



Investigation of the Prevalence of Brucella Diagnosis in Serum and Milk in Cattle

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10.18805/IJAR.B-1404

ABSTRACT

Background: Brucellosis is an infectious disease that affects both cattle and humans. Because brucellosis causes economic losses, seroalence studies are important in terms of eradication. Its diagnosis is integral to the design and implementation of preventive and management strategies for both cattle and humans. Various serological tests are commonly used in diagnostic processes.

Methods: This study was carried out in cattle farms located in Ankara, Çankırı, Kırşehir, Kayseri, Çorum, Yozgat, Kırıkkale, Nevşehir provinces in the Central Anatolia region of Turkey between January-2018 and December-2020. Bovines included in the study, simple random sampling method and 320 bovine blood serum and milk samples that were reported not to have Brucella vaccine were used. RBPT, SAT, CFT, I-ELISA tests were performed in the blood serum samples included in the study and I-Elisa, MRT tests were performed in the milk samples.

Result: The tests revealed that an average of 35.4% of the cattle had the bacteria while 64.5% of the raw milk samples were infected with brucellosis. The findings demonstrated the need to implement better strategies for managing and preventing brucellosis among animals, including vaccination. As a result, indirect ELISA (I-ELISA) method is considered as a useful, reliable, fast tool in the detection of Brucella from in milk and serum samples. However, it I-ELISA method can be used as a support for conventional tests in the initial isolation of waste materials and that its simultaneous use in field screening tests can be good diagnostic performance.

Key words: Brucellosis, Cattle, Milk, Serum, Seroprevalence.

INTRODUCTION

Brucellosis is an infectious and often subacute or chronic disease created by microorganisms belonging to the genus *Brucella*. *Brucella* bacteria are 0.6-1.5 µm in size, small, gram-negative, non-spore forming, aerobic or microaerophilic coccobacillus. It consists of 6 species: *B. melitensis*, *B. abortus*, *B. suis*, *B. canis*, *B. ovis* and *B. neotomae*. All species are catalase positive, but their oxidase and urease activities and H₂S formation are variable. The ideal growth temperature of *Brucella* bacteria is 37°C, but they can also grow at 20-40°C. They are sensitive to heat and disinfectants but resistant to penicillin (Alton, Forsyth, 1996). In domestic animals with economic value such as sheep, goats, cattle and pigs, settle in the genital organs such as testicles, breasts, uterus and infertility, resulting in large-scale economic losses. The disease is also known as fluctuating fever, Mediterranean fever and Maltese fever. It is also of great importance in terms of public health because of the contamination of infected animals with milk and dairy products, meat and meat products. One of the public health issues affecting the dairy cattle industry is the prevalence of brucellosis among the cows used to harvest milk especially in low-income regions and developing nations (Facciola *et al.*, 2018; Arenas-Gamboa *et al.*, 2021).

Brucella is a disease that causes offspring to be discarded in domestic animals such as cattle, sheep, goats and pigs and is also transmitted from animals to humans, economically damaging and important from a public health point of view.

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How to cite this article: Ülker, U. (2021). Investigation of the Prevalence of Brucella Diagnosis in Serum and Milk in Cattle. Indian Journal of Animal Research. DOI: 10.18805/IJAR.B-1404.

Submitted: 23-06-2021 **Accepted:** 23-09-2021 **Online:** 01-01-2022

In animals, this disease leads to serious losses both from the point of view of the breeder and the country's economy, such as offspring throwing, reduced milk yield, loss of breeding value, infertility (Khan and Zahoor, 2018). Controlling the condition still remains a challenge in many regions especially where effective vaccines are absent (Deka *et al.*, 2018). Bosilkovski (2019) notes how humans contract the condition after consuming animal fluids or food products. As such, detecting the *Brucella* bacteria in animal fluids can be an effective strategy for preventing its incidence among humans. Since *B. abortus* is the predominant agent, most diagnostic tests focus on its detection (Acharya *et al.*, 2016). In the prevention and control strategy of brucellosis, vaccination practices, control of animal movements and migrations, compliance with hygiene rules and culling of infected animals, including applications for diagnostic studies with screening tests in animals in the first place (Alton and Forsyth, 1996). Therefore, applications for performing

diagnostic studies with screening tests are of critical importance.

In the present study, various tests for *B. abortus* on the blood and milk samples obtained from cattle in central Anatolia region of Turkey were performed to ascertain the prevalence of the condition in the country.

MATERIALS AND METHODS

Before starting the current study, ethics committee approval was obtained from the Ethics Committee of the Etlik Veterinary Control Research Institute Directorate (Decision date and number: 01.01.2018/4). The research was carried out in line with ethical principles and rules, protecting animal welfare and rights. This study was carried out in cattle farms located in Ankara, Çankırı, Kırşehir, Kayseri, Çorum, Yozgat, Kırıkkale, Nevşehir provinces in the Central Anatolia region of Turkey between January-2018 and December-2020. Bovines included in the study, simple random sampling method and 320 bovine blood serum and milk samples that were reported not to have Brucella vaccine were used. RBPT, SAT, CFT, I-ELISA tests were performed in the blood serum samples included in the study and I-Elisa, MRT tests were performed in the milk samples (Fig 1).

Rose Bengal Plate Test (RBPT): Pendik Veterinary Procured from the Control Institute (PVKE), RBPT was performed using RBPT antigen Brucella Serum Agglutination Test (SAT): Tube agglutination test antigen prepared with *B. abortus* S99 strain obtained from PVKE was used. Brucella agglutination degree in serum was evaluated as IU per milliliter. 30 per milliliter and Serum containing more IU was considered positive. Complement Fixation Test (CFT): from PVCE was performed with the provided CFT antigen. 20 of the serums Those who reacted with titers of IKFTU/ml or higher were considered positive (Anonymous, 2009). Milk ring test (MRT), In the study, 320 serum samples were tested using MRT. I-ELISA is a type of the test that can be used to detect the presence of antibodies against certain

bacteriae in bovine blood serum or milk samples (Laborde, *et al.*, 2017). In the study, I-ELISA was conducted on both blood serum and milk samples since milk ELISA is internationally approved for lactating cows as an alternative to serum ELISA (Kumar *et al.*, 2018). In total, ELISA tests were performed on 320 serum and milk samples.

RESULTS AND DISCUSSION

It details the findings of serological tests on serum and milk samples taken from cattle that were not vaccinated against Brucella in various provinces in the Central Anatolia region of Turkey (Table 1). Table 1 shows the results of each of the tests performed RBPT, SAT, CFT, I-ELISA in serum samples and I-ELISA, MRT in milk samples, as well as serum numbers from each region.

Table 2 then depicts the results of the serological tests performed to examine the proportion of animals that proved positive and negative for *B. abortus* from their blood and milk samples. From Table 1, the various tests performed on the serum of the bovine samples revealed different results. From the RBPT test, 118 of the 320 serum samples were found to be positive, representing 36.8% of the samples. In contrast, from the SAT, only 22.1% of the tested samples (71 out of 320). The SAT results were close to those obtained from the CFT test which also revealed that 61 out of 320 serum samples (19.0%) were positive for brucellosis. The MRT presented a different result with 56.5% of the samples being positive. However, the ELISA serum test had the highest proportion of samples testing positive among the tests conducted. From the test 49.2% representing 156 of the 320 serum samples, were found positive for the antibodies.

The test results revealed that there was a high prevalence rate of brucellosis among non-vaccinated cattle in central Anatolia region of Turkey. Taking all the tests into consideration, the average value of the positive and negative results were as computed in Table 2. The average values portray that at least 35.4% of all the cattle in the regions

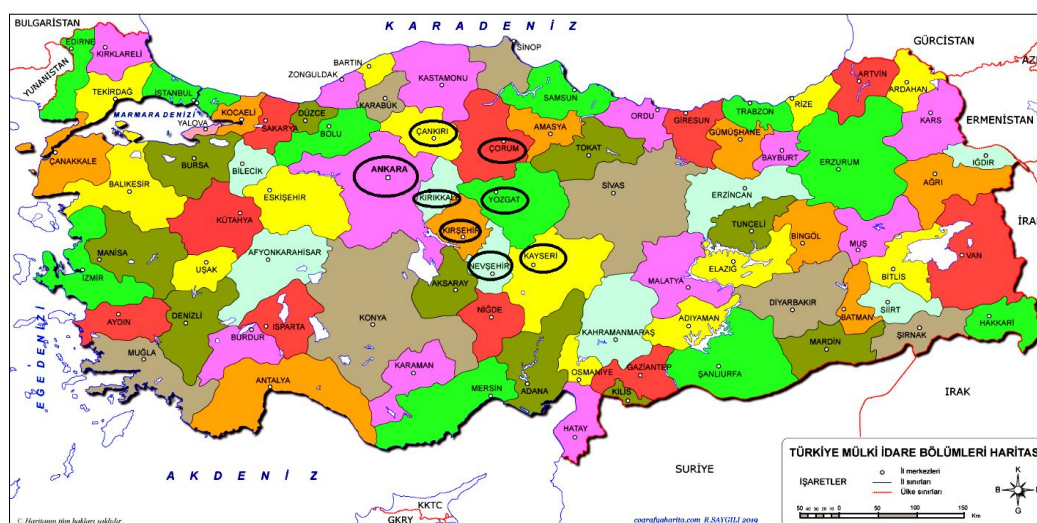


Fig 1: Locations where blood and serum samples are supplied.

Table 1: Antibodies according to provinces distribution of *B.abortus* by serological tests in bovine serum and milk samples

Location	Serum no (n)	RBPT (Serum)			SAT (Serum)			CFT (Serum)			I-ELISA (Serum)			I-ELISA (Milk)			MRT (Milk)								
		Positive		Negative	Positive		Negative	Positive		Negative	Positive		Negative	Positive		Negative	Positive		Negative						
		n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%						
Çankırı	63	25	40	38	60	10	17	53	83	8	14	55	86	20	32	43	58	28	45	35	55	12	22	51	78
Kırşehir	57	20	35	33	65	9	16	48	84	10	18	47	82	35	62	22	38	40	71	17	29	10	19	47	90
Kayseri	52	28	55	24	45	16	31	36	69	12	23	40	77	30	59	22	41	40	78	12	22	27	53	45	47
Çorum	39	16	42	23	52	12	33	37	67	13	35	26	65	19	51	20	49	18	48	21	52	15	40	24	60
Yozgat	35	11	32	24	68	5	15	30	85	6	17	29	83	13	38	22	62	15	43	20	57	9	26	26	74
Ankara	32	18	58	14	62	12	40	20	60	14	46	18	54	23	73	9	27	24	78	8	22	19	61	13	39
Kırıkkale	27	6	23	19	77	4	16	23	84	5	18	22	82	11	43	16	57	8	31	19	69	9	36	18	64
Neşehir	15	4	28	9	72	3	26	12	74	3	24	12	74	5	37	10	63	6	42	9	58	4	29	9	71
Total	320	118	36,8	202	63,2	71	22,1	249	77,9	61	19,0	259	81,0	156	49,2	164	50,8	179	56,5	141	44,5	94	29,3	226	70,7

where the samples were taken from are positive for the *B. abortus* bacteria, with only 64.5% of them testing negative. ELISA presents the most complete test even when other tests produce negative results (Molavi *et al.* 2014). The prevalence rate for the disease was highest in the cities of Ankara, Kırşehir and Kayseri which all recorded 73%, 62% and 59% positive results in serum ELISA tests. The proportion of samples that tested positive was lowest in the cities of Kırıkkale and Çankırı for which the serum ELISA test revealed that 43% and 32% of the cattle were positive for the *B. abortus* bacteria.

Brucellosis is caused by bacteria of the brucella genus; It is a zoonosis that can be transmitted to humans through meat of animals such as sheep, goats, cattle, buffalo and pigs, body fluids such as milk and urine, dairy products prepared with infected milk, pregnancy materials of infected animals (Young, 2000). Brucellosis is an important zoonotic public health problem that affects societies socially and economically. The seroprevalence of brucellosis in the world and in our country varies according to geographical situation, level of development and socioeconomic status. The Central Anatolian region, which is located in Ankara, the capital city of Turkey, is a region that is located on the route of animal movements and has a strategic importance in terms of population density, geopolitics and control of animal movements. The definitive diagnosis of brucellosis is made by obtaining the microorganism from blood, bone marrow and other tissues. Serological diagnosis gains importance in cases where the microorganism cannot be obtained for various reasons (Young, 2000). The diagnosis of brucellosis is still commonly made by serology (Gotuzzo *et al.*, 1992). Different rates are reported in studies to determine the prevalence of brucellosis. In the literature review, rates ranging from 1.8% to 25% are reported in our country (Abbasoğlu *et al.*, 1990; Kalkan *et al.*, 1999).

Within the scope of Brucella Eradication Project between 2000-2011 by the Ministry of Food, Agriculture and Livestock, General Directorate of Protection and Control, 5496 positive cases (8.2%) were reported in 66475 animals in 2011. In 2011, it was reported that herd prevalence was 7.8% in cattle and 22.5% in sheep (Yazıcıoğlu, 2014). Esendal *et al.* (2000) suspected of brucellosis 47.2% by RBPT, % by SAT in 250 bovine serum 51.6; % by RBPT in 250 sheep-goat serum. They found a positive reaction of 37.6%, 44.4% with SAT. In our current study, RBPT 36.8%, SAT 71 22.1%, CFT 19.0%, I-ELISA 49.2% were determined in line with the results of 320 bovine blood serum samples. According to Babaoglu *et al.* (2018), it was determined that 17.32% of the milk samples of both vaccinated and unvaccinated cows were Brucellosis positivity. In our current study, MRT was 29.3% and I-ELISA was 56.5%, in line with the results of 320 bovine milk samples. The positivity rate in MRT was 29.3%, 36.8% less than RBPT, but SAT was less than 22.1%. While the positive rates in SAT and CFT were 22.1% and 19%, similar results were found, RBPT was found to be high with 36.8%. The high rate of positivity in the results

Table 2: Average percentage results of *B.abortus* test results in milk and serum samples.

Table 2. Average percentage results of diagnostic tests used in milk and serum samples.							
<i>Brucella abortus</i>	Tests						Average
	Serum				Milk		
	RBPT	SAT	CFT	I-ELISA	MRT	I-ELISA	
Positive	118(36.8%)	71(22.1%)	61(19.0%)	156(49.2%)	94(29.3%)	179(56.5%)	113(35.4%)
Negative	202(63.2%)	249(77.9 %)	259(81.0%)	164(50.8%)	226(70.7%)	141(44.5%)	207(64.5%)
Total	320	320	320	320	320	320	320

of RBPT may be due to the low positivity. The fact that CFT results are decisive as average positivity results is due to the fact that this test is accepted as the Gold standard test. In the Brucella screening tests, positivity rates were found to be higher with the I-ELISA method compared to the sera analyzed by conventional methods. This may be related to the fact that I-ELISA detects lower and less Brucella antibodies and concludes. It is thought that the specificity and sensitivity of I-ELISA is higher than conventional tests. Despite the limitations of our current study, when the results obtained are examined, we think that the reason for the difference is the sample location, size, number of animals, the geopolitical situation in terms of animal mobility and the differences in the tests used in brucella disease.

CONCLUSION

The tests revealed that an average of 35.4% of the cattle had the bacteria while 64.5% of the raw milk samples were infected with the brucellosis. In conclusion, in order for Brucellosis prevention and eradication programs to be successful, animals should be regularly controlled and vaccinated and studies on the source of the disease should be expanded. In particular, we think that surveillance studies should be intensified and predominantly multicenter studies should be conducted.

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