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RESEARCH ARTICLE

Prevalence of Brucellosis in Dairy Herds with Abortion Problems

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Summary

Two thousand eight hundred sixty nine cattle blood serum samples collected from 55 dairy cattle herds selected by purposive sampling method were examined for brucellosis. Herd level seroprevalence was found to be 56.4%, 38.2% and 43.6% by Rose Bengal Plate Test (RBPT), Acidified Rose Bengal Plate Test (ARBPT) and Serum Agglutination Test (SAT), respectively. Individual animal seroprevalence was found as 6.8% by SAT. According to herd sizes, ≤20 cattle; 21-40 cattle; 41-60 cattle; 61-80 cattle and ≥81 cattle, herd level seropositivity detected by SAT were 14.3%, 30.8%, 50.0%, 66.7% and 71.4%, respectively. In majority of seropositive herds (45.8 %), within-herd seroprevalence was between 1 and 10%. By this study, it is stated that brucellosis infection is common in the dairy cattle herds with abortion problems in Burdur province and larger herds are at higher risk for brucellosis. Additionally, it is concluded that RBPT and ARBPT can be used together for diagnosis of brucellosis, but the serum samples found negative in ARBPT should be examined by SAT or another serological test since ARBPT could not detect the titers lower than 1/80.

Keywords: *Brucellosis, Burdur, Dairy cattle, Purposive sampling, Seroprevalence*

Yavru Atma Problemli Süt Sığırı Sürülerinde Brusellozis'in Görülme Sıklığı

Özet

Maksatlı örnekleme metodu ile seçilen 55 adet süt sığırı sürüsünden toplanan 2869 sığır kan serum örneği brusellozis yönünden incelendi. Sürü seviyesinde seroprevalans Rose Bengal Plate Test (RBPT), Asidifiye Rose Bengal Plate Test (ARBPT) ve Serum Aglütinasyon Testi (SAT) ile sırasıyla %56.4, %38.2 ve %43.6 olduğu belirlendi. SAT ile bireysel hayvan seroprevalansı %6.8 olarak bulundu. Sürü büyüklüklerine göre, ≤20 sığır, 21-40 sığır, 41-60 sığır, 61-80 sığır ve ≥81 sığır, SAT ile sürü bazında seroprevalans oranları sırasıyla %14.3, %30.8, %50.0, %66.7 ve %71.4 olarak belirlendi. Seropozitif sürülerin büyük bölümünün (%45.8) sürü içi seroprevalansının %1 ile %10 arasında olduğu görüldü. Bu çalışma ile, Burdur bölgesinde yavru atma problemli sürülerde brusellozis'in yaygın olduğu ve büyük sürülerin brucellosis için daha yüksek risk altında oldukları belirtildi. Ayrıca, Brusellozis'in serolojik tanısında RBPT ve ARBPT'in birlikte kullanılabileceği ancak ARBT'in 1/80 titrenin altındaki pozitif hayvanları belirleyemediğinden SAT ile sonuçların doğrulanması gerektiği sonucuna varıldı.

Anahtar sözcükler: *Brusellozis, Burdur, Süt sığırı, Maksatlı örnekleme, Seroprevalans*

INTRODUCTION

Bovine brucellosis is a chronic disease caused mostly by *B. abortus* and less frequently by *B. melitensis* and rarely by *B. suis* [1]. Abortion, especially in late gestation, is an important characteristic of the infection in cows [1]. Animals excrete the agent in uterine discharges during abortion and parturition and also in milk [1]. The diagnosis depends on the isolation of the agent from aborted fetus and udder secretion and detection of the antibodies against to *Brucella* sp. in cows by various serological tests [1-3].

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The serological and microbiological studies show that abortion is still important problem causing the devastating losses in dairy managements and brucellosis is among the main causes for abortion in cattle in Turkey [4-8]. In a nationwide seroprevalence study conducted in Turkey, the herd level prevalence of brucellosis in dairy cattle was found 11.4% and individual cattle prevalence was found 1.43% [9].

Burdur province, located in southwest of Turkey, has a large cattle population with approximately 140.000 cattle and almost 74.000 cattle were shipped to other parts of Turkey for breeding purpose in 2009 [10]. Thus, the control of brucellosis in Burdur province is very critical. In the nationwide seroprevalence study mentioned, the herd level prevalence in cattle in Burdur province was determined as higher than 1.0% and individual dairy cattle prevalence was found lower than 1.0% by RBPT and complement fixation test (CFT) [9]. In another regional study in Burdur province in 2003, seropositivity for brucellosis in cow milk samples was detected as 2.2% by the whey agglutination test (Whey-AT) and 1.0% by milk ring test (MRT) [11].

In this study, we aimed to detect the herd level and individual animal seroprevalence for brucellosis in dairy cattle herds with abortion problem in Burdur province. Also, we evaluated a new test for serological diagnosis of the infection.

MATERIAL and METHODS

Study Area and Animals

The present study was conducted in Burdur province, located in southwest of Turkey, where extensive dairy managements are applied and carried out on samples of blood collected from 2869 cows belonging to 55 herds in 6 districts (*Table 1* and *Table 2*). The herds were selected by purposive sampling and only the herds with history of abortion were included in the study. The cows were Holstein breed, older than 1 year of age and nonvaccinated before against to brucellosis. All cows having the criteria mentioned above in the selected herds were included in the study. The size of the herds varied between 5 and 332 animals.

The sample size of animals was determined using an expected field seroprevalence of 10% with a confidence level of 90% and error of 1%. The number of animals required by the method was 2435, however, 2869 animals belonging to 55 herds were sampled to increase the precision [12].

Blood Sample Collection

Blood samples were collected aseptically from the jugular or coccygeal vein of the animals using disposable needles and vacutainer tubes and allowed to clot at room temperature. The serum samples were separated by centrifugation at 4.000 rpm for 4 min and tested at the same day.

Serological Tests

All bovine sera were subjected initially to Rose Bengal Plate Test (RBPT) as screening test and then to Acidified Rose Bengal Plate Test (ARBPT) and Serum Agglutination Test (SAT).

RBPT and SAT were carried out according to Alton et al.[13]. Definite agglutination was considered as positive reaction where as no agglutination was considered as negative for RBPT. In SAT, the results of agglutination were recorded by reading the degree of clearing and sedimentation. A titer of 1:40 (50% agglutination) or above was accepted positive. Test antigens used in both tests were produced by a commercial company (Seromed, Istanbul).

ARBPT used in this study is an agglutination test in which the serial dilution of serum under test from 1:2 to 1:128 was performed on a plate with a diluent supplied by the kit (50 µL test serum and 50 µL diluent) (Seromed, Istanbul). These dilutions of ARBPT coincide to the SAT dilutions as follow: 1:2 to 1:80, 1:4 to 1:160, 1:8 to 1:320, 1:16 to 1:640, 1:32 to 1:1280, 1:64 to 1:2560, and 1:128 to 1:5120. Then, 50 µL acidified antigen (Seromed, İstanbul) was mixed with each dilution. The plate was rotated several times and read for agglutination and judged within 8 min. The agglutination in the first dilution (1:2) showed that the serum sample from an animal infected with *Brucella* sp. The last dilution with agglutination was accepted as antibody titers for brucellosis. All tests were performed with a positive and negative control serum.

Statistical Analysis

To be able to understand whether herd size is a risk factor to brucellosis, odd ratios (OR) for each herd size group were estimated and analyzed by logistic regression analysis *(Table 4)* [14]. For statistical significance, P value was accepted as 0.05.

RESULTS

Out of 55 herds tested, 31 herds (56.4%), 21 herds (38.2%) and 24 herds (43.6%) were detected seropositive by RBPT, ARBPT and SAT, respectively *(Table 1)*.

Of the 2869 sera tested, 290 sera (10.1 %) were found seropositive by RBPT, 164 sera (5.7%) were reacted positively in ARBPT with a titer ≥1:2 and 194 sera (6.8%) were reacted positively in SAT with a titer ≥1:40 (50% agglutination) *(Table 2)*.

Herd size groups, the number of the herds tested and

the number of positive herds for brucellosis detected by three tests in each group are presented in *Table 3*. As seen on the *Table 3*, as herd size increases the herd level positivity rate for brucellosis increases gradually. This pattern was seen in the results of all tests (ARBPT, RBPT and SAT).

In the present study, to be able to understand whether herd size can be considered as a risk factor to brucellosis, OR between the number of positive herds in each herd size group were estimated and analyzed by logistic regression analysis. The values of OR indicated that the herds with 21-40 animals were about 3.5 times and the herds with 41-60 animals were 8.0 times more likely to be seropositive than herds with 1-20 animals, but the differences were not significant (P>0.05). The herds with 61-80 animals were 16 times and the herds with more than 80 animals were 20 times more likely to be seropositive than the herds with

Table 1. *Location of herds tested and herd level seroprevalence*
Tablo 1. *Test edilen sürülerin yerleşimi ve sürü seviyesinde seroprevalans*

District	Herd Tested (n)	Herd Level Seroprevalence		
		RBPT n (%)	ARBPT n (%)	SAT n (%)
Bucak	12	5 (41.7)	2 (12.7)	2 (12.7)
Golhisar	5	5 (100)	3 (60.0)	4 (80.0)
Karamanli	7	4 (57.1)	2 (28.6)	3 (42.9)
Kemer	3	0 (0.0)	0 (0.0)	0 (0.0)
Merkez	24	13 (54.2)	11 (45.8)	12 (50.0)
Yesilova	4	4 (100)	3 (75.0)	3 (75.0)
Total	**55**	**31 (56.4)**	**21 (38.2)**	**24 (43.6)**

n: *the number of the herd*

Table 2. *Individual animal seroprevalence*
Tablo 2. *Bireysel hayvan seroprevalansı*

District	Animal Tested (n)	Individual Animal Seroprevalence		
		RBPT n (%)	ARBPT n (%)	SAT n (%)
Bucak	841	5 (0.6)	2 (0.2)	2 (0.2)
Gölhisar	268	42 (15.7)	24 (9.0)	30 (11.2)
Karamanlı	427	16 (3.8)	2 (0.5)	6 (1.4)
Kemer	100	0 (0.0)	0 (0.0)	0 (0.0)
Merkez	953	183 (19.2)	115 (12.1)	129 (13.5)
Yesilova	280	44 (15.7)	21 (7.5)	27 (9.6)
Total	**2869**	**290 (10.1)**	**164 (5.7)**	**194 (6.8)**

n: *the number of the animal*

Table 3. *Classification of herds based on size and herd level seroprevalence*
Tablo 3. *Sürü büyüklüğüne göre sürülerin sınıflandırılması ve sürü seviyesinde seroprevalans*

Herd Size	Herd Tested (n)	Positive Herds		
		RBPT n (%)	ARBPT n (%)	SAT n (%)
≤ 20 cattle (small herd)	9	2 (22.2)	1 (14.3)	1 (14.3)
21- 40 cattle (medium herd)	13	5 (38.5)	3 (23.1)	4 (30.8)
41- 60 cattle (medium herd)	20	13 (65.0)	9 (45.0)	10 (50.0)
61- 80 cattle (large herd)	6	4 (66.7)	3 (50.0)	4 (66.7)
≥ 81 cattle (large herd)	7	7 (100)	5 (71.4)	5 (71.4)
Total	**55**	**31 (56.4)**	**21 (38.2)**	**24 (43.6)**

n: *the number of the herd*

1-20 animals and the differences were significant (P<0.05) *(Table 4)*.

The within-herd seroprevalence in the herds (n: 55) showed a wide variability (from 0% to 34.4%). When the seropositive herds (n: 24) were classified according to the within-herd seroprevalence, 45.8% of the herds (11/24) had within-herd seroprevalence between 1 and 10%. The within-herd prevalence groups <1%, 11-20% and >30% had 3 herds each (3/24, 12.5%) and the within-herd seroprevalence group 21-30% had 4 herds (4/24, 16.7%) *(Table 5)*.

Table 4. *Herd size and odd ratios (based on SAT results)*
Tablo 4. *Sürü büyüklüğü ve odd oranları (SAT sonuçlarına göre)*

Variables Herd Size	Herd (n)	Positive Herds		Logistic Regretion		
		(n)	(%)	OR	P Value	95% CI
≤ 20 cattle	9	1	(14.3)	-	-	-
21- 40 cattle	13	4	(30.8)	3.5	0.298	0.33 - 38.78
41- 60 cattle	20	10	(50.0)	8.0	0.071	0.84 - 76.37
61- 80 cattle	6	4	(66.7)	16.0	0.043	1.09 - 234.26
≥ 81 cattle	7	5	(71.4)	20.0	0.027	1.42 - 282.46

CI: *Confidence Interval,* ***n:*** *the number of the herd*

Table 5. *The distribution of the positive herds (n: 24) according to within-herd seroprevalence (based on SAT results)*
Tablo 5. *Sürü içi seroprevalansa göre pozitif sürülerin (n: 24) dağılımı (SAT sonuçlarına göre)*

Within-herd Seroprevalence	Herd n (%)
< 1 %	3 (12.5)
1-10 %	11 (45.8)
11-20 %	3 (12.5)
21-30 %	4 (16.7)
> 30 %	3 (12.5)
Total	**24 (100)**

n: *the number of the herd*

DISCUSSION

RBPT is an agglutination test that utilizes *B. abortus* S99 strain antigen suspension stained with Rose Bengal dye buffered to pH 3.65. This acidity prevents the activity of IgM in the serum sample, helps IgG, especially IgG_1, to react with the test antigen [15]. Since IgG_1 is predominant in the later phase of brucellosis or in chronic brucellosis, RBPT can detect chronic cases. The disadvantage of the test is that the false positive results can occur due to cross reaction between *Yersinia enterocolitica* O:9, *Vibrio cholerae*, *Francisella tularensis*, *Salmonella* grup N, *Escherichia coli* O157:H7, *E. coli* O116:H21, *E. hermanni, Pasteurella haemolytica*, *Pseudomonas maltophilia*, *Rhizobium leguminosarum*, *Agrobacterium tumefaciens*, *Ochrobactrum anthropi*, *Phyllobacterium sp.* and *B. abortus* induced antibodies [16,17]. This is the reason in this study for detection of more seropositive herds by RBPT (56.4%) than by SAT (43.6%) and ARBPT (38.2%), and more seropositive individual animals by RBPT (10.1%) than by SAT (6.8%) and ARBPT (5.7%). Other studies also indicates that RBPT detects higher percentage of seropositive animals than SAT [18,19]. Thus, the positive results of RBPT need to be confirmed by SAT or another serological test to eliminate the false positive results. Nevertheless, RBPT is recommended as screening test in the field and it is adequate as a screening test to guarantee the absence of brucellosis [20].

The SAT is used as standard for evaluating new diagnostic tests [21]. Agglutinating antibodies against *Brucella* are found in the IgG-, IgA- and IgM- immunoglobulin fractions of animal serum and SAT detects IgM, IgG_2 and IgA. The antigen used in this test belongs to *B. abortus* S99 strain and antibodies to *B. abortus*, *B. melitensis* or *B. suis* can be detected by this antigen [20]. The SAT is suitable for diagnosis of acute and new cases but its use is limited in the differentiation between antibodies resulting from infection and vaccination. Since the test animals in our study had not been vaccinated before, this discrepancy did not occur in our data.

ARBPT is a serological test designed for determination of antibody titers for brucellosis in a short period of time. According to ARBPT protocol, the dilutions made coincide to the dilutions of SAT as follow 1:2 to 1:80, 1:4 to 1:160, 1:8 to 1:320, 1:16 to 1:640, 1:32 to 1:1280, 1:64 to 1:2560 and 1:128 to 1:5120 and positive reaction in the first dilution (1:2) in ARBPT proves that the serum sample from an infected cattle. The deficiency of the test is that ARBPT could not detect the titers lower than 1/80. On the other hand, a serum with 1/40 titer (50% agglutination) is accepted positive for brucellosis. Since ARBPT failures to detect the positive samples with lower than 1:80 titers, the herd level and individual animal seroprevalence rates were found lower than the seroprevalence rates found by RBPT and SAT (*Table 1* and *Table 2*). Thus, the serum samples found negative in ARBPT need to be tested by SAT or another serological test for possible infected animals with 1:40 titer (50% agglutination).

There have been several studies on herd size as a risk factor to brucellosis in cattle populations in different parts of the world, especially in the countries where brucellosis is highly prevalent in cattle populations and they found the similar results [22-25]. In a study in Zambia, the odds of *Brucella* infection were progressively higher in the larger herd categories (26-40 cattle, OR=2.6, CI: 0.70-10; 41-82 cattle, OR=4.9, CI: 0.93-26; >82 cattle, OR=9.4, CI: 1.7-51) compared to the smallest herd category (10-25) [23]. In another study conducted in Ethiopia, significant increase of seropositivity was also observed as herd size increases from small to medium (P<0.05) and then to large sizes (P<0.001) [20]. Aquiar et al.[24] stated that herd size of more than 25 cows was a significant factor associated with the seropositivity in the State of Rondonia in Brazil (OR=2.8). In a study in Jordan, Al-Majali et al.[25] also indicated that a larger herd size is a risk factor for cattle seropositivity for brucellosis. In our study, we had the similar results and as the herd size increased to above 60 animals, the risk to brucellosis significantly increased (P<0.05) *(Table 4)*.

In our study, individual animal seroprevalence was 6.8% (194/2869) by SAT. The seroprevalence studies have been conducted in different parts of Turkey by other investigators. In a study conducted on 720 cattle blood serum samples collected from the cattle with history of abortion in Kars province, located in northeast of Turkey, detected 338 (46.95%) positive animal by RBPT and 382 (53.89%) positive animal by SAT [5]. In the study on the serum samples from the cows in six dairy farms with abortion history in Afyonkarahisar province, located in west of Turkey, all serum samples (100%) gave positive results in RBPT [7]. These results are quite higher than our results (6.8%). This can be attributed to difference in study population and sampling protocols. We selected the herds with abortion history and we sampled all animals in each selected herds. In terms of Burdur province, the past seroprevalence studies conducted on brucellosis in cattle showed that the prevalence was 0.4% by RBPT and CFT [9] and 2.2% by Whey-AT and 1.0% by MRT [11]. They are also considerably different than our study's result (6.8%) because their purposes were to determine the prevalence of the disease in cattle population of Burdur province and they used the random sampling to select the animals. We conducted this study to determine the herd level and individual animal prevalence in the cattle herds with abortion history and we used the purposive sampling for herd selection and included all animals in the selected herds. In a bacteriological study with nearly similar sampling method conducted recently in Kars region by Celebi et al.[26], they collected vaginal swap and milk samples from unvaccinated 250 cows from dairy herds with abortion history and isolated *Brucella* sp. in 6.4% of vaginal swaps and 4.4% of milk samples.

Overall, almost half of the dairy herds with abortion problems in Burdur province were infected with Brucella infections. It can be stated that the within-herd seroprevalence in *Brucella* sp. infected herds in the region is mostly between 1 and 10% and larger herds are at higher risk for brucellosis. Additionally, we could state that RBPT and ARBPT can be used together for diagnosis of brucellosis, but the serum samples found negative in ARBPT should be tested by SAT or another serological test since ARBPT could not detect the antibody titers lower than 1/80. Since the cattle population in this region is unstable and the animals are shipped to other parts of Turkey, strict control and eradication program need to be carried out in the region and sero-surveys should be conducted regularly to evaluate the success of the program.

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