Original Article

Evaluation of patients with Tularemia in Bolu province in northwestern Anatolia, Turkey

Zafer Mengeloglu[1], Arif Duran[2], Ismail Necati Hakyemez[3], Tarik Ocak[2], Abdülkadir Kücükbayrak[4], Mustafa Karadag[5], Tekin Tas[1], Hayrettin Akdeniz[4]

[1] *Department of Medical Microbiology, Abant Izzet Baysal University, Faculty of Medicine, Bolu, Turkey*
[2] *Department of Emergency, Abant Izzet Baysal University, Faculty of Medicine, Bolu, Turkey*
[3] *Department of Infectious Diseases and Clinical Microbiology, Bezmialem University, Faculty of Medicine, Istanbul, Turkey*
[4] *Department of Infectious Diseases and Clinical Microbiology, Abant Izzet Baysal University, Faculty of Medicine, Bolu, Turkey*
[5] *Department of Contagious Diseases, Bolu Provinceal Health Directorate, Bolu, Turkey*

Abstract

Introduction: Tularemia is a zoonotic disease caused by *Francisella tularensis*. Here we present an epidemic occurring in Bolu province, located in northwestern Anatolia in Turkey, and some features of the cases.

Methodology: The data was provided by the Bolu Provincial Health Directorate. All of the antibody response tests were studied in the National Health Institute (formerly named Refik Saydam Hygiene Department), the reference laboratory of the Ministry of Health of the Turkish Republic. A total of 393 individuals were tested by microagglutination test (MAT) for tularemia between 2006 and 2011. A total of 218 patients whose demographical data were available were included in the study; 83 were accepted as the patient group and 135 were the controls. Of the patients, 31 (37.3%) were male and 52 (62.7%) were female.

Results: Fever ($p < 0.001$), URTI symptoms ($p = 0.047$), conjunctivitis ($p = 0.004$), and rash ($p = 0.026$) were significantly higher in the patient group. A positive association was found between MAT and fever ($r = 0.324$; $p < 0.001$), and a negative association was found between MAT and both lymphoadenopathy ($r = -0.25$; $p = 0.013$) and chills ($r = -0.218$; $p = 0.035$). Higher MAT titers were detected in oropharyngeal tularemia ($r = 0.306$; $p = 0.003$).

Conclusions: In conclusion, tularemia must be considered in differential diagnosis in patients presenting with fever and LAP in non-endemic regions. Furthermore, water sources and contact with rodents must be investigated.

Key words: tularemia; Turkey; Bolu; microagglutination

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Introduction

Tularemia, observed in the northern hemisphere in general, is a zoonotic disease caused by *Francisella tularensis*, a Gram-negative coccobacillus that can grow intracellularly. *F. tularensis* is quite resistant to environmental conditions and can live for a long time in cold and moist soil. *F. tularensis* is classified into four subspecies – *F. tularensis* subsp. *tularensis,* subsp. *holarctica,* subsp. *mediaasiatica* and subsp. *novicida* – all of which vary in terms of virulence in humans and rabbits. *F. tularensis* subsp. *tularensis*, the most virulent subspecies, is commonly observed in the United States, while *F. tularensis* subsp. *holarctica*, the least virulent subscpecies, is the most frequent subspecies in Turkey [1-3].

The causative pathogen of tularemia transmits to humans via direct contact with skin and mucosal surfaces, the consumption of contaminated food and water, and the inhalation of aerosols. Consumption of food or water contaminated with infected animal tissue is one of the major transmission ways of epidemics of tularemia [1,4].

The first tularemia epidemic in Turkey was reported in 1936, and since then, other epidemics have been seen, especially in the northwestern part of the country [5]. Here we present the first tularemia epidemic occurring in Bolu province, located in northwestern Anatolia, and some features of the cases.

Methodology

This study was conducted with permission of the Bolu Provincial Health Directorate of the Health Ministry of the Turkish Republic and was approved by the Abant Izzet Baysal University Clinic Research Ethical Committee. The data was provided by the Bolu Provincial Health Directorate. All of the antibody response tests were studied in National Health Institute (formerly named Refik Saydam Hygiene Department), the reference laboratory of the Ministry of Health of the Turkish Republic.

A total of 393 individuals were tested for microagglutination titer (MAT) for tularemia between 2006 and 2011 in the province of Bolu. A total of 218 patients whose demographic data were available were included in the study; 83 were accepted as the patient group and 135 were the controls. Presence of fever, syptoms and/or findings of upper respiratory tract infections (URTIs), lymphadenopathy (LAP), chills, fatigue, headache, skin ulcer and erythema, conjunctivitis, and diarrhea were evaluated in individuals whose sera were tested with a prediagnosis of tularemia. The prediagnosis of clinical forms and microagglutination test titers (MAT) of the patients were recorded.

Statistical analyses were conducted using the Statistical Package for the Social Sciences version 17.0 for Windows (SPSS, Inc., Chicago, IL, USA). Mean comparisons for continuous variables were done using independent group *t* tests. Proportion comparisons for categorical variables were done using Chi-square tests, although Fisher's exact test was used when data were sparse. P values < 0.05 were accepted as statistically significant.

Results

Of 83 patients whose data were available, 31 (37.3%) were male and 52 (62.7%) were female. The mean age was 39.94 ± 19.24 (0-78). In terms of jobs, 50% of the patients were housewives, 21.9% were farmers, and 18.8% were students (Table 1).

In terms of the symptoms and findings, fever was observed in 77.1% of the patients, URTI symptoms were observed in 75.9%, LAP in 66.3%, chills in 25.3%, headache in 18.1%, fatigue in 13.3%, and skin ulceration in 7.2%. Among the patients with LAP, 60.2% of them had cervical, 4.8% had submandibular, and one patient had preauricular LAPs (Table 1). All the data about symptoms, clinical forms, and MAT titers are shown in Table 1.

A total of 53 (63.9%) of the patients had an oropharyngeal clinical form of tularemia, 17 (20.5%) had glandular, 8 (9.6%) had ulceroglandular, and 5 (6.0%) had oculoglandular forms (Table 1). Most of the patients (31; 37.3%) had 1: 1280 titers, and 20 (24.1%) had 1: 640 titers (Table 1).

Fever ($p < 0.001$), URTI symptoms ($p = 0.047$), conjunctivitis ($p = 0.004$), and rash ($p = 0.026$) were found to be significantly higher in the patient group (Table 2).

A positive association was found between MAT and fever ($r = 0.324$; $p < 0.001$), and a negative association was found between MAT and both lymphoadenopathy ($r = -0.25$; $p = 0.013$) and chills ($r = -0.218$; $p = 0.035$). Higher MAT titers were detected in oropharyngeal tularemia ($r = 0.306$; $p = 0.003$) (Table 3).

Discussion

Tularemia is an endemic disease in Turkey. The first tularemia outbreak was reported in 1936 in the Trakya region. In the years following this outbreak, many epidemic or sporadic cases were reported from different sites in Turkey [6].

In our country, most of tularemia epidemics have been considered to be related to water. It is believed that similar epidemics have been prevented with consumption of water in hygienic conditions and chlorination of water at regular intervals [6]. In epidemics of our country, it has been reported that tularemia was observed in people living in rural areas. In these places, *F. tularensis* reservoirs may be caused by contamination of water sources and food. The survival of *F. tularensis* in water for months supports this hypothesis. Insufficient cleaning, chlorination, and precautions against small rodents in the water tanks have been reported in areas where tularemi have been observed. In addition, in these places, it is reported that water from uncontrolled rivers and sources is used [7]. Besides this, the presence of rodents in houses or surrounding areas is reported to be an important risk factor, as contact with them or their stools or urine is possible [5,7,8]. Similar to other studies, most of the patients in our study lived in rural areas of Bolu. Most of the transmissions are believed to be caused by contaminated water or contact with rodent products [5,7,8].

Similar with living in rural areas, jobs such as farming or animal husbandry that require spending much time in contact with materials that might be contaminated with infected animal products are reported to be risk factors for tularemia [9].

Table 1. Distribution of the patients in terms of symptoms, clinical forms, and MAT titers

Symptoms	%	Jobs	%	Clinical forms	%	MAT	n (%)
Fever	77.1	Housewife	50.0	Oropharyngeal	63.9	1:160	12 (14.5)
URTIS	75.7	Farmer	21.9	Glandular	20.5	1:320	16 (19.3)
LAP	66.3	Student	18.8	Ulceroglandular	9.6	1:640	20 (24.1)
Chills	25.3	Officer	6.2	Oculoglandular	6.0	1:1280	31 (37.3)
Fatigue	13.3	Retired	3.1			1:2560	4 (4.8)
Headache	18.1						
Skin ulcer	7.2						
Conjunctivitis	6.0						
Diarrheae	1.2						

MAT: microagglutination titer; URTIS: upper respiratory tract infection symptoms; LAP: lymphadenopathy

Table 2. Distribution of symptoms according to tularemia results

Variables	Results	Tularemia-positive		Tularemia-negative		p
		n	%	n	%	
Sex	Female	52	63	61	45	0.012
	Male	31	37	74	55	
Fever	Positive	19	23	72	53	< 0.001
	Negative	64	77	63	47	
URTIS*	Positive	20	24	50	37	0.047
	Negative	63	76	85	63	
Chills	Positive	62	75	105	78	0.60
	Negative	21	25	30	22	
Fatigue	Positive	72	87	112	83	0.46
	Negative	11	13	23	17	
Headache	Positive	68	82	122	90	0.10
	Negative	15	18	14	10	
Skin ulceration	Positive	77	93	130	96	0.25
	Negative	6	7	5	4	
Conjunctivitis	Positive	78	94	135	100	0.004
	Negative	5	6	0	0	
Rash	Positive	80	96	135	100	0.026
	Negative	3	4	0	0	
Diarrhaea	Positive	82	99	135	100	0.20
	Negative	1	1	0	0	
Lymphadenopathy	Positive	28	34	44	33	0.86
	Negative	55	66	91	67	

*upper respiratory tract infection symptoms

Table 3. Association between some symptoms and MAT

Symptoms	MAT*	
	r	P
Fever	0.324	< 0.001**
Chills	-0.218	0.035***
LAP	-0.250	0.013***

*microagglutination titer
** significance according to $p < 0.05$
*** significance according to $p < 0.01$

In addition, these jobs may cause increased risk in exposure of ticks or mosquitos, both of which are reported to be the most common vectors in the transmission of tularemia [9,10]. In the present study, all of the female patients were housewives, and most of the male patients were retired. This result makes us consider that these patient groups had a long duration of contact with the water sources that caused the epidemics, because they lived in epidemic areas. Additionally, most of housewives and retired men living in rural areas spend time engaged in animal husbandry; they accept animal husbandry as a lifestyle and they do not consider animal husbandry to be a kind of job that must be reported in questionnaires in medical centers [7,9]. Therefore, we consider that the high rate of housewives and retired men in our study is not an accurate result, and that the patients in these two groups may be part of the animal husbandry group. However, the farmers in our study cannot be considered to be part of this group, as they were never in contact with animals, while the students living in rural areas must be investigated if they spend their time out of school engaged in animal husbandry. These considerations seem accurate because no patient reported having animal husbandry as a job in the records we evaluated, which does not make sense and is extremely discordant with the literature. We believe that this topic needs to be clarified with further epidemiological studies.

Clinical symptoms and findings in tularemia vary depending on the virulence, inoculum dose, entrance way of the causative pathogen, and the immune condition of the host. Clinical manifestation can be asymptomatic as well, as it varies in range from mild disease to fatal infection. The disease has six clinical forms: ulceroglandular, glandular, oculoglandular, oropharyngeal, pneumonical, and typhoidal forms. The most common forms in Europe are ulceroglandular and glandular forms, while the oropharyngeal form is the most frequent one in Turkey [4,11]. Akinci *et al.* reported that all of their patients had the oropharyngeal form, and in addition, 25% of them also had the oculoglandular form [12]. It was reported that 62.5% of the patients had the oculoglandular form by Dikici *et al.* [7]. Helvaci *et al.* reported that 83% of 205 cases that they followed between 1988 and 1998 in Bursa had presented with oropharyngeal involvement [13]. In the present study, the oropharyngeal form was the most common clinical form in Bolu province.

Oropharyngeal tularemia is a manifestation characterized by membranous tonsillopharingitis and cervical LAP. Lymph nodes may be suppurated spontaneously in cases where treatment is delayed. LAP develops following tonsillopharyngitis and continues for weeks, or months in general. As a result, in cases of membraneous tonsillopharyngitis and LAP, which do not respond to therapy of bacterial angina in endemic regions, tularemia must be considered in differential diagnosis [1,2].

Helvaci *et al.* reported that LAP was observed in 85% of their patients, fever in 66%, and sore throat in 58% [13]. They found that most of the LAPs were cervical and unilateral [13]. In our study, LAP was found in 66.3% of the patients; the most common findings were fever (77.1%) and URTI symptoms (75.7%). In agreement with the study of Helvaci *et al.*, most of our cases with LAP had the cervical form, with a rate of 60.2% [13]. Dikici *et al.* reported that 90% of their patients had cervical LAP [7], and that the most common symptoms and findings were LAP (85%), sore throat, and chills [7]. All of the patients in the study of Akinci *et al.* had LAP, with unilateral cervical LAP found in 18 (56.3%) patients [12]. The literature from our country about LAP rates has reported rates of between 85% and 100%; our findings, in which LAP was commonly observed, were in agreement with the literature [7].

Oropharyngeal tularemia presents with sore throat, fever, erythema in oral and pharyngeal mucosal membranes, and hyperthrpy in tonsils and cervical lymph nodes [4,14]. The most common symptoms are reported to be fever and sore throat [15,16]. Akinci *et al.* found that 94% (30/32) of their patients had fever and 23 (72%) had sore throat [12]. In our study, fever was observed in 77.1% of patients, being the most common symptom, and other URI sypmtoms were observed in 75.9% of the patients.

Akinci *et al.* reported that all of the age groups and both genders were affected equally [12]. In contrast, in the study of Kilic *et al.*, which included 1,091 tularemia cases, most of the cases were in the 30-44 year age group, 10% of the patients were children, and women were affected 1.2 times more than men [17]. In the study of Dikici *et al.*, 62.5% of the patients were females, the mean age was 37.6, 12.% of the patients were children; these results were in accord with the results of Kilic *et al.* [7,17].

Ulu-Kilic *et al.* reported that being younger than 40 years of age was detected as a significant marker for the disease in their study [8]. They considered that this result depended on the longer exposure of young people to risk factors [8]. However, this result contradicts other studies, stating the increased sensitivity to the disease with advancing age.

We found a positive association between MAT and fever, and a negative association between MAT and both of lymphoadenopathy and chills. In addition, in the present study, higher MAT titers were detected in oropharyngeal tularemia. To the best of our knowledge, these associations with MAT have not been reported in previous studies.

We consider that tularemia must be considered in differential diagnosis in patients presenting with fever and LAP in non-endemic regions, and that the water sources and contact with rodents must be investigated. The young population is more exposed to risk factors. Contact with rodent waste in homes is a risk for tularemia. Tularemia spreads to non-endemic regions in our country and is a threat to public health.

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Corresponding author

Zafer Mengeloglu, M.D.
Assistant Professor of Microbiology
Faculty of Medicine, Department of Medical Microbiology
Abant Izzet Baysal University
14280 Bolu, Turkey
Phone: 90-374-2534656 / 3264
Fax: 90-374-2534559
Email: mengeloglu@gmail.com

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