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# Tularemia in Çanakkale province, Marmara region, Turkey: a 10 years' experience

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***ABSTRACT:***

— ***Objective:*** In this retrospective study, we aimed to perform a clinical and laboratory evaluation of the cases of tularemia in the last 10 years in Çanakkale province, Marmara region, Turkey, which is considered an endemic area for the disease.

— ***Patients and methods:*** Patients aged 11 years and older who were referred to our outpatient clinic between January 2011 and December 2020 and were diagnosed with Tularemia based on clinical findings and laboratory test results were included in the study. Sociodemographic, possible risk factors for this disease, physical examination findings, laboratory test results, treatments, and outcomes of the patients were analyzed.

— ***Results:*** During the study period, 264 people were tested for tularemia and 28 patients were diagnosed. The mean age was 47.42 (11-82) years, and 20 (71.4%) of them were women. 89.2% of the cases were diagnosed in 2019. Living in rural areas (n=18, 64.3%), using a neighborhood/village fountain as a water source (n =18, 64.3%), not knowing the chlorination status of drinking water (n=24, 85.7%), and the presence of a similar disease in the neighborhood/village (n=18, 64.3%) were the most common risk factors for tularemia. The most common form of tularemia is the oropharyngeal form (71.4%); presence of enlarged lymph nodes and/or painful lymph nodes (89.2%) and sore throat (75%) were the most common symptoms reported. The mean time to diagnosis was 40.5±2.9 days. The development of suppuration was detected in 49.9% of the cases.

— ***Conclusions:*** Tularemia outbreaks continue to be reported in the Marmara Region, where the first tularemia cases were detected in Turkey. Tularemia should be included in the differential diagnosis in patients with neck lymphadenopathy, sore throat, and fever, especially those living in endemic areas.

— ***Keywords:*** Tularemia, Marmara Region, *Francisella*.

## INTRODUCTION

Tularemia is a zoonotic disease caused by *Francisella species* and has different names such as Francis disease, Ohara disease, rabbit fever-plague, horse fly fever, Siberian ulcer, and hunter's disease. *Francisella* species are small, aerobic, thin-encapsulated, catalase-positive, pleomorphic, non-motile, non-spore-less gram-negative coccobacilli that require cysteine for reproduction. This pathogen mainly causes disease in animals and rarely in humans[1,2]. Transmission occurs either directly through contact with infected animals or through contact with infected animal flesh/body fluids (urine, feces, or blood) or by biting, or indirectly through contaminated drink-



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ing water. Direct contact with infected animals, drinking contaminated water or consuming infected animal raw meat are the most common routes of transmission in our country. The disease can also be transmitted by vectors such as flies or ticks that carry the bacteria. Inhalation of contaminated water or dust particles (aerosol transmission) is a rare mode of transmission. Very rare modes of transmission include fishing line injuries, especially during fishing season[2,3]. In addition, it is considered a biological weapon[4].

Tularemia usually causes non-specific systemic symptoms such as fever, chills, anorexia, and malaise that occur with a sudden or rapid onset approximately three to five days after exposure (between 1 and 21 days). Classically, the fever may subside after a few days but then quickly return. Other nonspecific symptoms include headaches, fatigue, pain in the chest or muscles, abdominal pain, vomiting or diarrhea. In some patients, these systemic symptoms may have decreased by the time of evaluation[2]. Secondary skin changes are common in all forms of Tularemia, with up to 50% reported in some series, and are frequently misdiagnosed or overlooked (33/36). Skin lesions reported to accompany Tularemia include erythema nodosum, erythema multiforme, Sweet's syndrome, and urticaria[3-7]. The most frequently involved organs in the disease are lymph nodes, lungs and pleura, spleen, liver, and kidney. The spread of bacterial growth in lymph nodes, skin and mucous membranes can be seen here[3]. The disease begins suddenly and loudly. It can cause complaints such as fever, chills, headache, sore throat, weakness, loss of appetite, weight loss, cough, myalgia, abdominal pain, vomiting, and diarrhea. Fever-pulse discordance (relative bradycardia) is seen in 42% of the cases[4]. It has been reported that the following symptoms vary according to the localization of the disease[2].

Regarding diagnosis, direct microscopic examination of gram stain is a rapid method. However, its diagnostic value is very low. Bacterial isolation is the gold standard in diagnosis. Serological tests have been the most commonly used method for diagnosing Tularemia for approximately 50 years[1,6]. Since the antibodies become positive after the second week and reach the highest level in the fourth-fifth weeks, serological examinations are limited in the early disease period[1,6]. With the serological method, antibodies or antigens of *F. tularensis* can be searched in the patient's serum in the acute stage. Searching for antibodies against *F. tularensis* in agglutination tests performed in tubes or microplates is the easiest diagnostic method. The Microagglutination test (MAT) is the most commonly used diagnostic laboratory method[1].

As for treatment, it is recommended to use aminoglycosides, tetracyclines or quinolons. Early initiation of treatment affects the success of treatment. Aminoglycosides (streptomycin or gentamicin) are the first line treatment. For alternative treatment, ciprofloxacin or doxycycline are recommended. Treatment failure and relapse are rare due to the bactericidal properties of aminoglycoside antibiotics[1,2,6]. Chloramphenicol and new quinolone derivatives are used if meningitis is present[1,6]. The duration of treatment should be 14-21 days with bacteriostatic antibiotics and 10-14 days with bactericidal antibiotics[1]. In cases of pediatric Tularemia, gentamicin is recommended for the treatment of mild or moderate infections. The usual duration of gentamicin is 10 days, but it can be shortened to 5-7 days if there is an adequate clinical response in children with mild disease and no complications. Doxycycline is not recommended for the treatment of Tularemia in children because it is associated with higher recurrence rates[7-10].

The epidemiology of Tularemia has changed worldwide as a result of disasters such as vector/reservoir population distribution changes, wars, migrations, which are thought to be due to global warming[1].

In many countries, Tularemia is not included in the list of notifiable diseases, and this could lead to underestimation of cases. In addition, due to the inability to diagnose the disease based on clinical findings and the need for advanced microbiological investigations, much fewer Tularemia cases are detected than expected[4]. Due to the increasing number of Tularemia cases reported in our country from different geographical regions, Tularemia was included in the list of "group C" notifiable diseases in the "Standard Diagnosis, Surveillance and Laboratory Guidelines for the Notification and Notification System of Infectious Diseases" in 2005. According to the Turkish Ministry of Health Legislation, "Group C" Notifiable Diseases are notified on an institution-based or sentinel basis[1]. In line with the data obtained from the examinations and notifications, it has been determined that *Francisella spp* are endemic in our country, especially in the Marmara Region and Black Sea Region, eventually causing epidemics. For these reasons, it has become a public health problem[1,5]. Moreover, outbreaks in Çanakkale province have been reported. It is of great importance to evaluate the clinical and epidemiological characteristics of the cases in our cities in order to take appropriate public health measures regarding Tularemia and to carry out appropriate follow-up and treatment for these patients.

We aimed to conduct a retrospective analysis collecting patients' characteristics, treatment outcomes and epidemiological data of all reported cases of Tularemia in the last ten years in Çanakkale province, Marmara region, Turkey.

## PATIENTS AND METHODS

Data from patients aged 11 years and older who attended the Infectious Diseases and Clinical Microbiology Outpatient Clinic of our tertiary hospital between January 2011 and December 2020 who were diagnosed with Tularemia were included. The sociodemographic characteristics of the patients (age, gender, occupation, residence,), risk factors (characteristics of the water sources consumed as drinking water, possible infected animal contact, tick and/or rodent contact history, etc.), physical examination findings, and results of laboratory tests, treatment offered and follow-up data were examined MAT titer of 1/160 and above was considered serologically positive. Patients' data forms of Tularemia patients and hospital information automation system data were collected. Drug prescriptions, radiological examinations

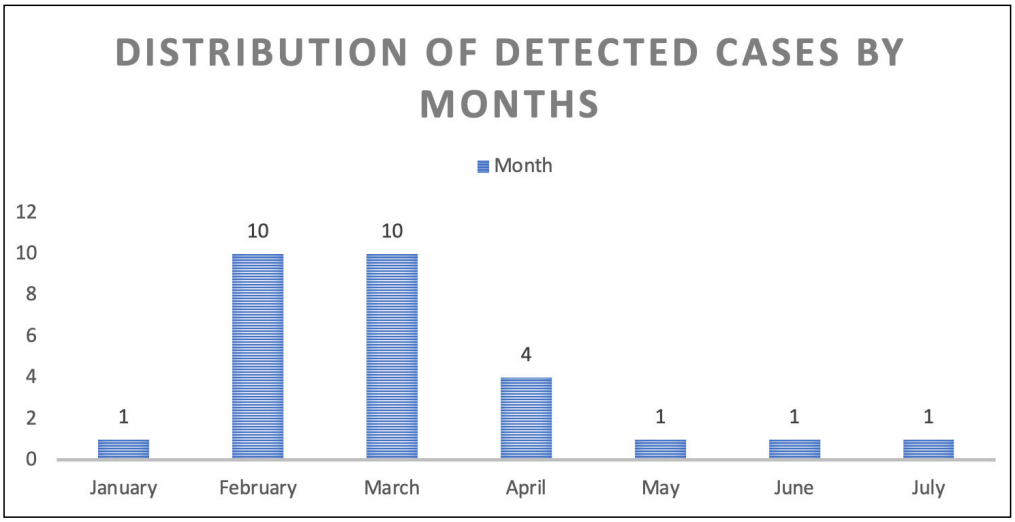


**Figure 1.** The number of cases of Tularemia reported in Çanakkale province, Marmara region, Turkey, by month.

and laboratory test results were included. Serum samples taken from patients with a pre-diagnosis of Tularemia were sent to the Turkish Public Health Institution National Tularemia Reference Diagnosis Laboratory to perform a MAT test. Other laboratory parameters were collected from biochemistry laboratory of our hospital.

The patients' data were collected in an Excel file designed for evaluation by the authors. A control group was not considered.

All of the tularemia 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.

## RESULTS

Between January 2011 and December 2020, a total of 264 people were tested with a preliminary suspect of Tularemia. Of these, 28 tested positive at MAT (titer ≥1/160). The mean age of the cases diagnosed with Tularemia was 47.42 (11-82) years, and 20 (71.4%) were women.

One case in 2012, two cases in 2015, and 25 cases in 2019 were reported. (85.7%) (24/28) cases resided in Çan district and the remaining ones resided in the central districts of Kepez, Bayramiç, and Yenice.

Considering the monthly distribution of the cases, the highest number of cases were detected in February and March (Figure 1).

18 (64.3%) of the cases were located in rural areas. A travel history to rural areas (n =18, 64.3%), a neighborhood/village fountain as a water source (n =18, 64.3%), the unknown chlorination status of drinking water (n =24, 85.7%) and the presence of similar disease in neighborhood village (n =18, 64.3%) were the most common risk factors reported in our study (Table 1).

Of the cases diagnosed with Tularemia, 9 (32.1%) had leukocytosis, 8 (25.6%) increased erythrocyte sedimentation rate (ESR), two (7.2%) Alanine Aminotransferase (ALT)/Aspartate Aminotransferase (AST) elevation, and one (3.6%) lactate dehydrogenase (LDH)) elevation. The mean laboratory values at the time of admission are summarized in Table 2.

The oropharyngeal form was the most common picture reported and the most common symptoms reported were lymph nodes enlargement and/or pain (89.2%), sore throat (75%), fatigue (78.6%), anorexia (50%), fever (46.4%), and muscle/ joint pains (39.3%). The mean time to diagnosis was 40.5±2.9 days. Suppuration developed in 49.9% of cases. Regarding treatment, all subjects received treatment for 14 days (Table 3). 82.1% of the cases we presented were treated

**Table 1.** Review of possible risk factors reported from 28 cases of Tularemia in the Çanakkale province, Marmara region, Turkey.

| | **n, % (total: 28)** |
|---|---|
| ***Place of residence*** | |
| – rural areas | 18 (64.3) |
| – village | 9 (32.1) |
| – town center | 1 (3.6) |
| ***Travel to rural areas*** | 18 (64.2) |
| ***Tick bite*** | |
| – unknown | 11 (39.3) |
| – no | 17 (60.7) |
| – yes | 0 (0) |
| ***Chlorination of drinking water*** | |
| – unknown | 24 (85.7) |
| – yes | 1 (3.6) |
| – no | 3 (10.7) |
| ***Drinking water source*** | |
| – neighborhood/village fountain | 23 (82.1) |
| – drinking water distribution systems | 5 (17.9) |
| ***Farming activity*** | 3 (10.7) |
| ***Similar illness in people living around (village/neighborhood)*** | 18 (64.2) |
| ***Animal husbandry or animal feeding*** | 5 (17.9) |
| ***Presence of mice, rabbits and rodents around the house*** | 1 (3.6) |
| ***Contact with lake-stream water*** | 0 (0) |
| ***Hunting/history of contact with/eating wild animals*** | 0 (0) |
| ***Travel history (to other endemic place)*** | 1 (3.6) |
| ***Activities in nature (picnic, sports, etc.)*** | 2 (7.2) |

**Table 2.** Summary of the laboratory parameters of 28 Tularemia cases in the Çanakkale province, Marmara region, Turkey in 2010-2020.

| Laboratory parameters | Mean |
|---|---|
| White blood cell (/mm$^3$) | 9941±4210.9 (4000-12000) |
| CRP (mg/dl) | 3.68±2.1 (<0.5) |
| Elevation in liver function tests | 3 |
| ALT (IU/lt) | 28.3±27.2 (<45) |
| AST (IU/lt) | 20.9±18.9 (<45) |
| Ürea (mg/dL) | 26.5 (10-40) |
| Creatinine (mg/dl) | 0.74 (0.59-1.35) |
| LDH (IU/l) | 225.3 (105-333) |
| Erythrocyte Sedimentation Rate (mm/hr) | 50.8 (1-20) |
| Total bilirubin (mg/dL) | 0.35 (0.3-1.2) |
| CK (U/L) | 60.7 (22-198) |

*CRP: C-Reactive Protein; ALT: Alanine Aminotransferase, AST: Aspartate Aminotransferase, LDH: Lactate dehydrogenase, CK: Creatine Kinase.

with streptomycin. Other treatments are summarized in Table 3. We also preferred gentamicin for the treatment of the only pediatric case in our series. No relapse was detected.

**Table 3.** Summary of clinical and laboratory findings, treatment, and outcome of 28 tularemia cases in the Çanakkale province, Marmara region, Turkey, 2010-2020.

| Clinical findings | n, % (total:28) |
|---|---|
| ***Tularemia subtype*** | |
| – glandular form | 7 (25) |
| – oculoglandular form | 1 (3.6) |
| – oropharyngeal form | 20 (71.4) |
| ***Lymph nodes enlargement and/or painful lymph node*** | 25 (89.2) |
| ***Sore throat*** | 21 (75) |
| ***Weakness*** | 22 (78.6) |
| ***Muscle and joint pain*** | 11 (39.3) |
| ***Fever*** | 13 (46.4) |
| ***Anorexia*** | 14 (50) |
| ***Abdominal pain and/or diarrhea*** | 2 (7.2) |
| ***Nausea and/or vomiting*** | 4 (14.4) |
| ***Eye redness and swelling*** | 1 (3.6) |
| ***Ulcer and/or wound on the skin*** | 0 (0) |
| ***MAT titer*** | |
| – 1/1280 and above | 4 (14.4) |
| – 1/640 | 7 (25) |
| – 1/320 | 7 (25) |
| – 1/160 | 10 (65.2) |
| ***Treatments*** | |
| – ciprofloxacin | 2 (7.2) |
| – doxycycline | 1 (3.6) |
| – streptomycin | 23 (82.1) |
| – no information | 2 (7.2) |
| ***Outcome*** | |
| – scar development | 13 (46.4) |
| – suppuration | 14 (49.9) |
| – no information | 1 (3.6) |

## DISCUSSION

A total of 28 cases diagnosed with tularemia in our outpatient clinic between January 2011 and December 2020 were included in our study. In the national literature review, tularemia cases were first reported from our country in 1936 in the Thrace part of Marmara region[11]. Three epidemics were reported, in Van, Kırklareli, and Antalya provinces between 1938 and 1954. The largest tularemia epidemic reported in our country was experienced in Antalya in 1953. This epidemic, in which more than two hundred cases were detected, developed as a result of the contamination of the village fountain water[1]. After that, no more cases were reported until 1988, when 250 cases were encountered in Bursa between 1988 and 1998[11]. Few cases were reported from Marmara region, Central and Western Black Sea regions (Istanbul, Samsun, and Bolu outbreaks) in recent years. Moreover, cases have been reported from Central Anatolian provinces (Sivas, Yozgat, Konya, Ankara, Eskişehir) in Turkey[12-31]. Gürcan[28] reported the annual number of tularemia cases diagnosed in our country has been increasing: 431 cases were reported in 2005 and 2151 cases in 2011 from Turkey, showing an increasing trend, actually. According to the data of the Ministry of Health of our country, 6452 new cases of tularemia were reported between 2008 and 2017. Of interest, most of the reported cases occurred in 2010 (1531 cases) and 2011 (2151 cases). No tularemia-related deaths were reported. However, data after 2017 were not included[24]. The increasing trend could be partially explained by the fact that in 2004 tularemia was included in the list of "group C" diseases in the "Standard Diagnosis, Surveillance, and Laboratory Guidelines for the Notification and Notification System of Infectious Diseases"[1].

In the province of Çanakkale, where our study was conducted, two epidemics were reported in two villages in the Biga district in 2009[11] and in Çan district in 2019[12]. In these occasions, the disease was kept under control with effective epidemic management[11,12]. In our study, we aimed to evaluate the general characteristics of the patient diagnosed with tularemia between 2011 and 2020. Since data from our hospital before 2011 could not be collected, only the cases with positive MAT tests who attended our outpatient clinic in the last 10 years were evaluated. There was a peak in the number of cases in 2019, and no cases were found in 2020. The reason why no cases were detected in 2020 may be due to the fact that our hospital has been a pandemic hospital since March 2020 due to the Coronavirus Disease 2019 (COVID-19) pandemic. In addition, the reason for the peak in 2019 can be attributed to the heavy rainfall that year and the contamination of water sources.

Tularemia is most common in the Northern Hemisphere. In the Americas and continental Europe (Sweden and Finland), this disease is endemic. While vector (tick and mosquito)-related cases increase in warm months, there is an increase in wild animal hunting-related cases such as rabbits in the winter months[2,29-33]. Tularemia outbreaks in our country are generally caused by infected food and water, and are generally reported between August and March. This situation is thought

to be connected to the increased number of rodents after the rains. The water-borne outbreaks are a possible consequence of rodent contact with the water[28-34]. In our series, two peaks in February and March were detected. *F. tularensis* survives for weeks in cold and humid environments, while it cannot survive in the sunlight or high temperatures[1,6]. This may explain why the number of cases peaked in March and February in our study.

In our country, cases are most common in rural areas, mostly among farmers, hunters, and forestry workers. Although all age groups can be affected, it has been reported that the disease is seen more frequently above the age of 30 because at-risk activities involve mostly adults[1,29,35]. However, there are also pediatric case reported[32]. Moreover, previous studies[5,17,24,26,27,32] in Turkey report that 51-75% of tularemia cases occur in females. Accordingly, in our study 71.4% of the cases occurred in females. However, in a study conducted in France[33], the incidence of the disease was 1.2 times higher in men, and in a study conducted in the USA, the disease was found more frequently in men over 65 years of age[34]. The reason for this gender difference has not been found in the available literature. In addition, we reported a wide age range (11-82), and the average age of the cases was 47.42 years, while only two cases over 65 years old were reported.

While the most common mode of transmission in our study was consumption of non-chlorinated drinking water, the most common mode of transmission reported in the literature is through contact with infected animals[1,2,26,35]. This can be explained by the fact that the public's consumption of non-chlorinated water is widespread, especially in rural areas.

In patients with tularemia, there may be leukocytosis or the leukocyte count may be normal. It has been reported that sedimentation, CK, ALT, AST elevation, thrombocytopenia, low sodium, renal failure, or pyuria/myoglobinuria may occur[36-40]. Routine laboratory tests, however, are not specific for tularemia[39]. In our study, 9 (32.1%) had leukocytosis, 8 (28.5%) had increased ESR, two (7.2%) had elevated ALT/AST, and one (3.6%) had elevated LDH levels. In our study, a routine biochemical and hematological test result that may be specific for tularemia was not found, as MAT test was not studied in our hospital and MAT test was studied in the reference laboratory. Statistical analysis could not be performed due to insufficient number of cases.

In our study, the oropharyngeal form (71.4%) was the most commonly reported. This is in accordance with national data[5,17,24,26,36] although the most common form in the rest of the world is the ulcer glandular.

In our series, the most common symptoms were lymph node enlargement and/or painful lymph node (89.2%), sore throat (75%), fatigue (78.6%), anorexia (50%), fever (46.4%), and muscle/joint pain (39.3%). Skin involvement, pneumonia or cardiac involvement were not detected in any of the patients. However, diarrhea/abdominal pain, and eye(s) redness and swelling were reported. Clinical picture of tularemia may be non-specific. Therefore, patients could be misdiagnosed. Gürcan highlighted that the disease is often confused with upper respiratory tract infection or tuberculosis leading to inappropriate antimicrobial therapy and delayed diagnosis[44]. He reported that one fifth of the patients diagnosed with tuberculosis have tularemia instead[44]. These data can be used as a reminder that physicians should suspect tularemia even if the clinical picture is blurred, especially in an endemic area such as Marmara region. In addition, because the diagnosis of this disease is often made based on serological methods, there may be delays in the diagnosis[5,27,32]. In the series published in our country, it has been shown that the diagnosis of patients can take up to 20-60 days. According to previous studies, suppuration and scar development rates were found to be significantly higher in cases with late presentation and late diagnosis[5,27]. Moreover, delayed diagnosis can lead to inappropriate use of antibiotics. In our study, the time to diagnosis was quite long (mean, 40.5±2.9 days). This can explain the high complication rates (approximately 50%), including development of scarring and suppuration. This may be due to the fact that our patients were initially misdiagnosed and received another treatment.

Our study has some limitations. It is a single-center and retrospective study. It cannot reflect the entire region. We think there are many missed cases because of misdiagnosis or because they are offered to other centers. Moreover, an active screening is missing. On the other hand, possible overestimation must be considered for cross-reactions with other infections.

## CONCLUSIONS

In recent years, the number of cases of tularemia has increased. This disease should be considered in the differential diagnosis of many clinical pictures, especially in endemic regions such as our country. A timely diagnosis can guarantee effective treatment, and reduced complications and it is crucial to identify possible outbreaks.

**Ethics Approval:**
The study was conducted in accordance with the principles in the Helsinki Declaration revised in 2013. The study was approved by the Ethics Committee of Çanakkale Onsekiz Mart University (date: 09.06.2021, number: 2021-06).

**Informed Consent:**
Informed Consent was not obtained from the patients as it is a retrospective study.

**Availability of Data and Materials:**
All data are available upon reasonable request by contacting the Corresponding Author of the manuscript.

**Conflict of Interests:**
None.

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**Author Contributions:**
All authors were involved in every aspect of the study.

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