BRIEF REPORT

Seroprevalence of *Brucella* antibody titer in rural population of Abhar, Iran

Bahram Amini[*], **Hamid Baghchesaraie**, **Davod Taimori Jelodar**

Department of Microbiology, Zanjan University of Medical Sciences, Zanjan, Iran

ABSTRACT

Background: Brucellosis is a major health problem worldwide, especially in developing countries. Following infection with *brucella*, antibodies appear in the serum and its titer detection can help us to evaluate the course and epidemic status of the infection. The aim of this study was to determine the seroprevalence of anti-brucella antibody titer in rural population of Abhar.

Patients and methods: In this descriptive study, 300 individuals were randomly selected for whom blood samples were screened to determine anti-brucella antibody titter, using standard tube agglutination (STA) and Coombs tests.

Results: The seroprevalence of anti-brucella antibody titer was 1.25% for STA and 4.58% for Coombs test. Totally, 14 (5.83%) individuals had titers of 1/80 or higher in STA and/or Coombs tests.

Conclusions: Our results emphasizes on the necessity of conducting comprehensive and scheduled program of seroprevalence survey, particularly in rural area, aimed at reducing brucella prevalence as well as to guide planning and resource allocation of decision makers for future interventions.

Keywords: *Brucellosis, Coombs test, Seroprevalence, Standard tube agglutination test.*
(Iranian Journal of Clinical Infectious Diseases 2010;5(3):152-155).

INTRODUCTION

Brucellosis is an important and widely prevalent zoonotic disease of man and animals (cattle, buffaloes, sheep, goats, dogs, etc) caused by *Brucella* organisms (1). The disease is considered by the Food and Agriculture Organization (FAO), the World Health Organization (WHO) and the Office International des Epizooties (OIE) as one of the most widely spread zoonoses in the world (2). Brucellosis is diagnosed either by isolation of *Brucella* organism in culture or by a combination of serological tests and clinical findings consistent with brucellosis. Isolation of the *Brucella* organism is the definitive means of diagnosis but in practice it is difficult due to the early tissue localization, exacting culture requirements of the organism and also prolonged time required for isolation. In practice, blood cultures are positive in 10-30% of brucellosis and the remainders are diagnosed serologically (3). Therefore, in the absence of bacteriological confirmation, a presumptive diagnosis can be made on the basis of a single high rising titer of specific antibodies. Among a variety of serological tests, STA (Standard Tube Agglutination) is the most widely used (4).

This disease can have a considerable impact on human and animal health as well as a

socioeconomic status, especially in rural areas that largely rely on livestock breeding and dairy products for their livelihood (5). Hence, attempt should be applied to screen the seroprevalence of brucella antibody, especially in rural area, for planning more efficient health promoting programs in the affected areas. For the same reason, in the present study, seroprevalence of brucella-antibody titer, using STA and Coombs test, was evaluated in rural population of Abhar in Zanjan province.

PATIENTS and METHODS

Study protocol was reviewed and approved by the Ethical Committee of Zanjan University of Medical Science. Participants were enrolled after giving informed consent according to local ethics committee guidelines.

In this descriptive study, performed during spring 2007, totally 300 individual, were randomly selected from Sonbolabad, Amidabad, Usefabad, Khairabad and hosainabad villages in Abhar district of Zanjan province. Following completion of questionnaire on name, age, sex and place of living, blood samples were collected and subsequently transferred, in a cool box, to Microbiology Department of Zanjan University of Medical Science. Serum was removed by centrifugation of coagulated blood sample, at 3000rpm for 10 min and stored at -20 ºC for later serological analysis.

Wright agglutination test (STA): All sera were routinely diluted from 1:20 to 1:1280 and STA test was performed, as previously described (6), on serum dilutions using *Brucella abortus* standard antigen obtained from the Pasteur Institute of Tehran, Iran. Serial dilutions of sera were mixed with the standard tube agglutination antigen and then incubated at 37°C for about 24 hours. Each batch of the test, included a positive control and a negative control.

Coombs Wright: The anti-human globulin (Coombs) test was performed, as an extension of

SAT, for detection of incomplete, blocking or non-agglutinating IgG antibodies, as previously described (7) by using anti-human globulin reagent (anti-IgG; Ortho Diagnostic Systems, N.J.). Positive results were defined as any sample showing agglutination with SAT and/or Coombs at any level in comparison with the control tube.

In the present study, titrations of 1:80 was accepted as exposure to *Brucella* (8), and 1:160 or greater as presumptive evidence of infection with *Brucellae*, which should be clinically conformed by clinician (9,10). Therefore, in our setting, the titrations of 1:80 and greater was accepted as seropositive.

RESULTS

Of 300 samples, 60 were discarded as a result of hemolysis. Remaining 240 samples belong to 98 (40.8%) males and 142 (59.2%) females. Tables 1 demonstrates results of serological tests (STA, Coombs) and frequency distribution in 240 samples.

In this study, the seroprevalence of anti-brucella antibody titer was 1.25% for STA and 4.58% for Coombs test .Totally, 14 (4.58%) individuals had titers of 1/80 or higher in STA and/or Coombs tests.

Table 1. Results of serological tests in 240 samples

Titer	STA No (%)	STA M/F ratio	Coombs No (%)	Coombs M/F ratio
Negative	207(86.3)	84/123	172(71.7)	71/101
1/20	27(11.3)	11/16	40(16.7)	18/22
1/40	3(1.3)	2/1	17(7.1)	5/12
1/80	2(0.7)	1/1	8(3.3)	3/5
1/160	-	-	2(0.8)	1/1
1/320	1(0.4)	1/0	-	-
1/640	-	-	1(0.4)	0/1

STA: Standard Tube Agglutination; **M/F:** Male to female

DISCUSSION

The control of brucellosis in animals, and thus prevention of disease in humans, depends mainly upon the use of efficient diagnostic procedures

(11). Although a number of techniques have been developed for measuring brucella antibodies, STA is probably the most widely used (9).

In the present study, the seroprevalence of brucella antibody titer using STA and Coombs test were 1.25% and 4.57%, respectively, which was slightly higher than a study conducted in Turkey (12). In our country, brucellosis represents a major health problem and continuously reported with increasing frequency from various parts of the country (4). In a study performed among nomads in Khozestan province (south-west of Iran), the reported seroprevalence of brucella was 6.3% (13). In another study conducted on blood donor in Boshehr province (South of Iran), of 10,500 samples, only six sera, with different titers (1/20–1/40), were detected (14).

Limited report has been released on seroprevalence of brucella antibody determined by Coombs test. In a study performed on agricultural workers in Spain, of 482 subjects, 15(3.1%) had titers of 1/40 or higher to Wright and/or Coombs tests (15). Since serological tests are aimed to find any foot trace of *brucella* in affected individuals, in addition to main serological test, conduction of supplementary tests are recommended for relatively precise estimation of its seroprevalence.

Our results emphasizes on the necessity of conducting comprehensive and scheduled program of seroprevalence survey, particularly in rural area, aimed at reducing the prevalence of brucellosis as well as to guide planning and resource allocation of decision makers for future interventions.

ACKNOWLEDGEMENT

REFERENCES

1. Chachra D, Saxena HM, Kaur G, Chandra M. Comparative efficacy of Rose Bengal plate test, standard tube agglutination test and Dot ELISA in immunological detection of antibodies to Brucella abortus in sera. J Bacteriol Res. 2009;3:030-033.

2. Swail ES, Schoonman L. Human brucellosis: Seroprevalence and risk factors related to high risk occupational groups in Tanga municipality, Tanzania. Zoonoses Public Health. 2009;56:183–87.

3. Agasthya AS, Isloor S, Prabhudas K. Brucellosis in high risk group individuals. Indian J Med Microbiol. 2007;25:28-31.

4. Rajaii M, Naghili B, Pourhassan A. Comparison of ELISA and STA tests in diagnosis of brucellosis. Iranian Journal of Clinical Infectious Diseases 2006;1(3):145-47.

5. Al-Majali AM, Talafha AQ, Ababneh MM, Ababneh MM. Seroprevalence and risk factors for bovine brucellosis in Jordan. J Vet Sci. 2009;10:61-65.

6. Alton CG, Jones IM, Pietz DE, editors. Laboratory techniques in brucellosis. 2nd edition. WHO: Geneva, Switzerland, 1975.

7. Araj GF, Kattar MM, Fattouh LG, Bajakian KO, Kobeissi SA. Evaluation of the PANBIO Brucella Immunoglobulin G (IgG) and IgM enzyme-linked immunosorbent assays for diagnosis of human brucellosis. Clin Diag Lab Immunol. 2005;12:1334-35.

8. Cetinkaya Z, Aktepe OC, Ciftci IH, Demirel R. Seroprevalence of human brucellosis in a rural area of western Anatolia, Turkey. J Health Popul Nutr. 2005;23:137-41.

9. Ertekm M, Yazgi H, Özkurt Z, Ayyildiz, Parlak M. Comparison of the diagnostic value of the Standard Tube Agglutination Test and the ELISA IgG and IgM in patients with brucellosis. Turk J Med Sci. 2006;36:159-63.

10. Yildiz F, Tanyel E, Hatipoğlu CA, Ertem GT, Tülek N. Evaluation of Brucella tube agglutination test in patients with brucellosis, patients with bacterial infections other than brucellosis and healthy subjects. Mikrobiyol Bul. 2005;39:211-7.

11. Maha H, Mohamed TR, Khoudair RM. Serodiagnosis of brucellosis in cattle and humans in Egypt. Immunology. 2009,241:223-26.

12. Vancelik S, guraksin A, Ayyildiz A. Seroprevalence of human brucellosis in rural endemic areas in eastern Turkey. Trop Doct. 2008;38:42-3.

13. Alavi M, Rafiei A, Nikkhooi A. The effect of lifestyle on brucellosis among nomads in Khuzestan province of Iran. Pak J Med Sci. 2007;23:358-60.

14. Khorasgani MR, Esmaeili H, Pourkarim MR, Mankhian AR, Salehi TZ. Anti-brucella antibodies in blood donors in Boushehr, Iran. Comp Clin Pathol. 2008;7:267–69.

15. Villamarín-Vázquez JL, Chiva-Nebot F, Arnedo-Pena A. Seroprevalence of brucellosis in agricultural workers in the coastal zones of Castellón, Spain. Salud Publica Mex. 2002;44:137-9.