

Short Communication

Tularemia Re-Emerging in European Part of Turkey after 60 Years

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SUMMARY: The aim of this study was to investigate a tularemia outbreak in the Thrace region of Turkey. The outbreak occurred in Demirkoy village of Edirne, in 2005. Of 400 villagers, 266 were examined and their sera were taken. Throat swabs and lymph node aspirates were cultured. Specific antibodies in patients and domestic animals were screened by a microagglutination test. PCR assays and cultures of the samples of patients, animal tissues, and water sources were performed, along with active surveillance to identify risk factors. Seven out of 10 cases were diagnosed as oropharyngeal form; the remaining three patients were asymptomatic. The cultures for tularemia were negative; however, PCR assays were positive in one lymph node aspirate and in water from one spring. Some animals had the specific antibody at low levels. Increased rodent population in the vicinity, exposure to wild rabbits, and drinking from one of the springs were identified as risk factors with the risk ratios (and 95% confidence interval) of 10.5 (10.3 - 10.7), 6.5 (5.43 - 7.57), and 2.1 (1.1 - 2.5), respectively. Therapeutic and preventive measures were taken. When tularemia cases have been detected in a region even a few decades earlier, tularemia should be considered in the differential diagnosis of patients.

In Turkey, two outbreaks of tularemia occurred in the European part of Turkey (Thrace) in 1936 and 1945. A total of 150 and 18 patients were reported in the first and second outbreaks, respectively (1,2). There has been no case report in the last 60 years from the European part of Turkey, until February 2005. The most recent Thracian outbreak of tularemia was seen in one of the villages of Edirne near the Bulgarian border in 2005. A total of 10 tularemia cases were diagnosed during this outbreak. The aim of our study was to investigate the source and size of the outbreak and to analyze risk factors for tularemia.

Patients with a history of stomatitis, pharyngitis or tonsillitis in the last 3 months plus a cervical lymphadenitis and serum antibody titer to *Francisella tularensis* antigen at a dilution of 1:160 or more were diagnosed as having oropharyngeal tularemia. If patients were seropositive (≥ 160 dilutions) without symptoms, they were considered to have asymptomatic tularemia. A microagglutination test was performed on a V-plate as previously described (3). BD *Francisella tularensis* antigen (Becton Dickinson, Sparks, Md., USA) was used in the tests.

A female patient was diagnosed as having oropharyngeal tularemia on 28th of February, 2005 (4). At this time, there were patients with similar complaints in her village (Demirkoy). Of 400 villagers composed of 102 families, 266 were examined and their sera were taken within the same week by a team. Seven villagers had an oropharyngeal form of tularemia. Four and three patients had the antibody against *F. tularensis* at 1:5,120 and 1:10,240 dilutions, respectively. Also, three asymptomatic cases were detected with 1:160-

1:5,120 antibody dilutions. Four of the 10 cases belonged to the same family; the other 6 were each from a different family. In addition, 124 students, who came from adjacent villages but attended elementary school in Demirkoy were examined; however none of them had clinical symptoms and/or seropositivity. No patients with tularemia were found in the nearby villages.

Swabs from palatine tonsils of patients and aspirate from lymph nodes of one patient were obtained. Water samples were collected from one reservoir for one pipe water and from three springs in the vicinity of the village. The reservoirs of two springs (Mesut and Aliaga) were almost superficial. Chlorination of the pipe water was routinely performed once every 2-3 months, but the other sources had been never chlorinated. All water sources were contaminated with coliforms at health hazard levels. Samples of the swabs, one lymph node aspirate and water filters were cultured in blood agar supplemented with 1% glucose and 0.1% cystine. The lymph node aspirate was also put directly into tubes containing 500 μ l lysis buffer (guanidium isothiocyanide [5 M], Na acetate [1/10 (v/v)], sarcosyl 0.5%). Water samples (2 litres from each source) were put through 0.45 μ m filters, and filters were washed with 10 ml distilled water for polymerase chain reaction (PCR) assays. *Tul4* (encoding 17 kDa lipoprotein) and *fopA* (encoding 43 kDa outer membrane protein) primers, 5'-FAM and 3'-TAMRA labeled probes for *F. tularensis* and TaqMan 5' nuclease used for PCR assays. All tests were performed as indicated elsewhere (5).

Farming and stock breeding are the main sources of income of the villagers, and there is a rabbit farm in the village. Sera of the rabbits, sheep, and cattle belonging to the patients were analyzed for anti-*F. tularensis* antibodies. One of the 25 rabbits tested and 19 of the 27 cows had the antibody against *F. tularensis* at low dilutions (1:20 - 1:80). None of the 19 sheep tested had sarcosyl specific antibody. Also, three ticks

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Table 1. Analysis of risk factors for tularemia among the respondents

Risk factor	Population at risk	Patients	AR (+) ¹⁾	AR (-) ²⁾	RR ³⁾	95% CI
Exposure to wild rabbits	16	3	18.8	2.9	6.5	5.43-7.57
Eating wild rabbits	52	3	5.8	3.3	1.8	0.82-2.87
Drinking Aliaga Spring	85	5	5.9	2.8	2.1	1.1-2.5
Increased mouse population at surroundings	124	9	7.3	0.7	10.5	10.3-10.7
Drinking Mesut Spring	113	2	1.8	5.2	0.4	
Drinking Demirbaba Spring	114	4	3.5	4.0	0.9	
Increased mouse population at home	51	2	3.9	3.7	1.1	

¹⁾: Attack rates for the population who have factor (drinking water, exposure to animal, etc.).

²⁾: Attack rates for the population who have no factor (drinking water, exposure to animal, etc.).

³⁾: Risk ratio.

sampled from the animals were crushed and inoculated into bacteriological media. The liver and spleen tissues of a rabbit which died spontaneously during the investigation were cultivated. Liver and spleen samples of eight *Rattus rattus* carcasses found at the homes of the patients were also cultivated and investigated with PCR assay.

F. tularensis could be isolated in none of the cultures; however, PCR assay for *tul4* was positive in one lymph node aspirate and in a water sample from Mesut Spring. PCR assays in animal samples were negative. The amount of DNA copies found in the node aspirate and water sample were 3.8×10^3 and 1.4×10^2 , respectively. PCR assay for *fopA* was negative in these samples.

All people were interviewed with a structured questionnaire to determine possible exposure routes. The resulting analysis of risk factors is shown in Table 1. Oropharyngeal tularemia was one of the most common clinical forms in the outbreaks in Turkey (1-3,6). The ingestion of infected foodstuffs or bacteria in drinking water may result in oropharyngeal or gastrointestinal tularemia, depending on the site of colonization in the host tissues (3). In some tularemia cases, no signs of the disease appear. The rates of asymptomatic cases reported in outbreaks in Turkey have ranged between 4 and 19% (3,6). We detected three asymptomatic cases in the Demirkoy outbreak. The high rate of asymptomatic cases may be related to the number of population screened.

A definitive diagnosis of tularemia requires isolation of the causative agent, which is rather difficult. Thus, serology is often used for the diagnosis of tularemia. We detected high-titer antibody in the sera. We could not isolate the causative agent, but we demonstrated its presence in the lymph node aspirate of a patient using PCR.

Important vectors of the illness are blood-sucking arthropods and mosquitoes. The major animals infecting humans are hares, beavers, and microtine rodents (3). A large outbreak in Kosovo occurred because of the existence of large numbers of rodents in the peridomestic environment (7). Tularemia outbreaks in Europe have been correlated with increasing numbers of rodents (8,9). Tularemia frequently appeared in villagers hunting hare in the first and the most recent Thrace outbreaks of tularemia (Table 1) (1). Some of the domestic rabbits in Demirkoy village died in the last 2 months. However, cultures and serology were negative, so we thought that the rabbit farm was not the source. Significant increases in rodent populations were detected in Luleburgaz between 1930 and 1936. Also, rodents increased in the entire Thrace region of Turkey in 2004 both in fields and around houses. Although the causative agent could not be detected in eight

rat carcasses, rodents might have contaminated water sources and thus caused the outbreak. Sometimes domestic animals play a role in transmission of the disease to humans. Senol et al. (10) reported a case transmitted from sheep. Tokgöz and Golem (11) showed that hedgehogs, pigeons, partridges, donkeys, goats, and sheep were orally or parenterally susceptible to *F. tularensis* in Turkey. We searched antibodies against *F. tularensis* in domestic animals of the patients. A number of cows and one rabbit had low-level tularemia antibodies. These seropositivities were evaluated as nonspecific; we concluded that domestic animals were unimportant for this outbreak.

The first outbreak of tularemia in the Thrace region of Turkey occurred in villages near a brook, and emerged in a summer with heavy rain. Also, employees of rice fields and consumers of brook and canal water suffered from the disease. Thus, water was considered the source of the infection (1). *F. tularensis* was first isolated in Turgutbey, Yenitaslı, Hamzabey, and Ceylan Brooks in 1936 (1). In recent outbreaks, water was also considered as the source of tularemia (3,5). We showed that the risk ratio was higher among consumers of Aliaga Spring water while the PCR assay was positive for *F. tularensis* DNA in Mesut Spring water. Additionally, water cultures confirmed that there was microbiological contamination in all water sources of the village. Thus, we concluded that the sanitation of water was important for the control of outbreak.

Symptomatic cases were treated by daily administration of doxycycline 200 mg plus streptomycin 1 g for 10 days. Preventive measures were taken to get the tularemia outbreak under control such as the chlorination of pipe water, the education of villagers in avoiding of risk factors and hand-washing especially after exposure to risk factors, and the alerting of medical doctors to be aware of tularemia. No single case was detected after these control measures were taken.

Clearly, tularemia is a re-emerging disease in Turkey. There has been a recent tularemia report from Bulgaria, also (9). Together with the Kosovo outbreak, these results suggest that the Balkans have become a new focus for tularemia (7).

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