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Research article

Serological investigation of tularemia and brucellosis in Tazi sighthounds in Şanlıurfa, Türkiye

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Abstract

Tularemia and brucellosis are zoonoses of One Health importance. However, exposure to their agents has not been investigated in the Tazi, the native Turkish sighthound traditionally used for hunting. This study investigated antibodies against *Francisella tularensis* and *Brucella* spp. in 53 hunting Tazi from Şanlıurfa, southeastern Türkiye. Samples were collected between 2023 and 2025 during routine clinical examinations or evaluations of various health problems. Sera were screened for anti-*F. tularensis* antibodies using an in-house enzyme-linked immunosorbent assay (ELISA) and the microagglutination test (MAT). Anti-*Brucella* antibodies were tested using in-house ELISAs based on smooth (*B. abortus*/*B. melitensis*) and rough (*B. canis*) antigens, together with the Rose Bengal plate test (RBPT). Five dogs (9.4%) were seropositive for tularemia by ELISA, and all five were confirmed by MAT. For brucellosis, five dogs (9.4%) were seropositive by rough-antigen ELISA, consistent with exposure to *B. canis*. Three dogs (5.7%) were seropositive by smooth-antigen ELISA, of which one was also RBPT-positive. These findings are the first serological evidence of exposure to *F. tularensis* and *Brucella* spp. in Tazi sighthounds. Likely sources include contact with wild hares and other wildlife during hunting, contaminated water and environments, and livestock-derived material. Because these dogs live closely with their owners, they may act as sentinels for zoonotic risk to humans. Molecular and culture-based studies with larger samples are needed to identify the species involved and clarify the epidemiology of these infections in this population.

Keywords: Brucellosis, Serology, Tazi sighthounds, Tularemia, Türkiye, Zoonosis

Introduction

Tularemia and brucellosis are important zoonotic diseases that threaten animal and human health and pose significant economic and public health challenges. The causative agents of both diseases are considered potential biological agents because they can be transmitted via aerosols and have low infectious doses [1, 2]. Brucellosis was first reported in Türkiye in 1915 [3], and tularemia was identified during an outbreak in 1936 [4]. Since then, both infections have been reported in humans and in various animal species across different regions of Türkiye [5, 6, 7, 8, 9].

Tularemia is an infection caused by *Francisella* (*F.*) *tularensis* and occurs in many countries in the Northern Hemisphere [1]. The subspecies responsible for infection in Türkiye is *F. tularensis* subsp. holarctica. The majority of human tularemia outbreaks in Türkiye are waterborne, and *F. tularensis* DNA has been detected by the Polymerase Chain Reaction (PCR) in drinking water sources within outbreak areas [1, 8]. Transmission among animals may occur when other animals consume carcasses of animals infected with *F. tularensis*. In addition, infected animal carcasses may contaminate water sources, contributing to environmental contamination and waterborne tularemia outbreaks [10].

Lagomorphs are among the major animal groups affected by tularemia and play a key role in transmitting the agent to other animals and humans. Multiple mammalian species, including voles, mice, gray shrews, sheep, dogs, foxes, and wolves, are involved in *F. tularensis* epidemiology [11, 12]. Arthropod vectors such as ticks and mosquitoes may also contribute to transmission [10, 12]. Tularemia in domestic animals may present with nonspecific clinical signs or remain asymptomatic [1]. Nevertheless, particularly in dogs, an antibody response may develop after infection, and clinical disease may also occur [11, 13].

Brucellosis is caused by several *Brucella* (*B.*) species [2], and infections in Türkiye have been reported in many mammal species, including livestock, wild mammals, and aquatic species [3]. The *Brucella* species primarily associated with canine brucellosis is *B. canis*, which possesses rough lipopolysaccharide (LPS). However, canids can also be infected with species possessing smooth LPS, such as *B. melitensis, B. suis,* and *B. abortus* [14, 15, 16]. As in other mammalian species, clinical signs associated with *Brucella* infection in dogs are not pathognomonic. Clinical presentations may range from asymptomatic infection to abortion, epididymitis, prostatitis, and orchitis [17]. The M-strain antigen used in the serological diagnosis of *B. canis* minimizes cross-reactions with other Gram-negative and Gram-positive bacteria, thereby improving the diagnostic accuracy of the test [17]. Tests prepared using the M-strain antigen have

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been reported to exhibit 100% sensitivity and 98.8% specificity [18].

Tazis are traditional hunting sighthounds raised in various regions of Türkiye, particularly in Şanlıurfa and Konya, and they pursue prey primarily by sight and speed [19]. Despite their traditional use in hunting, information on infectious and zoonotic diseases in this canine population is limited. During hunting, dogs may be exposed to *Brucella* spp. through contact with infected wildlife, raw animal tissues, carcasses, or reproductive materials [20, 21]. These activities may also increase contact with wildlife and arthropod vectors, which is particularly relevant to tularemia, as *F. tularensis* circulates among wildlife and seroepidemiological studies have previously reported exposure in hunting dogs [10, 11, 13, 22]. Therefore, Tazi's hunting lifestyle, together with the scarcity of epidemiological data on zoonotic infections in this population, provides a rationale for investigating serological exposure to both rough and smooth *Brucella* antigens and to *F. tularensis*.

This study aimed to investigate antibodies against *F. tularensis* in Tazi sighthounds using enzyme-linked immunosorbent assay (ELISA) and microagglutination test (MAT), and antibodies against *Brucella* spp. using the Rose Bengal plate test (RBPT), and *Brucella* ELISA with smooth and rough *Brucella antigens*.

Materials and methods

Sampling

This retrospective study used archived serum samples from Tazi sighthounds presented to the Animal Hospital of Harran University's Faculty of Veterinary Medicine between 2023 and 2025 for routine clinical examinations or evaluations of various health problems. No additional blood samples were collected specifically for this study.

Serum samples were stored at −20 °C until analysis. All animals included in the study lived in Şanlıurfa Province, southeastern Türkiye, and ranged in age from 6 months to 11 years (Figure 1). Of the 53 Tazi sighthounds, 24 (45.3%) were female, and 29 (54.7%) were male. Positive and negative control sera and antigens used in the study were obtained from previously prepared and stored stocks at the Microbiology Laboratory of the Harran University Faculty of Veterinary Medicine.



Figure 1: Location of Şanlıurfa Province, southeastern Türkiye, and a representative image of the Tazi sighthounds included in the study. The map was originally created using QGIS, and the photograph of the Tazi sighthounds was taken by the authors.

Serodiagnosis of brucellosis

Because canids may be exposed to both smooth (e.g., *B. melitensis, B. abortus,* and *B. suis*) and rough (e.g., *B. canis*) *Brucella* species [2], serological testing was performed using both smooth and rough LPS antigens. ELISA was used for both antigen types, and RBPT was used as an additional agglutination test for smooth *Brucella* ELISA-positive samples.

For the ELISA analyses, native smooth LPS from *B. abortus* S99 was used to detect antibodies against smooth *Brucella*, whereas native rough LPS from *B. canis* M(−) was used to detect antibodies against rough *Brucella*. The antigens were prepared according to the methods recommended by the World Organization for Animal Health (WOAH). The antigens were diluted to appropriate concentrations in coating buffer, dispensed into wells of flat-bottom polystyrene microplates at a volume of 100 µL per well, and incubated overnight at 4 °C. Following incubation, the plates were washed once using an automatic plate washer with phosphate-buffered saline (PBS) containing 0.5% Tween 20 (PBST) and then dried. The plates were subsequently blocked for 2 h with PBS containing 5% non-fat dry milk and 0.5% Tween 20. After washing, test and reference sera diluted 1:100 in PBST were added at 100 µL per well, and test sera were assayed in duplicate. Three positive and three negative control sera, as well as two blank wells, were included in the assay. The plates were incubated for 30 min at room temperature on a shaker at 160 rpm. Following incubation, the plates were washed four times with PBS. Horseradish peroxidase (HRP)-conjugated recombinant Protein A/G (Pierce 32490) was added as the secondary antibody at 100 µL per well and incubated for 30 min. After washing and drying, 100 µL of substrate solution containing 0.015% H_2O_2 and 0.5 mg/mL ABTS [2,2′-azinobis (3-ethylbenzothiazoline-6-sulfonic acid)] prepared in pH 4.0 buffer was added to each well. After approximately 15 min of incubation, the reaction was stopped by adding 100 µL of 1 mM sodium azide to each well. Optical density (OD) values were measured at 405 nm using an ELISA plate reader. The ELISA cut-off value was calculated as the mean OD value of the negative control sera plus three standard deviations (SD), and OD values above the cut-off were considered positive.

Serum samples positive by smooth ELISA were further analyzed using the RBPT in accordance with the WOAH guidelines. For the assay, 30 µL of serum was mixed with an equal volume of Rose Bengal antigen (SOY-MedVet, Şanlıurfa, Türkiye) and evaluated for agglutination.

Serodiagnosis of tularemia

To detect antibodies against *F. tularensis*, we used a previously developed in-house ELISA protocol [23]. LPS antigen from the *F. tularensis* live vaccine strain (LVS), obtained by the Tri-Reagent method, served as the solid-phase antigen. The antigen was coated onto 96-well microplates in carbonate-bicarbonate buffer (pH 9.6), and the plates were incubated overnight at 4 °C. Serum samples were diluted 1:100 and added to the wells. HRP-conjugated recombinant Protein A/G was used to detect bound antibodies. Color development was achieved using o-phenylenediamine (OPD) as the substrate, and absorbance was measured at 490 nm. The ELISA cut-off value was calculated as the mean optical density (OD) of the negative sera plus three standard deviations (SD), and OD values above the cut-off were considered positive.

ELISA was used to initially detect antibodies against *F. tularensis*. Serum samples that tested positive for tularemia by ELISA were further analyzed by the microagglutination test (MAT) to assess agglutinating antibodies and determine antibody titers. Using both tests provided complementary serological information on exposure to *F. tularensis*. Serum samples were serially two-fold diluted in physiological saline in U-bottom microplates. An equal volume of tularemia agglutination test antigen suspension (SOY-MedVet, Şanlıurfa, Türkiye) was added to each well and mixed by pipetting. The plates were incubated overnight at 37 °C and subsequently evaluated for agglutination.

Statistical analysis

Statistical analyses were performed using SPSS Statistics v22.0. Descriptive data were expressed as numbers (n) and percentages (%). The associations between sex and seropositivity for *Brucella* spp. and *F. tularensis* were evaluated using Fisher's exact test. A p-value of < 0.05 was considered statistically significant.

Results and discussion

Serological screening of Tazi dogs (n = 53) revealed that three samples (5.7%) were positive by smooth *Brucella* ELISA, of which only one also agglutinated in the RBPT. Seven samples (13.2%) were positive by rough *Brucella* ELISA; however, two of these were also reactive by smooth *Brucella* ELISA. Because antibodies elicited by smooth *Brucella* species can cross-react with rough antigens, these two dual-reactive samples were excluded from the rough *Brucella* seropositive category. Accordingly, five samples (9.4%) that reacted exclusively by rough *Brucella* ELISA were classified as rough *Brucella* seropositive.

On the other hand, five serum samples (9.4%) were positive for anti-*F. tularensis* antibodies by ELISA, and all five also agglutinated in the MAT, with titers of 1:5 (n = 2), 1:20 (n = 1), and 1:40 (n = 2). Two of these tularemia-seropositive samples were also reactive by rough *Brucella* ELISA. By contrast, none of the samples positive by smooth *Brucella* ELISA were tularemia-seropositive.

Smooth *Brucella* ELISA results were positive in 8.3% (2/24) of female dogs and 3.4% (1/29) of male dogs.

Rough-only *Brucella* seropositivity was 8.3% (2/24) among females and 10.3% (3/29) among males; Table 1 shows the initial rough Brucella ELISA results (3/24, 12.5%, and 4/29, 13.8%, respectively), before excluding the two dual-reactive samples. Tularemia ELISA positivity was 4.2% (1/24) in females and 13.8% (4/29) in males (Table 1). Sex was not significantly associated with positivity on smooth or rough *Brucella* ELISA, or with *F. tularensis* ELISA ($p > 0.05$).

Table 1: Distribution of brucellosis and tularemia seropositivity according to sex.

			***Brucella*_sELISA**		**Total**	***Brucella*_rELISA**		**Total**	**Tularemia_ELISA**		**Total**
			Negative	Positive		Negative	Positive		Negative	Positive	
Sex	Female	Number	22	2	24	21	3	24	23	1	24
		Gender	91.7%	8.3%	100%	87.5%	12.5%	100%	95.8%	4.2%	100%
	Male	Number	28	1	29	25	4	29	25	4	29
		Gender	96.6%	3.4%	100%	86.2%	13.8%	100%	86.2%	13.8%	100%
Total		Number	50	3	53	46	7	53	48	5	53
		Gender	94.3%	5.7%	100%	86.8%	13.2%	100%	90.6%	9.4%	100%

Rough *Brucella* ELISA (rELISA) results represent the initial seven positive samples, including two samples also positive by smooth *Brucella* ELISA (sELISA) that were excluded from the final rough-only seropositivity classification.

Previous studies have documented brucellosis in humans and livestock in Şanlıurfa Province through serological testing and bacterial isolation [7, 24]. According to the Turkish Ministry of Health, Şanlıurfa Province had one of the lowest reported incidence rates of human tularemia (0.09 per 100,000 population) during 2005–2019 [8]. Although serological studies of *Brucella* spp. and *F. tularensis* have been conducted in dogs from different regions of Türkiye [5, 20, 25, 26], data specifically addressing these infections in Tazi sighthounds remain limited.

Francisella spp. and *Brucella* spp., which cause significant health problems in animals and humans, are classified as hazardous pathogens. Serological tests play an important role in diagnosis because isolating and culturing these agents poses biosafety risks. According to WOAH, ELISA is among the recommended serological methods for determining the prevalence of tularemia and brucellosis and conducting surveillance studies [1, 2]. In this study, serum samples from 53 Tazi sighthounds were tested by ELISA for antibodies against both diseases. Positive samples were then confirmed using agglutination tests.

Among the 53 Tazi sighthound serum samples tested for tularemia, 5 (9.4%) were positive by ELISA. All five also showed agglutination at varying titers by MAT. Additionally, ELISA detected rough *Brucella* in five samples (9.4%) and smooth *Brucella* in three (5.7%). Cross-reactions in tularemia agglutination tests have been reported in the presence of antibodies against smooth *Brucella* species and *Legionella* spp. [1].

In a study conducted in Türkiye using only MAT in shepherd and pet dogs, titers of 1:80 or higher were considered diagnostic, and the overall seropositivity was reported as 4.1% [5]. Tularemia is a zoonotic infection that occurs naturally in lagomorphs such as rabbits and hares [1]. The use of Tazi sighthounds for hunting these animals in Türkiye [19], together with serological evidence of *F. tularensis* infection in hares [27], suggests that Tazi sighthounds may be more likely to be exposed to *F. tularensis* through contact with infected prey than dogs not used for hunting. Indeed, the detection of antibodies against *F. tularensis* in hunting dogs during a tularemia outbreak associated with a hare hunt in Germany supports the possibility that hunting dogs may be exposed to the agent through contact with infected lagomorphs [22]. The 9.4% seropositivity for tularemia observed in the present study may be associated with the hunting lifestyle of Tazi sighthounds, which could increase their exposure to infected wildlife, vectors, and contaminated environmental sources.

Serological tests are commonly used to investigate *Brucella* infections in dogs [20, 28], and antibody responses against both rough and smooth *Brucella* species can be detected serologically [16, 26]. In the present study, three (5.7%) serum samples were positive by smooth *Brucella* ELISA, whereas agglutination by RBPT was observed in only one of these samples. Seven (13.2%) samples were positive by rough *Brucella* ELISA. Accordingly, two samples positive by rough ELISA were also positive by smooth ELISA and were therefore evaluated for possible cross-reactivity and excluded from the classification of rough *Brucella* seropositivity. Although the LPS structures of smooth and rough *Brucella* species differ, shared antigenic epitopes in the core oligosaccharide region present in both structures have been reported to cause serological cross-reactivity [20]. Thus, RBPT positivity and/or smooth ELISA positivity were considered smooth *Brucella* seropositivity, whereas the five samples positive only by rough ELISA were considered rough *Brucella* seropositive.

Different rates of *B. canis* seropositivity have been reported in dogs. Seropositivity was reported at 17.3% in shelter dogs in Italy [28], while ELISA-based seropositivity rates of 7.45% and 2.7% were reported in shelter dogs from İstanbul and İzmir [29] and in dogs from Kayseri [30], respectively. In a study of shelter dogs in Van Province, ELISA determined rough and smooth *Brucella* seropositivity rates were 5.3% and 18%, respectively [26]. In the present study, rough and smooth *Brucella* seropositivity rates were 9.4% and 5.7%, respectively. These findings indicate that seropositivity rates vary considerably depending on the population examined and the geographical region. In a previous study conducted in Şanlıurfa, *B. canis* seropositivity of 11.5% was reported by ELISA in dogs suspected of genital tract infections [25]. In the present study, the detection of 9.4% rough *Brucella* seropositivity in Tazis used for hunting in the same region demonstrates serological reactivity against rough *Brucella* antigens in a different canine population.

A key limitation of this retrospective study is the absence of bacteriological and molecular confirmation. Although culture and PCR are valuable diagnostic methods, collecting additional samples from Tazi sighthounds is challenging because of their high value to owners. Therefore, the findings should be interpreted as serological evidence of possible exposure rather than as evidence of confirmed infection. Published data on brucellosis and tularemia in Tazi sighthounds, particularly outside Türkiye, remain limited.

Conclusion

This study provides serological evidence of exposure to *F. tularensis* and *Brucella* spp. among Tazi sighthounds used for hunting in Şanlıurfa, Türkiye. Notably, two serum samples showed concurrent seropositivity to *F. tularensis* and rough *Brucella* antigens, suggesting possible exposure to multiple zoonotic agents. These findings highlight the potential epidemiological importance of hunting dogs in surveillance for tularemia and brucellosis.

Declarations and supplementary information

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Author contributions: Conceptualization, SÖ; Methodology, SÖ; Investigation, SÖ, MSK, and SEG; Resources, SÖ, MSK, SEG, and OYT; Data curation, SÖ; Formal analysis, SÖ; Writing—original draft, SÖ; Writing—review and editing, SÖ, MSK, and SEG; Project administration, SÖ and OYT; Supervision, SÖ. All authors have read and agreed to the published version of the manuscript.

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Ethics statement: The use of these previously collected and archived samples for the present study was evaluated by the Harran University Local Ethics Committee for Animal Experiments (HRÜ-HADYEK), which determined that specific ethical approval was not required (session no. 2026/006, decision no. 01-31, July 9, 2026).

Data availability: The data supporting the findings of this study are available from the corresponding author upon reasonable request.

Supplementary materials: Not applicable.

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