Evaluation of tularaemia courses: a multicentre study from Turkey

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Abstract

In this multicentre study, which is the largest case series ever reported, we aimed to describe the features of tularaemia to provide detailed information. We retrospectively included 1034 patients from 41 medical centres. Before the definite diagnosis of tularaemia, tonsillitis (n = 653, 63%) and/or pharyngitis (n = 146, 14%) were the most frequent preliminary diagnoses. The most frequent clinical presentations

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were oropharyngeal (n = 832, 85.3%), glandular (n = 136, 13.1%) and oculoglandular (n = 105, 10.1%) forms. In 987 patients (95.5%), the lymph nodes were reported to be enlarged, most frequently at the cervical chain jugular (n = 599, 58%), submandibular (n = 401, 39%), and periauricular (n = 55, 5%). Ultrasound imaging showed hyperechoic and hypoechoic patterns (59% and 25%, respectively). Granulomatous inflammation was the most frequent histological finding (56%). The patients were previously given antibiotics for 1176 episodes, mostly with β-lactam/β-lactamase inhibitors (n = 793, 76%). Antituberculosis medications were provided in seven (2%) cases. The patients were given rational antibiotics for tularaemia after the start of symptoms, with a mean of 26.8 $\pm$ 37.5 days. Treatment failure was considered to have occurred in 495 patients (48%). The most frequent reasons for failure were the production of suppuration in the lymph nodes after the start of treatment (n = 426, 86.1%), the formation of new lymphadenomegalies under treatment (n = 146, 29.5%), and persisting complaints despite 2 weeks of treatment (n = 77, 15.6%). Fine-needle aspiration was performed in 521 patients (50%) as the most frequent drainage method. In conclusion, tularaemia is a long-lasting but curable disease in this part of the world. However, the treatment strategy still needs optimization.

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Introduction

Tularaemia is a zoonotic infection caused by *Francisella tularensis*, and the disease has been seen in many parts of the northern hemisphere [1,2]. *F. tularensis* is a very potent human pathogen that can produce infection with as few as ten organisms. The microorganism is highly infectious, and may enter the human body through the skin after contact with an infected animal; transmission through the mucosal membranes of the mouth, throat, eye or bronchus may also occur. Furthermore, ticks can also transmit the pathogen [3]. The disease has various clinical presentations, including ulceroglandular, glandular, oculoglandular, oropharyngeal, pneumonic and typhoidal forms [3]. The ulceroglandular form of the disease has been reported to be the most common form in the USA and European countries such as Bulgaria, Hungary, Austria, and Germany [4,5]. The bacterium is known to persist in water, hay or mud for weeks, and waterborne epidemics have been reported in eastern Europe and Turkey [6–9].

F. tularensis ssp. *tularensis* (type A) and *F. tularensis* ssp. *holarctica* (type B) are the two major subspecies causing human disease. Type A is considered to be a potential agent of biological warfare, as it is highly infectious and can cause severe disease with high fatality rates [10]. On the other hand, type B causes mild disease with low fatality rates in Europe and Asia, and is occasionally related to waterborne tularaemia outbreaks. Both climate change and global warming have been suggested to have contributed to the spread of the disease [11]. Recent outbreaks and sporadic case notifications of tularaemia have been observed in Europe [12]. Sporadic cases of tularaemia or local outbreaks have been reported since 1936 in Turkey, and the disease has been increasingly seen in Turkey since 1988 [13]. The clinical manifestations of tularaemia have been reported to range from asymptomatic illness to septic shock [3]. As tularaemia has been known to be potentially fatal if left untreated [14], proper management of the disease is of paramount importance for the patient.

There are relatively small case series for tularaemia in the medical literature detailing the features and the management issues for the disease. Thus, in this multicentre study, which is the largest case series ever reported, we aimed to delineate the potential impacts of this multifaceted disease, and to provide detailed information concerning the clinical, diagnostic and therapeutic implications of tularaemia in a region of the northern hemisphere, Turkey.

Materials and Methods

Study design and patient population

This multicentre study pooled patients with any form of tularaemia from 41 medical centres in Turkey. The cities of the participant centres are shown in Fig. 1. The study had a retrospective design, and included patients treated between 2000 and 2013. No control groups were included for this study. Fatih Sultan Mehmet Training and Research Hospital's Review Board in Istanbul approved the study.

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FIG. 1. The cities of the participant centres.

Microbiological, serological, PCR and other routine test methods

Blood and serum samples, throat swabs and lymph node aspirates were used for laboratory diagnosis of tularaemia.

Cultures. Clinical specimens, including throat, conjunctival and wound swabs, and/or lymph node aspirates, were cultured on antibiotic-supplemented Cysteine Heart Agar Base with sheep blood agar (8%) plates (VCNT inhibitor, REF 212408; Becton Dickinson and Company, Sparks, MD, USA) or blood agar supplemented with 1% glucose, 0.1% cystine and 100 000 U/mL penicillin plates, and incubated at 37°C with 5% CO_2 for 10 days. *Francisella* colonies were diagnosed by the use of an agglutination test with specific antibody (*F. tularensis* Anti-serum; BD, Sparks, MD, or Difco, Detroit, MI, USA) and then PCR.

Agglutination test

A microagglutination test (MAT) was performed with a commercial antigen as described by the manufacturer (BD *F. tularensis* Antigen; Becton Dickinson, Sparks, MD, USA) or with a home-made *F. tularensis* antigen obtained from the strains isolated from patients with tularaemia in Turkey. In the presence of compatible symptoms, sustained high titres of 1/160 or greater in the sera were accepted as indicating a presumptive diagnosis of tularaemia. For definitive serological diagnosis of all cases, sera were sent to the reference laboratories in Bursa and in Ankara by the local health directorates. At the beginning of 2005, a new and completely revised communicable disease notification was launched nationwide in Turkey, and tularaemia was given notifiable status in Turkey [15].

ELISA. An anti-*F. tularensis* ELISA test kit (Seramun, Wolzig, Germany) was used [16].

PCR analysis. Primer and probe sets targeting ISFtu2 were used for Real Time TaqMan PCR analysis as described previously. Reactions were performed in 25-μL volumes containing 2 μL of sample DNA. TaqMan PCR conditions were as follows: activation for one cycle at 95°C for 3 min, and amplification and detection for 40 cycles at 94°C for 10 s and 65°C for 30 s. In this study, both negative and positive controls (10-fold dilutions of *F. tularensis* ssp. *holarctica* NCTC 10857) were used in terms of quality assurance [17].

In all patients, a complete blood cell count was performed, and the erythrocyte sedimentation rate (ESR) was measured by the Westergren method and the C-reactive protein (CRP) level by the nephelometric method in blood samples.

Inclusion criteria

Only adult patients treated for tularaemia and aged >17 years were enrolled. The laboratory diagnosis of tularaemia was established by one of the following: (i) isolation of *F. tularensis* from the clinical specimens; (ii) positive PCR test result; (iii) positive ELISA result; or (iv) a MAT or tube agglutination test titre of ≥1/160 or a four-fold increase in the MAT or tube agglutination test titres within 2 weeks.

Treatment

The patients were treated randomly with one of the antimicrobial regimens, including parenteral streptomycin (15 mg/kg daily) or gentamicin (5 mg/kg daily), oral doxycycline (2 × 100 mg), or tetracycline (4 × 500 mg), or ciprofloxacin (2 × 500 mg), or moxifloxacin (1 × 500 mg) or combined or sequential drug regimens with these antibiotics.

Definitions

Standard definitions were used for leukopenia, leukocytosis, and ESR [18,19].

Inadequate medical treatment. Poor compliance with therapy, irrational antibiotic use for tularaemia and inadequate doses and duration of rational antibiotics were placed in this category.

Therapeutic failure. The presence of at least one of the following was considered to indicate therapeutic failure: (i) the absence of a decrease in fever in a minimum period of 72 h despite rational treatment, or recurrence of fever in the

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course of therapy; (ii) persistence of constitutional symptoms (sore throat, conjunctivitis, fatigue, myalgia, and other complaints) related to tularaemia despite adequate medical treatment; (iii) unresolved complaints despite 2 weeks of medical treatment; (iv) absence of shrinkage in the lymph nodes despite medical treatment; (v) the appearance of new lymphadenomegaly or lymphadenomegalies in a patient under treatment; (vi) the occurrence of lymph node suppuration and/or abscess formation after the start of treatment; and (vii) the absence of decreases in the blood leukocyte count, serum CRP level and ESR despite medical treatment.

Relapse. Relapse was defined as the reappearance of clinical signs and symptoms of tularaemia after the end of treatment.

Ultrasonography (US) investigations

The US investigations were performed by the experienced radiologists in the participant centres with 7.5–12-MHz linear transducers. The morphology of the enlarged lymph nodes (long axis to short axis diameter ratios) and echo characteristics were determined and recorded for each patient. Data on colour Doppler US and power Doppler US were not included, to prevent confusion.

Data collection and statistical methods

A standard questionnaire was sent to the participant centres via the Internet, and data were collected by use of a computer database. Demographics, clinical data, including preliminary diagnosis, the form of tularaemia, comorbid conditions, routine and tularaemia-specific laboratory analyses, US findings, and treatment (therapeutic regimens used and surgical operations performed), and outcome data, including therapeutic failure, cure, and relapse, were recorded for each patient.

Frequencies and percentages were used for categorical variables, and mean ± standard deviation (range) was used for continuous variables. Categorical parameters were tested with Fisher's exact test or the chi-square test as appropriate. Numerical data were first tested for normality. Parametric variables were analysed with Student's *t*-test, and non-parametric variables were analysed with the Mann–Whitney *U*-test. Statistical analysis was performed with standard statistical software (SPSS version 16.0; SPSS, Chicago, IL, USA). Tests were two-tailed, and a p-value of <0.05 was considered to be significant.

Results

In this study, the mean age of the patients was 41.21 ± 16.70 years, and 588 of 1034 patients were females (57%). The patients were more likely to live in rural areas ($n = 737$, 71%) than in urban centres ($n = 297$, 29%) The distribution of the occupations was as follows: farmers, 483 (47%); housewives involved in daily agricultural activities, 266 (26%); students, 51 (5%); workers, 32 (3%); shepherds, 30 (3%); hunters, 22 (2%); government employees, 21 (2%); retired personnel, 18 (2%); soldiers, five (0.4%); foresters, four (0.3%); veterinarians, three (0.2%); and miscellaneous, 104 (10%).

Preliminary diagnoses

Before the definite diagnosis of tularaemia, the preliminary diagnoses were as follows: tonsillitis in 653 patients (63%) and/or pharyngitis in 146 (14%); unspecified lymphadenopathy in 132 (13%); conjunctivitis in 19 (2%); tuberculous lymphadenopathy in 19 (2%); abscess formation in 16 (2%); mumps/parotitis in 14 (1.4%); upper respiratory system infections in 13 (1%); influenza in 13 (1%); malignancy in 11 (1%); deep neck infections in seven (1%); and thyroiditis in three (0.2%).

Symptoms and findings

The complaints and the findings of the patients are shown in Table 1. In 987 patients (95.5%), the lymph nodes were reported to be enlarged. The most frequently involved site was the cervical lymphatic system, which included the jugular, submandibular, occipital and preauricular chains. The distribution of enlarged lymph nodes is shown in Table 2. One-hundred and three skin lesions were detected in 101 (10%) patients. The distribution of skin eruptions was as follows: maculopapular, 33; erythema multiforme, 32; erythema nodosum, 30; papular, three; erythematous, two; vesicular, one; pustular, one; and Sweet syndrome, one.

Laboratory test results

F. tularensis was recovered from culture specimens from 149 patients (14%). The distribution of cultures in which the microorganism was isolated was as follows: 68 blood cultures, 45 lymph node aspirate cultures, 44 throat cultures, and three conjunctival cultures. The diagnosis was established with the MAT in 980 cases (95%), and with tube agglutination in 54 (1%) cases. The distribution of agglutination test titres is shown in Fig. 2. PCR provided the diagnosis in 440 (40%) patients, and ELISA was performed in only eight (1%) patients, all of whom were positive. An increased ESR was found in 754 of 903 patients (83%) tested. In 23 (3%) patients, the ESR was >100 mm/h. Serum CRP levels increased in 763 of 905 patients (84%) tested (>1 mg/dL). Leukocytosis was detected in 272 (29%) of 923 patients tested. The leukocyte count was within the normal range in 650 (70%) cases, and leukopenia was found in only one patient (0.1%).

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TABLE 1. Symptoms and findings of patients according to clinical presentation

	Total, *n* (%)	Oropharyngeal, *n* = 832	Glandular, *n* = 136	Oculoglandular, *n* = 105	Ulceroglandular, *n* = 43	Pneumonic, *n* = 1	Typhoidal, *n* = 1
Symptoms, no. (%)							
Lymphadenopathy[a]	987 (95)	758 (91)	129 (95)	94 (90)	40 (93)	–	–
Fever	883 (85)	746 (90)	83 (61)	91 (87)	34 (79)	1	1
Sore throat	872 (84)	771 (93)	82 (60)	78 (74)	20 (47)	–	–
Muscle pain	680 (66)	591 (71)	49 (36)	71 (68)	25 (58)	1	1
Conjunctivitis	103 (10)	59 (7)	–	100 (95)	2 (5)	–	–
Headache	43 (4)	25 (3)	15 (11)	9 (9)	–	–	1
Skin ulcer	41 (4)	30 (4)	–	10 (10)	11 (26)	–	–
Weight loss	35 (3)	25 (3)	11 (8)	7 (7)	–	–	–
Weakness	32 (3)	20 (2)	8 (6)	9 (9)	3 (7)	–	–
Anorexia	31 (3)	18 (2)	9 (7)	6 (6)	1 (2)	–	–
Cough	24 (2)	20 (2)	4 (3)	4 (4)	–	1	–
Arthralgia	21 (2)	12 (1)	4 (3)	7 (7)	2 (5)	–	–
Nausea/vomiting	17 (2)	8 (1)	5 (4)	1 (1)	3 (7)	–	–
Dysphagia	7 (1)	6 (1)	1 (1)	–	1 (2)	–	–
Rash	7 (1)	5 (1)	–	1 (1)	1 (2)	–	–
Sputum	6 (1)	3 (0.3)	3 (2)	1 (1)	–	1	–
Oral aphthae	3 (0.2)	3 (0.3)	–	–	–	–	–
Findings, no. (%)							
Fever	76 (7)	66 (8)	2 (1)	5 (5)	3 (7)	1	1
Tonsillopharyngitis	482 (47)	466 (56)	–	37 (35)	7 (16)	–	–
Mucosal membrane	315 (30)	305 (37)	–	13 (12)	5 (12)	–	–
Oral mucosal lesion	53 (5)	51 (6)	–	11 (10)	1 (2)	–	–
Conjunctivitis	56 (5)	26 (3)	–	55 (52)	–	–	–
Rash	101 (10)	87 (10)	–	14 (13)	10 (2)	–	–

[a]Detected by palpation.

TABLE 2. The results of lymph node investigations

Lymphadenopathy location (*n* = 987, 95%)	Right/left (%/%)	Bilateral (%)
Cervical (*n* = 599, 61%)	365/359 (37/36)	125 (13)
Submandibular (*n* = 401, 41%)	270/220 (27/22)	89 (9)
Periauricular (*n* = 55, 6%)	33/24 (3/2)	2 (0.2)
Axillary (*n* = 26, 3%)	14/15 (1/2)	3 (0.3)
Inguinal (*n* = 7, 1%)	5/5 (1/1)	3 (0.3)
Occipital (*n* = 5, 1%)	0/5 (0/1)	–
Other areas (*n* = 24, 2%)	–	–

US pattern (*n* = 725)	*n*	%
Hyperechoic pattern	431	59
Hypoechoic pattern	183	25
No change in echogenicity	43	6
Hypoechoic and hyperechoic pattern	3	0.4
Unknown	33	5

Shape of lymph node[a] (*n* = 825)	*n*	%
L/S ratio of <2 (round node)	545	66
L/S ratio of >2 (elongated node)	330	40

Cytology findings (*n* = 157)	*n*	%
Granulomatous inflammation	88	56
Suppurative inflammation	77	49
Caseous necrosis	12	8
Necrosis (without caseification)	12	8
Multinuclear giant cells	11	7
Reactive	5	3

US, ultrasonography; L/S, long axis to short axis diameter.
[a]US/computed tomography/magnetic resonance imaging.

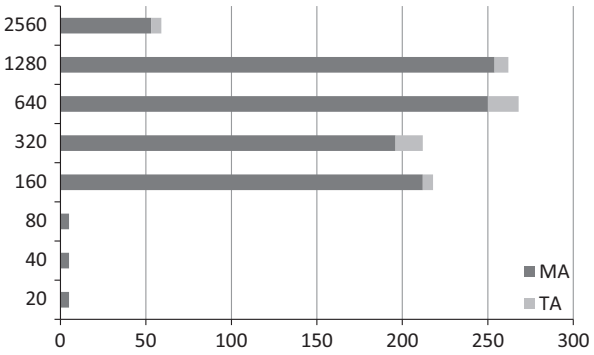


FIG. 2. The distribution of agglutination test titres. MA, microagglutination; TA, tube agglutination.

Previous treatments

In this study, 212 (20.5%) patients did not receive previous antibiotics for the treatment of tularaemia. However, the rest of the patients were given antibiotics for 1176 episodes, which included a single episode in 468 (45.3%) patients, and a second episode in 354 (34.2%) patients. In the previous treatment, β-lactam/β-lactamase inhibitors were given to 793 patients (76%), clindamycin or metronidazole combined with another antibiotic to 45 (2%), macrolides to seven (0.7%), antituberculosis treatment to seven (0.7%), and other antibiotics to 13 (1%).

Specific treatments for tularaemia

The patients were given rational antibiotics for tularaemia after the start of symptoms with a mean of 26.8 ± 37.5 days (minimum–maximum, 1–135 days; median, 21 days). The antibiotic choices are shown in Table 3. In this cohort, 713 patients were given single antibiotics, 299 patients were given combination regimens, and 11 patients were given sequential therapy. For 11 patients, data on the antibiotic choices were

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TABLE 3. Reasons for therapeutic failure after the start of antibiotics in tularaemia patients

Therapeutic failure parameters (n = 495)	Overall n (%)	Monotherapy n (%)	Combination n (%)
Suppuration of the lymph nodes	426 (86)	248 (58)	178 (60)
Formation of new lymphadenopathies	146 (29)	93 (22)	53 (18)
Persisting complaints despite 2 weeks of treatment	77 (16)	37 (9)	40 (13)
Absence of shrinkage in the lymph nodes	63 (13)	45 (10)	18 (6)
Absence of regression in acute-phase reactants[a]	13 (3)	6 (1)	7 (2)
Unresolving fever for 72 h despite treatment	4 (1)	2 (0.4)	2 (1)
Total	495	431	298

[a]Erythrocyte sedimentation rate, C-reactive protein, leukocytosis.

missing from the hospital records. Five of eight pregnant women were treated with gentamicin. The remaining three were treated after the delivery.

Therapeutic outcomes

Treatment failure was considered to have occurred in 495 patients (48%). The most frequent reasons for failure were the production of suppuration in the lymph nodes after the start of treatment (n = 426, 86.1%), the formation of new lymphadenomegalies under treatment (n = 146, 29.5%), and persisting complaints despite 2 weeks of treatment (n = 77, 15.6%). Therapeutic failure parameters are shown in Table 4 and Fig. 3. Lymph nodes were seen to be fistulizing in 108 (10%) patients on admission. After the start of antibiotics, lymph node size decreased in 519 patients (50%), lymph nodes were impalpable in 101 (10%), lymph node size was unchanged in 167 (16%), lymph node size increased in 108 (10%), and lymph nodes fistulized in 53 (5%) (Fig. 4). The last three parameters indicated the unresponsiveness of lymph node inflammation to antibiotic treatment (n = 328, 35%). In this study, lack of compliance with treatment, leading to persistence of the complaints, was observed in 15 (1.5%) patients. Other complications included nephropathy (n = 8, 0.8%), hearing loss (n = 3, 0.3%), vertigo (n = 2, 0.2%), hepatitis (n = 2, 0.2%), and tinnitus (n = 1, 0.1%). It was not certain whether these other rare complications were directly related to tularaemia or were attributable to other reasons. Relapse was observed in 24 patients (2%). Table 5 shows the antibiotic choices in patients with relapses. Mortality was not observed.

Surgical procedures

Fine-needle aspiration was performed in 521 patients (50%), incision and drainage in 121 (12%), and excision of the lymph node in 36 (3%).

Discussion

This study, the largest case series ever reported, has shown that tularaemia in Turkey is mostly of the oropharyngeal form. The presentation of the patient was mostly based on enlarged lymph nodes (92%), which can be translated as swollen neck along with fever (85%), and sore throat (84%). However, symptoms and

TABLE 4. Distribution of antibiotics with respect to duration of treatment

			Treatment duration (days)				
Antibiotics	**n (%)**	**Missing**	**<7**	**7–14**	**15–21**	**22–30**	**>45**
Monotherapy, no. (%)							
Streptomycin	291 (28)	2 (0.2)	–	266 (26)	18 (2)	4 (0.4)	1 (0.1)
Ciprofloxacin	188 (18)	–	–	141 (14)	47 (5)	–	–
Doxycycline	127 (12)	2 (0.2)	–	41 (4)	80 (8)	2 (0.2)	2 (0.2)
Gentamicin	85 (8)	–	–	–	83 (8)	2 (0.2)	–
Moxifloxacin	17 (2)	–	–	10 (1)	6 (1)	1 (0.1)	–
Tetracycline	5 (0.5)	1 (0.1)	–	–	–	1 (0.1)	3 (0.3)
Combination therapy, no. (%)							
Streptomycin + doxycycline	104 (10)	–	–	41 (4)	54 (5)	8 (1)	1 (0.1)
Ciprofloxacin + doxycycline	71 (7)	–	1 (0.1)	12 (1)	57 (6)	–	1 (0.1)
Gentamicin + doxycycline	30 (3)	–	–	2 (0.2)	27 (3)	1 (0.1)	–
Moxifloxacin + doxycycline	10 (1)	–	–	1 (0.1)	9 (1)	–	–
Streptomycin + ciprofloxacin	35 (3)	–	–	29 (3)	4 (0.4)	–	2 (0.2)
Gentamicin + ciprofloxacin	41 (4)	–	1 (0.1)	40 (4)	–	–	–
Doxycycline + rifampicin	3 (0.3)	–	–	1 (0.1)	–	2 (0.2)	–
Streptomycin + doxycycline + ciprofloxacin	5 (0.5)	–	–	2 (0.2)	3 (0.3)	–	–
Sequential therapy (total days), no. (%)							
Streptomycin–doxycycline	4 (0.4)	–	–	1 (0.1)	1 (0.1)	2 (0.2)	–
Ciprofloxacin–doxycycline	3 (0.3)	–	–	3 (0.3)	–	–	–
Streptomycin–ciprofloxacin	2 (0.2)	–	–	1 (0.1)	–	1 (0.1)	–
Gentamicin–ciprofloxacin	2 (0.2)	–	–	2 (0.2)	–	–	–
Missing data, no. (%_	11 (1)	11 (1)	–	–	–	–	–
Total, no. (%)	1034 (100)	16 (0.5)	2 (0.2)	593 (57)	389 (38)	24 (2)	10 (1)

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TABLE 5. Distribution of antibiotics in the patients with relapses

Antibiotics	*n* (%)	Missing	Treatment duration (days) <7	7–14	15–21	22–30	>45
Monotherapy							
Streptomycin	7	–	–	5	–	2	–
Ciprofloxacin	2	–	–	2	–	–	–
Doxycycline	1	–	–	–	–	1	–
Gentamicin	2	–	–	2	–	–	–
Combination therapy							
Streptomycin + doxycycline	4	–	–	1	–	3	–
Ciprofloxacin + doxycycline	2	–	–	1	1	–	–
Gentamicin + doxycycline	1	–	–	1	–	–	–
Doxycycline + rifampicin	2	1	–	–	–	1	–
Streptomycin + doxycycline + ciprofloxacin	1	–	–	–	1	–	–
Sequential therapy (total days)							
Ciprofloxacin–doxycycline	1	1	–	–	–	–	–
Missing data	1	1	–	–	–	–	–
Total	24	3	–	12	2	7	–

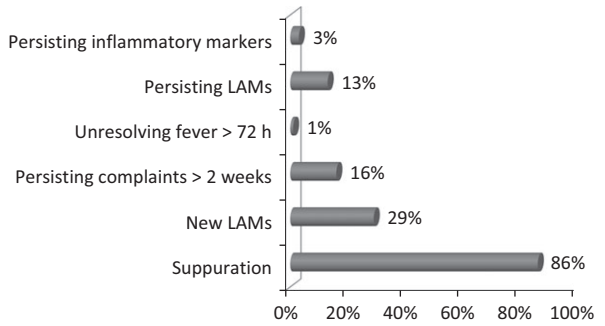


FIG. 3. The distribution of therapeutic failure parameters. LAM, lymphadenomegaly.

findings were seen in a diverse pattern. Waterborne spread is far more common in Europe and Turkey than in the USA. In recent Turkish studies, drinking natural spring water was found to be the leading risk factor for the development of tularaemia [17,20]. The surveillance of infection has been strengthened in Turkey since 2004 by mandatory notification of the disease [21], and cases are now better recognized throughout the country. Skin rashes were of particular interest, and inconsistent data were reported in relatively small case series comprising a few cases with dermatological manifestations [7,22,23]. In our study, 10% of the patients had skin rashes such as maculopapular eruptions, erythema multiforme and erythema nodosum as the most frequent lesions. Accordingly, acute-phase reactant levels were high in most of our patients. In this study, more than three-quarters of the patients were found to suffer from tonsillitis and/or pharyngitis preceding the diagnosis of tularaemia.

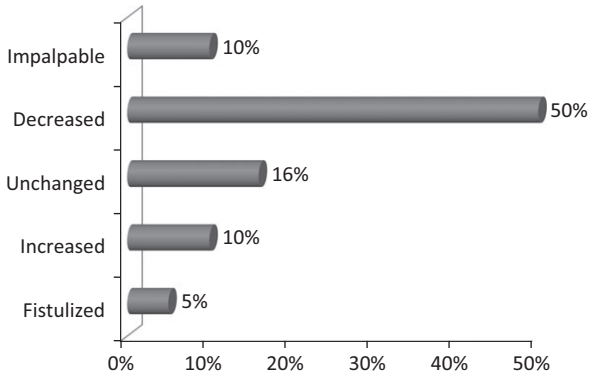


FIG. 4. The course of the enlarged lymph nodes after the start of antibiotics.

In this study, the US findings of the cervical lymph nodes were quite non-specific, as they presented as either hyperechoic or hypoechoic changes. Focal or diffuse abnormal (hyperechoic, hypoechoic, or mixed) echo changes of cervical lymph nodes were found in nearly 85% of our cases with tularaemia. Being the most prevalent pattern, hyperechoic changes were probably attributable to increased interfaces of inflammation, which reflected the sound beam in all directions [24]. The second form, hypoechogenity, may be related to necrosis [24,25]. Affected lymph nodes were unilateral in up to two-thirds of all cases, and long axis to short axis diameter ratios were preserved in two-thirds of the cases. There are several small case series in the literature in which the lymphatic enlargement was unilateral [22,26,27]. The probable explanation is the high infectivity of the bacterium, as the present organisms are able, with low bacterial counts, to invade the oropharyngeal mucosa wherever feasible. Although variability existed, the mean diameter of the short axes of the dominant lymph nodes was found to be 1.7 cm and that of the long axes 3.2 cm in another study [25]. Consequently, tularaemia must be considered in the differential diagnosis of a febrile disease with oval or round enlarged cervical lymph nodes with hypoechoic/hyperechoic cortical changes.

Although the diagnosis in our patients was commonly established with the MAT, culture was positive in 14% of cases and PCR disclosed tularaemia in 40% of cases. Despite the special requirements, such as biosafety level 3 laboratories, for the culture of *F. tularensis* [28], the high positivity rate in our study seems to be directly correlated with the increasing recognition in the country leading to notable collaboration with the national reference laboratory. Accordingly, the MAT was used in 95% of our cases. The probable reasons for the widespread MAT use were the awareness of tularaemia and the free-of-charge testing that the government provided. However, the MAT titres in our patients were very high, with

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a mean of 1/640. These exceedingly high titres were probably attributable to delayed diagnosis of the cases. Maximum antibody titres are known to occur 6 weeks after the onset of symptoms, and they decline 2–3 months after treatment [7]. Thus, in a patient with sore throat, fever, and lymphadenopathy, the MAT can be easily used in the differential diagnosis of tularaemia in endemic regions. However, in the first 2 weeks of infection, MAT titres were reported to be low, and thus the use of PCR should be considered at the early stages of tularaemia [2,28,29].

The data related to the histopathological features of lymphadenomegaly in tularaemia are restricted to small case series [25,30,31]. Usually, reactive changes without necrosis have been reported in the early stages. After the second week, abscess formation with or without epithelioid cell reactions has been reported. A great number of small epitheloid granulomas that contain necrosis at the centre appear at approximately 2–6 weeks, and multinuclear giant cells have been noted. Finally, caseous necrosis has generally been detected after the fourth week of disease [32,33]. Tuberculosis must be kept in mind, particularly in the differential diagnosis when caseous material exists. When lymph nodes are inflamed, abscess formation can occur in tuberculous lymphadenitis [33]. Thus, in a patient with febrile lymphadenopathy, the clinical and histopathological assessment of the disease may be confusing, and may favour tuberculous adenitis. In this study, 157 patients were evaluated histopathologically. The most frequent histopathological diagnosis was granulomatous inflammation (56%), followed by suppurative inflammation in half of the cases. Caseous necrosis was seen in only 8% of our patients. Consequently, clinicians have a tendency to manage febrile patients who are unresponsive to β-lactam antibiotics with antituberculosis medications in cases of granulomatous or caseous lymphadenitis [33,34]. As tularaemia can be treated with antituberculosis drugs such as streptomycin, these patients can be erroneously treated in favour of tuberculosis for months. In a large cohort of cervical tuberculosis patients, 7% of the cases were found to be positive for tularaemia with the MAT [34]. In contrast, 1% of the cases were treated for tuberculosis in this study. Thus, in countries where tularaemia is endemic, the disease should be considered primarily in the differential diagnosis of tuberculosis to prevent irrational treatment.

Owing to the rapid development of infection in the course of tularaemia, therapeutic success will depend on early clinical suspicion and start of therapy [28]. According to the WHO guideline, the first choices for treatment are bactericidal antibiotics such as streptomycin or gentamicin for 10 days. Doxycycline or ciprofloxacin are the alternative choices, particularly for non-severe cases. When doxycycline is used as a therapeutic regimen, the duration of treatment should be 15 days, owing to its bacteriostatic nature. Penicillins, cephalosporins, macrolides, rifampicin, trimethoprim–sulphamethoxazole and clindamycin are not effective against *F. tularensis*, and these drugs should not be used in the management of tularaemia [http://whqlibdoc.who.int/publications/2007/9789241547376_eng.pdf]. The antibiotic susceptibility patterns of *F. tularensis* in local Turkish studies were in accordance with these general concepts [35,36]. In this study, three-quarters of our patients who previously attended health institutions had used various antibiotics, most frequently β-lactams. It appears that the diagnosis of the disease is generally delayed. However, early treatment of the disease is known to result in better outcomes [25,37], and therapeutic delays exceeding 3 weeks have resulted in frequent failures [23]. In this study, it took a mean of 4 weeks to provide rational antibiotics to tularaemia patients, and early treatment was not always feasible, owing to the subtle nature of the disease. One of the therapeutic failure parameters was detected in half of our patients. Consequently, our data suggested that tularaemia was more severe and more frequently associated with complications than is commonly perceived. The most frequent reasons for failure were related to lymph nodes, such as the absence of shrinkage, suppuration, and the formation of new lymphadenopathies, despite treatment. Unresolving complaints despite treatment and the persistence of high levels of inflammatory markers were relatively rare in this study. However, at the end, although the use of antibiotics was extended to >2 weeks in one-quarter of the cases, all of the patients were cured completely. Thus, the course of the disease was both protracted and related to therapeutic failures, and, when medical treatment was extended in combination with surgical procedures, the infection was completely eradicated even in problematic cases. According to our data, up to half of the patients with therapeutic failures were treated with combination regimens, although combination therapy is not advocated in current guidelines, other than for particular conditions such as meningitis and endocarditis. However, the clinicians in this study probably preferred to use combined antibiotics in hard-to-treat cases or in patients with extended disease courses frequently related to therapeutic failures. Accordingly, five pregnant women were cured with gentamicin in our study, and there are reports in the literature of the successful use of gentamicin during pregnancy [38]. Finally, although the relapse rates were as low as 2% with combined medical and surgical approaches, some of those who relapsed may have gone to other centres, and the relapse rate may therefore have been under-reported.

Controversy still exists regarding the selection of the drainage procedure. Fine-needle aspiration may have particular

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advantages, as it does not leave scar tissue, in contrast to incisional drainage or excision. On the other hand, septum formation of the lymph node may obscure the efficacy of the method chosen, and further data are needed on this issue. In this study, the most frequent drainage procedure was fine-needle aspiration, which was performed in half of all patients, followed by incision, which was performed in one-tenth of the cases.

The major limitation of our study was its retrospective design. However, in this study, which is the largest case series ever reported, we have presented a wealth of information. In conclusion, tularaemia is a long-lasting but a curable disease in this part of the world. Our study indicated that, in our region, the most common clinical form was oropharyngeal tularaemia. Cervical lymphadenomegaly was the most common clinical sign, and granulomatous inflammation of lymph nodes was the most common histopathological finding. In addition, the most common complication of oropharyngeal tularaemia was lymph node suppuration. Hence, the differential diagnosis of tuberculosis is an important concern for tularaemia patients. In this study, the most commonly used antibiotics were streptomycin and doxycycline. However, the treatment strategy still needs optimization.

Transparency Declaration

We have no competing interests to declare.

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