

## Original Article

## An outbreak of tularemia in southwestern Turkey

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**Introduction:** Tularemia has reemerged and spread throughout Turkey, and the number of cases has increased. In this study, we report on a waterborne outbreak of tularemia in the spring of 2013 in a region which was previously disease-free, and we investigated the reasons for the outbreak.

**Methodology:** The index case, a 17-year-old male, was diagnosed with oropharyngeal tularemia. An outbreak investigation was initiated after receiving information from other patients with similar symptoms from the same village along with Balkica, Tavas, and Denizli. An epidemiological and environmental investigation was conducted. Tonsil swab specimens/lymph node aspirates collected from patients, and water samples collected from unchlorinated drinking water sources, were cultured. Additionally, a real-time polymerase chain reaction (RT-PCR) was performed on these samples. Serum samples from patients were analyzed for antibody response.

**Results:** A total of 7 patients were found in this outbreak investigation. The attack rate was found to be 1% among the people of the village and 25% among patients' family members. The drinking-water system was contaminated with *F. tularensis* during this outbreak.

**Conclusions:** Lack of appropriate water infrastructure and sanitation was the primary reason for this tularemia outbreak in Turkey. Improving the water source infrastructure and sanitation should be the primary approach to preventing tularemia outbreaks.

**Key words:** Bacterial pathogens; drinking-water sanitation; emerging diseases; waterborne.

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**Introduction**

Tularemia is a zoonotic disease that presents with different clinical outcomes depending on the site through which the bacteria *Francisella tularensis* entered the body. Animals, vectors, and contaminated water and food are the major factors influencing bacterial transmission. The disease may occur sporadically or through outbreaks, which can alter the epidemiology of the disease across regions and/or countries [1,2].

Since 1988, tularemia has reemerged in Turkey, with recurring outbreaks [2-7]. The majority of outbreaks occurred in the 2000s in the northern and northwestern parts of Turkey [4,6,8-12]. Numerous cases and outbreaks have occurred since 2009, particularly in the central part of Turkey, and tularemia has spread across a large part of the country since then [2,7,13-16]. In 2004, it was added to the list of notifiable diseases by the Turkish Ministry of Health (Figure 1). A few case reports of tick-borne and probable contaminated food-related familial tularemia

have been seen in Turkey [17-19]; however, the main clinical form of tularemia in Turkey is oropharyngeal, and numerous waterborne tularemia outbreaks have been reported in the last few decades [8-16].

In this study, we present a tularemia outbreak and investigate the reasons and features of the outbreak in a previously disease-free region in southwestern Turkey.

**Methodology***Epidemiological investigation*

The index case, in early May 2013, was a 17-year-old male admitted to an outpatient clinic with a mass on the left side of his neck. The patient had a throat infection and fever at the end of March. Despite cefuroxime treatment, his fever persisted, and then the swelling in the neck appeared. Physical examination revealed a 5×4-cm mass in the left submandibular region (Figure 2). He was admitted to the hospital with a preliminary diagnosis of tularemia; the work-up included mass aspirate, throat swabs, and serum samples, which confirmed the tularemia diagnosis.

After information was received about other patients with similar symptoms from the same village as well as from Balkıca, Tavas, Denizli, an outbreak investigation was initiated.

We set up an outbreak investigation team that consisted of officers from the Communicable Diseases Branch of the Denizli Provincial Health Directorate and two medical experts from Pamukkale University—one medical microbiology specialist and one infectious diseases specialist. The following case definition was established: people living in Balkıca with a sore throat, fever, and neck swelling since the beginning of February 2013 were considered possible cases.

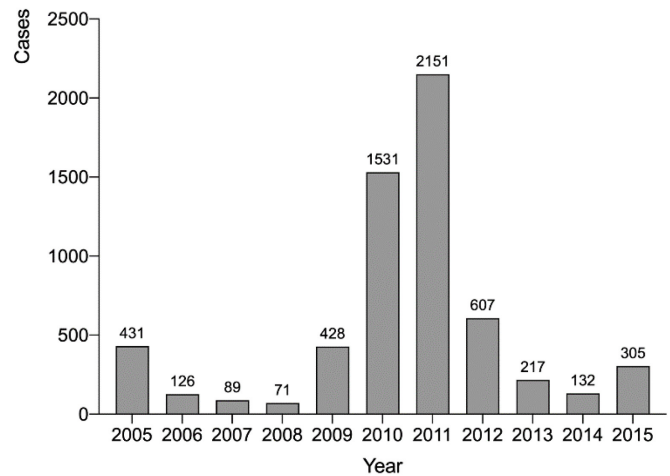
The team first visited Tavas State Hospital, and a patient who had purulent tonsillopharyngitis compatible with the defined case was identified. The patient was hospitalized due to resistant fever and tonsillopharyngitis despite seven days of beta-lactam antibiotic treatment. The demographic information about the patient was recorded, and a questionnaire was conducted to identify the risk factors. A serum sample and throat swabs were collected for serological assays and real time polymerase chain reaction (RT-PCR).

The team then visited Balkıca, a small village with 850 inhabitants. The village is located northwest of Bozdağ Mountain, which is 2,421 meters high and covered with pine forests. Steppes and agricultural areas are found west and north of the village, and there are no rivers or lakes that may pose a risk for tularemia in or around the village. The team visited the village clinic, and possible cases were determined through consultation with the nurse. The team visited the houses of the possible cases, and a questionnaire was administered to the patients and their family members. The data of people with symptoms and/or clinical

**Figure 2.** The neck abscess in the 17-year-old male.



**Figure 1.** The annual distribution of tularemia cases in Turkey, 2005–2015.



findings were recorded. Throat swabs and serum samples were obtained from all possible cases and their household members for diagnosis of the disease.

#### *Environmental investigation*

An environmental investigation was conducted to determine if the cases' homes had food that had been contaminated by rodents. Additionally, the water sources, water reservoir, and water supply were assessed. The water reservoir and water sources were located between the village and Bozdağ Mountain. Additionally, a construction site for a ski center was located about 1,500–2,000 m from the water sources. We gathered water samples from four water sources, the main water reservoir, and the left and right water supplies.

#### *Microbiological investigation study*

All tonsil swab specimens/lymph node aspirates collected from patients were subjected to culturing and RT-PCR. The samples were cultured for 4–10 days on cystine heart agar and supplemented with a VCNT inhibitor (6 mg/L vancomycin, 15 mg/L colistin, 10 mg/L trimethoprim, and 25 mg/L nystatin, Becton Dickinson, Sparks, US). All samples and cultures were handled in a Class III safety cabinet (Labconco, Kansas City, US). In total, 10 tonsil swab specimens/lymph node aspirates and 8 serum samples from patients were analyzed for an antibody response.

In addition, 11 specimens of water (0.3–1.5 L) were collected from unchlorinated drinking water sources or village fountains in the outbreak region. The specimens were transported at +4°C to a microbiology laboratory at Kocaeli University. Water samples were filtered through cellulose acetate membranes (pore size: 0.22

µm), and filters were placed on an antibiotic (Oxoid SR147)-enriched cystine heart agar base with sheep blood and incubated at 37°C in a humidified atmosphere containing 5% CO<sub>2</sub> for 4–10 days. Water filtration was carried out in a Class II safety hood, and cultivation was performed in a separate laboratory under Class III biological safety conditions.

After the water specimens were filtered, the surfaces of filters were washed with sterile distilled water for 15 min in a shaker. Then, DNA was isolated from the specimens using a commercial kit (QIAamp DNA Mini Kit, Hilden, Germany). DNA from clinical tonsil swabs was also extracted, and PCR using primer and probe sets targeting ISFtu2 was conducted as previously described [20]. Negative and positive controls (the latter consisting of 10-fold dilutions of *F. tularensis* subsp. *holarctica* LVS; NCTC 10857) were amplified in parallel.

## Results

A total of 7 patients were identified in this outbreak investigation. These patients and their 21 household members were examined and surveyed. The patients' ages ranged from 17 to 55 years. The time between the first case's disease onset and the diagnosis of the index case was nearly 40 days. The attack rate was found to be 1% among the people of the village and 25% among family members. The appearance of neck masses following tonsillopharyngitis was detected in 6 cases, and these patients had positive serological tests for tularemia. Throat swabs and aspiration samples of the masses were determined to be positive in 3 cases by RT TaqMan PCR for *F. tularensis* subsp. *holarctica*. *F. tularensis* did not grow in the cultures of the clinical samples in any of the cases. All the cases were treated

with streptomycin or ciprofloxacin for 10–14 days. Four cases were hospitalized, and cervical mass aspiration in addition to antibiotic therapy was performed in 3 cases.

None of the patients nor their household members had risk factors such as a tick bite, utilization of contaminated water (except for tap water), or contact with/ingestion of a hunted animal. We did not detect any rodents or rodent excrement in the houses and/or in the close environment. The tap water of the village, which was obtained from 4 different water sources, was first collected in a reservoir and then provided to the water supply. The covers of the water sources were not fitting, were broken, were level with the ground, and were not protected against contamination (Figure 3). We observed that the window of the reservoir building was broken, and the water in the reservoir was exposed to many environmental factors. Moreover, the water in the reservoir was not regularly chlorinated. The tap water analysis before the outbreak showed contamination of the water with coliform bacteria. The water in the reservoir and the supply was chlorine-free, and 3 water samples tested positive for *F. tularensis* by RT TaqMan PCR.

## Discussion

In this study, we conducted an outbreak investigation, and we found that the cause of the outbreak was the contaminated drinking-water system. We obtained a positive RT-PCR result for *F. tularensis* subsp. *holarctica* in clinical samples of three investigated cases and three water samples.

In recent decades, tularemia outbreaks have been widespread throughout Turkey. Although no report of tularemia was made between 1953–1988, the number of tularemia outbreaks in Turkey has increased after 1988, similar to some European countries (such as Germany and Bulgaria) [21,22].

Drinking-water contamination is one of the major causes of tularemia outbreaks in Turkey; and many variable factors are major causes of drinking-water contamination, including the lack of an appropriate water infrastructure. Problems with the tap water network infrastructure —such as unprotected water sources, open transfer of water, and superficial lining of the water supply pipes— have been reported in outbreaks [3,8,13,23,24]. Physically insufficient or dirty water reservoirs have also been reported in previous tularemia outbreaks [13,23-25,28]. Previous investigations have found that infrastructural and sanitation problems persisted in recurrent outbreaks [11,25]. In one outbreak report, there was a complete

**Figure 3.** Photo showing one example of the status of water sources.



lack of tap water infrastructure [12]; furthermore, the use of water sources other than tap water—such as spring water, well water, and village fountains—is commonly documented in many outbreaks [13,16,23,25-27].

Another important factor causing outbreaks is the lack of proper chlorination. As reported previously, irregular or no chlorination was a common problem in other tularemia outbreaks in Turkey, especially in outbreaks in small settlements [8,13,23,27,28]. However, tularemia outbreaks have also been reported in settlements with automated chlorination systems. In one of these outbreaks, the automated system malfunctioned; in the other outbreak, another water source different from the tap water system was used by the patients [4,6]. In the outbreak studied here, we detected that the water sources and the main water tank were unprotected (Figure 3); furthermore, in Provincial Health Directorate documents, we found that in routine drinking-water analyses, coliform bacteria contamination in the tap water samples—a marker of poor water sanitation—was recorded in the pre-outbreak period. Drinking-water contamination with coliform bacteria and poor drinking-water sanitation have been observed in a number of previous outbreak investigations [4,16,23-28].

Additionally, heavy precipitation and floods are shown to be primary factors of drinking-water contamination [29-30]. Heavy precipitation before an outbreak was previously reported in some studies in Turkey [3,6,8,9,23]. Additionally, turbid and dirty tap water was reported before outbreaks without documented flooding [3,8,27]. Turkey saw 62 reports of heavy precipitation and flooding in 2007 and 42 in 2008, but 128 in 2009 and 156 in 2010 [31], with a considerable increase in the number of tularemia cases in the latter period (Figure 1). In a recent study on tularemia cases in the Kayseri province, the authors reported that the number of cases increased in 2010 and 2011, which were wet years that followed several dry years [16]. Although heavy precipitation or flooding was not observed before the outbreak reported here, the water sources were not protected against potential contaminants (Figure 3).

In the current investigation, we also assumed that the construction of a ski center close to a water source may have served as a risk factor for the outbreak. Changes in the environment may alter the exposure among humans, vectors, and reservoirs. New settlement areas and dam constructions have been considered as important factors that increased the exposure in several outbreaks [32,33].

The increase in the rodent population and the contamination of water and food during the war were important factors in tularemia outbreaks in Kosovo [34]. An increasing rodent population in the environment has been documented in several outbreak reports in Turkey [3,16,26], and voles were recently demonstrated via molecular analysis to act as tularemia vectors [35]. Since the rodent population is negatively affected in the winter by cold temperatures, we think that, in addition to the factors we discussed above, global warming and climate change may play essential roles in the epidemiology of tularemia, both in Turkey and in the rest of the world [36,37]. In Turkey, the long-term average temperature in winter has been 3.7°C, although this has increased by 3°C in recent years [38]. Deutz *et al.* [39] showed a relationship between higher winter temperatures and more tularemia cases in hares and estimated that the expected temperature increases in the future may cause widespread occurrence of the disease.

Approximately 20,000 amoebiasis cases and 15,000 giardiasis cases are reported annually in Turkey, which may be indicative of a prevalence of waterborne infectious diseases [40]; however, there are relatively few reports of waterborne outbreaks of these diseases [41-44]. Nevertheless, numerous waterborne tularemia outbreaks have been reported in the last few decades. Therefore, tularemia has become a disease indicator of water quality, especially in rural areas of Turkey.

## Conclusions

A lack of appropriate water infrastructure and sanitation is the major reason for waterborne tularemia outbreaks in Turkey. Improving the water source infrastructure and the sanitation should be the primary approach for preventing tularemia outbreaks, especially in rural areas.

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