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SPOTTED FEVER RICKETTSIOSIS CAUSED BY *RICKETTSIA RAOULTII* - CASE REPORT

GORĄCZKA PLAMISTA WYWOŁANA PRZEZ *RICKETTSIA RAOULTII* - OPIS PRZYPADKU

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STRESZCZENIE

CELEM PRACY. jest przedstawienie przypadku riketsjowej gorączki plamistej, wywołanej najprawdopodobniej przez *Rickettsia raoultii,* nowopojawiający się patogen, poprzednio opisywany u pacjentów z limfadenopatią po pokąsaniu przez kleszcze (tick-borne lymphadenopathy -TIBOLA lub *Dermacentor*-borne necrosis erythema and lymphadenopathy -DEBONEL). W piśmiennictwie nie ma wielu opisów przypadków zakażeń wywołanych przez *R. raoultii.* Badanie kleszczy w Polsce (*Ixodes ricinus* i *Dermacentor reticulatus*) pokazało, że *R. raoultii* występuje we wszystkich regionach i przeważa nad innymi riketsjami z grupy gorączek plamistych (*R. slovaca* i *R. helvetica)*.

OPIS PRZYPADKU. Do Kliniki Chorób Odzwierzęcych i Tropikalnych została przyjęta 17-letnia, dotychczas zdrowa pacjentka, z powodu gorączki, zmiany skórnej o typie owrzodzenia pokrytego strupem i wysypki w postaci licznych drobnych zmian rozsianych na tułowiu, kończynach, także na dłoniach i stopach oraz na błonie śluzowej ust. Większość zmian skórnych była nieznacznie wyniesiona, z obwódką, zaczerwienieniem i martwiczym środkiem, pojedyncze zmiany miały wygląd pęcherzyków. Z odchyleń w badaniu przedmiotowym stwierdzono ponadto powiększone węzły chłonne szyjne po stronie prawej.

WYNIKI BADAŃ. W badaniach laboratoryjnych stwierdzono leukopenię (z pałeczkami w rozmazie krwi w odsetku 22%), trombocytopenię, niewielki wzrost stężenia CRP, przy wzroście także prokalcytoniny.

WYNIKI LECZENIA. Obserwowano szybką poprawę po rozpoczęciu leczenia ceftriaksonem i doksycykliną. W dwa tygodnie od początku choroby wykryto przeciwciała w klasie IgG w mianie 1:128 w reakcji z antygenem *R. rickettsii.* Następnie w testach IFA z antygenami

ABSTRACT

We report the case of rickettsial eschar-associated spotted fever, most probable due to *Rickettsia raoultii*, an emerging pathogen, which was previously described in patients with tick-borne lymphadenopathy (TIBOLA), also called *Dermacentor*-borne necrosis erythema and lymphadenopathy (DEBONEL). The pathogenicity of *R. raoultii* is not well established. The survey of ticks from Poland (*Ixodes ricinus* and *Dermacentor reticulatus*) revealed that *R. raoultii* occur in all regions of Poland and predominate over other rickettsiae of spotted fever group – *R. slovaca* and *R. helvetica.*

A 17-year-old otherwise healthy girl was admitted to Department of Zoonotic and Tropical Diseases because of fever, eschar and rash. Multiple disseminated small lesions were present on the skin of her head, trunk and limbs, also palms and soles, and mucosa of her lips. The majority of them had necrotic center slightly elevated with redness around, single ones had vesicular appearance. The lymph nodes on the right side of her neck were enlarged. Laboratory investigations revealed: leukopenia (with 22% of bands in differential), thromocytopenia, slightly elevated C-Reactive Protein, as well as procalcytonin. The quick improvement was observed with a treatment with ceftriaxone and doxycycline.

Two weeks after the onset of disease, IgG serum antibodies titer of 128 reacting with *R. rickettsii* antigen only was detected. IFA tests with six SFG rickettsial species demonstrated the strongest reaction with *R. raoultii* group antigens in a titer of 64.

The case we report, resembling boutonneuse fever, with leukopenia, thrombocytopenia and septic parameters indicates possible higher virulence of *R. raoultii* than it was previously observed.

grupowymi sześciu gatunków riketsji gorączek plamistych najsilniejszą reakcję wykazano dla antygenów grupowych *R. raoulti* w mianie 1: 64.

Wniosek: Przedstawiony przypadek riketsjozy, przypominający gorączkę śródziemnomorską, z leukopenią, trombocytopenią i parametrami sepsy sugeruje potencjalnie większą patogenność *R. raoultii* niż dotychczas opisywano.

Słowa kluczowe*: Rickettsia raoultii, gorączka plamista, choroby przenoszone przez kleszcze*

Key words: *Rickettsia raoultii, spotted fever, tick-borne diseases*

INTRODUCTION

Spotted fever group (SFG) rickettsioses are diseases of diverse clinical course with highly virulent *R. rickettsii* and relatively benign *R. acari* or *R. conorii* infections (1, 2, 3, 4). *R. raoultii* has been recently described as an unique species among SFG rickettsiae (5). It was detected in ticks throughout Europe and identified as one of etiologic agents of a syndrome named tick-borne lymphadenopathy (TIBOLA) or *Dermacentor*-borne necrosis erythema and lymphadenopathy (DEBONEL) or scalp eschar and neck lymphadenopathy after tick bite (SENLAT) (6, 7, 8). However the pathogenicity of *R. raoultii* is not well documented and considered as rather low (7).

THE CASE

In April 2011 a 17-year-old otherwise healthy girl was admitted to Department of Zoonotic and Tropical Diseases because of fever, eschar and rash.

Six days earlier on the left site of her neck she noticed a red spot and felt to be feverish. The fever continued the following days, dry cough and enlargement of the lymph nodes on the right side of her neck occurred and primary lesion on the left side progressed in size. The generalized rash appeared five days after the onset of symptoms.

The patient lived in a village in central Poland and did not notice to be bitten by a tick. Nobody at home or at school had similar symptoms. She did not travel abroad the previous year. Her past medical history was not significant.

On admission: her axillary temperature was 38.6°C, her pulse, blood pressure and respiratory rate were normal. An eschar, about 3 cm in diameter was present on the left side of her neck, the second one similar in size was located on the left calf (Figure 1, panel A, B). Multiple disseminated small lesions were present on the skin of her head, trunk and limbs, also palms and soles, and mucosa of her lips (Figure 1, panel C, D). The majority of them had necrotic center slightly elevated with redness around, single ones had vesicular appearance. The lymph nodes on the right side of her neck were enlarged.

The examination of chest and abdomen was normal. No neurological abnormalities were found.

Laboratory investigations revealed: leukopenia (white blood cells 3,700/μL with 22% of bands in differential, normal range 4,000-10,000/μL, with bands <5%), thromocytopenia (with a platelet count 65,000/μL, normal range 128,000- 348,000/μL), C-Reactive Protein was slightly elevated- 29 mg/l (normal range <5 mg/l), as well as procalcytonin 0,85 ng/ml (normal range < 0,5 ng/ml), sodium concentration was slightly decreased- 133 mmol/l (normal range 137- 145 mmol/l), liver function tests, concentration of potassium, D-dimer and other coagulation tests yielded normal results. The ultrasound scan of the abdomen did not reveal enlarged lymph nodes in the abdominal cavity nor spleen enlargement. The chest X-ray was normal. Blood, a skin sample and a swab from skin lesions were taken for culture. The treatment with amoxycilin-clavulanate, which had been started before admission (the previous day) was switched to ceftriaxone (1x2 g i.v.) and doxycycline (2x 100 mg orally).

Considering clinical symptoms, infection with *Rickettsiae* sp. was suspected. The serum samples collected ten days and four weeks after onset of symptoms were sent to a referential laboratory.

The quick improvement was observed with normalization of body temperature and beginning of skin changes healing within 48 hours. In control laboratory tests on the fifth day of treatment no abnormalities in total blood cell count or biochemistry were detected. The results of cultures were negative. The follow up after three weeks revealed completed recovery.

Detection of antibodies to spotted fever group (SFG) rickettsioses.

The IgG *Rickettsia* spp. level of serum antibodies, were detected with microimmunofluorescence (Rickettsia IFA IgG, Focus diagnostic, USA). A two-step procedure was applied for *Rickettsia* spp. antibody evaluation. The first step was the detection and the differentiation of antibodies specific to typhus and spotted

fever groups. The screening was performed at a titer of 1:16. Then, serum samples positive for SFG rickettsiae were assayed for the presence of antibodies to six SFG rickettsial species (Rickettsia Screen IFA IgG Antibody Kit, Fuller Laboratories, Fullerton, California). Purified, acetone-fixed, antigens of *R. conorii*, *R. helvetica, R. felis, R. slovaca, R. sibirica, R. raoulti* were applied as diagnostics antigens. Highest titer of a given rickettsial species is considered indicative for the infection with this specific *Rickettsia* species.

To exclude other tick-borne diseases such as Lyme borreliosis and bartonellosis additional tests were performed. Levels of *Bartonella* spp. IgG antibodies were determined by indirect immunofluorescence assay (Bartonella IFA IgG, Focus diagnostics, USA). *B. burgdorferi* IgM and IgG antibodies were checked with ELISA tests (Borrelia 14kD+OspC IgM and Borrelia IgG +VlsE, DRG Diagnostics, Germany).

PCR

DNA from blood and skin samples, collected ten days after the onset of symptoms, were extracted with QIAamp Tissue Kit (Qiagen, Hilden, Germany). Bacterial DNA was examined by PCR method for presence of *Rickettsia* sp. citrate synthase (*gltA*) with *Rp*CS.409d and *Rp*CS.1258n primers for an initial screening to amplify fragment characteristic for all rickettsiae [9]. PCR test included negative (water) and positive (*R. conori* strain H24 from collection of NIPH-NIH) controls. Amplicons were analyzed in electrophoresis in 1,5% agarose gel stained with ethidium bromide.

RESULTS

Two weeks after the onset of disease, IgG serum antibodies titer of 128 reacting with *R. rickettsii* antigen only was detected. Reaction with *R. typhi* was negative. IFA tests with six SFG rickettsial species demonstrated the strongest reaction with *R. raoulti* group antigens in a titer of 64.

DNA of *Rickettsia* spp. was not detected in blood and skin samples with PCR.

Specific antibodies to *B. henselae* and *B. burgdorferi*, were not detected either. Three weeks later, consequtive serologic tests showed IgG antibodies to *R. rickettsii* antigen in titer of 32 and to *R. raoulti* group antigens in a titer of 16 .

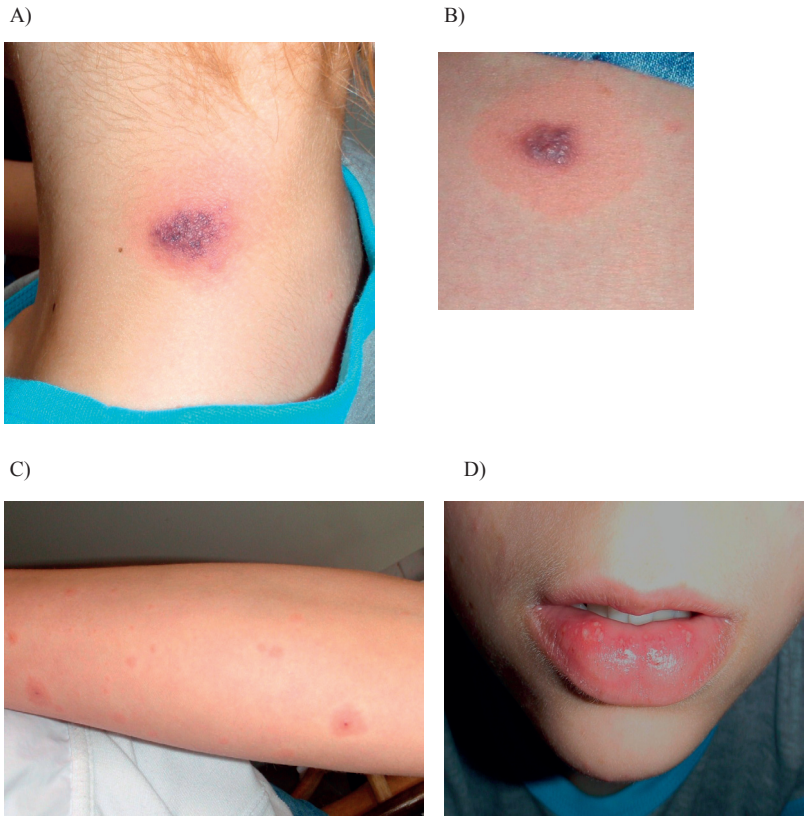

Fig. 1. Lesions of a patient with eschar-associated spotted fever caused by *Rickettsia raoultii* A) Eschar on the neck; B) eschar on the calf; C) papular rash on the limb; D) erosions on the lower lip.

Ryc.1. Zmiany skórne związane z wystąpieniem gorączki plamistej wywołanej przez *Rickettsia raoultii* A) strup na szyi; B) strup na łydce; C) wysypka plamista kończyn górnych; D) zmiany nadżerkowe wargi dolnej.

CONCLUSIONS

R. raoulti is an emerging pathogen recently isolated and described (6, 7). The survey of ticks from Poland (*Ixodes ricinus* and *Dermacentor reticulatus*) revealed that *R. raoulti* occur in all regions of Poland and were noted in 18.2% of *I. ricinus* and 56.7% of *D. reticulatus* and predominate over other SFG rickettsiae – *R. slovaca and R. helvetica* (10).

Due to the not characteristic symptoms of rckettsioses their diagnosis requires an extended set of serological tests. Their interpretation may be difficult due to cross reactions, which occur between the various species of rickettsiae antigens. IFA tests routinely used with a single antigen do not allow for a final determination of an etiologic agent and using several species-specific antigens are needed. The highest level of antibodies to one of the species tested, allows to determine the etiologic agent. In addition, molecular testing can be performed by PCR to detect the DNA of individual species of the genus *Rickettsia* (11). In presented study, cuttings taken from the changed skin and blood were negative with PCR, probably due to initiation of treatment with antibiotics (fifth day of the therapy).

The study of TIBOLA/DEBONEL suggested low virulence of *R. raoulti*, because in patients with asymptomatic tick bites, all ticks positive by PCR harbored *R. raoulti* (7). The case we report, resembling boutonneuse fever, with leukopenia, thrombocytopenia and septic parameters indicates possible higher virulence of *R. raoulti* than it was previously observed. Clinicians should be alert for rickettsial spotted fevers as emerging diseases.

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