Northwestern Med J. 2023;3(1):45-49
doi: 10.54307/NWMJ.2023.97269

Original Article

Seroprevalence of Francisella tularensis in patients with neck mass complaints

Boyunda kitle şikayeti olan hastalarda Francisella tularensis seroprevalansı

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Atıf/Cite as: Toprak SF, Ayral M, Dedeoğlu S, Özbek E, Temiz H. Seroprevalence of Francisella tularensis in patients with neck mass complaints. Northwestern Med J. 2023;3(1):45-49.

ABSTRACT

Background: Tularemia is a zoonotic disease endemic in the northern hemisphere. The causative agent of the disease is *Francisella tularensis*. *F.tularensis* is endemic in Turkey, predominantly in the Marmara and Black Sea regions, and causes small outbreaks. This study aimed to investigate the seroprevalence of *F.tularensis* in patients admitted to the Otorhinolaryngology outpatient clinic with the complaint of neck mass by using two different methods.

Methods: Serum samples were collected from patients who were admitted to the Otorhinolaryngology outpatient clinic of Dicle University Faculty of Medicine Hospitals between January 2021 and December 2021 with the complaint of neck mass. A commercially available immunochromatographic lateral flow test (ICT) and a single-assay chemiluminescence test (CHT) were used to detect *F.tularensis* antibodies. Rose-Bengal test was performed on all sera to determine cross-reactions with antibodies produced in brucellosis. Brucella immunocapture agglutination tests (BCT) were performed on the sera of patients with positive screening tests.

Results: The ages of patients diagnosed with neck mass ranged between 14–70 years, with a mean age of 44.5±12.1 years. Sixty two (62%) of the patients were male, and 38 (38%) were female. When the test results were evaluated, two sera were positive for *F.tularensis* by both ICT and CHT methods. The first serum tests were negative for Brucella. The titer of the second serum in the chemiluminescence test was low positive.

Conclusion: Tularemia should be considered in the differential diagnosis of patients presenting with neck mass complaints, especially in patients living in rural areas, and specific diagnostic tests should be performed. In addition, a more comprehensive seroprevalence study supported by molecular testing techniques to be conducted in Diyarbakır will provide clearer data on the extent to which tularemia affects our region and which subspecies is the causative agent.

Keywords: Neck mass, tularemia, seroprevalence

ÖZ

Giriş: Tularemi, kuzey yarımkürede endemik olarak görülen zoonotik bir hastalıktır. Hastalığın etkeni Francisella tularensis'tir. Francisella tularensis, Türkiye'de, Marmara ve Karadeniz bölgelerinde endemiktir ve küçük salgınlara neden olmaktadır. Bu çalışmada, Kulak Burun Boğaz polikliniğine boyunda kitle şikayeti ile başvuran hastalarda Francisella tularensis seroprevalansının iki farklı yöntem kullanılarak araştırılması amaçlandı.

Yöntem: Dicle Üniversitesi Tıp Fakültesi Hastaneleri Kulak Burun Boğaz polikliniğine Ocak 2021-Aralık 2021 tarihleri arasında boyunda kitle şikayeti ile başvuran hastalardan serum örnekleri toplandı. F. tularemia karşı oluşan antikorlar ticari olarak temin edilebilen immunokromatografik (ICT) ve kemilüminesans (CHT) yöntemle çalışılan testlerle tespit edildi. Brusellozda oluşan antikorlarla çapraz reaksiyonları belirlemek için tüm serumlara Rose-Bengal tarama testi yapıldı. Tarama testleri pozitif olan hastaların serumlarına Brucella immunocapture aglütinasyon testleri (BCT) yapıldı.

Bulgular: Boyun kitlesi tanısı alan hastaların yaşları 14-70 arasında değişmekte olup, ortalama yaş 44,5±12,1'di. Hastaların 62'si (%62) erkek, 38'i (%38) kadındı. Test sonuçları

Received: 11.01.2023
Accepted: 25.01.2023
Publication date: 01.02.2023

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değerlendirildiğinde, hem ICT hem de CHT yöntemiyle iki farklı serum F. tularemia için pozitif bulundu. Brucella antikorları için ilk serumun testleri negatifti. Kemilüminesans yöntemle çalışan testte ikinci serumun titresi düşük derecede pozitif saptanmıştır.
Sonuç: Boyunda kitle şikayeti ile başvuran ve özellikle kırsal kesimde yaşayan hastalarda ayırıcı tanıda tularemi düşünülmeli ve spesifik tanı testleri yapılmalıdır. Ayrıca Diyarbakır'da yapılacak moleküler test teknikleriyle desteklenmiş daha kapsamlı bir seroprevalans çalışması, tulareminin bölgemizi ne ölçüde etkilediği ve hangi alt türün etken olduğu konusunda daha net veriler sağlayacaktır.

Anahtar kelimeler: Boyunda kitle, tularemi, seroprevalans

INTRODUCTION

Tularemia is a zoonotic disease seen as endemic in the northern hemisphere. The causative agent of the disease is *F.tularensis*. This bacterium is an aerobic, non-spore-forming, non-motile, Gram-negative, and facultative intracellular coccobacillus (1). The first case of tularemia in Turkey was detected in Lüleburgaz in 1936. In the following years, sporadic cases and small-point tularemia outbreaks were reported from different regions (2). Studies have shown that tularemia outbreaks in Turkey are waterborne. All these data show that *F.tularensis* is endemic in Turkey, predominantly in the Marmara and Black Sea regions, and causes small outbreaks (3,4). It can be transmitted to humans by rodents such as rats, mice, beavers, and rabbits, which are reservoirs, after contact with vectors such as ticks that have contact with these organisms, or by using water sources harboring the bacteria or consuming contaminated food (5,6). Humans can transmit it to others through the skin, mucosal, oral, or respiratory route (7). While the most common transmission route in the world is contact with infected animals and ticks, our country's most important transmission route is the consumption of spring water or unchlorinated drinking water (3).

In 2005, standardization was developed for tularemia, which was included in the list of notifiable diseases; sampling and submission rules, laboratory criteria for diagnosis, and case definition were established. *F.tularensis* has become a zoonotic infection that has regained importance in Turkey recently (6). Tularemia cases are rare in this region and there is no adequate study and epidemiological data on seroprevalence.

This study aimed to investigate the seroprevalence of *F.tularensis* in patients admitted to the Otorhinolaryngology outpatient clinic with the complaint of neck mass by using two different methods.

MATERIALS AND METHODS

In this study, serum samples were collected from the patients who were admitted to the Otorhinolaryngology outpatient clinic of Dicle University Faculty of Medicine Hospitals between January 2021 and December 2021 with the complaint of a neck mass. Approximately 5cc of venous blood was collected under sterile conditions from the patients, who were informed about the study beforehand and whose informed consent was obtained. The blood samples were centrifuged at 5000 rpm for 5 minutes and the serum was separated. These sera were transferred to 2cc Eppendorf tubes and stored at -80°C until the day of the study. Antibodies to *F.tularensis* in 100 sera collected at the end of one year were determined by various tests.

Immunochromatographic and chemiluminescence tests (CHT) were used to detect F.tularensis antibodies. Samples with indexes above 1.1 were considered to have antibodies against *F.tularensis*.

Rose-Bengal test was performed on all sera to determine cross-reactions with antibodies produced in brucellosis. Samples with agglutination were considered positive, and those with no agglutination were considered negative. Brucella immunocapture agglutination tests (BCT) (Brucellacapt, Vircell, Spain) were performed on the sera of patients with positive screening tests by the manufacturer's recommendations. The Dicle University Faculty of Medicine Non-

Interventional Clinical Research Ethics Committee approved the study protocol (03.09.2020 / 286).

RESULTS

The ages of patients diagnosed with neck mass ranged between 14–70 years, with a mean age of 44.5±12.1 years. Sixty two (62%) of the patients were male, and 38 (38%) were female. When the test results were evaluated, two sera were positive for *F.tularensis* by both ICT and CHT methods. The first serum tests were negative for Brucella. The titer of the second serum in the chemiluminescence test was low positive (Index=1.2). The results of the patients' sera are given in Table 1.

Table 1. Test Results of Patients' Serum.

	CHT	ICT	RBT	BCT
1. Serum	Positive	Positive	Negative	Negative
2. Serum	Positive	Positive	Positive	Negative

CHT: chemiluminescence test, ICT: Immunochromatographic test, RBT: Rose-Bengal test, BCT: Brucella immunocapture agglutination tests.

DISCUSSION

Anamnesis, presence of suspicious clinical findings, isolation of the agent from clinical samples, demonstration of the presence of antigen or antibody by serological methods, and determination of the genetic structure by molecular methods diagnose tularemia. The isolation of *F.tularensis* from clinical samples is the gold standard for diagnosis (4). *F.tularensis* is a bacterium that does not grow on sheep blood and EMB agar but can grow on enriched, cystine, or cysteine-enriched media at 35°C in 2-5 days. Due to the slow growth of the bacterium and the high risk of contamination, culture is difficult in practice (8). Investigation of antibodies against the causative agent in the patient serum or bacterial antigens in the acute phase by serological methods is one of the most commonly used and easy-to-apply methods in diagnosis (4,6).

Specific IgM, IgG, and IgA antibodies developed against *F.tularensis* can be detected in serum approximately one week the after infection. Antibodies reach their highest levels in the second month and can be detected for up to 11 years (9). Tube agglutination (TA) and microagglutination test (MAT) are usually used for antibody detection. The indirect enzyme linked immunosorbent assay (ELISA) is particularly suitable for routine serodiagnosis and seroepidemiological studies because it is highly sensitive and specific. However, these tests should be evaluated together with the clinical findings (10,11).

In this study, two sera (2%) of 100 patients presenting with a cervical mass in the neck were positive for *F.tularensis*. Since the onset, clinical signs, and symptoms of tularemia are not specific, it can be confused with many diseases (4). The clinical picture varies according to the route of entry, virulence of the bacteria, and the immune status of the host. The disease may occur in six forms, including ulceroglandular, glandular, oculoglandular, pneumonic, typhoidal, and oropharyngeal tularemia (12). Most tularemia cases in Turkey occur in the oropharyngeal form due to ingesting contaminated water and food. In the case series with the highest number of patients reported in Turkey, the oropharyngeal form was seen in 83% of the patients, and lymphadenopathy was found in 85% (13,14). Tularemia can be mingled for a range of other diseases. Among these, there are various conditions presenting with fever and lymph node enlargement (15). Lymphadenitis may frequently be unilateral. These findings may be confused with tuberculous lymphadenitis, streptococcal tonsillitis, and infectious mononucleosis (13,16). In cases where the etiology of cervical lymphadenitis cannot be determined completely, patients may not recover for a long time and have to consult many different physicians and hospitals because specific treatment cannot be given (17). Therefore, making a differential diagnosis in patients presenting to otorhinolaryngology clinics with a mass in the neck is very important. It should be kept in mind that patients may present with only lymphadenopathy due to late presentation and delays in diagnosis, and fever may not be seen (18). The fact that both immunochromatographic

and chemiluminescence methods were positive in the same sera indicates that the tests provide compatible results. Some researchers prefer the ELISA method because it is more sensitive in detecting antibodies formed long ago, many materials can be studied quickly, and the possibility of cross-reaction is lower (19).

In this study, seropositivity was found to be 2%. In a study conducted in 2006 by Kılınç et al.[20] tularemia antibodies were detected at a rate of (0.3%) in the Thrace Region. For this reason, it is estimated that the agent is still present in the Thrace Region. In a study conducted by Esmaeili et al.[21] in Iran in 2013, 250 serum samples were screened by the ELISA method. While antibody seroprevalence was 14.4%, the highest seroprevalence was found in hunters, with 18%. In a study conducted by Yazgı et al.[22] a total of 240 volunteer sera from villages in the Erzurum city center and Pasinler district of Erzurum, where there was no previous case reported, were examined. The seropositivity was found at 2.1% by ELISA. In a study by Bayram et al.[6] in Van, 495 human serum samples were analyzed. *F.tularensis* seropositivity rate was found to be 3.6%.

In the study conducted by Atmaca et al.[23] 20 patients who presented to the Ear-Nose-Throat outpatient clinics with a mass in the neck were analyzed. Of these, 7 (35%) were positive with the MAT test. Sixty-four volunteer hunters participated in a study by Yeşilyurt et al.[24] in 2010. *F.tularensis* antibodies were investigated by MAT and ELISA. A total of four cases (6.3%) were found to be seropositive, and all cases were found to be negative in the Brucella agglutination test. In a study conducted on blood donors in our region in 2015, 1.3% seropositivity was found (25). Our results are consistent with this and indicate that the causative agent is present in the region, and people may be at risk. In one serum, low titer positivity was detected in CHT; agglutination was observed in Rose-Bengal test (RBT) but was negative in BCT. It was also found in another study and it was reported that cross-reactions between Brucella species and *F.tularensis* were observed at low titers (19,23,26). In a study conducted in Van province, it was reported that cross-reactions with antibodies against Brucella were observed more frequently since brucellosis was frequently observed in that region (6). The 4-amino, 4-6 dideoxy-mannose (N-acyl-D-perosamine) region in the O polysaccharide chain attached to lipopolysaccharides of Brucella species (B.abortus, B.melitensis, and B.suis) shows common antigenic properties with some Gram-negative bacteria such as *F.tularensis*. This is responsible for the cross-reactions (27,28).

In this study, we aimed to determine the seropositivity rate of *F.tularensis* in patients presenting with complaints of cervical mass to shed light on further studies and to raise awareness about tularemia disease. Tularemia should be considered in the differential diagnosis of patients presenting with neck mass complaints, especially in patients living in rural areas, and specific diagnostic tests should be performed. Misdiagnosis leads to inadequate treatment and financial loss.

In addition, a more comprehensive seroprevalence study supported by molecular testing techniques to be conducted in Diyarbakır and its neighboring provinces will provide clearer data on the extent to which tularemia affects our region and which subspecies is the causative agent.

Ethics Committee Approval: The study protocol was approved by the Dicle University Faculty of Medicine Non-Interventional Clinical Research Ethics Committee (03.09.2020 / 286).

Conflict of Interest: The authors have declared that they have no conflict of interest.

Funding: The authors stated that the work funded by the Dicle University scientific research projects (Project No: TIP.21.001).

REFERENCES

1. Ellis J, Oyston PCF, Green M, Titball RW. Tularemia. Clin Microbiol Rev. 2002; 15(4): 631-46. https://doi.org/10.1128/CMR.15.4.631-646.2002
2. Gürcan S. Epidemiology of tularemia. Balkan Med J. 2014; 31(1): 3-10. https://doi.org/10.5152/balkanmedj.2014.13117
3. Gürcan S, Otkun MT, Otkun M, Arikan OK, Ozer B. An outbreak of tularemia in Western Black Sea region of Turkey. Yonsei Med J. 2004; 45(1): 17-22. https://doi.org/10.3349/ymj.2004.45.1.17
4. T.C. Sağlık Bakanlığı. Tularemi Hastalığının Kontrolü için Saha Rehberi. Ankara: T.C. Sağlık Bakanlığı, Temel Sağlık Hizmetleri Genel Müdürlüğü, Zoonotik Hastalıklar Daire Başkanlığı; 2011. Avilable at: https://web.archive.org/web/20110928232437/http://www.saglik.gov.tr/TR/dosya/1-71840/h/tularemi-saha-rehberi.pdf/
5. Şahin İ. Tulareminin bulaş yolları. In: Gürcan Ş, ed. Francisella tularensis ve Tularemi. İstanbul: Nobel Tıp Kitabevleri; 2009: 89-97.
6. Bayram Y, Özkaçmaz A, Parlak M, Başbuğan Y, Kılıç S, Güdücüoğlu H. Seroprevalence of tularemia in risk groups of humans and animals in Van, eastern Turkey. Mikrobiyol Bul. 2015; 49(4): 532-41. https://doi.org/10.5578/mb.9966
7. Tärnvik A, Priebe HS, Grunow R. Tularaemia in Europe: an epidemiological overview. Scand J Infect Dis. 2004; 36(5): 350-5. https://doi.org/10.1080/00365540410020442
8. Uludağ Altun H, Tetik H. Unilateral cervical mass: Tularemia. Ege Journal of Medicine. 2013; 52(1): 43-4.
9. Koskela P, Salminen A. Humoral immunity against Francisella tularensis after natural infection. J Clin Microbiol. 1985; 22(6): 973-9. https://doi.org/10.1128/jcm.22.6.973-979.1985
10. Çetinkaya Z. Francisella ve Brucella. In: Başustaoğlu A, Kubar A, Yıldıran ŞT, Tanyüksel M, trans. Klinik Mikrobiyoloji. Ankara: Atlas Kitapçılık; 2009: 815-34.
11. Wawszczak M, Banaszczak B, Rastawicki W. Tularaemia - a diagnostic challenge. Ann Agric Environ Med. 2022; 29(1): 12-21. https://doi.org/10.26444/aaem/139242
12. Arslanyılmaz M, Aslan D, Akın L, Aktaş D. Tularemi: güncel değerlendirmeler. Turk Hij Den Biyol Derg. 2014; 71(2): 99-106. https://doi.org/10.5505/TurkHijyen.2014.50490
13. Karadenizli A, Gurcan S, Kolayli F, Vahaboglu H. Outbreak of tularaemia in Golcuk, Turkey in 2005: report of 5 cases and an overview of the literature from Turkey. Scand J Infect Dis. 2005; 37(10): 712-6. https://doi.org/10.1080/00365540510012125
14. Helvaci S, Gedikoğlu S, Akalin H, Oral HB. Tularemia in Bursa, Turkey: 205 cases in ten years. Eur J Epidemiol. 2000; 16(3): 271-6. https://doi.org/10.1023/a:1007610724801
15. World Health Organization. WHO Guidelines on Tularaemia. Switzerland: WHO Press; 2007. Available at: https://apps.who.int/iris/bitstream/handle/10665/43793/9789241547376_eng.pdf?sequence=1&isAllowed=y
16. Akalın H. Ülkemizde Tularemi sorunu. 7. Ulusal İç Hastalıkları Kongresi, Antayla, 2005. Available at: http://www.tihud.org.tr/uploads/content/kongre/7/7.24.pdf
17. Karagöz E, Ulçay A, Hatipoğlu M, Turhan V. Tuberculosis and tularemia as part of the differential diagnosis in cervical lymphadenitis. Mikrobiyol Bul. 2014; 48(4): 707-8. https://doi.org/10.5578/mb.7657
18. Benli A. First Tularemia Outbreak in the Province of Muş: Evaluation of 50 Cases. Klimik Derg. 2022; 35(2): 74-7. https://doi.org/10.36519/kd.2022.3623
19. Gürcan Ş. Serolojik Tanı Yöntemleri. In: Gürcan Ş, ed. Francisella tularensis ve Tularemi. İstanbul: Nobel Tıp Kitabevleri; 2009: 269-72.
20. Dedeoğlu GK, Gürcan S, Eskiocak M, Kılıç H, Kunduracılar H. Trakya bölgesi köylerinde tularemi seroprevalansının araştırılması. Mikrobiyol Bul. 2007; 41(3): 411-8.
21. Esmaeili S, Gooya MM, Shirzadi MR, et al. Seroepidemiological survey of tularemia among different groups in western Iran. Int J Infect Dis. 2014; 18: 27-31. https://doi.org/10.1016/j.ijid.2013.08.013
22. Yazgi H, Uyanik MH, Ertek M, et al. Erzurum merkez ve kırsalında yaşayan riskli gruplarda tularemi seroprevalansı. Mikrobiyol Bul. 2011; 45(1): 67-74.
23. Atmaca S, Leblebicioğlu H, Ünal R, et al. Samsun ve çevresinde görülen tularemi olgulari. KBB-Forum. 2005; 4(4): 171-2.
24. Yeşilyurt M, Kılıç S, Çelebİ B, Gül S. Tularemi: Avcılar Gerçekten Bir Risk Grubu mu? Mikrobiyol Bul. 2012; 46(1): 153-5.
25. Bozdağ H, Özekinci T. Tularemia in Diyarbakır Province and its Surroundings: A Rarely Known Disease. İKSSTD. 2020; 12(1): 52-6. https://doi.org/10.5222/iksstd.2020.48243
26. Yıldırım M. Türkiye'de Tularemi: Seroprevalans Çalışmaları, Francisella tularensis ve Tularemi. Gürcan S, editor. İstanbul: Nobel Tıp Kitabevleri; 2008: 299-304.
27. Porsch-Ozcürümez M, Kischel N, Priebe H, Splettstösser W, Finke E-J, Grunow R. Comparison of enzyme-linked immunosorbent assay, Western blotting, microagglutination, indirect immunofluorescence assay, and flow cytometry for serological diagnosis of tularemia. Clin Diagn Lab Immunol. 2004; 11(6): 1008-15. https://doi.org/10.1128/CDLI.11.6.1008-1015.2004
28. Kılıç S, Çelebi B, Bayram Y, Çitil B. Francisella tularensis antikorları ile Brucella çapraz reaksiyonlarının araştırılması. Turk Hij Den Biyol Derg. 2013; 70(2): 65-70. https://doi.org/10.5505/TurkHijyen.2013.70893