

Short Communication

Comparison of the 2000 and 2005 Outbreaks of Tularemia in the Duzce Region of Turkey

Davut Ozdemir*, Irfan Sencan¹, Ali Nihat Annakkaya², Aynur Karadenizli³,
Ertugrul Guclu, Erhan Sert⁴, Mustafa Emeksiz⁵ and Ali Kafali⁵

Department of Clinical Microbiology and Infectious Diseases and ²Department of Chest Diseases, Duzce University Medical School; ⁴Department of Clinical Microbiology and Infectious Diseases, Tandogan Tokgoz Duzce State Hospital; ⁵Primary Health Care Unit, The Office of Ministry of Health, Duzce; ¹Department of Clinical Microbiology and Infectious Diseases, Diskapi Yildirim Beyazit Training and Research Hospital, Ankara; ³Department of Microbiology and Clinical Microbiology, Kocaeli University Medical School, Kocaeli, Turkey

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SUMMARY: Tularemia caused by *Francisella tularensis*, which is considered a biological warfare agent, is a widely distributed zoonosis. In this study, we aimed to compare a 2005 outbreak of tularemia that was confirmed as waterborne by PCR to outbreak of tularemia that was reported as waterborne in 2000 and to investigate the changes of epidemiological characteristics between these two outbreaks occurring in the same region. In the present study, a total of 11 patients were diagnosed with tularemia. In the 2000 outbreak, oropharyngeal type was observed in 19 patients, and ulceroglandular type in 3 patients. In the 2005 outbreak, oropharyngeal type was observed in 8 patients, and oculoglandular type in 3 patients. However, our cases are not sufficient to make a conclusion that the characteristics of tularemia seem to be changing.

Tularemia caused by *Francisella tularensis*, which is considered a biological warfare agent, is a widely distributed zoonosis. Transmission of *F. tularensis* to humans occurs through the bite of an insect or animal, contact with contaminated animal products or aerosol droplets, or contact with contaminated water. The clinical manifestations depend on the portal of entry of the organism. Patients usually present with at least one of six classic types of tularemia: ulceroglandular, glandular, oculoglandular, pharyngeal, typhoidal, or pneumonic. A presumptive diagnosis of tularemia is supported by an acute microagglutination titer of 1:128 or more, in the face of compatible disease. Methods for rapid diagnosis that have been reported include polymerase chain reaction (PCR). PCR assays offer high specificity and sensitivity (1).

In this study, we aimed to compare a 2005 outbreak of tularemia that was confirmed as waterborne by PCR to an outbreak of tularemia that was reported as waterborne by Sencan et al. (2) in 2000 and to investigate the differences in epidemiological characteristics between these two outbreaks occurring in the same region.

In February 2005, seven patients, all from Beyhanlı Village (a forest region of Duzce-Akcakoca, in northwestern Turkey, neighboring two villages where the tularemia emerged in 2000) were admitted to Tandogan Tokgoz Duzce State Hospital, with a complaint of swollen neck, fever, and sore throat that had lasted for 20 days. A medical history revealed that all patients had been administered a course of beta-lactam antibiotics and did not respond. No patient had received a tularemia vaccine. Routine throat cultures did not yield any pathogenic bacteria. An enzyme-linked immunosorbent assay (ELISA) for anti-EBV IgM antibody was found to be

negative in all cases. Tularemia was suspected in these patients, and thus a microagglutination test (MAT) for diagnosis of tularemia was performed at the Department of Microbiology and Infectious Diseases, Uludag University School of Medicine by Gedikoglu et al. (3).

Some of the patients living in this village were found to be positive for tularemia MAT. One week later, Beyhanlı Village was visited by the investigation team in order to investigate the source of the outbreak. In the course of the visit, four patients having symptoms clinically compatible with tularemia were found. These patients had been misdiagnosed before our visit. Blood samples were drawn from these patients. In addition, two 2-liter samples were collected from each of the tap water and a village fountain. PCR was performed for *F. tularensis* at the Department of Microbiology and Clinical Microbiology, Kocaeli University Medical School by Karadenizli et al. (4). Recommendations were made to control the outbreak.

Detailed information about all 11 patients was obtained by a questionnaire and physical examination. All sera were also examined by Rose-Bengal (Refik Saydam Hygiene Center, Ankara, Turkey) test for brucellosis to eliminate cross-reactions (2).

The patients consisted of 7 females (63.6%) and 4 males (36.4%) with an age range from 25 to 48 years (35 ± 8). The inhabitants of the village were using tap water and village fountain water which had not been treated with any disinfection or chlorination procedures. The reservoir that supplied the tap water and the village fountain water to the village was supplied by a stream that was not adequately protected from the entry of animals. Additionally, village fountain water was collected in a pool that had no protection from the environment before coming to the village by pipes. The villagers reported that there was a considerable quantity of mud and dirt in the drinking water, but they had not found any excrement or any animal carcasses in the water reservoirs. All of

*Corresponding author: Mailing address: Department of Clinical Microbiology and Infectious Diseases, Duzce University Medical School, Duzce 81620, Turkey. Tel: +90-3805414107/2462, Fax: +90-3805414105, E-mail: davutozdemir@hotmail.com

the patients had drunk village fountain water in a marriage ceremony 7 or 8 days before the symptoms first appeared.

The time between the onset of the symptoms and the diagnosis of tularemia was 35.7 ± 21.8 (5-90) days. The most frequent symptoms were swollen neck (100%), malaise (90.9%), chills (90.9%), fever (81.8%), and sore throat (72.7%). Before the diagnosis of tularemia, 6 patients were misdiagnosed with tonsillitis (54.5%), 3 (27.3%) with conjunctivitis, and 2 with adenitis (18.2%). Lymphadenopathy (100%) was the most frequent sign. Eight patients were considered to have oropharyngeal tularemia, and three oculoglandular tularemia. The majority of all of the lymphadenopathies were localized in the left side (63.6%) in both patients with oropharyngeal and oculoglandular tularemia. The results of laboratory tests of the patients were normal, except for increases in the mean erythrocyte sedimentation rate (ESR) (38 ± 28 mm/h) and white blood cell count (WBC) ($10.3 \times 10^9/L \pm 2.5 \times 10^9/L$). Serological diagnosis was made on the basis of the following finding: antibody titer against *F. tularensis* was found to be 1:640 in 1, 1:320 in 2, and 1:160 in 3 patients' sera. A fourfold increase in the antibody titer over a period of at least 14 days was observed in 5 patients. *Francisella* spp. was detected in the village fountain water by using the PCR amplification of the *tul4* gene and was not detected in tap water. The sera water samples were negative for the *Fop A* gene by PCR.

Tularemia is an endemic disease, and the main mode of transmission of *F. tularensis* in Turkey is thought to be water (2-4). By PCR analysis of a water sample from the village fountain, we verified that *F. tularensis* was the cause of the waterborne tularemia outbreak in 2005. However, the outbreak of tularemia in 2000 was reported as waterborne by Sencan et al. (2) because the spring water of villages was contaminated with the excrement of rattus and other wild rodents. In addition, they reported the finding of a rattus carcass in the spring water of a village. Similar to our findings, Pazdiora et al. (5) also detected *Francisella* spp. in water samples by using PCR in their study.

It is interesting that the *F. tularensis* isolate was found and identified by PCR only in the village fountain water. Although no excrement or animal carcasses were observed in the water reservoirs, the water may have been contaminated in the pool.

A rare variation of ulceroglandular disease is oculoglandular tularemia, where the conjunctiva is the initial site of infection, usually as a result of the transfer of bacteria on the fingertips (6). It is interesting that in the present oropharyngeal tularemia outbreak, we also found the oculoglandular tularemia form. This situation may be related to the transmission of oculoglandular tularemia with contaminated splashes and aerosols (1). Although the most frequent symptom in the study of Sencan et al. (2) was sore throat, in our study the most frequent symptom was swollen neck. This difference might be related to a different in the form of tularemia between the two outbreaks or to a change in the epidemiological characteristics.

Eliasson et al. (7) reported that swollen lymph nodes and

wound infections were more common in the emergent area, while pneumonia was more common in the disease-endemic area. Endemic areas are heavily forested with small plots of arable land interspersed, but the emergent areas have more open, continuous farmland. The villages that had been affected in our study and Sencan et al. (2) had the same properties as the area reported by Eliasson et al. (7). In the 2000 outbreak, wound infection and swollen lymph nodes were reported. However, in the present outbreak, wound infection was not observed.

It is interesting that the lymphadenopathies were usually localized in the left side both in 2000 and the more recent outbreak. This situation may be related to the localization of oropharyngeal inflammation, but we cannot sure at this time. Ohara et al. (8) reported that lymph nodes predominantly on the left side, and explained that this may have been due to the fact that right-handed persons held the slaughtered animal firmly with the left hand while preparing for cooking.

The number of days elapsed between the start of symptoms and the diagnosis was lower in the 2005 than in the 2000 outbreak. The amount of time between the first medical consultation and the time tularemia is clinically suspected based on the form of disease is related to the prevalence of tularemia in the geographical area (9).

However, the size of our patient cohort was not sufficient for drawing a conclusion that the characteristics of tularemia are changing. Therefore, health-care providers and public health officials should have knowledge of the local epidemiology of tularemia. On the basis of this knowledge, public health officials can recommend locally appropriate prevention and control measures, such as avoiding the consumption of untreated water.

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