ORİJİNAL ARAŞTIRMA ORIGINAL RESEARCH

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Tularemia Outbreak in Western Part of Turkey; Revenge of 'Mount Ida'

Türkiye'nin Batı Kesiminde Tularemi Salgını; İda Dağı'nın İntikamı

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ABSTRACT Objective: Tularemia is a zoonotic disease. Sporadic cases and outbreaks occur in humans. Here, we report a waterborne outbreak, its clinical presentation, and the results of treatment. **Material and Methods:** An increase in the frequency of patients presenting with lymphadenopathy from the same region was detected. Patients with serological diagnosis of tularemia were included in the study between December 2018 and April 2019. This case series were evaluated as an outbreak, clinical and laboratory parameters, demographic characteristics, clinical forms of tularemia and laboratory findings such as immunoglobulin M antibody titer for *F. tularemia*, inflammatory markers (C-reactive protein, leukocytosis, erythrocyte sedimentation rate), choice of first antibiotic treatment, total duration of treatment, initial clinical/laboratory response day, relapse and reinfection ratios have been investigated. The bacteria was investigated from drinking water fountains by polymerase chain reaction (PCR) (16sRNA Nanobiz® *F. tularemia*). **Results:** Tularemia was diagnosed serologically in 35 patients. Distribution of clinical forms was glandular (21 cases-60%); ulceroglandular (11 cases-31.4%); oropharyngeal (2 cases-5.7%) and oculoglandular (1 case-2.8%), respectively. First serologic test positivity was 80%. Inflammatory markers such as C-reactive protein, leukocytes, erythrocyte sedimentation rate were high. Antibiotic choice for treatment was streptomycin in 19 cases (54.3%), ciprofloxacin in nine cases (25.7%), gentamicin in four cases (11.4%); doxycycline in three cases (8.6%) respectively. The common suspicious contact was the use of drinking water from street fountains. *F.tularemia* PCR (16sRNA) was positive in 35 of these fountains (35/80, 43.75%). **Conclusion:** Tularemia should always be considered in head and neck lymphandenopathies that do not heal or show late recovery. We should keep in mind tularemia in a water-borne outbreak, were high it is rare.

Keywords: Tularemia; lymphadenopathy; outbreak

ÖZET Amaç: Tularemi, zoonotik bir hastalıktır. İnsanlarda sporadik vakalar ve salgınlar meydana gelir. Burada bir su kaynaklı salgını, klinik sunumunu ve tedavi sonuçlarını sunuyoruz. **Gereç ve Yöntemler:** Aynı bölgeden lenfadenopati ile başvuran hastaların sıklığında artış tespit edildi. Aralık 2018 ve Nisan 2019 tarihleri arasında serolojik tularemi tanısı alan hastalar çalışmaya dâhil edildi. Bu vaka serisi salgın olarak değerlendirildi, klinik ve laboratuvar parametreleri, demografik özellikler, tularemenin klinik formları ve *F. tularemia* için immünglobulin M antikor titresi gibi laboratuvar bulguları olarak değerlendirildi, inflamatuar belirteçler (C-reaktif protein, lökositoz, eritrosit sedimantasyon hızı), ilk antibiyotik tedavisi seçimi, toplam tedavi süresi, ilk klinik/laboratuvar yanıt günü, nüks ve yeniden enfeksiyon oranları araştırıldı. Bakteriler, içme suyu çeşmelerinden polimeraz zincir reaksiyonu (PCR) (16sRNA Nanobiz® *F. tularemia*) ile araştırıldı. **Bulgular:** Tularemi, 35 hastada serolojik olarak teşhis edildi. Klinik formların dağılımı glandüler (21 vaka-%60) şeklindeydi; ülseroglandüler (11 vaka-%31,4), orofaringeal (2 vaka-%5,7) ve okülоglandüler (1 vaka-%2,8). İlk serolojik test pozitifliği %80 idi. C-reaktif protein, lökositler, eritrosit sedimantasyon hızı gibi inflamatuar belirteçler yüksekti. Tedavi için antibiyotik seçimi; 19 vakada (%54,3) streptomisin, 9 vakada siprofloksasin (%25,7), 4 vakada gentamisin (%11,4), sırasıyla 3 vakada (%8,6) doksisiklin. Yaygın şüpheli temas, sokak çeşmelerinden içme suyu kullanılmasıydı. Bu çeşmelerin 35 (35/80, %43,75)'inde *F. tularemia* PCR (16sRNA) pozitifti. **Sonuç:** Tularemi, geç iyileşme göstermeyen veya iyileşmeyen baş-boyun lenfadenopatilerinde her zaman düşünülmelidir. Nadir de olsa su kaynaklı bir salgında tularemiyi akılda tutmalıyız.

Anahtar Kelimeler: Tularemi; lenfadenopati; salgın

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Tularemia is a zoonotic disease that occurs only in the northern hemisphere in the world. Causative microorganism *Francisella tularensis,* is a facultative intracellular gram-negative bacterium. Human infection occurs following contact with infected animals, invertebrate vectors and contaminated water with animal excrement or urine in food storage areas.[1] When patients admit to hospitals, they usually have clinical manifestations associated with one of the six major clinical forms of tularemia, depending on the entry such as; ulceroglandular, glandular, oculoglandular, pharyngeal (oropharyngeal), pneumonic and typhoidal.[2]

Tularemia began to appear frequently in Turkey in recent years.[3] There are some differences in the tularemia cases seen in Turkey; oropharyngeal involvement is more common. This is mostly due to the transmission by contaminated water.[3] Therefore, tularemia is seen in epidemics rather than sporadic cases in our country.[4,5]

Tularemia can exist in nature for long periods of time. It affects more than 100 mammals, birds, cold-blooded animals and arthropods. A positive correlation is usually found in the relationship between the numbers of human cases and the numbers of animals infected with *F. tularensis*.[2,3] Ida Mountain, besides its natural structure is famous for its water resources. In recent years, it has become popular with gold mines. Excavations and tree felling have changed the natural water basins. And it is also famous in mythology, for example; the gods live on Mount Olympus and have houses on Mount Ida. Zeus, god of gods, was born on Mount Ida. Can is a district city, located in the foothills of Mount Ida and has natural water fountains. These fountains have been used as drinking water by the inhabitants of the region for centuries. After the spring water is collected and chlorinated, it is offered for use as drinking water in the fountains in the city. In this study, we report an outbreak seen after contamination of drinking water with *F. tularensis* and clinical characteristics of patients.

MATERIAL AND METHODS

Firstly, in December 2018, a case of unhealed tonsillopharyngitis admitted to the outpatient clinic; oropharyngeal tularemia was serologically diagnosed. Then, until April 2019, a total of 35 patients from the same region admitted to the policlinic. These patients were analyzed retrospectively by investigating the demographic characteristics, clinical forms of tularemia and laboratory findings [ELISA immunoglobulin M (IgM) antibody titer, inflammatory markers such as C-reactive protein, leukocytosis, erythrocyte sedimentation rate], choice of first antibiotic treatment (after diagnosis), duration of treatment, first clinical/laboratory response day, relapse and reinfection ratios. Relapse was defined as the appearance of the same clinical form three months after discontinuation of antibiotic therapy. The appearance of the same and/or different clinical form six months after discontinuation of antibiotic treatment was defined as reinfection. In all cases, serological titer positivity of 1/160 and above was required. First clinical/laboratory response day was defined as regression of first defined symptoms and/or clinical findings and reduction in the markers of inflammation described above as laboratory tests.

In the field analysis, *F. tularensis* DNA (16sRNA-Nanobiz®, Ankara, Türkiye) was investigated by polymerase chain reaction (PCR) in water samples for obtaining possible transmission way. Water samples were collected from all fountains in the area for this reason.

Ethics committee approval: This study has been approved by Onsekiz Mart University Clinical Research Ethics Committee in 2019 (24.07.2019/2019-14).

The study was conducted in accordance with the Declaration of Helsinki principles.

RESULTS

A total of 35 cases were diagnosed with tularemia based on clinical and laboratory findings. These patients are summarized in Table 1. Only six of these patients did not show positivity at the first serological test. All the glandular tularemi patients did not show ulceration (Figure 1, Figure 2, Figure 3, Figure 4). The highest serological titer was obtained (1/1,280) in oropharyngeal tularemia cases. Most of the cases with ulceroglandular (Figure 5) tularemia had a positive titer ≥1/160 (16/21, 76.2%). One of the six patients whose first serological test was negative,

TABLE 1: Study group characteristics (N=35).

Gender (M/F)	10/25
Age-years old±SD	43±15.7
(minimum-maximum)	(19-81)
Clinical form	
Glandular	21 (60%)
Ulceroglandular	11 (31.4%)
Oropharyngeal	2 (5.7%)
Oculoglandular	1 (2.8%)
Serological first test positivity	28 (80%)
(minimum-maximum)	(1/160-1/1280)
WBC±SD	9.260±2.468
(minimum-maximum)	(4.700-17.800)
PML%	57±10
(minimum-maximum)	(36-75)
CRP*±SD	3.67±6.25
(minimum-maximum)	(0.1-26)
ESR**±SD	48.17±22.13
(minimum-maximum)	(2-117)
First antibiotic treatment choice (after diagnosis)	
Streptomycin	19 (54.3%)
Ciprofloxacin	9 (25.7%)
Gentamicin	4 (11.4%)
Doxycycline	3 (8.6%)
Total specific antibiotic treatment day±SD	19±9.8
(minimum-maximum)	(10-50)
First clinical/laboratory response day±SD	9±6.7
(minimum-maximum)	(3-32)
Total recovery time (day)±SD	62.8±43.2
(minimum-maximum)	(10-150)

SD: Standard deviation; WBC: White blood cell; PML: Polymorphonuclear leukocyte; CRP: C-reactive protein; ESR: Erythrocyte sedimentation rate.
Serologically negative all patients showed positivity after 3 weeks interval; White blood cell counting, Normal value=4.50-11.00x10^3/mL; Polymorphic leukocyte, Normal value=40-60%; *C-reactive protein, Normal value=0-0.5 mg/L; **Erythrocyte sedimentation rate, Normal value=0-20 mm/h.

had oculoglandular tularemia and five of them had glandular tularemia. Secondary serological tests repeated after two weeks later revealed positivity in all of these patients. Mean diagnosis time was 21 days after first onset of symptoms in study group. The most common symptom was enlarged neck lymph nodes with fever and chills. Antibiotic use due to sore throat was the most common related condition in the medical history of the patients. The most commonly used antibiotic was moxifloxacin (27/35, 77.14%). The second most commonly used antibiotic was amoxicillin clavulanate (2/35, 5.7%). Only six patients had no history of antibiotic usage (6/35, 17.14%) before diagnosis. Relapse was seen in only two patients, two of them had glandular tularemia, and both had negative serological test initially. Reinfection was not seen in any case. The mean recovery time was 60 days. The common suspicious contact was the use of drinking water from street fountains.

FIGURE 1: Glandular tularemia.

FIGURE 2: Glandular tularemia, before fistulisation.

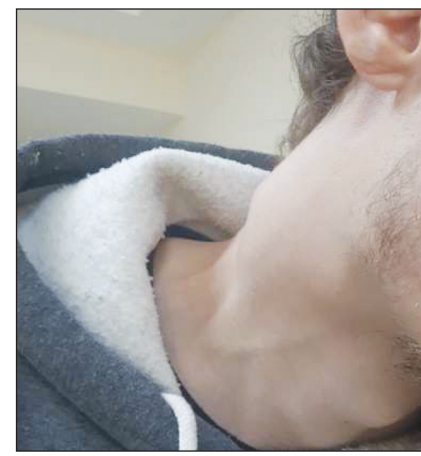

FIGURE 3: Glandular tularemia.

FIGURE 4: Glandular tularemia.

FIGURE 5: Ulceroglandular tularemia.

In fact, the water in these fountains originated from the spring waters from the Mount Ida. There were about 80 fountains in this area. Chlorination of spring water was also applied before transfer to the fountains. Chlorine level was measured monthly. Although chlorine content was sufficient (0.5 PPM), *F. tularensis* PCR was positive in 35 of these fountains (35/80, 43.75%). It was shown that this was actually a water-borne outbreak. Samples from the reservoirs also showed positivity for *F. tularensis*. The reservoirs of spring waters were discharged and the remaining sediment was removed. High dose chlorination was performed after cleaning (1 PPM). Control samples of tanks and fountains were taken after two weeks and all of them were found to be negative for *F. tularensis* DNA by PCR.

DISCUSSION

In our country, *F. tularensis* is a common bacteria in nature and causes human disease either by vectors or water.[2,6] It rarely causes outbreaks in different regions in the country.[7,8] Interestingly, the outbreak notified in this article is the first waterborne outbreak reported from our provience since 2010.[9] This is the second outbreak report from our region.[4] In the first report, rains and sanitation conditions were determined as possible risk factors as a matter of fact first outbreak area was far away from Mount Ida. This time, we estimate that mining activity might cause changing in natural habitats and groundwater flow direction. But in fact this is only an estimation. *F. tularensis* contamination has not been reported in natural spring waters which have been used in this region in this century. The fact that mining activity in the last five years has expanded to natural water resources reinforces this possibility. There are studies showing changes in the location and direction of diseases and even changes in antibacterial resistance as a result of change in geographic area.[10] In fact, this issue was firstly brought up by the World Health Organization in 2015 with the Ebola epidemic.[11]

Although ulcerated glandular and oropharyngeal tularemia are the most commonly described clinical manifestations in the literature, glandular tularemia is more common in our study.[1,2] Most of our patients had a history of antibiotic usage for sore throat such as moxifloxacin and amoxiciline clavunate before the diagnosis of tularemia. Ciprofloxacin was the only quinolone, has been shown to be effective in the treatment of tularemia.[5] The efficacy of levofloxacin in treatment of tularemia was firstly described by Limaye et al.[12] In vitro efficacy of moxifloxacin has been shown recently.[13] Based on this literature, we think that moxifloxacin usage in these patients provides a partial improvement in oropharyngeal complaints. Therefore, while the first clinical picture might be oropharyngeal tularemia, before admission with glandular tularemia after partial recovery under antibiotic pressure. The average duration time for diagnosis might be long, due to this situation.

F. tularensis is an obliged intracellular microorganism especially in macrophages. Although many factors such as lipopolysaccharide change at bacterial cell surface, inhibition of nitrous oxide entry into the cell, the presence of capsules, and slow doubling time; were main cause of ability to live in the cell and

stay in the long term is still unclear.[14] Because of the diversity of microbiological factors that are effective in bacterial pathogenesis, relapse and reinfection concepts are controversial in tularemia. Relapse can be seen in tularemia after any treatment, it is more common due to the bacteriostatic properties of tetracyclines and when used for less than 14 days. In tularemia, relapse may develop in the presence of lymph node and/or empyema that does not respond to medical treatment. In this status, surgical drainage or excision should be considered in the treatment.[15] In our study, relapse was seen in only two patients, two of them had glandular tularemia, and both had negative serological test initially.

CONCLUSION

We should keep in mind tularemia in a water-borne outbreak, although it is rare. It should be kept in mind that the oropharyngeal type, which is the most common type of tularemia in our country, may present only with cervical lymphadenopathy. Therefore, tularemia is essential in the differential diagnosis of cervical lymphadenopathy that does not improve with medical treatment in patients living in endemic regions.

Source of Finance

During this study, no financial or spiritual support was received neither from any pharmaceutical company that has a direct connection with the research subject, nor from a company that provides or produces medical instruments and materials which may negatively affect the evaluation process of this study.

Conflict of Interest

No conflicts of interest between the authors and / or family members of the scientific and medical committee members or members of the potential conflicts of interest, counseling, expertise, working conditions, share holding and similar situations in any firm.

Authorship Contributions

***Idea/Concept:** .Alper Şener, Taylan Önder, Sevil Alkan Çeviker; **Design:** Alper Şener, Esen Eker; **Control/Supervision:** Alper Şener, Sevil Alkan Çeviker, Ebru Doğan; **Data Collection and/or Processing:** Sevil Alkan Çeviker, Alper Şener, Safiye Bilge Güçlü Kayta; **Analysis and/or Interpretation:** Esen Eker, Sevil Alkan Çeviker, Alper Şener; **Literature Review:** Taylan Önder, Sevil Alkan Çeviker; **Writing the Article:** Sevil Alkan Çeviker, Alper Şener, Safiye ;Bilge Güçlü Kayta; **Critical Review:** Ebru Doğan, Sevil Alkan Çeviker, Alper Şener; **References and Fundings: Sevil Alkan Çeviker,** Alper Şener, Safiye Bilge Güçlü Kayta, Esen Eker, Taylan Önder, Ebru Doğan; **Materials:** Alper Şener, Ebru Doğan, Taylan Önder, Safiye Bilge Güçlü Kayta.*

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