Journal of Animal Research: v.7 n.6, p. 1083-1087. December 2017

DOI: 10.5958/2277-940X.2017.00161.9

Comparative Efficacy of Serological Tests for Detection of *Brucella* Antibodies in Sheep and Goats

Kirit B. Patel*, S.I Patel, H.C Chauhan, A.K Thakor, B.R Pandor, S.S Chaudhari, P.H Chauhan, B.S Chandel

Department of Animal Biotechnology and Veterinary Microbiology, College of Veterinary Science and Animal Husbandry, Sardarkrushinagar Dantiwada Agricultural University (SDAU), Sardarkrushinagar, Gujarat, INDIA

**Corresponding author: KB Patel; Email: kiritpatel23@gmail.com*

Received: 19 Aug., 2017 **Revised:** 16 Nov., 2017 **Accepted:** 28 Nov., 2017

ABSTRACT

Brucellosis is an important zoonosis and a significant cause of reproductive losses in animals. In view of the considerable problems related to direct diagnosis of brucellosis in animals, the present study envisaged the appraisal of seroepidemiology of brucellosis in sheep and goats by detection of brucella specific antibodies, comparison of two serological tests, *viz.,* i-ELISA (Indirect Enzyme Linked Immunosorbent Assay) and RBPT expand for detection of Brucella-specific antibodies. Out of 1012 sheep and goat sera screened, 88 (8.70%) and 75 (7.41%) were detected positive by RBPT and i-ELISA, respectively. Species-wise seroprevalence was detected 12.26% and 10.97% in sheep and 5.67% and 4.39% in goats by RBPT and i-ELISA, respectively. During present investigation, RBPT detected more number of samples positive for brucella antibodies. However, compared to i-ELISA, overall sensitivity and specificity of RBPT were 80.00% and 97.01%, respectively. Species-wise sensitivity of RBPT found was 82.35% in sheep and 75.00% in goat, whereas specificity was 96.38% in sheep and 96.41% in goats.

Keywords: Brucellosis, *B.melitensis,* RBPT, i-ELISA, seroprevalance

Brucellosis due to *Brucella melitensis* is widespread in India and is considered to be the major cause of abortion in small ruminants incurring severe economic loss. Free grazing and movement with frequent mixing of flocks of sheep and goats also contribute to the wide distribution of brucellosis in these animals. In Sheep and Goat, that average economic annual loss due to brucellosis per animal was found to be ₹ 1180 and ₹ 2121.82, respectively. (Sulima and Venkataraman *et al*., 2010). Appreciating the economic losses of brucellosis, Department of Biotechnology (DBT), New Delhi has initiated a nationwide network project on brucellosis. Sardarkrushinagar Dantiwada Agricultural University, Sardarkrushinagar is one of the co-opting centre. The Brucellosis diagnosis (and surveillance) almost entirely rely on serological tests, *e.g.,* Rose Bengal Plate Test (RBPT), Standard Agglutination Test (STAT), indirect Enzyme Linked Immunosorbent Assay (i-ELISA), and Complement Fixation Test (CFT), that detect antibodies against Brucella antigens including lipopolysaccharides (LPS) and give indirect evidence of Brucella infection (Godfroid *et al.,* 2002; Adone and Pasquali, 2013). The major drawbacks of these assays are that they are not always specific and can cross react with other gram negative bacteria like *Yersinia enterocolitica*, *Vibrio cholerae, Campylobacter fetus, Bordetella bronchiseptica* and *Salmonella* spp. (Corbel and Brinley-Morgan, 1984) and antibodies are not produced in the acute stage of infection (Moussa *et al.,* 2011). So, the main aim of present study is which is the better serological test in sense of sensitivity and specificity from RBPT and i-ELISA for diagnosis of Brucellosis.

MATERIALS AND METHODS

The present work on presence or absence of brucella antibodies in serum samples collected from sheep and goats. A total of 1012 serum samples were collected

from rural areas and organised farms belonging to five districts (Banaskantha, Patan, Navsari, Vapi and Kutchch) of Gujarat from 2014 to 2016. About 9 ml of blood was collected aseptically from the jugular vein of individual animal in a vacuette with serum clot activator (Greiner bio-one, Austria). The vacuettes were kept in upright position at room temperature for about 2 hrs. The separated serum samples were collected and stored at -20ºC till further use.

Rose bengal plate test (RBPT)

The RBPT antigen was procured from the Institute of Animal Health and Veterinary Biologicals (IAH and VB), Hebbal, Bangalore, Karnataka-560 024. The test was carried out by mixing 0.03 ml of serum and 0.03 ml of *B. abortus* Rose Bengal coloured antigen on a slide and mixed thoroughly with sterile tooth picks and then the slide was rotated and observed for reaction upto four min. The results were recorded. Definite clumping / agglutination was considered as positive reaction, where as no clumping/ agglutination was considered negative.

Indirect-enzyme linked immunosorbant assay (i-ELISA)

Brucella Antibody Test Kit, ELISA along with the user's manual was procured from National Institute of Veterinary Epidemiology and Disease Informatics (NIVEDI) formerly Project Directorate on Animal Disease Monitoring and Surveillance (PD-ADMAS), Bangalore was used in the present study. The test was performed as per the protocol outlined in the user's manual.

RESULTS AND DISCUSSION

Brucellosis is an infectious bacterial disease caused by genus *Brucella* and affecting a number of animal species. It is a worldwide zoonotic disease that is recognised as a major cause of heavy economic losses to the livestock industry and poses serious human health hazard (Ocholi *et al.,* 2005). Overall seroprevalence of brucellosis was detected, 8.70 and 7.41% in goats and sheep by RBPT and i- ELISA, respectively. Seroprevalence of brucellosis was 5.67 and 4.39% in goats and 12.26 and 10.97% in sheep by RBPT and i- ELISA, respectively.

Comparative efficacy of serological tests

In the present study, i-ELISA was found to detect low seroprevalence as compared to RBPT in goats and sheep. In goat, 4.39% of seropositivity was detected by i-ELISA against 5.67% by RBPT. On the other hand, 10.97% of seropositivity by i-ELISA and 12.26% by RBPT were detected in sheep. Overall, in both the animals, comparison to 7.41% of seropositivity was detected by i-ELISA as compared to 8.70% by RBPT. Similar results were observed by Rahman *et al.,* (2011[b]) for testing of goats and sheep samples, who found highest seroprevalence of brucellosis by RBPT followed by STAT and i-ELISA. Din *et al.* (2013) found RBPT (11.33%) to be more sensitive than SPAT (9.33%) and STAT (7.66%) for testing goat samples. In contrast, Kotadiya (2012) found higher seropositivity of 11.38% by RBPT than 9.44% by STAT but the seropositivity of 18.20% by i-ELISA was highest as compared to these two tests for testing sheep samples from Gujarat. Sonawane *et al*. (2011) also observed higher seroprevalence of 15.60% by i-ELISA as compared to 5.92% by RBPT in samples of sheep and goat from Rajasthan.

Christopher *et al.* (2010); Godfroid *et al.* (2010) and Diaz *et al.* (2011) were of the view that these variations may be due to the ability of each test to detect different antibody classes.

Comparison of sensitivity and specificity of i-ELISA and RBPT

The comparative efficacy of RBPT to i-ELISA was determined with regards to their sensitivity, specificity and overall agreement in the diagnosis of caprine and ovine brucellosis for detecting antibodies, with regards to seroprevalence of *Brucella* infection.

In this study, the sensitivity of RBPT was 75.00% and specificity was 96.41% in goat (Table 1).

Sharma *et al.* (2006) recorded slight lower sensitivity (67.85%) and higher specificity (99.51%) of RBPT in goat samples of Mehsana and Patan district of Gujarat when compared with dot-ELISA. Rahman *et al.* (2013) recorded the sensitivity (80.2%) and specificity (99.6%) of RBPT to be high in comparison to present study and Ekgatat *et al.,* (2010) also found higher diagnostic sensitivity (99.2%) and specificity (100%) of RBT. Reddy *et al.* (2014) found

Table 1: Sensitivity, specificity and overall agreement of RBPT by comparing with i-ELISA for detection of *Brucella* antibodies in goat

Test		i-ELISA		Total	Sensitivity (%)	Specificity (%)	Overall Agreement (%)
		Positive	Negative				
RBPT	Positive	18	13	31	75.00	96.41	96.53
	Negative	06	510	516			
	Total	24	523	547			

Table 2: Sensitivity specificity and overall agreement of RBPT by comparing with i-ELISA for detection of *Brucella* antibodies in sheep

Test		i-ELISA		Total	Sensitivity (%)	Specificity (%)	Overall Agreement (%)
		Positive	Negative				
RBPT	Positive	42	15	57	82.35	96.38	94.84
	Negative	09	399	408			
	Total	51	414	465			

Table 3: Overall sensitivity specificity and overall agreement of RBPT by comparing with i-ELISA for detection of *Brucella* antibodies in goat and sheep

Test		i-ELISA		Total	Sensitivity (%)	Specificity (%)	Overall Agreement (%)
		Positive	Negative				
RBPT	Positive	60	28	88	80.0	97.01	95.75
	Negative	15	909	924			
	Total	75	937	1012			

low relative sensitivity (54.16%) while high specificity (100%) for RBPT. Hence, i-ELISA was found to be a better serological test as compared to RBPT and it could be advocated for screening of goat.

In case of sheep the sensitivity and specificity of RBPT were found to be 82.35% and 96.38%, respectively as compared to i-ELISA (Table 2).

Rahman *et al.* (2013) found similar sensitivity (82.8%) and specificity (98.3%) of RBT whereas Sharma *et al.* (2006) recorded lower sensitivity (55.55%) and similar specificity (94.59%) of RBPT for sheep samples from Mehsana and Patan districts of Gujarat as compared to dot- ELISA. Barbuddhe *et al.* (1994) found lower relative sensitivity and higher relative specificity of 42.85 and 100.00% of RBPT, respectively for goat samples when CFT was considered as gold standard test. Al-Mariri *et al.* (2011) found higher sensitivity (91%) of RBT for Syrian female sheep samples when CFT was considered as gold standard test. Kotadiya (2012) recorded lower sensitivity (65.83%) and higher specificity (100%) for RBPT, considering i-ELISA as a gold standard test for sheep samples. Hence, i-ELISA was found to be a better serological test as compared to RBPT and could be advocated for screening of animals.

In the present study, overall the sensitivity and specificity of RBPT were found to be 80.00% and 97.01%, respectively as compared to i-ELISA in sheep and goat (Table 3).

Hence, i-ELISA was found to be a better serological test as compared to RBPT for screening of animals. Almost similar results were obtained by Tayshete (2001) who found the sensitivity of RBPT to be 71.42% in contrast to this study in which specificity of RBPT was slight high (100%), considering i-ELISA as a gold standard test. On the other hand, Coelho *et al.,* (2008) who found higher sensitivity (97.6%) and lower specificity (77.6%) values of RBT. Al-Gardia *et al.* (2011) noted higher sensitivity (89.04%) and specificity (99.06%) of commercial RBPT and Khalek *et al.* (2012) also recorded higher sensitivity (92.90%) and specificity (83%) for RBT.

In this study, overall agreement of RBPT with i-ELISA was 95.75% for samples from small ruminants (Table 3); 96.53% for goats (Table 1) and 94.84% for sheep (Table 2) respectively. Hence, i-ELISA was found to be better serological test as compared to RBPT and it could be advocated for screening of goats and sheep for brucellosis. Almost similar results were recorded by Sadhu *et al.* (2015) in small ruminants with overall agreement between RBPT and i-ELISA of 92.50% and concluded i-ELISA to be a better serological test as compared to RBPT and STAT. Ekgatat *et al.* (2009) conclude i-ELISA to be a simple and rapid test that was highly sensitive and specific for antibody detection and could be a reliable alternative for presumptive serological diagnosis of *Brucella* sp. infection in cattle and goats. Nielsen *et al.,* (2005) also concluded that i-ELISA performed better than the c-ELISA and the FPA in goats. Sharma *et al.* (2006) recorded slight higher 97.49% concordance of RBPT with dot-ELISA in goats but slight lower (86.95%) in sheep.

In diagnosis of caprine and ovine brucellosis, the efficacy of RBPT and STAT was considered doubtful (WHO, 2004). As per WHO (2006), it should be noted that although the ELISA is more sensitive than the RBPT, but sometimes, it does not detect infected animals which are RBPT positive.

CONCLUSION

The present study indicate the brucellosis prevalent in Gujarat, However in view of consideration of cost, feasibility and reliability as field diagnostic test, RBPT has been found to be much cheaper, easier and convenient to perform than ELISA. According to sensitivity and specificity ELISA is more sensitive than the RBPT. Hence, i-ELISA was found to be better serological test as compared to RBPT and it could be advocated for screening of goats and sheep for brucellosis.

ACKNOWLEDGEMENTS

We are highly thankful to Department of Biotechnology, Government of India for financial assistance for the DBT Network project on brucellosis. The authors are also thankful to the Department of Animal Biotechnology, College of Veterinary Science and A.H., SDAU, SK Nagar for providing the facilities for this work.

REFERENCES

Adone, R. and Pasquali, P. 2013. Epidemiosurveillance of brucellosis. *Revue Scienti. Tech., (Int. office Epizoo.)* **32**(1): 199-205.

Al-Gardia, M.A., Khairani-Bejo, S., Zunita, Z. and Omar, A.R. 2011. Detection of *Brucella melitenisis* in blood samples collected from goats. *J. Anim. Vet. Adv.,* **10**(11): 1437-1444.

Al-Mariri, A., Ramadan, L. and Akel, R. 2011. Assessment of milk ring test and some serological tests in the detection of *Brucella melitensis* in Syrian female sheep. *Trop. Anim. Health Prod.,* **43**(4)**:** 865-870.

Barbuddhe, S.B., Yadavam, V.K. and Singh, D.K. 1994. Comparison of dot ELISA with conventional serological tests for diagnosing ovine brucellosis. *Indian J. Comparative Microbiol. Immunol. Infectious Dis.,* **15**(1 and 2): 1-5.

Christopher, S., Umapathy, B.L. and Ravikumar, K.L. 2010. Brucellosis: review on the recent trends in pathogenicity and laboratory diagnosis. *J. Lab. Physicians,* **2**(2): 55.

Coelho, A.M., Coelho, A.C., Gois, J., Pinto, M. de L. and Rodrigues, J. 2008. Multifactorial correspondence analysis of risk factors for sheep and goat brucellosis seroprevalence. *Small Ruminant Res*., **78**(1-3): 181-185.

Corbel, M.J. and Brinley-Morgan, W.J. 1984. Genus Brucella. Meyer and Shaw 1920, 173AL, In Krieg, N.R., Holt, J.G., Bergey's Manual of Systematic Bacteriology, Vol.1, Williams & Wilkins, Baltimore-London, 377-388.

Diaz, R., Casanova, A., Ariza, J. and Moriyon, I. 2011. The Rose Bengal Test in human Brucellosis: a neglected test for the diagnosis of a neglected disease. *PLOS Negated Trop. Dis.,* **5**(4): 950.

Din, A.M.U., Khan, S.A., Ahmad, I., Rind, R., Hussain, T., Shahid, M. and Ahmed, S. 2013. A study on the seroprevalence of brucellosis in human and goat populations of district Bhimber, Azad Jammu and Kashmir. *The J. Anim. Plant Sci*., **23**(1): 113-118.

Ekgatat, M., Kanitpun, R., Khunchit, P., Thammasart, S. and Wongkasemjit, S. 2009. The Accuracy of an indirect ELISA for diagnosis of *Brucella* spp. infection in cattle and goats. *Kasetsart Veterinarians,* **19**(1): 1-8.

Ekgatat, M., Kanitpun, R., Kunchit, P., Arampong, W., Raksajit, S., Thammasart, S., Trenuntawan, U., Tumcha P. and Wongkasemjit, S. 2010. Comparison of serological tests for antibody detection against *Brucella melitensis* infection in goats. *Kasetsart Veterinarians,* **20**(1): 19-26.

Godfroid, J. 2002. Brucellosis in wildlife. *Revue Scienti. Tech., (Int. office Epizoo.)* **21**(2): 277-286.

Godfroid, J., Nielsen, K. and Saegerman, C. 2010. Diagnosis of brucellosis in livestock and wildlife. *Croatian Med. J.,* **51**(4): 296-305.

Khalek, M.M.A., Ramadan, K.M., Hazem, S.S. and Khairy, E.A. 2012. Evaluation of Immunochromatographic Assay for Serodiagnosis of Brucella among Cattle, Sheep and Goats in Egypt. *Global Veterinaria,* **8**(5): 511-518.

Kotadiya, A.J. 2012. Serological, cultural and molecular detection of brucella infection of sheep in Gujarat. M. V. Sc. thesis submitted to S.D. Agricultural University, Sardarkrushinagar, Gujarat.

Moussa, I.M., Omnia, M.E., Amin, A.S., Ashgan, M.H. and Selim, S.A. 2011. Evaluation of the currently used polymerase chain reaction assaya for molecular detection of Brucella species. *African J. Microbiol. Res*., **5**(12): 1511-1520.

Nielsen, K., Gall, D., Smith, P., Bermudez, R., Moreno, F., Renteria, T., Ruiz, A., Aparicio, L., Vazquez, S., Dajer, A., Luna, E., Samartino, L. and Halbert, G. 2005. Evaluation of serological tests for detection of caprine antibody to *Brucella melitensis*. *Small Ruminant Res*., **56**(1-3): 253-258.

Ocholi, R.A., Kwaga, J.K., Ajogi, I. and Bale, J.O. 2005. Abortion due to *Brucella abortus* in sheep in Nigeria. *Revue Scienti. Tech., (Int. office Epizoo.)* **24**(3): 973-979.

Rahman, A.K., Saegerman, C., Berkvens, D., Fretin, D., Gani, M.O., Ershaduzzaman, M., Ahmed, M.U. and Emmanuel, A. 2013. Bayesian estimation of true prevalence, sensitivity and specificity of indirect ELISA, Rose Bengal Test and Slow Agglutination Test for the diagnosis of brucellosis in sheep and goats in Bangladesh. *Preventive Vet. Med.,* **110**(2): 242-252.

Rahman, M.S., Ali Hahsin, M.F., Ahasan, M.S., Her, M., Kim, J.Y., Kang, S. and Jung, S.C. 2011[b]. Brucellosis in sheep and goat of Bogra and Mymensingh districts of Bangladesh. *Korean J. Vet. Res.,* **51**(4): 277-280.

Reddy, D.A., Kumari, Gita, Rajagunalan, S., Singh, D.K., Kumar Ashok and Kumar Pavan P. 2014. Seroprevalence of caprine brucellosis in Karnataka. *Vet. World*, **7**(3): 182-188.

Sadhu, D.B., Panchasara, H.H., Chauhan, H.C., Sutariya, D.R., Parmar, V.L. and Prajapati, H.B. 2015. Seroprevalence and comparison of different serological tests for brucellosis detection in small ruminants. *Vet. World*, **8**(5): 561-566.

Sharma, V.K., Savalia, C.V., Selvam, D.T. and Darekar, S.D. 2006. Seroprevalence of caprine and ovine brucellosis in Mehsana and Patan districts of Gujarat. *Intas Polivet.,* **7**(2): 316-318.

Sonawane, G.G., Tripathi, S. and Dubey, S.C. 2011. Sero-incidence of brucellosis in small ruminants of semiarid Rajasthan. *The Indian J. Anim. Sci*., **81**(4): 327–29.

Sulima, M. and Venkataraman, K.S. 2010. Economic losses due to *Brucella melitensis* infection in sheep and goats. *Tamilnadu J. Vet. Anim. Sci*., **6**(4): 191-192.

Tayshete, S.R. 2001. Seroprevalence of brucellosis in North Gujarat. M. V.Sc. thesis submitted to S. D. Agricultural University, Sardarkrushinagar, Gujarat.

WHO. 2004. World Health Organization Laboratory Biosafety Manual, Third Edition. WHO, Geneva, Switzerland.

WHO. 2006. Brucellosis in humans and animals. World Health Organization in collaboration with the Food and Agriculture Organization of the United Nations and World Organisation for Animal Health. WHO, Geneva, Switzerland.