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Comparative efficacy of RBPT, STAT, Dot-ELISA, Plate ELISA and serum PCR indiagnosis of caprine brucellosis

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Abstract
Present study was aimed at comparing efficacy of serological tests including RBPT, STAT, Dot-ELISA, Plate ELISA and serum PCR in diagnosis of caprine brucellosis using unvaccinated goat population as target. Pair-wise comparison of different tests employed for diagnosis of brucellosis revealed almost perfect agreement was observed between RBPT and Dot-ELISA (k = 0.8366), while substantial agreement was observed between STAT and Plate ELISA (k = 0.7364), Dot ELISA and PCR (k = 0.6414) and Plate ELISA and PCR (k = 0.6642). Results from STAT vs Dot ELISA (k = 0.6009), STAT vs PCR (k = 0.5981), Dot ELISA vs Plate ELISA (k = 0.5572), RBPT vs Plate ELISA (k = 0.5412) and RBPT vs STAT (k = 0.4487) revealed moderate agreement between tests. RBPT and PCR showed a fair agreement with kappa value of 0.3924. Evaluation of relative sensitivity and specificity of different diagnostic tests revealed highest sensitivity (98.78%) for Dot ELISA followed by RBPT (85.3%) while they lack specificity. However specificity of Plate ELISA (99.8%) and PCR (99.4%) was highest but with low sensitivity. The results suggested use of a combination of serological tests for screening, supplemented with direct test like isolation and/or PCR for confirmation and species level identification.

Keywords: Dot-ELISA, Plate ELISA, serum PCR, caprine brucellosis

Introduction
Brucellosis is a widespread zoonosis affecting a variety of livestock and wildlife, caused by gram-negative, non-spore-forming, facultative, intracellular bacteria of genus *Brucella.* In goats brucellosis is caused mainly by *Brucella melitensis biovars 1, 2, and 3*, which is the most important cause of abortion and zoonosis. Due to their high virulence to humans, *B. abortus, B. melitensis* and *B.suis* are considered potential bioterrorist agents, having been classified as major biodefense/biothreat pathogens, and their possession and use is strictly regulated in the United States (Rossetti *et al.,* 2009) [23]. The major concern for *B.melitensis* infection in goats is that it causes abortion in the goats. Since the aborted placenta and fetus are massively contaminated with *Brucella,* the migrating flocks present a major risk by dissemination of the organisms in the environment and to exposure of uninfected animals to the disease (Minas, 2006) [18]. Also, *B. melitensis* is excreted in the milk. The fact that brucellosis can revert to latency suggests that a certain amount of animals could be silent carriers of the disease. Many of the gestating dams can, therefore, transfer the disease in utero to the fetuses or post-natally to the offspring throughout lactation. In humans, brucellosis has been essentially recognized as an occupational hazard, but infections can also occur from ingesting contaminated dairy products (Seleem *et al.*, 2010) [25]. Thus proper control and efficient diagnostic techniques is the prime requisite for elimination of disease.

Diagnostic tests can be applied with different goals: confirmatory diagnosis, screening or prevalence studies, certification, and, in countries where brucellosis is eradicated, surveillance in order to avoid the reintroduction of brucellosis through importation of infected animals or animal products. Diagnostic methods include direct tests, involving microbiological analysis or DNA detection by polymerase chain reaction (PCR)-based methods; and indirect tests, which are applied either *in vitro* (mainly on milk or blood) or *in vivo* (intradermal allergic test) (Godfroid *et al.*, 2010) [25]. The choice of a particular testing strategy depends on the prevailing epidemiological situation of brucellosis in susceptible animals (livestock and wildlife) in a

country or a region. Present study was aimed at comparing efficacy of serological tests including RBPT, STAT, Dot-ELISA, Plate ELISA and serum PCR in diagnosis of caprine brucellosis using unvaccinated goat population as target.

Materials and methods

A total of 680 samples were collected from 8 different areas viz. Kharnak, Sumdho, Chuchul, Stakna, Digger and Kargyam of Ladakh irrespective of age and sex. About 5 ml of blood was collected from each animal aseptically from the jugular vein in vacutainers containing clot activator. The serum was separated by centrifugation at 5000 rpm for 10 min and stored in multiple aliquots at -20 ^{0}C till further use. Different diagnostic tests applied on these samples were: Modified Rose Bengal Precipitation Test-(RBPT), Standard Tube Agglutination Test – (STAT), Dot ELISA, Plate ELISA and serum PCR.

Modified Rose Bengal Precipitation Test (MRBPT): The coloured antigen procured from the Indian Veterinary Research Institute (I.V.R.I.), Izatnagar, Uttar Pradesh was used. The test was performed according to the manufacturer's literature and as recommended by OIE (OIE, 2009; Mariam *et al*. 2017) [20, 17].

Standard Tube Agglutination Test (STAT): The plain antigen for STAT was obtained from the I.V.R.I., Izatnagar, Uttar Pradesh and the test performed according to the manufacturer's literature as described by Mariam *et al.* (2017) [17].

Dot-Enzyme Linked Immunosorbent Assay (Dot-ELISA): The test was performed using *B. melitensis* detection kit for sheep and goats (Central Institute for Research on Goats, Makhdoom, U.P) as per the manufacturer's protocol (Mariam *et al*. 2017) [17].

Plate Enzyme Linked Immunosorbent Assay (ELISA): Plate ELISA was performed using *B. meltensis* whole antigen as per the method described by Mariam *et al.* (2017) [17].

Polymerase Chain Reaction (PCR): Wizard® Genomic DNA Purification Kit was used for DNA extraction from serum samples. Standard cultures available in the Division were used as positive control. Brucellosis was confirmed by carrying out geneus specific PCR for amplification of 282 bp fragment of omp2 gene using the primer sequences described by Bardenstein *et al*., 2002 [6]. [5'TGGAGGTCAGAAATGAAC3' (Forward) and 5'GAGTGCGAAACGAGCGC3' (Reverse)]. For confirmation of *B. melitensis*, the primer sequences described by Redkar *et al*. (2001) [21] viz. forward Primer (5'CATGCGCTATGTCTGGTTAC3') derived from the 3' end of the genetic element IS711 and reverse Primer (5'AGTGTTTCGGCTCAGAATAATC3') derived from signature sequences of *B. melitensis*, were used to amplify a 252 bp fragment. PCR amplifications were performed in 25µL reactions as per the method described by Samadi *et al*. (2010) [24] and AL-Garadia *et al*. (2011). Amplified products were analyzed by electrophoresis in ethidium bromide stained 1.5% agarose gel in TBE buffer and documented using a Bio-Rad® gel document system. The detailed procedure has been described earlier (Mariam *et al*. 2017) [17].

Statistical Analysis

Statitical analysis was done using 2x2 contingency tables of different tests using all combinations and the relatedness of tests was predicted using kappa statistic (Viera and Garrett, 2005) [27]. Relative sensitivity, specificity, positive and negative predictive values were calculated taking one of the tests as gold standard (one by one) and comparing other tests with it using XLStat-2014 software.

Sensitivity= a/ n1; Specificity= d/ n0

Positive predictive value= a/m1; Negative predictive value= d/m0

% of false negative= c/n1; % of false positive= b/n0

Test 2/Test 1	+ve	-ve	Total
+ve	a	b	m_1
-ve	c	d	m_0
Total	n_1	n_0	n

$$\text{Kappa Value} = \frac{Po - Pe}{1 - Pe}$$

$$P_{o=} \frac{a+d}{N}$$

$$pe = [(n1/n) * (m1/n)] + [(n0/n) * (m0/n)]$$

Interpretation: Kappa Agreement
< 0: Less than chance agreement
0.01–0.20: Slight agreement
0.21– 0.40: Fair agreement
0.41–0.60: Moderate agreement
0.61–0.80: Substantial agreement
0.81–0.99: Almost perfect agreement

Results

Pair-wise comparison of different diagnostic tests applied to same set of samples is presented in table 1. Comparative evaluation of RBPT and STAT showed that out of 680 samples screened, 489 were negative in both; 72 positive by both; 95 negative by STAT, positive by RBPT; and 24 negative by RBPT, positive by STAT. Similarly, comparison between RBPT and dot ELISA, showed 487 samples were negative and 151 positive by both; 16 were negative by dot ELISA, positive by RBPT; and 26 were negative by RBPT, positive by dot ELISA. Comparison between dot ELISA and STAT showed 499 samples were negative and 92 positive by both tests; 85 were negative by STAT, positive by dot ELISA; while 4 were negative by dot ELISA, positive by STAT. RBPT vs plate ELISA results revealed 507 samples negative and 77 positive by both Tests; 6 were negative by RBPT, positive by plate ELISA; and 90 were negative by plate ELISA, positive by RBPT. Comparison between plate ELISA and STAT showed 570 samples negative and 69 positive by both tests; 14 samples were negative by STAT, positive by plate ELISA; and 27 negative by plate ELISA, positive by STAT. Comparison between plate ELISA and dot ELISA showed 502 samples were negative and 82 positive by both tests; 1 was negative by dot ELISA, positive by plate ELISA; and 95 were negative by plate ELISA, positive by dot ELISA. Comparative evaluation between PCR and RBPT showed 479 were negative and 68 positive by both tests; 34 were RBPT negative PCR positive; and 99 were PCR negative RBPT

positive. Comparison between PCR and STAT showed 547 were negative and 65 positive by both tests; 37 were negative by STAT, positive by PCR; and 31 negative by PCR, positive by STAT. Comparison between PCR and dot ELISA revealed 500 samples were negative and 99 positive by both tests; 3 negative by dot ELISA, positive by PCR; and 78 negative by PCR, positive bydot ELISA. Comparison between PCR and Plate ELISA revealed 560 samples were negative and 66 positive by both tests; 36 was negative by plate ELISA, positive by PCR; and 18 were negative by PCR, positive by plate ELISA.

The kappa values depicting the relatedness of different diagnostic tests for brucellosis are in table 2. Almost perfect agreement was observed between RBPT and Dot-ELISA (k = 0.8366), while substantial agreement was observed between STAT and Plate ELISA (k = 0.7364), Dot ELISA and PCR (k = 0.6414) and Plate ELISA and PCR (k = 0.6642). Results from STAT vs Dot ELISA (k = 0.6009), STAT vs PCR (k = 0.5981), Dot ELISA vs Plate ELISA (k = 0.5572), RBPT vs Plate ELISA (k = 0.5412) and RBPT vs STAT (k = 0.4487) revealed moderate agreement between tests. RBPT and PCR showed a fair agreement with kappa value of 0.3924.

The relative sensitivity, specificity, predictive values, etc. of various tests, considering RBPT as gold standard, are given in table 3. The sensitivity and specificity of STAT, Dot ELISA, Plate ELISA and PCR with respect to RBPT were 0.431 (95% CI, 0.358-0.507) and 0.953 (95% CI, 0.931-0.969); 0.904 (95% CI, 0.849-0.941) 0.949 (95% CI, 0.926-0.965); 0.461 (95% CI, 0.387-0.537); 0.988 (95% CI, 0.974-0.995); 0.407 (95% CI, 0.336-0.483); 0.934 (95% CI, 0.908-0.952), respectively.

The relative sensitivity, specificity, predictive values, etc. of various tests, considering STAT as gold standard, are given in table 4. The sensitivity and specificity of RBPT, Dot ELISA, Plate ELISA and PCR with respect to STAT were 0.750 (95% CI, 0.654-0.826) and 0.837 (95% CI, 0.805-0.865); 0.958 (95% CI, 0.893-0.987) and 0.854 (95% CI, 0.823-0.881); 0.719 (95% CI, 0.621-0.799) and 0.976 (95% CI, 0.960-0.986); 0.677 (95% CI, 0.578-0.762) and 0.937 (95% CI, 0.914-0.954), respectively.

The relative sensitivity, specificity, predictive values, etc. of various tests with respect to Dot ELISA are given in table 5. The sensitivity and specificity of RBPT, STAT, Plate ELISA and PCR were 0.853 (95% CI, 0.793-0.898) and 0.968 (95% CI, 0.948-0.981); 0.520 (95% CI, 0.447-0.592) and 0.992 (95% CI, 0.979-0.998); 0.463 (95% CI, 0.391-0.537) and 0.998 (95% CI, 0.987-1.000); 0.559 (95% CI, 0.486-0.630) and 0.994 (95% CI, 0.982-0.999), respectively.

The relative sensitivity, specificity, predictive values, etc. of various tests with respect to Plate ELISA are given in table 6. The sensitivity and specificity of RBPT, STAT, Dot ELISA and PCR with respect to Plate ELISA were 0.928 (95% CI, 0.847-0.969) and 0.849 (95% CI, 0.818-0.876); 0.831 (95% CI, 0.735- 0.897) and 0.955 (95% CI, 0.935-0.969); 0.988 (95% CI, 0.927-1.000) and 0.841 (95% CI, 0.809-0.868); 0.786 (95% CI, 0.685-0.860) and 0.940 (95% CI, 0.917-0.956), respectively.

The relative sensitivity, specificity, predictive values, etc. of various tests with respect to Plate ELISA are given in table 86. The sensitivity and specificity of RBPT, STAT, Dot ELISA and plate ELISA were 0.667 (95% CI, 0.570-0.751) and 0.829 (95% CI, 0.796-0.857); 0.637 (95% CI, 0.540-0.724) and 0.946 (95% CI, 0.925-0.962); 0.971 (95% CI, 0.912-0.993) and 0.865 (95% CI, 0.835-0.891); 0.647 (95% CI, 0.550-0.733) and 0.969 (95% CI, 0.951-0.980), respectively.

Table 1: Comparison of Rbpt, Stat, Dot Elisa, and Pcr (2x2 Contingency Table)

Test		Stat			Dot Elisa			Pcr		
		+Ve	-Ve	Total	+Ve	-Ve	Total	+Ve	-Ve	Total
RBPT	+VE	72	95	167	151	16	167	68	99	167
	–VE	24	489	513	26	487	513	34	479	513
Total		96	584	680	177	503	680	102	578	680
Stat	+VE				92	4	96	65	31	96
	–VE				85	499	584	37	547	584
Total					177	503	680	102	578	680
Dot ELISA	+VE							99	78	177
	–VE							3	500	503
Total								102	578	680

Table 2: Comparison of RBPT, STAT, Dot ELISA, and PCR (Kappa Values)

Test	Stat	Dot Elisa	PCR
RBPT	0.4487	0.8366	0.3924
Stat		0.6009	0.5981
Dot Elisa			0.6414

Table 3: Estimates of relative attributes of STAT, Dot ELISA and PCR with RBPT as gold standard (95% Confidence Interval)

	Stat	Dot Elisa	PCR
Sensitivity	0.431 (0.358-0.507)	0.904 (0.849-0.941)	0.407 (0.336-0.483)
Specificity	0.953 (0.931-0.969)	0.949 (0.926-0.965)	0.934 (0.908-0.952)
Prevalence	0.246 (0.213-0.278)	0.246 (0.213-0.278)	0.246 (0.213-0.278)
False positive rate	0.047 (0.029-0.065)	0.051 (0.032-0.070)	0.066 (0.045-0.088)
False negative rate	0.569 (0.495-0.643)	0.096 (0.052-0.140)	0.593 (0.519-0.666)
PPV (Positive Predictive Value)	0.750 (0.663-0.837)	0.853 (0.801-0.905)	0.667 (0.575-0.758)
NPV (Negative Predictive Value)	0.837 (0.807-0.867)	0.968 (0.953-0.984)	0.829 (0.798-0.859)

Table 4: Estimates of relative attributes of RBPT, Dot ELISA, and PCR with Stat as gold standard (95% Confidence Interval)

	RBPT	Dot ELISA	PCR
Sensitivity	0.750 (0.654-0.826)	0.958 (0.893-0.987)	0.677 (0.578-0.762)
Specificity	0.837 (0.805-0.865)	0.854 (0.823-0.881)	0.937 (0.914-0.954)
Prevalence	0.141 (0.115-0.167)	0.141 (0.115-0.167)	0.141 (0.115-0.167)
False positive rate	0.163 (0.133-0.193)	0.146 (0.117-0.174)	0.063 (0.044-0.083)
False negative rate	0.250 (0.165-0.335)	0.042 (0.003-0.081)	0.323 (0.231-0.415)
PPV (Positive Predictive Value)	0.431 (0.356-0.506)	0.520 (0.446-0.593)	0.637 (0.544-0.731)
NPV (Negative Predictive Value)	0.953 (0.935-0.971)	0.992 (0.984-1.000)	0.946 (0.928-0.965)

Table 5: Estimates of relative attributes of RBPT, STAT and PCR with Dot ELISA as gold standard (95% Confidence Interval)

	RBPT	STAT	PCR
Sensitivity	0.853 (0.793-0.898)	0.520 (0.447-0.592)	0.559 (0.486-0.630)
Specificity	0.968 (0.948-0.981)	0.992 (0.979-0.998)	0.994 (0.982-0.999)
Prevalence	0.260 (0.227-0.293)	0.260 (0.227-0.293)	0.260 (0.227-0.293)
False positive rate	0.032 (0.017-0.047)	0.008 (0.000-0.016)	0.006 (0.000-0.013)
False negative rate	0.147 (0.095-0.198)	0.480 (0.407-0.553)	0.441 (0.368-0.513)
PPV (Positive Predictive Value)	0.904 (0.860-0.949)	0.958 (0.918-0.998)	0.971 (0.938-1.000)
NPV (Negative Predictive Value)	0.949 (0.930-0.968)	0.854 (0.826-0.883)	0.865 (0.837-0.893)

Table 6: Estimates of relative attributes of RBPT, STAT, and Dot ELISA with PCR as gold standard (95% Confidence Interval)

	RBPT	STAT	Dot ELISA
Sensitivity	0.667 (0.570-0.751)	0.637 (0.540-0.724)	0.971 (0.912-0.993)
Specificity	0.829 (0.796-0.857)	0.946 (0.925-0.962)	0.865 (0.835-0.891)
Prevalence	0.150 (0.123-0.177)	0.150 (0.123-0.177)	0.150 (0.123-0.177)
False positive rate	0.171 (0.141-0.202)	0.054 (0.035-0.072)	0.135 (0.107-0.163)
False negative rate	0.333 (0.244-0.423)	0.363 (0.271-0.454)	0.029 (0.000-0.062)
PPV (Positive Predictive Value)	0.407 (0.333-0.482)	0.677 (0.584-0.771)	0.559 (0.486-0.632)
NPV (Negative Predictive Value)	0.934 (0.912-0.955)	0.937 (0.917-0.956)	0.994 (0.987-1.000)

Discussion

Pair-wise comparison of different tests employed for diagnosis of brucellosis revealed almost perfect agreement was observed between RBPT and Dot-ELISA. Similar findings have been reported by Das *et al*. (2004). Substantial agreement was observed between STAT and Plate ELISA, Dot ELISA and PCR and Plate ELISA and PCR. Results from STAT vs Dot ELISA, STAT vs PCR, Dot ELISA vs Plate ELISA, RBPT vs Plate ELISA and RBPT vs STAT revealed moderate agreement between tests. Similar results have been for ELISA and RBPT and PCR and Dot ELISA by Malik *et al.* (2013) [16]. However, contrary to present finding, Yohannes *et al*. (2012) [29] reported perfect agreement between RBPT and STAT. However, Chachra *et al.* (2009) [7] reported that out of 18 serum samples from cattle suspected for brucellosis, 9 were positive by RBPT and only 1 was positive by STAT. In present study RBPT and serum PCR showed the least (fair) agreement. Al-Garadia *et al.* (2011) also reported worst correlation between RBPT and real-time PCR. The results suggest that a combination of serological tests may be used for screening, which should be supplemented with direct test like PCR for confirmation and species level identification (Al-Garadia *et al.*, 2011; Arabaci and Oldacay, 2012) [5]. A combination of the RBPT and ELISA has been suggested to achieve accurate diagnosis of brucellosis (Abuharfeil and Abo-Shehda, 1998; Yohnnes *et al.*, 2012) [91].

Evaluation of relative sensitivity and specificity of different diagnostic tests revealed highest sensitivity (98.78%) for Dot ELISA followed by RBPT (85.3%) while they lack specificity. However specificity of Plate ELISA (99.8%) and PCR (99.4%) was highest but with low sensitivity No single serological test has been shows 100% sensitivity and specificity simultaneously (Munoz *et al*., 2005). Sulima *et al.*, (2010) [26] reported that i-ELISA was more sensitive with, followed by RBT and STAT for diagnosis of brucellosis in sheep and goat. The lower specificity of RBPT and STAT may be attributed to cross reactivity of the anti-brucella antibodies (Al-Attas *et al.,* 2000) [2]. ELISA has been reported to have higher sensitivity. It is recommended by OIE (OIE, 2009) [20] and is considered a better test in early detection of infection than classical diagnostic tests like complement fixation, agglutination and precipitation (Rojas and Alonso, 1995) [22]. El-Razik *et al*. (2007) [10] have suggested its efficiency as a screening and confirmatory diagnostic test in goats and sheep. Hendy (1996) [14] reported highest sensitive for SAT and ind ELISA in case of cattle, Indirect-ELISA in case ofBuffaloes and goats and Indirect-ELISA, BAPAT and SAT in sheep. Anisur Rahman *et al.*, (2013) [4] reported that the sensitivity and specificity of 92.9% and 96.5% in goats and 92.0% and 99.5% in sheep for iELISA; 80.2% and 99.6% in goats and 82.8% and 98.3% in sheep for RBT; and 57.1% and 99.3% in goats and 72.0% and 98.6% in sheep for SAT. Chanda *et al.*, (2005) [8] reported that the specificity of milk-ELISA in brucellosis free flock was 100% and sensitivity and positive predictive value were 96.11% and 94.28%, respectively, in infected flocks. The low sensitivity but high specificity approaching 100% for PCR has been reported by previous workers (Leyla *et al.,* 2003; Gupta *et al.*, 2006; Hajia *et al*., 2013) [15, 12, 13].

The results suggested use of a combination of serological tests for screening, supplemented with direct test like isolation and/or PCR for confirmation and species level identification.

References

1. Abuharfeil N, Abo-Shehada MN. A comparison between three serological tests for Brucella melitensis infection in sheep. Turkish Journal of Veterinary and Animal Sciences. 1998; 22:119-122.
2. Al-Attas RA, Alkhalifa M, Alqurashi AR. Evaluation of PCR, culture and serology for the diagnosis of acute

human brucellosis. Annals of Saudi Medicine. 2000; 20:224-228.
3. AL-Garadi MA, Khairani-Bejo S, Zunita Z, Omar AR. Detection of *Bucella melitensis* in blood samples from Goats. Journal of Animal and Veterinary Advances. 2011; 10(11):1437-1444.
4. Anisur Rahman AKM, Saegermanb C, Berkvensc D, Fretind D, Ganie MO *et al*. 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 Veterinary Medicine. 2013; 110(2):242-252.
5. Arabaci F, Oldacay M. Evaluation of serological diagnostic tests for human Brucellosis in an endemic area. Journal of Microbiology and Infectious Diseases. 2012; 2(2):50-56.
6. Bardenstein S, Mandelboim M, Ficht TA, Baum M, Banai M. Identification of the *Brucella melitensis* vaccine strain Rev.1 in animals and humans in Israel by PCR analysis of the PstI site polymorphism of its omp2 gene. Journal of Clinical Microbiology. 2002; 40(4):1475-80.
7. Chachra D, Saxeana HM, Kaur G, Chandra M. Comparative efficacy of RBPT, STAT and Dot ELISA in immunological detection of antibodies to *Brucella abortus* in sera. Journal of Bacteriological Research. 2009; 98:1270-1281.
8. Chand P, Rajpurohit BS, Malhotra AK, Poonia JS. Comparison of milk-ELISA and serum-ELISA for the diagnosis ofB*rucella melitensis* infection in sheep. Veterinary microbiology. 2005; 108(3-4):305-11.
9. Das AM, Kasalkar AB, Sherikar AA, Majee SB, Gandge RS, Rathod PN. Application of Dot-Elisa in the serological survey of Brucellosis among buffaloes. The Journal of Bombay Veterinary College. 2004; 12(1-2):25-27.
10. El-Razik KA, Ghazi YA, Salama EM. Monitoring of Brucella reactor does following milk examination using different techniques. Pakistan Journal of Biological Sciences. 2007; 10:240-244.
11. Godfroid J, Nielsen K, Saegerman C. Diagnosis of brucellosis in livestock and wildlife. Croatian medical Journal. 2010; 51(4):296-305.
12. Gupta VK, Verma DK, Rout PK, Singh SV, Vihan VS. Polymerase chain reaction (PCR) for detection of *Brucellamelitensis* in goat milk. Small Ruminant Research. 2006; 65(1):79-84.
13. Hajia M, Fallah F, Angoti G, Karimi A, Rahbar M, Gachkar L *et al*. Comparison of Methods for Diagnosing Brucellosis. Laboratory Medicine. 2013; 44(1):29.
14. Hendy AM. *Comparative Serological Studies on Brucellosis in Animals*. M.V.Sc. thesis submitted to Department of Microbiology, Faculty of Veterinary Medicine, Assiut University, Egypt, 1996.
15. Leyla G, Kadri G, Umran O. Comparison of polymerase chain reaction and bacteriological culture for the diagnosis of sheepbrucellosis using aborted fetus samples. Veterinary Micobiology. 2003; 93:53-61.
16. Malik RUI, Mohinder PG, Pritam KS, Gursimran F, Hari MS, Tawheed AS. Comparative evaluation of indirect enzyme linked immunosorbent assay, rose bengal plate test, microagglutination test, and polymerase chain reaction for diagnosisof brucellosis in buffaloes. Turkish Journal of Veterinary & Animal Sciences. 2013; 37(3):306.
17. Mariam A, Mir MS, Gupta V, Kashoo ZA, Rather MA, Baba OK *et al*. Prevalence of Brucellosis in Changra goats of cold arid desert, Ladakh, Jammu and Kashmir. International Journal of Livestock Research. 2017; 7(12):124-142.
18. Minas A. Control and eradication of brucellosis in small ruminants. Small Ruminant Research. 2006; 62(1):101-107.
19. Mu˜noz PM, Marn CM, Monreal D, Gonzlez D, Garin-Bastuji B, Daz R *et al*. Efficacy of several serological tests and Antigens for diagnosis of bovine brucellosis in the presence of false-positive serological results due to *Yersinia enterocolitica* O: 9. Clinical and Diagnostic Laboratory Immunology. 2005; 12(1):141-151.
20. OIE. Bovine brucellosis. In Manual of standards for diagnostic tests and vaccines. Office International de Epizooties (OIE), Paris, 2009.
21. Redkar R, Rose S, Bricker B, Del Vecchio V. Real-time detection of *Brucella abortus*, *Brucella melitensis* and *Brucella suis*. Molecular and Cellular Probes. 2001; 15(1):43-52.
22. Rojas X, Alonso O. ELISAs for the diagnosis and epidemiology of Brucella abortus infection in cattle in Chile. Archivos de Medicina Veterinaria. 1995; 27:45-50.
23. Rossetti CA, Galindo CL, Lawhon SD, Garner HR, Adams, LG. *Brucella melitensis* global gene expression study provides novel information on growth phase-specific gene regulation with potential insights for understanding Brucella:host initial interactions. BMC Microbiology. 2009; 9:81.
24. Samadi MA, Ababneh MK, Giadinis ND, Lafi SQ. Ovine and Caprine Brucellosis (*Brucella melitensis*) in Aborted Animals in Jordanian Sheep and Goat Flocks. Veterinary Medicine International. 2010, 458-695.
25. Seleem MN, Boyle SM, Sriranganathan N. Brucellosis:a reemerging zoonosis. Veterinary Microbiology. 2010; 140(3-4): 392-8.
26. Sulima M, Venkataraman KS, Rishikesavan R. Seroprevalence of brucellosis in different age groups of sheep and goats. Indian Journal of Field Veterinarians 2010; 6(2):25-26.
27. Viera AJ, Garrett JM. Understanding Interobserver Agreement: The Kappa Statistic. Family Medicine. 2005; 37(5):360-363.
28. WHO. Fact sheet N173. 1997. (www.who.int/inf-fs/en/fact 173.hrml).
29. Yohannes M, Gill JPS, Ghatak S, Singh, DK, Tolosa T. Comparative evaluation of the Rose Bengal plate test, standard tube agglutination test and complement fixation test for the diagnosis of human brucellosis. Rev. sci. tech. Office Internationale. Epizootics. 2012; 31(3):979-984.