ORIGINAL ARTICLE

The seroprevalence of brucellosis among undiagnosed family members of brucellosis positive patients

A Alim, M Oguzkaya-Artan[1], C Artan[2]

Department of Pharmaceutical Microbiology, Faculty of Pharmacy, Cumhuriyet University, Sivas, [1]Halil Bayraktar Health Services Vocational College, Erciyes University, [2]Chest Clinic, Kayseri Research and Training Hospital, Kayseri, Turkey

Abstract

Aim: This study investigated the seroprevalence, complications and risk factors of *Brucella* infection in rural areas of Sivas, Turkey.

Materials and Methods: The study was conducted in three hyperendemic counties for brucellosis known as Gurun, Altinyayla and Kangal in Sivas between April and October in 2011. A total of 1,430 subjects were consulted.

Results: Of the 1,430 subjects, 217 (15.2%) with clinical findings compatible with brucellosis were examined by taking blood samples to study both standard tube agglutination test (STAT) and enzyme-linked immunosorbent assay (ELISA) (Genzyme Virotech GmbH, Rüsselsheim, Germany). The seroprevalance of *Brucella* was found to be 8.0%. *Brucella* seropositivity was detected in 114 (52.5%) of the 217 subjects with STAT. There was no significant difference between female and male subjects with regard to *Brucella* seropositivity ($P = 0.214$). The seropositivity of subject 16–65 age group was significantly higher than those of subjects in <16 and >65 age groups ($P = 0.001$). In *Brucella* ELISA test results, 123 (56.7%) subjects had positive IgG antibodies and 96 (44.2%) IgM antibodies. Skeletal complications were the most frequent; joint, muscle, and waist pain were found in 87.1%, 79.7%, and 74.6% of subjects respectively. Most subjects (90.8%) gave a history of frequent consumption of fresh cheese directly from the cattle they own and contact with animals (77.8%) for risk factors of brucellosis.

Conclusion: *Brucella* seropositivity is high in Gurun, Altinyayla and Kangal counties and primary care physicians should keep in mind the clinical and laboratory findings of brucellosis especially in family members of brucellosis patients.

Key words: Brucellosis, complications, enzyme-linked immunosorbent assay, risk factors, seropositivity

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Introduction

Brucellosis is one of the world's major zoonoses that continue to have public health and economic concern in many parts of the world. The heaviest disease burden lies in countries of the Mediterranean Basin and Arabian Peninsula. The disease is usually transmitted from infected animals to man by direct contact or by consumption of raw milk infected with *Brucella* organisms.[1-3]

Address for correspondence:
Dr. Muge Oguzkaya-Artan,
Halil Bayraktar Health Services Vocational College, Erciyes University, Kayseri, Turkey.
E-mail: martan38@gmail.com

Brucellosis, like tuberculosis, is a chronic granulomatous infection caused by intracellular bacteria and requires combined, protracted antibiotic treatment.[4] Human brucellosis is notoriously a multisystem disease with varied manifestations, and the onset may be either acute or insidious.[5] Goats, sheep, cattle, swine, dogs, and

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buffalo may be infected with *Brucella*. Farmers, animal caretakers, veterinarians, butchers and slaughterhouse workers have an increased risk of infection because of their professional activities.[6,7] The clinical features of Brucellosis are not disease-specific; almost every organ can be affected.[1,3,8]

Although many countries have eradicated *Brucella abortus* from cattle, in some areas *Brucella melitensis* and *Brucella suis* have emerged as causes of this infection in cattle, leading to human infections.[9,10] Human brucellosis is a significant public health problem in Sivas city located in mid-Anatolia, Turkey Figure 1.[11-13] In this region, farming is one of the most common activities, and people usually consume dairy products. Majority of the people live in rural areas and work in animal farming and agriculture. Although the prevalence of *Brucella* is not exactly known in Turkey, the seropositivity has been reported to be about 2–6%.[11] The seroprevalance of brucellosis in sheep and cows in this country has been reported to be 1.97% and 1.43% respectively.[5] The records of the Public Health Laboratory, Sivas, revealed that 5,316 persons were diagnosed with *Brucella* infection in 2009, and cumulative incidence was 7.8%.[12]

The gold standard for the diagnosis of brucellosis is the isolation and identification of *Brucella* species from clinical specimens by culture. However, it is time-consuming and hazardous to the laboratory personnel. Therefore, most cases are diagnosed by serological testing. The most frequently used method is the standard tube agglutination test (STAT).[14] Enzyme-linked immunosorbent assay (ELISA) typically uses the cytoplasmic proteins as antigens and measures IgM, IgG, and IgA, which allow for better interpretation. It has been reported to be superior to other serologic tests due to its higher sensitivity and specificity.[1,15,16]

In this study, we aimed to determine the seroprevalance of *Brucella* in three hyperendemic counties: Gurun, Altinyayla and Kangal in Sivas, Turkey by STAT and *Brucella* ELISA, and we also intended to evaluate the complications and risk factors of brucellosis in seropositive subjects.

Material and Methods

Sivas is one of the central Anatolian cities of Turkey. It had a population of 600,323 in 2010.[17] Sivas has 16 counties and 28,197 people are living in Gurun (9,136), Altinyayla (5,840) and Kangal (13,221) which are three hyperendemic counties for brucellosis.[18] This study was conducted in these counties between April and October in 2011. A total of 1,430 subjects who had a family member diagnosed brucellosis; who had drunk raw (unpasteurized) milk or had eaten dairy products made from raw milk of infected animals; who had consumed Turkish raw-meat ball; or who had contact with animals infected with *Brucella* were chosen to screen for the clinical findings of brucellosis. The 1,430 subjects were informed about the nature of the study, and written informed consent was obtained. The approval was taken from the Local Ethics Committee of the Sivas Health Directorate. Of 1,430 subjects with a possibility of brucellosis, 217 (15.3%) had a diagnosis of brucellosis according to clinical findings after examinations of primary care physicians. They were divided into three groups according to their ages as <16 years, 16–65 years and >65 years.

In 217 (15.2%) subjects with clinical findings compatible with brucellosis, after taking blood samples, we performed STAT and ELISA for *Brucella*. The STAT is the most popular diagnostic tool for brucellosis though sometimes yielding misleading results.[19] All the sera were tested by the *Brucella* STAT as described by Bilgehan.[20] *Brucella* STAT was obtained from "Refik Saydam Hygiene Center Contagious Diseases Research Department" in Ankara in Turkey. Serum was harvested from blood collected from the peripheral venous vessels and stored at −20°C until usage in serological tests. All sera were routinely diluted from 1/20 to 1/1280. Each batch of the test included a positive control and a negative saline control. The antigen and serum were mixed gently in the tube and then incubated at 37°C for 48 h. After the incubation period, tubes were examined against a dull black background. Samples were considered positive if there were 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. A definite agglutination of the suspension was read as a positive reaction. For positive samples, the lowest positive titer was determined. The diagnostic criterion was the titer of 1/160 or more.[21-24] The presence of antibodies against *Brucella* was used as a proxy for exposure to *Brucella* infection.

All the sera were tested by *Brucella* ELISA for *Brucella* IgM and IgG antibodies. Previous studies found ELISA to be an effective, reliable and sensitive testing method for diagnosis of brucellosis.[15,25] ELISA (Genzyme Virotech GmbH, Rüsselsheim, Germany) was performed according to the instructions provided by the manufacturer. The test is rapid, easy to perform and can be automated. The cut-off values recommended by the manufacturer were used to determine positive, negative, and borderline results representing cases of brucellosis, cases without brucellosis, and cases of unknown status, respectively. Total 100 μL of prediluted serum samples were added to each of wells. The micro-plate was incubated at room temperature for an hour. The wells were washed three times with washing solution. Total 100 μL of conjugate was added to each of the wells. The micro-plate was incubated for 30 min at room

temperature. The wells were washed again as described in stage. Substrate and chromogen solution was added to each well. After incubation (10 min), the stopping solution was added and then absorbances were measured in micro-plate reader (Thermo Scientific Multiskan Ascent).

Statistical Package for the Social Sciences (SPSS) for Windows (Version 13.0; SPSS, Chicago, IL, USA) software was used for the statistical analysis of the data. Descriptive data were expressed as simple frequencies and percentages. Univariate analyses of categorical variables were done with the Chi-square test and Fisher's exact test when appropriate. The level of significance was set at 0.05 using the two-tailed method.

Results

Totally, 217 subjects enrolled in the study were aged between 10 and 80 (median 42.2) years. One hundred and twenty-nine were female, and 88 were male. The *Brucella* seropositivity was detected in 114 (52.5%) of 217 subjects. Table 1 shows the *Brucella* seropositivity according to the age groups of the subjects. There was no significant difference between the female and male subjects with regard to the *Brucella* seropositivity ($P = 0.214$). The *Brucella* seropostivity of subjects in the 16–65 age group was significantly higher than those in the <16 and >65 age groups ($P = 0.001$). There was no significant difference among the subjects in <16, 16–65, and >65 age groups with regard to the *Brucella* seropositivity ($P = 0.214$). The mean age of females with the *Brucella* seropositivity was significantly higher (45.0 ± 13.1 vs. 38.7 ± 20.0; $P = 0.039$) than that of the females with the *Brucella* seronegativity. The mean age of males with the *Brucella* seropositivity was significantly higher (49.6 ± 15.3 vs. 33.3 ± 22.2; $P = 0.001$) than that of the males with the *Brucella* seronegativity.

Table 2 shows the *Brucella* ELISA test results, 123 (56.7%) subjects had positive IgG antibodies and 96 (44.2%) IgM antibodies. All borderline results were considered as negative. The *Brucella* ELISA IgG and IgM positivities were higher among the females than the males, but these differences did not reach statistical significance ($P = 0.102$ and $P = 0.326$). Overall, in all the subjects, the positivity of IgG ELISA was more than IgM ELISA.

Table 1: Distribution of seropositivity of *Brucella* according to clinical parameters

	n (%)		*P*
	Seropositivity	Total	
Gender			
Male	51 (57.9)	88 (40.6)	0.214
Female	63 (48.8)	129 (59.4)	
Age (years)			
<16	0 (0)	21 (9.7)	0.001
16-65	99 (59.3)	167 (77.0)	
>65	15 (51.7)	29 (13.4)	

Table 2: The distribution of tests performed on serum samples

Tests performed	Males			Females			Total	*P*
	n	Percentage of males	Percentage of total	*n*	Percentage of females	Percentage of total	*n* (%)	
STAT	51	39.5	23.5	63	71.6	29.0	114 (52.5)	0.119
IgM ELISA	44	34.1	20.3	52	59.0	23.9	96 (44.2)	0.102
IgG ELISA	52	40.3	23.9	71	80.7	32.7	123 (56.7)	0.326

STAT=Standard tube agglutination test; ELISA=Enzyme-linked immunosorbent assay

Table 3: Complications of brucellosis

Complication	Males			Females			Total	*P*
	n	Percentage of males	Percentage of total	*n*	Percentage of females	Percentage of total	*n* (%)	
Joint pain	83	94.3	38.2	115	89.1	53.0	189 (87.1)	0.140
Fever	64	72.7	40.6	119	92.2	59.4	183 (84.3)	0.000
Muscle pain	71	80.7	32.7	102	79.1	47.0	173 (79.7)	0.456
Night sweating	67	76.1	30.9	104	80.6	47.9	171 (78.8)	0.265
Waist pain	64	72.7	40.6	98	76.0	59.4	162 (74.6)	0.350
Weight lost	44	50.0	20.3	66	51.2	30.4	110 (50.7)	0.488
Joint distension	29	33.0	13.4	44	34.1	20.3	73 (33.6)	0.489
Limitation of the joint	20	22.7	9.2	28	21.7	12.9	48 (22.1)	0.493
Lymphadenopathy	21	23.9	9.7	27	20.9	12.4	48 (22.1)	0.363
Hepatolmegaly	7	8.0	3.2	11	8.5	5.1	18 (8.3)	0.545
Hepatosplenomegaly	7	8.0	3.2	11	8.5	5.1	18 (8.3)	0.208
Splenomegaly	6	6.8	2.8	5	3.9	2.3	11 (5.1)	0.254
Epididymo-orchitis	2	2.3	0.9	-	-	-	2 (0.9)	0.163

Table 4: Distribution of risk factors associated with brucellosis

Risk factors	Males			Females			Total	*P*
	n	Percentage of males	Percentage of total	*n*	Percentage of females	Percentage of total	*n* (%)	
Consumption of fresh cheese	78	88.6	35.9	119	92.2	54.8	197 (90.8)	0.251
Consumption of raw milk	12	13.6	5.5	21	16.3	9.7	33 (15.2)	0.370
Consumption of Turkish raw-meat ball	33	37.5	15.2	36	27.9	16.6	69 (31.8)	0.090
Have contact with animals	72	81.8	33.1	97	75.2	44.7	169 (77.8)	0.180

Table 5: OR of risk factors related to brucellosis in all subjects

	Subjects (*n*=217)		
	Seropositive (*n*=114)	Seronegative (*n*=103)	OR
Consumption of fresh chase			
Yes	107	90	2.21 (95% CI: 0.84-5.77; *P*=0.106)
No	7	13	
Consumption of raw milk			
Yes	17	16	0.99 (95% CI: 0.47-2.08; *P*=0.987)
No	93	87	
Consumption of Turkish raw-meat ball			
Yes	39	30	1.26 (95% CI: 0.71-2.24; *P*=0.422)
No	75	73	
Positive contact with animals			
Yes	90	79	1.13 (95% CI: 0.59-2.16; *P*=0.69)
No	24	24	

OR=Odds ratio; CI=Confidence interval

Signs and symptoms of brucellosis in these series reflected a combination of systemic illness with certain manifestations. Joint pain, fever, muscle pain, night sweating, waist pain were the main presenting symptoms. Table 3 shows the most common abnormalities on physical examination which were joint pain (87.1%), fever (84.3%), muscle pain (79.7%), night sweating (78.8%), waist pain (74.6%), weight lost (50.7%), joint distension (33.6%), limitation of the joint (22.1%), lymphadenopathy (22.1%), hepatomegaly (8.3%), hepatosplenomegaly (8.3%), splenomegaly (5.1%), and epididymo-orchitis (0.9%).

Risk factors related to brucellosis are shown in Table 4 according to the gender of subjects, and the ratios of risk factors (consumption of fresh cheese, consumption of raw milk, consumption of Turkish raw-meat ball, and have contact with animals) of females and males were found as comparable ($P > 0.05$). Most patients gave a history of consumption of fresh cheese (90.8%) and contact with animals (77.8%). We calculated odds ratios of the risk factors related to brucellosis, and there was no risk factor with a statistically significant odds ratio [$P > 0.05$; Table 5].

Figure 1: The location of Sivas on the map of Turkey

Discussion

Brucellosis complications are major medical problem in countries where brucellosis is still endemic, as in our region of central Anatolia. Sivas has three hyperendemic counties for *Brucella* known as Gurun, Altinyayla, and Kangal. In this study, we found the prevalence of brucellosis in these counties as 8.0%. *Brucella* seropositivity was detected in 114 (52.5%) of the 217 subjects with STAT.

Various studies on *Brucella* seropositivity have been conducted in Turkey and in other countries. Oguzkaya-Artan and Baykan.[26] evaluated 2,295 patients' sera suspected by brucellosis and found the prevalence as 8.2% in another mid Anatolian city, Kayseri. In another study from Kayseri, seropositivity was found 3.3%, in the general population of a village.[26] Sümer *et al.*[11] evaluated 750 subjects, 65 years old and found 3.2% *Brucella* seropositivity. Cetinkaya *et al.*[5] studied 1,052 subjects in a rural area of western Anatolia and found 4.8% seropositivety. Kose *et al.*[27] found 0–4.0% in Wright agglutination test at titer equal or higher than 1:100 in west and South-East Anatolia. In a study from India among high-risk group individuals, Agasthya *et al.*[28] found 14.23% seropositivity in veterinary professionals and 1.45% of the samples were positive among supporting staff and others. Bikas *et al.*[29] from a neighbor country Greece found 7.5% positivity. From Ethiopia, Kassahun *et al.*[30] found the seroprevalence of brucellosis 4.8%. There are some differences between the studies. In our opinion, these differences are because of the groups studied. In studies done by Oguzkaya-Artan and Baykan, Agasthya *et al.*, Bikas *et al.* and our study, the groups were including the suspected subjects but the others were on the whole population and so the prevalence are different from each other.[26,28,29]

In the current study, all the sera were tested by *Brucella* ELISA for *Brucella* IgM and IgG antibodies. For *Brucella* ELISA test results, 123 (56.7%) subjects had positive IgG antibodies and 96 (44.2%) IgM antibodies in our study. Heydari *et al.* from west Azerbaijan Province of Iran found seroprevalance of brucellosis 51.1% with STAT and 62.1% with IgG and IgM ELISA.[24] Cakan *et al.* from Turkey found positivity 87.3% IgG and 36.8% IgM ELISA.[31]

In the subjects of the current study, skeletal complications were the most frequent. Joint, muscle and waist pain were found as 87.1%, 79.7%, 74.6%, respectively. Followed by fever (84.3%), night sweating (78.8%), weight loss (50.7%), lymphadenopathy (22.1%), and other hematological complications. Geyik *et al*.[32] from Turkey evaluated 195 patients' symptoms and found fever 81.0%, splenomegaly 28.0%, and hepatomegaly 26.0%. Ertek *et al*.[22] studied the complications of *Brucella* infection on 216 adult cases of brucellosis and found that the most frequent complication was on the skeletal system (68.1%). Symptoms were as follows: Arthralgia (84%), malaise (80%), sweating (78%), anorexia (73%), and fever (72%), and the signs: Splenomegaly (35%), hepatomegaly (27%), and lymphadenopathy (5%). Our findings were not different from this, but there were some frequency differences. Kokoglu *et al*.[33] found the most frequent symptoms of brucellosis as fever (40.6%), splenomegaly (36.2%), and hepatomegaly (26.8%). Hasanjani Roushan *et al*.[34] showed that fresh cheese (22.4%), animal husbandry (11.3%), laboratory worker (8.1%) and veterinary profession (1.5%) were the main risk factors and sweating, fever, and arthralgia were the most frequent clinical symptoms. Sacar *et al*.[35] detected the most frequent complaints and symptoms in 30 patients. They found that at first admission, they were arthralgia, perspiration, myalgia, backache, and fever. The most frequently affected systems were osteoarticular and hematopoietic systems. Gür *et al*.[36] reported that the frequency of *Brucella* complications was variable in different age groups in Anatolia of Turkey.

In our study, most subjects (90.8%) gave a history of frequent consumption of fresh cheese directly from the cattle they own or tend, and contact with animals (77.8%) for risk factors related to brucellosis. Cetinkaya *et al*.[5] studied on risk factor of brucellosis on 1052 adult cases and found that the most frequent risk factor was contact with animals (71.4%). Risk factors were as follows: Consumption of fresh cheese (36.1%) and consumption of raw cream (30.0%). Turkish raw-meat ball also has a suggestive role in its epidemiology, but this role is not clear yet. Socioeconomic and educational factors were also independent risk factors. Occupational, food, and socioeconomic risk factors significantly confounded one another.

In our study, there were differences in *Brucella* seropositivity among age groups ($P = 0.000$). *Brucella* seropositivity was higher in older age groups in male and female subjects in our study. *Brucella* seropositivity of female and male subjects was comparable. Cetinkaya *et al*. found differences among age groups and also according to the sex.[37] Sumer *et al*.[11] found no differences both in sex and age groups. In this region of our country, human-animal contact and high number of cattle population is usual. Adherence to traditional farming practices and lifestyle and preference for fresh dairy contribute to the high seroprevalence of brucellosis. Vaccination of livestock is of utmost importance and consumption of fresh milk and dairy products prepared from unpasteurized milk should be halted. In conclusion, *Brucella* seropositivity is high in Gurun, Altinyayla and Kangal counties and primary care physicians should keep in mind the clinical and laboratory findings of brucellosis. Human brucellosis acquired from milk is preventable, and legislation should be enacted to strictly require pasteurization of milk and dairy products. Nevertheless, public health education assumes an important role in preventing the transmission of brucellosis from animals to humans.

These results showed that physicians consulted in endemic regions for brucellosis should keep in mind the family members of these patients that they are at a high risk for brucellosis, and they must screen the family members of these patients.

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