Original Article

Investigation of *Francisella tularensis* Seroprevalence and Determination of Risk Factors for Tularemia among Hunters in Northern Cyprus

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Abstract

Background: Tularemia is a zoonotic disease (reservoir is usually rodents) caused by *Francisella tularensis*, especially seen in the northern hemisphere. Hunters are in the risk group for this disease. In this study, it was aimed to determine the seroprevalence of tularemia among hunters and determine the risk factors of tularemia in our country.

Methods: The Turkish Republic of Northern Cyprus (TRNC) is divided into four regions (Nicosia, Kyrenia, Famagusta/Trikomo, and Morphou/Lefka) and 100 volunteer hunters randomly selected from these regions were included in our study. Tube agglutination test (TAT) and *F. tularensis* IgG and IgM (ELISA method) were applied in all sera. All hunters were filled with a pre-prepared questionnaire to determine risk factors for tularemia.

Results: TAT positivity was found in 11%. While *F. tularensis* ELISA IgG positivity was 17%, IgM positivity was not found in any hunters. Hunters with positive *F. tularensis* ELISA IgG test (17%) were accepted as seropositive in terms of tularemia. There was no statistically significant difference between the mean age of IgG-positive and negative hunters (p= 0.915). Of the 86 hunters who kept at least one hunting dog in their garden, 15 (17.4%) were IgG-positive. There was no significant relationship between feeding hunting dogs and tularemia (p= 0.561).

Conclusion: Our study showed that the seroprevalence of tularemia was high (17%) among hunters, who are considered a risk group, in our country. We think that more epidemiological research should be done on tularemia infection and it should not be overlooked in the clinic.

Keywords: Tularemia; *Francisella tularensis*; Northern Cyprus; Seroprevalenve; Hunters

Introduction

Tularemia is a zoonotic disease caused by the intracellular, gram-negative Coccobacillus bacterium *Francisella tularensis*. The bacteria can survive in soil, water, and dead animals for months and also has a wide range of hosts including birds and domestic and wild mammals (1, 2). Its natural reservoir is usually rodent animals such as hares, squirrels, voles, beavers, deer, and raccoons. Small rodents and lagomorphs (rabbits and hares) are mainly responsible for human infections. Humans and domestic animals are the accidental hosts of *F. tularensis* and no human-to-human transmission has been reported. Tularemia is an infection that can be seen all over the world, although its prevalence is higher, especially in the northern hemisphere (between 30–71° latitudes) (3–5). Tularemia cases have recently been reported in Europe (6), Turkey (7), Iran (4), Jordan (1) and also Cyprus (8). The dis-

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ease is referred to by different names in various geographical regions such as Francis' Disease, Pahvant Valley plaque, hunters' disease, rabbit fever, deer fly fever, and tick fever (9, 10).

Francisella tularensis is transmitted to humans by direct contact with the infected animals or their tissues, consumption of raw or undercooked meat of infected animals (for example rabbits), drinking contaminated water, animal bite or scratching, arthropod bite (mainly ticks, and mosquitoes in specific areas), and by inhalation of contaminated aerosols or dusts. Risk groups for the disease are laboratory workers, farmers, ranchers, hunters, veterinarians, nature conservation officers, butchers, and slaughterhouse workers (4, 11).

There are six clinical forms depending on the virulence of the agent, the site of entry, and the immune system of the host: ulceroglandular, glandular, oropharyngeal, oculoglandular, typhoidal, and pulmonary. The most common form in Europe is ulceroglandular, however oropharyngeal form with waterborne outbreaks has been reported in Turkey recently (12). Clinical findings depend on factors such as the patient's immune resistance, degree of systemic involvement, virulence of the bacteria, diagnosis, and treatment at the right time. The incubation period of the bacteria is 3–5 days on average and the disease onset is within 14 days after the infection (13). Tularemia usually begins acutely with symptoms such as fever, chills, weakness, headache, and loss of appetite. Afterward, the disease presents flu-like symptoms including, sore throat, dry cough, and retrosternal pain. Regional lymph nodes grow rapidly and present a bubonic appearance. Progressive drowsiness, weight loss, and persistent lymphadenopathies are observed in untreated cases (3).

In the report published by the European Center for Disease Prevention and Control (ECDC) in 2016, it was stated that no tularemia cases were observed in the island of Cyprus between 2010–2014 (14). According to the data of the Turkish Republic of Northern Cyprus (TRNC) Ministry of Health, only one (1) tularemia case was observed in Northern Cyprus between 2014–2018. Tularemia is among the notifiable diseases in TRNC (15).

There is only one epidemiological data about the seroprevalence of tularemia disease caused by *F. tularensis* in Cyprus (16). Hunting is permitted for six months throughout the year in our country. Considering the TRNC Hunting Federation's feature of being the largest non-governmental organization in the country with 89 registered hunting unions and 22.000 members (17), it's of great importance to determine the seroprevalence of the disease for hunters who are the first risk group. In our study, it was aimed to determine the seroprevalence of *F. tularensis* for hunters living in TRNC and to determine the effect of the disease on public health. In addition, identifying the behaviors that may increase the risk of transmission are among our goals.

Materials and Methods

Study area

Cyprus Island is located on the east of the Mediterranean, between 34°33'–35°42' north latitudes and 32°16'–34°36' east longitudes. It is the third largest island (9.251 km2) in the Mediterranean, after Sicily and Sardinia. The island is divided into two parts: TRNC in the North and Northeast, and the Greek Cypriot Administration of Southern Cyprus (GCASC) in the South and Southwest (18). There are Turkey (70 km) in the North, Syria (102 km) and Lebanon (165 km) in the East, Greece (835 km) in the Northwest, and Egypt (347 km) in the South of the island. TRNC has six cities as capital Nicosia, Kyrenia, Famagusta, Morphou, Lefka, and Trikomo (Fig. 1) (19).

TRNC reflects a typical Mediterranean climate. Summers are hot and dry, winters are mild and rainy. In terms of vegetation, Mediterranean flora prevails and pine, cypress, oak, juniper, and eucalyptus (which are grown on the island) are the most common trees. In ad-

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dition to pines, citrus fruits, and olives alongside maquis and steppe constitute the general vegetation group of the TRNC (18, 19).

Study group and sampling

TRNC is divided into four regions (Nicosia, Kyrenia, Famagusta/Trikomo, and Morphou/Lefka) and a total of 100 male hunters were selected randomly from these regions and have been included in our study. All of the hunters are 21 years of age or older and have a confirmed hunting license. Between January–December 2020, approximately 5 mL of blood samples were taken from each of the hunters into jelled dry tubes. The blood samples taken were delivered to the microbiology laboratory of Near East University (NEU) Hospital immediately, and their serums were separated by centrifugation. The serum samples obtained were transferred to 2–3 different eppendorf tubes and stored at -80 °C until the time of use.

Questionnaire form

A pre-prepared tularemia questionnaire was conducted among all of the hunters included in our study. Information on sociodemographic characteristics (age, area of residence) was obtained from the hunters. In addition, information that could be a risk factor for *F. tularensis* was obtained. Risk factors include consuming hunted animals, getting into contact with a dead or alive mouse, history of mosquito and/or insect bites, collecting food from nature and feeding pets in the household and/or the garden, the presence and number of hunting dogs, a place where the hunted animal is handled and if the hunters feed the internal organs of the hunted animal to their dogs.

Serological tests

Specific antibodies for *F. tularensis* in all serum samples belonging to the hunters were investigated using the Ferbil Antigen Agglutination Test (Becton, Dickinson and Company, 8810131jaa (02), USA). A tube agglutination test (TAT) was applied to all serums following the manufacturer's recommendations, and the results were evaluated after 20 hours of incubation in a 37 °C water bath visually. The titer of ≥ 1/80 was accepted as positive for TAT (20).

Afterward, *F. tularensis* IgG and IgM (Serion®ELISA, Institute Virion/Serion GmbH, Würzburg, Germany) were studied in all sera by ELISA method following the manufacturer's recommendations. For this purpose, 'U' 96-well ELISA plates were utilized and the results were obtained by reading in a spectrophotometer set at 405 nm wavelength. The retrieved OD values were calculated and evaluated according to the Se-rion/Verion protocols.

To detect the presence of *Brucella* spp. that can cross-reaction with *F. tularensis*, a Rose Bengal (Lorne Laboratories Ltd., United Kingdom) agglutination test was performed on all TAT and/or ELISA IgG positive serum samples.

Statistical analysis

Statistical analysis of the data obtained was conducted with SPSS (Statistical Package for the Social Sciences) Demo Ver 22.0 (SPSS Inc., Chicago, IL, USA) program. Person Chi-Square, Fisher's Exact test, and Binary Logistic Regression Analysis were used to determine statistical significance and the significance was evaluated at $p< 0.05$.

Results

The average age ± standard deviation/SD (age range) of 100 hunters in our study group was 35.67±11.42 (21–81). In our study, 11% *F. tularensis* antibody positivity was detected with TAT. Serums of 2 (two) (2%) TAT-positive persons (Patient no: 92, 1/40 titer and Patient no: 75, 1/80 titer) cross-reacted with the *Brucella* Rose Bengal test. Both IgM and IgG tests of these two hunters were negative. While 17 % of the hunters were *F. tularensis* IgG positive, none of the sera were found to be positive for *F. tularensis* IgM. All antibody tests were negative for the other 81 sera. The average age± SD (age range) of hunters with positive *F. tularensis* IgG test was 35.94±10.99 (21–61). There

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was no statistically significant difference between the mean age of IgG-positive and negative hunters (p= 0.915). Hunters with positive *F. tularensis* ELISA IgG test (17%, 17/100) were accepted as seropositive in terms of tularemia. Table 1 shows the tests used for the detection of *F. tularensis* antibodies and the results of these tests.

The IgG results were compared with the regions where the hunters live. Accordingly, 16.7 % (3/18) in Nicosia, 14.3% (2/14) in Kyrenia, 18.5% (5/27) in Famagusta/Trikomo, and 17.1% (7/41) in Morphou/Lefka IgG positivity has been detected. No relationship was found between IgG positivity and regions (p= 0.990) (Fig. 2).

It was determined that the hunters included in our study have been actively hunting between 1–60 years (average 13.47±10.95). Seropositive individuals reported that they had a hunting career of 2–40 (average 15.06±11.52) years. There was no statistical significance between tularemia seropositivity and hunting length (p= 0.514).

In our country, there is a habit of feeding hunting dogs with the internal organs of the hunted animals. 57% (n: 57) of the hunters participating in our study reported that they feed their dogs with the internal organs of hunted animals. Nine (15.8%) of these people were IgG-positive. However, this action was not found to be a factor that increases tularemia infection (p= 0.711).

It was observed that 24 (24%) of the people who were included in the questionnaire had a history of dead or alive mouse contact and 48 (48%) reported an increase in the number of mice in their houses or environment. In addition, 44 (44%) reported that they encountered mouse waste in their homes or the environment. Sixty-four (64%) of the participants stated that they had a history of mosquito and/or tick bites. Besides, it has been determined that 56 (%56) hunters were nature occupations and 68 (68%) of them have habits of gathering food from nature. None of these parameters were found to be associated with tularemia seropositivity (Table 2). It has been determined that all hunters (100 %) are in contact with hunted animals at all times of the year and consume hunted animal meat.

Table 1. The blood samples of 100 volunteer hunters living in TRNC were collected between January 2020–December 2020 and TAT (tube agglutination test), *F. tularensis* IgG, and *F. tularensis* IgM tests were performed. List of hunters with positive *F. tularensis* antibody test results

Patient no	Age	TAT titer	*F. tularensis* IgG	*F. tularensis* IgM	Rose Bengal
5	43	1/160	Positive	Negative	Negative
14	21	Negative	Positive	Negative	Negative
27	53	1/320	Positive	Negative	Negative
32	24	Negative	Positive	Negative	Negative
34	29	1/160	Positive	Negative	Negative
36	40	1/160	Positive	Negative	Negative
39	45	1/160	Positive	Negative	Negative
46	24	Negative	Positive	Negative	Negative
48	61	Negative	Positive	Negative	Negative
66	38	1/80	Positive	Negative	Negative
68	28	Negative	Positive	Negative	Negative
78	30	Negative	Positive	Negative	Negative
80	45	1/160	Positive	Negative	Negative
81	31	1/80	Positive	Negative	Negative
82	37	Negative	Positive	Negative	Negative
84	36	Negative	Positive	Negative	Negative
88	26	1/80	Positive	Negative	Negative
75	47	1/80	Negative	Negative	Positive

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Table 1. Continued ...

92	50	1/40	Negative	Negative	Positive

TAT: Tube aglutination test

Fig. 1. Study area: Turkish Republic of Northern Cyprus and its cities

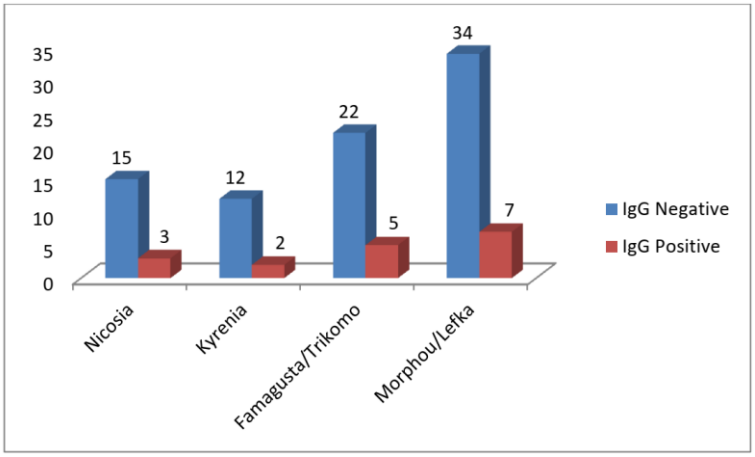

Fig. 2. Distribution of ELISA (enzyme-linked immunosorbent assay) *Francisella tularensis* IgG results obtained from our study conducted between January 2020 and December 2020 among TRNC cities

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Table 2. Evaluation of *F. tularensis* IgG and *F. tularensis* IgM positivity with the survey questions applied in our study, n (%)

Question	*F. tularensis* IgG positive	*F. tularensis* IgG negative	p-value
Do you have a history of contact with a mouse (dead or alive)?			
No	13 (17.1)	63 (82.9)	0.616
Yes	4 (16.7)	20 (83.3)	
Is there an increase in the number of mice in the environment or at home?			
No	8 (15.4)	44 (84.6)	0.746
Home	1 (16.7)	5 (83.3)	
Environment	7 (22.6)	24 (77.4)	
Both	1 (9.1)	10 (90.9)	
Is there any rat waste in the environment or at home?			
No	8 (14.3)	48 (85.7)	0.267
Home	1 (7.7)	12 (92.3)	
Environment	8 (25.8)	23 (74.2)	
Do you have a history of insect and/or mosquito bites?			
No	4 (11.1)	32 (88.9)	0.643
Mosquito	12 (21.8)	43 (78.2)	
Tick	0 (0.0)	3 (100.0)	
Both	1(16.7)	5 (83.3)	
Do you have a habit of collecting food (vegetables, fruits, mushrooms) from nature?			
No	5 (15.6)	27 (84.4)	0.802
Yes	12 (17.6)	56 (86.4)	
Do you have a habit of doing things in nature?			
No	9 (20.5)	35 (79.5)	0.415
Yes	8 (14.3)	48 (85.7)	

Discussion

Tularemia has been reported in many countries of the northern hemisphere, but it is rarely seen in the southern hemisphere. The disease is generally seen as sporadic cases in North America, Asia, and Central and Northern European countries (21, 22). Most of the cases are reported from Sweden, Finland, and Turkey. In Turkey, tularemia outbreaks have been reported in Marmara, Thrace, Central Anatolia, and Western Black Sea regions (23). When the tularemia cases were examined in Turkey, it was seen that the cases peaked (n: 2.151) in 2011 (24). The number of cases in Turkey alone was higher than tularemia cases seen in all European Union countries during the same year. In the following years, the number of cases started to decrease continuously (24). To date, only one epidemiological study on tularemia has been conducted in our country. In this study by Karataş Yeni et al. 430 human serum samples from the general population were examined and *F. tularensis* seropositivity was found to be 0.93% (4/430) (16). Moreover, there is one case (a 5-year-old girl) of tularemia reported in the

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literature so far, and it has been reported that this case was in contact with an infected wild rabbit. In the patient's anamnesis, there was a history of contact with wild rabbits hunted by his father who was a hunter (8). Our study is the first to carry out research that showed tularemia seroprevalence in our country among hunters who are considered to be risk groups and showed a high seroprevalence (17%).

The prevalence of tularemia varies in different parts of the world. This variability depends on the culture and lifestyle of the people in the community, as well as ecological conditions (4). In a study conducted by Obaidat et al. the seroprevalence of *F. tularensis* was found to be 7.7% in 828 randomly selected individuals in Jordan (1). The seroprevalence of tularemia among hunters was determined as 1.7% in Germany (25). In both Finland and Belgium, *F. tularensis* seroprevalence in randomly selected populations was reported to be 2% (26, 27). This rate was 0.5% in Austria (28). In a study conducted with 240 volunteers who are in the risk group for tularemia in Erzurum City of Turkey, 5 (2.1%) individuals were found seropositive in terms of tularemia (29). *Francisella tularensis* seropositivity was 3.6% in Van (22). The seroprevalence of tularemia among hunters was found to be 6.3% in Yozgat Province located in the Central Anatolia region of Turkey (30). In another study conducted on hunters in Poland, *F. tularensis* antibodies were not found in any hunter (31). Contrary to these researches, higher seroprevalence rates are observed in our study. A total of 250 serum samples were examined in Iran and anti-tularemia antibody positivity was found 14.4%. In the same study, it was determined that the highest tularemia seropositivity was in hunters with a percentage of 18% and continuous contact with wildlife is a significant risk factor (11). Therefore, hunters may be in contact with infected wild animals, they are generally considered to be in the high-risk group for tularemia infection. We assume that the high tularemia seroprevalence rate in our study is due to the high hunting culture in our country. In addition, the Turkish Cypriot population generally being intertwined with nature is a factor that increases the risk of tularemia.

In our study, no relationship was found between age and tularemia infection. Similarly, studies showed that tularemia infection and age are unrelated in Jordan (1) and Finland (26). In addition, in a study examining tularemia cases in Turkey, it was emphasized that there was no relationship between age and tularemia (24). Contrary to this, a positive correlation was found between tularemia and age in Azerbaijan. According to this, tularemia seropositivity increases as the age gets older (32). In relation to age, the length of hunting activity is another risk factor for tularemia. Esmaeili et al. found that the length of hunting activity and old age had a significant relationship with the rate of tularemia seroprevalence (11). However, no significant difference was detected between the hunting length of seropositive and seronegative hunters in our study.

A humoral response develops as a result of contact with *F. tularensis*, and this humoral response is a good marker of the infection. Antibodies (IgM, IgG, and IgA) against *F. tularensis* lipopolysaccharide appear within 6–10 days after the onset of the symptoms (33). The agglutination reaction for *F. tularensis* was first reported in 1926 using the TAT and this test was used for decades for diagnosis of tularemia. Then, the TAT test was replaced by the microagglutination test (MAT) which has many advantages (34, 35). Studies have reported that there may be a cross-reaction between *F. tularensis* and *Brucella* species (33, 35). In our study, *F. tularensis* TAT and Rose Bengal tests were found to be positive in two patients. However, *F. tularensis* IgG and *F. tularensis* IgM ELISA results of these two patient samples were negative. Therefore, *F. tularensis* and *Brucella* cross-reactivity rate in our study was 2%. In Behan and Klein's study, cross-reactivity to *Brucella abortus* antigen was found in 42 of 128 (32.8%) tularemia serum

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samples, and cross-reactivity titers to *F. tularensis* antigen were found in 8 of 34 (23.6%) brucellosis serum samples (36). In the study of Çelebi et al. cross-reactions with *Brucella abortus* antigen were detected in 49 of 260 (18.8%) tularemia-positive samples, while cross-reactions with *F. tularensis* antigen were observed in 23 of 252 (9.1%) brucellosis-positive samples (33).

Conclusions

The fact that there is only one epidemiological study and one case study reported in our country so far and despite this, the high seropositivity rate in hunters shows that this disease exists in our country and understanding the patients do not receive the correct diagnosis. There is a high probability that most patients have an asymptomatic or mild tularemia infection. Because tularemia is a rare disease, it is overlooked by the clinicians and not given enough attention. Therefore, we think that most patients were treated blindly with antibiotics and recovered. As a result, more epidemiological studies should be conducted on tularemia infection that we have detected in our country. In addition, clinicians and laboratory personnel should keep in mind this disease, especially in risk groups such as hunters.

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Ethical considerations

Ethical approval for our study was obtained at the meeting of the NEU Institutional Review Board on 02.05.2019 with project number NEU/2019/68-789. In addition, the consents of all the hunters were obtained.

Conflict of interest statement

The authors declare there is no conflict of interests.

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