

Assessment and comparison of serum biochemical parameters of *Brucella* infected and healthy ewes

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Abstract

The present study was undertaken to assess and compare the serum biochemical parameters of healthy ewes and ewes infected with *Brucella*. A total of forty blood samples were collected from the sheep of different flocks in villages nearby College of Veterinary Science, Korutla in which the abortions were frequent. Blood samples were screened for brucellosis by Rose Bengal Plate Test and later analyzed for serum biochemical parameters like total protein, globulin, albumin, glucose, blood urea nitrogen, Aspartate transaminase and Alanine transaminase. Ten samples gave positive result whereas the remaining thirty samples gave negative result for brucellosis. The results of biochemical parameters shown that globulin, cholesterol, Aspartate transaminase and Alanine transaminase significantly ($P<0.05$) increased whereas albumin and glucose significantly decreased ($P<0.05$) in *Brucella* infected ewes compared to healthy ewes. Total protein and blood urea nitrogen concentrations in the serum decreased in *Brucella* infected ewes compared to healthy ewes though significance ($P<0.05$) was not found. These changes in blood biochemical parameters indicate that brucellosis causes deteriorative effects on health of ewes infected with *Brucella*.

Keywords: Brucellosis, ewes, Rose Bengal Plate Agglutination Test, biochemical tests.

Introduction

Ovine and caprine brucellosis is a zoonotic infection, which has important effects for both human health and animal reproduction. Brucellosis in sheep can be divided into two types, one is classical brucellosis and second one is ram epididymitis. Ram epididymitis is caused by non-zoonotic agent *Brucella ovis*, while classical brucellosis is caused by *Brucella melitensis* and constitutes a major public health threat equal to goat brucellosis (Acha and Szyfres, 2003). *Brucella melitensis* is an economically important infectious disease of small ruminants worldwide and the predominant symptoms of *Brucella melitensis* infection are reproductive disorders such as abortion, stillbirth, delivery of weak offspring, and placenta retention in females, and orchitis and epididymitis in males (Lone *et.al.*, 2013, Hamada *et.al.*, 2013). Abortion occurs during the last two months of gestation. World Health Organization rated the *Brucella* as one of most important zoonosis due to its high pathogenicity to humans.

There are many methods for diagnosis of brucellosis such as cultural, serological and molecular methods (Sahin *et.al.*, 2008). Traditional isolation and characterization of organism by cultural methods are still considered as the "Gold standard" method, but these are laborious, time consuming as well as dangerous to laboratory workers. Molecular techniques are very costly, sensitive, accurate and requires 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. The test is highly sensitive for individual diagnosis and can be performed in the field. The RBPT can be used in all animal species but positive results should be confirmed by a quantitative test. The changes in the concentrations of blood biochemical parameters and enzyme profiles shows the impact of brucellosis on the vital organs' functioning in the body and thereby helps to understand the health status of individual (Radostits *et.al.*, 2007). In keeping view of the above facts, the present work was undertaken to assess and compare the serum biochemical parameters of *Brucella* infected sheep with healthy sheep.

Materials and Methods

Collection of samples: Blood samples were collected from the sheep of different flocks in the nearby villages of College of Veterinary Science, Korutla where the abortions were a frequent problem. The samples were collected aseptically in a test tube and kept in slant position for one hour and later serum was collected in the tubes. The serum containing tubes were transferred on ice to the laboratory and kept under refrigeration till further analysis.

Screening of samples: Sera samples were screened for brucellosis by Rose Bengal Plate Agglutination Test using Rose Bengal Antigen obtained from Division of Biological Products at Indian Veterinary Research Institute, Izatnagar. These samples were also screened by using *Brucella* Abortus Coloured (Plate) Antigen obtained from IVP at Ranipet, Tamil Nadu. Serum (30 ml) was mixed with an equal volume of Rose Bengal antigen on a white tile to produce a zone approximately 2 cm in diameter. The mixture was agitated gently for four minutes at ambient temperature, and then observed for agglutination. Any visible reaction was considered to be positive. The same method was followed for the coloured antigen of IVP also (Corbel, 2006).

All the positive and negative sera samples for brucellosis by Agglutination Test were analyzed for biochemical parameters using the standard protocols given in the commercial kits of ERBA.

Statistical analysis

The results of serum biochemical parameters were analyzed for the paired "t" test (Snedecor and Cochran, 1994)

Results and Discussion

Rose Bengal Plate Test

This test was used to identify the *Brucella* positive (Fig 1) cases among sheep. Among the forty animals that were tested for the *Brucella*, 10 cases shown positive reaction for the RBPT (25%). The percentage of animals negative for Brucellosis using RBPT was 75% *i.e.*, 30 out of 40 cases recorded. This is in accordance with the results found in the previous report (Al-Hussary *et.al.*, 2010).

Serum Biochemical Parameters

The results of the serum biochemical parameters are presented in the Table 1.

Total Protein: There is a decrease in concentration of serum total proteins in the *Brucella* effected ewes (59.62 ± 0.3315 g/L) compared to the normal ewes (62.67 ± 0.9909 g/L) though there is no significance. Al-Hussary *et.al.*, (2010) and Arslan *et.al.*, (2011^a) reported that decrease in the serum total protein concentration in ewes and goats infected with brucellosis. Rita Nath *et.al.* (2014) found significant increase in serum total protein in cattle infected with brucellosis The decrease in the Serum Total Protein concentration may be attributed to the kidney damage and hence loss of proteins through the urine due to improper glomerular filtration (Hamada *et.al.*, 2013).

Table 1: Mean \pm S.E of serum biochemical parameters of healthy and *Brucella* infected sheep

S. N.	Biochemical parameter	Healthy sheep	Sheep with <i>Brucella</i> infection
1	Total Protein (g/L)	62.67 \pm 0.9909 ^a	59.62 \pm 0.3315 ^a
2	Albumin (g/L)	27.86 \pm 0.4296 ^a	22.43 \pm 0.2749 ^b
3	Globulin (g/L)	43.91 \pm 0.9194 ^a	50.61 \pm 1.7394 ^b
4	Glucose (mg/dL)	74.52 \pm 1.0907 ^a	64.84 \pm 2.5334 ^b
5	Cholesterol (mg/dL)	63.91 \pm 1.3248 ^a	70.35 \pm 2.1609 ^b
6	Blood Urea Nitrogen (mmol/L)	11.28 \pm 0.4142 ^a	9.52 \pm 0.9193 ^a
7	Aspartate Transaminase (U/L)	63.69 \pm 1.2535 ^a	89.82 \pm 5.1080 ^b
8	Alanine Transaminase (U/L)	29.04 \pm 0.3456 ^a	30.51 \pm 0.4362 ^b

Values carrying the different letters are significantly different at $P < 0.05$.

Albumin: There is a significant lowering of serum albumin concentration in the affected animals (22.43 \pm 0.2749 g/L) compared to the serum albumin concentrations of the unaffected sheep (27.86 \pm 0.4296 g/L). The finding correlates with the results of (Arslan *et.al.*, 2011^a). This lowering of Serum Albumin concentration in the *Brucella* affected ewes may be due to the outcome of many reasons like loss of albumin through urine due to kidney damage, less feed intake by the affected ewes and lessened production of albumin by the liver due to hepatic damage (Al-Hussary *et.al.*, 2010)

Globulin: Compared to the healthy sheep (43.91 \pm 0.9194 g/L), serum Globulin concentration in the *Brucella* affected ewes (50.61 \pm 1.7394 g/L) significantly increased. These results are in accordance to the works of (Hamada *et.al.*, 2013). This increase is obvious that the globulin concentration increases due to the *Brucella* organism by triggering the immune system's response of the sheep (Hamada *et.al.*, 2013, Abenga and Anosa, 2005).

Glucose: Glucose concentration in *Brucella* effected ewes decreased significantly. The results of the present study were in contrast to findings of (Al-Hussary *et.al.*, 2010), Arslan *et.al.*, 2011^b) in ewes, native goats (Arslan *et.al.*, 2011^a), cattle (Rita Nath *et.al.* 2014, Kushwaha *et.al.*, 2014). The decrease in the present study can be attributed to the decrease in lowered feed intake due to *Brucella*.

Cholesterol: Serum cholesterol concentration of the effected ewes (70.35 \pm 2.1609 mg/dL) is significantly more than that in healthy ewes (63.91 \pm 1.3248 mg/dL) same as that depicted by the works of (Al-Hussary *et.al.*, 2010, Arslan *et.al.*, 2011^a, Rita Nath *et.al.* 2014, Arslan *et.al.*, 2011^b and Kushwaha *et.al.*, 2014) in ewes, native goats, cattle, sheep and cattle respectively. This increase in the serum cholesterol concentration is due to the hepatic damage. Normal metabolizing activity and excretory activity as bile acids and steroid hormones, of the liver gets hampered. So the Cholesterol gets accumulated.

Blood Urea Nitrogen: Serum Blood Urea nitrogen levels were significantly higher in the healthy ewes (11.28 \pm 0.4142 mmol/L) in compare to *Brucella* effected ewes (9.52 \pm 0.9193 mmol/L). Similar results were reported previously (Rita Nath *et.al.*, 2014). The reduced BUN levels in the *Brucella* effected ewes may be answered by the fact that damaged liver tissue cannot form Urea from the ammonia (Hamada *et.al.*, 2013, El-Boshy *et.al.*, 2009).

ALT and AST: ALT and AST are considered as the Hepatic markers though AST is a non-specific marker. Due to liver damage, the ALT and AST enzymes of the hepatic parenchyma get leaked and the serum levels of these enzymes increased. The present study stands as a proof to the above said fact with its results. ALT activity and AST activity in healthy animals are 63.69 \pm 1.2535 U/L and 29.04 \pm 0.3456 U/L respectively. ALT activity and AST activity in affected animals are 89.82 \pm 5.1080 U/L and 30.51 \pm 0.4362 U/L respectively as in accordance to the facts in the research works of (Al-Hussary *et.al.*, 2010, Arslan *et.al.*, 2011^a, Rita Nath *et.al.* 2014, Arslan *et.al.*, 2011^b and Kushwaha *et.al.*, 2014) in ewes, native goats, cattle, sheep and cattle respectively.

Conclusion

Though Brucellosis is mainly considered as the cause of abortions in the ewes, the present study revealed hepatic and kidney damage which leads to the considerable economic losses. Hence it is concluded that the study of biochemical parameters in the ewes can determine the extent of damage caused by the *Brucella* organism.

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