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Original Article

Tularemia as a result of outdoor activities for children in the countryside*

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Aim: To investigate the features of a new tularemia outbreak that occurred in the Thrace region.

Materials and methods: The research team visited the village after the identification of the index case. Serum and throat samples were taken from 41 villagers who were examined, and environmental samples were taken in order to identify the source of the outbreak. Culture, serology, and molecular methods were used to search for *Francisella tularensis* in these samples.

Results: A total of 8 children were diagnosed with tularemia. The adults and all of the other children were seronegative for tularemia. All of the patients had a history of swimming in a pool filled with water from a local stream, and contact with stream water was calculated to increase the risk of developing the disease 9.3-fold. Polymerase chain reaction analysis was positive in a lymph node aspirate of the index case and in the home tap water of 3 patients as well as in the spring water and stream water in the village. *Francisella tularensis* could not be isolated from any culture of samples. Interestingly, the waterborne tularemia outbreak affected only children.

Conclusion: Although tularemia has been not reported from Tekirdağ Province for 74 years, the disease reemerged in the region due to the removal of hygienic measures. These clues may signify that the agent had maintained its presence in the region for many years.

Key words: *Francisella tularensis*, tularemia, outbreak, waterborne diseases, Thrace region of Turkey

Introduction

In Turkey, tularemia was first identified in the Thrace region. A total of 168 patients were diagnosed with tularemia in 2 outbreaks in this region in both 1936 and 1945 (1). Although tularemia had not been reported in this region for 60 years, a new tularemia outbreak, which affected 10 patients, was identified in a village close to Bulgaria in 2005 (2). This outbreak shows that the agent persists in this region or that it spreads by the movements of rodents and other animals in the area. When similarities between the Turkish and Bulgarian strains were examined by multiple-locus variable-number tandem repeat analysis (MLVA) (6 loci), the microorganisms were determined to be the same genetically. Attempts have been made to explain these similarities by proposing that the spread of strains to the region stems from the movements of various animals (3). Indeed, it was suggested in a previous study that the agent might be transferred from one region to another by birds (4).

In the literature, available data are very limited about the long-term survival of *Francisella tularensis*

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after settling in a region (5,6). One of the studies shows that *F. tularensis* continues its presence in nature in a viable but nonculturable state. This is an important fact explaining the long-lasting presence of the causative agent in nature. The survival of bacteria in protozoa may allow *F. tularensis* to remain in mud and water (7). Taking these findings into account, it becomes easier to understand recurrent outbreaks of tularemia in a region and how it becomes endemic.

In 2005, tularemia was included in the notification system of communicable diseases by the Turkish Ministry of Health in order to obtain reliable data about the disease in Turkey. According to this notification system, a total of 431 tularemia cases were reported in various cities of Turkey in 2005 (1). In the following years, the annual number of cases gradually decreased and the reports of tularemia fell to 70 patients in 2008. Unfortunately, in 2009, the number of cases increased again and reached a total of 428 (8). In the following year, the increase in the annual number of cases accelerated and the number of cases exceeded 1500. In 2009-2010, outbreaks were reported in many provinces where there had previously been no reported cases of tularemia (9,10). The Thrace region of Turkey was also affected during these tularemia outbreaks. In 2010, tularemia was diagnosed in the village of Muzruplu, near the area where the first outbreak of the disease was identified. In the present study, our aim was to examine the characteristics of the latest outbreak.

Materials and methods

In the last week of September 2010, a male child was admitted to the hospital of Trakya University with a fever, sore throat, and suppurating neck mass; he was taken to the Department of Pediatric Infectious Diseases. The tularemia agglutination of the patient was detected to be positive in a dilution of 1/5120 and it was found that there were similar cases occurring at the same time in his village, Muzruplu, in Tekirdağ-Hayrabolu. An investigation team was formed consisting of specialists in the fields of microbiology, pediatric diseases, and public health, as well as a clinician from the Provincial Health Directorate and aides for surveillance. The village was visited in the same week.

First, an epidemic map was created. The house of the first patient was marked on the maps, as were the patients in contact with him and the water resources for the village (stream, pond, and village water). The common features of the patients were investigated. The population of the village was 76. All the villagers were invited for check-ups and 41 volunteers were examined by the team. The tularemia case inquiry form, developed by the researchers, was applied to all participants in order to determine the source of the outbreak. The inquiry form was composed of personal data, patient complaints, epidemiological history, physical examination findings, former diagnoses, and laboratory findings. Blood samples were taken from all patients for serological examination and throat samples were taken for culture examination. In order to identify the source of the outbreak, environmental studies were carried out and water samples were taken for the detection of *F. tularensis* by culture and molecular methods. Residual chlorine analysis was also performed on the village water.

Samples for culture were inoculated into a blood agar supplemented with 1% glucose, 0.1% cystine, and 10^5 U/mL penicillin. A tularemia microagglutination test was used for the serological analysis. The antigen (patent no.: TPE-2008 01623 B-Şaban Gürcan) used in the test was obtained from the strains isolated from the patients with tularemia (3). The microagglutination test and polymerase chain reaction (PCR) analysis were performed as described previously (2,11).

In accordance with the information obtained from the questionnaires, a risk analysis was performed for the factors that may affect the emergence of the disease and to determine the demographic characteristics of those who were included in the study. Afterwards, a descriptive statistical analysis was performed. Attack and risk rates of the patients were calculated depending upon their exposure to the risk factors (2). All analyses were evaluated at a 95% confidence interval and bidirectionally. Fully informed consent was obtained. Participation was on a voluntary basis and in accordance with the guidelines of the Helsinki Declaration in 1995 (as revised in Tokyo in 2004). The necessary permits were obtained by the Tekirdağ Provincial Health Directorate.

The villagers were educated about tularemia and how to avoid the disease. The patients with positive serology were invited to the hospital for further examination. A report was presented to the Provincial

Health Directorate on the necessary environmental precautions and hygienic drinking water.

This study is descriptive and cross-sectional in nature.

Results

The index child patient was diagnosed with oculoglandular and oropharyngeal tularemia. A total of 42 villagers, including the index patient, were examined by the team. Of the study group, 30 (71%) were female and 23 (55%) were under 14 years of age. One of the patients was diagnosed with an ulcer on the back. Within the pathological biopsy of the ulcer, granulomatous inflammation was identified and the ulcer recovered without treatment. Another patient was diagnosed with a viral infection in another hospital where she was admitted for an upper respiratory tract infection that could not be treated with beta-lactam antibiotics. She also recovered without specific treatment. Less severe disease symptoms were determined in 5 other patients. Tularemia microagglutination tests were positive in dilutions of 1/160-1/5120. As a result, 8 children were clinically and serologically diagnosed with tularemia in the outbreak. The age of the patients ranged between 7 and 12 (median age: 9) and 5 of them (63%) were female. All of the patients had swum in a pool filled with water from the stream several times. *F. tularensis* could not be isolated in any throat samples of the patients. The adults and all of the other children were seronegative for tularemia.

It was found that the water for the villagers was supplied by tap water and well water. Drinking water was drawn from a depth of 200-300 m and transported to a reservoir 1-2 km away by pipe. Drinking water was distributed to 4 villages from this reservoir without being chlorinated. Residual chlorine was not detected in the village water. The villagers usually consumed tap water as drinking water, but well water was used for other purposes. There were no findings associated with tularemia in the 3 other villages to which the water was distributed.

According to the information received from villagers, there had been no difference in the number of rodents in recent years; however, there had always been rodents around the village area. No dead rodents were encountered in water sources. On the other hand, the number of mosquitoes had increased dramatically the previous summer.

Sewage from the village was gathered in a reservoir about 150-200 m away by a system. However, we observed that the sewage overflowed around this reservoir, forming a convenient habitat for rodents and other pests.

F. tularensis could not be isolated in the culture of the first patient's lymph node aspirate, spring water, tap water from the patients' houses, or water samples collected from the reservoir, the stream in the village, or the lake. DNA of *F. tularensis* was detected by real-time TaqMan PCR in the lymph node aspirate of the first patient, as well as in tap water from 3 patients' houses and stream water.

All of the subjects lived in the village. The main sources of income for the villagers were agriculture, hunting, and livestock breeding. All of these people used the tap water. Of the villagers, 35 (83%) consumed raw vegetables; 34 (81%) fed animals at home, in the garden, or barn; 30 (71%) saw rodents around the home; 27 (64%) had had contact with rodents; 27 (64%) had seen a rodent around; 20 (48%) used well water in addition to tap water; 18 (43%) had had contact with lake and/or stream water; and 7 (17%) had had contact with hunting animals.

When evaluated in accordance with the risk factors, all of the patients with tularemia (n = 8) stated that they used tap water, saw rodents around, consumed raw vegetables, and were included in activities such as rural picnics and games. All of the child patients swam in an ornamental pool filled with stream water in the village. The rates attributable to special risk were calculated depending on the other risk factors, and risk rates are shown in the Table.

Discussion

In 1936, 33 villages from 2 adjacent cities in the Thrace region (Kırklareli and Tekirdağ) were affected by a tularemia outbreak (Figure) (1). The village of Muzruplu in Tekirdağ, which experienced the outbreak in 2010, is near the villages that reported tularemia in 1936. The fact that the latest outbreak occurred in the same area that was affected during the previous outbreak suggests that the causative agent has maintained its presence in this region for 74 years. It is interesting that there had not been any

Table. Risks and risk rates according to the calculation of the risks attributed to tularemia.

Risk	PAR*	Patient	AR (Patient)†	AR (Healthy)‡	RR§	95% Confidence interval
Contact with lake or stream water	18	7	38.9	4.2	9.3	(8.5-10.7)
Animal feed	34	7	20.6	12.5	1.7	(0.71-3.09)
Presence of rodents around the home	27	5	18.5	21.4	0.8	(0.67-0.91)
Contact with hunting animals	7	1	14.3	20.0	0.7	(0.61-0.76)
Contact with rodents	28	5	10.0	41.6	0.2	(0.09-0.43)

*PAR: population at risk; †AR (Patient): attack rate of the patient group; ‡AR (Healthy): attack rate of the healthy group; §RR: risk ratio, as calculated using the formula AR (Patient) / AR (Healthy).

reported cases of tularemia for 74 years. Because of this, it may be that the agent responsible may have been transported to the region by ticks, rodents, or other animals (4). When the *F. tularensis* strains isolated between 1945 and 2002 in Russia were analyzed using the multiple-locus variable-number tandem repeat method, it was found that some clones with similar genotypic features survived for a long time. Based on this finding, researchers concluded that the strains can persist for a long time in the environmental foci of a region (6). Unfortunately, due to the fact that older strains are not available in the Thrace region of Turkey, conclusive evidence regarding the persistence of *F. tularensis* could not be obtained.

There are limited data about the natural life cycle of *F. tularensis*. The bacteria can be detected in approximately 250 species in nature. Its persistence in water can be associated with its survival in protozoa (5). Such survival of bacteria in nature can cause tularemia in appropriate conditions. The lack of reported cases of tularemia in the Thrace region can be explained in 2 ways: first, no cases of tularemia occurred; second, health care workers did

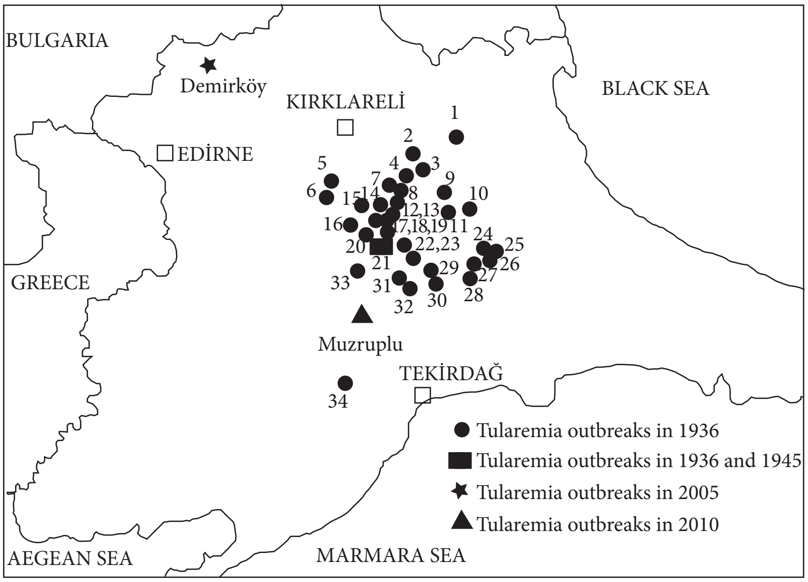

Figure. Tularemia outbreaks in the Thrace region of Turkey: 1 - İslambeyli, 2 - Kaynarca, 3 - Pınarhisar, 4 - Yancıklar, 5 - Karabayır, 6 - Yeniköy, 7 - Osmancık, 8 - Ceylanköy, 9 - Cevizköy, 10 - Topçuköy, 11 - Yenimandıra, 12 - Hamzabeyli, 13 - Eskitaşlı, 14 - Celaliye, 15 - Hamitabat, 16 - Kırıkköy, 17 - Tatarköy, 18 - Yenitaşlı, 19 - Turgutbey, 20 - Ayvalı, 21 - Lüleburgaz, 22 - Umurca, 23 - Evrensekiz, 24 - Servi, 25 - Sinanlı, 26 - Bahçedere, 27 - Beyazköy, 28 - Ahimehmet, 29 - Küçükkarıştıran, 30 - Büyükkarıştıran, 31 - Karamusul, 32 - Çiftlikköy, 33 - Düğüncübaşı, 34 - Kaşıkçı.

not realize the possibility of tularemia's presence in a definitive diagnosis. Indeed, the index patient for the most recent outbreak was admitted to other health facilities first, but tularemia could not be diagnosed. Therefore, the patient was admitted to the hospital of Trakya University.

It was thought that the stream water would be the source of the disease because all of the patients in the Muzruplu outbreak were children who had had contact with water from the stream, which increased the risk of tularemia 9.3 times. That there had been no cases of tularemia among the adults of the village in spite of the bacteria detected in the drinking water and the fact that all of the adults were seronegative suggests that the disease may not occur despite the presence of the bacteria in the body. Indeed, some researchers observed the presence of bacteria in the water sampled from an endemic area although there were no cases reported in that period of time (5). In conclusion, the presence of the bacteria in the body is not sufficient to cause an infection. In the emergence of tularemia, other factors, such as the number of the bacteria and host immunity, are considered to be influential.

The fact that there had been no reported cases of tularemia in Tekirdağ Province for 74 years despite the presence of the bacteria in that region can be explained by the fact that the Thrace region is one of the most socially, culturally, and economically developed regions of Turkey. When the villagers living in this region are compared with villagers from other regions of Turkey, they are found to be more educated and informed and to make more use of modern technology. In addition, this rural region has relatively better infrastructure, including healthy drinking water and sewage systems. Although it is an important failure that there is no chlorination in the village water of Muzruplu, its supplement from ground waters minimizes bacterial contaminations. After being informed about the importance of chlorination, the villagers, showing their sensitivity to the issue, established an automatic chlorination system. According to these findings, it would not be wrong to say that *F. tularensis* could persist in the Thrace region and the disease could occur again in certain conditions.

The ulceroglandular form of tularemia resulting from blood-sucking insects or arthropods is the most common type of tularemia in the world (12). Oropharyngeal tularemia, on the other hand, is caused by the consumption of contaminated water or food. In Turkey, tularemia is encountered in the oropharyngeal form as a result of drinking contaminated water (1). In the outbreak of Muzruplu, most of the cases were of the oropharyngeal form, suggesting that the source of the disease could be drinking water. The samples taken from the houses of some patients and from stream water have PCR positivity for *F. tularensis*, which proves the suggested theory. However, as stated before, the presence of the bacteria is not enough to cause infection in hosts. The fact that the adult villagers were not affected by the disease while only the children who came into contact with the pool water got infected suggests that the number of bacteria in the local drinking water is insufficient to cause tularemia. The presence of approximately 10^8 bacteria in the body can cause oropharyngeal tularemia (13). The number of bacteria could be more easily increased in the bodies of children because of the fact that they had not only drunk village water but had also had contact with the pool water. The investigation team could not analyze a sample of water from the pool, however, because it had been cleaned out. Still, there is some evidence indicating that the pool water was contaminated:

- The PCR positivity of the stream water is one of the most important clues suggesting that the pool water was contaminated as well.
- The fact that the index patient had both the oropharyngeal form and the oculoglandular form suggests that a large number of bacteria had entered the gastrointestinal system by aspiration of both drinking water and pool water during swimming and that the contaminated pool water came into contact with the eyes of the patient.
- The presence of granulomatous inflammation on the back skin of another patient who swam in the pool indicates that the bacteria had entered the body through some scratches that occurred while swimming.
- The fact that the risk of infection for the people who had had contact with the pool/stream water was 9.3 times higher than that of the others is an important clue regarding the contamination of the pool water.

The earlier tularemia outbreak in the Thrace region occurred during years when the number of rodents increased (1,2). During the latest outbreak, the villagers mentioned a significant increase in the number of mosquitoes rather than the number of rodents. It is known that mosquitoes can also cause some outbreaks of tularemia (12,14). Although the granulomatous inflammation that occurred on the skin on the back of a patient suggests that it is ticks, mosquitoes, or other insects that caused the outbreak, this is unlikely because they usually bite uncovered body surfaces. For this reason, the main source of the outbreak is probably rodents contaminating the stream and drinking water.

Waterborne tularemia outbreaks usually affect people in all age groups, including children. In a review of 1091 tularemia cases recorded between 2005 and 2009 in Turkey, tularemia cases were fewer in the elderly group above 65 (9.3%) and in children between 1 and 14 (10.4%) (8). Celebi et al. (15) reported that tularemia patients under 10 years of age were extremely rare in Turkey. Interestingly, the disease affected only children in the Muzruplu outbreak.

In Turkey, tularemia was first reported in 1936-1945 from the Thrace region and it reemerged in 2005 and 2010, so it can be said that *F. tularensis* persists in that region (1,2). In clinically compatible cases, it is important to regard tularemia as one of the first possible diagnoses in order to take the necessary precautions. For this reason, it is necessary to educate health care workers about the symptoms of tularemia and the ways in which it is transmitted, and to distribute brochures about the disease in order to increase people's awareness of it. More importantly, appropriate conditions in terms of hygiene and sanitation should be ensured for the prevention of infectious diseases. The absence of residual chlorine in the tap water of Muzruplu village is a facilitating factor for the emergence of tularemia. In order to take the necessary measures, after the chlorine analysis was conducted, an automatic chlorine machine was installed. In conclusion, appropriate measures for general health and the supply of safe drinking water should never be compromised.

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