. At the time of the hospitalization, his lymph nodes were only moderately swollen, not painful and the patient was afebrile. The blood laboratory analysis showed a lower number of neutrophils (NEU 44.2 %; reference range 46–73 %), an increased number of lymphocytes (LYM 48.9 %; reference range 18–44 %), and a higher mean platelet volume (MPV 13.5 fL; reference range 7.5–12 fL). In urine, the presence of white blood cells (26.7/µl), squamous epithelial cells (16.7/µl), and mucus were recorded. All other blood and urine parameters were within the reference range.

Blood sera samples were taken and sent for serological examination of the presence of anti-*Borrelia burgdorferi*, anti-*Toxocara*, and anti-*Toxoplasma gondii* IgM and IgG antibodies, with negative results. However, sera tested positive for the presence of IgG against EBV (Epstein-Barr virus) and CMV (cytomegalovirus). Some of the clinical signs (e.g. fatigue, fever, head and body aches, swollen lymph nodes, and swollen liver) which were present, are specific also for the acute and/or chronic infectious mononucleosis caused by the EBV. Due to abdominal pain, a stool sample was taken for the parasitological examination, resulting in the exclusion of protozoal and/or helminthic infection. The patient was discharged from the hospital with several diagnoses, namely tension cefalea, tetanic syndrome, chronic fatigue syndrome, nodal syndrome of unknown origin, and suspected closely unspecified non-Hodgkin's lymphoma. After discharge from the hospital, the patient was on sick leave at home.

In May 2015, the patient continuously complained of persistent fatigue, malaise, headache, and repeated lymphadenopathy. The blood sample was sent to the Institute of Parasitology SAS, where nested PCR targeting the *gltA* gene of *Rickettsia* spp. (Regnery et al. 1991; Choi et al. 2005), and PCRs targeting the *msp2* gene of *Anaplasma phagocytophilum* (Massung and Slater 2003) and *18 S* rRNA gene of *Babesia* spp. (Casati et al. 2006) were performed. Genomic DNA extracted from the blood sample tested negative for the presence of *A. phagocytophilum* and *Babesia* spp. Nested PCR assay targeting *gltA* gene of *Rickettsia* spp. gave positive results, with a 337 bp long fragment of the gene amplified. The nucleotide sequence of the *gltA* gene of *Rickettsia* sp. (MN722421) obtained in this study was 99.37 % identical to several *Rickettsia* species, namely e.g. *R. aeschlimannii* (MK732478), 15 isolates of *Candidatus* Rickettsia barbariae (e.g. MH675633, MF002503, DQ423369, KY233232, JF803910), *R. sibirica* (MF098405) and *R. raoultii* (KR131756). According to the acquired sequence of the *gltA* gene, it was not possible to determine the causative agent of the infection at the species level. We present the case as a *Rickettsia*-like infection.

Subsequently, the patient underwent treatment with doxycycline (200 mg/day) for 10 days. After the therapy, malaise and fatigue passed off and the clinical status of the patient improved significantly. Second and third blood samples, taken 10 days and 30 days after the treatment, respectively tested negative for *Rickettsia* spp.

Discussion

Early diagnosis of rickettsial diseases is difficult because early signs and symptoms are usually nonspecific or mimic benign viral illnesses (Huntzinger 2007). Moreover, for the classification of rickettsial isolates, multiple gene sequencing is recommended (Fournier et al. 2003). As in the present study, the obtained nucleotide sequence was identical to several *Rickettsia* species, it was impossible to determine the causative agent of infection on the species level. Similarly, Thu

et al. (2019) detected 15 *gltA* genotypes of *Rickettsia* spp. in a nationwide study in Japan, however, sequencing of additional five genes of each *gltA* genotype (*16 S* rRNA, *ompA*, *ompB*, *htrA*, and *sac4* genes) did not allow to assign nine *gltA* genotypes into the species due to lack of consensus between the trees and/or absence of sequences from previously validated rickettsial species in the same phylogenetic clusters.

The patient in the present study negated the tick bite but indicated the bite of an unknown flying insect to both ear lobes in December 2014 with subsequent development of health problems. Attempts to specify the flying insect more closely, based on the discussion with the patient, were not successful. Members of the genus *Rickettsia* are very variable and except ticks, they were amplified from a huge variety of host organisms (mites, lice, fleas, beetles, flies, true bugs, leeches, and amoebae) (Perlman et al. 2006). Despite the link between the bite of an unknown flying insect and the onset of rickettsiosis, in this case, might be speculative (due to the winter season), the personal anamnesis of the patient could not be ignored during the investigation, and this possibility thus could not be excluded. At the same time, since the patient lives in the village, spends time outdoor, and breeds a dog, the possibility that he was bitten also by ticks, fleas, or mites during the early spring should not be excluded as well. It happens quite often that persons who are in frequent contact with animals or have pets with a history of tick bites are at increased risk. In addition, patients may not report a specific personal history of tick bites because most do not realize they have been bitten and bite marks may be difficult to detect or distinguish from other bites (e.g., spider or chigger bites) (Huntzinger 2007).

In 2014, the National Reference Centre for Rickettsioses in Slovakia was established to support the surveillance of rickettsioses in the country. From 2016 to 2018, two cases of "unspecified spotted fever" assigned as A77.9 diagnosis in the International Diseases Classification, ten cases of diagnosis "other specified rickettsioses" (A79.8), and one case of "unspecified rickettsiosis" (A79.9) were recorded. The diagnoses were based on anamnesis of patients, reported symptoms, and results of ELISA tests. However, since people often do not exhibit symptoms specific for rickettsiosis and the diagnosis is complicated, delayed, and/or frequently neglected by doctors, the real number of human rickettsioses in Slovakia might probably be higher (Sekeyová et al. 2012a, b, c).

In conclusion, this study reports a molecularly confirmed human rickettsiosis, caused by *Rickettsia*-like organism in a human patient with several associated health problems. Considering that the patient did not report the tick bite before the development of clinical symptoms, the possibility of rickettsial infection should be considered also when the symptoms that might be typical for the diseases appear after the bite of a flying insect (e.g. louse/hippoboscid flies, stable flies, horse flies, and/or several mosquito species – *Anopheles* spp., *Aedes* spp., *Uranotaenia sapphirina* (Osten Sacken, 1868)) (Hornok et al. 2011; Baldacchino et al. 2013; Barua et al. 2020).

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Declarations

Ethical standards The patient agreed with all examinations and publication of case report and signed the informed consent. No identifying data are presented in the paper. Study was performed in accordance with the ethical standards as laid down in the Declaration of Helsinki of 1975 and revised in 2008.

Conflict of interest Authors declare that they have no competing interests.

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