

The Seroprevalence of *Brucellosis* Among Blood Donors in Hamadan Province, West of Iran

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Brucellosis is a widespread zoonotic disease. Person to person transmission is rare, whereas, blood transfusion and tissue transplantation are known sources of brucellosis especially in endemic areas. This study aimed to determine the prevalence of brucellosis in blood donors in Hamadan province, west of Iran. Blood specimens were collected from 600 blood donors who referred to Hamadan Blood Organization between January and June 2014. Antibody titer $\leq 1:80$ in wright and coombs wright and $\leq 1:20$ in 2ME tests were considered positive. Anti-*brucella* IgG checked with Enzyme-Linked Immunosorbent Assay (ELISA) that titer >12 U/mL were considered positive. Among 600 specimens, 30% were inhabitants of rural areas. 62.7% had history of consumption of unpasteurized dairy products and 10.5% had history of contact with animals. In our blood donor population, 2 cases (0.3%) with wright and coombs wright test, 3 cases (0.5%) with the 2ME test and 76 cases of 544 (14%) with ELISA were found positive for *Brucella* antibodies that 13% of them showed signs and symptoms of brucellosis before the last two years. Based on our findings and due to interrupt transmission of blood-borne infections, we suggest questioning from all blood donors about symptoms of brucellosis before blood donation.

Key words: Seroprevalence, *Brucellosis*, Blood donors, Iran.

Brucellosis is an important zoonotic disease that infects humans and several animals and it is a significant economic and major public health concern worldwide (Pappas *et al.*, 2006; Nouri *et al.*, 2014; Alavi *et al.*, 2014; Khani *et al.*, 2015). The prevalence of this disease among animals reduced in developed countries because of routine screening and vaccination program of animals but it still remains common in some areas such as Iran (Lopes *et al.*, 2010). *Brucella* transmission to humans occurs by ingestion of infected raw milk and milk products or by exposure

to infected animals. Person to person transmission of brucellosis is rare, however, blood transfusion and tissue transplantation are known sources of brucellosis especially in endemic areas that persistence of *brucella* in organs can cause subsequent problems such as osteoarticular complications (Hashemi *et al.*, 2007; Nouri *et al.*, 2014; Alavi *et al.*, 2014; Khani *et al.*, 2015). Akcokus *et al.* (2005) reported that *Brucella melitensis* in blood cultures of two newborns after exchange transfusion, however, the antibody titer against *Brucella* were not elevated and the newborns did not showed brucellosis. Polat *et al.* (2012) reported a 15-year-old boy with brucellosis after liver transplantation that *Brucella* antibody titer was 1:320. However, blood culture was negative for

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Brucella but Bone marrow culture was positive. Ting *et al.* (2013) in china showed that brucellosis 3 years after renal transplantation in a 45 year old man.

Several studies show that the risk factors of brucellosis in Iran are occupations of people, consumption of unpasteurized dairy products and direct contact with animals (Sofian *et al.*, 2013). Since, serological tests provide a very safe, fast and economical screening method of brucellosis diagnosing in human beings than the culture methods and because of the sensitivity and specificity of serological tests affect prevalence of brucellosis, this epidemiological study performed based on wright, coombs wright, 2-mercaptoethanol (2ME) tests, and Indirect Enzyme Linked Immunosorbant Assay (I-ELISA) (Corbel, 2010). Recently, ELISA is well suited for the rapid, routine diagnostic and also, it is a simple method which enjoys some advantages as portability of the equipment, hand-holding validation, and reliability for the analysis of samples (Mozaffari Nejad *et al.*, 2013; 2014; Eslami *et al.*, 2015).

Since, Hamadan province of Iran is an endemic area of brucellosis (Jow-Afshani *et al.*, 2005) and because there are few studies on the prevalence of brucellosis among blood donors; this study aimed to determine the prevalence of brucellosis in blood donors in Hamadan province, Iran.

MATERIALS AND METHODS

In our study, blood specimens were obtained from volunteer blood donors who referred to Hamadan blood organization between January and June 2014. The demographic data of each person like belonging to rural area, exposure to animals, consumption of unpasteurized dairy products, history of brucellosis, history of antibiotics therapy, occupation, age and sex documented. All blood donations were screened for five infectious diseases: HBV (hepatitis B virus) surface antigen and antibodies against HCV (Hepatitis C virus), HIV (Human Immunodeficiency Virus) and *Treponema pallidum* (syphilis) and *brucella*.

All of the 600 clotted blood specimens were centrifuged at $3000 \times g$ for 10 minutes due to obtained the sera and the wright, coombs wright

and 2ME tests were performed on all of them to detect *Brucella* antibodies. Both of the Wright and Coombs Wright tests measure the total amount of agglutinating IgM and IgG antibodies but any agglutination that occurs in 2ME test, is caused by IgG antibodies because of IgG resistance to action of 2ME. The tube agglutination test was carried out as follow: First, serial dilutions of the serum specimens were made (from 1:10 to 1:1, 280) and equal volumes of serial dilutions of the serums, *Brucella* antigen was added to test tube and incubated in an incubator at 37 °C for 24 h (Park *et al.*, 2012).

After incubation, the tubes were kept at room temperature for 30 min and the degree of agglutination recorded. The total number of Unit / milli Liter (U/mL) of the serum were calculated. 80 U/mL was considered as positive, 40 U/mL as doubtful and 20 U/mL as negative. Anti human globulin solution was added due to strong agglutination in the coombs wright test, and 2-mercaptoethanol solution was added in the 2-ME test due to determine acute brucellosis.

Brucella IgG ELISA kit (IBL co, Germany) was used for the accurate measurement of IgG class antibodies against *Brucella* in patient serums. In this method, controls or specimens were poured to the wells of a 96 wells plate which has been precoated with *Brucella* antigens to bind specific antibodies of the serum specimens and incubated. Following washing the wells to remove all unbound specimen material, anti-Human IgG conjugate labelled with horseradish peroxidase (HRP) was poured to the wells, duo to binds to the *Brucella*-specific antibodies. Tetramethyl benzidine (TMB) substrate, which is catalyzed by the HRP to produce a blue color product that changes to yellow after adding an acidic stop solution, was added. The density of yellow coloration is directly proportional to the amount of *Brucella* IgG sample captured in plate. Absorbance at 450/620 nm was measured in an ELISA reader. The absorbance values were converted into U/mL using the standard curve designed on controls and the results were considered as follow: <8 U/mL as negative, 8-12 U/mL as equivocal and >12 as positive (Varshochi *et al.*, 2011).

Statistical significance was determined by χ^2 and Fisher's Exact tests. The P value less than 0.05 was considered meaningful.

RESULTS AND DISCUSSION

Six hundred blood specimens were obtained from volunteer blood donors who referred to Hamadan blood organization between January and June 2014. The age range of blood donors population was between 20 to 63 years (mean age 37.78 ± 9.997). In our blood donor population, 565 (94.2%) male, 35 (5.8%) female and 329 (54.8%) self-employment, 140 (23.3%) staffer, 95 (15.8%) rancher, 33 (5.5%) house keeper, 3(0.5%) butcher and also, 371 (61.8%) illiterate, 154 (25.7%) diploma, 68 (11.3%) undergraduate, 6(1%) postgraduate, 1 (0.2%) physician were found. One hundred and eighty of our cases (30%) were inhabitants of rural areas, 376 (62.7%) had history of consumption of unpasteurized dairy products and 63 (10.5%) had history of contact with animals, 24(4%) had history of brucellosis that 7(29%) of them had at least one brucellosis patient in their family, qua 3 cases had 1patient, 2 cases had 2 patient and 2 cases had 5 patient in their family.

Serological tests including wright, Coombs Wright and 2-mercaptoethanol (2ME) were accomplished for all specimens. Of 600 specimens checked by the wright and coombs wright tests, 27 (4.5%) by both of the methods were positive that 2 cases were positive at dilution 1:80 and 573 (95.5%) were negative. All of the twenty seven agglutinated specimens in varies dilutions, were also investigated for antibodies by the 2ME test that 3 (0.5%) were positive. The ELISA test on 544 specimens showed that 434 (79.8%) were negative, 34 (6.2%) equivocal and 76 (14%) positive. Table 1 shows the distribution of *Brucella* antibodies results by serological methods. On Fisher2 s Exact Test, there were meaningful relationships between the ELISA test results and Occupation, contact

with animals, unpasteurized dairy consumption, history of brucellosis and antibiotics therapy ($P < 0.05$) but there was not any relationship between other serological tests (Wright and Coombs Wright tests) and these items ($P > 0.05$). On χ^2 test, there was not any meaningful relationship between all of the above serological tests and blood groups of blood donors ($P > 0.05$). All blood specimens were negative for HIV, HBV, HCV and syphilis infectious diseases.

Brucellosis is a zoonotic disease transmitted by cattle to man by direct contact with infected animals or indirectly by consumption of dairy products, especially in the developing countries. There are some investigations about prevalence of Brucellosis from the voluntary blood donors in all over the word. For example, in Mexico by Torres Padilla *et al.* (2004) reported the seroprevalence of brucellosis among 18 of 500 (3.6%) blood specimens from blood donors. The previous survey in India by Vaishnavi *et al.* (2007) of 292 specimens checked by the slide method, reported one positive (80 IU/mL) and another doubtful (40 IU/mL). Of the above 292 samples, 273 were also investigated for antibodies by Standard Tube Agglutination (SAT) that a lone specimen was found to be positive at 160 IU/mL and 46 (16.8%) had insignificant levels. There are some cases have been reported about our study that conducted by other researchers in Iran. In Ahvaz, southwest of Iran Shakurnia *et al.* (2013) reported the seroprevalence of brucellosis among 1450 blood donors as follow: 10(0.70%) by Rose Bengal Plate (RBP) test, 7 (0.34%) by STA test that 2 cases at dilution 1:40, 4 cases at dilution 1:80, 1 case at dilution 1:160 and 3 (0.20%) by the 2ME test were positive. Also, the same study at the Central Province of Iran by Sofian *et al.* (2013) among 897 serum specimens of healthy blood donors, reported 3 (0.33%) *Brucella* antibodies by STA at dilution 1:20 and only one sample (0.11%) at dilution 1:80 that this case was a 26 years old male who lives in city with history of consumption of unpasteurized dairy products and without any history of brucellosis infections. The 2ME test and blood culture were negative in all 4 subjects and none of them showed signs and symptoms of brucellosis in 6 months follow-up. Ghilian *et al.* (2009), among 300 serum specimens of blood donors, reported 19 (6.3%) and 17 (5.7%)

Table 1. Comparative results of serological tests on serum specimens from blood donors

Test	1:20	Dilution	
		1:40	1:80
		No (%)	
Wright test	15(2.5%)	10(1.7%)	2(0.3%)
Coombs Wright test	12(2%)	13(2.2%)	2(0.3%)
2ME test*	3(0.5%)	0	0

*2ME : 2-mercaptoethanol

Brucella antibodies by STA at dilution 1:80 and ELISA method, respectively, and only 2 cases (0.6%) were positive at low titer (1:20) by the 2ME test. The previous result in Boushehr province of Iran, Rabbani Khorasgani *et al.* (2007) among 10,500 serum specimens of blood donors, reported 0.076% and 0.057% *Brucella* antibodies by RBP and STA test, respectively and only one specimen (~0.01%) at low titer (1:20) by 2ME test. In our study, the level of *Brucella* antibodies was 4.5% and 14% by the tube agglutination and the ELISA tests, respectively. However, this level in the other studies is low but use of a high sensitivity and specificity test such as the ELISA can indicate the exact prevalence of brucellosis (Bashiri *et al.*, 2013).

Brucellosis is mainly an occupational disease and it is higher in rural areas where agriculture is the main occupation, therefore, in human varied based on occupations of people (2.5-18.6%) as regards it was reported in livestock farmers (2.6-21.6%), milkers (18.6%), butchers (2.5%) and veterinarians (5.3-11.1%) who have direct contact with animal and its products or who consume unsafe raw milk, therefore, consumption of dairy products and direct contact with animals are the risk factors of brucellosis, which showed in the current study too (Islam *et al.*, 2013; Alikhani *et al.*, 2005). Whereas, no *Brucella* screening test for blood donors exists, however, The Council of Europe guidelines recommend deferral, for at least 2 years following full recovery, investigation of blood donor population give us an overview of the endemicity of this disease. Since the disease has a distribution with higher prevalence in Hamadan province of Iran than the others, therefore, this study carried out to investigate the background prevalence of *brucella* antibodies in blood donors' population, so it is suggested that, in endemic areas for brucellosis, blood donors should be questioned about symptoms of brucellosis before donation, and if suspected, serological tests for brucellosis should be performed before blood transfusion.

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