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Comparison of Standard Seroagglutination Tests and ELISA for Diagnosis of Brucellosis in West Azerbaijan Province, Iran

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Abstract: Brucellosis is still a major health concern worldwide. The aim of the present study was to compare sensitivity and specificity of the STA (Standard Tube Agglutination) test and IgM and IgG ELISA (Enzyme Linked Immunosorbent Assay) in diagnosis and follow up of the brucellosis. A total of 280 patients were studied. STA test materials were prepared by Razi Institute (Tehran, Iran), while IgM and IgG ELISA kits were manufactured by GmbH (IBL, Germany). Totally, 143 cases were positive in both STA and ELISA evaluation, hence, 102 cases were positive in STA test. ELISA was demonstrated to be more sensitive when compared with STA. Although, STA is a widely applied test, it isn't precise and it cannot differentiate acute and chronic states of brucellosis. Our data suggest that IgM and IgG ELISA may be a suitable test for diagnosis and follow up of brucellosis.

Key words: Brucellosis, ELISA, standard tube agglutination

INTRODUCTION

Brucellosis is an important public health concern in developing countries. The disease is found globally, however is more common in the Mediterranean countries, the Arabian Peninsula, the Indian subcontinent and part of Mexico and central and South America (Young, 1994; Rajaii *et al*., 2006). In Iran, Brucellosis represents a major health problem and continuously reported with increasing frequency from various parts of the country (Karimi, 2000). Particularly in the northwest, traditional eating habits, including the consumption of unpasteurized milk in Iran 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 cause of the spend of brucellosis via the digestive tract in humans.

Brucellosis is a zoonotic infection with a variety of clinical pictures and may be fused with a number of other illnesses in diagnosis. Hence, several serological tests have been developed for the diagnosis of human brucellosis, including the standard agglutination tube (STA) test, anti-human globulin (Coombs) test, indirect fluorescence antibody (IFA) test and enzyme-linked Immunosorbent assay (Araj *et al*., 2005).

The purpose of this study was to investigate, the seroprevalence of human brucellosis in the general population. In west Azerbaijan province of Iran, by using Rose-Bengal Agglutination (RBA) test, STA, Coombs, 2 Mercapthoethanol (2ME) test and *Brucella* IgG and IgM ELISA Kits (IBL, Germany) and comparison of this methods to laboratory of human brucellosis.

MATERIALS AND METHODS

Clinical specimens, from May 2007-2008, a total of 280 peripheral samples taken from suspected patients with Brucellosis in the central diagnostic laboratories of west Azerbaijan province, Iran, before starting appropriate antibiotic treatment.

The diagnosis of Brucellosis was established according to one of the following criteria:

- Isolation of *Brucella* sp. (Moyer and Holcomb, 1995) in blood culture.
- The presence of a compatible clinical picture together with the demonstration of specific Antibodies at significant titers or sero conversion. Significant titers were considered to be a Wright's seroagglutination titer of ≥1/160.

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Standard Tube Agglutination (STA) test material by Razi Institute (Tehran, Iran), while IgG and IgM ELISA Kit were manufactured by GmbH (IBL, Germany).

Bacteriological and serological techniques: Two blood cultures, as well as a serological battery including the Rose Bengal plate agglutination test, Wright's seroagglutination test, 2-Mercapthoethanol (2ME), Coomb's Wright, IgG and IgM ELISA test were done for all of the serum samples.

Bacterial analysis: Blood culture were processed according to standard techniques (Moyer and Holcomb, 1995), With incubation being maintained for 30 days and blind subcultures performed on chocolate agar and *Brucella agar* (Merck, Germany) after 10, 20 and 30 days.

These subcultures were incubated at 37°C in a 5-10% CO_2 atmosphere for 3 days. If growth appeared, the suspected colonies were identified by colonial morphology; Gram staining; oxidase, catalase and urease test and positive agglutination with specific antiserum.

Wright agglutination test:

A = Preparation of 2 fold serial dilution of serum samples (starting dilution of 1/20).
B = Total 0.5 mL of antigen solution (*Brucella abortus*) was added to each tube.
C = The tubes were shake gently and were incubated in 37°C for overnight.
D = The latest tubes that showed agglutination was considered the titer of serum antibody.

2ME (2-Mercapthoethanol): This test was performed like Wright agglutination but the solution of antigen was mixed with a reductive chemical agent (2ME) that reduces the S = S bound in IgM molecules.

Thus, ultimately acute, sub acute and chronic status of brucellosis could be distinguished.

Coomb's wright: This test was also performed like wright agglutination however, antihuman globulin (AHG) was added to each tube to appear in complete or blocking antibodies in serum samples following 3 times washing and centrifugation.

IgG and IgM ELISA test:

A = Total 100 μL of prediluted serum samples were added to each of wells that coated *Brucella melithensis.*
B = The micro-plate was incubated at room temperature for an hour.
C = The wells were washed three times with washing solution.
D = Total 100 μL of conjugate was added to each of the wells.
E = The micro-plat was incubated for 30 min at room temperature.
F = The wells were washed again as described in stage.
G = Substrate and chromogen solution was added to each well.
H = After incubation (10 min), the stopping solution was added and then absorbencies were measured at 415-620 nm in Micro plate reader (Stat Fax ® 2100, USA).

RESULTS AND DISCUSSION

Among the 280 cases, 106 had no any titers with STA and ELISA test. But 143 cases were positive in both STA and ELISA evaluation, hence, 31 revealed low titers with STA (≥1/160), while a variety of positive titers have been recorded with IgM and IgG ELISA. Meanwhile, 4 cases were positive with STA but negative with IgM and IgG ELISA test. Table 1 represents STA and IgG ELISA results of 8 high-titer group patients.

Brucellosis, a zoonotic infection, is still a major health concern among Iranian population. Vaccination of ruminants (especially sheep and cows) against *Brucella* and use of pasteurized milk or milk products could decrease the rate of infection in the human societies (Arime *et al*., 2005). On the other hand, prompt diagnosis and treatment of infection is another efficient strategy (Ker *et al*., 1968).

Isolation of microorganism from blood culture is a qualified monitoring technique, but requires relatively long time or experienced Lab technician (Simmaro *et al*., 2001).

In the present study, none of the patients revealed to be blood culture-positive. However, we have encountered the following limitations: the cross-reaction between *Brucella* and other microorganisms, the presence of blocking or excessive level of antibodies that ensue false negative reactions.

Table 1: STA (Standard Test Agglutination), IgM and IgG ELISA results of 8 high-titer group *brucellosis* patients

			Agglutination		
No.	IgM ELISA	IgG ELISA	Wright	Coomb's	2 ME
1	2.01	3.512	1/640	1/640	1/640
2	1.8	2.4	1/1280	1/1280	1/640
3	1.85	3.56	1/1280	1/1280	1/1280
4	3.12	2.78	1/1280	1/1280	1/940
5	2.55	3.46	1/160	1/640	1/160
6	1.45	0.045	1/640	1/640	1/40
7	1.265	3.495	1/640	1/1280	1/640
8	0.1	3474	1/320	1/320	1/320

CONCLUSION

In conclusion, ELISA was demonstrated to be more sensitive when compared with STA, indeed, 31 cases that were negative with STA were revealed to be positive with ELISA, however, 4 cases that were positive with STA, revealed to be negative with IgM and IgG ELISA test, therefore, we concluded that it is advisable to perform both IgG and IgM ELISA technique in order to achieve higher accuracy.

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REFERENCES

Araj, G.F., M.M. Kattar, L.G. Fattouh, K.O. Bajakian and S.A. Kobeissi, 2005. Evaluation of the PANBIO *Brucella* Immunoglobulin G (IgG) and IgM Enzyme-Linked Immunosorbent Assays for Diagnosis of Human Brucellosis. CDLI, 12 (11): 1334-1335. DOI: 10.1128/CDLI.12.11.

Arime, S.M., E. Koroti and E.K. Kangethe, 2005. Risk of infection with *Brucella abortus* and *Escherichia coli* O157: H7 associated with marketing of unpasteruized milk in Kenia. Acta Tropica, 91 (1): 1-8. DOI: 10.1016/j.actatropica.2005.05.012.

Karimi, A., 2000. Active case finding of communicable diseases in the south of Islamic Republic of Iran. EMHJ, 6 (2/3): 487-493. PMID: 11556041.

Ker, W.R., J.D. Mccaughey and D.J. Coglban, 1968. Techniques and interpretations in the serologic diagnosis of brucellosis in man. J. Med. Microbiol., 1: 181-193. PMID: 4989595.

Moyer, N. and P.L.A. Holcomb, 1995. *Brucella*. Manual of Clinical Microbiology. In: Murray, P.R., E.J. Baron, M.A. Pfaller, F.C. Tenover and R.H. Yolken (Eds.). 6th Edn. Am. Soc. Microbiol., Washington, D.C., pp: 382-386. ISBN: 1-55581-086-1. DOI: 10.1016/S0966-842X (00) 89003-4.

Rajaii, M., B. Naghili and A. Pourhassan, 2006. Comparison of ELISA and STA tests in diagnosis of Brucellosis Iranian. IJCID, 1 (3): 145-147. www.idtmrc.ac.ir/Journal/JournalArticleList.aspx?uid=9-26k.

Simmaro, E., J. Perrez, J. Ruiz and J. Gomes, 2001. Failure to detect *Brucella melitensis* in 3 hemo culture systems. J. Clin. Microbiol. Infect. Dis., 19 (1): 35-36. PMID: 11256249.

Young, E.J., 1994. An overview of human brucellosis. Clin. Infect. Dis., 21: 283-290. PMID: 8562733.