

# *Francisella tularensis*/*Rickettsia* spp. co-infections in patients with skin changes and lymphadenopathy

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## Abstract

**Introduction:** Tularemia and spotted fever group rickettsioses (SFG) can be transmitted by ticks and have a number of common clinical symptoms. Most characteristic are a maculopapular or vesicular rash or an eschar at the site of the tick or insect bite accompanied by painful lymph nodes. The aim of this study was to determine whether *Rickettsia* spp./*Francisella tularensis* mixed infections occurred in patients with similar symptoms who were diagnosed with either *Rickettsia* spp. or *F. tularensis* infection.

**Material and methods:** Thirty-six cases from 2011–2014, including 15 individuals with clinically and serologically recognized SFG and 21 with tularemia, were analyzed retrospectively using immunofluorescence for detection of *Rickettsia* spp. or ELISA for detection of *F. tularensis*.

**Results:** Of the 36 cases examined, specific high titers of antibodies to *Rickettsia* spp. were found in 1 (4.4%) patient with tularemia and specific high titers of antibodies to *F. tularensis* were detected in 1 (6.7%) patient with SFG.

**Conclusions:** The results of our study show that in infections with fever, enlarged lymph nodes and skin lesions after tick and insect bites, laboratory testing of both diseases – SFG rickettsiosis and tularemia – should be implemented. Identification of *F. tularensis* and *Rickettsia* spp. mixed infections is crucial in order to administer appropriate antibiotics and to avoid treatment failure and relapse.

**Key words:** rickettsiosis, tularemia, co-infection.

## Introduction

Tularemia and spotted fever group rickettsioses (SFG) can be transmitted by ticks and have a number of common clinical symptoms. In Poland, both diseases are not often recognized.

Tularemia is caused by *Francisella tularensis* – a Gram-negative bacterium, pathogenic for many wild animals and humans. They are able to survive in a humid environment for a long time. The infection is transmitted by insect bites of various species of horseflies and mosquitoes. The other possibilities are contacts between hunters, foresters and farmers and the infected animals, inhalation of infected aerosols or dust or by consumption of contaminated water [1, 2]. Spotted fever group rickettsioses (SFG) are caused by *Rickettsia* spp. [3, 4]. In Poland three species are considered to be the etiological agent of the infections: *R. slovaca*, *R. raoultii* and *R. helvetica*. They are transmitted by *Ixodes* and *Dermacentor* ticks [5].

Both infections usually affect individuals with a history of being in a wooded area.

The incubation time of tularemia from infection to onset of symptoms ranges from 3 to 5 days. A painless ulcer appears at the site of the bite and evolves to a scar. Local lymph nodes are enlarged and they are usually painful. In about one third of the cases lymph nodes suppurate. Ulceroglandular and glandular forms are the most common for this infection. Systemic symptoms include fever, chills, malaise, fatigue and sleepiness. Patients may also complain of a headache and a sore throat [2].

The incubation time of SFG rickettsioses is usually 1 week (1–15 days). The characteristic symptoms are a maculopapular or vesicular rash or an eschar at the site of the tick bite accompanied by painful lymph nodes. General symptoms associated with acute rickettsial infections are nonspecific and include fever, headache, malaise, and sometimes nausea and vomiting [4].

Due to the similar clinical picture of both diseases, the aim of our study was to determine whether *Rickettsia* spp./*Francisella tularensis* mixed infections occurred in patients who were diagnosed with either *Rickettsia* spp. or *F. tularensis* infection.

## Material and methods

Thirty-six cases from the years 2011–2014, including 15 individuals with clinically and serologically recognized SFG and 21 with tularemia, were analyzed retrospectively. The mean age of the patients was 36 years; 28 (78%) of the patients were male and 8 (22%) female. Detailed anamnesis was obtained from 23 patients. All of them presented with skin changes and lymphadenopathy.

For detection of IgM and IgG *Rickettsia* spp. antibodies, microimmunofluorescence (MIF) was used as the reference method for serodiagnosis of rickettsioses, with inactivated *R. typhi* and *R. rickettsii* antigens (Rickettsia IFA IgM, Rickettsia IFA IgG, Focus diagnostic, USA).

Specific IgM and IgG antibodies to *F. tularensis* were tested with Serion ELISA classic *Francisella tularensis* IgG/IgM (Institut Virion/Serion GmbH, Wurzburg, Germany).

## Results

We tested the serum from 36 patients who had a confirmed diagnosis of either SFG or tularemia infection. Of these 36, 15 individuals had a confirmed SFG infection and 21 had a confirmed tularemia infection.

Of the 21 patients with a confirmed tularemia infection, we detected antibodies against *Rickettsia* spp. in 10 (47.6%) patients. Of these, 6 (28.6%) patients had non-significant, low-level antibodies (titer of 1 : 32) and 3 (14.3%) patients had low-level antibodies (titer of 1 : 64) against *Rickettsia* spp. (data not shown). A single patient (4.4%) had a specific high titer of 1 : 128 to *Rickettsia* spp. (Table I).

Of the 15 patients with confirmed SFG infection, 3 (20%) had antibodies to *F. tularensis*. Of these patients, 1 had a specific high titer to *F. tularensis* (Table I).

Co-infections of *F. tularensis* and *Rickettsia* spp. were recognized on the basis of a high level of antibodies in the 2 (5.5%) patients described above. The first patient had been recognized as a tularemia case in June 2013 (case 1) and SFG had been recognized in the second patient in August of the same year (case 2).

**Table I.** Clinical symptoms and results of diagnostic tests in patients with mixed infection

Case	Clinical diagnosis (symptoms of disease)	Day of symptoms <sup>1</sup>	<i>F. tularensis</i> ELISA <sup>2</sup>		<i>R. rickettsii</i> IFA <sup>3</sup>		Treatment
			IgM	IgG	IgM	IgG	
1	Fever up to 39°C, ulcer in the left popliteal fossa with regional lymphadenopathy, tachycardia, chills, significant weakness and malaise, fatigue	35	165	100	Negative	1 : 128	Ceftazidime (2.0 g/day) and clarithromycin (500 mg/day) Meropenem (1.5 g/day) with vancomycin (1.5 g/day) Doxycycline (200 mg/day)
	Asymptomatic – treatment control	105	36	130	Negative	1 : 64	
2	Fever up to 39°C, suppurating eschar on the right lower leg, regional lymphadenopathy	14	104	100	Negative	1 : 512	Doxycycline (200 mg/day)

<sup>1</sup>Patients were tested on various days of disease symptoms. <sup>2</sup>Level of specific antibodies to *F. tularensis* according to ELISA test: IgM antibodies positive > 25, IgG antibodies positive > 12. <sup>3</sup>IgG serum antibodies endpoint titers of *R. rickettsii* according to IFA test: 1 : 256 and greater are considered presumptive evidence of recent or current infection, < 1 : 256 and ≥ 1 : 64 past or early infection, < 1 : 64 no antibody detected.

Case 1: A 39-year-old man was admitted to the hospital in June of 2013 for fever up to 39°C with chills, significant weakness and malaise and fatigue for 2 weeks. A few days before the appearance of symptoms the patient has been bitten by an insect in the left popliteal fossa with subsequent swelling and inflammatory infiltration. Physical examination on admission revealed tachycardia to 120 beats/min, ulcer in the left popliteal fossa and packages of enlarged (up to several cm) lymph nodes in the left groin. In laboratory blood tests, moderate leukocytosis (13 000 cells/ $\mu$ l) and elevated C-reactive protein (CRP) (40 mg/l) were noted. The patient was treated intravenously with ceftazidime (2.0 g/day) and clarithromycin (500 mg/day). The results of Lyme disease serological tests were negative. The treatment was modified using meropenem (1.5 g/day) with vancomycin (1.5 g/day) after 3 days due to the lack of improvement. Fever and chills subsided and an ulcer in the popliteal fossa healed up, but tachycardia, perspiration occurring 3–5 times a day and swollen lymph nodes in the left groin persisted. After a few days, despite antibiotic treatment, one of the enlarged lymph nodes suppurated, and was surgically removed. During 3 weeks of hospitalization the intensity of all symptoms decreased. Prior to discharge, blood tests for tularemia and SFG rickettsiosis were performed. Both diseases were confirmed with serology. Treatment with doxycycline (200 mg/day) was ordered. Over the next week the symptoms resolved completely.

Case 2: A 51-year-old man was admitted to the hospital in August of 2013 for fever up to 39°C with a suppurating eschar on the right lower leg. In the right inguinal area multiple enlarged lymph nodes were found. He reported that he had been bitten by an insect a few days earlier. SFG rickettsiosis or tularemia was suspected. The immunofluorescence test with *R. rickettsii* antigen showed a high titer of IgG class of antibodies. Specific IgG and IgA antibodies to *F. tularensis* were detected as well. Doxycycline (200 mg/day) was used in treatment.

## Discussion

The results of our serological testing indicate that *Rickettsia* spp./*F. tularensis* co-infections may occur in humans in Poland. Infections caused by both pathogens separately have been described as the SENLAT syndrome – scalp eschar and neck lymphadenopathy after tick bite [6–9].

In nature, *Rickettsia* spp. and *F. tularensis* have been found in host-feeding and questing ticks in Germany [10], Austria [11], and Czechoslovakia [12]. Both pathogens have been found in ticks of the genera *Dermacentor* and *Ixodes* – the most prevalent arthropod vectors capable of infection

dissemination in Poland as well as in Europe [5]. The occurrence of *Rickettsia* spp. in ticks depends on the species and geographical location in the country. They were detected in up to 26% of *I. ricinus* and 57% of *D. reticulatus* ticks [5, 10, 13, 14].

Co-infections with *F. tularensis* and other tick-borne pathogens have been described so far in ticks and reservoir animals. However, it depends on bacterial species and strains as well as the ability to survive and to transmit the disease agent to a susceptible host [15, 16]. The most potent in circulation in ticks seem to be *Borrelia burgdorferi* sensu lato, *Anaplasma* spp., and *F. tularensis*. Species specificity of small mammals as the reservoir for various tick-borne pathogens determines the possibility of co-infections. It has been found that *Rattus rattus* could be a host for both *B. burgdorferi* and *F. tularensis*, whereas in *Apodemus agrarius* and *Mus musculus* these three pathogens can occur separately only. *Rickettsia* spp./*F. tularensis* co-infection in animals thus far was detected in *Clethrionomys glareolus* and *Microtus arvalis*, but there are no data on such cases in humans [17, 18].

The outcome of the distribution of these microorganisms in ticks is human infections, particularly in professional groups with high risk of acquiring tick-borne disease. The presence of antibodies to SFG rickettsiae has been detected in 14.7% to 36.0% of forest workers in Poland [19]. The prevalence of antibodies to *F. tularensis* among forest workers is 2.1% to 3.2% of tested individuals in Poland [20–22]. Seroprevalence data on risk groups indicate that neither SFG rickettsioses nor tularemia is a rare disease, as has been reported in many countries of Europe [1, 2, 23, 24], and mixed infection may occur not only in Poland but in also other geographical areas outside of Europe.

Performing serological tests, cross-reactions should be taken into consideration. It has been found that in some patients examined for tularemia, false positive results were obtained due to the presence of cross reacting antibodies to *Yersinia*, *Brucella*, *Salmonella* and *Legionella* [25]. Until now, cross reactions in rickettsial infections have been detected among antigens of bacteria within the genus *Rickettsia* only [3].

Recognition of rickettsiosis/tularemia co-infections in humans is of great importance for correct antibiotic treatment. Doxycycline (200 mg/day) remains the treatment of choice for tick-transmitted rickettsioses. In patients with severe hypersensitivity to tetracyclines, chloramphenicol can be considered as an alternate therapy, but its use is limited by side effects. Other broad-spectrum antibiotics, including penicillins, cephalosporins, and aminoglycosides, are ineffective for rickettsial diseases [7].

The drugs of choice for tularemia in humans are aminoglycosides. Due to their ototoxicity and nephrotoxicity they are now rarely used (in the most serious cases only). Chloramphenicol is also effective but seldom used due to its possible side effects on hematopoiesis. Fluoroquinolones, such as ciprofloxacin, have been shown to be highly efficacious drugs in the treatment of uncomplicated tularemia, especially in infected children and pregnant women. Tetracyclines including doxycycline are effective, but are associated with treatment failures [26].

Treatment with doxycycline of both patients resulted in rapid improvement of the health status. This indicates the effectiveness of this antibiotic in confirmed mixed infections of *F. tularensis*/*Rickettsia* spp., although this thesis requires wider studies and a larger group of patients.

In conclusions, the results of our study show that in infections with fever, enlarged lymph nodes and skin lesions after tick and insect bites, laboratory testing of both diseases – SFG rickettsiosis and tularemia – should be implemented. Fast, accurate identification of *F. tularensis* and *Rickettsia* spp. mixed infections is crucial in order to administer appropriate antibiotics and to avoid treatment failure and relapse.

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### Conflict of interest

The authors declare no conflict of interest.

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