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# Comparative Evaluation of Two Serological Tests for Screening of Bovine Brucellosis

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**Abstract:** Brucellosis is an economically important disease of livestock causing reproductive problems and economic losses from international trade bans. The present study was conducted to evaluate the efficacy of Rose Bengal Plate test (RBPT) and Enzyme linked Immunosorbent assay (ELISA) for the detection of antibodies against brucella infection and to compare between the serum ELISA and the milk ELISA for diagnosis of bovine brucellosis. Blood samples of 7879 dairy cattle raised in two farms located at Eastern Province, Saudi Arabia were examined. The results revealed that RBPT could detect more seropositive animals (16.7%) than ELISA test (13.6%). In addition, RBPT and ELISA showed perfect agreement (kappa value=0.87). By comparing between the milk ELISA and the serum ELISA for diagnosis of bovine brucellosis, the results reveled that high percentage of positive animals could be detected by serum ELISA than milk ELISA. Bovine brucellosis is endemic disease and has public health concern; the periodical serological screening has great value to discover the status of the herd.

**Key words:** Bovine brucellosis · RBPT · ELISA · Serum · Milk · Cattle

## INTRODUCTION

Brucellosis is a highly contagious zoonotic disease affecting various livestock animals and human [1-3]. The disease is worldwide distributed, has public health concern and of great economic importance [4-6].

Brucella infection can be transmitted mainly via direct contact with infected animals and their discharges or indirect through ingestion of contaminated milk with the microorganism [7, 8]. Brucellosis can affect both sexes causing abortion, metritis, stillbirth, retained placenta and mastitis in females while cause orchitis and arthritis in males [1,9].

The control and eradication program of brucellosis depends mainly on serological examination [10-12]. Nevertheless, the isolation of *Brucella* spp. from positive serological animals is considered as the most appropriate diagnostic method. At present, there is no single serological test able to detect a positive animal at different stage of infection and a combination of confirmatory and screening test is the most appropriate method for detection of infected animal [13-15].

In addition, several serological tests can be used as screening tests for the detection of brucellosis among dairy cattle such as Rose Bengal plate test (RBPT) and indirect enzyme-linked immunosorbent assays (ELISAs) [16,17]. RBPT is simple agglutination test, used as screening test but give false positive results due to cross reaction with other bacteria. So, low pH of antigen reduces agglutination of IgM and non-specific reaction [18].

Moreover, ELISA test is highly sensitive and can be applied on large scale as screening test for Brucellosis and can be used for detection of antibodies either in milk or serum of animals [18-20].

The present work attempt to evaluate and compare between RBPT and ELISA as screening test for detection of bovine brucellosis.

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## MATERIALS AND METHODS

**Samples:** A total of 7879 blood samples were collected from dairy cattle which were raised in two farms at Eastern Province, Saudi Arabia during 2018. Cows were screened to the prevalence of bovine brucellosis. All animals had not vaccinated against Brucellosis and were apparently healthy.

Blood samples (5ml) were collected from jugular vein of each animal using vacuum tubes containing ethylenediaminetetraacetic acid (EDTA) as an anticoagulant, followed by centrifugation at 13000xg for 10 min to separate the sera and preserved it at -20°C until the serological analysis.

Also, 200 milk samples were collected from bulk tank milk of the same animals (7879 cattle) with unknown status of brucellosis to detect presence of anti-brucella antibodies using the ELISA test.

**Serological Analysis:** All serum samples of all animals were examined serologically using RBPT and commercial ELISA kit, Brucella abortus antibody test (IDEXX, Main, USA).

Concerning Rose Bengal plate test, equal volume from serum of examined animal and antigen were mixed for 4 min and observed for the occurrence of agglutination. The presence of agglutination considered positive.

Moreover, the antibodies against *B. abortus* were detected in serum samples of the same animals using Brucella abortus antibody test kit (IDEXX, Main, USA) according to manufacturer's instruction. The optical density was measured at 450nm using ELISA micro-plate reader. The sample with OD <80% was considered negative while ≥ 80% considered positive.

The results of both serological tests (RBPT and ELISA) were compared to determine the agreement between them.

In addition, the presence of antibodies against *B. abortus* were determined in milk of the same examined animals using IDEXX Brucellosis Milk X2 test Kit (IDEXX, Main, USA) according to manufacturer's instruction. The optical density was measured at 450nm using ELISA micro-plate reader. The sample with OD <60% considered negative while ≥ 60% considered positive

**Statistical Analysis:** The data of serological examination were analyzed using Chi-square (SPSS, IBM) while the comparison and agreement between RBPT and ELISA were evaluated on the basis of kappa (κ) value.

## RESULTS

The presence of anti-brucella antibodies was determined using RBPT and ELISA test. Out of 7879 samples, 1320 (16.7%) were positive with RBPT while ELISA test was able to detect antibodies only in 1074 animals (13.6%; Table 1).

RBPT detected more animals (246 positive and 20 negative) than ELISA. Although, there was perfect agreement (K=0.87) between RBPT and ELISA test (Table 2).

In addition, the level of antibodies against *B. abortus* varied in milk and serum of the same animals where ELISA test was positive with fewer milk samples of 120 (1.7%) cattle in comparison with 1074 (13.6%) cattle showed antibodies in sera (Table 3).

Table 1: Results of serological examination of serum samples

| | RBPT | | | ELISA | | |
|---|---|---|---|---|---|---|
| Total serum samples | Positive (%) | Negative (%) | 95% CI | Positive (%) | Negative (%) | 95% CI |
| 7879 | 1320 (16.7) | 6559 (83.2%) | 15.9-17.5 | 1074 (13.6) | 7129 (90.5) | 12.8-14.41 |

Confidence interval (CI)

Table 2: Correlation between RBPT and ELISA for detection of antibodies in sera

| | ELISA | | | | |
|---|---|---|---|---|---|
| RBPT | Positive | Negative | Total | Kappa Value | 95% CI |
| Positive | 1074 | 246 | 1320 | 0.8701 | 0.8701-0.8827 |
| negative | 20 | 6539 | 6559 | | |
| Total | 1094 | 6785 | 7879 | | |

Confidence interval (CI)

Table 3: Detection of antibodies against B. abortus in milk and sera using ELISA test

| | Milk | | | Serum | | |
|---|---|---|---|---|---|---|
| Number of examined animals | Positive (%) | Negative (%) | 95% CI | Positive (%) | Negative (%) | 95% CI |
| 7879 | 120 (1.5) | 7759 (98.4) | 1.2-1-8 | 1074 (13.6) | 7129 (90.5) | 12.8-14.41 |

## DISCUSSION

Bovine brucellosis is worldwide distributed disease and endemic in several countries including Saudi Arabia, cause severe economic losses and have public health concern [21]. Moreover, infected cows were shedding brucella organism in milk up to nine years after recovery [22]. The isolation of bacteria still the standard method for diagnosis of brucellosis but the serological tests can be used for diagnosis of the disease on large scale [23].

Therefore, the present study compared efficacy of the two serological tests for detection of antibodies against brucella infection and compared the level of antibodies in milk and serum of infected animals. By comparing the overall results of these serological tests for brucellosis, it can be seen that RBPT gave the highest positive percentage (16.7%), whereas ELISA showed the lowest rate (13.6%). In the present study, ELISA was found to be more sensitive, which is in concurrence with the previous study [24]. On the contrary, [25] reported that RBPT is more sensitive than ELISA, when applied to buffalo sera.

In addition, RBPT can detect more animals than ELISA, detected 246 while ELISA detected 20 positive animals those were negative with RBPT. There was a perfect agreement between RBPT and ELISA test. The serum samples which were positive by the RBPT were negative by ELISA. This might result from the cross-reacting antibodies in the RBPT. Interestingly, twenty of serum samples were positive with ELISA were negative with RBPT. This may refer to the higher sensitivity of the ELISA than the conventional serological tests such as RBPT [26, 27].

The level of antibodies against *B. abortus* varied in milk and serum of the same animals where ELISA test was positive with fewer milk samples (1.7%) of cattle in comparison with bovine sera of the same animal (13.6%). Similarly, the Brucella antibodies increased in sheep milk sample (13.8%) compared sera (2.33%) [28]. These finding could be attributed to nature of samples (colostrum, mastitis milk or clotted milk) which might affect the sensitivity of milk-ELISA [29]. Also, clinical and physiological status of examined animals might influence the results of milk-ELISA where the transport of IgG from blood to milk varied between animals [30].

Deviation of this study with earlier researchers might refer to the presence or the absence of antibodies in the samples from selected animals in various clinical and physiological conditions, problem in IgG transport from blood to milk against brucellosis and nature sample influence over each diagnostic test (colostrum, mastitis milk, clotted milk and blood) and individual variations in diagnostic sensitivity and specificity of each test.

## CONCLUSION

The present study confirms the circulation of *B. abortus* among dairy cattle in Saudi Arabia. ELISA is more sensitive and large-scale test for screening of bovine brucellosis in comparison with RBPT. In addition, milk ELISA can be replaced serum ELISA for diagnosis of brucellosis in dairy cattle but it is still less sensitive than serum ELISA.

## REFERENCES

1. Abd El-Wahab, E.W., Y.M. Hegazy, W.F. El-Tras, A. Mikheal, A.F. Kabapy, M. Abdelfatah, M. Bruce, M.M. Eltholth, 2019. A multifaceted risk model of brucellosis at the human–animal interface in Egypt. Transbound Emerg Dis, 66: 2383-2401.
2. Khan, M.Z.M. Zahoor, 2018. An overview of brucellosis in cattle and humans and its serological and molecular diagnosis in control strategies. Trop. Med. Infect Dis., 3: 65.
3. Seleem, M.N., S.M. Boyle and N. Sriranganathan, 2010. Brucellosis: a re-emerging zoonosis. Vet. Microbiol., 140: 392-398.
4. Madut, N.A., A. Muwonge, G.W. Nasinyama, J.B. Muma, J. Godfroid, A.S. Jubara, J. Muleme, C. Kankya, 2018. The sero-prevalence of brucellosis in cattle and their herders in Bahr el Ghazal region, South Sudan. PLoS Negl Trop. Dis., 12(6): e0006456.
5. Santos, R.L., T.M. Martins, Á.M. Borges and T.A. Paixão, 2013. Economic losses due to bovine brucellosis in Brazil. Pesq Vet. Bras., 33: 759-764.

6. Lindahl-Rajala, E., T. Hoffman, D. Fretin, J. Godfroid, N. Sattorov, S. Boqvist, A. Lundkvist and U. Magnusson, 2017. Detection and characterization of *Brucella* spp. in bovine milk in small-scale urban and peri-urban farming in Tajikistan. PLoS Negl Trop Dis., 11(3): e0005367.
7. Ali, S., S. Akhter, H. Neubauer, F. Melzer, I. Khan, E.N. Abatih, H. El-Adawy, M. Irfan, A. Muhammad, M.W. Akbar and S. Umar, 2017. Seroprevalence and risk factors associated with bovine brucellosis in the Potohar Plateau, Pakistan. BMC Res Notes, 10(1): 73.
8. Adesokan, H.K., P.I. Alabi, J.A. Stack and S.I. Cadmus, 2013. Knowledge and practices related to bovine brucellosis transmission amongst livestock workers in Yewa, south-western Nigeria. J. S Afr., 84: 1-5.
9. Aparicio, E.D., 2013. Epidemiology of brucellosis in domestic animals caused by Brucella melitensis, Brucella suis and Brucella abortus. Rev. Sci. Tech., 32: 53-60.
10. Franc, K., R. Krecek, B. Häsler and A. Arenas-Gamboa, 2018. Brucellosis remains a neglected disease in the developing world: a call for interdisciplinary action. BMC Public Health, 18: 1-9.
11. Islam, M.A., M.M. Khatun, S.R. Were, N. Sriranganathan and S.M. Boyle, 2013a. A review of Brucella seroprevalence among humans and animals in Bangladesh with special emphasis on epidemiology, risk factors and control opportunities. Vet. Microbiol., 166: 317-326.
12. Nielsen, K. and W. Yu, 2010. Serological diagnosis of brucellosis. Prilozi, 31: 65-89.
13. Al-Dahouk, S. and K. Nöckler, 2011. Implications of laboratory diagnosis on brucellosis therapy. Expert Rev Anti Infect Ther., 9: 833-845.
14. Gwida, M.M., A.H. El-Gohary, F. Melzer, H. Tomaso, U. Rösler, U. Wernery, R. Wernery, M.C. Elschner, I. Khan and M. Eickhoff, 2011. Comparison of diagnostic tests for the detection of Brucella spp. in camel sera. BMC Res Notes, 4: 525.
15. McGiven, J., J. Tucker, L. Perrett, J. Stack, S. Brew and A. MacMillan, 2003. Validation of FPA and cELISA for the detection of antibodies to Brucella abortus in cattle sera and comparison to SAT, CFT and iELISA. J. Immunol. Methods, 278: 171-178.
16. Al-Mashhadany D.A., 2019. Application of Rose Bengal Test and ELISA in Meat Juice for Monitoring of Brucellosis among Cattle Carcasses at Erbil City, Iraq. Bulletin of University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca Animal Science and Biotechnologies, 76: 14-20.
17. Nielsen, K., 2002. Diagnosis of brucellosis by serology. Vet. Microbiol., 90: 447-459.
18. Terzi?, G., O. Buyuktanir, O. Genc, A. Gucukoglu and N. Yurdusev, 2010. Detection of Brucella antibody and DNA in cow milk by ELISA and PCR methods. Kafkas Univ. Vet. Fak. Derg., 16: S47-S52.
19. Matope, G., J. Muma, N. Toft, E. Gori, A. Lund, K. Nielsen and E. Skjerve, 2011. Evaluation of sensitivity and specificity of RBT, c-ELISA and fluorescence polarisation assay for diagnosis of brucellosis in cattle using latent class analysis. Vet Immunol Immunopathol, 141: 58-63.
20. Shafee, M., M. Rabbani, A.A. Sheikh and A. Razzaq, 2011. Prevalence of bovine brucellosis in organized dairy farms, using milk ELISA, in Quetta City, Balochistan, Pakistan. Vet. Med. Int, 2011.
21. Aloufi, A.D., Z.A. Memish, A.M. Assiri and S.J. McNabb, 2016. Trends of reported human cases of brucellosis, Kingdom of Saudi Arabia, 2004–2012. J. Epidemiol. Glob Health, 6: 11-18.
22. Higgins, J.L., M. Gonzalez-Juarrero and R.A. Bowen, 2017. Evaluation of shedding, tissue burdens and humoral immune response in goats after experimental challenge with the virulent Brucella melitensis strain 16M and the reduced virulence vaccine strain Rev. 1. PLoS One 12.
23. Asadi, F.T., S.H. Hashemi, M.Y. Alikhani, A. Moghimbeigi and Z. Naseri, 2016. Clinical and diagnostic aspects of brucellosis and antimicrobial susceptibility of Brucella isolates in Hamedan, Iran. Jpn. J. Infect Dis., 2016: 2133.
24. Islam, M.R.U., M.P. Gupta, P.K. Sidhu, G. Filia, H.M. Saxena and T.A. Shafi, 2013b. Comparative evaluation of indirect enzyme linked immunosorbent assay, rose bengal plate test, microagglutination test and polymerase chain reaction for diagnosis of brucellosis in buffaloes. Turk J. Vet. Anim. Sci., 37: 306-310.
25. Mittal, V., M. Kumar and T. Ambwani, 2005. Seroepidemiological pattern of brucellosis among livestock of district Udham Singh Nagar in Uttaranchal. Indian J. Vet. Med., 25: 28.
26. Ferreira, A., R. Cardoso, I. Dias, I. Mariano, A. Belo, I. Preto, A. Manteigas, A. Fonseca and M.I.C. De Sá, 2003. Evaluation of a modified Rose Bengal test and an indirect Enzyme-Linked Immunosorbent Assay for the diagnosis of *Brucella* melitensis infection in sheep. Vet. Res., 34(3): 297-305.

27. Erdenliğ Gürbilek, S., O.Y. Tel and O. Keskin, 2017. Comparative evaluation of three serological tests for the detection of Brucella antibodies from infected cattle herds. J. Appl. Anim. Res., 45: 557-559.
28. Nofal, A.S., A.A. El-Leboudy, H.S.A. El-Makarem and A.H.A. El-Rahman, 2017. Prevalence of Brucella Organism in Milk and Serum Samples of Some Lactating Dairy Animals. Alex J. Vet. Sci., 55: 2.
29. Chand, P., B. Rajpurohit, A. Malhotra and J. Poonia, 2005. Comparison of milk-ELISA and serum-ELISA for the diagnosis of Brucella melitensis infection in sheep. Vet. Microbiol., 108: 305-311.
30. Capparelli, R., M. Parlato, M. Iannaccone, S. Roperto, R. Marabelli, F. Roperto and D. Iannelli, 2009. Heterogeneous shedding of Brucella abortus in milk and its effect on the control of animal brucellosis. J. Appl. Microbiol., 106: 2041-2047.