

Seroprevalence of Brucellosis in Human, Sheep, and Cattle Populations in Kırıkkale (Turkey)

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Abstract: The seroprevalence of Brucellosis in human, sheep, and cattle populations was investigated in rural regions of Kırıkkale in Turkey. Serum samples were obtained from 1436 humans, and 3.2% (46) of the population was found to be positive by Rose Bengal Plate test (RBPT) and 3.0% (43) gave positive results with the standard tube agglutination test (STAT). Immunoglobulin class analysis of RBPT-positive sera using the ELISA test gave positive results for 33 people (75%) with Brucella ELISA IgG and for 11 people (25%) with Brucella ELISA IgM. The evaluation of 804 serum samples, which were obtained from 301 cattle and 503 sheep, showed 6.47% (52) seropositivity with RBPT. Additionally, all animals were found to be positive with STAT. The seropositivity was 1% and 8 %, respectively, in serum samples obtained from 301 cattle and 503 sheep with the complement fixation test (CFT), which was used as the confirmatory test.

Key Words: Brucellosis, seroprevalence, human, cattle, sheep

Kırıkkale (Türkiye)'de İnsan, Koyun ve Sığır Topuluklarında Bruselloz Seroprevalansı

Özet: Türkiye'de Kırıkkale'nin kırsal kesimlerinde insanlarda, koyun ve sığır topluluklarında Brusella seroprevalansı araştırıldı. Serum örnekleri 1436 kişiden elde edildi (% 28,41 erkek ve % 71,59 bayan). Toplumun % 3,2 (46)'si Rose Bengal Plate testi (RBPT) ile ve % 3,0 (43)'ü standard tüp aglutinasyon testi (STAT) ile pozitif olarak sonuç verdi. RBPT ile pozitif olarak bulunan serumlar ELISA testi kullanılarak % 75 (33 kişi) Brucella ELISA IgG ile ve % 25 (11 kişi) Brucella ELISA IgM ile pozitif olarak sonuç verdi. RBPT ile 301 sığırdan ve 503 koyundan olmak üzere toplam 804 serum örneğinin araştırılması sonucu % 47 (52) seropozitiflik gözlemlendi. RBPT ile pozitif olarak tespit edilen tüm hayvanlar STAT ile de pozitif olarak sonuç verdi. 301 sığır ve 503 koyunda doğrulama testi olarak kullanılan Kompleman Fiksasyon Testi (CFT) ile seropozitiflik sırasıyla % 1 ve % 8 olarak bulundu.

Anahtar Sözcükler: Bruselloz, seroprevalans, insan, sığır, koyun

Introduction

Brucellosis has a worldwide distribution among humans as well as animals, with a high prevalence in Mediterranean countries (1,2). It can be acquired via exposure to infected animals or infected food. Farmers, ranchers, veterinarians, and meat inspectors are the groups at highest risk. People of all ages are susceptible. Childhood brucellosis is more common in countries where *Brucella melitensis* is the prevalent species (3,4). The diagnosis of brucellosis is based on clinical features and the results of laboratory tests (5). The standard tube agglutination test (STAT) is the most commonly used

serological test and detects antibodies against *B. abortus*-, *B. suis*-, *B. melitensis*- and *B. canis*-specific antigens. The 2-mercaptoethanol test detects immunoglobulin G (IgG) and titers higher than 1:80 define active infection. A high IgG antibody titer or a titer that is higher following treatment suggests persistent infection or relapse. Presently, enzyme immunoassay (ELISA) is the most sensitive method for detection of immunoglobulin M (IgM), immunoglobulin A (IgA), and IgG anti-*Brucella* antibodies. Therefore, the laboratory diagnosis of brucellosis is usually based on serological tests. These tests are easy to perform and the results can be obtained within a short time (6,7).

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The aim of the present study was to determine the seroprevalence of brucellosis in human, sheep, and cattle populations in rural regions of Kirikkale province in Turkey.

A total of 1436 sera were obtained from humans (28.41% male and 71.59% female) who were in close contact with cattle or sheep. People who presented to the Medical School of Kirikkale University and the Government Hospital of Kirikkale were asked to answer a questionnaire. The age of these patients ranged from 5 years to 75 years (mean: 34 years). STAT was performed on all 1436 human sera samples, which is the standard method for serological diagnosis of brucellosis (5). A total of 804 sera were collected from cattle (301 sera) and sheep (503 sera). The sera obtained from animals for this study included male cattle and sheep that were exploited for meat, and cows and female sheep that were exploited for their milk. The cattle were vaccinated with *B. abortus* S19, and sheep were vaccinated with *B. melitensis* Rev1 vaccine.

Rose Bengal plate test antigen (RBPT)

RBPT for *Brucella* was obtained from Pendik Veterinary Control Research Institute, İstanbul. RBPT was performed by a rapid slide screening method, as described by Diaz et al. (6).

Tube agglutination test (STAT)

With STAT, a formalin- and heat-killed *B. abortus* suspension (Diagnostics Pasteur, France) was used. The test was carried out according to the manufacturer's instructions and as described by Cox (7).

Complement Fixation Test (CFT)

CFT was performed on the sera that showed no agglutination by STAT. The procedure was performed as described by Edwards et al. (8).

ELISA Test

ELISA (Genzyme Virotech GmbH Löwenplatz 5, D 65428 Rüsselsheim, Germany) was performed according to the instructions provided by the manufacture.

Statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS, for Windows 9.0). All parametric results were expressed as mean ± SD for the groups. Differences between the parametric data were analyzed by Student's t test and Mann-Whitney U test, and differences between the non-parametric data were analyzed by chi-squared and Fisher's exact tests where appropriate.

The serum samples obtained from humans were 28.41% (n = 408) male and 71.59% (n = 1028) female. The distribution of tests performed on human serum samples is shown in Table 1. Among the 1436 human serum samples, 3.20% were found to be positive with RBPT. All 46 serum samples positive according to RBPT were investigated for *Brucella* ELISA IgG and IgM levels. The distribution of RBPT results according to age and sex is given in Table 2.

Of the 46 samples with positive RBPT results, 44 (95.7%) were also positive with ELISA IgG (n = 33, 75%) and IgM (n = 11, 25%), whereas seropositivity was not confirmed with ELISA for positive RBPT samples (Table 2).

The 46 positive human samples were confirmed with STAT. Statistically there was no significant difference found between sexes (P = 0.01; $\chi^2 = 1.00$). STAT test titers of humans, cattle, and sheep are given in Table 3.

Of the 804 sera obtained from 301 cattle and 503 sheep, 6.47% (52) were positive according to RBPT. Among the 301 cattle sera, 2.67% showed seropositivity with RPBT; this rate was 8.73% (n = 44) for the sheep. All 52 positive results obtained with RBPT were also positive (100%) with STAT.

Brucellosis has a worldwide distribution and remains a major problem in humans and animals in Middle Eastern and Mediterranean countries, where the prevalence is high. Brucellosis is also a health problem for humans and animals and causes economic loss due to the loss of

Table 1. The distribution of tests performed on human serum samples.

	SERUM NUMBER	RBPT +	STAT +	CFT +	ELISA IgM +	ELISA IgG +
HUMANS	1436	46 (3.2%)	43 (3.0%)		11 (0.8%)	33 (2.3%)
ANIMALS	804	52 (3.2%)	52 (3.2%)	51 (3.2%)		

Table 2. The distribution of Rose-Bengal (RBPT), and Brucella IgG and IgM tests in 46 human serum samples. n (%), M: male, F: female.

	AGE						Total
	≤ 20	21-30	31-40	41-50	51-60	≥ 61	%
RBPT (M/F)	1 / 2	6 / 5	0 / 4	3 / 13	1 / 3	5 / 3	16 /30
Brucella IgG (71.74%)	1	7	3	13	2	7	33
Brucella IgM (23.91%)	2	3	1	3	1	1	11

Table 3. STAT and CFT titers among humans, cattle, and sheep. n (%), M: male, F: female.

	TITERS						TOTAL
	1/20	1/40	1/80	1/160	1/320	1/640	
Humans: STAT (M/F)	4/4	3/3	2/7	1/5	1/7	4/2	15/28
Cattle: STAT	5	1	1			1	8 (15.38)
Cattle: CFT	5		2		1		8 (15.38)
Sheep: STAT	25	9	7	2	1		44 (4.62)
Sheep: CFT	25	9	4	4	1		43 (4.62)

animals. Isolation of the *Brucella* organism is the definitive means of diagnosis, but in practice it is difficult due to early tissue localization and the exacting culture requirements of the organism. In practice, blood cultures are positive in 10%-30% of brucellosis cases, (9) and the remainder are diagnosed serologically. Although no single test provides 100% sensitivity and specificity, STAT still remains the test of choice for diagnosis. In the presence of appropriate signs and symptoms, a presumptive diagnosis of brucellosis is usually defined serologically as a STAT titer of 1:160 or greater (9,10). Previous studies in Turkey indicated that brucellosis occurs in the 20-40-year-old age group and is seen mostly in females (11-13). Our study showed that brucellosis occurred in the same age group and was seen in females (3%) and males (4%) equally. Research included only people who fed animals, including cattle and sheep. To the best of our knowledge, this is the first study in Turkey to have included animals and humans at the same time. We comprehensively compared the RBPT, STAT, and ELISA methods in

humans, and RBPT, STAT, and CFT methods in animals (cattle and sheep) in order to evaluate their applicability as alternative methods in surveillance programs.

We found that when RBPT was used as the reference test for humans there was no difference between STAT and ELISA results ($P = 0.273$; $\chi^2 = 1.204$). When RBPT was used as the reference, the sensitivity of STAT was 96.9%. The specificity and sensitivity of CFT were superior to those of STAT, being in the range of 95% and 80%, respectively, in most countries where a brucellosis control program has been implemented (14-16).

In human brucellosis, we found that ELISA IgG and ELISA IgM showed acute and chronic disease, and using only one of them did not reveal all the patients with brucellosis. Therefore, ELISA IgG and ELISA IgM tests must be performed together to accurately determine seropositivity. ELISA is a sensitive method for the detection of IgM and IgG anti-*Brucella* antibodies, if applied together (6,17-19).

The infection prevalence rate was 3.6% in cattle and 1.3% in sheep (12,13). Our data are in accordance with previous studies in Turkey among human and animal populations. Baum et al. (20) concluded that CFT, RBPT, and STAT are among the most useful tests for routine diagnosis. STAT was evaluated by using *Brucella*-free sheep and goats prior to and after vaccination. The specificity of STAT was 100% (20).

The data of the present study showed significant brucellosis seropositivity levels in humans and animals in

Kırıkkale, Turkey. According to the results obtained, it was concluded that vaccination is important and should be continued fastidiously for animals.

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