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Title: Retrospective analysis of tularemia cases in Tokat

Running Title: The prevalence of tularemia in Tokat

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ABSTRACT

Objective: Tularemia is a rare and overlooked zoonotic infection. While ulceroglandular and glandular types are observed most frequently in epidemics originating from Europe, oropharyngeal type is most common in Turkey. The most common clinical findings are lymphadenopathy, skin rash and tonsillitis. In our study, we aimed to investigate the frequency of tularemia in Tokat, which is located in the Central Black Sea Region of Turkey.

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Materials and Methods: The data of patients diagnosed with tularemia in XXX University Faculty of Medicine Research and Application Hospital between January 2011 and July 2021 were retrospectively analyzed. Laboratory-confirmed cases were included in the study. Sociodemographic characteristics, risk factors, clinical and laboratory findings, treatments, and post-treatment follow-up of the cases were evaluated.

Results: Tularemia was detected in 20 patients. A total of 80% patients lived in the villages, 65% were engaged in agriculture and 60% in animal husbandry. The most important possible sources of contamination were rodents around the house (40%) and non-chlorinated drinking water (50%). Among 20 cases 57% were oropharyngeal tularemia, 95% were treated with monotherapy or combinations containing aminoglycosides, and 50% surgical lymph node drainage.

Conclusion: Tularemia is a rare infection in Tokat province. But it should be considered in the differential diagnosis of patients living in rural areas and presenting with fever, sore throat and cervical lymphadenopathy unresponsive of beta-lactam agents.

Keywords: Central Black Sea, Francisella tularensis, Tokat, Tularemia

INTRODUCTION
Tularemia is a zoonotic infectious disease caused by Francisella tularensis, an aerobic and small Gram-negative coccobacillus. F. tularensis is an extremely contagious bacterium. It can spread to humans by bite of infected arthropods, connection with infected animal materials, ingestion of contaminated food or water, and inhalation of contaminated aerosols (1). There are four subtypes: F. tularensis subsp. tularensis (type A), subsp. holarctica (type B), subsp. mediiasiatica and subsp. novicida. Type A is more commonly transmitted through tick bite and rodent contact, whereas type B is more commonly transmitted through contaminated water and foods (2,3).

The clinical manifestations of tularemia may range from asymptomatic illness to septic shock and death, in part depending on the virulence of the bacterium, portal of entry, amount of inoculum, and the immune status of the host (4). The infection typically manifests as regional lymphadenopathy and skin / mucosal ulceration following contact with infected animal

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materials; pharyngitis and tonsillitis with lymphadenopathy following ingestion of contaminated food and water; or pneumonia and systemic disease following inhalation of contaminated aerosols (1,4)

Tularemia is classified into six clinical forms according to the route of entry of the bacterium to the host. Ulceroglandular form; regional lymphadenopathy and cutaneous ulcer, glandular form; regional lymphadenopathy without ulcer, oculoglandular form; conjunctivitis and preauricular lymphadenopathy, oropharyngeal form; cervical lymphadenopathy with stomatitis, pharyngitis or tonsillitis, pneumatic form; primary pleuropulmonary disease and the typhoid form is characterized by febrile disease without early localized signs and symptoms. Ulceroglandular form is the most commonly reported worldwide (20-81%). Although the oropharyngeal form is seen at a rate of 1% worldwide, it is the most common clinical form of tularemia with a rate of 77% in Turkey (5).

Tularemia is generally seen as sporadic cases in many parts of North America, especially in the Northern Hemisphere, in Asia, Central and Northern Europe, especially in Scandinavian countries, and occasionally causes epidemics. However, the distribution of tularemia in the Northern Hemisphere is not homogeneous and shows a fragmented distribution (6). The first tularemia epidemic in Turkey was reported from Lüleburgaz in 1936, and sporadic cases and small point outbreaks were reported from different regions in the following years. The biggest tularemia epidemic in our country was experienced in Antalya in 1953. Tularemia came to the fore again in our country with the detection of an epidemic of 64 cases in Bursa in 1988, 35 years after this epidemic. The previous studies showed that F. tularensis is endemic in Turkey, mainly in the Marmara, Black Sea and Central Anatolia Regions, and causes small epidemics (6,7) Tularemia is among the C group mandatory to report diseases in the Ministry of Health communicable diseases notification system in Turkey since 2004 (6).

Tularemia may be confused with many diseases and especially sporadic cases may be missed. So, tularemia cases may receive misdiagnosis and mistreatments, delay in the diagnosis and treatment may be seen (8). In this study, it was aimed to evaluate the epidemiological data, clinical findings, risk factors and treatments of laboratory-confirmed tularemia cases between 2011-2021 in the province of Tokat, located in the Central Black Sea Region of Turkey.
MATERIALS and METHODS

This study is an observational descriptive study. In this study, the patients were diagnosed with tularemia who applied to XXX University Research and Application Hospital, between January 2011 and July 2021 were analyzed. Data were obtained from hospital automation system and General Directorate of Public Health (GDPH) via the Tularemia Information System. Laboratory-confirmed cases were included in the study. According to Centers for Disease Control and Prevention (CDC), isolation of *F. tularensis* from a clinical sample and seroconversion of antibodies to *F. tularensis* in serum samples taken at 2-3 week intervals were determined as definitive diagnostic criteria. In addition, detection of antibodies to *F. tularensis* in a single serum or detection of *F. tularensis* by PCR in patients with compatible clinical findings is also diagnostic (9). According to the Tularemia Field Guide of the Turkish Ministry of Health, in the presence of clinical suspicions and findings, the result of a tube agglutination test performed on a single serum ≥1:160 is also diagnostic (6). In this study a laboratory-confirmed tularemia was defined as having at least one of the following criteria:

- Presence of antibodies to *F. tularensis* in the serum with a titer of > 1/160
- Detection of *F. tularensis* by molecular tests (polymerase chain reaction (PCR) assay)
- Isolation of *F. tularensis* from clinical specimens

Sociodemographic characteristics of the patients (age, gender, occupation, place of residence, characteristics of water resources in rural areas), risk factors (animal contact, history of tick and/or rodent bites, etc.), clinical and laboratory findings, treatments, and post-treatment follow-up were evaluated.

Tularemia clinical presentations were categorized as ulceroglandular, glandular, oculoglandular, oropharyngeal, typhoidal, or pneumonic based on patient's symptoms and physical examination findings in the patient records.

**Statistic analysis**

Statistical Package for Social Sciences (SPSS) 22 (Inc. Chicago, Illinois, USA) statistical package program was used to calculate the data. The conformity of the variables to normal
distribution was examined by visual (histogram and probability graphs) and analytical methods (Kolmogorov-Smirnov/Shapiro-Wilk tests). Descriptive statistics were made as numbers and percentages for categorical variables, mean ± standard deviation (SD) for normally distributed continuous variables, and median (minimum-maximum) for non-normally distributed continuous variables. Pearson Chi-square test and Fisher's test were used for comparison of categorical variables. Variables were compared between groups using Student's T test and Mann-Whitney U test. The situations where p value was less than 0.05 were considered statistically significant.

Ethic statement
The study was approved by the local ethics committee (Clinical Research Ethics Committee of XXX University, Faculty of Medicine, 20-KAEK-189).

RESULTS
The diagnosis was confirmed in 20 (13.6%) of 146 patients with a preliminary diagnosis of tularemia. Thirteen (65%) of the patients were male and the mean age was 42 (min 4, max 81). The diagnosis was confirmed with detection of *F. tularensis* by PCR in one patient (5%) and antibodies to *F. tularensis* in 19 patients (95%).

Data about the years and seasons in which the cases were detected was presented in Figure-1 and Figure-2, and information about the place they live was presented in Figure-3. Thirteen (65%) of the patients were engaged in agriculture and 12 (60%) were engaged in animal husbandry. Eight patients (40%) had a history of activity in nature (such as picnics, camps) before the onset of symptoms. Nine (45%) had rodents around houses, while four (20%) had direct contact with a rodent. There was a history of close contact with game animals in five patients (25%), tick bites in three patients (15%), and history of swimming in a lake or stream in four patients (20%). The drinking water sources of 14 patients (70%) were tap water, four patients (20%) were spring water, and two patients (10%) were well water. The drinking water of 10 patients (50%) was chlorinated. While there were cases with similar symptoms in the neighborhood/village where five patients (25%) lived, only one patient (5%) had someone with the same complaints in their household.
The median time between the onset of symptoms and the diagnosis was 30 days (min 7, max 185 days). The most common symptoms were, in order; lymph node enlargement and/or pain (n=15, 90%), sore throat (n=11, 55%), malaise (n=11, 55%), and fever (n=10, 50%). The most common physical examination findings were, in order; lymphadenopathy (n=18, 90%), fever (n=7, 35%), pharyngitis/tonsillitis (n=6, 30%), and oral mucosal lesions (n=4, 20%). Lymphadenopathy was detected in all patients in neck ultrasonography (USG) or computed tomography (CT). Twelve (60%) lymph ganglia were located in the submandibular, seven (35%) in jugulodigastric, and one (5%) in posterior cervical triangle. There was oropharyngeal tularemia in 15 (75%), glandular in four (20%), and typhoidal tularemia in one (5%) of the patients. Leukocytosis (>10,000/mL) was detected in six (30%), and elevated CRP levels (>5 mg/L) in 13 (65%) patients (Table 1).

Fourteen of the patients (70%) used beta-lactam antibiotics for lymphadenopathy before the diagnosis of tularemia, but did not benefit. Eleven (55%) patients with confirmed tularemia diagnosis were treated with aminoglycoside monotherapy (9 patients streptomycin, 2 patients gentamicin), and four (20%) patients received streptomycin + ciprofloxacin, three (15%) patients received gentamicin + ciprofloxacin, one (5%) patient received streptomycin + doxycycline, and one (5%) patient received ciprofloxacin + doxycycline combined therapy. Surgical lymph node drainage was performed in 10 (50%) of the patients, while spontaneous drainage developed in two (10%) patients. There was no case with a mortal course.

DISCUSSION

The first tularemia epidemic in Tokat province occurred in December 2005. In this mentioned study, an epidemic of tularemia, thought to be transmitted by contaminated food from mice, was reported in eight members of the same family (10). In the literature, we could not find any study on tularemia in Tokat in the following years. In October 2004, a tularemia epidemic occurred in the neighboring province of Amasya, Suluova district, and 86 cases were reported. The attack rate was reported as 2.3 per 1000. In Samsun-Havza, another province in the Central Black Sea region, 75 tularemia cases were detected between 2005 and 2007 (11). Two more outbreaks occurred in Amasya with 28 cases in 2008 and 31 cases in 2009-2011 (12,13).
Tokat is a province located in the Central Black Sea region of Turkey, with large lands on the valley, and suitable vegetation for wild and domestic animals used as hosts by ticks. This area has also been recognized as an epicenter for outbreaks of Crimean Congo Hemorrhagic Fever (CCHF), a tick-borne zoonosis (14). Ticks are known to be both vectors and reservoirs in the life cycle of tularemia (15). Ticks are not at the forefront in the transmission of tularemia in our country and it is known that the transmission is mostly through contaminated water and food. However, in some case series, it has been shown that ticks also cause the transmission of tularemia in Turkey. Yeşilyurt et al. reported two cases of tick-borne tularemia in Yozgat (16). It has been reported that tularemia developed after tick contact in three of 22 tularemia cases reported from Düzce (17). In Kars, *F. tularensis* was isolated from ticks by mouse inoculation experiment and it was proven that this kind of ticks are vectors for the transmission of tularemia in sheep (18). In this presented study three patient had a history of tick bite.

In rural areas water resources are not regularly chlorinated, neglected water tanks and village fountains are at risk for tularemia. Although tularemia is generally seen in rural areas where animal contact is more and hygienic conditions are not suitable, the disease is rarely encountered in those living in cities. Independent variables such as consumption of spring water, hunting and eating wild rabbit meat, contact with rodent interests, unhygienic food consumption, a significant increase in the number of rodents in and around the house, and nature-related activities are among the epidemiological risk factors (6). Tokat is located in the Central Black Sea Region of Turkey. It has a total population of 597,861 and 33.2% of the people live in villages (19). In our study, 80% of the cases lived in rural areas, 40% had a history of nature-related activities (such as picnics, camps). The drinking water of 50% was not chlorinated, 20% of them had a recent history of swimming in a lake or stream. 65% of them were engaged in animal husbandry, 45% of them had rodents around their house and 20% of them had close contact with these animals. 25% had a history of contact with game animals and 15% had a history of tick bite.

Oropharyngeal form is the most common clinical form of tularemia in different regions of Turkey (5). The most common form was the oropharyngeal form (%75.9) in the study of Engin et al. in Sivas, which is in the close neighborhood of Tokat, although it is in the Central
Anatolian Region (20). Similarly, in our study, the most common form was the oropharyngeal form with a rate of 75%. The second most common form in our study was glandular tularemia (20%). Similarly, in the study of Alkan et al. in Samsun, the glandular form was the second most common after the oropharyngeal form (21).

Although the disease can be seen in all seasons, rodent-related infections are more common in the winter months due to hunting, and tick-related infections are more common in the summer months (6). The epidemic, which was reported in 2005 in Tokat province, occurred in December (10). In our study, the most cases were encountered in summer and winter months. In this presented study, the median time between the onset of symptoms and the diagnosis was 30 days. It is noteworthy that the diagnosis period was longer than 3 weeks in previous studies from Turkey (20,21). It is thought that the long time elapsed between the onset of the symptoms and the diagnosis of tularemia is due to the fact that they applied to the primary or secondary level health institutions and were given non-specific antibiotics and nonsteroidal anti-inflammatory drugs there. Fourteen of the our patients (70%) used beta-lactam antibiotics for lymphadenopathy before the diagnosis of tularemia. Since beta lactam antibiotics are not effective against tularemia, patients whose symptoms did not improve may have applied to the university hospital as a last resort. The other reasons for delay in diagnosis may be the physician's failure to think of tularemia because of its rareness, and the patient's late admission to the physician.

Antibiotics effective against tularemia are streptomycin and gentamicin from the aminoglycoside group, fluoroquinolones, chloramphenicol and tetracyclines. Aminoglycosides (gentamicin or streptomycin) should be used in the treatment in the presence of severe infection (typhoidal and pneumonic tularemia and other forms of long-term and systemic symptoms, development of renal failure or sepsis). In the treatment of mild or moderate infection, oral fluoroquinolone is recommended as the first choice, and doxycycline as an alternative treatment option. Gentamicin is recommended as first-line therapy in pediatric patients (4). Although monotherapy is sufficient, some authors also recommend combined therapy (22). In our study, 55% of the cases were treated with aminoglycoside, while 45% were treated with any combination of aminoglycoside, ciprofloxacin and doxycycline treatments.
The most important limitation of our study is the retrospective design. Since it is a rare infection, it is possible that there were cases that the clinician never thought of and did not test for tularemia. Prospective studies are needed to determine the real prevalence in the province and region.

CONCLUSION

In our study, the prevalence of tularemia was investigated over a 10-year period in a university hospital in the Central Black Sea Region. Only 20 cases were positive. It can be concluded that tularemia is a rare infection in Tokat province. However, it is possible that there are cases that have never been thought of and have not been examined for tularemia. Tularemia should be considered in the differential diagnosis of patients living in rural areas and presenting with fever, cervical lymphadenopathy and tonsillitis unresponsive to the beta-lactam agents or in the presence of negative routine cultures.

References


9. Centers for Disease Control and Prevention, National Center for Emerging and Zoonotic Infectious Diseases (NCEZID), Division of Vector-Borne Diseases (DVBD). Tularemia. USA: Centers for Disease Control and Prevention, 2021.


Table Legends
Table 1: Symptoms, physical examination findings and laboratory findings of tularemia cases

Figure Legends
Figure 1: Distribution of tularemia cases according to years

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Figure 2: Distribution of tularemia cases according to seasons
Figure 3: Distribution of tularemia cases by districts where they live

Table 1: Symptoms, physical examination findings and laboratory findings of tularemia cases

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Figure 1: Distribution of tularemia cases according to years
**Figure 2:** Distribution of tularemia cases according to seasons
Figure 3: Distribution of tularemia cases by districts where they live