Research Article

Serum Biochemical Parameters of *Brucella* Infected Rams

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Abstract
A Study was conducted to evaluate the serum biochemical parameters of *Brucella* affected rams compared to healthy animals in and around villages near Proddatur region of Andhra Pradesh. A total of twenty (20) serum samples were collected from rams showing orchitis out of which eight (8) animals showed positive result for Rose Bengal Plate test (RBPT) which is used to screen *Brucella* infection. These samples were analyzed for serum parameters like total protein, albumin, globulin, A: G ratio, glucose, cholesterol, triglycerides, urea, blood urea nitrogen (BUN), Alanine transaminase (ALT) and Aspartate transaminase (AST). The results showed significant ($p<0.05$) increase in glucose, cholesterol, triglycerides, globulin, ALT and AST and parameters like total protein, albumin, urea, BUN and A: G ratio were significantly ($p<0.05$) reduced in *Brucella* infected rams compared to healthy animals. These changes indicate that *Brucella* infection in rams cause deteriorative effects to health thereby disturb the function of vital organs of the animal.

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Introduction

Brucellosis is a bacterial zoonotic infection that affects sheep and goat population and has an impact on the economy in the developing countries [1]. It is a contagious disease caused by bacteria of genus *Brucella*. Among the nine species of *Brucella* identified, *Brucella meletensis* is the most pathogenic and invasive species for human [2]. Brucellosis in sheep can be divided into ram epididymitis and classical brucellosis. Ram epididymitis is caused by *B.ovis*, while classical brucellosis by *B.melitensis* and constitutes a major public health concern [3]. *B.melitensis* occurs worldwide and major symptoms include abortion, still birth, delivery of weak offspring and placenta retention in females and orchitis and epididymitis in males [4] and [5]. World Health Organization rated the *Brucella* as one of the most important zoonotic disease due to its high pathogenicity to humans.

There are many methods for diagnosis of brucellosis such as cultural, serological and molecular methods [6]. Cultural tests are laborious, time consuming and dangerous. Molecular techniques are very costly, sensitive and require expertise. Most of the serological methods are easy to perform and can be done in the field itself. Rose Bengal Plate Test (RBPT) is a spot agglutination test used to screen the flocks. It is highly sensitive test for individual diagnosis but positive results should be confirmed by a quantitative test.

The study of blood constituents can provide valuable information about the general health of the animal and therefore, can be used for evaluating the health status of the animal. Brucellosis has serious affects on animal health because it infect vital organs in the body leading to their damage, impairment of their function and increase or decrease the release of their enzymes according to the stage of infection [7, 8]. There is little information available about the serum biochemical profile of *Brucella* infected rams. Keeping in view the above facts, this study was conducted with the objective to investigate the changes in biochemical metabolites of the rams diagnosed with brucellosis that reflect the effects of the disease in animals health performance.

Materials and Methods
Collection of sample

In the present study, blood samples were collected from rams with orchitis in the nearby villages of Proddatur region. A total of 20 samples were collected aseptically in clot activator serum vials and kept in slant position for one hour and later serum was separated which was stored in -20^{0}C till further analysis.

Screening of samples

The serum samples were screened for Brucellosis by RBPT using Rose Bengal Antigen obtained from IVPM at Ranipet, Tamil Nadu. Serum (30 µl) was mixed with an equal volume of Rose Bengal Antigen on a slide with white background to produce a zone approximately 2 cm in diameter. The mixture obtained was agitated gently at ambient temperature for four minutes and agglutination was observed. Any visible reaction was considered to be positive.

Biochemical tests

All the positive and negative sera samples for Brucellosis by agglutination test were analyzed for biochemical parameters like glucose, cholesterol, triglycerides, total protein, albumin, globulin, A:G ratio, urea, BUN, ALT and AST using the standard protocols given in the commercial kits of ERBA.

Statistical analysis

The results obtained were analyzed for paired 't' test using SPSS version 20.0 [9]. Differences were considered as significant at $p < 0.05$.

Results and discussion
Rose Bengal Plate Test

This test was used to identify the *Brucella* positive cases among the rams. Out of the 20 animals tested for *Brucella*, 8 cases showed positive reaction for agglutination of RBPT and the remaining 12 were negative which was depicted in **Figure 1**. The results obtained are in accordance with the previous report [10].
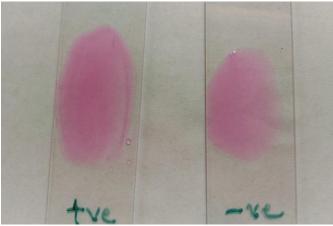

Figure 1 Rose Bengal plate test depicting Positive and Negative reaction for Agglutination

Serum biochemical parameters

The mean values of serum biochemical parameters were presented in the **Table 1**.

Total protein

In the present study the total protein levels of *Brucella* affected rams (6.03 ± 0.25 g/dL) were significantly ($p<0.05$) reduced as compared to healthy animals (6.46 ± 0.33 g/dL). Our findings are in accordance with [10] who reported reduced total protein levels in *Brucella* affected ewes and goats. The decrease in serum total protein may be due to kidney damage and hence loss of proteins through urine due to improper glomerular filtration [4].

Albumin

A significant ($p<0.05$) reduction in the serum albumin levels of *Brucella* affected rams (2.10 ± 0.02 g/dL) was noticed compared to healthy rams (3.03 ± 0.03 g/dL) and our results correlate with [11] who also reported reduced albumin levels in sheep affected with *Brucella*. The reduced levels may be due to loss of albumin through urine due to kidney damage or reduced production by the liver due to hepatic damage [10].

Table 1 Mean± S.E. of serum biochemical parameters of healthy and Brucella affected rams

Parameters	Healthy rams (N=12)	Brucella affected rams (N=8)
Total Protein (g/dL)	6.46 ± 0.33[a]	6.03 ± 0.25[b]
Albumin (g/dL)	3.03 ± 0.03[a]	2.10 ± 0.02[b]
Globulin (g/dL)	3.43 ± 0.04[a]	3.92 ± 0.01[b]
A:G ratio	0.88 ± 0.02[a]	0.53 ± 0.01[b]
Glucose (mg/dL)	57.68 ± 0.22[a]	74.43 ± 0.55[b]
Cholesterol (mg/dL)	79.76 ± 0.24[a]	90.01 ± 0.38[b]
Triglycerides (mg/dL)	17.49 ± 0.27[a]	44.58 ± 2.44[b]
Urea (mg/dL)	36.55 ± 0.22[a]	26.36 ± 0.34[b]
Blood urea nitrogen (mg/dL)	16.92 ± 0.10[a]	12.21 ± 0.16[b]
AST (U/L)	71.43 ± 0.32[a]	94.43 ± 0.41[b]
ALT (U/L)	26.41 ± 0.32[a]	30.37 ± 0.26[b]
Means with different superscripts differ significantly within the row (p < 0.05)		

Globulin

A significant (p<0.05) increase in globulin levels of *Brucella* affected rams (3.92 ± 0.01 g/dL) compared to healthy animals (3.43 ± 0.04 g/dL) was observed in the present study. Similar findings were observed by [12] in *Brucella* affected ewes compared to control. The increase in globulin level may be due to triggering of immune mechanism by the *Brucella* organism [13].

A: G ratio

The A: G ratio of *Brucella* affected rams (0.53 ± 0.01) was significantly (p<0.05) reduced when compared to control rams (0.88 ± 0.02) and our results are in accordance with [14] who reported reduced A: G ratio in *Brucella* affected cattle compared to healthy animals which is due to increased globulin and reduced albumin levels.

Glucose

Glucose levels of *Brucella* affected rams (74.43 ± 0.55 mg/dL) were significantly (p<0.05) higher compared to control animals (57.68 ± 0.22 mg/dL). The result obtained was in accordance with the findings [10] and [11] who reported elevated glucose levels in *Brucella* affected ewes compared to healthy animals. Elevated glucose levels were also reported by [15] in *Brucella* affected cattle.

Cholesterol

The serum cholesterol levels were significantly (p<0.05) more in affected rams (90.01 ± 0.38 mg/dL) compared to healthy rams (79.76 ± 0.24 mg/dL). Similar findings were depicted by [12] in ewes, [14] in sheep and [15] in cattle affected with *Brucella*. The increase in serum cholesterol is due to hepatic damage. The normal metabolizing and excretory activity as bile acids and steroid hormones, of the liver gets hampered leading to cholesterol accumulation.

Triglycerides

A significant (p<0.05) increase in triglyceride levels was seen in *Brucella* affected rams (44.58 ± 2.44 mg/dL) compared to control (17.49 ± 0.27 mg/dL). Our results are in accordance with [4] and [14] who reported elevated triglyceride levels in *Brucella* affected sheep and cattle respectively. The elevated levels are explained by the fact that *Brucella* produces tumor necrosis factor α (TNF-α) which inhibits the lipoprotein lipase which in turn results in elevated serum triglyceride levels [16].

Urea and BUN

The urea and BUN levels in *Brucella* affected rams (26.36 ± 0.34 and 12.21 ± 0.16 mg/dL respectively) were significantly (p<0.05) lower compared to control animals (36.55 ± 0.22 and 16.92 ± 0.10 mg/dL respectively). Similar findings were reported by [12] in *Brucella* affected ewes compared to control. The liver damage caused by *Brucella* infection causes reduced formation of urea and BUN from ammonia thereby reduced levels are found [4].

ALT and AST

The enzymes ALT and AST are considered as the hepatic markers. In the present study the levels of ALT and AST in *Brucella* affected rams (30.37 ± 0.26 and 94.43 ± 0.41 U/L respectively) were significantly ($p<0.05$) higher compared to healthy rams (26.41 ± 0.32 and 71.43 ± 0.32 U/L respectively) and our results are in accordance with the research works of [11], [12] and [15] in sheep, ewes and cattle and respectively. The damaged liver parenchyma causes leakage of the enzymes into the serum thereby elevating their levels.

Conclusion

Thus it can be concluded that *Brucella* infection in rams leads to altered serum biochemical parameters due to damage of vital organs like liver and kidney. The assaying of these parameters may be helpful in elucidating the adverse affects associated with Brucellosis in rams.

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