

# A Case of Human Monocytic Ehrlichiosis in Serbia

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## SUMMARY

**Introduction** Ehrlichiosis is a bacterial zoonosis transmitted by hematophagous arthropods – ticks. In humans, it occurs as monocytic, granulocytic, and *ewingii ehrlichiosis*. Pathological process is based on parasitic presence of Ehrlichia organisms within peripheral blood cells – monocytes and granulocytes.

**Case Outline** Fifty-two year old patient was admitted to hospital due to high fever of over 40°C that lasted two days, accompanied with chills, muscle aches, malaise, loss of appetite, headache, confusion, breathing difficulties, and mild dry cough. The history suggested tick bite that occurred seven days before the onset of disease. Doxycycline was introduced and administered for 14 days, causing the disease to subside. Indirect immunofluorescence assay was used to analyze three serum samples obtained from this patient for *Ehrlichia chaffeensis* antibodies, and peripheral blood smear was evaluated for the presence of Ehrlichia and Ehrlichia aggregation into morulae.

**Conclusion** Ehrlichiosis should be considered in each case where there is a history of tick bite together with the clinical picture (high fever, chills, muscle aches, headache, generalized weakness and malaise, and possible maculopapular rash). The presence of *Ehrlichia chaffeensis* antibodies was confirmed in a patient with the history of tick bite, appropriate clinical picture and indirect immunofluorescence assay. This confirmed the presence of human monocytotropic ehrlichiosis, a disease that is uncommonly identified in our country.

**Keywords:** *Ehrlichia chaffeensis*; human ehrlichiosis; fluorescent antibody technique; indirect; doxycycline

## INTRODUCTION

Ehrlichiosis, as human infectious disease, was described in 1986, although Ehrlichia has been studied as a cause of disease in animals until 1968, when *Ehrlichia canis* was identified as the cause of disease of a large number of military dogs in Vietnam. The similarity between *E. canis* and *E. sennetsu* (previously known as *Rickettsia sennetsu*), causing fever in humans in Japan, was then observed. Recent findings have suggested that *Ehrlichiae*, together with *Rickettsiae*, form a subgroup of Proteobacteria [1]. Bacteria from the Ehrlichia genus were classified to the Anaplasmataceae family [2]. Based on their genetic characteristics, Ehrlichia organisms were divided into 3 genogroups. Genogroup I includes *E. chaffeensis* (cause of human monocytotropic ehrlichiosis – HME), *E. canis*, and *E. ewingii*. Genogroup II (Anaplasma group) includes *Anaplasma phagocytophilum* – human granulocytic ehrlichiosis – HGE, and *E. equi*. Genogroup III (called *Neorickettsiae*) includes *E. sennetsu* and *E. risticii* [1].

The first case of human ehrlichiosis, similar to *E. canis*, was described in the USA in late 1980s [3]. Patients with specific antibodies to *E. chaffeensis* were later described in other continents and countries: Israel [4], Thailand [5], and Argentina [6]. *E. chaffeensis* was isolated from the tick *Ixodes ricinus* in Germany [7]

and Bulgaria [8]. The human infections caused by *E. chaffeensis* were confirmed by numerous seroepidemiological studies in Italy [9], Czech Republic [10], Portugal [11], Slovenia [12], and Croatia [13], while there has been one case reported in Serbia [14]. Retrospective studies showed that Ehrlichiae, as causes of infectious diseases, had been present in human population even before 1980s [15].

The aim of this paper was to present clinical characteristics of the serologically confirmed case of ehrlichiosis in a patient who had previously been bitten by a tick.

## CASE REPORT

Fifty-two year old patient was admitted to Department for Infectious Diseases of General Hospital in Subotica due to high fever of over 40°C that lasted for two days and was accompanied by chills, muscle aches, malaise, loss of appetite, headache, confusion, breathing difficulties, and mild dry cough. Seven days before the onset of disease, the patient noticed a tick in his scrotal region and removed it himself. Before admission to hospital, the patient took antipyretics only. On admission, he had high fever, was adynamic to prostration, and confused. The remaining physical findings appeared normal, as well as local findings at the site of tick bite.

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Laboratory findings immediately upon admission were the following: WBC  $2.9 \times 10^9/L$ , platelets  $72 \times 10^9/L$ , RBC  $4.44 \times 10^{12}/L$ , hemoglobin 129 g/L, AST 82 U/L (normal level: up to 37), ALT 55 U/L (normal level: up to 42), CK 1890 U/L (normal level: up to 195), and CRP 29.2 mg/L. Urine examination showed normal results. Blood and urine cultures were negative to *Mycobacterium tuberculosis*. Serologic reactions to *Rickettsia prowazekii*, *Coxiella burnetii*, *Brucella abortus*, *Borrelia burgdorferi*, as well as TPHA were negative. Furthermore, serologic reactions to EBV, CMV and HSV were negative. RF and ANA were also negative. Lung X-ray, and the abdominal and urinary tract ultrasound suggested normal findings. Peripheral blood smear showed morulae in small number of monocytes (less than 10%) staining darker than the rest of the monocytes.

On the second day of hospital stay, doxycycline was introduced at a dose of  $2 \times 100$  mg orally, and on the following day, the patient's fever subsided. Treatment lasted 14 days, after which the patient had no subjective complaints and the physical findings were normal.

Laboratory findings at discharge: WBC  $7.5 \times 10^9/L$ , platelets  $220 \times 10^9/L$ , RBC  $4.1 \times 10^{12}/L$ , hemoglobin 120 g/L, AST 32 U/L, ALT 34 U/L, CK 155 U/L, CRP 15.2 mg/L. During the following six months, the patient used to come for regular controls and he had no subjective complaints, while his physical and laboratory findings remained normal.

At the Department for Immunology of the Military Medical Academy, indirect immunofluorescence assay was used to analyze three serum samples from this patient for *E. chaffeensis* antibodies, and peripheral blood smear was evaluated for the presence of Ehrlichia and Ehrlichia aggregation into morulae. Serum samples were taken on days 7 (July 24, 2006), 14 (July 31) and 50 (September 5) of the illness, and the titers of IF antibodies were < 1:32, 1:64, and 1:128, respectively. The peripheral blood smear was dated July 19, 2006.

### Indirect Immunofluorescence Assay (IFA)

The antigen for identification of *E. chaffeensis* antibodies (Lot no. 02-0266N) was obtained as a suspension of *E. chaffeensis* replicated in canine monocytes. The suspension was spread over 8-field microscopy slides, dried at room temperature for at least three hours, and fixed with cold acetone (Merck pro analysi) for 10 minutes at  $-18^\circ\text{C}$ . The slides were then dried 10 minutes at room temperature, and immediately used as an antigen, or stored for later use at  $-20^\circ\text{C}$ .

The immune control serum was human serum obtained from convalescents infected with *E. chaffeensis* with titers of 128 or higher. The control negative serum was human serum negative to *E. chaffeensis* antibodies at a dilution of 1:16.

### The IFA reaction

After brief rehydration, patient's sera were applied to prepared antigen in series of PBS dilutions ranging from

1:16 to 1:256. After 30 minutes of humidity chamber incubation at  $37^\circ\text{C}$  and PBS wash-out, in order to remove antibodies not bound to antigen, a mixture of fluorescein labeled goat antihuman IgG (INEP, Belgrade) was added, as well as Evans blue for better contrast when reading the reaction. After 30 minutes of humidity chamber incubation at  $37^\circ\text{C}$ , slides were washed out, dried, and after adding glycerin, the IFA reaction was studied using Leitz fluorescent microscope (at  $400\times$  magnification). The last serum dilution showing observable immunofluorescence in monocyte cytoplasm with good positive and negative control was considered to be the titer of *E. chaffeensis* antibodies.

### DISCUSSION

The disease of the humans known as humane monocytic or monocytotropic ehrlichiosis is caused by *Ehrlichia chaffeensis*. It dwells and replicates in the cytoplasm of certain host cell types, mainly neutrophil leukocytes, leukocytes and monocytes, within vacuoles that are bound to cellular membrane. The name of the disease HME suggests the tropism of the organism toward peripheral blood monocytes [13]. Until now, only one report on HME in Serbia has been published. Dokić et al. [16] presented the case of HME in a patient admitted to Military Medical Academy.

The HME is a complex zoonosis. The causing organism persists in the nature through the infection of various mammals. Dogs, wolves and deer are considered to be the primary reservoirs of *Ehrlichia chaffeensis*. Fox and goats have been experimentally infected as well. The primary vector is a tick *Amblyomma americanum*. *Ehrlichia chaffeensis* may also be transmitted by a tick *Dermacentor variabilis* [17]. Most ehrlichiosis cases have history of tick bites (as in our patient), although in 32% of cases this information may be missing [18]. Therefore, the absence of definite tick attachment should never dissuade physician from considering the diagnosis of HME.

There is no specific clinical sign that would help in distinguishing this condition. In both HME and HGE, the symptoms may vary in their intensity, ranging from mild disease to life-threatening conditions. The most common symptoms are elevated body temperature (>90%), headache (>85%), fever, muscle aches (>80%), nausea (40%), vomiting (40%), loss of appetite (40%), overall weakness and malaise (>80%), and confusion (20%). The diffuse skin changes are uncommon (10%), and if present, they are manifested as maculopapular rash without any predilection. The central nervous system infection is present in 20% of patients with HME and may manifest as aseptic meningitis with lymphocytic pleocytosis and elevated protein level in cerebrospinal fluid, cranial nerve neuropathy or encephalopathy [19, 20]. Because the signs and symptoms are nonspecific, the clinicians must frequently incorporate clues from the clinical and epidemiologic history and consider other features. Most of these symptoms and signs were present in our patient, and the history of tick bite made our diagnosis of HME easier.

The etiological diagnosis of human ehrlichiosis is based on serological diagnostics or identification of causative microorganism in the elements of peripheral blood. In the peripheral blood smear, morulae are found in granulocytes (25-80%) and in monocytes (less than 10%). Morulae are cytoplasmic vacuoles in which Ehrlichia replicates. The most commonly used staining method is Wright's stain. The serological reaction that is used for diagnosis is indirect immunofluorescence (IF) assay [21]. According to the Center of Disease Control (CDC), the first sample should be taken as early as possible, preferably in the first week of symptoms, and the second sample should be taken 2 to 4 weeks later [22]. The IF assay is considered positive if there is an increase or decrease in titer of antibodies 4 times or more during the disease, with minimum titer of 1:80. In our patient, the IF assay was in accordance with these data, and the expected higher levels of antibody titer were not obtained, most probably due to early antibiotic treatment (5<sup>th</sup> day of the disease).

Doxycycline is a treatment of choice for any type of human ehrlichiosis. It is very efficient; it stops disease progression and leads to recovery [23]. An optimal duration of therapy has not been established; current recommendations for HME treatment are at least 3 days after the fever

subsides and until the clinical improvement is evident, which typically accounts for a minimal total course of 5-7 days [22]. After treatment for 14 days with doxycycline, our patient had no subjective complaints, while his physical and laboratory findings were normal.

The human ehrlichiosis is a newly identified disease that is still being rarely diagnosed. Ehrlichiosis should be considered in each case where there is a history of tick bite together with clinical picture (high fever, chills, muscle aches, headache, generalized weakness and malaise, and possible maculopapular rash). The diagnosis is made with high certainty using serological assays, and the disease is effectively treated with doxycycline.

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## REFERENCES

- Paddock CD, Childs JE. Ehrlichia chaffeensis: a prototypical emerging pathogen. Clin Microbiol Rev. 2003; 16(1):37-64.
- Dumler JS. Anaplasma and Ehrlichia infection. Ann NY Acad Sci. 2005; 1063:361-73.
- Fishbein DB, Sawyer LA, Holland CJ, Hayes EB, Okoroanyanwu W, Williams D, et al. Unexplained febrile illnesses after exposure to ticks. Infection with an Ehrlichia? JAMA. 1987; 257(22):3100-4.
- Keysary A, Amram L, Keren G, Sthoeger Z, Potasman I, Jacob A, et al. Serologic evidence of human monocytic and granulocytic ehrlichiosis in Israel. Emerg Infect Dis. 1999; 5(6):775-8.
- Heppner DG, Wongsrichanalai C, Walsh DS, McDaniel P, Eamsila C, Hanson B, et al. Human ehrlichiosis in Thailand. Lancet. 1997; 350(9080):785-6.
- Ripoll CM, Remondegui CE, Ordóñez G, Arazamendi R, Fusaro H, Hyman MJ, et al. Evidence of rickettsial spotted fever and ehrlichia infections in a subtropical territory of Jujuy, Argentina. Am J Trop Med Hyg. 1999; 61(2):350-4.
- Baumgarten BU, Röllinghoff M, Bogdan C. Prevalence of Borrelia burgdorferi and granulocytic and monocytic ehrlichiae in ixodes ricinus ticks from southern Germany. J Clin Microbiol. 1999; 37(11):3448-51.
- Christova I, Van De Pol J, Yazar S, Velo E, Schouls L. Identification of Borrelia burgdorferi sensu lato, Anaplasma and Ehrlichia species, and spotted fever group Rickettsiae in ticks from Southeastern Europe. Eur J Clin Microbiol Infect Dis. 2003; 22(9):535-42.
- Nuti M, Serafini DA, Bassetti D, Ghionni A, Russino F, Rombola P, et al. Ehrlichia infection in Italy. Emerg Infect Dis. 1998; 4(4):663-5.
- Hulinska D, Kurzova D, Drevova H, Votypka J. First detection of Ehrlichiosis detected serologically and with the polymerase chain reaction in patients with borreliosis in the Czech Republic. Cas Lek Cesk. 2001; 140(6):181-4.
- Morais JD, Dawson JE, Greene C, Filipe AR, Galhardas LC, Bacellar F. First European case of ehrlichiosis. Lancet. 1991; 338(8767):633-4.
- Arnez M, Luznik-Bufon T, Avsic-Zupanc T, Ruzic-Sabljić E, Petrovec M, Lotrič-Furlan S, et al. Causes of febrile illnesses after a tick bite in Slovenian children. Pediatr Infect Dis J. 2003; 22(12):1078-83.
- Topolovec J, Puntarić D, Antolović-Pozgain A, Vuković D, Topolovec Z, Milas J, et al. Serologically detected "new" tick-borne zoonoses in eastern Croatia. Croat Med J. 2003; 44(5):626-9.
- Samardžić S, Marinković T, Marinković D, Djuričić B, Ristanović E, Simović T, et al. Prevalence of antibodies to Rickettsiae in different regions of Serbia. Vector Borne Zoonotic Dis. 2008; 8(2):219-24.
- Dawson JE, Fishbein DB, Eng TR, Redus MA, Green NR. Diagnosis of human ehrlichiosis with the indirect fluorescent antibody test: kinetics and specificity. J Infect Dis. 1990; 162(1):91-5.
- Djokić M, Čurčić P, Nožić D, Lako B, Begović V, Rajić-Dimitrijević R, et al. Human ehrlichiosis. Vojnosanit Pregl. 2006; 63(4):403-8.
- Skotarczak B. Canine ehrlichiosis. Ann Agric Environ Med. 2003; 10(2):137-41.
- Fishbein DB, Dawson JE, Robinson LE. Human ehrlichiosis in the United States, 1985-1990. Ann Intern Med. 1994; 120(9):736-43.
- Lotrič-Furlan S, Avšič-Zupanc T, Petrovec M, Nicholson WL, Sumner JW, Childs JE, et al. Clinical and serological follow-up of patients with human granulocytic ehrlichiosis in Slovenia. Clin Diagn Lab Immunol. 2001; 8(5):899-903.
- Wallace BJ, Brady G, Ackman DM, Wong SJ, Jacquette G, Lloyd EE, et al. Human granulocytic ehrlichiosis in New York. Arch Intern Med. 1998; 158(7):769-73.
- Brouqui P, Salvo E, Dumler JS, Raoult D. Diagnosis of granulocytic ehrlichiosis in humans by immunofluorescence assay. Clin Diagn Lab Immunol. 2001; 8(1):199-202.
- Chapman AS, Bakken JS, Folk SM, Paddock CD, Bloch KC, Krusell A, et al. Diagnosis and management of tickborne rickettsial diseases: Rocky Mountain spotted fever, ehrlichioses, and anaplasmosis - United States: a practical guide for physicians and other health-care and public health professionals. MMWR Recomm Rep. 2006; 55(RR-4):1-27.
- Horowitz HW, Wormser GP. Doxycycline revisited: an old medicine for emerging diseases. Arch Intern Med. 1998; 158(2):192-3.

## Случај хумане моноцитне ерлихиозе у Србији

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### КРАТАК САДРЖАЈ

**Увод** Ерлихиоза је бактеријска зооноза која се преноси хематофагним артроподама – крпељима. Код људи се јавља као моноцитна, гранулоцитна и *ewingii* ерлихиоза. Патолошки процес је последица унутарћелијског паразитирања ерлихије у моноцитима и гранулоцитима периферне крви.

**Приказ болесника** Болесник стар 52 године примљен је на одељење због високе дводневне фебрилности (преко 40 °C) која је праћена дрхтавицом, боловима у мишићима, малаксалошћу, губитком апетита, главобољом, конфузношћу, отежаним дисањем и оскудним сувим кашљем. У анамнези је добијен податак о уједу крпеља седам дана пре пријема. Лабораторијски налази су указали на тромбоцитопенију, леукопенију, анемију и повећање активности трансаминеза у серуму. Болесник је лечен доксициклином 14 дана, након чега су се тегобе повукле. Методом индиректне иму-

нофлуоресценције анализирана су три узорка серума овог болесника на присуство антитела на бактерију *Ehrlichia chaffeensis* и прегледан је узорак размаза периферне крви на присуство ерлихија и конгломерата ерлихије у моруле, које представљају цитоплазматске вакуоле.

**Закључак** Код болесника с податком о уједу крпеља, одговарајућом клиничком сликом и серолошким тестом индиректне имунофлуоресценције доказана су антитела за бактерију *Ehrlichia chaffeensis*, што указује на хуману моноцитотропну ерлихиозу, болест која се код нас ретко доказује. На ерлихиозу треба мислити када уз клиничку слику (висока фебрилност, грозница, болови у мишићима, главобоља, општа слабост и малаксалост, евентуално макулопапулозна оспа) постоји податак о уједу крпеља.

**Кључне речи:** *Ehrlichia chaffeensis*; хуман ерлихиоза; индиректна имунофлуоресценција; доксициклин

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