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# Prevelance of Brucellosis in the Turkish Republic of North Cyprus

## Kuzey Kıbrıs Türk Cumhuriyeti'nde Bruselloz Prevalansı

**Mehmet ÖZDOĞAÇ[1], Meryem GÜVENİR[2], Emrah GÜLER[1], Aslı AYKAÇ[3], Murat SAYAN[4], Tamer ŞANLIDAĞ[5], Kaya SÜER[1]**

*[1]Near East University Faculty of Medicine, Department of Infectious Diseases and Clinical Microbiology, Lefkosia, Cyprus*
*[2]Near East University, Health Services Vocational School, Lefkosia, Cyprus*
*[3]Near East University Faculty of Medicine, Department of Biophysics, Lefkosia, Cyprus*
*[4]Kocaeli University Faculty of Medicine Hospital, Central Laboratory PCR Unit, Kocaeli, Turkey*
*[5]Near East University, Experimental Health Sciences Research Center, Lefkosia, Cyprus*

## Abstract

**Introduction:** Brucellosis causes a necrotic and inflammatory infection in humans and animals, and is among the world's most common zoonotic diseases. The aim of this study was to determine the seroprevalence of *Brucella* antibodies and compare serological methods in Turkish Republic of North Cyprus, where animal husbandry is common.
**Materials and Methods:** The study was conducted between December 2017 and February 2018 and included veterinarians (n=50), animal caregivers (n=109), butchers (n=65), and a control group of individuals who had no connection with animals (n=100). Serum samples from the participants were analyzed with serological techniques including Rose Bengal test (RBT), standard tube agglutination test (STA) and ELISA; IgG and IgM methods. The sensitivity and specificity of the methods used were estimated by considering ELISA (IgG and/or IgM) results as reference/golden standard.
**Results:** Of 27 patients (8.3%) patients who had positive results from at least one of the serological tests, 21 (6.5%) had positive RBT, 15 (4.6%) had positive STA and 10 (3.1%) had positive ELISA (IgG and/or IgM). Six (28%) patients with negative RBT results were found to be positive in ELISA. Seventeen samples (80.9%) were RBT-positive but ELISA- negative.
**Conclusion:** These data suggest that *Brucella* infection is at low rate in the Turkish Republic of North Cyprus. Laboratory diagnosis should be supported by tests such as seroconversion for low titers on STA, or tests such as ELISA. Only two cases were reported to the Ministry of Health Statistics Unit in 2013.
**Keywords:** *Brucella melitensis*, serological tests, epidemiology, prevalance, ELISA

## Öz

**Giriş:** Bruselloz, insanlarda ve hayvanlarda nekrotik ve yangısal enfeksiyonlara neden olan, dünyanın en fazla yayılım alanına sahip zoonotik hastalıklarından biridir. Hayvancılığın yaygın olduğu Kuzey Kıbrıs Türk Cumhuriyeti'nde (KKTC) özellikle *Brucella* spp. antikorlarının seroprevalansının saptanması ve serolojik yöntemlerin karşılaştırılması amaçlanmıştır.
**Gereç ve Yöntem:** Çalışmaya Aralık 2017-Şubat 2018 tarihleri arasında veterinerler (n=50), büyük ve küçükbaş hayvan yetiştiricileri (n=109), mezbaha işçileri ve canlı hayvan kesimi yapan kasaplar (n=65) ve hayvan ile ilişkisi olmayan halk topluluğu (n=100) dahil edildi. Olguların serumlarında Rose Bengal testi (RBT), standart tüp aglütinasyon testi (STA), ELISA çalışıldı. Yöntemlerin karşılaştırılması için ELISA test sonuçları referans alınarak duyarlılık ve özgüllükler hesaplandı.
**Bulgular:** Serolojik testlerin en az biri ile olumlu sonuç veren 27 hasta (%8,3) serumunda RBT ile 21 (%6,5), STA ile 15 (%4,6) ve ELISA (IgG ve/veya IgM) ile 10 (%3,1) olumluluk bulunmuştur. RBT olumsuz olan altı (%28) hastanın ELISA olumlu olduğu tespit edilmiştir. RBT olumlu olan fakat ELISA sonuçları olumsuz bulunan 17 (%80,9) örnek saptanmıştır.
**Sonuç:** Bu veriler KKTC'de *Brucella* enfeksiyonunun düşük olduğunu desteklemektedir. STA'nın düşük titreleri için serokonversiyon takibi veya ELISA gibi testlerle laboratuvar tanı desteklenmelidir. KKTC Sağlık Bakanlığı İstatistik Birimi'nden 2013 yılında sadece iki olgu bildirilmiştir.
**Anahtar Kelimeler:** *Brucella melitensis*, serolojik testler, epidemiyoloji, prevalans, ELISA

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**Address for Correspondence/Yazışma Adresi:** Meryem Güvenir MD,
Yakın Doğu Üniversitesi, Sağlık Hizmetleri Meslek Yüksekokulu, Lefkoşa, KKTC
**Phone:** +90 392 680 20 00/3430 **E-mail:** meryemguvenir@hotmail.com **ORCID:** orcid.org/ 0000-0002-9702-9947
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## Introduction

Brucellosis, caused by microorganisms of the *Brucella* genus, causes necrotic and inflammatory infections in humans and animals. Brucellosis is one of the world's most widespread zoonotic diseases. Although *Brucella* spp. infections have been completely eradicated in many developed countries, brucellosis continues to be an important public health problem in developing countries[1,2]. According to the World Health Organization, brucellosis is widespread in developing countries. It causes economic problems and affects food safety directly[3,4].

In Cyprus, the prevalence of brucellosis in animals was claimed to be 0.1% since 2007 as a result of the eradication programs conducted at various time periods[1]. Brucellosis (in both animals and humans) is a notifiable disease in the Turkish Republic of Northern Cyprus (TRNC). According to TRNC Ministry of Health records, its prevalence in humans is on the rise. This increase has been attributed to improved diagnosis, a higher reporting rate, and inadequate disease control[5]. The aim of this study was to determine the seroprevalence of *Brucella melitensis* and *Brucella abortus* antibodies in TRNC, where livestock farming is widespread. The study included high-risk groups such as veterinarians, animal care workers, and butchers. Due to the lack of previous research on *Brucella* spp. in TRNC, we believe that this study will contribute to our national data.

## Materials and Methods

The study subjects comprised 4 groups; group 1: community members not associated with livestock farming (n=100), group 2: cattle breeders (n=109), group 3: veterinarians (n=50), group 4: slaughterhouse workers and butchers who practiced live animal slaughter (n=65). Sample size was determined according to data obtained from TRNC Veterinary Bureau about the number of people engaged in livestock farming in TRNC and the acceptable prevalence value. Cattle breeders, veterinarians, or slaughterhouse workers who had been engaged in livestock farming for more than 5 years were excluded from the study while asymptomatic people were included. The groups' distributions based on the country's population were determined, and statistically relevant number of blood samples representative of each region (Nicosia, Kyrenia, Güzelyurt, Famagusta, and İskele) was collected. Approximately 10 cc of venous blood was collected from the study participants. The sera were separated by centrifuging the samples at 3000 rpm for 10 minutes and stored at -20 °C until analysis.

The serum samples were analyzed using Rose Bengal test (RBT) (Pendik Veterinary Control and Research Institute, Turkey), standard tube agglutination test (STA) (Pendik Veterinary Control and Research Institute, Turkey), and *Brucella* IgM and IgG tests with the ELISA method (VIRCELL, Santa Fe, Granada, Spain). ELISA cut-off control values were accepted as >0.55 and <1.5 in accordance with the manufacturer's recommendations and considered the gold standard.

Agglutination in RBT and titers of 1/160 and above in the STA test were considered positive. *Brucella* ELISA IgG and IgM levels >11 (VIRCELL, Santa Fe, Granada, Spain) were considered positive. The study was approved by the Near East University Scientific Research Assessment Ethics Committee (decision number: 2017/466 dated: 24.02.2017).

### Statistical Analysis

The results were evaluated by using the SPSS version 15.0 (Statistical Package for the Social Sciences, United States) software. The sensitivity and specificity of the RBT and STA were calculated using ELISA IgG and IgM positivity as reference/golden standard results.

## Results

The study included a total of 324 people, who volunteered to participate between December 2017 and February 2018 (Annex 1). Of the 27 (8.3%) patients who tested positive for *Brucella* in at least one of the serologic tests, 25 (92.5%) were male and 2 (7.4%) were female, and the mean age was 39.4±12.8 (13-84) years. Fifteen (55.5%) of the patients resided in urban areas and 12 (44.4%) resided in rural areas. The distribution of the patients included in the study by sex and age is shown in Table 1. Livestock farming (60%) was the most common occupation among ELISA-positive patients.

**Table 1. Demographic characteristics**

| | RBT positive | | ELISA IgG positive | | STA positive | |
|---|---|---|---|---|---|---|
| | n | % | n | % | n | % |
| **Age (years)** | | | | | | |
| 21-30 | 4 | 19 | 3 | 30 | 4 | 26.7 |
| 31-40 | 10 | 47.6 | 4 | 40 | 4 | 26.7 |
| 41-50 | 4 | 19 | - | - | 4 | 26.7 |
| 51-60 | 3 | 14.2 | 3 | 30 | 3 | 26.7 |
| **Gender** | | | | | | |
| Male | 19 | 90.4 | 10 | 100 | 13 | 86.7 |
| Female | 2 | 9.6 | - | | 2 | 13.3 |
| **Occupation** | | | | | | |
| Animal husbandry | 13 | 61.9 | 6 | 60 | 11 | 73.3 |
| Veterinarian | 2 | 9.5 | 1 | 10 | 2 | 13.3 |
| Butcher | 3 | 14.2 | 3 | 30 | 2 | 13.3 |
| Control group | 3 | 14.2 | - | | - | 0 |

RBT: Rose Bengal test, STA: Standard tube agglutination

Of the 27 patients with positive serologic test results, RBT was positive in 21 (6.5%), STA was positive in 15 (4,6%), and ELISA (IgG and/or IgM) was positive in 10 (3.1%) of the patients (Table 2, 3). Six RBT-negative patients (28%) had positive ELISA results.

**Table 2. Distribution of serologic test results**

| Serologic test | Number of positive samples | | Number of negative samples | | Total number of serum samples |
|---|---|---|---|---|---|
| | n | % | n | % | |
| RBT | 21 | 6.48 | 303 | 93.5 | 324 |
| STA | 15 | 71.4 | 6 | 28.0 | 21 |
| ELISA IgG | 10 | 3.1 | 314 | 96.9 | 324 |
| ELISA IgM | 0 | 0 | 324 | 100 | 324 |

RBT: Rose Bengal test, STA: Standard tube agglutination

**Table 3. Distribution of patients with positive laboratory test results**

| Patient number | RBT | STA | | *Brucella* IgG | *Brucella* IgM |
|---|---|---|---|---|---|
| | | *Brucella abortus* | *Brucella melitensis* | | |
| 20 | + | 1/10 | 1/10 | + | - |
| 29 | + | 1/20 | 1/20 | - | - |
| 31 | + | 1/20 | 1/20 | + | - |
| 71 | - | - | - | + | - |
| 73 | - | - | - | + | - |
| 76 | - | - | - | + | - |
| 124 | + | 1/20 | 1/10 | + | - |
| 125 | + | 1/20 | 1/10 | - | - |
| 127 | + | 1/20 | 1/10 | - | - |
| 128 | + | 1/10 | 1/10 | - | - |
| 135 | + | 1/10 | 1/10 | + | - |
| 136 | - | - | - | + | - |
| 139 | + | 1/20 | 1/10 | - | - |
| 144 | + | 1/10 | 1/10 | - | - |
| 147 | + | 1/10 | 1/10 | - | - |
| 155 | + | 1/10 | 1/20 | - | - |
| 158 | + | 1/80 | 1/80 | - | - |
| 163 | + | 1/20 | 1/40 | - | - |
| 198 | + | 1/20 | 1/20 | - | - |
| 199 | + | 1/10 | 1/20 | - | - |
| 206 | + | 1/40 | 1/20 | - | - |
| 207 | + | 1/10 | 1/20 | - | - |
| 218 | - | - | - | + | - |
| 224 | - | - | - | + | - |
| KB25 | + | 1/10 | 1/10 | - | - |
| KB63 | + | 1/10 | 1/10 | - | - |
| KB77 | + | 1/10 | 1/10 | - | - |

STA: Standard tube agglutination

Seventeen samples (80.9%) were RBT-positive but ELISA-negative. Tables 2 and 3 shows the distributions of serological test results in the serum samples analyzed in our study. There was no statistically significant correlation between presence of *Brucella* IgG and sex, age, or years of livestock farming experience ($p<0.05$). Titers did not differ between rural and urban residents.

When ELISA was considered to be the gold standard, the sensitivity and specificity of Rose Bengal and STA tests were calculated as 40%, 60% and 60%, 72%, respectively.

## Discussion

Despite being primarily an animal disease, brucellosis is counted among the most important zoonoses due to the more than 500,000 human cases reported annually worldwide[6,7]. Endemic regions include the Mediterranean basin, Arabian Peninsula, Central Asia, Africa, Mexico, and Central/South America[7,8]. Risk groups consist of people such as veterinarians, animal breeders, shepherds, slaughterhouse workers, artificial insemination technicians, food industry workers, and laboratory workers, who are in constant contact with infected animal tissues and animal products such as hides and wool[9]. Therefore, we included three different occupational groups living in TRNC in this study: livestock breeders, butchers, and veterinarians.

Patients do not always present characteristic signs and symptoms, and the disease may manifest clinically in different forms (acute, subacute, chronic, and localized), thus making diagnosis challenging[10]. Since asymptomatic patients were included in our study group, none were *Brucella* IgM-positive. Ten participants tested positive for *Brucella* IgG, 60% of which were animal breeders. Transmission to humans occurs through consumption of non-pasteurized milk and dairy products, contact with infected animal tissues, and inhalation or mucosal inoculation (nose, eyes, mouth) of infected aerosols[7]. We think that direct contact with infected material, one of the primary routes of brucellosis transmission, proves the results of IgG-positive participants.

Today, STA is the most commonly used serologic test worldwide[8]. In our study, 21 serum samples with positive RBT results underwent STA testing and 15 had titers higher than 1:160. False negative results should also be taken into account in the STA test. False negativity can result from testing within the first week of infection (titers <1:160 in the early bacteriological period); the presence of blocking antibodies (chronic brucellosis); invisible/masked agglutination at low dilutions due to excess antibody in patient serum (prozone phenomenon); *Brucella canis* infection; and agammaglobulinemia[9]. We believe that these factors might have affected the positivity rate in our study by resulting in false

negatives, and that it may be related to differences among the participants in regional means of livelihood and employment in livestock production. Since our study group consisted of asymptomatic participants, seroconversion study was not performed. Our findings of low sensitivity and specificity in the STA compared to other studies is likely due to the fact that the STA test could not be repeated 2 weeks later.

## Conclusion

Laboratory criteria for the diagnosis of brucellosis are as follows: (1) Supportive: Antibody positivity in serum samples with RBT, (2) Confirmatory: The isolation of *Brucella* spp. from clinical samples and/or STA antibody titer >1:160 in a single serum sample in a previously untreated patient and/or >4-fold increase in STA *Brucella* antibody titer in serial serum samples collected at least 2 weeks apart[8]. Only two cases were reported in 2013 according to the TRNC Ministry of Health Statistics Bureau[11]. These data suggest that *Brucella* infection is at low rate in the Turkish Republic of North Cyprus. Laboratory diagnosis should be supported by tests such as seroconversion for low titers on STA, or tests such as ELISA.

### Ethics

**Ethics Committee Approval:** The study was approved by the Near East University Scientific Research Assessment Ethics Committee (decision number: 2017/466 dated: 24.02.2017).

**Informed Consent:** Consent form was filled out by all participants.

**Peer-review:** Externally and internally peer-reviewed.

### Authorship Contributions

Design: K.S., T.Ş., M.S., Data Collection or Processing: M.G., M.Ö., E.G., A.A., Analysis or Interpretation: K.S., M.G., M.Ö., Literature Search: M.G., E.G., M.Ö., Writing: K.S., E.G., M.G., M.Ö., A.A.

**Conflict of Interest:** No conflict of interest was declared by the authors.

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**Annex 1. Study participant survey form**

Protocol number:
Date:
Region:
Name/surname:
Birth date:
Gender:
How many years you have worked in animal husbandry:
Breeder/butcher/vetrinarian:
Phone number: