

First report of human ehrlichiosis in Turkey

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Received: 26th January 2018, Revised: 26th February 2018, Accepted: 3rd March 2018

SUMMARY: Emiroğlu M, Çelebi B. First report of human ehrlichiosis in Turkey. *Turk J Pediatr* 2019; 61: 267-270.

Ehrlichiosis, a tick-borne infection, can cause severe and fatal disease. A 6-year-old boy was admitted with fever, chills, malaise, headache, anorexia, rhinorrhoea, and cough lasting two days. He had had contact with a dog 10 days prior, and a tick had been removed the day before. Fever, minimal conjunctival injection, oropharyngeal hyperemia and cracked, hyperemic lips were observed. Laboratory tests were normal except for lymphopenia and hyponatremia. Cytoplasmic morulae in the monocytes and granulocytes were seen on peripheral blood smear. Doxycycline was started immediately, and the fever subsided within 48 hours. Given the *Ehrlichia* was positive but *Anaplasma* negative by real-time PCR, he was diagnosed as ehrlichiosis, subspecies identification could not be performed. This is the first human ehrlichiosis case in Turkey.

Key words: ehrlichiosis, zoonoses, tick-borne diseases, Turkey, morulae.

Tick-borne zoonotic infections due to different microorganisms can cause fatal infections, including rickettsiosis, ehrlichiosis, anaplasmosis and Crimean-Congo haemorrhagic fever (CCHF).¹ Ehrlichiae are obligate intracellular Gram-negative bacteria that grow in membrane-bound vacuoles in leukocytes. They can infect humans and other mammals, including dogs, cattle, sheep, goats, rodents, and deer, via tick bite.² Although, CCHF cases have been described in neighboring countries before, the first clinical CCHF cases in Turkey were not recognised until 2002³; at present, anyone with a tick bite history and fever is evaluated for CCHF, but not ehrlichiosis. We report a child with ehrlichiosis, representing the first case in Turkey.

Case Report

In November 2017, a 6-year-old boy from Konya was admitted with fever, chills, malaise, headache, anorexia, rhinorrhoea and cough lasting two days. He had had contact with a street dog 10 days before, and his mother had removed a tick the previous day. The patient's axillary temperature was 39.5°C, and minimal conjunctival injection, oropharyngeal hyperemia and cracked, hyperemic lips were observed.

The laboratory test results were as follows: white blood cell (WBC) count 11,800/mm³, neutrophils 10,240/mm³ (72.4%), lymphocytes 600/mm³ (5.12%), haemoglobin, 13.1 g/dl, platelets 236,000/mm³, international normalised ratio (INR) 1.22, erythrocyte sedimentation rate 7 mm/h, C-reactive protein 7.62 mg/dl, procalcitonin, 0.598 ng/ml, serum albumin 4.5 g/dl, serum sodium 130 mEq/L, aspartate aminotransferase 46 U/L, alanine aminotransferase 19 U/L, and lactic dehydrogenase 291 U/L. Chest X-ray and echocardiography were normal. Peripheral blood smear was performed, and cytoplasmic morulae were seen in the both of monocytes and granulocytes (Fig. 1.). Doxycycline was started immediately; blood samples were sent to the Public Health Institution of Turkey, National High Risk Pathogens Reference Laboratory.

The patient had diarrhea for one day and intense malaise continued; the WBCs dropped to 3,500/mm³, whereas the lymphocytes rose to 1,620/mm³. Blood, throat and urine cultures were negative. The fever subsided in 24-48 hours. Given that he tested positive for *Ehrlichia*, but negative for *Anaplasma* on real-time polymerase chain reaction (PCR)⁴, he was diagnosed with ehrlichiosis. *Rickettsiae conorii*

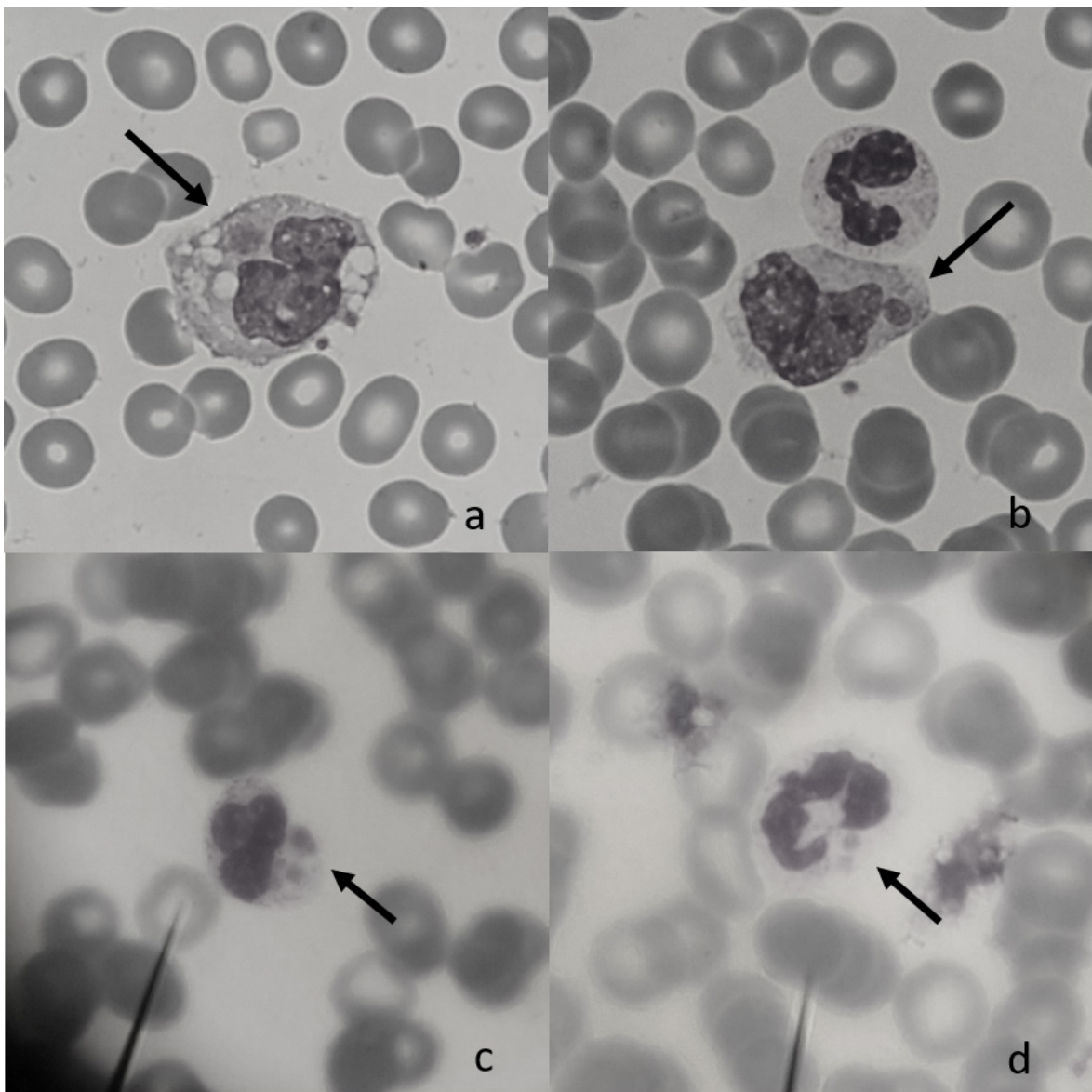


Fig. 1. Peripheral blood ($\times 1000$). Giemsa staining shows a monocytes (a, b) and granulocytes (c, d) with an intracytoplasmic morulae (arrow).

immunoglobulin (Ig) M and IgG by enzyme linked immunosorbent assay, and CCHF IgM by indirect immunofluorescence antibody (IFA) and CCHF PCR were also negative. Doxycycline was completed in 10 days.

Informed consent was received from the family.

Discussion

Ehrlichiosis is an infection caused by bacteria of the *Ehrlichia* genus in the family Anaplasmataceae, which include *E. chaffeensis*, *E. ewingii*, *E. canis* and *E. muris*-like agent¹. *E.*

chaffeensis involves monocytes and macrophages and causes human monocytic ehrlichiosis, whereas *E. ewingii* involves granulocytes and causes *Ehrlichia ewingii* ehrlichiosis. These two agents cause most human ehrlichiosis.² *A. phagocytophilum*, another member of the family Anaplasmataceae, also involves granulocytes, causing human granulocytic anaplasmosis (HGA).¹

Although it was previously known in animals, the first described cases of human ehrlichiosis involved *Ehrlichia chaffeensis* and *E. ewingii*, identified in 1991 in 1999, respectively.²

Tick-borne diseases are closely related with geographic location of vector ticks. *E. chaffeensis* and *E. ewingii* are transmitted to humans by *Amblyomma americanum*, while *A. phagocytophilum* comes from *Ixodes scapularis*.¹ We were unable to retrieve the dog or tick.

Human ehrlichiosis is increasingly reported in North America and Europe. During 2008–2012, 4,613 cases of *E. chaffeensis* and, 55 cases of *E. ewingii* infections in humans were reported from United States.² In Europe, the prevalence of the *A. phagocytophilum* genogroup in *I. ricinus* ticks is 0.4–66.7%. However, in seroepidemiological studies, the HGA seroprevalence rates were 0–2.9% in blood donors and 1.5–21% in tick-exposed individuals. *E. ewingii* and *E. chaffeensis* cases have not been described.⁵ In Turkey, molecular and serological studies have been evaluated Ehrlichia species in ticks, dogs, ruminates and humans. Christova et al.⁶ found that 5% of non-Ixodes ticks from Turkey yielded a signal with the Ehrlichia/Anaplasma genus probe on the reverse line blot. The seropositivity rates for *A. phagocytophilum* in *I. ricinus* ticks was reported as 3.93%⁷, while in cattle and sheep it was 10.1–15.3% and 9.9–13.6%, respectively.⁸ In cattle, Ehrlichia spp. seropositivity was detected as 1.66%⁸. Only two studies have evaluated humans for *A. phagocytophilum* in persons with a history of tick exposure; Ongut et al.⁹ reported a seropositivity of 8% in Antalya, while Kilic et al.⁸ found a seropositivity of 25% in the Thrace Region. Unfortunately, no Turkish study has evaluated *A. americanum*, *I. scapularis* or mammals or humans for *E. chaffeensis* and *E. ewingii* seropositivity. We think that the discordance between seropositivity rates in the population and lack of reported ehrlichiosis cases is due to physicians' lack of awareness.

Ehrlichiosis mostly occurs in the months of May to August. The common symptoms are fever, chills, malaise, myalgia, headache, rash, confusion, conjunctival injection and gastrointestinal symptoms like nausea, vomiting, diarrhoea and anorexia.¹ Rash and gastrointestinal symptoms are less common in *E. ewingii* infection. Without treatment, symptoms typically resolve after 1–2 weeks, but prompt antimicrobial therapy will shorten the duration and reduce the risk of serious manifestations¹⁰, such as acute respiratory

distress syndrome, encephalopathy, meningitis, disseminated intravascular coagulation, spontaneous hemorrhage and renal failure.¹ The estimated fatality rate for *E. chaffeensis* infections is approximately 1–3%, while for anaplasmosis, it is less than 1%; no fatal cases of *E. ewingii* ehrlichiosis have been reported.¹⁰ Generally, paediatric patients have a mild infection, but children aged <10 years and geriatric or immunosuppressed persons have the highest case-fatality rates¹. Our patient was admitted in November with flu-like symptoms and tick bite history; global warming may have affected the tick activity.

Characteristic routine laboratory findings for ehrlichiosis are thrombocytopenia, leukopenia, elevated liver enzymes and hyponatremia², similar to anaplasmosis.

After the bacteria infects blood leukocytes, it multiplies in cytoplasmic membrane-bound vacuoles forming membrane-bound intracytoplasmic bacterial inclusions (morulae, Latin for “mulberry”), which stain dark blue or purple and are stippled in appearance.¹ Cytoplasmic morulae in the WBCs on peripheral blood smear, bone marrow, or cerebrospinal fluid may indicate infection with Anaplasmataceae; morula positivity in the blood smear is reported in about 17% of immunocompetent patients and all of immunocompromised hosts.¹¹ Interestingly, morulae have not been observed yet in peripheral blood cells in patients with *Ehrlichia muris*-like agent infection¹. Although leukocyte tropism has been well described for different ehrlichiae, it is not a strict rule; *E. chaffeensis* most commonly involves monocytes, while *E. ewingii* mainly involves granulocytes.¹⁰ The fourfold increase in the IgG-specific antibody titre by IFA assay between the acute and convalescent period is meaningful. Since *E. ewingii* has never been grown in culture, and no antigens are available for diagnostic use, it must be confirmed via molecular detection. Detection of specific DNA in a clinical specimen by PCR assay is highly specific (60–85%) and sensitive (60–85% for *E. chaffeensis* infection and 67–90% for *A. phagocytophilum* infection) during acute infection.¹² Although, PCR is the only definitive diagnostic test for *E. ewingii* infection, the sensitivity and specificity of this test is unknown.¹² A high rate of false positive

serology usually occurs due to cross reactive antigens shared by Ehrlichia and Anaplasma, but there is no report about false positivity with PCR, but sequence confirmation of the amplified product is necessary to identify infection with certain species.² Blood samples for PCR must be collected before antibiotic therapy is initiated. We collected our patient's blood two days after the doxycycline treatment started; thus, further sequencing for species identification failed. We could not perform IgG-specific antibody titre analysis. Although, identification of morulae in WBC is insensitive, it is highly suggestive of ehrlichiosis and can serve to early diagnosis¹¹, as in our patient. It is impossible to distinguish *E. chaffeensis* infections from *E. ewingii* based on history, clinical signs and routine tests²; in our patient with tick bite history; fever, flu-like symptoms, lymphopenia and mild hyponatremia were present at the admission, we suspected ehrlichiosis because of morulae seen in both of the monocytes and granulocytes cytoplasm and, diagnosed by real-time PCR but, could not be differentiated as *E. chaffeensis* or *E. ewingii* infection.

Doxycycline is the drug of choice for human ehrlichiosis and anaplasmosis treatment, regardless of patient age. The recommended paediatric dosage is 4 mg/kg per day, divided every 12 hours (maximum 100 mg/dose). Treatment should continue for at least 3 days after the fever subsides and until there is evidence of clinical improvement.¹⁰ If fever persists for >48 hours after initiation of therapy, an alternative or additional diagnosis must be considered.¹ Minimum course of treatment is 5 to 10 days.

In conclusion, patients with a history of tick bite and fever and flu-like symptoms, with variable degrees of anemia, thrombocytopenia, leukopenia and elevated liver enzymes, should be evaluated for tick-borne infections, including ehrlichiosis. We think that the frequency of Ehrlichia infection diagnoses will rise in Turkey with increased awareness of the disease and diagnostic availability.

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