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## Blood biochemical indices lack diagnostic significance in subclinical caprine brucellosis: Evidence from non-vaccinated *Brucella melitensis* positive Changra goats in Northern Himalayan region of Ladakh

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#### Abstract

Caprine brucellosis caused by *Brucella melitensis* is prevalent globally causing severe economic losses in terms abortions and still births, maintenance of animals with sub-clinical or latent infection and associated labour, besides being an important zoonosis. Cohort serum biochemical profiling aimed at elucidating any possible diagnostic efficacy in caprine brucellosis was undertaken in unvaccinated Pashmina goats. Positive and negative reactors from farmer's flocks were identified by RBPT and STAT, and *Brucella melitensis* infection confirmed by species specific Dot-ELISA and Plate-ELISA and serum based PCR. Evaluation of serum biochemical parameters with respect to positive vs negative reactor goats revealed non-significant differences in blood glucose (76.697±4.747 vs 67.534±4.019); total protein (5.959±0.106 vs 5.947±0.104); albumin (3.191±0.048; 3.272±0.062); globulin (2.769±0.107 vs 2.675±0.100); AST (62.722±3.839 vs 67.972±4.925); ALT (16.753±1.340 vs 15.684±1.197); ALP (250.670±20.067 vs 266.960±20.100); Creatinine (0.638±0.072 vs 0.713±0.073); urea (30.717±1.443 vs 30.397±1.808); cholesterol (137.214±3.406 vs 138.322±3.363). The serum biochemical indices seemed to be stable with respect to brucellosis at least in sub-clinical or latent phase of infection in goats.

**Keywords:** Blood biochemical, subclinical caprine brucellosis, *Brucella melitensis*, Northern Himalayan

#### Introduction

Brucellosis is one of the leading global zoonoses (Seleem *et al.*, 2010) [43] and the casual agents *Brucella abortus*, *B. melitensis* and *B. suis* have been classified as major biodefense/biothreat pathogens, and their possession and use is strictly regulated in the United States (Rossetti *et al.*, 2009) [39]. The pathogen has a wide host range including terrestrial and aquatic mammals (de Figueiredo *et al.*, 2015) [10]. Brucellosis is endemic in many developing regions of the globe, including the Middle East, Asia, Africa, and South America, and in the United States where foci of disease remain because of persistent infection in wildlife species (Cutler *et al.* 2005) [9]. *Brucella* spp being enlisted as category B pathogen, the cost of surveillance in areas declared brucellosis free remains very high (de Figueiredo *et al.*, 2015) [10]. The annual incidence of brucellosis among humans has been estimated to be more than 500,000 (Pappas *et al.*, 2006) [35]. In endemic areas the reported incidence varies from <0.01 to >200 per 100,000 population, which has been regarded as gross underestimation of number of persons infected due to absence or low levels of surveillance and reporting (McDermott and Arimi, 2002; Mantur and Amarnath, 2008) [29, 27]. Besides zoonosis, brucellosis has marked socio-economic impact (Wadood *et al.*, 2009; Maadi *et al.*, 2011) [46, 25]. The disease is widespread in India, affecting 5% cattle and causing Rs 350 million losses (Renukaradhya *et al.*, 2002; PD-ADMAS, 2012; Kushwaha *et al.*, 2014) [38, 36, 22].

*B. melitensis*, the major cause of brucellosis among goats has high virulence to humans causing systemic infection with multiple organ pathology. Clinically the acute disease resembles with malaria and typhoid with undulant fever, anorexia, fatigue, headaches, depression, and weight loss. However the disease progresses to chronic form with serious Musculoskeletal (arthritis), cardiovascular (endocarditis), visceral (hepatitis), reproductive (orchitis) and central nervous System (encephalomyelitis) complications (OIE, 2009) [39].

In animals the disease has significant economic impact in terms of compromised productive and reproductive capabilities (Unger, 2003) <sup>[45]</sup>, buttressed by zoonotic threat enforcing culling of the animals following prolonged maintenance. Lack of recognizable clinical signs in animals prior to productive stage constitutes an important limiting factor in its early diagnosis with direct and indirect economic consequences and maintenance of higher zoonotic risk. Besides, following late abortion in first pregnancy, female animals remain carriers throughout life which remain an important risk factor. This is especially important among goats. *B. melitensis* infection in goats causes abortion with heavy bacterial load in aborted placenta and fetus facilitating dissemination to animals and human (Minas, 2006) <sup>[30]</sup>. Besides, transmission of the infection to buck during breeding facilitates rapid spread within the flock and also between the flocks when bucks are exchanged or sold out. Thus pre-clinical, sub-clinical, and carrier stages of the disease may be recognized as a subtle threat challenging researchers and field veterinarians alike. Present study was undertaken with an aim to explore possible pre-clinical significance of serum biochemical indices in goats positive for *B. melitensis* infection.

### Materials and Methods

**Study protocol:** The sampling for laboratory investigation of brucellosis and serum biochemical indices, were carried out from non-vaccinated Changra goats reared in Kharnak, Sumdho, Chushul, Mughlib and Kargyam (traditional rearing belt) and Digger, Turtuk and Stakna (non-traditional belt) area of Ladakh. Modified Rose Bengal Precipitation Test (MRBPT) and Standard Tube Agglutination Test (STAT) were used as screening tests for brucellosis followed by confirmation using *Brucella melitensis* species specific Dot-ELISA and Plate-ELISA and serum based PCR. Animals were allocated to brucellosis positive (positive by at least two confirmatory tests) and negative groups (negative by all tests) for evaluation. 10 animals for each group from each area were included in final study.

**Collection of blood and serum:** Approximately 10 mL of blood samples were collected by venipuncture from the jugular vein using standard techniques, during early morning before animals were let out for grazing. The blood was immediately transferred to sterile clot activator tubes. The serum was separated by centrifugation at 5000 rpm for 10 min and stored in multiple aliquots at -20°C until used.

### Screening for Brucellosis:

**Modified Rose Bengal Precipitation Test (MRBPT):** The antigen obtained from the Indian Veterinary Research Institute (I.V.R.I.), Izatnagar, and Uttar Pradesh was used and the test was performed according to the manufacturer's literature and as per OIE recommendations (OIE, 2009; Mariam *et al.*, 2017) <sup>[39, 28]</sup>.

**Standard Tube Agglutination Test (STAT):** The antigen obtained from the I.V.R.I., Izatnagar was used and the test performed according to the manufacturer's literature as described by Mariam *et al.* (2017) <sup>[28]</sup>.

### Confirmation of *B. melitensis* infection

#### Dot-Enzyme Linked Immunosorbent Assay (Dot-ELISA):

Dot ELISA was performed using kit for *B. melitensis* detection in sheep and goats (Central Institute for Research on Goats, Makhdoom, U.P) as per the manufacturer's protocol (Gupta *et al.*, 2002; Mariam *et al.*, 2017) <sup>[28, 18]</sup>.

#### Plate Enzyme Linked Immunosorbent Assay (ELISA):

Plate ELISA was performed using *B. melitensis* whole antigen as per the method described by Mariam *et al.* (2017) <sup>[28]</sup>.

#### Polymerase Chain Reaction (PCR):

The DNA was extracted from serum samples using a Wizard® Genomic DNA Purification Kit. Standard cultures available in the Division were used as positive control. Genus specific primers for omp2 gene, 5'TGGAGGTCAGAAATGAAC3' (Forward) and 5'GAGTGCAGAAACGAGCGC3' (Reverse), were used to amplify a 282 bp fragment (Bardenstein *et al.*, 2002) <sup>[7]</sup>. For *B. melitensis*, the forward Primer (5'CATGCGCTATGTCTGGTTAC3') derived from the 3' end of the genetic element IS711 and reverse Primer (5'AGTGTTCGGCTCAGAATAATC3') derived from signature sequences of *B. melitensis*, were used to amplify a 252 bp fragment (Redkar *et al.*, 2001) <sup>[37]</sup>. PCR amplification was performed in 25µL reaction as per the method described by Samadi *et al.* (2010) <sup>[41]</sup> 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) <sup>[28]</sup>.

#### Serum Biochemistry:

The biochemical indices analyzed included blood glucose (GOD POD method); serum proteins including total protein (Biuret method), albumin (BCG Dye binding method) and globulin (difference method); Serum enzyme activity including aspartate transaminase (AST), alanine transaminase (ALT) (IFCC method/ Reitman and Frankel's method) and alkaline phosphatase (Modified DGKC method); Kidney function test viz. blood urea nitrogen (BUN) (Berthelot method), and creatinine (modified Jaffe's method); and total cholesterol (CHOD-POD method) using diagnostic kits (Aspen Laboratories Pvt. Ltd, Rapid Diagnostic Group of Companies, Karnal Road Industrial Area, Delhi, India) and semi-automatic blood chemistry analyser (model ERBA CHEM-PRO) as per manufacturer's literature.

**Statistical Analysis:** Data were analyzed by t-test, using SPSS software.

### Results

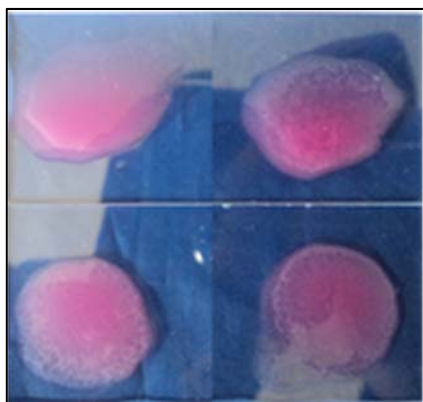
Brucella positive reactors were first identified by MRBPT and STAT followed by confirmation using Dot ELISA, Plate ELISA and PCR (Fig 1-4). Samples negative by all tests were taken as non-reactors. The comparison of various serum biochemical indices in brucellosis-positive and brucellosis-negative reactor Changra goats is presented in table-1.

**Table 1:** Comparison of various serum biochemical indices in brucellosis-positive and brucellosis-negative reactor Changra goats reared in different areas of Changthang, Ladakh (Mean±SE)

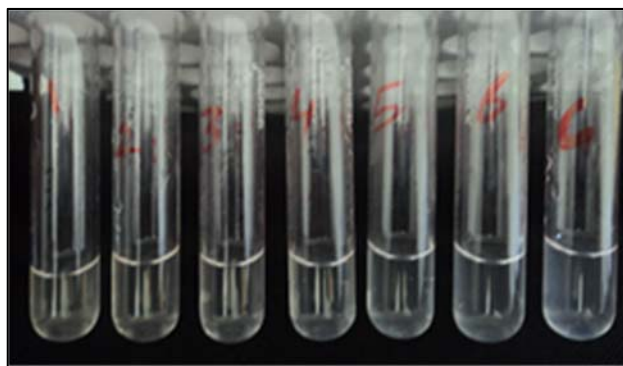
Parameter	Status	Kharnak	Sumdho	Chushul	Kargyam	Stakna	Mughlib	Digger	Turtuk	TOTAL
Glucose	+ ve	54.618 ± 2.877	41.742 ± 2.752	147.748 ± 1.874 <sup>a</sup>	76.830 ± 4.070	70.968 ± 2.110	76.802 ± 1.882	72.072 ± 1.428	72.794 ± 2.246	76.697 ± 4.747
	- ve	55.500 ± 1.307	34.966 ± 1.647	82.100 ± 27.567 <sup>b</sup>	83.720 ± 6.406	66.272 ± 1.951	73.274 ± 0.887	72.276 ± 2.347	72.160 ± 2.226	67.534 ± 4.019
Total protein	+ ve	5.462 ± 0.317	5.248 ± 0.102	5.562 ± 0.243	6.692 ± 0.160	6.486 ± 0.193	5.742 ± 0.119	6.062 ± 0.219	6.420 ± 0.290	5.959 ± 0.106
	- ve	5.292 ± 0.245	5.446 ± 0.128	5.584 ± 0.087	6.772 ± 0.220	6.340 ± 0.295	5.710 ± 0.216	6.054 ± 0.124	6.376 ± 0.306	5.947 ± 0.104
Albumin	+ ve	3.064 ± 0.122	3.180 ± 0.125	2.802 ± 0.117	3.332 ± 0.141	3.360 ± 0.086	3.452 ± 0.125	3.194 ± 0.133	3.140 ± 0.052	3.191 ± 0.048
	- ve	3.136 ± 0.270	3.232 ± 0.063	2.994 ± 0.043	3.634 ± 0.253	3.444 ± 0.146	3.360 ± 0.087	3.100 ± 0.181	3.274 ± 0.144	3.272 ± 0.062
Globulin	+ ve	2.398 ± 0.401	2.068 ± 0.055	2.760 ± 0.300	3.360 ± 0.090	3.126 ± 0.208	2.290 ± 0.051	2.868 ± 0.312	3.280 ± 0.305	2.769 ± 0.107
	- ve	2.156 ± 0.158	2.214 ± 0.173	2.590 ± 0.097	3.138 ± 0.281	2.896 ± 0.327	2.350 ± 0.244	2.954 ± 0.280	3.102 ± 0.295	2.675 ± 0.100
AST	+ ve	69.954 ± 5.273	51.076 ± 8.377	25.672 ± 5.721	78.128 ± 10.258	70.224 ± 7.385	56.288 ± 8.932	64.004 ± 11.255	86.430 ± 6.146	62.722 ± 3.839
	- ve	72.716 ± 5.692	66.472 ± 14.592	24.178 ± 0.928	73.000 ± 4.213	69.444 ± 4.662	56.312 ± 9.662	112.982 ± 18.878	68.668 ± 11.093	67.972 ± 4.925
ALT	+ ve	5.222 ± 1.315	7.512 ± 1.447	25.496 ± 4.762	14.930 ± 2.382	16.830 ± 1.919	24.776 ± 1.375	19.986 ± 1.187	19.272 ± 2.461	16.753 ± 1.340
	- ve	4.716 ± 0.651	6.288 ± 0.904	18.720 ± 1.221	15.184 ± 1.697	15.158 ± 2.447	21.102 ± 2.255	24.672 ± 1.795	19.628 ± 2.426	15.684 ± 1.197
ALP	+ ve	281.610 ± 26.809	316.012 ± 42.955	191.280 ± 19.872	256.614 ± 75.997	252.154 ± 42.935	283.864 ± 99.610	191.372 ± 80.516	232.450 ± 31.471	250.670 ± 20.067
	- ve	356.956 ± 28.367	283.584 ± 43.971	198.622 ± 24.077	309.096 ± 93.361	217.032 ± 25.923	353.670 ± 71.745	198.41 ± 71.125	218.308 ± 25.005	266.960 ± 20.100
Creatinine	+ ve	0.520 ± 0.058	0.340 ± 0.081	0.200 ± 0.055	0.940 ± 0.201	1.300 ± 0.202	0.460 ± 0.093	0.780 ± 0.242 <sup>a</sup>	0.560 ± 0.144	0.638 ± 0.072
	- ve	0.560 ± 0.163	0.360 ± 0.051	0.360 ± 0.068	1.040 ± 0.136	1.080 ± 0.174	0.760 ± 0.220	1.120 ± 0.269 <sup>b</sup>	0.420 ± 0.128	0.713 ± 0.073
Urea	+ ve	24.936 ± 0.682	47.826 ± 2.389 <sup>a</sup>	35.050 ± 2.852	22.404 ± 0.562	29.850 ± 0.759	23.792 ± 0.343	38.84 ± 0.369 <sup>a</sup>	23.034 ± 0.262	30.717 ± 1.443
	- ve	23.984 ± 0.786	54.008 ± 5.562 <sup>b</sup>	31.078 ± 1.538	21.650 ± 0.980	25.982 ± 1.352	24.180 ± 0.327	39.406 ± 0.282	22.890 ± 0.557	30.397 ± 1.808
Cholesterol	+ ve	147.994 ± 2.688 <sup>a</sup>	149.614 ± 1.467 <sup>a</sup>	147.832 ± 1.907 <sup>a</sup>	123.622 ± 10.956 <sup>a</sup>	154.274 ± 3.057 <sup>a</sup>	110.456 ± 6.381	154.580 ± 2.745	109.340 ± 7.131	137.214 ± 3.406
	- ve	147.854 ± 1.195 <sup>a</sup>	151.344 ± 0.763 <sup>a</sup>	149.220 ± 1.567 <sup>a</sup>	111.214 ± 6.719 <sup>a</sup>	163.514 ± 3.224 <sup>a</sup>	111.316 ± 7.035 <sup>a</sup>	150.836 ± 1.591 <sup>a</sup>	121.278 ± 6.254	138.322 ± 3.363

+ ve: Brucellosis Positive ; - ve : Brucellosis Negative

Mean between two groups (+ve & -ve) within an area bearing different superscript differ significantly,  $P \leq 0.05$



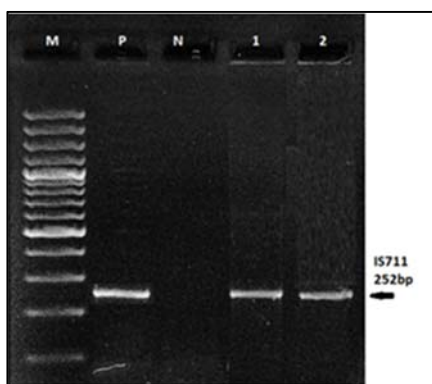
**Fig 1:** Rose Bengal Plate Agglutination test (RBPT): Note the clear clump formation depicting agglutination reaction



**Fig 2:** Standard Tube Agglutination Test (STAT): Tubes 1 to 6: dilution 1:10, 1:20, 1:40, 1:80, 1:160 & 1:320; Tube C: Control; Note the mat formation upto tube-4 (1:80 dilution), turbidity in tubes 5,6 and control.



**Fig 3:** Dot-ELISA for *Brucella melitensis*: Note blue dot formation in positive samples



**Fig 4:** PCR amplification of *Brucella melitensis* specific IS711 (252bp): M: Molecular weight marker (100bp+); P: Positive control; N: Negative Control; 1&2: Positive samples

**Serum Glucose:** In general no significant differences were observed in positive vs negative reactor goats with respect to blood glucose ( $76.697 \pm 4.747$  vs  $67.534 \pm 4.019$ ). Similar trends were observed in different areas except at chushul where glucose levels were significantly higher in positive reactors ( $147.748 \pm 1.874$  vs  $82.100 \pm 27.567$ ). Also, comparatively higher values in positive reactors were observed at Sumdho ( $41.742 \pm 2.752$  vs  $34.966 \pm 1.647$ ), but the mean values were statistically comparable. In general, the values were lower than other areas. Also, lower glucose levels were observed in goats at Kharnak ( $54.618 \pm 2.877$  vs  $55.500 \pm 1.307$ ).

**Serum Proteins:** The overall mean serum total protein ( $5.959 \pm 0.106$  vs  $5.947 \pm 0.104$ ); albumin ( $3.191 \pm 0.048$ ;  $3.272 \pm 0.062$ ); and globulin ( $2.769 \pm 0.107$  vs  $2.675 \pm 0.100$ ) levels were comparable between brucella positive and negative Changra goats. The mean total protein and globulin levels, were comparatively higher at Kargyam ( $6.692 \pm 0.160$  vs  $6.772 \pm 0.220$ ;  $3.360 \pm 0.090$  vs  $3.138 \pm 0.281$ ), Stakna ( $6.486 \pm 0.193$  vs  $6.340 \pm 0.295$ ;  $3.126 \pm 0.208$  vs  $2.896 \pm 0.327$ ), and Turtuk ( $6.420 \pm 0.290$  vs  $6.376 \pm 0.306$ ;  $3.280 \pm 0.305$  vs  $3.102 \pm 0.295$ ), whereas mean albumin levels were higher at Kargyam ( $3.332 \pm 0.141$  vs  $3.634 \pm 0.253$ ), Stakna ( $3.360 \pm 0.086$  vs  $3.444 \pm 0.146$ ), and Mughlib ( $3.452 \pm 0.125$  vs  $3.360 \pm 0.087$ ). However, the means did not differ significantly between brucella positive and negative goats.

**Enzymes:** The overall mean AST ( $62.722 \pm 3.839$  vs  $67.972 \pm 4.925$ ); ALT ( $16.753 \pm 1.340$  vs  $15.684 \pm 1.197$ ); and ALP ( $250.670 \pm 20.067$  vs  $266.960 \pm 20.100$ ) levels were comparable between brucella positive and negative goats. AST levels showed wide variation between the areas. Minimum AST levels were observed at Chushul

( $25.672 \pm 5.721$  vs  $24.178 \pm 0.928$ ). The mean value were markedly higher among brucella negative goats at Digger ( $64.004 \pm 11.255$  vs  $112.982 \pm 18.878$ ), but were comparable at all other places. Mean ALT levels were markedly lower at Kharnak ( $5.222 \pm 1.315$  vs  $4.716 \pm 0.651$ ) and Sumdho ( $7.512 \pm 1.447$  vs  $6.288 \pm 0.904$ ). However, differences between brucella positive and negative goats were non-significantly at all the areas. The mean ALP values varied widely between different areas ( $191.280 \pm 19.872$  to  $356.956 \pm 28.367$ ) but were comparable between the brucellosis positive and negative groups within the areas.

**Kidney Function Test:** The overall mean serum creatinine ( $0.638 \pm 0.072$  vs  $0.713 \pm 0.073$ ) and serum urea ( $30.717 \pm 1.443$  vs  $30.397 \pm 1.808$ ) levels were comparable between brucellosis positive and negative goats. Also, no significant differences were observed between the two groups within different areas except for significantly lower values of creatinine at Digger ( $0.780 \pm 0.242$  vs  $1.120 \pm 0.269$ ) and urea at Sumdho ( $47.826 \pm 2.389$  vs  $54.008 \pm 5.562$ ) in brucellosis positive goats.

**Cholesterol:** The overall mean serum cholesterol levels of brucellosis positive and negative reactor goats ( $137.214 \pm 3.406$  vs  $138.322 \pm 3.363$ ). Were comparable. Similarly, no significant differences were found between the two groups within different areas.

## Discussion

In present study MRBPT and STAT were used as screening tests followed by dot-ELISA, plate ELISA and PCR as confirmatory tests. Serological tests using either smooth lipopolysaccharide (sLPS) prepared by chemical extraction or a whole cell antigen are usually recommended for large-scale screening/surveillance (Nielsen, 2002) [32]. Virtually all serological tests utilize *B. abortus* antigen because common epitopes are present in *B. melitensis*, *B. suis* and *B. abortus* (OIE, 2009) [39]. Hence such tests cannot facilitate species level differentiation (Corbel, 1985) [8]. The serological response is influenced by several factors including- the type of exposure, the stage of gestation at the time of exposure, the vaccination status and the variable and long incubation period during which serotest results are negative (Lord *et al.*, 1989) [24]. Hence, the time-point post infection at which sampling and testing occurs has a major impact on the results and helps in distinction between acute and chronic infection (Godfroid *et al.*, 2010; Saegerman *et al.*, 2004) [16, 40]. Besides, sensitivity and specificity of serological tests mainly relies on the antigen and type of serological test used. The serological test results can be strongly influenced by the presence of false positive serological cross-reactions (FPSR) due to other gram-negative bacteria sharing antigenic determinants with the *Brucella* O-chain. Cross-reactivity has been observed between *Brucella* sLPS and sLPS of other bacteria such as *Yersinia enterocolitica* O:9, *Salmonella* group N (O:30), *Vibrio cholera* O1, *Escherichia coli* O:157, some strains of *Escherichia hermanni* and *Stenotrophomonas maltophilia* (Gerber *et al.*, 1997; Saegerman *et al.*, 2004) [16, 40]. Choice of serological diagnostic method depends on epidemiological situation (Godfroid *et al.*, 2010) [16]. One of the principle requirements of a screening test is that it has to be economical, rapid and must be as diagnostically sensitive as possible, but it needs not to be highly specific. This means a high number of false positive reactions may be expected warranting application of confirmatory tests for positive reactors (Nielsen, 2002; Stemshorn, 1985) [32, 4]. In goats the

sensitivity and specificity, respectively, has been reported to be 48.1% vs 96.1% (Arabaci and Oldacay, 2012) <sup>[4]</sup>, and 80.2% vs 99.6% (Anisur Rahman *et al.*, 2013) <sup>[3]</sup> for RBPT; 85.7% vs 100% (Sariguzel *et al.*, 2011) <sup>[42]</sup>, 82.6% vs 77.6% (Yohannes *et al.*, 2012) <sup>[47]</sup>, and 57.1% vs 99.3% (Anisur Rahman *et al.*, 2013) <sup>[3]</sup> for STAT; 91.7% vs 97.6% (Nielson *et al.*, 2004), and 92.9% vs 96.5% (Anisur Rahman *et al.*, 2013) <sup>[3]</sup> for iELISA; 75.0% vs 99.8% (Nielson *et al.*, 2004) for cELISA and 97.4% vs 100% (Leyla *et al.*, 2003) <sup>[23]</sup>, 90% vs 100% (Gupta *et al.*, 2006) <sup>[19]</sup> for PCR. RBPT is of value as a screening test especially in high risk rural areas whereas SAT remains the most popular and yet used worldwide diagnostic tool. ELISA is an excellent method for screening large populations for *Brucella* antibodies and for differentiation between acute and chronic phases of the disease. However, PCR is considered as a diagnostic substitute to culture (Franco *et al.*, 2007; Mantur and Amarnath, 2008; Geresu and Kassa, 2016) <sup>[13, 27, 15]</sup>.

The observation of non-significant differences in serum biochemical indices with respect to positive vs negative reactor goats is in accordance with Forbes *et al.* (1996) who reported that clinical chemistry and hematology was stable and similar to normal values in experimentally induced brucellosis in moose. Gul *et al.* (2013) <sup>[17]</sup> reported that brucellosis did not cause any significant change in the biochemical parameters of horses except for significant decrease in the values of alkaline phosphatase and a significant increase in alanine aminotransferase. Contrary to our findings, various workers have reported significant alterations in serum biochemical indices including glucose, total protein, albumin, globulin, AST, ALT, LDH, ALP, total and direct bilirubin, creatin phosphokinase (CPK) activities, urea, creatinine, triglycerides and cholesterol (Al-Hussary *et al.*, 2010; Arslan *et al.*, 2011; Mahboub *et al.*, 2013) <sup>[1, 5, 26]</sup>. However the results were not consistent.

Although, significant as well as non-significant increase in serum glucose levels was observed in few areas, in most areas no significant differences were observed. Various workers have reported increase in serum glucose concentration in brucellosis affected sheep, goat and cattle (Al-Hussary *et al.*, 2010; Arslan *et al.*, 2011a,b; Nath *et al.* 2014; Kushwaha *et al.*, 2014) <sup>[1, 22, 6, 31]</sup>. Contrarily, Kumar, *et al.* (2015) <sup>[21]</sup> reported decreased serum glucose in brucellosis affected ewes and attributed it to decreased feed intake.

While decrease in serum total protein and albumin has been reported by some workers and attributed to brucellosis induced nephropathy leading to impaired glomerular filtration, hepatopathy, and decreased feed intake (Al-Hussary *et al.*, 2010; Arslan *et al.*, 2011; Hamada *et al.* 2013; Kumar *et al.*, 2015) <sup>[1, 5, 20, 21]</sup> in sheep and goat, contrary results have been reported in cattle (Nath *et al.*, 2014) <sup>[31]</sup>. Contrary to present observation, serum globulin levels have been found to increase and attributed to immune response following *Brucella* infection (Hamada *et al.*, 2013; Kumar *et al.*, 2015) <sup>[20, 21]</sup>.

Earlier workers have reported increased AST and ALT activities in sheep, goats, and cattle, and attributed it to brucellosis induced hepatopathy (El-Boshy *et al.*, 2009; Al-Hussary *et al.*, 2010; Arslan *et al.*, 2011; Nath *et al.* 2014; Arslan *et al.*, 2011b; Kushwaha *et al.*, 2014; Kumar *et al.*, 2015) <sup>[1, 22, 6, 31, 11, 21]</sup>. However, no changes in serum enzyme activities were observed in present study.

In present study lowered levels of serum urea and creatinine were observed at few places whereas no changes were observed in general. However, earlier workers have reported significant decrease in serum urea following brucellosis and

attributed to hepatopathy associated decreased urea formation from ammonia (El-Boshy *et al.*, 2009; Hamada *et al.*, 2013, Nath *et al.* 2014; Kumar *et al.*, 2015) <sup>[31, 11, 20, 21]</sup>

The discrepancies may be attributed to the fact that during the present study all the reactor goats were possibly having latent phase of infection.

### Conclusions

The serum biochemical indices studied seemed to be stable with respect to brucellosis at least in sub-clinically affected or during latent phase of infection in goats. However, further studies using omics approach are needed to elucidate any discrepancies.

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