

CASE REPORT

Clinical Characteristics of Ulceroglandular Tularemia in Two Bulgarian Regions, 2014-2015: a Report of Five Cases**Liliya M. Pekova¹, Magdalena P. Baymakova², Pavlina O. Parousheva¹, Mariya D. Fartunova¹, Nikolay E. Dimitrov¹, Iskra A. Tomova³**¹ Department of Infectious Diseases, Stara Zagora University Hospital, Bulgaria² Department of Infectious Diseases, Military Medical Academy, Sofia, Bulgaria³ National Reference Laboratory High Medical Risk Infections, National Center of Infectious and Parasitic Diseases, Sofia, Bulgaria**Correspondence:**

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We present here the first five human cases with tularemia from two regions in South Bulgaria in which there had been no previous report of the infection. The cases occurred over a period of 8 months (December 2014 – August 2015). They were treated at the Department of Infectious Diseases in Stara Zagora University Hospital, Bulgaria. We present the clinical, epidemiological and laboratory data for four men and one woman (age range 52 to 73 years). Three men were hunters, four patients took part in handling, preparing/skinning and cooking the game animals. One man marked agricultural work and contact with straw stems. After a mean incubation period of 4.8 ± 1.4 days ulcers appeared, followed by local painful lymphadenitis. All patients presented with liver enlargement and elevation in acute phase reactants. The etiological diagnosis was made by tube agglutination test in all cases, PCR positive result was found in one. The administered antibacterial treatment was a combination of aminoglycosides and 4-quinolones with the outcome being favorable for all patients. The current report suggests presence of *Francisella tularensis* in South Bulgaria.

INTRODUCTION

Francisella tularensis is a fastidious Gram-negative primarily zoonotic pathogen that causes tularemia and occasionally affects humans.¹ The pathogen usually enters through small skin lesions although it is possible that it can penetrate through grossly intact skin. *F. tularensis* is highly infectious. A small number of bacteria of about 10 organisms can cause this form of the disease.² The infection could be life-threatening in most cases, but prompt diagnosis and proper treatment are the key to a successful outcome. Because of these etiological and epidemiological features tularemia has been considered a potential bioterrorist agent since 1970.¹ Depending on the route of infection the disease has six clinical forms: ulceroglandular, glandular, oculoglandular, pharyngeal, typhoidal, and pneumonic.¹

Ulceroglandular tularemia is the most common

presentation with tick bites or animal contacts being the usually recalled exposures.³ This form is the most quickly recognized as tularemia in endemic area. The initial specific complaint is often enlarged and tender localized lymphadenopathy. A skin lesion is the most common point of entry of the bacteria. Skin lesions may appear before, simultaneously with, or from one to several days after the adenopathy.² Vesicles also may be seen, and these may be mistaken for herpes simplex or varicella infection.¹ The papule undergoes necrosis, leaving a tender ulcer with a raised border. If left untreated, the ulcer may take weeks to heal and leave a residual scar.¹⁻³ Multiple lesions may occur, particularly in infections with animal sources. The location of the ulcer generally reflects the mode of acquisition. Animal contacts tend to yield ulcers on the hands and forearms while tick bites tend

to yield ulcers on the trunk, the perineum, the lower extremities, and the head and neck.¹ The distribution of lymphadenopathy also reflects the exposure history.

Tularemia (known as plague-like disease and hare fever) is a zoonotic disease in Northern hemisphere.⁴ Cases are reported in most countries, but occurrences vary from region to region.⁴ Endemic foci have been found in Russian Federation, Kazakhstan, Turkmenistan, also in Sweden and Finland.⁴ Annually most cases are reported from Eastern Europe compared with Western Europe.⁴

The first microbiologically proven appearance of tularemia in Bulgaria was in 1963 after importation of infected muskrats at the preserve territory of Srebarna lake near the Danube river, Northern Bulgaria.⁵ Only a small cluster of four human cases was reported at that time and the epizooty was quickly dealt with.⁵

The second appearance of tularemia in Bulgaria started in 1996 in the western part of the country and more than 280 cases were reported up to 2006.⁶ Many Bulgarian authors investigate the problem during this outbreak.⁷⁻¹⁵ Since 2007 only 0–3 single sporadic cases were reported in the country and as before – never from Stara Zagora and Haskovo districts.¹⁶

AIM

The aim of this study was to describe five human tularemia cases that occurred for the first time in South Bulgarian regions with emphasis on their clinical characteristics.

MATERIALS AND METHODS

CASE DEFINITION

The tularemia cases were diagnosed according to the World Health Organization (WHO) case definition.⁴ Clinically compatible cases with culture positive results for *F. tularensis* or with a four-fold or greater change in the serum antibody titer were considered confirmed; cases with a single elevated serum antibody titer or a clinical sample test positive by DNA detection were considered as probable (presumptive).⁴

DIAGNOSTIC TESTS

A retrospective study was conducted at the Department of Infectious Diseases, Stara Zagora University Hospital, Bulgaria. Patients with clinical, epidemiological and serological data for tularemia were selected from medical records (in-patients and out-

patients) over a period of eight months (December 2014 through August 2015).

Using records of clinical histories, an epidemiological questionnaire, medical history, risk factors, comprehensive physical examinations and clinical monitoring were obtained. Various laboratory tests including blood count, erythrocyte sedimentation rate (ESR), urine analyze, C-reactive protein (CRP), serum transaminases (AST and ALT), cholestatic enzymes (GGT and AP), glucose level, creatine, urea, total protein and albumin were performed. Haemostatic functions like fibrinogen levels and international normalized ratio (INR) were monitored. Abdominal ultrasound scan and X-ray were performed in all cases.

Clinical and laboratory follow-up was done after the etiological treatment and every month up to the six months from the beginning of the illness. Patients were considered clinically recovered if symptoms were successfully resolved and lymphadenopathy resolved without suppuration and complications.

The etiological tests were performed at the National Reference Laboratory High Medical Risk Infections, National Center of Infectious and Parasitic Diseases (NCIPD), Sofia, Bulgaria using serological tube agglutination test (BulBio-NCIPD, Bulgaria) with diagnostic titer of $\geq 1:160$. Attempts for culturing *F. tularensis* from one lymph node (fine-needle aspiration biopsy) was done on cystine heart agar with 9% chocolate blood (CHAB) at 37°C in humid atmosphere with 5% CO₂ in BSL 3 cabinet. The same node was investigated also with Real-time Taq Man PCR for *tul4* gene. Culturing or molecular investigations were not performed in four of the cases because they refused such tests.

ETHICS

The medical procedures of this study were approved by the Local Ethics Committee of Stara Zagora University Hospital, Bulgaria.

RESULTS

Case 1. A 56-year-old veterinarian and a hunter, resident of Radnevo town, Stara Zagora region was admitted to the Department of Infectious Diseases, Stara Zagora University Hospital, Bulgaria on 18th of December 2014. Epidemiological information revealed that on November 2014 he shot a hare near his hometown. When handling the hare he noticed that its fatty tissue had an uncommon dark-yellowish colour. Twelve days later a small macula (1 cm) appeared on the 4th finger of his left hand.

After a short period of time it became erythematous papula which progressed within hours to vesicula and pustule. Later on, ulceration and a spontaneous evacuation of a liquid whitish purulent scentless material occurred. In the next four days axillary and epitrochlear swollen painful lymph nodes appeared. Lymph nodes enlargement was accompanied by fever (39.2°C), chills, muscle and joint pains, headache, fatigue and anorexia. Upon subsequent questioning the patient reported that he had a small cut on the fourth finger of his left hand. He also reported that he had seen large number of dead hares in the range. At admission the man was in a generally bad condition with a painful ulcer (1 cm) with black base as well as swollen (2-3 cm) and painful regional lymph nodes enlargement. There was a mild hepatomegaly. Two days after the hospitalization the patient developed red macular rash (0.5-1.5 cm) localized on the forearms trunk and buttocks (**Fig. 1**). His fever lasted for 12 days. Lymphadenopathy and hepatomegaly persisted during the first week in the hospital. Laboratory findings included mild leukocytosis with neutrophils predominating and lymphopenia, moderately increased ESR. Diagnosis of tularemia was serologically confirmed with antibody titer 1:160 on admission and 1:640 two weeks later. The patient was treated with parenteral administration of amikacin 1.0 g once a day and ciprofloxacin 200 mg twice daily for seven days. After discharge oral clarithromycin 500 mg twice

a day was prescribed for seven days. The patient showed improvement in clinical signs within two weeks of treatment.

Case 2. A 57-year-old woman, a resident of town of Chirpan, Stara Zagora region, was admitted to the Department of Infectious Diseases, Stara Zagora University Hospital, Bulgaria on 12 January, 2015. She had a 7-day history of fever (38.5°C), chills, extreme weakness, muscle and joint pains, nausea and diarrhea, swollen and painful right axillary lymph nodes. The woman reported that her brother was a hunter who had shot a hare in a village near Chirpan. She took part in handling and cooking the hare meat. The onset of her clinical symptoms was five days later. The patient recalled that she had a small cut on the 3th finger of her right hand. On admission the patient was in a generally bad condition. A skin lesion was manifested as an ulcer (1 cm), with raised reddish edges and a brown base. Cervical and right axillary swollen painful lymph nodes (1-2.5 cm) were found. Liver and spleen were enlarged. Blood tests showed mild leukocytosis – $11.3 \times 10^9/L$ (normal range: $4.8-10.8 \times 10^9/L$), increased ESR – 91 mm/h (normal range: <12 mm/h), high level of CRP – 270 mg/L (normal range: <10 mg/L). The clinical diagnosis was confirmed serologically. On admission the antibody titer for *F. tularensis* infection was negative, but after 10 days it was 1:160 and 14 days after discharge – 1:640. Appropriate therapy with



Figure 1. A maculopapular rash in a 56-year-old man with tularemia.

intravenous ciprofloxacin 200 mg twice a day and gentamicin 160 mg once a day was administered. After ten days of active treatment the woman was discharged with therapy of ciprofloxacin of 500 mg twice a day for seven days.

Case 3. A 51-year-old hunter, resident of Sime-

onovgrad town, Haskovo region was admitted on 14th January 2015 in a generally bad condition. He reported that two weeks prior to the admission he suddenly developed fever (39°C), chills, fatigue, muscle/joints pains and dry cough. Three days later he noticed a swollen, painful and enlarged lymph



Figure 2. Enlarged axillary lymph node in a 51-year-old patient with *Francisella tularensis*.



Figure 3. Lesions and abrasions on the hand in a 51-year-old man with tularemia.

node in the left axilla (**Fig. 2**). His family doctor suspected pneumonia based on clinical signs without X-ray and prescribed cefuroxime orally. Despite the antibiotic therapy the clinical symptoms weren't resolved. Four days prior to the onset of illness the patient found a dead hare. He and his father-in-law handled the hare meat and noticed that the animal's spleen appeared very swollen and the fatty tissue was brownish. The patient had small cuts and abrasions on his hands (**Fig. 3**). Consequently, a scanty macular rash appeared and developed primary skin ulcer. He reported that there were large numbers of dead mice and some dead hares. On physical examination two swollen and painful solid left axillary lymph nodes (3-4 cm in diameter) were identified. There was hepatosplenomegaly. Chest X-ray was normal without pathological presentation. Blood tests found leukocytosis, $16.4 \times 10^9/L$ (normal range: $4.8-10.8 \times 10^9/L$), increased ESR, high level of AST and ALT. The epidemiological diagnosis of tularemia was confirmed with serological test. Fourteen days after the onset of the illness the antibody titer was 1:640. The patient was treated with intravenous combination of amikacin 1.0 g per day (10 days) and ciprofloxacin 200 mg twice daily (14 days). The man was discharged with therapy of doxycycline 100 mg twice daily for the following 10 days. After the suitable antibiotic combination the patient recovered.

Case 4. A 73-year-old man, father-in-law of case 3, and resident of Simeonovgrad town in Haskovo region presented at the Department of Infectious Diseases, Stara Zagora University Hospital, Bulgaria when he learned the diagnosis of his son-in-law. He was hospitalized about two weeks after the onset of the disease on 17th January 2015 in a generally bad condition. He handled the dead hare with his son-in-law. The patient denied participation in cooking and eating the hare meat. The symptoms developed seven days after the animal contact. Main signs were fever ($39.2^\circ C$), chills, fatigue, myalgia, joint pain, abdominal pain, nausea, vomiting, diarrhea and sore throat. A lot of cuts, abrasions and scabs were found on his fingers. Swollen and painful bilateral axillary lymph nodes (1-2 cm in diameter) were identified. The physical examination did not reveal exudative pharyngitis or tonsillitis, but hepatomegaly was present. Blood tests were within the normal range. The diagnosis was confirmed serologically 28 days after the onset of the disease with antibody's titer of 1:1280. This patient treated himself with oral ciprofloxacin for 7

days before admission. During the hospital stay of seven days an antibiotic combination of gentamicin 160 mg once daily and ciprofloxacin 200 mg twice a day was administered.

Active epidemiological investigation identified another six participants in the hare hunt which took place at Chirpan region. The hunters were 27 to 63 years old, residents of Chirpan town, including the husband and the brother of Case 2. They handled cooked and ingested hare meat. Handling the hare they noticed that the spleen and the liver appeared very swollen and the fatty tissue was dark-yellowish. These hunters had no complaints and all of them refused serological tests. They reported that there were large numbers of dead mice and hares in the range.

Case 5. A 48-year-old man from Kazanlak town (Stara Zagora region) presented at the Department of Infectious Diseases, Stara Zagora University Hospital, Bulgaria on 25th August 2015 with fever ($39.8^\circ C$), chills, fatigue and headache. He had a 5-day history of vomiting, diarrhea and severe muscle pain. At admission he had a single painful lymph node (3 cm) in the right inguinal region and liver enlargement (2-2-2.5 cm). This patient was an agronomist and he had a prick on his right ankle with straw stems 16 days before the onset of the illness. On physical examination he had a small local inflamed painful ulcer. His laboratory findings showed leukocytosis $17.5 \times 10^9/L$ (normal range: $4.8-10.8 \times 10^9/L$), elevated CRP – 134 mg/L (normal range <10 mg/L). The patient was treated from the beginning with intravenous doxycycline 100 mg twice daily for seven days and continued after that with oral doxycycline 100 mg twice a day for another five days. Serological investigation confirmed the diagnosis of tularemia with titer 1:640. Only in this case another material, a lymph node, was later sent for further microbiology tests. It showed negative result in culturing but presence of *tul4* gene in Real-time Taq Man PCR.

DISCUSSION

Tularemia is a re-emerging infection in Bulgaria. The previous outbreak affected the western part of the country and cases were never reported from the south regions including Stara Zagora and Haskovo districts. Unlike some north European countries where mosquitoes are common vectors, in Bulgaria the principal vectors are ticks and the natural hosts are lagomorphs and other rodents.^{1,7} In the present study, four patients mentioned epidemiological data

for previous contact with hares. They did skinning, handling and preparing the hare meat. The fifth patient in our report did agricultural work with probably contaminated straw. The mechanism of transmission in our patients is associated with infected animals and probably arthropod bites. We accepted ulceroglandular form of tularemia in presented cases based on clinical and epidemiological data. The previous Bulgarian studies reported oropharyngeal tularemia as the most common form in 96.5%.⁶ Similar data were reported by Turkish researchers.¹⁷⁻²¹ In contrast French outbreak described glandular form in 46% and ulceroglandular tularemia in 26% of cases.²² The variety of clinical forms is influenced by complex factors as transmission, mechanism of entry and infective dose. Our patients had animal contact, agricultural work, skin lesions and ulcers. Consequently the ulceroglandular form is the most suitable clinical form in these cases.

In the relevant literature the incubation period is said to depend on quantity of inoculum and may last from 1 to 21 days (mean: 2-6 days).²³ The mean incubation period for our patients was 10.3 days. Males are more infected than women, the same proportion was found in our study.^{4,22} Mean age is 57 years, which differs from other reports.²² A French study reported mean age of 49 years²² in contrast to a Turkish author who gave a mean age of 33 years.²⁴ The clinical manifestation may range from asymptomatic infection to septic shock and death.²⁵ Signs and symptoms include: a skin ulcer that forms at the site of infection – usually a small lesion, a painful regional lymphadenopathy, fatigue, weight loss, fever, chills, myalgia, joint pain, diarrhea.⁴ In our study, medical history and clinical presentation were typical for ulceroglandular form of tularemia. Patients mentioned contact with hares, a sudden onset of the disease, skin lesions, painful regional lymphadenopathy accompanied by fever, chills, headache, anorexia, fatigue, myalgia, joint pain. Diarrhea is reported as a quite uncommon symptom (2.1%) in literature.¹⁻³ In contrast three of our patient had gastrointestinal symptoms. In the present study the diagnosis was microbiologically proven by serological tests and in one case also with PCR. Negative culture in this case could be explained with the fastidious nature of *F. tularensis* but most probably this result is a consequence of the delayed patients' agreement for sending his lymph node for further tests and also the effect of the antibiotic treatment. On the other hand, at the same period of the year, materials from five hares

from Stara Zagora and Haskovo regions were sent to the National Reference Laboratory High Medical Risk Infections, NCIPD-Sofia (Bulgaria). All of them were positive in real-time PCR and isolation of *F. tularensis* was successful from lymph nodes and blood in three hares. Our cases had epidemiological link with hares from those regions.

The antibacterial treatment included aminoglycoside and 4-quinolones for mean period of 14 days. The administered therapy is appropriate in accordance with international recommendations.⁴ After six-month follow-up all patients recovered without complications and lymphadenopathy resolved.

The disease tularemia is difficult to diagnose. It is a rare disease and the symptoms can be mistaken. Diagnosis is suspected in humans at risk of infections who presents with a history of fever, chills, weakness, primary lesion and regional painful lymphadenopathy. We present five cases of ulceroglandular form of the diseases. They had typical clinical signs, physical findings and laboratory deviations. Epidemiological data for animal contact and handling hare meat, agriculture work and exposure are risk factors for the transmission of *F. tularensis*. In our cases only the regions of Stara Zagora and Haskovo are not corresponding to the known information for Bulgarian endemic area. After these five human confirmed cases and other animal cases we can add regions Stara Zagora and Haskovo in the field of *F. tularensis* transmission. Our report provided evidence of expansion of the natural area of tularemia in Bulgaria with involvement for the first time of south Bulgarian regions.

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CONFLICTS OF INTEREST

None of the authors have any associations that might be deemed a conflict of interest to the publication of this manuscript.

ABBREVIATIONS USED IN THIS ARTICLE

ALT: alanine transaminase; AST: aspartate transaminase; CRP: C-reactive protein; ESR: erythrocyte sedimentation rate; GGT: gamma-glutamyl transferase; AP: alkaline phosphatase; PCR: polymerase chain reaction.

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Клинические характеристики ульцерогляндулярной туляремии в двух болгарских районах за период 2014 – 2015 г.: доклад о пяти случаях

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Целью настоящего доклада является представление первых пяти случаев заболевания туляремией людей из двух регионов в южной Болгарии, откуда до этого не поступало информации о подобной инфекции. Случаи заболевания были диагностированы в течение восьмимесячного периода (декабрь 2014 – август 2015 гг.) и прошли курс лечения в Клинике инфекционных заболеваний в Университетской больнице в городе Стара Загора, Болгария. Нами представлены клинические, эпидемиологические и лабораторные данные о четырёх мужчинах и одной женщине в возрасте от 52 до 73 лет. Трое мужчин являются охотниками, четверо пациентов принимали участие в обработке, подготовке/ свеживании туш диких животных и приготовлении пищи из них. Один из пациентов упоминает о сельскохозяйственных работах и о контакте с соломой. По истечению среднего периода инкубации продолжительностью 4.8 ± 1.4 дней появляются язвы с последующим региональным болезненным лимфаденитом. У всех пациентов установлено увеличение печени и увеличение реагентов острой фазы. Этиологический диагноз был поставлен на основании пробирочного агглютинационного теста при всех случаях, PCR (Polymerase Chain Reaction - полимеразная цепная реакция) тест оказался положительным в одном из случаев. Антибактериальное лечение включало в себя комбинацию аминогликозидов и 4-хинолонов и исход лечения был благоприятным для всех пациентов. Настоящий доклад устанавливает существование *Francisella tularensis* в южной Болгарии.