

Characteristics of Tularemia in Kosovo during the period 2006-2011

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Abstract

Aim: First cases of tularemia in Kosovo were confirmed in 2000. During the 2000-2002 epidemic outbreak over 600 cases occurred whereas in 2010 over 320 tularemia cases were reported. Our aim was to present the epidemiological, clinical and diagnostic characteristics and treatment of patients with tularemia treated in the Clinic for Infectious Disease at University Clinical Center of Kosovo, during 2006-2011.

Methods: A total of 100 patients were examined. Medical records data including anamnesis, clinical examinations, laboratory tests and treatment were analyzed.

Results: Mean age of patients was 23.7 years. The average hospital stay was 15.7 days/patient. All patients were from rural areas and 66% of them had access only to well water. The predominant manifestation of the disease was the glandular form whereas 19% and 17% of cases presented pharyngeal and pulmonary forms too, respectively. High Erythrocyte Sedimentation rate was recorded in 95% of patients. 51% of cases had slight anemia and 47% had leukocytosis. Agglutination test in all cases was positive. The Polymerase Chain Reaction test was positive in all taken samples and *Francisella tularensis* subspecies *holarctica* was isolated as putative agent. Besides Gentamycin (88%) and Streptomycin (12%), incision and drainage of lymph nodes as accessory therapy was applied in 51% of patients.

Conclusion: Tularemia still represents a public health problem in Kosovo. The glandular form of the disease predominates. Incision and drainage of inflamed glands as accessory therapy has shown to be a good method of treatment for severe forms of disease in combination with antimicrobial therapy.

Keywords: agglutination test, incision and drainage, Kosovo, PCR, tularemia.

Introduction

Tularemia, a zoonotic disease caused by the highly infective, virulent, nonsporulating gram-negative coccobacillus *Francisella tularensis*, is found throughout most of the Northern Hemisphere in a wide range of animal reservoir hosts (1-6). In addition, the organism can be isolated from contaminated environmental sources such as water and mud. It is transmitted to humans by various modes, including direct handling of infectious carcasses, ingestion of contaminated food or water, arthropod bites, or inhalation of infectious dusts or aerosols. Person-to-person transmission is not known to occur (7). In January 2000, the Kosovo Institute of Public Health (IPH) in Prishtina implemented a new surveillance system for 20 communicable disease syndromes. On 22 March 2000, a public health physician in western Kosovo reported a cluster of patients with an unusual syndrome of fever, pharyngitis, and marked cervical lymphadenitis. Tularemia was clinically suspected, and the diagnosis was serologically confirmed on April 14 at the World Health Organization (WHO) regional reference laboratory in Rome. Active case-finding identified other patients at multiple sites in Kosovo who in the previous 6 months had a similar syndrome. Public health records from 1946 disclosed no prior reports of tularemia in Kosovo, but the disease has been reported, although infrequently, from other areas of Yugoslavia and other Balkan states (7). Tularemia has never before been documented in Kosovo in clear clinical picture or atypical form until the first cases of the disease were diagnosed in the south-western Kosovo, in Gjakova region, in April 2000. The disease had spread extensively: 22 cases were from Brovina village and they had consumed water from the local water supply of the village as well as one case each from villages Skivijan, Batuse and Korenica who had direct connections to the source of infection in Brovina (3,8,9). In total during the period of 2000-2010 in Kosovo there were reported 1221 cases with tularemia, respectively 5.2 cases per 100,000 inhabitants (8). Natural appearance of tularemia is rare. Its outbreaks have been documented in war situations including one in 1942 during the Battle of Stalingrad. The reporting of hundreds of cases in Kosovo indicate that the increased risk is associated

with sensitive environments, for example those areas in which agriculture, water and sanitation conditions are severely disturbed in general (3).

The purpose of this study was to present and analyze the epidemiological and clinical data of tularemia disease in Kosovo during the period of 2006-2011.

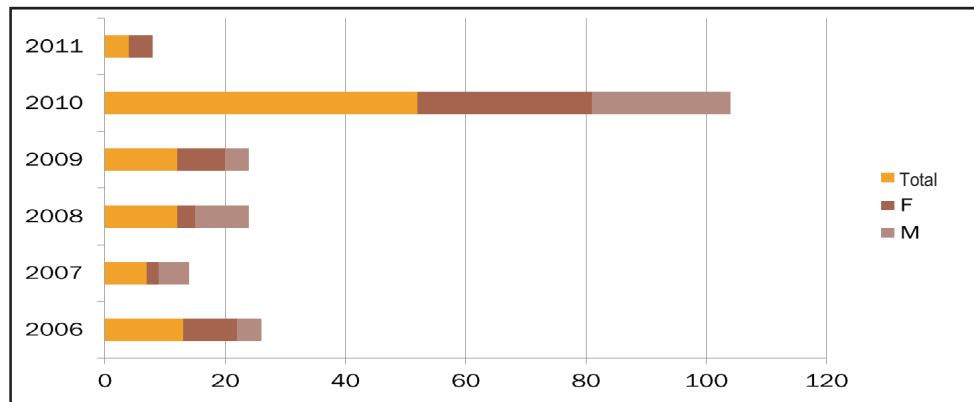
Methods

We have analyzed all patients diagnosed with tularemia and treated in the Clinic for Infectious Disease at University Clinical Center of Kosovo (UCCK), during the period of 2006-2011. Main inclusion criteria were the diagnosis of tularemia from the medical chart and being a Kosovo resident. In total 100 patients, from which 47 children, have been included in the study. Data from medical records including anamnesis of the disease, clinical examination, laboratory tests and treatment were analyzed. Serological test of agglutination was performed for all cases and also the Polymerase Chain Reaction (PCR) test for three samples taken from the wounds of three patients was performed in the National Institute of Public Health in Ljubljana, Slovenia. The main method for the confirmation of the diagnosis of disease was the serological examination.

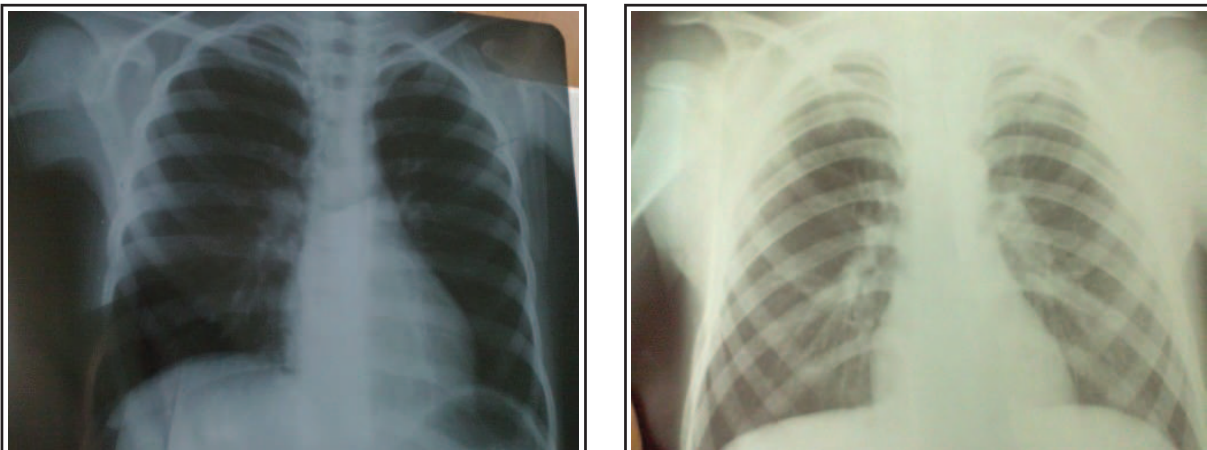
Data processing was done with the statistical package InStat 3. The structure index, the arithmetic mean, standard deviation, minimum and maximum values are calculated. For testing parametric data the student test was used, while for non parametric data the Mann Whitney test and Kruskal Wallis tests were employed. Also Fisher's exact test and chi square test was used to analyze categorical variables. Presentation of data is done through tables and graphs.

Results

The average age of patients was 23.7 years and 46% of patients were under the age of 18. The average hospitalization stay per patient was 15.7 days. All patients were living in rural areas and 66% of them reported water supply only wells. 55% of patients were females. The largest number of patients (52 in total) was treated during the year 2010 (Graph 1). The majority of cases (80%) were hospitalized during winter period (December-February).

Graph 1. Total number of cases by gender during the years

Regarding clinical manifestations, glandular form of tularemia was predominant and found in 97% of cases. Figure 1 visualizes such form of the disease. In 19% of cases and 17% of cases the pharyngeal and pulmonary form (Figure 2) of the disease was present too, respectively. 2% of patients had ulceroglandular form, 1% oculoglandular form and 3% of cases manifested typhoid form.

Figure 1. Clinical forms of tularemia**Figure 2. Pulmonary radio images of patients with tularemia (showing bronchopneumonia)**

The high temperature was present in 100% of cases, lymph gland swelling in 97%, phlegm in 93%, sore throat in 89% and fever in 81% of patients (Table 1).

Table 1. Clinical symptoms of the disease by gender

Symptoms	Females		Males		Total*		P-value†
	Number	Percentage	Number	Percentage	Number	Percentage	
Temperature	55	100.0	45	100.0	100	100.0	1.00
Sweating	19	34.5	23	51.1	42	42.0	0.142
Pain	50	90.9	39	86.7	89	89.0	0.723
Apathy	46	83.6	30	66.7	76	76.0	0.08
Fever	31	56.4	20	44.4	51	51.0	0.324
Swelling of the glands	53	96.3	44	97.7	97	97.0	1.00
Lymph Gland induration	13	23.6	14	31.1	27	27.0	0.541

* The total number of patients with the given symptom.

† P-value from the chi square test.

The laboratory data: Increased sediment of erythrocytes (SE) has been examined in 95% of patients, middle anemia was detected in 51% of cases and leukocytosis was present in 47% of cases.

Agglutination test was positive at the second week in 91% of patients. As shown in Table 2, in 9% of cases agglutination test was negative after two weeks, while during the second time after 4 weeks test has been positive. The first agglutination test titer was 1: 80 in 4% of cases, 1:160 at 2% of cases, and 1:320 in 74% of all cases.

Table 2. Level of the titer by weeks

Weeks	Level of titer	Negative	1:80	1:160	1:320	1:1280	1:2500	1:5100	Positive	Total
	I-II		9*	4	2	74	3	4	1	3
After week IV		1	2	3	61	0	1	2	30	100

* Number of patients with the respective level of titer. In this case the absolute number coincides with the respective percentage as there are 100 patients under study.

In the second testing after the first month: in one case agglutination test was negative, and in 61 cases test was positive with agglutination titer of 1:320. Diagnosis was confirmed by agglutination test in almost all cases (99%). Only in one case the diagnosis was first set by histopathological analysis, which was later confirmed with agglutination test (1,2,3,4). During the second test all samples were positive. For the first time in Kosovo, the examination of Polymerase Chain Reaction (PCR) test was positive in all three samples taken from wounds of three patients (100%). In all three examined samples

Francisella tularensis subspecies holartica was isolated as causative agent of tularemia.

Regarding the therapy, patients were treated with antibiotics: Gentamicin, Streptomycin, Erythromycin, Bactrim and Ampicillin. Gentamicin was applied in 88 cases (88%), while in 12 cases (12%) it was applied Streptomycin. In addition, in 6 cases (6%) other antibiotics were administered as well (Table 3). Incision and drainage of lymph nodes as accessory therapy was applied in 51% of patients, who were of older age, with long incubation and severe forms of the disease.

Table 3. Application of the treatment therapy

Medications	Gentamycin	Streptomycin	Other antibiotics	Total
No. of patients with basal therapy	88*	12	6	100
No. of patients with recidivist therapy	11	21	0	32

* Number of patients with the respective level of titer. In this case the absolute number coincides with the respective percentage as there are 100 patients under study.

Discussion

Tularemia is an epidemic disease in Kosovo, which occurs in sporadic forms over the years. In Kosovo the mean incidence of tularemia during 2006-2011 was 5.2 / 100,000 inhabitants, which is comparable to that in Sweden for the same period (3.2 per 100,000 inhabitants), which is known to be endemic for tularemia, but this incidence is much higher than that in Germany (0.013 per 100,000 inhabitants) (8). A study done in Norway reports that the number of cases with tularemia in 2010 was 1.4 cases per 100 000 inhabitants (10), while another study reports that in U.S. the number of cases with tularemia was 0.6 / 100 000 inhabitants during the period of 2001-2010 (9).

All patients treated in our study are from rural areas which is in accordance with reports that tularemia is a disease that is more common and occurs in people who live in rural areas (3,4,7,8).

The social epidemiological conditions are important factor for transmission of tularemia. In our study 66% of patients reported that their only supply with drinking water is from wells. A study from Bulgaria reports that the source of infection with tularemia was food and water contaminated from rodents (11). In our study most of the cases (80%) were hospitalized during the winter period (December-February), which is comparable to the reports of the cases in Turkey which mostly occurred during Autumn-winter period (3,12,13).

The predominant clinical signs of patients in our study were fever 100%, swelling of the lymph glands 97%, lethargy 76%, neck pain 89%, and sweating 42%. In a study report from Turkey, sore throat was present in 100% cases, temperature 93%, myalgia 100% lymphadenopathy 100%, and pharyngeal hyperemia 85% (14).

In our study, 19% of patients had oropharyngeal manifestation of tularemia. Oropharyngeal form of the disease was common in a study report from Turkey: in epidemics that occurred between 1936

and 2004, 387 out of 507 cases or 77% are referred like oropharyngeal forms (15). In another study in the city of Bursa in Turkey, 83% of patients had oropharyngeal form also. (16).

In our study 17% of cases had pulmonary manifestations. In one review in America, ulcer glandular tularemia was accompanied with pneumonia in 30% of cases (17).

Diagnosis was confirmed by agglutination test in almost all cases (99%). Only in one case the diagnosis was put by histopathological examination, which later was confirmed with agglutination test, too. During the first test 76% of samples were positive, 1:320 in 74%, and during the second test all samples were positive. In a study report in America all results of the first test were negative (1:40), but results of tests repeated after 14 days have been all positive, 1:320 (18).

For the first time in Kosovo, examination of Polymerase Chain Reaction (PCR) test was positive in all three samples taken from wounds of three patients (100%) and *Francisella tularensis subspecies holartica* was isolated as causative agent of tularemia. Helvacı has isolated the bacteria in 49% of his patients (16), while Bevanger has isolated the bacteria only in one of 57 patients (19).

In addition to treatment with antibiotics (88 cases were treated with Gentamicin, only 12 cases were treated with Streptomycin and 6 cases with other antibiotics), we applied incision and drainage of lymph nodes as accessory therapy in 51% of patients, who were of older age, with long incubation and severe forms of the disease. Although the literature points out the risk of staff infection during the application of the incision and drainage of lymph nodes interventions (1-6), we haven't experienced such cases during treatment of our patients. Therefore, it may be concluded that we found the incision to be a good method for treating severe forms of tularemia in combination with antimicrobial therapy.

Conclusion

Tularemia continues to be one of the diseases that pose a public health problem in Kosovo. Glandular form is predominant manifestation of the disease. (PCR) test was positive in all three samples taken from wounds of three patients (100%) and

Francisella tularensis subspecies holarctica was isolated as causative agent of tularemia in Kosovo. Incision and drainage of the inflamed glands as accessory therapy has shown to be a good method of treatment for severe forms of disease in combination with antimicrobial therapy.

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