Original Article

Tularemia in Bulgaria 2003-2004

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Abstract

Introduction: Tularemia is an uncommon but potentially fatal zoonosis. A second outbreak of tularemia in Bulgaria, about 40 years after the first, occurred in 1997 in two western regions, near the Serbian border. In 2003 tularemia reemerged in the same foci. This retrospective study aimed to evaluate the clinical characteristics and the efficacy of antibiotic therapy in a tularemia resurgence in the Slivnitza region in 2003-2004.

Methodology: A total of 26 cases were evaluated. Using medical records, the following data were collected for all patients: symptoms, physical signs, and microbiology results of agglutination tests, cultures and PCR assays.

Results: Twenty-four of 26 suspected tularemia patients were laboratory confirmed by agglutination test and/or culture. Fifteen (57.7%) patients had clinical presentation compatible with oropharyngeal, 8 (30.8%) with glandular, and 3 (11.5%) with oculoglandular tularemia. The most frequent symptoms were swollen neck (84.6%) and sore throat (76.9%). Lymphadenopathy (100%) was the most common finding. *Francisella tularensis* (*F. tularensis*) was detected by PCR, providing a definitive diagnosis in 82.3% of the cases. All the patients were treated with antibiotics considered effective against *F. tularensis*; however, therapeutic failure was observed in 23.1% of the cases, which was related to a delay in the initiation of antibiotics.

Conclusion: The tularemia outbreak in west Bulgaria near the Serbian border was probably food-borne, associated with a surge in the rodent population. The oropharyngeal form was the most common. Although the disease runs a benign course, late initiation of antimicrobial therapy might delay complete recovery.

Keywords: Francisella tularensis , oropharyngeal tularemia, therapeutic failure

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Introduction

Tularemia, caused by *Francisella tularensis (F. tularensis)*, is an uncommon but potentially fatal zoonosis in the northern hemisphere. *F. tularensis* has four subspecies, two of which are clinically and epidemiologically important for humans. *F. tularensis* subsp. *tularensis* (type A), mostly seen in North America, is more virulent, and is related to tick bite or contact with infected animals. *F. tularensis* subsp. *holarctica*, widely distributed through Eurasia, causes a milder disease and is associated with water and rodents living near water [1-3].

Some authors classify tularemia into two groups, which include the far more widespread ulceroglandular form (in which local or regional symptoms and signs predominate) and the more lethal typhoidal form (with systemic symptoms that dominate the clinical picture) [4]**.** More commonly, tularemia is divided into six types: ulceroglandular, glandular, oculoglandular, oropharyngeal, typhoidal, and pneumonic tularemia. Each form reflects the mode of transmission. While ulceroglandular tularemia occurs more frequently, oropharyngeal infection has increasingly been reported recently in Turkey and in other European countries, including Bulgaria [5-7].

The first tularemia outbreak was in the vicinity of the Srebarna reserve, in northeast Bulgaria in 1963 [8]. The second one, about 40 years later, occurred in 1997 in the adjacent Slivnitza and Pernik regions, west Bulgaria, near the Serbian border. The incidence increased again in 2003 in the same areas. While recent reports in Bulgarian publications have addressed mainly the epidemiological and microbiological features of tularemia [7,9], very little is known about the clinical presentation. This study

aimed to evaluate the clinical characteristics of tularemia in an emergent area in Bulgaria in 2003-2004**.**

Methodology

Twenty-six suspected tularemia cases were admitted to the Infectious Diseases Department, University Hospital Saint Anna, Sofia, Bulgaria, between March 2003 and November 2004. Data were collected from medical records and included demographics, history of illness, symptoms, clinical findings, laboratory results, and therapeutic response. Informed consent was obtained from all study participants.

Case definition

The tularemia cases were diagnosed according to the World Health Organization (WHO) case definition [10]. 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).

C*linical diagnosis*

Oropharyngeal tularemia was defined as the presence of pharyngitis or tonsillitis and cervical lymphadenopathy in a patient from an endemic region (Slivnitza), who had no response to ß-lactam antibiotics despite at least 10 days of therapy. Patients with enlarged and painful lymph nodes without apparent ulcer were diagnosed as having glandular tularemia, and those with conjunctivitis and regional lymphadenopathy were considered to have oculoglandular tularemia.

Diagnostic tests

Diagnosis was confirmed by the presence of at least one of the following test results: a four-fold or greater increase in the titer between two serum samples obtained two weeks apart with one above the 1:160 threshold with tube agglutination; a culture positive result; and a positive result in the polymerase chain reaction (PCR) assay.

Serology was performed using tube agglutination test (BulBio-NCIPD, Sofia, Bulgaria).

Aspirates from cervical lymph nodes and surgically extracted conjunctival granuloma (one patient) were collected for culturing and/or PCR detection.

In-house prepared solid medium with L-cystine, glucose, sodium tioglycolate, human blood, and penicillin was used for cultuvation of the bacteria. The incubation period was at least five days at 37ºC in a BSL III containment laboratory.

PCR with *tul4* and *RD1 F. tularensis* specific primers was performed according to the protocols by Johansson *et al*. [11] and Broekhuijsen *et al*. [12].

Treatment

All patients received appropriate antibiotics, which are defined as treatment with aminoglycoside or fluoroquinolone for at least 10 days or tetracycline for at least 15 days [10]. Streptomycin (1g/day i.m.) with doxycycline (200 mg/day p.o.) in combination was given to eight patients, and streptomycin (1g/day i.m) with chloramphenicol (50-100 mg/kg p.o.) was given to six patients. For eight cases, treatment started with ciprofloxacin (1000 mg/day i.v.) and for two patients, treatment was started with ciprofloxacin (1000 mg/day i.v.) and gentamicin (5 mg/kg i.v.) in combination. The patients with oculoglandular tularemia were also given topical antimicrobial treatment.

The mean delay time for the initiation of proper antibiotic treatment was as follows: 17.7 days (8 to 40 days) in 2003 and eight days (3 to 12 days) in 2004.

Therapeutic failure was defined by the presence of one of the following findings: suppuration and draining (spontaneously or by surgical means) of the involved lymph nodes during and after treatment, or an increase in the size of the existing lymphadenopathy, or the appearance of a new lymphadenopathy [13]. The treatment was considered successful if the signs and symptoms disappeared and lymphadenopathy resolved without suppuration. A second antibiotic course with a different regime was given in case of therapeutic failure.

The tularemia cases were followed up at two-month intervals over a six-month period.

Lymph node incision in one patient with oculoglandular tularemia resulted in the appearance of new granulomatous lesions on the affected conjunctiva.

Results

Twelve cases were defined as confirmed and 12 as probable. Another two patients with suggestive clinical symptoms had negative serological results and convalescent serum samples were not available

so they were excluded. Thus 24 patients were selected for further analyses.

The mean age of the cases was 35 years (range: 12 to 67 years). Tularemia patients were almost evenly distributed by gender (female 46.1%; male 53.9%). Six (25%) out of 24 cases were in the same households. The residents reported increased rodent populations both in and around their houses in the past two years. Furthermore, a few used to drink untreated water from their private wells. None of the patients had had a previous history of tularemia.

Before the diagnosis of tularemia all patients were treated with beta-lactam antibiotics, some of them with short-course gentamicin in addition to beta-lactams without effect.

Findings

Table 1 displays the clinical manifestations of the patients.

Table 1. Clinical manifestations in tularemia patients (n=24)

Characteristics	**N(%) of patients**
Symptoms	
Sore throat	20(83.3)
Swelling on the neck	22(91.6)
Fever[a]	5(19.2)
Ocular burning, itching	3 (12.5)
Signs	
Lymphadenopathy	24(100)
cervical[b]	23(958)
axillary	1(4,2)
Pharyngitis	12(50)
Tonsillitis	3(12.5)
Conjunctivitis	3 (12.5**)**
Treatment prescribed	
Streptomycin + Doxycycline	8 (33.3)
Streptomycin + Chloramphenicol	6 (25)
Ciprofloxacin	8 (33.3)
Ciprofloxacin + Gentamycin	2 (8.3)
Outcome	
No complication	18 (75)
Required surgical treatment	6 (25)

[a] - temperature >38.5ºC for more than 3 days
[b] – 3 patients with oculoglandular tularemia had cervical and preauricular lymphadenopathy

Fifteen (62.5%) patients had clinical presentation compatible with oropharyngeal tularemia, six (25%) with glandular tularemia, and three (12.5%) with oculoglandular tularemia. The most frequent symptoms were swollen neck (91.6%) and sore throat (83.3%). Lymphadenopathy (100%) was the most common finding. The lymphadenopathy was usually unilateral (80.71%) and with predominantly cervical localization (95.8%). The lymph nodes were palpable, slightly tender, and even visible. The three patients with oculoglandular tularemia presented with unilateral granulomatous conjunctivitis, chemosis, swelling of the eyelid, and tender preauricular and cervical lymphadenopathy

Figure 1. A patient with oropharyngeal tularemia

Microbiology results

Serology: Twenty-four cases had positive serological results. The agglutination test was diagnostic of tularemia (titer ≥.160) in 15 (62,5 %) patients from a single serum sample and a four-fold rise of the antibody titer was determined in 9 (37.5 %) cases.

Culture: Cultivation of the aspirates from three lymph nodes resulted in three *F. tularensis* isolates, which were further specified as *F. tularensis holarctica*. These three patients had a single elevated serum antibody titer on admission.

PCR

PCR results were positive in 14/17 (82.3%) samples, comprised of 13 lymph node aspirates and an extracted conjunctival granuloma. PCR confirmed the serological diagnosis in 14 patients.

Three out of 12 (50%) confirmed cases had positive cultures from lymph nodes and nine9 had a fourfold

or greater difference in paired serum antibody titers. The other 12 (50 %) patients with a single elevated serum antibody titer were considered probable.

Therapy response

Over a 3-month period 18 of 24 (75%) patients recovered completely. However, therapeutic failure (suppurated lymph nodes on admission or a few days later, requiring surgical intervention) was observed in the remaining 6 (25 %). They were given a second antibiotic course with gentamycin or ciprofloxacin without effect**.** Fine-needle aspiration was performed but no satisfactory improvement was achieved, followed by incision and drainage. Noteworthy, lymph node surgical drainage in one patient with oculoglandular tularemia resulted in the appearance of new granulomatous lesions on the affected conjunctiva.

While 9 of 10 (90%) patients treated with antibiotics active against *F. tularensis* within the first 3 weeks of their illness fully recovered, complete recovery was observed in only 9 of 14 (64,3 %) treated with appropriate antibiotics but delayed by more than 3 weeks. The difference was found to be significant with ch-square ($p < 0, 05$).

No mortality or severe complications was observed. Although a slight regression of lymphadenopathy was noted at the end of the hospital treatment**,** complete recovery took nearly three months. Due to the long interval between the follow-up visits, precise details of the recovery were not available.

Overall, the clinical presentation, course and outcome did not differ between confirmed and probable cases (data not shown).

Discussion

Tularemia outbreaks have recently been reported in a number of European countries. In Spain, ulceroglandular tularemia in human was first observed in 1998 [13]. In 2007 the second outbreak in the same region was with typhoid form predominance [14]. However, oropharyngreal tularemia is the most frequently observed type in some eastern European countries, particularly in Kosovo, Turkey, and Bulgaria [6,7,15]. In contrast to cases found in Sweden [16] and Finland [17], oropharyngreal tularemia is also the most common manifestation in Norway [18].

This retrospective study was conducted to describe the clinical characteristic of tularemia in an emergent area in Bulgaria. Oropharyngreal tularemia was the most common (62.5%) type found, with lymphadenopathy the usual single finding. Eighteen patients (75%) had an uneventful course with full recovery, six (25%) experienced treatment failure, such as suppuration**,** requiring incision and drainage.

As has been previously reported, fever, sore throat, and neck swelling are the most frequent complaints of oropharyngreal tularemia patients. Physical examination reveals exudative pharyngitis or tonsillitis accompanied by cervical lymphadenopathy, usually unilateral. Without appropriate treatment the enlarged lymph nodes may persist over a long time, even several years [19]. Most of our patients presented with lymphadenopathy, but only a few with tonsillitis and fever on physical examination. These findings might be explained by the hospitalization of these patients in the late stage of the disease. Similar to our results, Helvaci *et al.* [5] and Merik *et al.* [20] have reported tularemia patients with lymphadenopathy as the most common finding on admission.

The increase in rodent populations seen by our patients over the last two years facilitated the environmental contamination with *F. tularensis*. A recent Bulgarian study has shown high prevalence of *F. tularensis* in rodents (22%) trapped in the neighboring Pernik region and tested by PCR [21]. Moreover, *F. tularensis* has been cultivated from four private wells in the same place [9]. We suggest that the route of transmission might be by ingestion of uncooked food or, less likely, contaminated water. By comparison, as in other European counties [6,17], most of the outbreaks reported in Turkey over the last 20 years were related to the consumption of contaminated water [5].

Apart from oropharyngeal tularemia, we also observed three patients with the rare oculoglandular form. This form may be acquired by rubbing the eyes with infectious animal materials. The appearance of new conjunctival granulomas was the consequence of surgical drainage of submandibular lyphadenopathy in one of our patients. The lesson learned was to avoid invasive procedures during the acute stage because of the risk of local spread of the infection [22].

The gold standard of diagnosis of tularemia is the isolation of the causative agent, which is both difficult and hazardous; therefore, *F. tularensis* should be cultured only at biosafety level 3 laboratories. We succeeded in our attempts to isolate *F. tularensis* from lymph node aspirates drawn from

three patients. These isolates appeared to be the first human *F. tularensis* isolates in Bulgaria.

In practice, definitive diagnosis is usually established by serological tests. According to WHO guidelines on tularemia [10], in this study of the 12 (50%) confirmed cases, nine had a four-fold or greater increase of titer between acute and convalescent specimens and three had positive culture results. All 12 probable cases were diagnosed based on a single elevated serum antibody titer to *F. tularensis.* We have included both the confirmed and the probable cases in our study as their epidemiological and clinical features were very similar. Furthermore, none of the cases were vaccinated and the region was known as a non-endemic area for tularemia. Thus the single elevated titer was considered as a sign of recent rather than of past infection. As antibody response against *F. tularensis* is usually detectable 10 to 20 days post infection [23], empiric antimicrobial therapy should not be delayed pending laboratory confirmation.

PCR-based methods provide positive results in the early stage of tularemia, even after antibiotic therapy has been initiated [24, 25]. In this study *F. tularensis* was detected by PCR in the majority of lymph node aspirates and even in surgically extracted conjunctival granuloma [26].

Regarding treatment, there was a long interval between the onset of symptoms and the commencement of effective antimicrobials: 17.7 days in 2003 and two times shorter in 2004. All patients received at least one appropriate antibiotic to treat tularemia [10]. Nevertheless, six (25%) patients experienced therapeutic failure**,** which was related to the delay in the initiation of antibiotics, 10% of the patients when antibiotics started within the first 3 weeks and 35.7% when they received treatment later than the third week. Similar observations have been reported elsewhere [27]. The findings of the current study also correspond with previous reports from Turkey, where the diagnosis of oropharyngreal tularemia was delayed and suppurating lymph nodes developed in 40% and 60% of the cases, respectively [5,15].

Recently, quinolones, especially ciprofloxacin, have emerged as a new treatment option for tularemia type B. The first clinical reports from Scandinavia exhibited excellent response among both children and adults with tularemia [28,29]. Perez-Castrillon *et al.* [13] have revealed that the efficacy of ciprofloxacin was higher than that of streptomycin or doxycycline and was associated with fewer adverse effects. Meric *et al.* [20] later confirmed these results. All our patients treated with ciprofloxacin recovered completely; however, the lack of randomization of the treatment and the limited number of patients prevent us from drawing conclusions. Notwithstanding the limitations, this study has taken a step in the direction of defining the clinical features of oropharyngreal tularemia, which are similar to those reported in previous investigations [5,20].

In conclusion, oropharyngeal tularemia is considered the most common form, as shown in our study. Adequate antibiotic therapy has a limited benefit if it is initiated in the late stage of the disease. Tularemia should be suspected whenever a severe sore throat and cervical lymphadenopathy are present, particularly in those patients not responding to ß-lactam treatment. Animal contact history could guide the diagnosis and empiric antimicrobials should be initiated awaiting laboratory confirmation.

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