Istanbul J Pharm 49 (3): 137-141
DOI: 10.26650/IstanbulJPharm.2019.19023
Original Article

Risk factors associated with the frequency of antibodies to *Francisella tularensis* in two areas from Turkey

Zeynep Özgen Özdemir[1], Turabi Güneş[2], Özlem Oyardı[3]

[1]Department of Pharmacy Services, Vocational School of Health Services, Aydın University, İstanbul, Turkey
[2]Vocational School of Health Services, Cumhuriyet University, Sivas, Turkey
[3]Department of Pharmaceutical Microbiology, Faculty of Pharmacy, Istanbul University, Beyazit-Istanbul, Turkey

ORCID IDs of the authors: Z.Ö.Ö. 0000-0002-7282-4549; T.G. 0000-0002-8011-7844; Ö.O. 0000-0001-9992-7225.

Cite this article as: Özdemir ZÖ, Güneş T, Oyardı Ö (2019). Risk factors associated with the frequency of antibodies to *Francisella tularensis* in two areas from Turkey. Istanbul J Pharm 49 (3): 137-141.

ABSTRACT

Francisella tularensis is a zoonotic microorganism that can infect different species of animals and sometimes humans. The aim of this study was to determine the seroprevalence of *F. tularensis* in people from two areas in Turkey (Sivas and Tokat). This is a retrospective study of the serum samples collected in 2006 from people living in rural areas (n:360) and from people living in urban areas (n:90). *F. tularensis*-IgG antibodies were investigated by ELISA method. Antibody positivity against *F. tularensis* was found in 7.5% of 360 serum collected from rural areas and 1.1% of 90 serum collected from urban areas (OR 7.216, 95% CI 0.967-53.836, p=0.025). While there was no difference in *F. tularensis* seropositivity between different genders (p=0.424), tick contacts (p=0.303) and occupational groups (p=0.807), *F. tularensis* seropositivity was found to be higher in the Tokat region than in the Sivas region (p=0.047). Moreover, risk factors were observed in people over 40 years of age (p=0.045) and in those who consume fresh cheese (p=0.036). Our findings revealed that tularemia cases can be seen in these regions even though tick bite cases in the Sivas and Tokat regions were not an important influence on the transmission of *F. tularensis* to humans on the dates of our research.

Keywords: *Francisella tularensis*, tick bite, tularemia, Turkey

INTRODUCTION

Francisella tularensis is a non-motile, non-spore and highly infectious microorganism in coccobacillus form. Tularemia, a disease caused by *F. tularensis*, is a zoonotic disease that is usually located between 30°-71° latitude north, particularly in rural enviroments. However, cases of tularemia in many European countries including Turkey have also been reported (Ellis et al. 2002; Hestvik et al. 2015).

F. tularensis has 4 subspecies in nature and the most common and clinically important subtypes are subtype *tularensis* (A) and subtype *holarctica* or *palaearctica* (B) (Ellis et al. 2002; Zellner and Huntley 2019; Kılıç 2010). Type-A with higher virulence is transmitted by vectors such as ticks and infected animals. Type-B causes mild to moderate infection in the northern hemisphere and is transmitted from water sources. *F. tularensis* (A), which has higher virulence, is quite common in nature. The natural reservoirs of *F. tularensis* are mostly small mammalian species. Several arthropods, such as ticks, lice, fleas and flies, have been reported to be infected with *F. tularensis* (Socolovschi et al. 2009). In Europe, *F. tularensis* was isolated from *Ixodes ricinus*, *Dermacentor reticulates* and *D. marginatus* species (Milutinović et al. 2008; Genchi et al. 2015). Since blood-sucking insects such as ticks are vectors of tularemia for transmission to mammals, people living in rural areas are at greater risk (Gürcan 2014).

Address for Correspondence :
Özlem OYARDI, e-mail: ozlemyrd@gmail.com

Received: 29.05.2019
Accepted: 05.11.2019

F. tularensis is transmitted to humans by infection-bearing animals, contact with infected tissue and body fluids, arthropod bites, consumption of contaminated waters and inhalation of aerosols capable of infection. The transmission of *F. tularensis* through tick bites is usually sporadic (Ellis et al. 2002). In Turkey, after the first tularemia case was seen in 1936, several outbreaks occurred and the number of cases gradually increased. Between 1936 and 2011, 1441 cases diagnosed as tularemia were reported (Gürcan 2014). According to recent studies, *F. tularensis* is mostly transmitted by consumption of contaminated water in Turkey (Kilic et al. 2015; Duzlu et al. 2016).

Symptomatic manifestation of infection may vary depending on the type of disease. The ulceroglandular clinical form of the disease is the most common form of infection with 45-80% worldwide (Maurin and Gyuranecz 2016). In Turkey, epidemics mostly occur in northwest regions and Central Anatolia and the most prevalent clinical form is oropharyngeal form (Ulu-Kilic and Doganay 2014). IgG antibodies developed to *F. tularensis*, whether symptomatic or asymptomatic, may be detectable up to 10 years in the patient's serum even at low titers (Koskela and Salminen 1985). In epidemiological studies, IgG antibodies can easily be determined by methods such as microagglutination and ELISA. Since *F. tularensis* and *B. abortus* have common antigens, serological cross-reactions might occur between both, especially in the agglutination test. The sensitivity and specificity of the ELISA test is much higher than is the case with the agglutination tests (Ellis et al. 2002; Porsch-Ozcürümez et al. 2004).

The Tokat and Sivas regions in Turkey are geographic areas where tick-borne zoonoses are especially common (Gunes et al. 2012). This study aimed to determine whether there is a difference in the positivity of *F. tularensis* antibody between different factors including living area, tick contact, age, gender, occupation, city, and consumption of fresh cheese. In addition, *B. abortus*-IgG antibodies were studied to detect cross- reacting antibodies.

MATERIAL AND METHODS

Study area

Sivas is located in the Central Anatolia Region of Turkey with a 28,458 km^2 surface area and it has a population of around 650 thousand people. Tokat, which is a city located in the Black Sea region of Anatolia, has a population of around 600 thousand people and its surface area is approximately 10,000 km^2 (Figure 1). Sivas and Tokat districts have a fauna that hosts many animal species and a large flora and whose economy is based mainly on agriculture and livestock farming.

Collection of blood samples

In June-September 2006, 56 villages from 14 districts in Tokat and Sivas where tick-borne infections were endemic were selected as the study area (Figure 1). Blood samples were taken from 1093 people who were engaged in livestock farming and lived in rural areas of the Sivas and Tokat regions, and from 90 people who were not related to rural areas and animal husbandry and who lived in the city center. A questionnaire was formed and the participants were questioned about their names, gender, age, contact with animals (especially ticks) and consumption of fresh cheese (Table 1). Serums of blood samples were obtained. To minimize the drawbacks of the freeze-thaw, each serum was divided into 5 separate tubes and stored at -80°C prior to analysis. A total of 450 serum samples were included in this study and 360 serum samples from 1093 serums stored at -80°C were selected with the random sampling method. Of the 360 subjects included in the study group, 180 were male (mean age: 40.79±19.62), and 180 were female (mean age: 40.750±16.26 years). In total, 125 people from the Sivas region and 235 from the Tokat region were included in the study (Table 1). The study was approved by the Non-interventional Clinical Research Ethics Board (Decision No: 2017-11 / 12).

Serological tests

F. tularensis IgG antibodies were investigated by ELISA method. For this purpose, Serion ELISA classic *F. tularensis* IgG kits produced by Virion/Serion company and classic *B. abortus* IgG kits produced by Nova-Tec Company were used. The *F. tularensis* ELISA-IgG kit used in this study was able to screen antibodies produced against both the *F. tularensis* subtype tularensis (type-A) and *F. tularensis* holarctica (type-B) subtype. The experiments were conducted in accordance with the user manual contained in the kits. In the final stage, the microplates were read on the ELISA microplate reader (EL 312, Bio-Tek Instruments, Inc., Winooski, Vermont, USA) at a wavelength of 405 nm and the absorbance values of serum samples and standard serums were determined. The absorbance values of serums were examined and the cut off value was calculated with the formula specified in the kit prospectus. The absorbance values determined for each group were compared with the calculated cut off value and the serums having a higher value than the cut off value were evaluated as positive. Furthermore, the antibodies to *B. abortus* were examined by ELISA (NovaLisa Brucella IgG) to determine whether there was a serological relationship between *F. tularensis* and *B. abortus* in terms of cross reactions (in 56 seronegative serums and 27 seropositive serums for *F. tularensis).*

Statistical analysis

The data were transferred to the computer and statistically analyzed by the licensed The Statistical Package for the Social

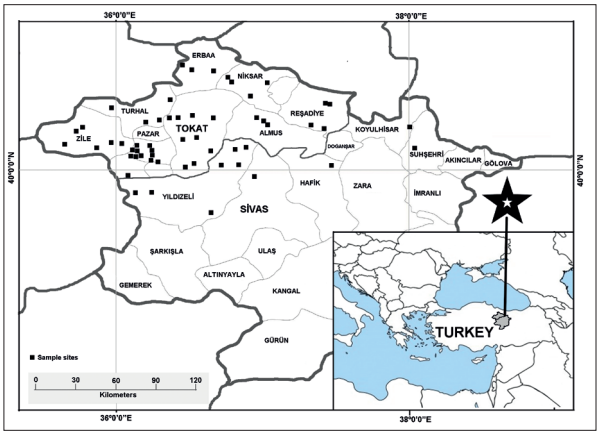

Figure 1. Map of the study area, 656x472mm (96 x 96 DPI).

Table 1. *F. tularensis* antibody findings in risk categories

Categories of risk factors	n	Positive (%)	p	Odds (95% CI)#
Total	450	28 (6.222)		
Village. City				
City residents*	90	1 (1.111)	0.025	7.216 (0.967-53.836)
Village residents	360	27 (7.555)		
Tick Contact				
Bitten by tick*	90	4 (4.444)	0.303	1.265 (0.328-4.871)
Cleaning ticks	90	5 (5.555)		2.687 (0.810-8.912)
Bitten by tick and cleaning ticks	90	10 (11.111)		2.098 (0.608-7.232)
No tick contact	90	8 (8.888)		
Residential Areas				
Sivas*	125	5 (4.000)	0.047	2.479 (0.915-6.715)
Tokat	235	22 (9.361)		
Gender				
Male*	180	11 (6.111)	0.424	1.499 (0.675-3.326)
Female	180	16 (8.888)		
Occupational Groups				
Livestock (only) *	23	1 (4.348)	0.807	2.870 (0.277-29.713)
Farming (only)	26	3 (11.539)		1.732 (0.222-13.524)
Livestock and farming	274	20 (7.299)		1.941(0.190-19.869)
Other	37	3 (8.108)		
Consumption of fresh cheese				
No*	86	2 (2.326)	0.036	4.217 (0.978-18.182)
Yes	274	25 (9.124)		
Age. 40				
Age ≤ 40 y*	189	9 (4.762)	0.045	2.353 (1.027-5.389)
Age > 40 y	171	18 (10.526)		

CI: Confidence interval, *: Reference category in Odds ratio

Sciences (SPSS) for Windows 14' program (SPSS, Inc., Chicago, IL, USA). Chi-square test method was used to evaluate risk categories such as tick bites, tick contacts, sex, age and occupational groups in terms of "p value". The chi-square test was used to calculate the odds value in two categorical variables (2x2), and a binary logistic regression analysis was used to calculate the odds value in more than one categorical variable (3x2, 4x2). Confidence interval was accepted as 95% and p value <0.05 for statistical significance.

RESULTS

According to the results, IgG antibodies were detected against *F. tularensis* in 27 (7.56%) of the 360 people living in rural areas and 1 (1.11%) of 90 people living in urban areas, and seroprevalence was found to be higher in people living in rural areas (p=0.025, odds ratio (OR)= 7.216, 95% confidence interval (CI)= 0.967-53.836) (Table 1).

When the presence of *F. tularensis* antibody was evaluated, antibody positivity was found in in 4 (4.4%) of those bitten by ticks, in 5 (5.6%) of those who were cleaning ticks from animals, in 10 (11.1%) of those who were both bitten by ticks and were cleaning ticks from animals, and in 8 (%8.9) of the subjects who lived in the village but had no contact with ticks. The results showed that seroreactivity against *F. tularensis* was not statistically significant between the groups in terms of contact with the ticks (p=0.303).

The rate of *F. tularensis* antibody positivity was 5 (4%) in serum samples of 125 from Sivas, and there were 22 (9.4%) positive results in 235 samples taken from Tokat (p=0.047, OR=2.479, 95% CI=0.915-6.715). When the results were evaluated in terms of sex, in 11 (6.1%) of 180 men, in 16 (8.9%) of 180 women, reactive antibodies against *F. tularensis* were found (p=0.424, OR=1.499, 95% CI=0.675-3.326).

Evaluation of the serums in terms of occupational groups showed that seropositivity against *F. tularensis* was found in 1 (4.4%) out of 23 people who fed only animals, in 3 (%11.5) out of 26 people engaged in farming, in 20 (7.3%) out of 274 people engaged in both livestock and farming, and in 3 (8.1%) out of 37 of the other occupational group employees. No significant difference was found in the frequency of tularemia infection among different occupational groups (p=0.807).

While *F. tularensis* seropositivity was observed in 25 (9.1%) out of 274 people who consumed fresh cheese, it was also found in 2 (2.3%) out of 86 people who did not consume it. The seroprevalence of *F. tularensis* in people consuming fresh cheese

was found to be high compared to those not consuming fresh cheese (p=0.036, OR=4.217, 95% CI=0.978-18.182).

In 18 (10.5%) of 171 people over 40 years of age and in 9 (4.8%) of 189 people under 40 years of age antibody positivity was found and the difference was statistically significant (p=0.045, OR=2.353, 95% CI=1.027-5.389).

IgG seropositivity against *B. abortus* was determined in 5 (18.5 %) of 27 serums that were positive for *F. tularensis* and in 14 (25 %) of the 56 seronegative samples for *F. tularensis*. The seroprevalence of *B. abortus* was not statistically different in *F. tularensis* negative and positive sera (p=0.587).

DISCUSSION

Tularemia is endemic in Europe, Finland and Sweden and cases have also been reported in Austria, Germany, Spain, Hungary, Bulgaria and Turkey (Ellis et al. 2002; Leblebicioglu et al. 2008; Gürcan 2014). This seroepidemiological study suggests that asymptomatic or symptomatic tularemia infections may be seen in the Tokat and Sivas regions.

13 % of 18,343 tularemia cases in Europe between 1992 and 2012 were reported by Turkey (Hestvik et al. 2015). In epidemiological studies conducted by different researchers after the tularemia outbreak in Turkey, *F. tularensis* seroprevalence was found between 2,6%-20.9% depending on the region. (Dedeoğlu Kilinç et al. 2007; Gürcan 2014). In this study, the prevalence of tularemia in people living in rural areas was approximately 7 times higher than in people living in urban areas (OR= 7.216). *F. tularensis* seroprevalence, which we found in 7.6% of the rural population, indicates that tularemia may pose a risk to the health of people living in rural areas in the Tokat and Sivas regions.

Since *F. tularensis* is a zoonotic bacterium, people engaged in livestock farming, farmers and especially hunters have higher seropositivity (Jenzora et al. 2008; Esmaeili et al. 2014). In our results, no statistically significant difference was observed in *F. tularensis* seroprevalence between different occupational groups (p=0.807). Of the 360 participants included in this study, 272 (76%) were engaged both in animal husbandry and farming. It is not typical for people living in rural areas to have only one occupation type. Considering that the transmission of *F. tularensis* subsp. holarctica in Turkey is generally waterborne, it can be assumed that people from different occupational groups living in the villages usually use similar water resources.

In Europe , *F. tularensis* has been detected in *Ixodes ricinus*, *Dermacentor reticulatus* and *D. marginatus* ticks. However, the prevalence of *F. tularensis* (0-3.8%) determined in ticks is quite low compared to other tick-borne agents (Milutinović et al. 2008; Reye et al. 2010; Karasartova et al. 2018). According to the scientific data, tick bites do not have a significant importance in the transmission of *F. tularensis* to humans (Clark et al. 2012). In our study, in terms of their contact to ticks, there was no difference in the seroprevalence of *F. tularensis* among 4 groups in rural areas (p=0.303). These results confirm that tick bites in humans is not very important in the transmission of *F. tularensis*. However, infected ticks may be of importance in the transfer of *F. tularensis* between wild reservoirs.

The Tokat region, compared to the Sivas region, is more suitable for the survival of many rodent species that can be a reservoir for *F. tularensis*. This is because the climate in the Tokat region is similar to that in the Black Sea region. According to this study, people living in the villages of Tokat compared to the villagers of Sivas, have a 2.5 times greater risk of contact with *F. tularensis* (OR:2.479). In terms of survival of small mammals such as rabbits, mice and squirrels, which may be reservoirs for tick-borne infectious agents, we think that the Tokat region is a more suitable geography than Sivas.

According to the scientific data, there is generally no difference in the seroprevalence of *F. tularensis* among women and men living in rural areas (Gutiérrez et al. 2003; Clark et al. 2012; Esmaeili et al. 2014). Similar results were obtained in our study, too (p: 0.424). The fact that men and women living in rural areas are dealing with similar jobs can be one of the reasons for this result. Considering that the *F. tularensis* infections seen in our country are generally waterborne, another reason for this is that the risk of using contaminated water is similar for both sexes.

In our study, a significant difference of the *F. tularensis* seroprevalence was found between the groups who consumed fresh cheese and those who did not (p=0.036; OR: 4.217). In general, non-pasteurized raw milk consumption in Turkey is not common, but in rural areas, in the production of dairy products such as cheese, the use of non-boiled milk can occur.. Consumption of the products produced with non-boiled milk causes many infections, especially brucellosis. We predict that the possibility of coming into contact with *F. tularensis* infection is high in individuals who consume fresh cheese due to the lack of awareness of hygiene and protection against infections.

With increasing age, there is also an increase in *F. tularensis'* seroprevalence (Clark et al. 2012; Esmaeili et al. 2014). According to our findings, *F. tularensis* seropositivity (10.5%) was statistically higher in individuals over 40 years of age than in those aged 40 and below (4.8%). The fact that people over the age of 40 are more interested in animal feeding and agricultural activities, and also the fact that IgG antibodies remain at a detectable level in serum for years after infection, may provide some reasons for this difference.

In serological tests between *B. abortus* and *F. tularensis*, false positive results may be observed due to cross-reactions. The possibility of cross-reaction is higher in microagglutination tests (Behan and Klein 1982). In this study, the seroprevalence of *B. abortus* was not found to be higher in *F. tularensis* positive serums than *F. tularensis* negative serums. We assume that the possibility of cross-reaction due to *B. abortus* in ELISA test is too low to have importance attached to it. In this study, *F. tularensis* and *B. abortus* co-seroprevalence were detected in 1.39% of 360 serum samples of individuals, and we believe that the active co-infection rate is probably even lower.

CONCLUSION

F. tularensis infection is an important zoonosis which can be seen in almost every region, especially in rural areas. In the presence of symptoms such as sudden high fever muscle pain, sore throat and swelling of lymph nodes in people dealing with agriculture and livestock farming, it is very important to make differential diagnosis by diagnostic tests for the treatment and control of the disease, given that there is a possibility of contracting tularemia.

Peer-review: Externally peer-reviewed.

Author Contributions: Concept – T.G.; Design – T.G.; Supervision – T.G.; Materials – Z.Ö.Ö.; Data Collection and/or Processing – Z.Ö.Ö.; Literature Search – Ö.O.; Writing – Ö.O.; Critical Reviews – T.G.

Acknowledgements: We thank Nurse Zübeyde GÜNEŞ, Prof. Dr Ömer POYRAZ and Dr Mehmet Ataş for contributed to the study.

Conflict of Interest: The authors have no conflict of interest to declare.

Financial Disclosure: This study was supported by the Cumhuriyet University Scientific Research Projects Commission (CUSRPC) (Project no = SHMYO-014).

REFERENCES

- Behan KA, Klein GC (1982). Reduction of *Brucella* species and *Francisella tularensis* cross-reacting agglutinins by dithiothreitol. *J Clin Microbiol* **16:** 756-757.
- Clark DV, Ismailov A, Seyidova E, Hajiyeva A, Bakhishova S, Hajiyev H, Hepburn MJ (2012). Seroprevalence of tularemia in rural Azerbaijan. *Vector Borne Zoonotic Dis* (Larchmont, N.Y.), **12:** 558-563. [CrossRef]
- Dedeoğlu Kilinç G, Gürcan S, Eskiocak M, Kiliç H, Kunduracilar H (2007). Investigation of tularemia seroprevalence in the rural area of Thrace region in Turkey. *Mikrobiyol Bul* **41:** 411-418.
- Duzlu O, Yildirim A, Inci A, Gumussoy KS, Ciloglu A, Onder Z (2016). Molecular investigation of *Francisella*-like endosymbiont in ticks and *Francisella tularensis* in ixodid ticks and mosquitoes in Turkey. *Vector Borne Zoonotic Dis* **16:** 26-32. [CrossRef]
- Ellis J, Oyston PCF, Green M, Titball RW (2002). Tularemia. *Clin Microbiol Rev* **15:** 631-646. [CrossRef]
- Esmaeili S, Gooya MM, Shirzadi MR, Esfandiari B, Amiri FB, Behzadi MY, Mostafavi E (2014). Seroepidemiological survey of tularemia among different groups in western Iran. *Int J Infect Dis IJID* **18:** 27-31. [CrossRef]
- Genchi M, Prati P, Vicari N, Manfredini A, Sacchi L, Clementi E, Bandi C, Epis S, Fabbi M (2015). *Francisella tularensis*: No evidence for transovarial transmission in the tularemia tick vectors *Dermacentor reticulatus* and *Ixodes ricinus*. *PLoS One* **10:** e0133593. [CrossRef]
- Gunes T, Poyraz O, Mehmet A, Turgut NH (2012). The seroprevalence of *Rickettsia conorii* in humans living in villages of Tokat Province in Turkey, where Crimean-Congo hemorrhagic fever virus is endemic, and epidemiological similarities of both infectious agents. *Turk J Med Sci* **42:** 441-448.
- Gutiérrez MP, Bratos MA, Garrote JI, Dueñas A, Almaraz A, Alamo R, Rodríguez Marcos H, Rodríguez Recio MJ, Muñoz MF, Orduña A, Rodríguez-Torres A (2003). Serologic evidence of human infection by Francisella tularensis in the population of Castilla y León (Spain) prior to 1997. *FEMS Immunol Med Microbiol* **35:**165-169.
- Gürcan Ş (2014). Epidemiology of tularemia. *Balk Med J* **2014:** 3-10. [CrossRef]
- Hestvik G, Warns-Petit E, Smith LA, Fox NJ, Uhlhorn H, Artois M, Gavier-Widen D (2015). The status of tularemia in Europe in a one-health context: a review. *Epidemiol Infect* **143:** 2137-2160. [CrossRef]
- Jenzora A, Jansen A, Ranisch H, Lierz M, Wichmann O, Grunow R (2008). Seroprevalence study of *Francisella tularensis* among hunters in Germany. *FEMS Immunol Med Microbiol* **53:** 183-189. [CrossRef]
- Karasartova D, Gureser AS, Gokce T, Celebi B, Yapar D, Keskin A, Taylan-Ozkan A (2018). Bacterial and protozoal pathogens found in ticks collected from humans in Corum province of Turkey. *PLoS Negl Trop Dis* **12:** e0006395. [CrossRef]
- Kilic S, Birdsell DN, Karagöz A, Çelebi B, Bakkaloglu Z, Arikan M, et al. (2015). Water as source of *Francisella tularensis* infection in humans, Turkey. *Emerg Infect Dis* **21:** 2213.
- Koskela P, Salminen A (1985). Humoral immunity against *Francisella tularensis* after natural infection. *J Clin Microbiol* **22:** 973-979.
- Leblebicioglu H, Esen S, Turan D, Tanyeri Y, Karadenizli A, Ziyagil F, Goral G (2008). Outbreak of tularemia: a case–control study and environmental investigation in Turkey. *Int J Infect Dis* **12:** 265-269. [CrossRef]
- Maurin M, Gyuranecz M (2016). Tularaemia: clinical aspects in Europe. *Lancet Infect Dis* **16:** 113-124. [CrossRef]
- Milutinović M, Masuzawa T, Tomanović S, Radulović Z, Fukui T, Okamoto Y (2008). *Borrelia burgdorferi sensu lato, Anaplasma phagocytophilum, Francisella tularensis* and their co-infections in host-seeking *Ixodes ricinus* ticks collected in Serbia. *Exp Appl Acarol* **45:** 171-183. [CrossRef]
- Porsch-Ozcürümez M, Kischel N, Priebe H, Splettstösser W, Finke E-J, Grunow R (2004). Comparison of enzyme-linked immunosorbent assay, Western blotting, microagglutination, indirect immunofluorescence assay, and flow cytometry for serological diagnosis of tularemia. *Clin Diagn Lab Immunol* **11:** 1008-1015. [CrossRef]
- Reye AL, Hübschen JM, Sausy A, Muller CP (2010). Prevalence and seasonality of tick-borne pathogens in questing *Ixodes ricinus* ticks from Luxembourg. *Appl Environ Microbiol* **76:** 2923-2931. [CrossRef]
- Socolovschi C, Mediannikov O, Raoult D, Parola P (2009). Update on tick-borne bacterial diseases in Europe. *Parasite* **16:** 259-273. [CrossRef]
- Ulu-Kilic A, Doganay M (2014). An overview: tularemia and travel medicine. *Travel Med Infect Di* **12:** 609-616. [CrossRef]
- Zellner B, Huntley JF (2019). Ticks and Tularemia: Do We Know What We Don't Know? *Front Cell Infect Microbiol* **9:** 146. [CrossRef]