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Outbreak of tularemia: a case—control study and environmental investigation in Turkey

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Summary
Objective: The aim of this study was to identify the potential factors associated with infection sources and modes of transmission during a recent outbreak (October 2004) of tularemia in Suluova, Turkey.
Methods: Following the diagnosis of five patients with tularemia in October 2004, active surveillance was initiated to identify further cases. This was a matched case—control study with analysis based on the first 43 cases of tularemia (probable or suspected) and 43 matched controls. A probable case was defined as a patient, resident in Suluova, who had signs and symptoms (regional lymphadenopathy and fever) compatible with tularemia and a positive serology or PCR for *Francisella tularensis* during the period October 21 to November 31, 2004. A suspected case was defined as a patient with compatible signs and symptoms who did not meet the laboratory criteria for a probable case, who also had no laboratory evidence of infection by other microorganisms, and who was resident in Suluova between the same dates. The microagglutination test was used for serological diagnosis. A standardized questionnaire was used to collect information on general demographics, exposure to all known sources of tularemia infection, potential risk factors related to water and animals (i.e., fishing, farming, hunting, and other activities), and the environmental conditions of the house. PCR was used to screen for evidence of the tularemia agents in clinical samples from patients and water samples.
Results: The overall attack rate was 2.3 per 1000 population (86/38 000). Twenty-eight suspected cases and 15 probable cases of tularemia were included in the study. The most common presenting symptom was lymphadenopathy present in 95.3%, followed by fever (83.7%) and sore throat (79.1%). Twenty-eight out of 43 were reported to have painful lymph nodes. *F. tularensis* was detected by PCR

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in samples obtained from the ulcerated lesions of two patients. In the multivariate logistic regression model, keeping a domestic animal in the garden was associated with an increased risk of contracting the disease (OR = 10.87; 95% CI: 1.26—93.65; $p = 0.03$). *F. tularensis* was detected by PCR in the water sample obtained from the rivulet that passes through Suluova.

Conclusions: The results of this study show that case—control studies may be useful for analyzing epidemics and for identifying the source of infection. In order to prevent water-related zoonotic infections, water and sewerage systems should be improved.

Introduction

Tularemia is a zoonotic disease caused by *Francisella tularensis*, a fastidious, small, Gram-negative rod that primarily infects a wide range of animal reservoir hosts, especially rodents and rabbits. *F. tularensis* can be transmitted to humans by tick bites, contact with contaminated water, handling infected materials, and inhalation.[1]

Five patients with fever and cervical lymphadenopathy were admitted to Ondokuz Mayıs University Hospital in Samsun, in the northern part of Turkey, in October 2004. All patients came from a province of Amasya (Suluova) and they did not respond to treatment with beta-lactam antibiotics. Tularemia was clinically suspected, and the diagnosis was serologically confirmed. Public health records revealed no prior reports of tularemia in Suluova, but the disease had previously been reported from surrounding cities. Suluova is situated near the Yesilirmak, which is one of the two major rivers in the Black Sea Region (the other is the Kizilirmak), and has a population of 38 000. Two small rivulets run through the county. Literally, Suluova means 'watery plain'. The main occupations in the area are agriculture (such as rice cultivation) and stockbreeding.

We describe herein the outbreak characteristics, field investigations on the source of infection, and a matched case—control study using a questionnaire to identify the potential factors associated with infection sources and modes of transmission.

Materials and methods

In October 2004, five patients were diagnosed with tularemia by microagglutination test. In the same week, our infection control team visited the epidemic site; the team initiated an active prospective tularemia surveillance and field detection of *F. tularensis* in collaboration with the local health authorities between October 21 and November 31, 2004. To identify common factors that might be the source of the outbreak, detailed interviews with patients were carried out. They were questioned on their occupation, recent travel history, home water supply, recreational activities, contact with rodents, and insect bites.

Case definition

A probable case was defined as a patient, resident in Suluova, with signs and symptoms (regional lymphadenopathy and fever) compatible with tularemia and positive serology or PCR for *F. tularensis* during the period October 21 to November 31, 2004. A suspected case was defined as a patient with compatible signs and symptoms who did not meet the laboratory criteria for a probable case, who also had no laboratory evidence of infection by other microorganisms, and who had been resident in Suluova between the same dates.

Case finding

To identify cases of tularemia, active surveillance was initiated for compatible cases in Suluova County. Interviews were conducted with the local health authorities to obtain relevant information. All local health services and hospitals in Suluova and surrounding cities were informed of the tularemia outbreak and asked to report cases. Patients presenting or referred to district hospitals with suspected tularemia were reviewed on hospital admission, and data were recorded on a standard case report form. Information collected included patient demographic characteristics, risk factors for tularemia, clinical symptoms and signs, and laboratory results. Also, all health records including information for the 2 weeks before the outbreak were reviewed.

Case—control study

Based on the findings of these interviews, a case—control study was initiated in November 2004. Forty-three patients with probable or suspected tularemia were enrolled. Forty-three controls were randomly sought from the primary health authority registration list on the same dates, and matched for age, sex, and district. Any control with a history of illness in the 30 days before the outbreak or during the outbreak period was excluded.

A standardized questionnaire was used to collect information on general demographics, exposure to all known sources of tularemia infection, potential risk factors related to water and animals (i.e., fishing, farming, hunting, and other activities), and the environmental conditions of the house. Questions were closed, allowing individuals to provide additional comments. Controls and cases were interviewed by healthcare personnel.

Statistical analyses

The exposure variables were analyzed by calculating univariate odds ratios (ORs) with 95% confidence intervals (CIs) to determine risk factors for disease. Variables that were associated with illness in the univariate analysis were included in the multivariate stepwise logistic regression analysis. Values of p less than 0.05 were considered statistically significant. SPSS for Windows 10.0 software was used for statistical analysis.

Environmental investigation

The early environmental investigations focused on municipal water depots in Suluova. The local public health authorities collected water samples from water depots and all working municipal taps in Suluova in order to undertake routine microbiological analysis for total and fecal coliforms, and for culturing of Shigella and Salmonella species. Water samples were also collected from the edge of the Ogulbagi rivulet that passes through Suluova.

Microagglutination test

Blood specimens were obtained at admission to the hospital from all hospitalized patients with suspected tularemia. Blood specimens were transported to Uludag University Microbiology Laboratory for testing for tularemia. The microagglutination test was used for serological diagnosis. The antigen used in the serological tests was prepared from an *F. tularensis* strain.[2] Antibody titers of 1:80 and above were accepted to be significant for diagnosis.[2]

PCR detection of *Francisella tularensis*

Clinical samples of the ulcerated lesions from two of the five hospitalized patients and water samples from the rivulet were screened for evidence of the tularemia agent by PCR. PCR amplification and product detection were performed using a real-time PCR system (Quantica; Thecne Inc., USA) at Kocaeli University Microbiology Laboratory. Regions targeted for TaqMan assay were specific for *F. tularensis* and included *tul4* and *fopA* genes, which encode outer membrane proteins.[3]

Results

During the epidemic, 86 cases were recorded by the health authorities in Suluova region. A map of the major affected area, including case distribution, is shown in Figure 1. The overall attack rate was 2.3 per 1000 population (86/38 000). Clinical data for 28 suspected and 15 probable cases were available. These cases were included in the study. Of these,

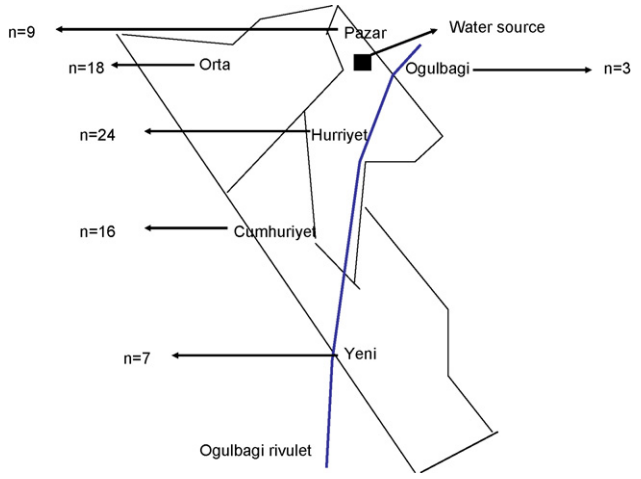

Figure 1 The water source, rivulet, and distribution of cases.

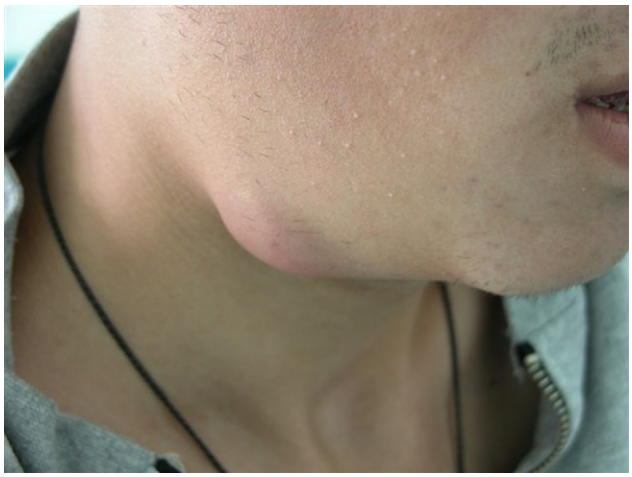

Figure 2 Cervical lymphadenopathy in a patient with tularemia.

21 (48.8%) were female and 22 (51.2%) were male. The difference in mean age between cases and controls was not statistically significant (29.5 ± 17.9 vs. 34.6 ± 14.7; $p = 0.15$).

Clinical presentation

The single most common presenting symptom was lymphadenopathy present in 95.3% (Figure 2), followed by fever (83.7%) and sore throat (79.1%). Twenty-eight out of 43 reported painful lymph nodes. Six of the patients had bilateral lymphadenopathy. The clinical forms of the disease are shown in Table 1.

Five patients underwent excision of the lesion because the discharge could not be controlled. The pathology results for four of these patients reported chronic granulomatous inflammation, and for the remaining one patient, chronic active inflammation. In all these cases, the tularemia agglutination test was positive. In two patients, *F. tularensis* was shown by PCR in samples obtained from the ulcerated lesion. No deaths were reported.

Case—control study

In the univariate analysis of recent exposure to potential risk factors, the presence of dead animals (rodents) in the neighborhood, keeping domestic animals (ducks, chickens) in the garden, and drinking spring water from the environment, all significantly affected the likelihood of disease (Table 2). All the significant variables and the type of water source, the functional status of the treatment plants, and geographic location were added to the final multivariate logistic regres-

Table 1 The clinical forms of tularemia

Clinical form	*n*	%
Glandular	26	60.4
Ulceroglandular	10	23.3
Oculoglandular	5	11.6
Pharyngeal	2	4.7

Table 2 Univariate analysis of risk factors for tularemia in case patients and control subjects

Risk factors	Cases ($N = 43$)		Controls ($N = 43$)		Odds ratio	95% CI	p
	n	%	n	%			
Pipe water as water source	42	97.7	41	95.3	2.0	0.18—23.5	1.00
Turbidity in water	28	66.7	22	56.4	1.50	0.63—3.81	0.47
Contact with a rodent	8	19.0	3	7.0	3.14	0.77—13.77	0.18
Presence of a dead animal in the neighborhood	7	16.7	1	2.3	8.4	0.98—71.60	0.03
Insect bite	0	-	2	4.7	-		0.49
Collecting food from the environment	3	7.3	0	-	-	-	0.11
Drinking spring water from the environment	7	16.7	0	-	-	-	0.005
Keeping a domestic animal in the house	3	7.1	0	-			0.12
Keeping a domestic animal in garden	9	21.4	1	2.3	11.45	1.38—95.2	0.007
Travel	3	7.3	4	9.3	0.77	0.16—3.77	1.00
Agricultural activities	7	17.1	6	14.3	1.23	0.37—14.05	0.96

sion model; only the keeping of domestic animals in the garden was associated with an increased risk of contracting the disease (OR = 10.87; 95% CI: 1.26—93.65; $p = 0.03$). When the regression analysis was carried out only for probable cases, it was found that the only risk factor for contracting the disease was keeping domestic animals in the garden ($p < 0.05$).

Environmental investigation

In two of the cultures obtained from water sources crossing the Ogulbagi rivulet, a high number of coliform bacilli were observed (Figure 1). The chlorine device used to disinfect this water source was found to be non-functioning, however no investigation could be made for *F. tularensis* in the water sources. *F. tularensis* was detected by PCR in the water sample obtained from the rivulet (Figure 3). The majority of the cases lived close to the Ogulbagi rivulet, and the areas where the patients lived were supplied with water from the water source with the non-functioning disinfection device. However, the disease was not observed in some other areas having their water supplied from this same source. It was found that the water and sewerage system had been repaired in May 2004.

Figure 3 The Ogulbagi rivulet.

Discussion

This case—control study evaluated the risk factors for tularemia during a tularemia epidemic in Turkey. To our knowledge, this is the first time *F. tularensis* has been shown with PCR in both lymph node aspiration fluid samples from patients and from the water source. An electronic Medline search was performed to identify all reports on tularemia epidemics in Turkey using the keywords '*Francisella tularensis*', 'tularemia', 'Turkey', and 'Turkiye'. One of the six records that were identified was related to an outbreak in Trakya region in 2002.[4] Two recent outbreaks reported from Duzce (2001)[5] and Kocaeli (2005)[6] were also found. Six additional outbreaks were published in national journals between 1936 and 2003. Also there were some case reports. Prior to 2004, tularemia was not included in the official list of reportable infectious diseases; in 2004, the list was revised and tularemia was added. Therefore there were no official statistics regarding tularemia in Turkey. The positive impact of recent outbreaks has been in increasing the awareness of tularemia.

The clinical presentations of the disease were found to be consistent with previous studies. The most common form is the glandular form. Helvaci et al.[2] reported that the most common form was the oropharyngeal form (83%). The concomitance of conjunctivitis with the glandular form in five cases suggests that the infectious agent spreads to the eye by rubbing or scratching the eye following contact with infected animals or water.

Beta-lactam antibiotics are those preferred for cases presenting with complaints of upper respiratory tract infection. *F. tularensis* is resistant to beta-lactam antibiotics,[7] and treatment of cases with beta-lactam antibiotics has resulted in a chronic disease process and delays in diagnosis.[2] The lack of response to beta-lactams may be an important clue in the diagnosis of tularemia. Upper respiratory tract infections, congenital disorders, tuberculosis, primary neoplasms, and metastasis are considered first in cases where there is a mass in the throat;[8] tularemia is

rarely considered unless there are epidemologic data. *F. tularensis* results in chronic granulomatous inflammation and mimics tuberculosis especially in cases progressing to the chronic state. In our country tuberculosis is common,[9] and this results in evaluation and treatment of patients for tuberculosis. One of the anti-tuberculosis drugs used for treating tuberculosis is streptomycin. Thus, tularemia might be indirectly treated in a portion of patients if the treatment has been initiated early. However, cases unnecessarily receive long-term anti-tuberculosis treatment.

The results of this case—control study support the hypothesis that animals play a role in tularemia epidemics. In addition, epidemiologic and environmental evidence seem to support the results of this case—control study and suggest that contamination of the rivulet water may have an impact on the occurrence of epidemics. In the areas of Suluova where the epidemic was observed, some families raise cattle, sheep, and poultry (hens, ducks) in order to feed themselves. Ducks in particular use the rivulet in the neighborhood for swimming. Rivulet water is also used for irrigation purposes. Children residing in the region use the rivulet area as a playground. Therefore contamination of the rivulet may play a role in the transmission of tularemia. Also the demonstration of *F. tularensis* by PCR in the water sample obtained from the rivulet supports the hypothesis that small domestic animals might play a role in the transmission of the disease. After cleaning, animals are cooked thoroughly before they are eaten. However, *F. tularensis* may be contracted by humans through the skin during plucking and cleaning. Another possible factor is the consumption of raw vegetables that were irrigated with the rivulet water without properly washing them. However contamination of the surrounding area and the rivulet by ducks or dead rodents cannot be ruled out (false positivity).

The agglutination test is frequently used for tularemia diagnosis; in our study, clinical diagnosis was first confirmed with the agglutination test. However, the antibody response occurs within the first two weeks of the disease.[1] This may not always be sufficient for early diagnosis. A four-fold rise in the microagglutination titer is diagnostic of infection, however we could not obtain paired sera from patients. Although culture is the gold standard for diagnosis, it is not usually used due to the requirements for special media and an equipped laboratory; it also poses a significant risk of infection for laboratory personnel. PCR is useful for diagnosis of human disease and as seen in our study, it is helpful for investigating *F. tularensis* both in tissue samples and samples obtained from the environment.

Conclusions

The results of this study show that case—control studies are useful for analyzing epidemics and for identifying the source of infection. In order to prevent water-related zoonotic infections, water and sewerage systems should be improved. Tularemia sometimes accompanied by epidemics, should definitely be considered in the differential diagnosis for patients presenting with upper respiratory tract infections and a history of mass in the throat, especially those who have been unresponsive to previous treatment with penicillin. For this purpose, healthcare personnel and the community should be educated on the diagnosis and treatment of tularemia.

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Conflict of interest: No conflict of interest to declare.

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