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Research Article

The seroprevalence of Francisella tularensis in horse herds in Turkey

Derya Karataş Yeni[1], Doğan Akça[2]

[1]Department of Microbiology, Faculty of Veterinary Medicine, Necmettin Erbakan University, 42310, Konya, TURKEY
[2] Department of Midwifery, Faculty of Health Science, Kafkas University, 36300, Kars, TURKEY

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Correspondence:
D. KARATAŞ YENİ
(vhekimderya@hotmail.com)

ORCID
D. KARATAŞ-YENİ : 0000-0001-7261-1394
E. AKÇA : 0000-0002-3986-8769

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ABSTRACT

Tularemia, caused by Francisella tularensis, can exist in nature over a long period of time. The disease can be transmitted by ticks, biting flies, contaminated food and water, and inhalation. F. tularensis infections are particularly common in North America, Europe, and Asia. Tularemia is often water-associated and affects humans and an array of animals, including domestic animals, small wild mammals and fish. The literature about tularemia in horses is limited; however, fever, dyspnea, incoordination and depression have been reported. This study aimed to estimate the seroprevalence of F. tularensis in horse herds in Turkey. A total of 109 horses, aged 36 months and older, were randomly sampled from different regions of Turkey. The serum samples were tested for the presence of antibodies to F. tularensis, using the Microagglutination Test (MAT), which has 51% sensitivity and 99% specificity. The overall (animal), within-herd and between-herd apparent seroprevalence values were calculated as 40.4% (95% CI = 31.6 to 49.8%), 41.1% (95 CI = 32.3 to 50.6%) and 81.8% (95% CI = 52.3 to 94.9%), respectively. The Rogan-Gladen estimator was then used to correct the apparent seroprevalence values to true seroprevalence values of 78.7% overall (95% CI = 61.3 to 97.5%), 80.2% within-herd (95 CI = 62.5 to 99.2%), and 161.6% between-herd (95 CI = 103 to 187.7%). The results provide useful information regarding the prevalence of tularemia in horse herds in Kafkas University Faculty of Veterinary Medicine Department of Microbiology, which it is hoped will attract the particular attention of veterinarians, enabling the establishment of an efficient control program.

INTRODUCTION

Tularemia is caused by a nonmotile, pleomorphic, Gram-negative coccobacilli bacteria called Francisella tularensis (F. tularensis), and is a common zoonotic infectious disease predominantly seen in the Northern Hemisphere (WHO, 2007; Mead, 2008). The disease is known by various names, such as Francis disease, Ohara disease, Rabbit fever-plague, Horsefly fever, Siberian ulcer and Hunter's disease (Kubelkova, 2015). F. tularensis is a resistant bacterium that can survive in cold and humid environments for weeks; however, it is not resistant to sunlight, high temperature or chlorination (Dikici, 2012). Transmission may occur by inhalation, ingestion, contact with infected animals, or via the bites of arthropod vectors (Arslanyılmaz,2014; CDC, 2018). Rodents (rats, mice and squirrels) and rabbits (Lagomorpha) are the most important reservoirs for tularemia (CDC, 2018). The existence of tularemia in sheep, cattle, pigs and horses was confirmed for the first time in the USSR (Pollitzer, 1963). Observation of tularemia in these animals led to the conclusion that Ixodes ticks, which were prevalent from 1939 to 1941 played an interepidemic role in the infection (Pollitzer, 1963). Tularemia is rarely reported in horses, but its occurrence is often accompanied by a severe tick infestation. Signs of tularemia in horses apparently include fever, shortness of breath (dyspnea), incoordination, depression, ataxia, and edema of the legs. Intense tick infestation and seroconversion have generally been observed in horses (Otlu, 2009). Death can occur within a day. In some cases, tularemia may be present in asymptomatic horses. Generally, animals with the disease are treated with agent-specific antibiotics such as streptomycin. Although it is difficult to control the disease in horses, this can be achieved by reducing tick infestation and rapid diagnosis and treatment. Animals that have recovered from the disease develop long-term immunity (Foley, 2019).

The diagnosis of tularemia can be performed with serological tests, such as the widely-used Microagglutination test (MAT), Hemagglutination test (HA) and the Enzyme-Linked Immuno-Sorbent Assay (ELISA). Among these, the MAT, using a stained F. tularensis antigen, is the most common tool for diagnosis (Arslanyılmaz, 2014).

The aim of this study was to determine the presence of antibodies to F. tularensis in horses by MAT and thus estimate the seroprevalence of tularemia in Turkey.

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MATERIAL and METHODS

Study design and Sample Collection

This study was approved by the local ethical committee. (Protocol no: 2021/17 Date: 05.11.2021 VKMAE). Blood samples for the study were collected from 11 different regions of Turkey, between 2021 and 2022, from ≥36 month-old horses, randomly selected from farms that implemented an extensive rearing system (stock farming mainly based on pasture and meadows) (Table 1).

A total of 109 blood samples, taken from horses with no history of vaccination against tularemia, were submitted to the laboratory. Serum samples were obtained by centrifugation at 3000 rpm for 10 min and stored at -20 °C, pending analysis.

Microagglutination Test (MAT)

A Microagglutination Test (MAT) was used for the detection of antibodies to F. tularensis in horse blood sera. MAT was performed with an antigen prepared from a standard strain of F. tularensis strain (NCTC 10857) (Arslanyılmaz, 2014). First, 40 µl saline buffer was put into the first well of the U-bottomed plate. Twenty-five µl of saline was put into the next 6 wells for sample sub-dilutions. Twenty-five µl of positive serum (1:160 titer) and 25 µl of saline were put into the 8th and 9th wells, respectively, for positive and negative control. Ten µl of test serum was introduced into the first well, and 25 µl of liquid content was transferred from the first well to the next, continuing to the 6th sub-dilution. Then, all of the wells, including the positive and negative controls, received 25 µl of stained antigen and, thus, 1:10 to 1:640 sub-dilutions were obtained. The test plate was put into a humidified box and incubated at 37°C overnight. Agglutination of the antigen-antibody complex in a net-like form, leaving a completely clear supernatant, was considered a positive reaction. Agglutination in a small, centrally-gathered smooth-edged form, surrounded by light red diluents, was evaluated as a negative reaction (Karataş Yeni, 2015; Kılıç, 2013).

The MAT test for F. tularensis has been shown to cross react with Brucella spp. at titers of up to 1:20 (Karataş yeni, 2015; Kılıç, 2013). Therefore, in this study, horse blood serum samples with a titer of 1:20, were subjected to the Brucella Microagglutination Test (Kılıç, 2013).

Statistical analysis

The data were statistically analyzed with the SPSS® Version 20. MAT results were evaluated by the Chi-square test (Preacher, 2001) and p-values smaller than 0.05 were assumed significant. The cut-off values established by Maurin (Maurin, 2020) were used for MAT sensitivity and specificity. The case definition and subsequent serial calculations of the apparent individual and mass prevalences (within-herd and between-herd) were carried out by the method reported by Buyuk et al. (2014). The true seroprevalence values for the animals overall, within-herd, and between-herd were calculated using the Rogan-Gladen estimator (Rogan, 1978).

RESULTS

Of the 109 horse blood serum examined for tularemia, 44 (40.4%) were found positive for F. tularensis with a titer of ≥1:20. When evaluated in terms of F.tularensis seropositivity, the p value was determined as >0.05. No statistical significance was observed among 11 different regions.The antibody titer distribution of the serum was 1:20 in 34 sera, 1:40 in 8 sera and 1:80 in 2 sera. In the Brucella Micro-Agglutination test results, a 1:10 titer was found in 4 samples. According to these results, the cross-reaction cut-off values were insignificant. The overall (animal), within-herd, and between-herd apparent seroprevalence values were calculated as 40.4% (95% CI = 31.6 to

Table 1. Sample distribution among the location and the results of the MAT survey for *F. tularensis*

Location	Number of samples	Number of seropositive samples	Apparent prevalence		True prevalence	
			Estimate, %	95% CI	Estimate, %	95% CI
Location 1	1	-	-	-	-	-
Location 2	1	1	100	20.7-100	100.7	-88-108
Location 3	1	-	-	-	-	-
Location 4	2	2	100	34.2-100	198	66.5-198
Location 5	2	2	100	34.2-100	198	66.5-198
Location 6	3	2	66.7	20.8-93.9	131.3	39.5-194.6
Location 7	7	1	14.3	2.6-51.3	26.6	-0.5-100.6
Location 8	10	2	20	5.7-51	38	9.3-100
Location 9	15	12	80	54.8-93	158	107.6-184
Location 10	25	12	48	30-67	94	58.1-131
Location 11	42	10	23.8	13.5-38.5	45.6	25-75.1
Total	109	44	40.4	31.6-49.8	78.7	61.3-97.5

49.8%), 41.1% (95 CI = 32.3 to 50.6%) and 81.8% (95% CI = 52.3 to 94.9%), respectively (Table 1).

True seroprevalence values were estimated by conversion from the apparent seroprevalence values using the Rogan-Gladen estimator. The true overall (animal), within-herd, and between-herd seroprevalence values were calculated as 78.7% (95% CI = 61.3 to 97.5%), 80.2% (95 CI = 62.5 to 99.2%) and 161.6% (95 CI = 103 to 187.7%), respectively (Table 2).

Table 2. Prevalence estimates of *F. tularensis* among animals, within-herds and between-herds

Prevalence type	Number tested	Number positive for F. tularensis	Apparent prevalence		True prevalence	
			Estimate, %	95% CI	Estimate, %	95% CI
Overall (Animal)	109	44	40.4	31.6-49.8	78.7	61.3-97.5
Within-herd	107	44	41.1	32.3-50.6	80.2	62.5-99.2
Between-herd	11	9	81.8	52.3-94.9	161.6	103-187.7

DISCUSSION

Tularemia is a zoonotic disease caused by F. tularensis, which is found worldwide, including in Turkey. It is also a potentially significant biological weapon. Amongst domestic animals, sheep are the primary host; however, tularemia has also been reported in dogs, cats, pigs, and horses (Otlu, 2009; Gese, 1997; Mörner, 1983). Some animals are highly susceptible to tularemia and, if infected, usually die before F. tularensis antibodies have even formed. However, antibodies are detectable in species such as cattle, sheep, dogs, pigs and horses by agglutination (MAT and tube agglutination tests) techniques (OIE, 2009; Bevanger, 1988; Arata,1973, Celebi, 2013). There are very few studies reporting clinical and pathological presentations of tularemia in horses infected experimentally or naturally. Generally, natural infection follows a heavy tick infestation and courses fever, dyspnea, incoordination, depression, and sudden death in horses (Tokgöz, 1938; Jellison, 1958; Claus, 1959; Cino, 2021). At autopsy, swelling and numerous necrotic foci in the lung, the liver and the spleen and diffuse necrosis in the intestinal lymph nodes are observed (Jellison, 1958; Cino, 2021). The existence of Tularemia in horses indicated by these clinical and pathological symptoms was made absolute by both the agent isolation and PCR or immunohistochemical methods (Jellison, 1958; Claus,1959; Cino, 2021).

Studies about the seroprevalence of tularemia in horses are very limited (Celebi, 2013; Tokgöz, 1938; Jellison, 1958, Stark, 1979). Horses, which are relatively more resistant to infection than other livestock, can develop a detectable antibody response to F. tularensis and can be detected for diagnostic purposes over a period. As a matter of fact, antibody titers decrease over time in horses, as in sheep cases, and turn negative within months (Jellison, 1958). The antibody titers have been reported in horses exposed to the agent or surviving between 1:10 and 1:640 (Celebi, 2013; Tokgöz, 1938; Jellison, 1958). A comprehensive study of the presence of F. tularensis antibodies in livestock, including horses, was conducted by Celebi et al. (2013) in the Kars Region of Turkey and a 50% (15/30 horses) seropositivity was obtained with antibody titer ranging from 1:10 to 1:40. In a study conducted in the USA, the presence of the causative agent in wild horses was demonstrated serologically with a rate of less than 11% (Stark, 1979). In the present study, the overall apparent and true seroprevalence was 40.4% and 78.7%, respectively. Although the cut-off value was taken as 1:20, the seropositivity rate was quite close to the rate reported by Celebi et al. (2013). However, the prevalence differences between the farm horses and the wild horses may have emerged from the geographical differences and the species and population intensity of ticks in these habitats. Considering the highly contagious nature of tularemia in both humans and animals, the different positivity rates of within-herd and between-herds, which are higher than the individual prevalence, are remarkable in this respect (Table 2).

Although serological methods, especially MAT, are widely used in the diagnosis of infections caused by such bacterial agents, which are difficult to culture, they have some disadvantages in terms of both cross-reaction possibilities with bacteria of close antigenic structure similarities and low diagnostic capabilities (OIE, 2018). Therefore, the corrected prevalence values calculated with the Rogan-Gladen estimator provide realistic diagnostic values that will enable us to accurately estimate the prevalence of the causative agent by eliminating such handicaps of the MAT. By its corrected version, the prevalence estimates of F. tularensis were detected at higher rates, revealing the common carriage of the causative agent in horses and their potential roles in possible transmissions.

CONCLUSION

As a result, a large-scale study representing the whole of Turkey was conducted for the first time in horses and F. tularensis seropositivity was found to be high. More comprehensive studies are needed to fully establish the degree of roles of horses in the eco-epidemiology of the disease.

DECLARATIONS

Ethics Approval

This study was approved by the local ethical committee (Protocol no: 2021/17 Date: 05.11.2021 VKMAE). Conflict of Interest

Authors declare that there are no conflicts of interest for this study.

Consent for Publication

Not applicable

Author Contribution

In all sections of the final article, each author contributed equally.

Data Availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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