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An Outbreak of Tularemia in Western Black Sea Region of Turkey

Şaban Gürcan[1], Müşerref Tatman-Otkun[1], Metin Otkun[1], Osman Kürşat Arikan[2], and Burçin Ozer[3]

Departments of [1]Microbiology and Clinical Microbiology and [3]Institute of Health Sciences, Trakya University Medical Faculty, Edirne, Turkey;
[2]Department of Otolaryngology-Head and Neck Surgery, Kırıkkale University Medical Faculty, Kırıkkale, Turkey.

The aim of this study was to investigate the source and the size of a tularemia outbreak in a village located in a non-endemic area. Five patients from the same village were admitted to hospital with the same complaints all within one week of September 2001. Tularemia was suspected and a diagnosis was made after physical and anamnesis examinations. The village was visited the same week that the patients were admitted to the hospital, in the January and April 2002. The villagers were examined and screened serologically by microagglutination method and the water sources were investigated bacteriologically. A total of 14 people were found to be infected from the outbreak and the oropharyngeal form was the only clinical presentation. Antibody titers ranged between 1 : 80 and 1 : 640. The patients responded well to the aminoglycoside plus tetracycline therapy. Examination of the pipewater and three springs revealed that all the water sources were contaminated by coliforms, however, *Francisella tularensis* could not be isolated in glucose-cystine medium. Antibody levels stayed stable or decreased seven months after. Tularemia had not been reported in this area before, so the first patients were misdiagnosed. In conclusion tularemia should be considered in differential diagnosis of patients with fever, sore throat and cervical lymphadenopaties.

Key Words: *Francisella tularensis*, tularemia, outbreak

INTRODUCTION

Tularemia caused by *Francisella tularensis* is a zoonosis frequently fatal in animals such as rats, guinea pigs, rabbits etc., but rarely fatal in humans.[1] Important vectors of the illness are blood-sucking arthropods and mosquitoes. The major animals infecting humans are hares, beavers and microtine rodents.[2] *F. tularensis* can survive in the carcasses of animals and contaminated water for many weeks.[3]

Tularemia epidemics have seasonal characteristics. While epidemics of *F. tularensis* var. tularensis are mainly seen in summer and are disseminated via ticks, *F. tularensis* var. palearctica mainly causes outbreaks during the fall and winter periods and is disseminated via contaminated water, rodents, and aquatic animals. Infection routes include the skin or mucosa by direct contact and inhalation.[4]

The clinical course of the illness may vary according to the site of entry and the virulence of the microorganism. Infected individuals may remain asymptomatic or the disease may progress to clinical forms, such as ulceroglandular, ocular, oropharyngeal, glandular, typhoid, pleuropulmonary, and gastrointestinal forms.[2,4]

Tularemia was suspected in five patients admitted to Gerede State Hospital (GSH) with swollen neck, fever and sore throat. The patients had not responded to previous therapy with penicillin V or amoxicillin-clavulanate. The aim of this study was to investigate the source and the size of the outbreak as well as to follow up the antibody levels in patients over the months following the outbreak.

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Reprint address: requests to Dr. Şaban Gürcan, Department of Microbiology and Clinical Microbiology, Trakya University Medical Faculty, 22030 Edirne, Turkey. Tel: 90-284-2357641-1607, Fax: 90-284-2361654, E-mail: sabangurcan@trakya.edu.tr

MATERIAL AND METHODS

Case-definition

The tularemia cases were diagnosed according to the case definitions of Centers for Disease Control and Prevention.[5] Clinically compatible cases with a fourfold, or greater, change in the serum antibody titer to *F. tularensis* antigen and those with elevated serum antibody titers without documented fourfold or greater change were considered as confirmed and probable tularemia cases, respectively.

Microagglutination method

The antigen was prepared from a *F. tularensis* strain which was isolated by Gedikoğlu, et al.,[4] which strongly agglutinates with Bacto *Francisella tularensis* antiserum (Difco 2241-47, USA). Microagglutination was applied in V-plate. Serial dilutions of sera (1:10, 1:20, etc) were prepared with saline in each well to a volume of 25-μl and then mixed with 25-μl antigen. The plates were incubated overnight at 37℃ by covering with parafilm and the agglutination was evaluated with the naked eye. The cut-off value for positivity was accepted as the 1 : 80 dilution.[4,6]

Culture for *F. tularensis*

For each water sample, 100 ml was centrifuged and the sediment cultured in blood agar supplemented with 1% glucose and 0.1% cystine.

Study design

In the first week of September 2001, five patients, all from a village in Gerede County, Bolu Province (Fig. 1), were admitted to GSH complaining of swollen neck, fever and sore throat. No pathogenic bacteria were cultured in routine throat cultures and the patients did not respond to beta-lactam antibiotics. Tularemia was suspected in these patients and they were serologically tested for the presence of tularemia antibodies using a microagglutination technique.[4]

The patients' village was visited during the same week in order to investigate the source of the outbreak (visit 1). An investigation team was established, which was led by an infectious diseases specialist (author no. 1), and included an otorhinolaryngology specialist (author no. 4), a general practitioner, an environmental health technician, and a nurse. They examined only the villagers who had symptoms, and patients clinically compatible with tularemia were treated with aminoglycoside plus tetracycline. In addition, water samples were collected from the reservoir pipe water and from three springs in the vicinity of the village and these were sent to Bolu Province Public Health Laboratory. Recommendations were made to control the outbreak.

The village was re-visited in January 2002 (visit 2) and all 108 villagers were examined. Each villager was given a questionnaire relating to the symptoms and the clinical findings in respect to tularemia, as well as the probable sources of infection (consumption of the pipe or spring waters, contact with a rodent or biting midges) and the

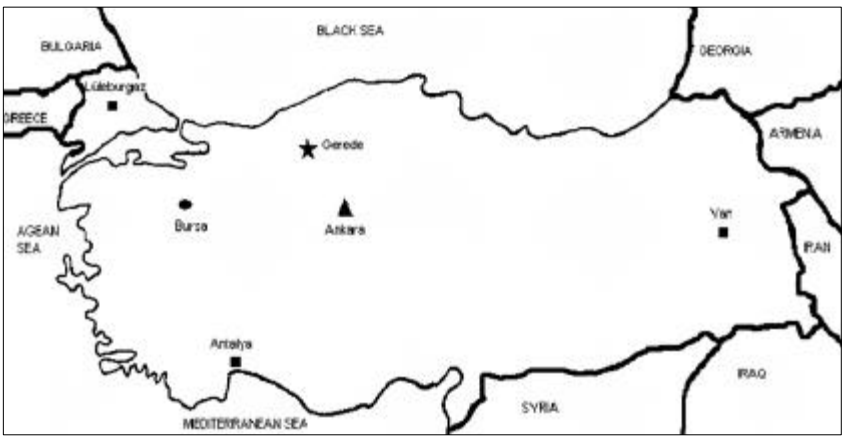

Fig. 1. The regions of reported tularemia outbreaks in Turkey. ■ The outbreak regions between 1928 and 1954. ● The outbreak region in 1988 - 2002. ▲ The outbreak region in 2000. ★ The outbreak region in this study.

history of vaccination for tularemia. The same water sources were sampled again, and cultured for tularemia. The tularemia antibody titers of the villagers were investigated in this visit and again in April 2002 (visit 3). All the sera were also examined by Rose-Bengal test (Diolab, Austria) for brucellosis to eliminate cross-reactions.[2] The study was performed with permission from the Local Committee of Ethics in Science. Participation was on a voluntary basis and in accordance with the guidelines of the Helsinki II declaration.

Fisher's chi-square test was used for statistical analysis.

RESULTS

When admitted to GSH in September 2001, five patients were antibody negative (Table 1). The patients could not be visited one month later, however, in the second visit in January 2002 seroconversion ($\geq$ 1:160) was detected in four of them. Thus, the diagnosis was confirmed as tularemia.

During the first visit to the village, three more cases with similar symptoms were found. The reservoir that supplied piped water to the village was surrounded by a wire fence, which was inadequate for preventing of the entry of small

Table 1. Serological Results of Tularemia Related Individuals

Case No	Age	On admission	Antibody titers		Definition
			(4^{th} month)	(7^{th} month)	
1*	19	1:20 -	1:320	1:160	Confirmed
2*	25	1:20 -	1:160	1:160	Confirmed
3*	40	1:20 -	1:20 -	1:20 -	†
4*	50	1:20 -	1:320	1:160	Confirmed
5*	56	1:20 -	1:160	1:80	Confirmed
6‡	30		1:160	1:160	Probable
7	7		1:640	1:160	Probable
8	10		1:160	1:160	Probable
9	14		1:640	1:20 -	Probable
10	19		1:640	1:320	Probable
11	21		1:80	1:40	Probable
12	45		1:80	Not available	Probable
13	55		1:160	1:80	Probable
14	56		1:20 -	1:20 -	†
15	60		1:80	1:80	Probable
16	63		1:320	1:320	Probable
17	70		1:20 -	Not available	†
18	10		1:80	1:40	§
19	10		1:80	1:40	§
20	55		1:80	1:80	§
21	70		1:80	1:40	§

*The first patients who had applied to GSH.
†Clinically compatible with tularemia but antibody negative patients.
‡Case living in Istanbul.
§Antibody positive for tularemia but there are no symptoms.

animals. In addition, the reservoir had not been cleaned for years and chlorination had never been performed. The villagers reported finding a rat carcass in the reservoir one month before the outbreak. Measures to prevent the entry of small animals to the reservoir, as well as periodic cleansing and chlorination, were recommended. In January 2002, the village was revisited (visit 2). A total of 108 people (48 males and 60 females) were living at the village. The average year of them was 40.5 ± 23.6 (minimum: 1, maximum: 83). All the villagers were examined and six more cases with similar clinical findings and history were found. Also, an individual from Istanbul who had stayed in the village during the first week of September, 2001 and who had similar symptoms, was included into the study. These additional ten patients (three patients in first visit plus six patients in second visit and the patient from Istanbul) were evaluated "probable tularemia" according to serological assays (Table 1). Additionally, tularemia antibodies were positive in 1:80 dilutions in four individuals with no symptoms, while three individuals (including one of the patients admitted to GSH initially) who had the same clinical findings during the outbreak were antibody negative. Rose-Bengal tests were negative in all patients and nobody was vaccinated for tularemia. The age and gender were not significant parameters for the disease (Table 2).

Both the reservoir pipe water and the spring water samples that were collected during visit 1, and subsequently sent to Bolu Province Public Health Laboratories, had coliform counts that were higher than those permitted by the Turkish Food Regulation for human drinking water. Therefore, these water sources were contaminated at a level perceived to be harmful to human health. Unfortunately, these samples were not cultured for *F. tularensis*. Although blood agar supplemented with glucose-cystine was used, *F. tularensis* did not grow in samples taken four months later during visit 2.

Of the 108 villagers, 52 used only the pipe water and 56 used both the pipe and spring water (Table 3). The source of water was not a significant risk factor for the disease. History of contact with rodents or biting midges could not be taken as being reliable although the villagers said that voles or rats were generally seen in the village. Also, it was noted that the measures recommended during the former visit were not taken.

In April 2002 (visit 3) the microagglutination tests for tularemia were performed in 19 of the 21 persons that were diagnosed with tularemia, including those that were asymptomatic or

Table 2. Distribution of the Villagers According to Age and Gender

Age	Sick		Healthy		Total
	Female	Male	Female	Male	
≤ 15	0	2	9	12	23
16-40	3	2	19	8	32
≥ 41	6	1	23	23	53
Total	9	5	51	43	108

Table 3. Distribution of the Villagers According to Usage of the Water Sources

Water sources	Sick		Healthy		Total
	Female	Male	Female	Male	
Only the pipe water	7	1	31	13	52
Both the pipe and spring water	2	4	20	30	56
Total	9	5	51	43	108

antibody negative. The results are shown in the last column of Table 1. The titers stayed stable or were slightly decreased.

DISCUSSION

The definitive diagnosis of tularemia requires the isolation of a causative agent, which is rather difficult. Thus, tularemia is diagnosed serologically, either on the basis of a high titer in a single serum specimen or, in most cases, by demonstration of significant increase in antibody titer in paired samples. However, antibody levels do not usually increase until the third week of illness.[2] The recommended titers for tularemia diagnosis in a single serum specimen are 1 : 160, in general, but several authors[4,6] suppose that positivity in the ≥ 1:80 dilution is sufficient. Since the causative agent could not be cultured, confirmation of the cases in this outbreak was made by antibody titers.

Three patients (case numbers 3, 14, 17) were antibody negative and clinically compatible with tularemia, although they did not meet our case definition criteria. Tularemia was suspected since their complaints started during the epidemic period, and they benefited from the therapy used for treating tularemia. Just as Helvacı, et al.[7] reported antibody negative but culture positive cases of tularemia, negative serology should not eliminate its diagnosis.[8]

The rate of asymptomatic cases reported in outbreaks in Turkey range between 4-13.4%.[4,7] We detected four individuals without symptoms, but with tularemia antibodies in the 1:80 dilution, in the outbreak region with some of these being confirmed and some probable tularemia cases. Since no one was vaccinated in the village, and the region was known as a non-endemic area for tularemia, the latter persons could also be asymptomatic cases.

Tularemia outbreaks were reported in the Thrace,[9] Van,[10] Antalya,[11] Bursa,[12] and Ankara[13] regions in Turkey (Fig. 1) and sporadic cases have only been continuing in the Bursa region. There have been no previously reported tularemia cases in the Black Sea Region of Turkey with this being the first of its kind. The onset of the outbreak for the majority of the cases was in September 2001, and then it continued at this intensity for four months (Fig. 2). There were no more outbreaks or sporadic tularemia cases in the vicinity afterwards.

The ulcero-glandular form is the most commonly reported clinical presentation in international literature,[2] but Helvacı, et al.[7] reported that the oropharyngeal form was the most common presentation of the tularemia outbreaks in Bursa with a prevalence of 83%. The causative organism enters the human body by consumption of contaminated water or food in the oropharyngeal form,[3] which was the clinical manifestation of all our cases. Since there were no gender and age differences, it was most likely that all the villagers were subject to a common source of infection such as water. In other reported outbreaks in Turkey recently, the water sources had been described as being the source of infection.[12,13] The consumption of the spring waters, not used by almost half of the villagers, was not a risk factor for this disease according to the case-control study. On the other hand, the reservoir, where a rat carcass had been found, might have been the source, since all the villagers used this water. The cultures carried out in September 2001 in Public Health Laboratories, confirmed that there was biological contamination in all water sources in the village. However, the water samples were not cultured for *F. tularensis* in the first sampling. In the second samples, collected four months later, there was an absence of growth of *F. tularensis* in specific media,

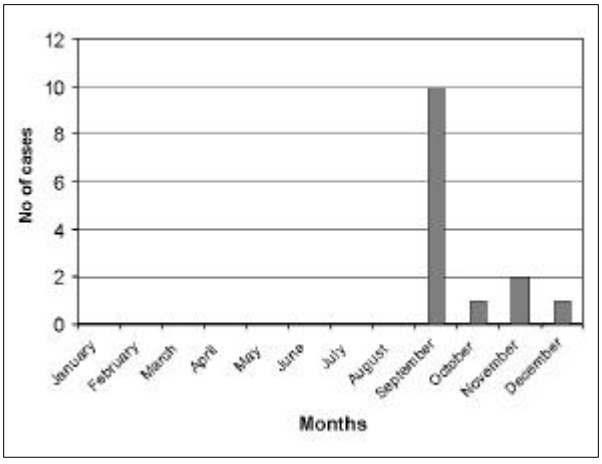

Fig. 2. Monthly distribution of tularemia cases.

perhaps due to the long period from start of the outbreak. The last case was detected one month before the second samples were taken. As reported by the villagers, the permanence of voles and rats in the environment suggest that reservoirs and other possible sources (spring waters and cellars) may be re-contaminated and, as a result, the risk of tularemia outbreaks will persist. The reason a large outbreak occurred in Kosovo after the war was because of the existence of large numbers of rodents in the peridomestic environment, according to Reintjes, et al.[8]

As a result, in patients with fever, sore throat and LAP, especially when they do not respond to non-specific therapies, tularemia must be considered in differential diagnosis. Mortality and morbidity will decrease in tularemia with early diagnosis.

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