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ORIGINAL ARTICLE

Brucellosis Seroprevalence in Southeast Turkey (Diyarbakır)

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Abstract: This study was conducted to determine brucellosis seropositivity in patients suspected of having brucellosis who sought treatment at the Central Laboratory of Dicle University Medical Faculty Hospital in southeast Turkey (Diyarbakır). 20,663 serum samples were collected during the study (1 August 2001-31 December 2002), and 14,480 sera were tested in a 12-month period on a seasonal basis by Rose-Bengal slide agglutination (RBSA), and positive sera were titrated by standard tube agglutination (STA). Titration values of 1/160 and above were considered positive.

Of the 20663 sera, 463 (2.2%) tested positive on RBSA. Of these 463 sera, 267 (57.6%) tested positive on STA, with titers of 1/160 and higher. Seasonally, hospital attendance was highest in the summer and lowest in the winter. On STA tests done on RBSA-positive samples, the highest concentration of titers of 1/160+ was in the spring. In order to eliminate brucellosis in southeast Turkey, an endemic region for the disease, precautions must be increased, the unregulated slaughtering and consumption of animals must be prevented, and the consumption of raw, unpasteurized milk and of dairy products made from such milk must be halted.

Key Words: Brucellosis, Seroprevalence, Southeast Turkey.

Introduction

Brucellosis is a major public health problem in Mediterranean countries (1-4). In Turkey, particularly in the southeast, traditional eating habits, including the consumption of unpasteurized milk and dairy products (the production of fresh cheese and butter from milk immediately after it is obtained from the animal is particularly common in this region) are among the primary causes of the spread of brucellosis via the digestive tract in humans.

Brucellosis is a zoonotic infection with a variety of clinical pictures, and may be confused with a number of other illnesses in diagnosis. Thus, the importance of serological tests such as Rose-Bengal slide agglutination (RBSA) and standard tube agglutination (STA) in definitive diagnosis is evident. In addition, it should be mentioned that the results of STA are in good agreement with those obtained by ELISA in the detection of IgG and IgM (5).

In the present study, the sera of patients attending Dicle University's Medical Faculty Central Laboratory, hospitalized patients, or polyclinic patients with clinical suspicion of brucellosis in southeast Turkey (Diyarbakır), an endemic region for brucellosis, were analyzed by RBSA and STA within 2 time periods. All results in the first period (1 August 2001-31 December 2002) were evaluated, while results were evaluated in terms of seasonal distribution in the second period (1 December 2001-30 November 2002).

Materials and Methods

Results were evaluated in 2 different periods. All results were assessed in the first period (1 August 2001-31 December 2002), while seasonal evaluations were made from serum samples taken between 1 December 2001 and 30 November 2002.

Dead and stained suspensions obtained from the *Brucella abortus* S. 99 strain by the Ministry of Agriculture and Rural Affairs in Turkey were used as antigens. Sera yielding agglutination on RBSA underwent STA, in which dilutions of 1/10, 1/20, 1/40, 1/80, 1/160, 1/320 and 1/640 were prepared with 0.9% saline

solution, and 200 µl of each was mixed with 200 µl of antigen, and mixtures at dilutions of 1/20, 1/40, 1/80, 1/160, 1/320, 1/640, and 1/1280 were incubated for 48 h at 37 °C. If agglutinations were observed under the agglutinoscope, and if macroscopically there was clearness on the top and sediment on the bottom associated with agglutination, the results for that dilution were considered positive. Sera in which agglutination was observed at titers of 1/160 and higher were considered seropositive. All results were assessed statistically by the χ^2 test.

Results

Of 20,663 sera, 463 (2.2%) were found positive by the RBSA, of which 267 (57.6%) were found by STA to be positive at titers of 1/160 and above (Table 1). Of these 267 positive sera, 168 were from men and 99 from women.

The results of 1 year (1 December 2001-30 November 2002) are shown in Table 2. The 14,480 sera suspected of brucellosis examined during this period were analyzed in terms of seasonal distribution, and it was determined that the highest rates of hospital attendance occurred in the summer ($P < 0.005$), and the lowest in the winter ($P < 0.005$). The number of serum specimens evaluated seasonally was compared to the number of positive results obtained by RBSA, and the highest number was found in the winter; this difference was statistically significant ($P < 0.005$). RBSA-positive sera were examined by STA, and titers of 1/160 and higher were concentrated in the spring (75%); this difference was statistically significant ($P < 0.005$) (Figure), and the lowest rate was in the autumn (45.9%) ($P < 0.005$).

Discussion

The incidence of brucellosis infection in people from different countries and regions of the world is associated with epidemic cases, animal raising, and local traditions and practices, and is closely related to the animal resources in a given region. The consumption of unpasteurized milk and dairy products, such as cheese and butter, made from such milk in regions with high concentrations of infected animal populations is a major factor in the incidence of infection. Particularly in developing or underdeveloped countries, the determination of the true prevalence of brucellosis, despite all epidemiological studies, is made impossible by the inadequacy of poorly organized health center, and the failure of these centers to report all of the cases that come to their attention. It has been shown that regulations controlling the raising of domestic animals such as sheep, goats, cattle and pigs, and the requirement

Table 1. Distribution and percentages of titers obtained by STA from among 463 brucellosis cases testing positive by RBSA (throughout the study).

	1/20	1/40	1/80	1/160	≥1/320	Total
Number	29	60	107	93	174	463
Percentage (%)	6.2	12.9	23.1	20	37.5	100

Table 2. The number and seasonal distribution of patients with a clinical suspicion of brucellosis between 1 December 2001 and 30 November 2002, number and percentage of patients testing positive on RBSA in each season, and titer distributions according to STA of RBSA-positive sera.

	Patients		RBLA(+)		STA(+)<160		STA(+)>160	
Season	Number	%	Number	%	Number	%	Number	%
Winter	2797	19.3	78	2.8	23	29.5	55	70.5
Spring	3442	23.8	72	2.1	18	25.0	54	75.0
Summer	4688	32.4	73	1.6	31	42.5	42	57.5
Autumn	3553	24.5	61	1.7	33	54.1	28	45.9
Total	14480		284	2.0	105	37.0	179	63.0

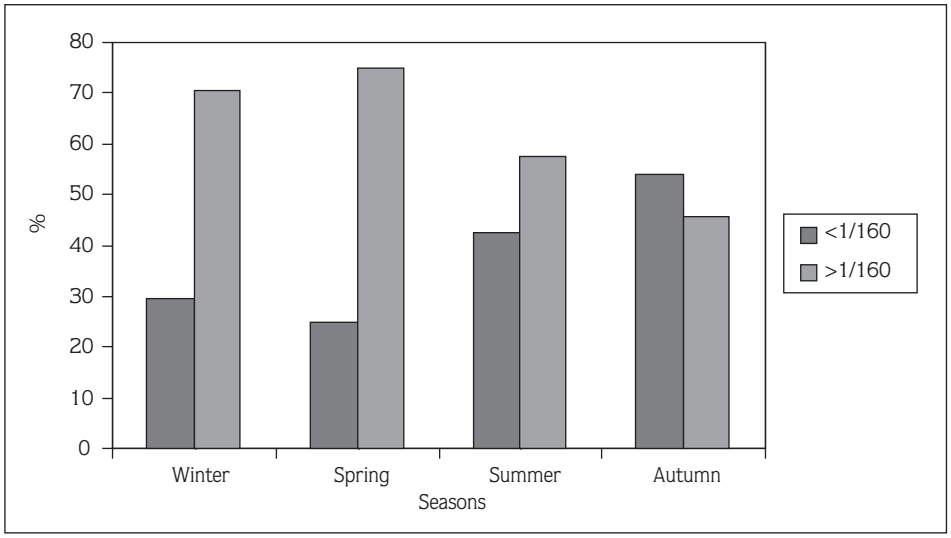

Figure. Seasonal percent distributions of by STA in sera testing positive on RBSA between 1 December 2001 and 30 November 2002.

of the pasteurization of milk reduce the incidence of human brucellosis. In Cyprus, for example, the coordinated efforts of medicine, public health, veterinarians, and state and non-state organizations have brought brucellosis entirely under control (6). In a similar way, its incidence is extremely rare in northern European countries. For example, only 4 cases of brucellosis were reported in Denmark between 1999 and 2000 (7). In Kentucky in the United States, only 3 cases were determined in a 4-year period (8). In a canton hospital in Switzerland, 11 cases were reported over a long period, but 9 of these involved workers from Mediterranean countries, particularly from rural areas (9).

Within Mediterranean countries, however, the incidence varies. In a 6-month epidemiological study conducted in southern Italy, 1294 serum samples were examined; seropositivity of 2.7% was determined in sera from Campania, and 3.8% in sera from Calabria (10).

Results obtained in studies performed serologically for clinical and epidemiological reasons in various countries at different times are shown broadly in Table 3. As is apparent in this table, efforts to bring brucellosis under control in underdeveloped countries as well as some Middle Eastern and Arab countries have not achieved the necessary success (11,12). Nonetheless, brucellosis is still an infectious agent for human health in the US in regions where pig husbandry is carried out, in slaughterhouse workers (25% of 1000 serum samples were found to be seropositive) (13) and in Hispanics in California, who commonly make cheese from unpasteurized milk (14).

If we evaluate seropositivity results from different regions of the world, we see that, in India, of 121 fever cases, 12 were brucellosis cases (4 acute, 8 chronic) (15). Again in India, in a study involving the workers of 2 different slaughterhouses, a seropositivity rate of 25.5% was determined in one (16), and of 20.6% in the other (17). While an incidence of nearly 15% was reported in Saudi Arabia in 2001 (18), a rate of 19.2% was determined in 1995 in the southern part of the country in a test of 4794 sera (19). In the Yemen, located on the Arabian peninsula, although there has been no comprehensive epidemiological study, brucella has been reported to be a major risk factor in terms of infection (20). In sera obtained from 66,962 people in 3 different regions of Mexico, seropositivity of 0.24%-13.5% was determined, with an overall average of 3.42%. Brucellosis was determined to be an endemic agent (21). In a study performed in different regions of Lebanon in high-risk groups (butchers, farmers, laboratory technicians, veterinarians), seropositivity rates of 34% were determined in Bigaa, 24% in Kisrwan, 16% in Sidon, 21% in Shouf and 12% in Aley (1). Seropositivities of 7.1% in farmers and 4.5% in

Table 3. Results from studies on the diagnosis of brucellosis performed serologically for clinical and epidemiological reasons in different countries during different time intervals.

Country	Year	Number of Cases or Percentage	References
Australia, Queensland	1989-1991	34	Robson et al., 1993 [23]
USA, Kentucky	4-year period	3	Dotson et al., 1990 [8]
USA, North Carolina	1992	18[a]	Trout et al., 1995 [24]
Jordan	1988-1997	7842	Abu., 2000 [11]
India, Kashmir	1992-1995	28[b]	Kadri et al., 2000 [25]
Israil, Taibe	1992	0.1766 %	Jaber et al., 1999 [26]
	1993	0.1750 %	
	1994	0.0570 %	
	1995	0.0010 %	
	1996	0.0025 %	
Saudi Arabia, Riyadh	1983-1995	0.013 %[c]	Khan et al., 2001 [27]
Saudi Arabia	Every year	8000	Memish et al., 2001 [12]
Greece, Northwestern	1979-1993	52[d]	Galanakis et al., 1996 [28]
Switzerland	1973-1992	11[e]	Canova et al., 1993 [9]
China	1991	61	Ding., 1993 [29]
Spain,Malaga	6-year period	339	Colmenero et al., 1990 [3]
Lebanon	1994-1998	63	Tohme et al., 2001 [4]
USA	1977-1978	25 %[f]	Gilbert et al., 1980 [13]
Denmark	1999-2000	4	Eriksen et al., 2002 [7]
Italy, Southern Region	1996	%2.7-3.8[g]	Terro et al., 1997 [10]

[a]All were working with pork, [b]3532 fever patients were examined, [c]Determined in a survey of pregnant women, [d]Performed among children 0-14 years old, [e]9 of the patients were foreign, from rural Mediterranean areas, [f]1000 serum samples from 3 different slaughterhouses were examined, [g]1294 serum samples from 2 different regions were examined.

veterinarians were found in a study in Eritrea (22). Even though the prevelance is not well known in Turkey, studies in different regions have shown that 2-6% seropositivity has been reported (30).

In the present study, seropositivity was 2.2% throughout the entire period, and 2% within 1 year. Our data bring up 2 interesting points. The first is that in winter, despite a low rate of patients seeking treatment, RBSA positivity is high, showing that brucellosis is a significant infectious agent epidemiologically in the Diyabakır region. The second is that spring, with the large number of sera of titers with 1/160 and above by STA, which is also the time during the consumption of raw milk and raw milk products is highest.

In conclusion, the 2.2% overall seropositivity clearly shows that the Diyarbakır region, although behind certain Middle Eastern, Far Eastern, and Arab countries, is an endemic region for brucellosis. We think that brucellosis will continue to be an endemic problem in this region for a long time to come because of the socioeconomic difficulties of the region, the lack of control over entries and exits of animals across the border or of animal movements within the region, unlawful and unregulated slaughtering of animals and selling of meat (the consumption of meat that has not been inspected by state officials), the consumption of raw milk and products made from raw milk without pasteurization, particularly in spring, the public's lack of information or the presence of incorrect information about the contagion of this agent, and the inadequacy of the disease reporting system.

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