

# Seroprevalence of brucellosis in high-risk groups in central Anatolia

## *Orta Anadolu'da, yüksek risk grubunda bruselloz seroprevalansı*

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### **Abstract**

**Aim.** We aimed to assess the seroprevalence of brucellosis in a rural area of central Anatolia. The study group consisted of subjects having high risk occupations (shepherds, animal-dealers and farmers) living in the randomly selected villages. **Methods.** The study was conducted in the rural area of Sivas city between May 2004 and March 2005. The study group consisted of 300 subjects having high risk occupations of shepherds, animal-dealers and farmers, living in the randomly selected villages. *Brucella* Wright agglutination test was used for serological testing. **Results.** The seropositivity of brucellosis was found 3.6%. There was no statistically significant difference between the seropositivity at *Brucella* Wright agglutination test and the characteristics of the sex, age groups and occupations of the subjects in the study group ( $p>0.05$ ). **Conclusions.** We conclude that although the seropositivity of brucellosis in our region was about the low limits of the previous reports in Turkey. Brucellosis is still an important public health problem. Preventive measures should be carried out precisely in developing countries.

**Keywords:** Brucellosis, seroprevalence, Turkey, risk groups, rural area.

### **Özet**

**Amaç.** Bu çalışmada, Anadolu'nun kırsal bölgelerinde yaşamakta olan insanların bruselloz açısından seroprevalansı araştırılmıştır. Çalışma grubu, rastgele örnekleme yöntemiyle seçilen köylerdeki bruselloz açısından yüksek riske sahip insanlardan (çobanlar, hayvan bakıcıları ve çiftçilerden) oluşturulmuştur. **Yöntem.** Çalışma, Mayıs 2004 ve Mart 2005 tarihleri arasında Sivas'ın kırsal bölgelerinde yapılmıştır. Rastgele örnekleme yöntemiyle seçilen köylerde yaşayan ve bruselloz açısından yüksek riske sahip olduğu düşünülen çobanlar, hayvan bakıcıları ve çiftçilerden oluşan 300 kişi üzerinde yapılmıştır. Test olarak *Brucella* Wright aglutinasyon serolojik testi kullanılmıştır. **Bulgular.** Bruselloz seropozitifliği %3,6 oranında bulunmuştur. *Brucella* Wright aglutinasyon testinin seropozitifliği yönünden cinsiyet, yaş ve meslekler arasında istatistiksel olarak anlamlı bir fark bulunamamıştır ( $p>0,05$ ). **Sonuçlar.** Bölgemizdeki bruselloz seropozitifliği ile ilgili Türkiye'de az sayıda çalışma yapılmıştır. Bruselloz hala önemli bir halk sağlığı problemi olarak görülmektedir. Bu konuda önleyici tedbirler gelişmiş ülkelerde olduğu gibi alınmalıdır.

**Anahtar Kelimeler:** Bruselloz, seroprevalans, Türkiye, risk grup, kırsal alan

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### **Introduction**

Brucellosis is a zoonotic disease with a worldwide distribution. It affects mainly domestic

animals. Human brucellosis is caused by accidental contamination from infected animals or animal products; in areas where the disease is endemic, such as Mediterranean Europe, the Middle East and many South American countries [1]. The main pathogenic species worldwide are *Brucella abortus*, *Brucella melitensis* and *Brucella suis*. These three *Brucella* spp. usually cause abortion in their natural hosts and also account for most cases of human brucellosis. *Brucella melitensis* is the most virulent species. Brucellosis has a wide clinical spectrum and presents various diagnostic difficulties because of mimicking many other diseases. There is still no optimal therapy for some particular clinical forms of brucellosis [2]. The World Health Organization (WHO) points out those 500.000 cases of brucellosis are reported each year from around the world. The reported incidence and the prevalence of the disease vary widely from country to country, with the fact that the true incidence of human brucellosis is unknown [2]. In this study we aimed to investigate the seroprevalence of brucellosis in a rural area of central Anatolia.

### Materials and methods

This cross sectional study was conducted in the rural area of Sivas city between May 2004 and March 2005. The study group consisted of 300 subjects having high risk occupations of shepherds, animal-dealers and farmers, living in the randomly selected villages. The ethical approval was taken from the local committee. The study group consisted of 131 male (43.7%) and 169 female (56.3%), with a median age  $38.7 \pm 16$  years, ranging between 13-80 years. When the subjects were classified to age groups, it was seen that 13% were in the 0-19 years, 39.3% were in 20-39 years, 32.7% were in 40-59 years, 14.3% were in 60-79 years, 0.7% were in 80 years and above. The occupations of the study group were animal handlers (3.3%), shepherds (17.0%), animal handlers and agricultural workers (79.3%), shepherds and agricultural workers (0.3%). Prevalence of *brucella*-specific antibodies was investigated in sera of the subjects. Sex, age, and occupations of the subjects were recorded. *Brucella* Wright agglutination was used for serologic testing as previously described [3]. The seroprevalence of *Brucella* specific antibodies were based on titers of 1/80 by standard *Brucella* Wright agglutination. Briefly, sera were harvested from collected blood from the peripheral venous vessels and stored at  $-20^{\circ}\text{C}$  until analysing. The test antigen (sterile killed *Brucella melitensis* whole-cell suspension) was diluted in the ratio of 1:100 in phenolized saline and allowed to stand for at least 12 hours before use. All sera were routinely diluted from 1:20 to 1:1280. Each batch of tests included a positive 1:1280 control and a negative saline control. A definite agglutination of the suspension was read (or regarded) as a positive reaction. The antigen and serum were covered with a rubber stopper, gently mixed in the tube and then incubated at  $37^{\circ}\text{C}$  for 48 hours. After the incubation period, tubes were examined against a dull black background. Samples were considered positive if there was clearing of the suspension and agglutination at the bottom of the tube before or after shaking gently. Negative samples remained milky, and agglutination could not be seen. For positive samples, the lowest positive titre was determined. A titration of 1/80 was taken as an indication of exposure to *Brucella* and one of 1/160 as an indication of brucellosis. Therefore, titration of 1/80 and over was accepted as positive [3]. Seropositivity intended to identify exposure rather than active infection. The sensitivity and specificity of the *Brucella* Wright agglutination test was 90.0% and 96.0% respectively [4]. In the statistical comparisons Chi-Square test was used. Statistical significance was assumed for a  $p < 0.05$ .

### Results

The seroprevalence of *Brucella* specific antibodies were based on titers of 1/80 by standard *Brucella* Wright agglutination. The seroprevalence was 3.6% (positive in 11 subjects). Nine of these 11 positive subjects had a positivity at 1/80 titer, while 2 of them had a positivity at 1/160 titer. Eight subjects (2.7%) had a positivity at 1/40 titer, while 5 subjects (1.7%) had a positivity at 1/20 titer. Samples of 276 subjects (92.0%) resulted negative in the agglutination test. There was no statistically significant difference

between the seropositivity at Standard *Brucella* Wright agglutination test and the characteristics of the sex, age groups and occupations of the subjects in the study group ( $p>0.05$ ). Seroprevalence of *Brucella* antibodies according to sex, age and occupation was shown in Table 1.

**Table 1. Seroprevalence of *Brucella* antibodies according to sex, age, and occupation.**

	Seropositivity		Total		Significance
	n	%	n	%	
Sex					
Male	5	45.5	131	43.7	$p>0.05$
Female	6	54.5	169	56.3	
Age groups					
0-19	2	18.2	39	13.0	$p>0.05$
20-39	3	27.3	118	39.3	
40-59	5	45.5	98	32.7	
60-79	1	9.1	43	14.3	
$\geq 80$	-	-	2	0.7	
Occupation					
Animal handler	-	-	10	3.3	$p> 0.05$
Shepherds	2	18.2	51	17.0	
Animal handlers plus farmers	-	-	238	79.3	
Shepherds plus farmers	9	81.8	1	0.3	
Total	11	3.6	300	100.0	

## Discussion

Turkey was reported as an endemic country for brucellosis [2]. The seropositivity of brucellosis was found to be 2-8% in the healthy population and 2.0- 25% in high risk occupational groups [5-9]. Similar studies done in our region reported that seropositivity of brucellosis in endemic villages and in high risk occupations varied between 0-12.5% [10-12]. The records of the Public Health Laboratory, Sivas, revealed that 5316 persons were diagnosed as brucellosis in 2002 and the cumulative incidence was 7.8% [13]. Our study was conducted at randomly selected villages and the subjects had high risk occupations for brucellosis. The incidence of seropositivity in our study, 3.6% was found to be between the reported incidences for our region. The main sources of brucellosis are consumption of unpasteurized milk and milk products such as fresh cheese, cream, butter and occupational risk of direct contact with infected animals [14]. In this study there was not a statistically significant difference between high risk occupations and seropositivity. Although seropositivity of brucellosis was reported higher in males [15-17], some studies reported no difference between genders [11, 18]. This might be due to the occupational choices between genders. We found no difference between sex and the seropositivity of the disease. Human brucellosis can occur in any age groups, but the majority of the cases were found in young men between the ages of 20 and 40 years, related to occupational hazards in this population [2, 19]. Similarly we found that 72.8% of the subjects having seropositivity of brucellosis were between 20-59 years age, resulting as an important economical loss in the productive period of young men. We conclude that although the seropositivity of brucellosis in our region was near the low limits of the previous reports in Turkey. Brucellosis is still an important public health problem. Preventive measures by education of the public about the disease and transmission ways, immunization of farm animals should be carried out precisely in developing countries.

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