Research Article

The Serological Study on Brucellosis of the Bulls and Comparison of the used Tests in the Northeast Anatolia Region of Turkey

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Abstract

The goal of this study was to use Rose Bengal Plate Test (RBPT), Serum Agglutination Test (SAT) and Complement Fixation Test (CFT) to determine the prevalence of brucellosis in bulls raised in Turkey's Northeast Anatolian Region (the provinces of Kars, Ardahan and Iğdır), where bovine brucellosis is endemic and compare these tests. The study material consisted of blood samples from bulls raised on cattle farms operated by families using extensive farming methods in the provinces of Kars, Ardahan and Iğdır, where animals exhibited no clinical symptoms of disease. This is the first study to use sampling to determine the prevalence of brucellosis in bulls. Of 227 bulls that were evaluated in the study, 21 (9.25%) were found positive for RBPT, 19 (8.37%) were positive for SAT and 20 (8.81%) were positive for CFT. In animals, venereal transmission that is thought to play the least role in transmission of disease considering founction of nonspecific barrier of vagina to infection is an issue that should be taken into consideration besides ingestion of infected tissues or body fluids, contact with mucous membranes, direct inoculation, and fomites that are the most common ways in transmission of bovine brucellosis.

Keywords: Brucellosis, Bull, Serological test, RBPT, SAT, CFT

Türkiye'nin Kuzeydoğu Anadolu Bölgesi'nde Boğaların Brusellozisi Üzerine Serolojik Araştırma ve Kullanılan Testlerin Karşılaştırılması

Özet

Bu çalışmada ineklerde brusellozisin endemik olduğu Kuzeydoğu Anadolu Bölgesinde (Kars, Ardahan, Iğdır illeri) yetiştirilen boğalarda brusellozis prevalansının Rose Bengal Plate Test (RBPT), Serum Agglutination Test (SAT) ve Complement Fixation Test (CFT) ile belirlenmesi ve bu testlerin karşılaştırılması amaçlanmıştır. Çalışmanın materyalini Kars, Ardahan ve Iğdır illerinde ailesel düzeyde ve ekstansif olarak yetiştirilen sığır işletmelerinde bulunan hastalığa ilişkin herhangi bir klinik belirti göstermeyen boğaların kan örnekleri oluşturdu. Örneklem alanında boğalarda brusellozis prevalansının belirlenmesine yönelik ilk olan bu araştırmada değerlendirilen 227 boğaya ait kan serumlarının 21'i (%9.25) RBPT, 19'u (%8.37) SAT ve 20'si (%8.81) CFT ile pozitif olarak saptanmıştır. Hayvanlarda, sığır brusellozisinin bulaşmasında en çok karşılaşılan doku ve vücut sıvılarının sindirimi, mukoz membranlarla temas, direkt inokülasyon ve fomitlerin yanı sıra vajinanın infeksiyonlara nonspesifik bariyer oluşturması göz önünde bulundurularak hastalığın aktarımında daha az rol oynadığı düşünülen veneral bulaşma da dikkate alınması gereken bir husustur.

Anahtar sözcükler: Brusellozis, Boğa, Serolojik test, RBPT, SAT, CFT

INTRODUCTION

Brucellosis is a disease, caused by *Brucella* species, that is characterized by abortions, infertility, joint inflammation and mastitis in cows and epididymitis, orchitis and seminal vesiculitis in bulls [1,2]. The disease is transmitted between animals via the digestive system, mating and direct contact with skin and conjunctiva, while it can spread to humans by direct contact with infected animals or the consumption of contaminated milk and milk products. For this reason, it is considered to be the most widespread zoonosis in the world. The disease is endemic in cattle populations in the Central Anatolia, Eastern Anatolia and Southeastern Anatolia regions of Turkey, which is

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geographically situated in a high risk area between Europe and the Middle East [3,4].

Although conclusive diagnosis of brucellosis requires isolation and identification of the causative agent, it is not practical for field and laboratory personnel to identify infected animals with cultural examinations when there are a large number of animals. Furthermore, these bacteria are delicate and grow slowly, and since isolating them requires a long incubation period, specific growth environments and subcultures, it may not always be possible to isolate the agent from the infected animal for a number of reasons [5]. Consequently, the control and eradication of brucellosis has been dependent on identifying reactors with serologic tests. Tests used for serological diagnosis of brucellosis include the Rose Bengal Plate Test (RBPT), Serum Agglutination Test (SAT), Rivanol Agglutination Test (RAT), Complement Fixation Test (CFT), Agar Gel Immunodiffusion (AGID) and Enzyme Linked Immunosorbent Assay (ELISA) [6-11]. Serologic tests are preferable in the diagnosis of brucellosis because it is easy to collect the samples (from blood, blood serum, milk serum, vaginal flow, seminal plasma, etc.) and the results are obtained quickly and produce high specificity and sensitivity. However, the recommendation has been made to evaluate the results obtained from at least two tests when attempting serological diagnosis of brucellosis. There are a number of reasons for this, including the fact that each test identifies different immunoglobulins, false positives can be observed, chronically and actively infected animals can give inconsistent responses to serologic tests, and immunization can be confused with a natural infection [7-12]. Although RBPT is a good screening test, it is not sensitive enough to distinguish between individual cases and immunized animals. SAT is cheap and easy to administer, but does not have enough specificity. CFT is very sensitive and specific for diagnosing the disease [7,9].
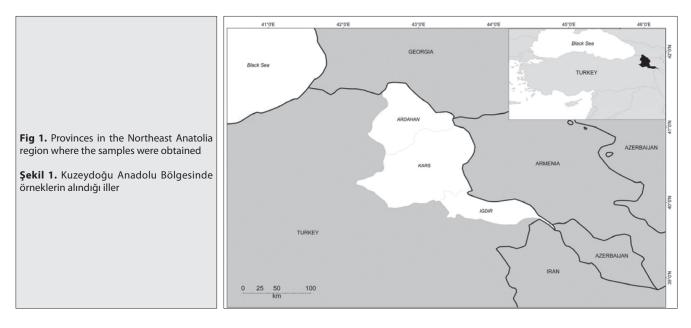
It is very important for the necessary steps to be taken to control and eradicate brucellosis in the regions where it is endemic because the disease poses a threat to human and animal health [4,13].

In the Northeastern Anatolia region of Turkey, cattle farming is largely done on family farms and with extensive methods. Artificial insemination is rarely used to produce calves and instead bulls from the herd or from other herds are used in this region. The goal of this study was to use RBPT, SAT and CFT tests to determine the prevalence of brucellosis in bulls raised in Turkey's Northeast Anatolian Region (the provinces of Kars, Ardahan and Iğdır), where bovine brucellosis is endemic and compare these tests.

MATERIAL and METHODS

Serum Samples

The study material consisted of blood samples from bulls raised on cattle farms operated by families using extensive farming methods in the provinces of Kars, Ardahan and Iğdır, where the mentioned animals exhibited no clinical symptoms of disease. The samples were collected from January 2010 to March 2015 and brought by the cattle farmers to the Microbiology Department of the Veterinary Faculty of Kafkas University to be checked for brucellosis. The sera from the blood samples was extracted and stored at -20°C until they were obtained subjected to serologic testing. The samples were taken from 227 bulls in 87 farms (98 from Kars, 74 from Ardahan and 55 from Iğdır), where the bulls ranged from 1-9 years old. According to the information that was gathered, cases of abortion were occurring on 23 of the farms, while no abortion was reported on the other farms. The provinces from which the samples were obtained have been shown on the map in *Fig. 1*.

Fig 1. Provinces in the Northeast Anatolia region where the samples were obtained

Şekil 1. Kuzeydoğu Anadolu Bölgesinde örneklerin alındığı iller

Serological Tests

All serum samples were evaluated with the RBPT, SAT and CFT. In order to prevent incorrect test readings, positive and negative control serums were used that had been procured from Institut Pourquier-Montpellier (France). Antigens used for RBPT and SAT were provided by the Istanbul Pendik Veterinary Control Institute, while a commercial kit (Virion/Serion CFT Reagents, Germany) was used for CFT.

RBPT was conducted according to the procedure described by Alton et al.[12]. Equal volumes (30 µl) of serum and antigen were mixed on a clean plate and gently agitated. If agglutination occurred within 4 min, the test was considered to be positive.

For SAT, sera were double-diluted from 1/5 to 1/320 in sterile physiological salt solution. An equal amount of antigen was added to all tubes, which were shaken hard and then incubated at 37℃ for 18-24 h. Agglutination that developed was evaluated at the end of this period, and sera producing a titer of 1/40 or more were considered to be positive [12,14].

CFT was conducted according to the procedure specified in a kit that was commercially obtained (Virion/Serion CFT Reagents, Germany). After the serum samples were diluted with veronal buffer, they were kept in a 62℃ water bath for 30 min in order to prevent anticomplementary activity that might occur in the serum. Samples that titrated at 1/5 ++ or higher (100% inhibition of hemolysis) after these procedures were considered to be positive. In order to eliminate this characteristic of serum samples were exhibit anticomplementary activity, a 5% solution of Bovine Serum Albumin (BSA) fraction V was prepared in VB [12]. The serum dilution used in the test was mixed with the aforementioned solution (at the ratio of 0.2 ml serum + 0.6 ml solution) and incubated for 30-60 min at 37℃. The sera were then inactivated for 30 min at 62℃ and the subsequent steps were followed exactly, after which they were evaluated.

RESULTS

Of 227 bulls that were evaluated in the study, 21 (9.25%) were found positive for RBPT, 19 (8.37%) were positive for SAT and 20 (8.81%) were positive for CFT. One serum sample found positive for RBPT and SAT was negative for CFT, while two samples found positive for RBPT and CFT were negative for SAT. The location in which the samples were taken, the number of samples and the results of the serologic tests have been shown in Table 1. Seventeen of the samples that were positive were from bulls on farms where abortions had been reported, and 16 of these 17 positive samples were positive for all three tests, while the remaining sample was positive for RBPT and CFT but negative for SAT.

DISCUSSION

Although Brucellosis has been eradicated in many developed countries in Europe as well as in countries like Australia, Canada, Israel, Japan and New Zealand, it is still one of the most significant problems in the world for animal farming and public health in Africa, Asia, Latin America, the Middle East and Mediterranean countries including Turkey [4,7]. The primary reasons that brucellosis is widespread and endemic in Turkey and in the Northeast Anatolia region in particular include the following: It is difficult to control the entry and exit of animals from the country because of its geographic location, there is a high volume of animal movement because they are on family-operated farms that use extensive farming methods, immunization programs are not fully implemented, the compensation paid for diseased animals is not sufficient or paid regularly, and calves are largely obtained by natural breeding.

Although many studies have been conducted that attempt to identify the prevalence of brucellosis in cattle in other countries, very few studies have been carried out with bulls. In a study conducted by Plant [15] that reported observations about two bulls infected with brucella, it was reported that although bull number 1 was found serologically positive for SAT and CFT from day 0 until day 141, the causative agent could not be isolated from its semen and the bull appeared clinically normal, while bull number 2 produced varying serologic results over a period of 203 days with the same tests and was found

Table 1. The location in which the samples were taken, the number of samples and the results of the serologic tests
Tablo 1. Serum örneklerinin alındığı yerler, serum örneği sayısı ve serolojik test sonuçları

Provinces	n	Serological Tests											
		RBPT				SAT				CFT			
		Positive	%	Negative	%	Positive	%	Negative	%	Positive	%	Negative	%
Kars	98	10	10.2	88	89.8	9	9.2	89	90.8	9	9.2	89	90.8
Ardahan	74	8	10.8	66	89.2	7	9.4	67	90.6	8	10.8	66	89.2
Iğdır	55	3	5.4	52	94.6	3	5.4	52	94.6	3	54.4	52	94.6
Toplam	227	21	9.3	206	90.7	19	8.4	208	91.6	20	8.8	207	91.2

negative for 74 days after that period. The researchers reported that orchitis followed by epididymitis developed in bull number 2 on day 122 and although no causative agent could be isolated from semen cultures conducted up until day 363, they isolated *B. abortus* biotype 1 from the seminal vesicle and ampulla obtained from an autopsy conducted on the animal. Based on the information they gathered, the researchers concluded that there are serious problems with diagnosing brucellosis in bulls, and that regardless of their serologic condition, all bulls in infected herds should be viewed with suspicion. Hill [16] conducted a histologic, bacteriologic and serologic investigation of 34 bulls and the serologic tests identified 17 of the 34 bulls as positive using CFT or the Indirect Hemolysis Test (IHLT). The author reported that *B. abortus* biotype 1 was isolated from various genital samples taken from 5 of the 17 bulls that were serologically positive. They reported that no causative agent could be isolated from genital samples of the remaining 12 bulls, and serologic testing of tissue fluids from these animals was also negative. Campos et al.[17] used the Huddleston and card tests to examine blood serum samples collected from 139 bulls on 60 farms. Two bulls were found positive with the Huddleston test, while the card test found all samples to be negative. The researchers reported that one of the bulls found positive was in a herd where abortions had been observed. Patel et al.[8] used RBPT and indirect-enzyme linked immunosorbent assay (i-ELISA) to investigate blood serum from 422 breeding buffalo bulls. They found 4 positive cases with RBPT and 12 positive cases with i-ELISA. The researchers found that seroprevalence can occur at different levels in studies that use various tests and that false positives and negatives can occur, so they recommend that i-ELISA be used together with other tests to identify brucellosis. Rhyan et al.[1] studied seven bison bulls that had been found serologically positive for brucellosis and isolated *B. abortus* biovar 1 from blood, lymph node, spleen and genital samples taken from six of the bulls. They reported that more studies need to be conducted to identify the role of the bulls in the spread of the causative agent.

In Turkey, brucellosis in cattle is endemic in the Northeastern Anatolian region, and many studies have discussed this situation. In a study conducted in the provinces of Kars and Ardahan, Genç et al.[18] employed Competitive Enzyme-Linked Immunosorbent Assay (C-ELISA), CFT, RBPT and SAT to evaluate the blood serum of 163 cattle that had not been immunized against brucellosis and had abortions, and found 68.1% (111), 65.6% (107), 58.9% (96) and 55.2% (90) of the animals tested positive for *B. abortus*, respectively. In a study carried out in the province of Kars, Sahin et al.[19] used RBPT, SAT and ELISA tests to investigate brucellosis in 626 blood serum samples collected between 2001 and 2006 from 27 herds with a history of abortion, finding positive results in 221 (35.30%), 206 (32.92%) and 247 (39.45%) of the samples. Otlu et al.[20] conducted a study from 2004 to 2006 in the province of Kars in which

they used RBPT and SAT to examine 407 serum samples they collected from 27 cattle herds with a history of abortions and found 134 (32.92%) and 141 (34.64%) positive results, respectively. However, there are very few studies that have investigated the disease in bulls. Ours is the first study carried out in the same region as the aforementioned studies but which investigates the prevalence of brucellosis in bulls. Of 227 bulls that were evaluated in the study, 21 (9.25%) were found positive for RBPT, 19 (8.37%) were positive for SAT and 20 (8.81%) were positive for CFT. Not only do the findings obtained in this study show that brucellosis is also quite widespread among bulls, it also suggests that this plays a significant role in the spread of the disease when we consider the high degree of prevalence in the cattle. Another notable finding is that the bulls found positive did not have any clinical symptom and that they were frequently part of herds that were experiencing abortions. These data are congruent with the aforementioned studies. However, the data supports the conclusions of reports indicating that a single test is not sufficient to effectively identify brucellosis [7,21]. Furthermore, when we consider the results of the serologic tests used in our study, the data supports studies which recommend using a simple serologic test like RBPT, which offers high sensitivity and rapid screening for identifying brucellosis in a herd, in addition to a verification test with high specificity such as CFT [7,9,22].

In conclusion, this is the first study to use sampling to determine the prevalence of brucellosis in bulls found in the Northeastern Anatolia region of Turkey. Of 227 bulls that were evaluated in the study, 21 (9.25%) were found positive for RBPT, 19 (8.37%) were positive for SAT and 20 (8.81%) were positive for CFT. The findings show that the prevalence of brucellosis in bulls is quite high. In the region in question, cattle farming is largely done on family farms and with extensive methods. For this reason, it is critical that periodic serologic tests be used on bulls employed for natural breeding, whether the bulls are from the same herd or brought in from another herd. When we consider that venereal transmission is one of the least significant horizontal transmission modes, it is clear that bulls must be monitored, identified and isolated if we are to control and eradicate this disease. We concluded that the cattle farmers themselves wanted to participate in the fight against brucellosis and that because of their interest, efforts to educate the farmers were effective. Bulls that tested positive were removed from breeding in a controlled fashion according to government regulations. This prevents venereal transmission of the disease by these bulls. The role of the bulls in *Brucella* transmission in cattle farms operated by families using extensive farming methods can be questionable and monitored, if we are to control and eradicate this disease.

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