Original Research Article

Comparative Sensitivity and Specificity of Various Serological Tests for Detection of Brucellosis in Small Ruminants

R.R. Padher*, J.B. Nayak, M.N. Brahmbhatt and R.A. Mathakiya

Department of Veterinary Public Health, College of Veterinary Science and Animal Husbandry,
Anand Agricultural University, Anand, 388001, Gujarat, India
*Corresponding author

A B S T R A C T

Brucellosis is a zoonosis known to be a major public health hazard of great economic importance globally. The present study was taken up to ascertain the seroprevalence of brucellosis in goats, sheep, and humans of Anand, districts of the Central Gujarat region. A total of 200 serum samples were collected from the goat and sheep of Anand district and subjected to different serological test i.e., Rose Bengal Plate test (RBPT), Standard Tube Agglutination Test (STAT) and Indirect ELISA to detect the *Brucella* antibody and also compared in terms of their sensitivity and specificity. A total 200 sera sample including 100 from goats and 100 from sheep collected from the Anand district. Among 200 sera samples of sheep and goats, 94 (47.00%) samples were found to be positive. 93 sera samples were positive by I-ELISA, 27 by RBPT while 16 by STAT. One RBPT positive goat serum sample was found to be negative by I-ELISA. Fifty six goat sera samples were found to be sero-positive, out of which 55 samples were found to be positive by I-ELISA while 16 by RBPT and 10 by STAT. Among 38 sera samples of sheep found to be positive by I-ELISA while 11 by RBPT and 6 by STAT. In case of goats, the sensitivity of RBPT and STAT was 27.27 and 18.18 per cent while specificity was 97.82 and 100 per cent, respectively. An overall agreement of RBPT and STAT with I-ELISA was 66.00 and 55.00 per cent, respectively. Thus, sensitivity of RBPT was more than STAT but specificity of RBPT and STAT was somewhat similar. In case of sheep, the sensitivity of RBPT and STAT was 28.95 and 15.78 per cent while specificity was 100 per cent for both test. An overall agreement of RBPT and STAT with I-ELISA was 73.00 and 68.00 per cent, respectively. Thus, sensitivity of RBPT was more than STAT but specificity of RBPT and STAT was similar.

Introduction

Brucellosis is one of the world's major diseases. It is neglected zoonoses (WHO, 2009) largely due to lack of public awareness and especially in pastoral and mixed crop-livestock farming systems (McDermott and Arimi, 2002). Serological evidence suggest that brucellosis is highly endemic in the most part of India (Raju *et al.,* 2004; Thavamathi,

2006; Valarmathy *et al.,* 2007 and Singh *et al.,* 2013). Polding (1942) first reported the isolation of *B. melitensis* from goat in India. Subsequently, B. abortus was isolated from cases of abortion in Haryana (Mathur, 1967).In small ruminants, most clinical infection is manifested as fertility-related issues. However, the most common symptoms

are usually abortions during the trimester including retained placenta, weak offspring and metritis which may result in temporary infertility. Others include drop in milk production due to the infection of the udder. Rams experience orchitis and epididymitis. In addition, animals with polyarthritis have been observed in endemic flocks (Radostits, Gay, Hinchcliff, & Constable, 2006). Seropravelance studies from the backbone of epidemiology investigation and are used to identify herd infected with *Brucella* (Renukaradhya *et al.,* 2002). The serological tests include Rose Bengal Plate Test, Complement Fixation Test, Serum Agglutination Test and Enzyme Linked Immunosorbent Assay (ELISA) test (Pal, 2007). RBPT test has proven useful as a herd screening test and is highly sensitive (MacMillan, 1990).

Materials and Methods

The study was conducted to detect *Brucella* antibodies for 200 sera samples comprising goat sera (100) and sheep sera (100) collected from various areas of Anand district, under aseptic precautions. These sera samples were tested for *Brucella* antibodies using RBPT, STAT, and I-ELISA for detecting *Brucella* antibodies from serum. Rose Bengal Plate Test was carried out by using rose Bengal plate test antigen. Using *B.abortus* agglutinating antigen carried out standard tube agglutination test. Both antigens were procured from Indian Veterinary Research Institute, Izatnagar. Indirect enzyme linked immunosorbent assay was carried out by using the smooth lipopolysaccharide (S-LPS) I-ELISA kits (for sheep and goat sera) procured from ICAR-National Institute of Veterinary Epidemiology and Disease Informatics (ICAR-NIVEDI) Bengaluru. The samples were collected in vacutainer with serum clot activator and transported to the departmental P. G. research laboratory on

icebox for further processing and serological analysis. The vacutainer was kept in upright position at room temperature for about 2 hr. Then the tubes were centrifuged at 3000 rpm for 10 minutes to facilitate separation of serum, which was collected in a screw capped plastic vials. The sera were stored at -20ºC till subjected to I-ELISA.

Results and Discussion

Overall seroprevalence of brucellosis in small ruminants

Among 200 sera samples of sheep and goat overall, 94 (47.00%) samples were found to be positive for brucellosis. 27 (13.50%) sera samples were positive by RBPT while 16 (8.00%) by STAT and 93 (46.50%) by I-ELISA, One RBPT positive goat serum sample were found to be negative by I-ELISA. The seropositivity was the highest by I-ELISA, intermediate by RBPT and lowest by STAT (Table 1&2, Fig. 1)

Comparative efficacy of serological tests

Comparison of I-ELISA as compare to RBPT and STAT

In the present study, overall seropositivity for *Brucella* antibodies was detected by RBPT, STAT and I-ELISA to compare their efficacy. Total 200 serum samples comprising of 100 from goats and 100 from sheep, were tested for the presence of *Brucella* antibodies by RBPT, STAT and I-ELISA. Overall, comparison to 46.50% of seropositivity in I-ELISA, 13.50% and 8.00% of the samples were found seropositive by RBPT and STAT, respectively. In goat, comparison to 55.00% of seropositivity in I-ELISA, 16.00% and 10.00% of the samples were found seropositive by RBPT and STAT, respectively while in sheep comparison to 38.00% of seropositivity in I-ELISA, 11.00%

and 6.00% of the samples were found seropositive by RBPT and STAT, respectively. Thus, I-ELISA detected highest seroprevalence than RBPT and STAT and RBPT detected more seroprevalence than STAT in both goats and sheep.

Comparison of sensitivity and specificity of I-ELISA, RBPT and STAT

Overall the sensitivity of RBPT and STAT was 27.95% and 17.20%, respectively, considering I-ELISA as a gold standard test while specificity was 99.06% and 100%, respectively. An overall agreement of RBPT and STAT with I-ELISA was 66.00% and 61.50%, respectively (Table 4.8 and Figure 4.13). Thus, sensitivity of RBPT was more than STAT but specificity of RBPT was little less then STAT. Thus, RBPT was more sensitive than STAT but specificity of both tests was more or less similar. Hence, I-ELISA was a better serological test as compared to RBPT and STAT and it could be advocated for screening of small ruminants (Table 3 and Fig 2).

In case of goat, the sensitivity of RBPT and STAT was 27.27% and 18.18%, respectively, considering I-ELISA as a gold standard test while specificity was 97.82% and 100%, respectively. Thus, sensitivity of RBPT was more than STAT but specificity of RBPT and STAT was somewhat similar. The overall agreement of RBPT and STAT with I-ELISA was 60.00% and 55.00%, respectively. Hence, I-ELISA was a better serological test as compared to RBPT and STAT and it could be advocated for screening of goats (Table 4 and Fig. 3).

In case of sheep, the sensitivity of RBPT and STAT was 28.95% and 15.78%, respectively, considering I-ELISA as a gold standard test while specificity was 100% for both test. Thus, sensitivity of RBPT was more than STAT but specificity of RBPT and STAT was

similar. An overall agreement of RBPT and STAT with I-ELISA was 73.00% and 68.00%, respectively. Hence, I-ELISA was a better serological test as compared to RBPT and STAT and it could be advocated for screening of sheep (Table 5 and Fig. 4). Overall seroprevalence of brucellosis in small ruminants

In the present study, the overall seroprevalence of brucellosis was 13.50%, 8.00% and 46.50% in small ruminants by RBPT, STAT and I-ELISA, respectively (Table 1&2, Fig. 1)

The finding of present study was similar with the finding of Tegeg n*et al.,*(2016)who reported overallseroprevalencewas13% with RBPT in small ruminants. Similar result was also reported by Valarmathy *et al.,* (2007) which was 13.80% by RBPT and 8.28% by STAT and Shadhu *et al.,* (2015) who also reported the seroprevalence of 11.30% and 11.10% by RBPT and STAT, respectively of small ruminants in Banaskantha district. In addition, Kumar *et al.,* (2015) who found 7% seroprevalence by STAT in animals in and around Junagadh district of Gujarat state. In addition, Din *et al.,* (2013) who reported 13.33%, 11.33% and 9.33% by RBPT, SPAT and STAT, respectively which was more or less similar to the present study.

Comparative efficacy of serological tests

Overall, comparison to 46.50% of seropositivity in I-ELISA, 13.50% and 8.00% of the samples were found seropositive by RBPT and STAT, respectively. In goat, comparison to 55.00% of seropositivity in I-ELISA, 16.00% and 10.00% of the samples were found seropositive by RBPT and STAT, respectively while in sheep comparison to 38.00% of seropositivity in I-ELISA, 11.00% and 6.00% of the samples were found seropositive by RBPT and STAT, respectively.

Table.1 Overall seroprevalence of brucellosis in small ruminants (sheep and goats)

Species	Total number of sera tested	No. of positive sera samples	Percentage (%)
Goats	100	56	56.00%
Sheep	100	38	38.00%
Total	200	94	47.00%

Table.2 Overall sensitivity, specificity and agreement of RBPT and STAT by comparing with I-ELISA (Gold Standard Test) for detection of *Brucella* antibodies in goats

Serological Test		I-ELISA		Total	Sensitivity (%)	Specificity (%)	Overall Agreement (%)
		Positive	Negative				
RBPT	Positive	15	1	16	27.27%	97.82%	60.00%
	Negative	40	45	85			
	Total	55	46	100			
STAT	Positive	10	0	10	18.18%	100%	55.00%
	Negative	45	45	90			
	Total	55	45	100			

Table.3 Overall sensitivity, specificity and agreement of RBPT and STAT by comparing with I-ELISA (Gold Standard Test) for detection of *Brucella* antibodies in small ruminants

Serological Test		I-ELISA		Total	Sensitivity (%)	Specificity (%)	Overall Agreement (%)
		Positive	Negative				
RBPT	Positive	26	1	27	27.95%	99.06%	66.00%
	Negative	67	106	173			
	Total	93	107	200			
STAT	Positive	16	0	16	17.20%	100%	61.50%
	Negative	77	107	184			
	Total	93	107	200			

Table.4 Overall sensitivity, specificity and agreement of RBPT and STAT by comparing with I-ELISA (Gold Standard Test) for detection of *Brucella* antibodies in sheep

Serological Test		I-ELISA		Total	Sensitivity (%)	Specificity (%)	Overall Agreement (%)
		Positive	Negative				
RBPT	Positive	11	0	11	28.95%	100%	73.00%
	Negative	27	62	89			
	Total	38	62	100			
STAT	Positive	6	0	6	15.78%	100%	68.00%
	Negative	32	62	94			
	Total	38	62	100			

Figure.1 Overall test wise seroprevalence of brucellosis in small ruminants (sheep and goats)
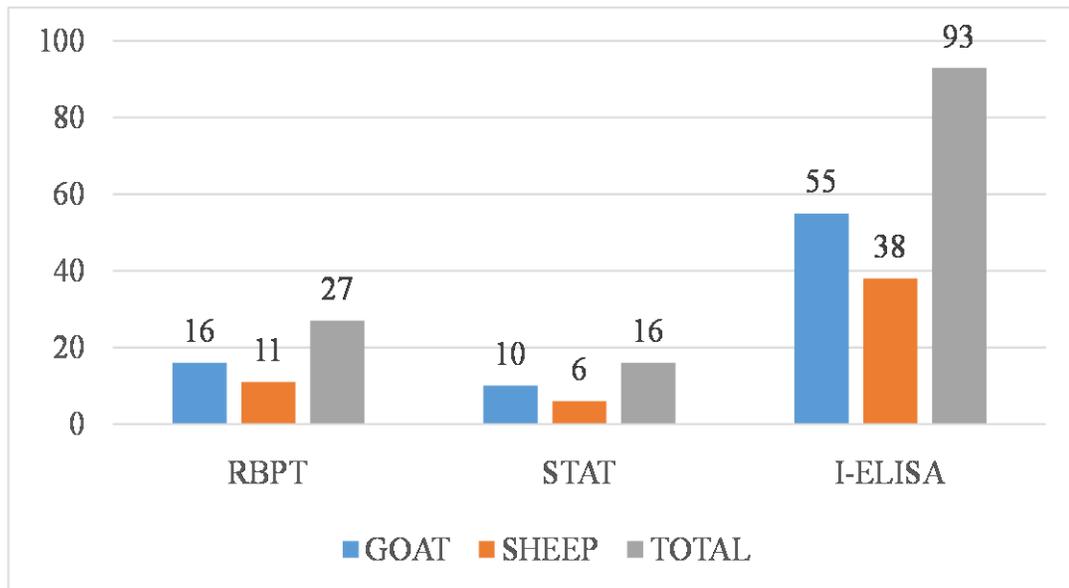
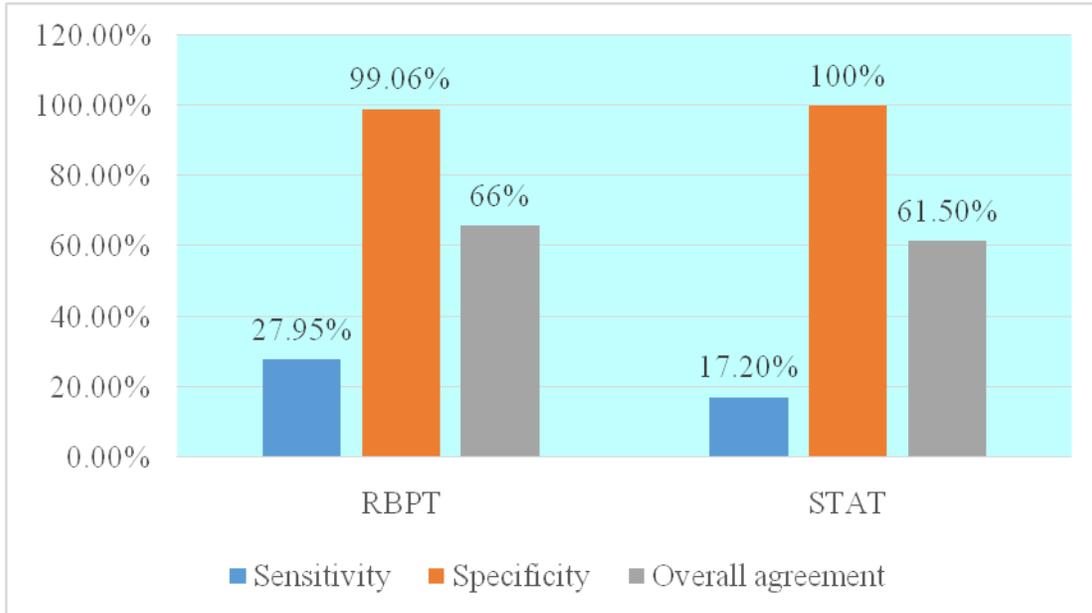**Figure.2** Overall sensitivity, specificity and agreement of RBPT and STAT by comparing with I-ELISA

Figure.3 Overall sensitivity, specificity and agreement of RBPT and STAT by comparing with I-ELISA (gold standard test) for detection of *Brucella* antibodies in goats
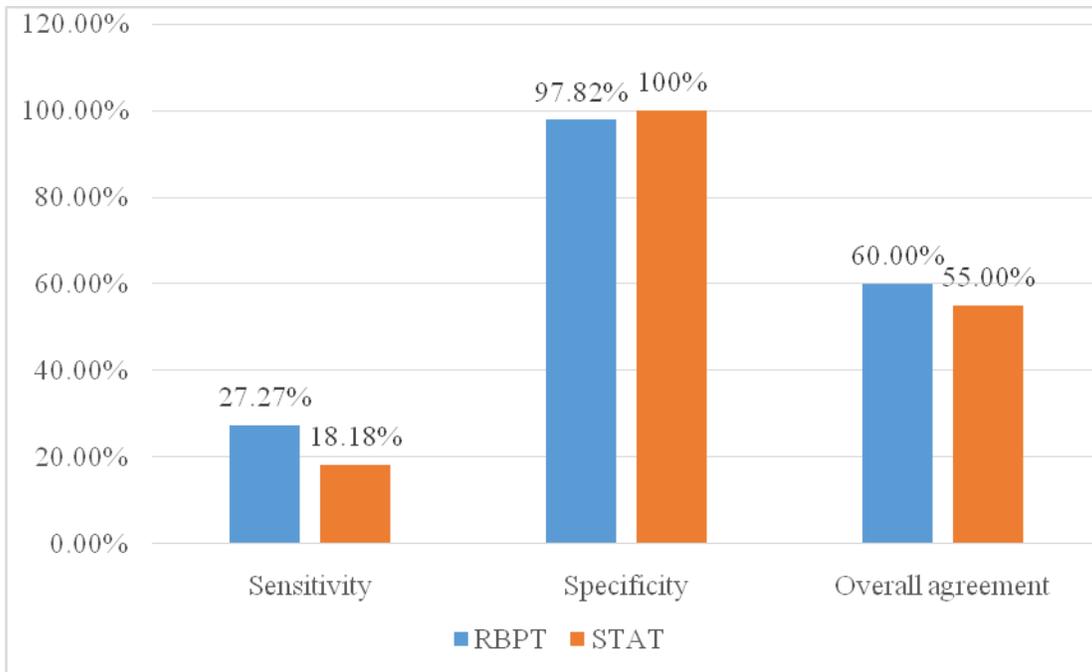
Figure.4 Overall sensitivity, specificity and agreement of RBPT and STAT by comparing with I-ELISA (gold standard test) for detection of *Brucella* antibodies in sheep
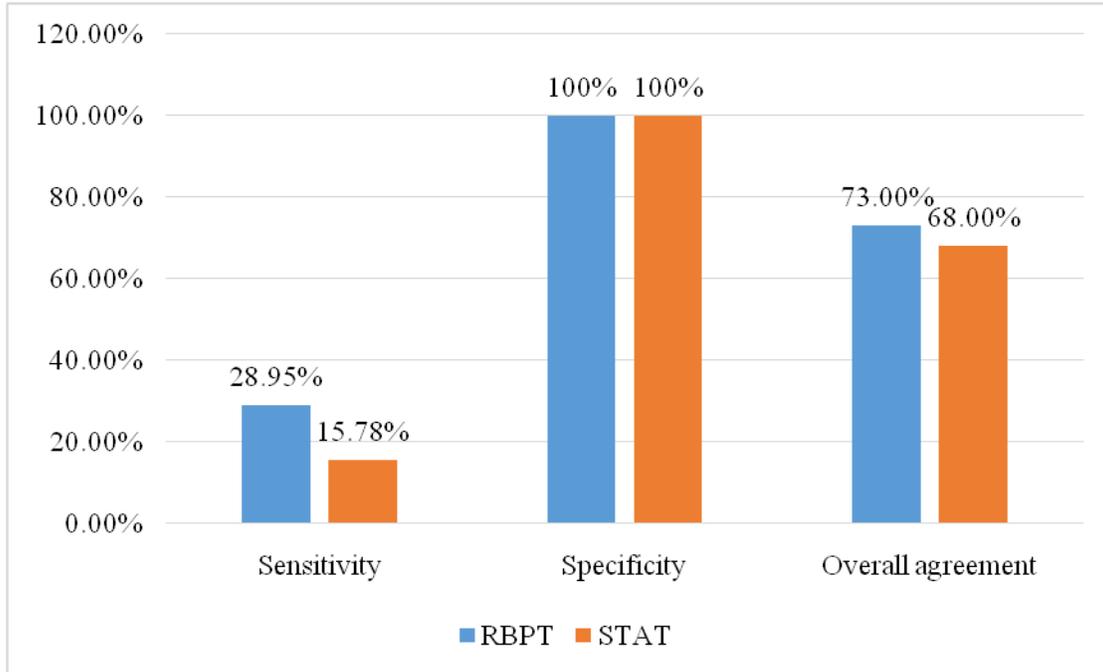

Table No. 2 Detection of *Brucella* antibodies by RBPT among goats and Sheep

Species	Total number of sera tested	Various serological methods		
		RBPT	STAT	I-ELISA
Goats	100	16 (16.00%)	10 (10.00%)	55 (55.00%)
Sheep	100	11 (11.00%)	6 (6.00%)	38 (38.00%)
Total	200	27 (13.50%)	16 (8.00%)	93 (46.50%)

In comparison to the present study similar seroprevalence was obtained by Din *et al.,* (2013) who found (11.33%) and (7.66%) with RBPT and STAT, respectively in goats, similarly, Kotadiya, (2012) who found higher seropositivity of 11.38% by RBPT as compared to 9.44% by STAT in sheep. In addition Sonawane*et al.,* (2011) also observed higher seroprevalence of 15.60% by I-ELISA as compared to 5.92% by RBPT in sheep and goat from Rajasthan.

In contrast, Rahman *et al.,* (2011) in goats and sheep who found highest seroprevalence of brucellosis by RBPT followed by STAT and I-ELISA. Shome *et al.,* (2006) in sheep and

goats who found lower seropositivity of 7.36% by I-ELISA as compared to 9.95% by RBPT from Rajasthan, Gujarat and Karnataka. These variations may be due to the ability of each test to detect different antibody classes. The variation results of different tests could be because of the various factors such as stage of infection, presence of false positive or negative animals and cross-reacting organism beside many more.

Comparison of I-ELISA as compare to RBPT and STAT

Overall sensitivity, specificity and agreement of RBPT and STAT by comparing with I-

ELISA (Gold Standard Test) for detection of *Brucella* antibodies in small ruminants

Overall the sensitivity of RBPT and STAT was 27.95% and 17.20%, respectively, considering I-ELISA as a gold standard test while specificity was 99.06% and 100%, respectively. An overall agreement of RBPT and STAT with I-ELISA was 66.00% and 61.50%, respectively (Table 3 and Figure 2).

Similar results was obtained by Tayshete, (2001) who found specificity of RBPT and STAT was 100 % but in contrast to present study sensitivity of RBPT and STAT was 71.42% which was higher to present study, considering I-ELISA as a gold standard test in small ruminants. In addition Al-Gardia *et al.,* (2011) who noted similar the specificity of commercial RBPT was 99.06% but in contrast to present study sensitivity of RBPT was 89.04% which was higher than present study.

In contrast, Coelho *et al.,* (2008) who found higher sensitivity 97.6% and lower specificity 77.6% values of RBT in small-ruminant. Similarly Abdel-Khalek *et al.,* (2012) who recorded higher sensitivity 92.9% for RBT and 90.7% for TAT while lower specificity 83% for RBT and 71.4% for TAT.

Overall sensitivity, specificity and agreement of RBPT and STAT by comparing with I-ELISA for detection of *Brucella* antibodies in goats

In case of goat, the sensitivity of RBPT and STAT was 27.27% and 18.18%, respectively, considering I-ELISA as a gold standard test while specificity was 97.82% and 100%, respectively. The overall agreement of RBPT and STAT with I-ELISA was 60.00% and 55.00%, respectively (Table 4 and Fig.3)

Barbudhe *et al.,* (1994) obtained lower the relative sensitivity and similar specificity to

be of 42.85% and 100.00% for RBPT, respectively and lower the relative sensitivity and relative similar specificity to be of 28.57% and 94.17% for STAT in goats when CFT was considered as gold standard test which was somewhat similar with present finding.

Sharma *et al.,* (2006) recorded the sensitivity (67.85%) which was higher than present study and specificity (99.51%) which was similar to present study of RBPT in goat of Mehsana and Patan. district of Gujarat when compared with dot-ELISA. Similarly Ekgatat *et al.,* (2010) who found higher diagnostic sensitivity (99.2%) which was higher than present study and diagnostic specificity (100%) which was similar to present study of RBT in goat.

In addition Reddy *et al.,* (2014) found higher relative sensitivity (54.16%) of RBPT and higher relative sensitivity (66.66%) of STAT while similar specificity (100%) for both tests, considering I-ELISA as a gold standard test in goat of the Karnataka.

Overall sensitivity, specificity and agreement of RBPT and STAT by comparing with I-ELISA for detection of *Brucella* antibodies in sheep

In case of sheep, the sensitivity of RBPT and STAT was 28.95% and 15.78%, respectively, considering I-ELISA as a gold standard test while specificity was 100% for both test. Thus, sensitivity of RBPT was more than STAT but specificity of RBPT and STAT was similar. An overall agreement of RBPT and STAT with I-ELISA was 73.00% and 68.00%, respectively (Table 5 and Fig. 4).

Hoverer, Kotadiya, (2012) who recorded higher the sensitivity of RBPT (65.83%) and STAT (62.02%) while similar the specificity (100%) for both the tests, considering I-

ELISA as a gold standard test in sheep although found similar results that RBPT was more sensitive than STAT but specificity was similar for both tests.

In contrast, Kotadiya, (2012) in sheep, who found overall agreement of RBPT and STAT with I-ELISA were 93.78% and 91.25%, respectively which was relative higher than present study. Similarly, Shadhu *et al.,* (2015) who found overall agreement of RBPT and STAT with I-ELISA was 92.50% and 92.30% in small ruminants which was higher than present study.

However in view of cost consideration, feasibility and reliability as field diagnostic test RBPT has been found to be much cheaper, easier and convenient to perform than ELISA.

Acknowledgement

References

Abdel-Khalek, M., Ramadan, K., Hazem, S., and Khairy, E. 2012. Evaluation of immunochromatographic assay for serodiagnosis of *Brucella* among cattle, sheep and goats in Egypt. *Global Vet.,* 8: 511-518.

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

Barbuddhe, S.B., Yadava, V.K. and Singh, D.K. 1994. Detection of IgM and IgG antibodies against *Brucella* by dot-ELISA in humans. *The J. Communicable Dis.,* 26(1): 1-5.

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. *J. Anim. Plant Sci.,* 23(1): 113-118.

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

Kotadiya, A.J. 2012. Serological, cultural and molecular detection of *Brucella* infection of sheep in Gujarat. M. V. Sc. thesis submitted to SardarkrushinagarDantiwada Agriculture University, Sardarkrushinagar, Gujarat.

Kumar, A.V., Srikanth, N.R., Naresh, G. and Vidya, B. 2015. Assessment and comparison of serum biochemical parameters of *Brucella* infected and healthy ewes. *J. Livestock Sci.,* 6: 100-103.

MacMillan, A.A. 1990. Conventional serological tests. *Animal brucellosis,* 206: 153-197.

Mathur, T.N. 1967. Isolation of *Brucella* abortusfrom goats and sheep in the Punjab. *Indian J. Vet. Sci.,* 37: 277-286.

McDermott, J.J. and Arimi, S.M. 2002. Brucellosis in sub-Saharan Africa: epidemiology, control and impact. *Vet. Microbiol.,* 90(1): 111-134.

Pal, M. 2007. Zoonoses. (2nd ed.) Satyan Publishers, Jaipur, India. pp. 98-99.

Polding, J.B. 1942. Brucellosis in India. *Ind. J. Vet. Sci.,* 13: 27-34.

Radostits, O.M., Gay, C.C., Hinchcliff, K.W. and Constable, P.D. (Eds.). 2006. Veterinary Medicine: A textbook of the diseases of cattle, horses, sheep, pigs and goats. Elsevier Health Sciences.

Rahman, M.S., Faruk, M.O., Her, M., Kim, J.Y., Kang, S. and Jung, S.C. 2011.

Prevalence of brucellosis in ruminants in Bangladesh. *Veterinarni Medicina,* 56(8): 379-385.

Raju, S., Kolhe, R.P., Raut, C., Shinde, S.V. and Zade, N.N. 2004. Sero-epizootological studies on brucellosis in goat and sheep. *J. Vet. Public Health,* 2(1/2):1-6.

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

Renukaradhya, G.J., Isloor, S. and Rajasekhar, M. 2002. Epidemiology, zoonotic aspects, vaccination and control/eradication of brucellosis in India. *Vet. Microbiol.,* 90(1): 183-195

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.

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.

Shome, R., Shome, B.R., Deivanai, M., Desai, G.S., Patil, S.S., Bhure, S.K. and Prabhudas, K. 2006. Seroprevalence of brucellosis in small ruminants. *Indian J. Comp. Microbiol. Immunol. Infect. Dis.*, 27(1): 13-15.

Singh, S.V., Chaubey, K.K., Gupta, S., Gupta, V.K., Agrawal, N.D. and Kumar, N. 2013. Co-infection of Mycobacterium avium subspecies paratuberculosisand *Brucella melitensis* in a sirohi breed goats in India. *Adv. Anim. Vet. Ci.*, 1(6): 188-190.

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

Tayshete, S.R. 2001. Seroprevalence of brucellosis in North Gujarat. (M. V. Sc. thesis submitted to S. D. A. University, S. K. Nagar, Gujarat).

Tegegn, A.H., Feleke, A., Adugna, W. and Melaku, S.K. 2016. Small ruminant brucellosis and public health awareness in two districts of Afar Region, Ethiopia. *J. Veterinar. Sci. Technol.,* 7(335): 2.

Thavamathi, A. 2006. Seroepidemiology of Caprine Brucellosis and Evaluation of Recombinant omp28 as a diagnostic antigen. Thesis, M. V. Sc (Epidemiology) submitted to Deemed University, Indian Veterinary Research Institute, Izatnagar, UP.

Valarmathy, K., Kumar, M., Singh, J.L. and Ananda, B.V. 2007. Seroepidemiological study of brucellosis in sheep and goats in Uttarakhand. *J. Vet. Public Health,* 5(1): 45-47.

WHO. 2009. Integrated Control Of Neglected Zoonotic Diseases In Africa: Applying The "One Health Concept". WHO Document Production Services, Geneva, Switzerland. Epidemiology Records. 84(17): 147-148.