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Outbreaks of Tularemia in Turkey

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Tularemia, casued by *Francisella tularensis,* is a zoonotic disease presenting various clinical forms. In the present study, three outbreaks of tularemia occurred from January to March and September in 2004 (first and second) and January to March in 2005 (third) are reported from the north-eastern part of Turkey. All cases originated from the same geographical location. In total, 56 patients having complaints of fever, malaise, chills and shivering, painful sore throat with swollen tonsils and enlarged cervical lymph nodes were affected and the patients were different in all cases. Forty-four, 7 and 5 people were affected in the first, second and third outbreak, respectively. The sera from all patients were analysed for the presence of *F. tularensis* antibodies using a microagglutination assay. Overall, of the 56 sera analysed, 39 (33, 3 and 3 were from the first, second and third outbreak, respectively) showed antibody titres of 1/160 and/or more against *F. tularensis*. The current report suggests that tularemia exists in north-eastern part of Turkey. The clinical manifestation of the current cases were similar to those of oropharyngeal form of tularemia. It is considered that this region should be accepted as an endemic area for tularemia and kept under control for a long period.

Tularemia, caused by *Francisella tularensis*, is primarily a disease of wild lagomorphs and rodents and regarded as a zoonotic disease (3,14). These animals are also natural reservoirs of this bacterium (8). *F. tularensis* is a small, Gram negative, pleomorphic, intracellular and nonmotile bacterium (21). It was first isolated from human cases in 1907 and from rodents in 1912 (17,18). The disease is transmitted to humans by ticks, mosquitoes and biting flies. Other transmission routes of tularemia to humans are foodstuffs, drinking water, infected animals, handling of infected meat and aerosols (3,8,12,13,15,21).

Tularemia is known to appear only in northern hemisphere. It most frequently occurs in Scandinavia, America, Russia and Japan. Recent cases of tularemia have also been increasingly reported from other countries such as Turkey, Kosovo, and Yugoslavia, which implicates that tularemia is widely distributed throughout the world (8,21).

Tularemia is a debilitating disease. Depending on the portal of entry of the bacterium, the clinical manifestations of tularemia vary in humans. The most common clinical form of the disease is ulceroglandular and/or glandular form characterized by the enlargement of lymph nodes with and without an ulcer, which usually occurs after a bite by an infected vector (8, 21). This form of the disease is rarely fatal (less than 3%) (20). The other form of the disease, typhoidal tularemia characterized by an acute septicemia without lymphadenopathy or with an appearance of an ulcer, is more fatal with a mortality rate of 30 to 60% (10). Oropharyngeal or gastrointestinal form of the disease is also seen in humans after the

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ingestion of food and/or water (3, 8). The oropharyngeal form occuring more frequently in Eastern Europe appears as a painful sore throat with enlargement of the tonsils accompanied by swollen cervical lymph nodes (19, 21). Gastrointestinal form of the disease is characterized from a mild but persistent to an acute fatal disease with extensive ulceration of the gastrointestinal system. The most acute form of tularemia is the pneumonic form, also known as respiratory form, which is presumably transmitted by farming activities such as handling of hay and inhalation of dust containing these bacteria (8).

Since *F. tularensis* is a fastidious and slow growing organism, it is difficult and time-consuming to isolate and identify bacteria in routine clinical laboratories (7). Therefore, most tularemia cases are diagnosed on the basis of clinical observation and/or serology. In addition, due to low infective dose of the organism, culture is not undertaken by most routine laboratories in order to avoid risks of laboratory infection (5,21), making serological diagnosis preferable. The serum antibodies raising against this organism are usually detected by agglutination and/or ELISA (4, 15). Detection of *F. tularensis* is also achieved directly in clinical specimens using molecular techniques such as polymerase chain reaction (14,15). *F. tularensis* is also accepted as a potential biological warfare agent and listed within the most likely bioterrorist agents (16,21).

Persistent painful sore throat with enlarged tonsils and swollen cervical lymph nodes were observed in a number of patients admitted to clinics of the state hospital in the years 2004 (repeated twice) and 2005 in Turkey. The aim of the present study was to diagnose theses cases both clinically and serologically and to share the experience gained out of this study with the scientific world.

MATERIALS AND METHODS

A total number of 56 patients having complaints of fever, painful sore throat with swollen tonsils and enlarged cervical lymph nodes were admitted to the State Hospital of Kars located in the north-eastern part of Turkey in the years 2004 and 2005. Affected persons were different in all cases and all the cases were from the same geographical location, Sarikamis area in Kars city. The first outbreak that affected 44 people (18 male and 26 female) occured between January and March, 2004. The second outbreak that comprised 7 people (4 male and 3 female) was seen in September, 2004. Between January and March in 2005, a third outbreak affecting five patients (3 male and 2 female) was encountered. Blood specimes from all the patients were obtained and the sera were analysed for the presence of *F. tularensis* (tularemia) antibody using a microagglutination test by the Department of Microbiology, Faculty of Medicine, Uludag University, Bursa, Turkey as described earlier (13). *F. tularensis* whole-cell antigen was employed for the test. Two-fold serial dilutions of the serum samples starting from 1/20 were made and used in order to perform the assay. The sera that showed no and/or lower antibody titres than 1/160 were accepted as negative for *F. tularensis* in all cases.

In addition, all the sera were tested for *Brucella* using rose-bengal plate test (RBT) and tube agglutination test (TAT).

RESULTS

Clinical symptoms of the patients. Two types of clinical manifestations depending on the clinical stage of the disease were observed in all affected persons. In initial stage of the disease, all the patients showed general symptoms such as fever (generally 38ºC and over), chills, shivers, headache, malaise and sore throat with enlarged tonsils, which lasted for

about two weeks. After two weeks, the general symptoms disappeared but the cervical lymph nodes started swelling unilaterally in all patients. Enlarged lymph nodes of the two patients developed suppuration and pistula.

Serological results. Serological investigation of the 56 sera from all three outbreaks showed that 39 had antibody titres of 1/160 and/or more against *F. tularensis* by the microagglutination test (Table 1).

Thirty sera of the 44 examined from the first 2004 outbreak were found positive. The antibody titers against *F. tularensis* from these patients have ranged between 1/160 and 1/2560. Distribution of the antibody titers were as follows: 1/2560 (2 sera), 1/640 (14 sera), 1/320 (10 sera) and 1/160 (4 sera). Two sera had 1/20 antibody titers. The remaining 12 sera did not display any detectable antibody titres. Of these 14 negative sera, 3 were found to be positive with antibody titres of 1/320 against *F. tularensis* in re-collected serum samples 6 weeks later.

Of the 7 sera obtained from the second outbreak patients, 3 were positive for *F. tularensis*. The distribution of the antibody titers were 1/640 (1 serum sample) and 1/320 (2 sera). Two serum samples had 1/80 (1) and 1/20 (1) antibody titres and 2 did not have any detectable antibody level against *F. tularensis* in the second outbreak.

Out of 5 serum samples from the third outbreak (2005 outbreak), 3 were found positive with antibody titers ranging as: 1/640 (2 sera) and 1/320 (1 serum). One serum had 1/40 antibody titre and the other showed less than 1/20 titer. The convalescence period samples were not able to be collected from the seronegative patients in the second and third cases.

All the 56 sera obtained from affected persons were negative for the presence of *Brucella* antibodies by RBT and TAT.

In order to treat patients, streptomycin were given intramuscularly for 14 days. All patients responded to the treatment but it took nearly a month for complete resolvement.

TABLE 1. The summary of serological investigation of the 56 sera from all three outbreaks.

Cases	**Number of seropositve sera (1/160 and/or more titers)**	**Number of seronegative sera (1/160 less titers)**
First	33	11
Second	3	4
Third	3	2
TOTAL	**39**	**17**

DISCUSSION

Tularemia outbreaks have previously been reported from various geographical locations in Turkey (9, 11, 13). However, to our knowledge, this is the first report of tularemia outbreak reported from north-eastern part of the country, where climate is very harsh with about 6 months of winter time with an average temperature of -15ºC. Interestingly, the cases were from the same area in three outbreaks. High titres of *F. tularensis* antibody detected in the sera of infected persons and clinical symptoms observed in the patients implicates the existence of the disease in the area.

The oropharyngeal form of tularemia is known to be common particularly in Eastern European countries including Turkey (13,19). The clinical manifestations observed in the current cases appeared similar to those of oropharyngeal form presenting with sore throats, enlarged tonsils and mostly unilateral cervical and/or retropharyngeal lymphadenitis (13).

The source(s) of the current tularemia outbreak was not properly determined since no attempts were made in order to isolate and/or detect bacteria from any possible source. However, the outbreak affected many people living in the same area and at the same time period. These may suggest that there might be a common source such as water for transmission to humans. In fact, people were using the same water reservoir for drinking and other needs and the water used at the time of the current outbreaks was not properly chlorinated. In addition, the drinking water supplying canals were open to surface in some parts, which may cause contamination of the water sources from the environment. Furthermore, the outbreak of the disease subsided upon chlorination of the drinking water in the area where outbreaks were present. In the current outbreaks, the number of patients might be many more than those given in this report since not all of the patients were applied to hospital clinics for treatment. Outbreaks of waterborne tularemia have previously been reported from several Eastern European countries including Turkey (2,3,12,13). In addition, the oropharyngeal form of tularemia is acquired by ingestion of contaminated water or food. The clinical nature of the present cases may also implicate that there may be a common source. *F. tularensis* was found to survive in water for several months (2). It is also noteworthy to state that *F. tularensis* is also capable of replicating in protozoans under experimental conditions suggesting that they might be existing in water-living protozoans (1,21). The natural source of the infection and ecology of *F. tularensis* in north-eastern part of Turkey need to be further studied by isolation or detection of the organism from various sources such as water, protozoa and wild rodents.

Small outbreaks of tularemia diagnosed bacteriologically and serologically were reported between 1936 and 1953 in Turkey. However, no cases of tularemia were reported between 1953 and 1988 (21). Since 1988, endemic and sporadic cases of tularemia were reported mainly from Marmara and Blacksea Region of Turkey (6,11,13). Helvaci et al. (13) reported that 205 cases of tularemia were diagnosed in north-western part of Turkey since 1988. The current outbreaks were from the north-eastern part of the country, which is far from the north-western part both geographically and by climate. It is reported that all tularemia cases reported from Turkey after 1988 were in winter months (6) as seen in the current report.

In this report, 17 of the 56 sera obtained from patients were found seronegative (no detectable antibodies and/or lower than 1/160 antibody levels) for *F. tularensis* although these patients were presenting the clinical symptoms suggestive of tularemia as in seropositive patients. The reason for this might be that the sera were obtained in early stages of the disease (within first week) and therefore antibody levels might not be sufficient to be detected by the microagglutination test. Positivity of the three sera that had been initially found negative may support this opinion. Moreover, these cases were present at the same time with tularemia outbreaks and the symptoms resolved when treated with streptomycin.

In conclusion, three outbreaks of tularemia in 2004 and 2005 were observed in humans in north-eastern part of Turkey. The clinical manifestations of the current outbreaks seemed similar to those of oropharyngeal form of tularemia. Due to nature and clinical magnitude of the present outbreaks, possible source of contamination might be implicated as water although isolation and/or detection of the etiological agent was not achieved. Early diagnosis of tularemia is important in preventing morbidity and mortality due to this infection.

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