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The prevalence of tularemia in occupational groups that have contact with animals

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Background/aim: The aim of the current study was to investigate the presence of antibodies against *Francisella tularensis* in individuals in different occupations that have contact with animals in the Kars region of northeastern Turkey.

Materials and methods: A total of 201 blood samples specifically including 103 farmers, 45 clinical veterinarians, 42 butchers, and 11 hunters were analyzed. The results of the study were reported in relation to some sociodemographic features (age, sex, occupation, and experience) of the volunteers. The presence of antibodies was determined by a microagglutination (MA) test. In addition, positive sera were confirmed using an ELISA kit.

Results: Fifteen (7.46%) individuals, including fourteen farmers and one clinical veterinarian, were found to be positive for *F. tularensis* by both MA and ELISA with a titer range of 1/10 to 1/160. The highest seroprevalence rate was observed in farmers (13.59%), followed by clinical veterinarians (2.22%). The occurrence of tularemia was found to increase with age.

Conclusion: Though the main route of tularemia outbreaks is water-borne in Turkey, it was determined that people whose occupations bring them into contact with animals are at risk. Similar studies are recommended in order to further clarify the epidemiology of the disease in the northeast of Turkey.

Key words: *Francisella tularensis*, tularemia, occupational groups, seroprevalence

1. Introduction

Tularemia is a zoonotic disease caused by *Francisella tularensis*. This bacterium is found widely in diverse animal hosts and habitats. Therefore, tularemia is a disease of many faces, a chameleon that adapts to various environments (1,2). The transmission of tularemia to human beings occurs mostly through the arthropod bite. Other means of transmission to humans include contaminated animal products, water, and mud (3). Tularemia is more common in individuals from certain occupational groups, especially hunters, foresters, farmers, laboratory workers, and veterinarians because they are more frequently in contact with both infected animals and the habitat of tularemia (4,5). The disease can display various clinical presentations in humans, including glandular, ulceroglandular, oculoglandular, oropharyngeal, pneumonic, and typhoidal tularemia (6). In addition to these symptomatic forms of the disease, cases of asymptomatic tularemia occur (the rate is 4%–19% in Turkey) and they may be detected only

by serological examination through microagglutination (MA) or ELISA tests (7).

Tularemia was first reported in the mid-1930s in Turkey, and the reporting of the disease has gained momentum throughout the country over time (8–12). Most outbreaks are water-borne, while cases of tularemia via other routes have rarely been reported in Turkey (3,13). The region of Kars lies in the northeast of Turkey and is an area where family farming is common (Figure 1). Some zoonotic diseases, mainly brucellosis, anthrax, and leptospirosis, have been reported in farmers and related occupational groups in this region over the years (14–17). The zoonotic nature of tularemia is also well known, and an outbreak between 2004 and 2005 was reported in one study in the Kars region, in patients with some obvious clinical signs. Moreover, the researchers suggested that tularemia flourished in the region (11). However, no related study is available about subclinical tularemia with significant antibody titers of *F. tularensis* in people who

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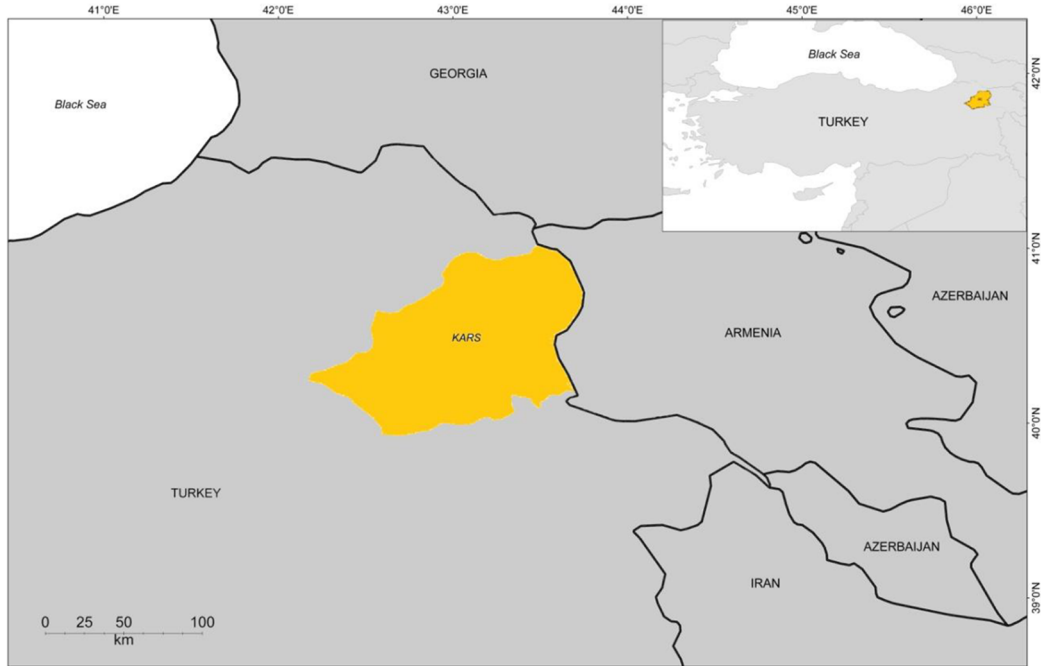


Figure 1. The sampling area of the study.

have been in close contact with animals in this region. Thus, in this study, we aimed to investigate the presence of antibodies against *F. tularensis* in people who have direct contact with animals in the region of Kars.

2. Materials and methods

This study was mostly conducted in the Kars region of northeastern Turkey. Sampling and MA tests were performed at Kafkas University. Confirmation tests of MA positive samples were conducted in the National Tularemia Reference Laboratory, Public Health Institution of Turkey. Ethical approval for the gathering of serum samples and their subsequent analysis for the presence of *F. tularensis* specific antibodies was obtained from the Ethics Committee of the Faculty of Medicine, Erciyes University (Kayseri, Turkey). A total of 201 blood samples were collected from volunteers who had been in direct contact with farm and/or wild animals. Serum samples were

obtained by centrifugation, kept at $-20\text{ }^{\circ}\text{C}$, and transferred to the reference laboratory under cold conditions.

No clinical complaints related to tularemia had been reported previously by the volunteers. The study sample was composed of 103 farmers, 45 clinical veterinarians, 42 butchers, and 11 hunters. The sociodemographic composition of the sample (age, sex, occupation, and experience) is given in Figures 2–5.

The presence of antibodies against *F. tularensis* was investigated by an MA test (18). Formalin-inactivated *F. tularensis* whole-cell suspension containing 0.005% Safranin-O was used as an antigen in the test. Five-fold serum dilutions were obtained in a 96-well round bottom microtiter plate by adding MA buffer solution containing 1% rabbit serum and 0.4% formaldehyde. Portions (20 μL) of five-fold serial dilutions of serum were mixed with an equal volume of antigen, and the reactions in the plates were observed 18 h after incubation at $37\text{ }^{\circ}\text{C}$ for agglutination.



Figure 2. Sample distribution by location.

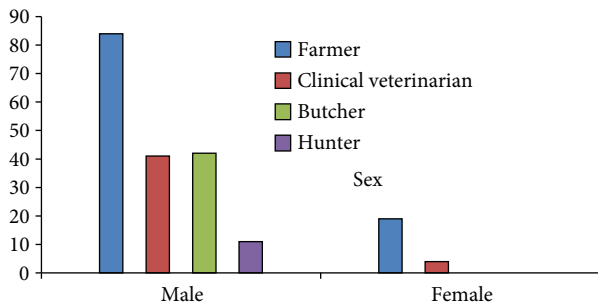


Figure 3. Sample distribution by sex.

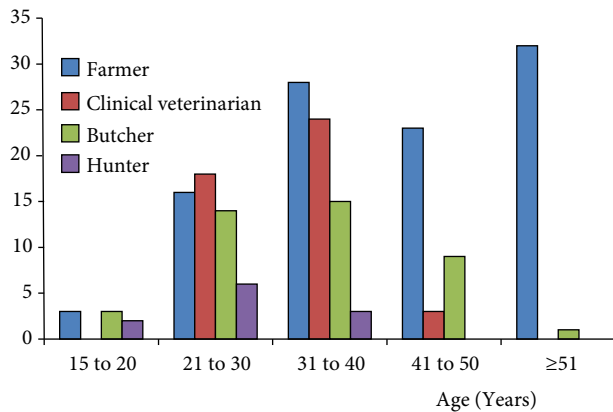


Figure 4. Sample distribution according to age ranges.

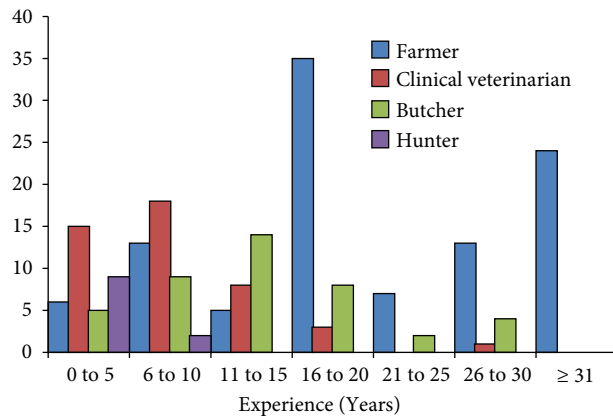


Figure 5. Sample distribution by length of occupational experience.

The agglutination titers were expressed as reciprocals of the highest serum dilution showing agglutination with the antigen. Agglutination at dilutions of 1:10 or higher was considered MA positive (19).

For confirmation testing of the MA positive samples, a commercial ELISA kit (Serazyme ELISA, anti-*F. tularensis*

GAM, Seramun, Germany) was used according to the manufacturer's recommendations. Samples were accepted as seropositive for tularemia in consequence of both the MA tests and ELISA results. To reveal cross-reactions with *Brucella* spp., all the samples were analyzed by the Rose Bengal Plate test (RBPT) using buffered *Brucella* antigens.

Statistics were performed using software with an interactive calculation tool for chi-square tests to compare the variables during the analysis (20). $P < 0.05$ was considered statistically significant.

3. Results

Antibodies against *F. tularensis* were detected by the MA test in 15 (7.46%) of the 201 individuals in the study, in titers that ranged from 1/10 to 1/160. Confirmation of the cases possessing antibody titers was conducted by ELISA, which yielded the same amount of positivity for total antibodies. Since the MA test gave high cross-reactive results, the 15 individuals (7.46%) who were found to be positive by ELISA were evaluated as seropositive for tularemia. Analysis of the cases of tularemia seropositivity confirmed by ELISA in relation to their sociodemographic features is undertaken in the discussion section below. Furthermore, two samples with MA titers of 1/40 and 1/160 reacted positively for *Brucella* antibodies by RBPT (Table).

According to the ELISA results, out of 201 samples tested, 15 (7.46%) were found to be positive. The seropositive individuals comprised 14 farmers and 1 clinical veterinarian. All volunteers from the other occupational groups were found to be negative for *F. tularensis* antibodies. By occupational group, there was a statistically significant difference in the seroprevalence of tularemia between farmers (13.59%) and clinical veterinarians (2.22%) ($P = 0.017$). Among the positive samples, the MA titers were 1/10 in 2 samples (both of farmers), 1/20 in 7 (all farmers), 1/40 in 4 (all farmers), 1/80 in 1 (clinical veterinarian), and 1/60 in 1 sample (farmer). None of the butchers or hunters was determined to be positive for *F. tularensis* antibodies (Table).

In this study, 135 samples (70 farmers, 32 clinical veterinarians, 22 butchers, and 11 hunters) were from the center of Kars, 23 (14 farmers and 9 butchers) were from Sarikamış, 15 (6 farmers, 4 clinical veterinarians, and 5 butchers) were from Susuz, 13 (2 farmers, 5 clinical veterinarians, and 6 butchers) were from Selim, 11 (all farmers) were from Arpaçay, and 4 (all clinical veterinarians) were from the Digor district (Figure 2).

By location, out of the 15 positive samples, 14 (13 farmers and one clinical veterinarian) were from Kars center and 1 (farmer) was from the Kars-Arpaçay district. No sample was reported to be positive for *F. tularensis* antibodies from any of the other districts of the Kars region

Table. The MA titer distribution of ELISA positive samples.

Sample groups	Sample size	Number of seropositive samples and titers				
		1/10	1/20	1/40	1/80	1/160
Farmer	103	2	7	4*	-	1*
Clinical veterinarian	45	-	-	-	1	-
Butcher	42	-	-	-	-	-
Hunter	11	-	-	-	-	-
Total	201	2	7	4	1	1

* One sample from each titer group gave a positive reaction for *Brucella* spp. by RBPT.

studied. Moreover, there was no statistically significant difference in the seropositivity of tularemia between Kars center and the neighborhoods ($P = 0.355$).

Twenty-three serum samples from females (19 farmers and 4 clinical veterinarians) and 178 serum samples from males (84 farmers, 42 butchers, 41 clinical veterinarians, and 11 hunters) were analyzed from all study groups (Figure 3).

By sex, out of the 15 positive cases, 12 (all farmers) were from male subjects and 3 (2 farmers and 1 clinical veterinarian) came from females. There was no statistically significant difference in the seropositivity of tularemia between the female and male individuals ($P = 0.325$).

In this study, 8 individuals (3 farmers, 3 butchers, and 2 hunters) were between the ages of 15 and 20 years, 54 individuals (16 farmers, 18 clinical veterinarians, 14 butchers, and 6 hunters) were between 21 and 30 years old, 70 individuals (28 farmers, 24 clinical veterinarians, 15 butchers, and 3 hunters) were between 31 and 40 years old, 35 individuals (23 farmers, 3 clinical veterinarians, and 9 butchers) were between 41 and 50 years of age, and 33 individuals (32 farmers and 1 butcher) were over 51 years of age (Figure 4).

By age, of the 15 positive subjects, 1 (farmer) was 27 years of age, 3 (2 farmers and 1 clinical veterinarian) were between the ages of 31 and 40, 6 (all are farmers) were between the ages of 41 and 50, and 5 (all are farmers) were over 51 years of age. The median age of seropositive individuals was 43.3 years (age range 27–62 years), compared with a median of 38 years (age range 12–75 years) in seronegative individuals. There was a statistically significant difference in the seropositivity of tularemia among the different age groups ($P = 0.045$).

In this study, various numbers of serum samples were analyzed from subjects with different lengths of experience in their occupations. The distribution of samples by length of occupational experience was 35 samples from subjects (6 farmers, 15 clinical veterinarians, 5 butchers, and 9

hunters) with 0 to 5 years of experience, 42 (13 farmers, 18 clinical veterinarians, 9 butchers, and 2 hunters) with 6 to 10 years, 27 (5 farmers, 8 clinical veterinarians, and 14 butchers) with 11 to 15 years, 46 (35 farmers, 3 clinical veterinarians, and 8 butchers) with 16 to 20 years, 9 (7 farmers and 2 butchers) with 21 to 25 years, 18 (13 farmers, 1 clinical veterinarian, and 4 butchers) with 26 to 30 years, and 24 (all farmers) with over 31 years of experience (Figure 5).

By length of occupational experience, out of 15 positive subjects, 2 subjects (1 farmer and 1 clinical veterinarian) had from 6 to 10 years of experience, 2 (all farmers) had 11 to 15 years; 3 (all farmers) had 16 to 20 years, 3 (all farmers) had 21 to 25 years; 3 (all farmers) had 26 to 30 years, and 2 (all farmers) had over 31 years of experience. There was no statistically significant difference in the seropositivity of tularemia with respect to the length of occupational experience ($P = 0.092$).

4. Discussion

Tularemia is a reemerging disease in Turkey, where quite a few cases have been reported recently (2,10,11,13,21–24). Most of the studies were conducted on persons with clinical signs, and therefore detected significant antibodies titers (13,24). One outbreak in three humans with obvious clinical signs was reported as occurring in the Kars region in 2004 and 2005 (11). However, no seroprevalence studies have been reported on subclinical infection in risk groups in this region. This study is the first carried out on tularemia in different occupational groups with a history of animal contact during their business life and it demonstrated moderate seroprevalence in the Kars region. At 7.46%, the rate of seroprevalence for tularemia found in this study is somewhere around that reported by others in Germany (2%) (25), Canada (2%) (26), Turkey (0.3%–6.3%) (21–23), and the United States (9%) (27). Scientists have alleged that exposure to the agent does not usually lead to severe or significant clinical symptoms (28) and that

the populations in endemic areas have measurable rates of antibodies to tularemia (27). The lack of clinical tularemia cases, despite the moderate rate of seroprevalence found in our study, could be explained by an inadequacy in the diagnosis of cases, indiscriminate treatment of probable patients with antibiotics, and other constructive factors such as the infective dose and virulence of the organism.

In the present study, antibodies against *F. tularensis* were detected in 15 (7.46%) out of 201 individuals by the MA test and all cases were confirmed as tularemia by ELISA. However, two samples with different titers reacted positively for *Brucella* antibodies by RBPT. It is well known that the common surface antigens presented by related bacteria result in cross-reaction between *F. tularensis* and *Brucella* species and that the agglutinin titers are due primarily to IgM antibodies (29). It was also demonstrated by ELISA in our study that IgM antibodies to *F. tularensis* cross-react with *Brucella* positive samples. On the other hand, due to the endemic and prevalent nature of brucellosis in the Kars region, significant *Brucella* titers may be expected in individuals without obvious clinical signs, as in this study. The paradox in the classification of disease cases based on this cross-reaction is not considered to be a problem because of the clinical differences between the two diseases. Nonetheless, this finding shows the requirement of further investigations to distinguish the cross-reacting epitopes between the two organisms.

In this study, 14 farmers and 1 clinical veterinarian were found to be positive for tularemia and the highest level of seroprevalence (13.59%) was observed in farmers, even though hunters as a group are considered to be at a high risk of tularemia infection (23). There was a significant correlation between the study groups and tularemia seroprevalence, which is similar to the findings of other studies (21,22). This can be explained by the fact that people who have contact with animals are always at risk of tularemia. The lack of seropositivity of tularemia in hunters can be explained by the lower number of samples, the ethnic situation of the study area where the consumption of meat from hunted wild animals (especially rabbits) is less usual, and the hunters' awareness of the zoonotic nature of tularemia.

In this study, 14 (13 farmers and 1 clinical veterinarian) individuals from Kars center and 1 (farmer) individual from the Kars-Arpaçay district were found to be positive. The difference between the districts was not statistically

significant. It is expected that rural residents will have a higher rate of tularemia seroprevalence than urban residents (21,22,30); however, no such comparative analysis could be undertaken in this study due to the lack of samples from urban environments.

In this study, 12 (all farmers) samples from male and 3 samples (2 farmers and 1 clinical veterinarian) from female individuals were found to be positive for tularemia. The findings were in parallel with the report of Dedeoglu Kilinc et al. (21); however, the difference between males and females was not statistically significant, in line with other studies (28,30). It was to be expected that the seroprevalence of tularemia will be higher in males as a result of more frequent contact with animals and with habitats that are reservoirs of the disease.

In the present study, of all tularemia positive subjects, 1 individual (1.85%) (farmer) was 27 years old, 3 (4.28%) (2 farmers and 1 clinical veterinarian) were between 31 and 40 years of age, 6 (17.14%) (all farmers) were between 41 and 50, and 5 (15.15%) (all farmers) were over 51 years old. There was a significant positive correlation between age group and tularemia seroprevalence, which is similar to the findings of other studies (28,30). The majority of positive cases were composed of people of elderly age in this study. This can be explained by the fact that tularemia antibodies remain for a long time in life and that the probability of exposure to pathogens increases with age (31,32).

In this study, 2 individuals (1 farmer and 1 clinical veterinarian) with 6 to 10 years of occupational experience, 2 (all are farmers) with 11 to 15 years of experience, 3 (all are farmers) with 16 to 20 years of experience, 3 (all are farmers) with 21 to 25 years of experience, 3 (all farmers) with 26 to 30 years of experience, and 2 (all farmers) with over 31 years of occupational experience were found to be positive for tularemia. Although it was expected that there would be a positive correlation between the rate of tularemia seroprevalence and the length of employment, no statistically significant difference was found in this study.

Overall, the results of the present study confirm the presence of tularemia in the Kars region. Similar studies in other parts of the country and on different occupational groups or animals will help to clarify the epidemiology of tularemia in the northeastern part of Turkey.

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