Assessment of Haemato-biochemical Parameters and Therapeutics on *Brucella* Infected Cattle

**Abstract**

Brucellosis is a contagious systemic bacterial disease of livestock. Antibiotics commonly being used have limited efficacy against the disease. In the present study, therapy with antibiotics on *Brucella* infected cattle was performed and subsequently haemato-biochemical parameters, detection of anti-*Brucella* antibodies in both serum and milk by different diagnostics and shedding of *Brucella* in milk were assessed. Treatment with antibiotics; streptomycin and long acting tetracycline alone and in combination were given for four weeks. Hb, TEC, TLC, neutrophils, eosinophils, lymphocytes, monocytes count, AST, ALT and SD values were lower whereas, PCV, serum glucose, total protein, albumin and creatinine were higher in *Brucella* infected cattle. Treatment with combination therapy showed promising results in these animals. Haemato-biochemical parameters were become near to normal in most of animals that received combination therapy. However, not all animals were returned to normal after treatment but a significant numbers in combination therapy were reduced anti-*Brucella* antibody titre, both in serum and milk. The level of shedding of *Brucella* in milk had also gone undetectable.

**Keywords**

Brucellosis; Cattle; Haemato-biochemical parameters; Oxytetracycline; Streptomycin

**Abbreviations**

RBPT: Rose Bengal Plate Test; STAT: Standard Tube Agglutination Test; ABR: Abortus Bang Ring; ELISA: Enzyme Linked Immunosorbent Assay; MRT: Milk Ring Test; PCR: Polymerase Chain Reaction; Hb: Haemoglobin; PCV: Packed Cell Volume; TEC: Total Erythrocytes Count; TLC: Total Leucocytes Count; DLC: Differential Leucocytes Count; AST: Aspartate Aminotranferase; ALT: Alanine Aminotranferase; SD: Sorbitol Dehydrogenase; PD-ADMAS: Project directorate on animal disease monitoring and surveillance; RAR: Research Animal Resources

**Introduction**

Brucellosis is a most common zoonosis in the world, caused by different *Brucella* species [1]. In cattle, it is caused by *B. abortus*, also known as contagious abortion [2]. Abortion in last trimester, birth of unthrifty newborn, retained placenta in female and orchitis, epididymitis in male animals are the common manifestations of brucellosis in animals [3-5] and it may lead to temporary or permanent infertility [6]. Diagnosis on the basis of abortion is, however, equivocal since many pathogens can induce abortion; thus laboratory testing is essential [7]. Various tests viz; Rose Bengal Plate Test (RBPT), Standard Tube Agglutination Test (STAT), Enzyme Linked Immunosorbent Assay (ELISA) and Milk Ring Test (MRT) are commonly used for the detection of anti-*Brucella* antibody [5]. Isolation of the causative agent is most accepted tool for confirmatory diagnosis, but it is time consuming and has reduced sensitivity in chronic stage of infection. Handling of suspected clinical samples will require containment level for group 3 pathogens [5]. Numerous Polymerase Chain Reaction (PCR) based assays have been developed for the rapid identification of *Brucella*. It is advantageous to detect DNA of pathogen that rendered biologically safe and hence, reduced the risk of infection to laboratory workers. In addition, PCR is more sensitive than traditional culture method [8].

There is limited evidence on treatment aspect of *Brucella* infected cattle as well as their haemato-biochemical parameters. *Brucella* is inaccessible to antibiotics as they are facultative intracellular pathogens. Many antimicrobials are active against *Brucella* species; however, clinical efficacy does not always correlate with *in vitro* susceptibility [9]. Treatments for brucellosis have been tried previously but none was found successful [10,11]. Streptomycin acts synergistically with penicillin or tetracycline to inhibit the growth of *B. abortus* within bovine cells cultured *in vitro* [12]. Considering the above facts, the present work was intended to determine the efficacy of antibiotic therapy and alteration in the haemato-biochemical parameters if any, in *Brucella* infected cattle after treatment.

**Materials and Methods**

A total of 27 cattle which showed antibodies both in serum and milk as well as *Brucella* DNA in milk were selected for the study. ELISA, RBPT and STAT were employed on serum whereas, MRT and ELISA on milk to detect the anti-*Brucella* antibodies. Milk samples were processed for detection of pathogen itself in form of its DNA (16S rRNA) by PCR [13]. Reference antigens and serum for RBPT, STAT and, for MRT, Abortus Bang Ring Antigen (ABR Antigen) were procured from the Indian Veterinary Research Institute, Izatnagar (India). AniGen *B. Brucella* Ab ELISA kit (Cat. No. EB43-01) (i-ELISA and Milk-ELISA) was procured.
from Bionote (Korea). Haemato-biochemical parameters, antibody titre in milk/serum and shedding of pathogen in milk were evaluated before and after the therapeutic trial.

**Antibody detection**

RBPT was performed according to the method prescribed in OIE [5]. Equal volume of both antigen and serum sample was mixed, definite agglutination was taken as positive whereas, no agglutination as negative. STAT was performed as per the method described by Alton et al. [14]. All serum samples were tested up to minimum of five dilutions. Considering the special significance of 50% end point, a control tube was set up to simulate 50% clearing by mixing 0.5 ml antigen with 1.5 ml of phenol saline (0.5%, v/v) in an agglutination tube. All tubes were incubated at 37 °C for 20 hrs before result was observed. The highest serum dilution showing 50% or more agglutination (50% clearing) was considered as the titre of the serum.

MRT was performed on individual milk samples according to the method described in OIE [5]. Dark pink ring above the white milk column was taken as positive whereas, pink colour of the underlying milk exceeds that of the cream layer as negative. i-ELISA and Milk-ELISA was performed by using AniGen B. Brucella Ab ELISA kit. Method mentioned in manual provided with kit was followed. OD of ELISA plate was taken at 450 nm to calculate the percent positivity (%P) of both serum and milk samples. Positive and negative samples were determined based on percent positivity (%P) value. Sera which have %P value ≥25 were taken as positive whereas, samples having %P value <25 were negative. %P of serum was calculated from OD as follow.

\[
\% P = \left( \frac{OD \text{ of serum sample}}{Average \ OD \ of \ standard \ strong \ positive \ control} \right) \times 100
\]

The milk samples gave %P value ≥15 were taken as positive whereas, samples with <15%P value as negative. It was calculated as follows:

\[
\% P = \left( \frac{OD \ of \ milk \ sample}{Average \ OD \ of \ standard \ positive \ control \ milk} \right) \times 100
\]

**Polymerase chain reaction**

Specific DNA sequence of 905 bp, which belongs to 16S rRNA of *B. abortus* [13], was targeted for amplification. Oligonucleotides (F4; 5'-TCGAGCGCCCGCAAGGGG-3' and R2; 5'-AACCATAGTGTCTCCACTAA-3') required for amplification of rRNA of *B. abortus* [13], was targeted for amplification. MRT was performed on individual milk samples according to the method described in OIE [5]. Dark pink ring above the white milk column was taken as positive whereas, pink colour of the underlying milk exceeds that of the cream layer as negative. i-ELISA and Milk-ELISA was performed by using AniGen B. Brucella Ab ELISA kit. Method mentioned in manual provided with kit was followed. OD of ELISA plate was taken at 450 nm to calculate the percent positivity (%P) of both serum and milk samples. Positive and negative samples were determined based on percent positivity (%P) value. Sera which have %P value ≥25 were taken as positive whereas, samples having %P value <25 were negative. %P of serum was calculated from OD as follow.

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\]

The milk samples gave %P value ≥15 were taken as positive whereas, samples with <15%P value as negative. It was calculated as follows:

\[
\% P = \left( \frac{OD \text{ of milk sample}}{Average \ OD \ of \ standard \ positive \ control \ milk} \right) \times 100
\]

**Haemato-biochemical parameters**

Haematological parameters; Haemoglobin (Hb), Packed Cell Volume (PCV), Total Erythrocytes Count (TEC), Total Leucocytes Count (TLC) and Differential Leucocytes Count (DLC) were estimated as methods described by Jain [17]. Biochemical parameters; Glucose, Total Protein, Albumin, Creatinine, Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST) and Sorbitol Dehydrogenase (SD) were estimated in serum by commercially available kits (Span Diagnostics and Sigma).

**Therapeutic trial**

Cattle which had anti-*Brucella* antibodies both in serum and milk as well as presence of pathogen in milk were chosen for therapeutic trial. Three groups were formed; each contained nine cattle and maintained for 4 weeks. Long acting oxytetracycline at a dose rate of 25mg/kg intramuscularly, every other day for 4 weeks was given in Group A. Similarly, streptomycin at a dose rate of 20mg/kg intramuscularly, every other day for 4 weeks was given in Group B. While in Group C, long acting oxytetracycline at a dose rate of 25mg/kg combined with streptomycin at a dose rate of 20mg/kg were injected intramuscularly every other day for two weeks and further, oxytetracycline at the same dosage without streptomycin for two weeks. A control group of three animals, all were negative for anti-*Brucella* antibodies both in serum and milk as well as no pathogen found in milk were maintained for 4 weeks without any treatment given.

**Statistical analysis**

Statistical analysis between respective means for various parameters was performed using ANOVA and t-test, as per the method described by Snedecor and Cochran [18] at 5% level of significance.

**Results**

Cattle found positive for antibodies both in serum and milk as well as presence of pathogen in milk were selected for the therapeutic trial. Treatment efficacies were evaluated on the basis of haemato-biochemical parameters, detection of antibodies both in milk and serum and shedding of pathogen in milk, before and after the treatments.

*Brucella* infected cattle showed; lower Hb, TEC, TLC, neutrophils, eosinophils, lymphocytes and monocytes count whereas, PCV was higher than the respective value from control group (Table 1). The biochemical study in the *Brucella* infected cattle showed higher level of serum glucose, total protein, albumin and creatinine but, AST, ALT and SD values were lower than the respective value from control group (Table 2).

Group A, which received long acting oxytetracycline treatment, showed significant increase in level (p<0.05) of Hb, lymphocyte and AST (Table 1 and 2). But, other haemato-biochemical parameters; PCV, TEC, TLC, glucose, total protein, creatinine, albumin, ALT and SD values were lower than the respective value from control group (Table 2).

Group A, which received long acting oxytetracycline treatment, showed significant increase in level (p<0.05) of Hb, lymphocyte and AST (Table 1 and 2). But, other haemato-biochemical parameters; PCV, TEC, TLC, glucose, total protein, creatinine, albumin, ALT and SD values were lower than the respective value from control group (Table 2).

Group A, which received long acting oxytetracycline treatment, showed significant increase in level (p<0.05) of Hb, lymphocyte and AST (Table 1 and 2). But, other haemato-biochemical parameters; PCV, TEC, TLC, glucose, total protein, creatinine, albumin, ALT and SD values were lower than the respective value from control group (Table 2).
Treatment with streptomycin was given in Group B; reduced the anti-Brucella antibodies levels in cattle to undetectable levels in some of them. RBPT and STAT were unable to detect anti-Brucella antibody in three cattle (Table 3). However, two cattle, as screened by ELISA, seem to be free of antibody. Antibodies in milk were not detected by MRT and ELISA respectively, in five, six and five animals (Table 3). Furthermore, PCR failed to detect Brucella DNA in five cattle’s milk after therapy. All the haematological and biochemical parameters under study were not altered significantly except TEC (p<0.05) (Table 2).

In group C, there was reduction in anti-Brucella antibody level in serum and milk to such an extent, RBPT, STAT and ELISA were unable to detect respectively, in five, six and five animals (Table 3). Similarly, MRT and ELISA on milk from six and five animals were negative for antibody, respectively. Furthermore, six cattle shed undetectable amount of pathogen in milk, as it was confirmed by PCR (Table 3). Animals which had received combination therapy showed, significant increased level (p<0.05) of Hb, TEC, TLC and lymphocytes counts (Table 1). However, alteration in PCV, neutrophils, eosinophils and monocytes count was confirmed by PCR (Table 3).

**Discussion**

Brucellosis has wide socio-economic impact, especially in countries in which rural income relies largely on animal husbandry [19,20]. In India, it causes approximately Rs. 350 million economic losses [21]. Several studies have confirmed widespread prevalence in different States of India [22-25].

### Table 1: Means of respective haematological parameter of animals from respective groups, before and after the therapeutic trial.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Before treatment</th>
<th>After treatment</th>
<th>Group A (n=9)</th>
<th>Group B (n=9)</th>
<th>Group C (n=9)</th>
<th>Group A (n=9)</th>
<th>Group B (n=9)</th>
<th>Group C (n=9)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hb (g/L)</td>
<td></td>
<td></td>
<td>Control (n=3)</td>
<td>Group A (n=9)</td>
<td>Group B (n=9)</td>
<td>Group C (n=9)</td>
<td>Group A (n=9)</td>
<td>Group B (n=9)</td>
</tr>
<tr>
<td></td>
<td>±11.00</td>
<td>±10.48*</td>
<td>98.0</td>
<td>99.5</td>
<td>99.5</td>
<td>144.0</td>
<td>99.5</td>
<td>105.1</td>
</tr>
<tr>
<td>PCV (L/L)</td>
<td>0.38 ±0.61</td>
<td>0.39 ±0.20</td>
<td>0.40</td>
<td>0.39</td>
<td>0.39</td>
<td>0.39</td>
<td>0.40</td>
<td>0.41</td>
</tr>
<tr>
<td>TEC (X 10^12/ L)</td>
<td>7.90 ±0.85</td>
<td>6.41 ±0.53</td>
<td>6.42</td>
<td>6.37</td>
<td>7.96</td>
<td>7.95</td>
<td>6.44</td>
<td>6.51</td>
</tr>
<tr>
<td>TLC (X 10^8/ L)</td>
<td>8.2 ±0.56</td>
<td>3.26 ±0.24</td>
<td>3.35</td>
<td>3.85</td>
<td>8.26</td>
<td>8.25</td>
<td>3.27</td>
<td>3.38</td>
</tr>
<tr>
<td>Neutrophil (X 10^3/ L)</td>
<td>2.96 ±0.21</td>
<td>2.62 ±0.52</td>
<td>2.6</td>
<td>2.73</td>
<td>3.03</td>
<td>3.11</td>
<td>2.67</td>
<td>2.62</td>
</tr>
<tr>
<td>Eosinophil (X 10^3/ L)</td>
<td>1.06 ±0.15</td>
<td>0.84 ±0.11</td>
<td>0.88</td>
<td>0.85</td>
<td>1.1</td>
<td>0.82</td>
<td>0.85</td>
<td>0.89</td>
</tr>
<tr>
<td>Lymphocyte (X 10^3/ L)</td>
<td>3.63 ±0.38</td>
<td>1.97 ±0.40</td>
<td>1.96</td>
<td>1.94</td>
<td>3.88</td>
<td>3.86</td>
<td>2.02</td>
<td>2.00</td>
</tr>
<tr>
<td>Monocyte (X 10^3/ L)</td>
<td>0.30 ±0.10</td>
<td>0.15 ±0.03</td>
<td>0.15</td>
<td>0.15</td>
<td>0.33</td>
<td>0.33</td>
<td>0.15</td>
<td>0.16</td>
</tr>
</tbody>
</table>

*Figures having same superscripts are significant at 5% level of significance for respective parameters, n=number of animals.

### Table 2: Means of respective biochemical parameters of animals from respective groups, before and after the therapeutic trial.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Before treatment</th>
<th>After treatment</th>
<th>Group A (n=9)</th>
<th>Group B (n=9)</th>
<th>Group C (n=9)</th>
<th>Group A (n=9)</th>
<th>Group B (n=9)</th>
<th>Group C (n=9)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose (mmol/L)</td>
<td>3.33 ±0.33</td>
<td>3.07 ±1.00</td>
<td>3.04</td>
<td>3.06</td>
<td>5.027</td>
<td>5.04</td>
<td>5.04</td>
<td>5.02</td>
</tr>
<tr>
<td>Total Protein (g/L)</td>
<td>74.67 ±4.93</td>
<td>92.96 ±6.15</td>
<td>93.33</td>
<td>92.67</td>
<td>92.46</td>
<td>92.46</td>
<td>93.05</td>
<td>93.05</td>
</tr>
<tr>
<td>Albumin (g/L)</td>
<td>29.00 ±2.65</td>
<td>37.77 ±1.07</td>
<td>38.53</td>
<td>39.03</td>
<td>3.86</td>
<td>37.31</td>
<td>37.99</td>
<td>37.99</td>
</tr>
<tr>
<td>Creatinine (µmol/L)</td>
<td>10.26 ±35.22</td>
<td>179.24 ±19.47</td>
<td>179.53</td>
<td>180.65</td>
<td>178.50</td>
<td>178.50</td>
<td>178.65</td>
<td>178.65</td>
</tr>
<tr>
<td>ALT (units/L)</td>
<td>15.26 ±3.24</td>
<td>7.00 ±2.10</td>
<td>6.72</td>
<td>6.35</td>
<td>7.10</td>
<td>7.10</td>
<td>6.78</td>
<td>6.78</td>
</tr>
<tr>
<td>AST (units/L)</td>
<td>91.3 ±4.53</td>
<td>61.53 ±7.73*</td>
<td>60.88</td>
<td>59.73</td>
<td>91.99</td>
<td>62.04</td>
<td>64.19</td>
<td>61.49</td>
</tr>
<tr>
<td>SD (µmol/L)</td>
<td>9.90 ±1.25</td>
<td>2.92 ±0.33</td>
<td>2.82</td>
<td>2.75</td>
<td>9.93</td>
<td>2.95</td>
<td>2.85</td>
<td>2.85</td>
</tr>
</tbody>
</table>

*Figures having same superscripts are significant at 5% level of significance for respective parameters, n=number of animals.

PCR: Polymerase Chain Reaction; MRT: Milk Ring Test; ELISA: Enzyme Linked Immunosorbent Assay; i-ELISA: Indirect-Enzyme Linked Immunosorbent Assay

Despite the advances made in the diagnosis, vaccination and therapy to control the disease, it is still widespread and prevalent in many developing countries. There are limited researches on treatment and haemato-biochemical parameters of infected animals. Lowered haematological values such as Hb, TEC, TLC, neutrophils, eosinophils, lymphocytes and monocytes counts were recorded in the present study. Most of earlier studies didn’t explore the all haematological parameters in Brucella infected animals. RAR [29] reported lower Hb value than the reference value in the Brucella infected cattle. Intra-cellular position of the Brucella spp. might cause reduction of Hb percentage [30]. Animals such as moose, infected experimentally with B. abortus showed slight lower Hb concentration [31] or within the range of reference value [30]. Some worker has also reported higher Hb concentration in Brucella infected animals [32]. Lymphocyte count was also lowered in Brucella infected animals [32,33]. However, Säder et al. [30] found higher values of neutrophil, monocyte and eosinophil counts in the B. abortus positive cattle than the standard values. He also found Hb, PCV, TEC, TLC, lymphocytes and basophils values of infected cattle within the reference range. Lymphoid depletion in the thymic cortex in natural and experimental animals might cause lymphopenic condition [33].

Biochemical parameters such as serum glucose, serum total protein, serum albumin, and creatinine, revealed higher value in infected cattle but, other parameter such as AST, ALT and SD were lower than the reference value in the present study. El-Boshy et al. [32] reported significant increases (P<0.05) in serum SD, AST and ALT levels and non-significant variations in creatinine in Brucella infected animals. Forbes et al. [31] confirmed stable and similar haemato-biochemical parameters in moose experimentally infected with B. abortus biovar 1.

Several treatment trials for brucellosis have been previously attempted, but none was entirely successful. The Brucella bacterium is protected from antibiotics since it survives within phagocytic cells of the reticuloendothelial system. Successful treatment needs permeability of drug across the cell wall of bacteria. Present therapeutic trial on Brucella infected cattle showed; long acting oxytetracycline and streptomycin combination gave better results than the drugs given alone. However, they did not cure infection completely. Animals either stopped shedding of pathogen or reduced its level to such an extent to diagnose by test implied. Similarly, antibody level in some of animals reduced to such a level as it was undiagnosed by different diagnostics used both on serum and milk.

Oxytetracycline and streptomycin are capable of penetrating the bacterial cell wall, inhibiting protein synthesis and providing long lasting concentrations in the plasma and hence considered most effective in the treatment of brucellosis [34]. Combination of both has demonstrated synergistic effect *in vitro* [12]. Long term treatment with high doses of oxytetracycline and streptomycin combination in Brucella infected Neumann’s gazelles resulted in eradication of the infection [35]. But, relapse and abortion were noticed frequently in animals which were received antibiotic treatments [36,37]. Streptomycin-tetracycline could be the choice of therapy for brucellosis, particularly in severe cases [38].

Combination therapy of long acting oxytetracycline and streptomycin revealed a significant increase (p<0.05) in Hb, TEC, TLC, lymphocytes count, ALT, AST and SD and significant decrease (p<0.05) in the values of glucose, total protein and albumin and non-significant alteration (p>0.05) in other parameters. Omer et al. [34], found significant changes in Hb, ALT and glucose, after combination therapy in Brucella infected gazelles. But changes in other haemato-biochemical parameters following the treatment were non-significant.

### Conclusion

Long acting oxytetracycline and streptomycin in combination could be used for treatment of Brucella infected cattle, but it needs further validation. Shedding of pathogen in milk or other body secretions of animal should be monitored for longer duration, not only by PCR but also by isolation of bacterium on suitable media. Relapse of infection or abortion in subsequent pregnancy should also be monitored to validate the treatments. Use of haemato-biochemical parameters as indicator of Brucella infection is not warranted, as alterations in these parameters are also evidenced in other bacterial infections. Therefore, these alterations should be carefully interpreted to give a final decision. It is better to complement a serological or molecular test to attain a final conclusion.

### Acknowledgement

The authors are thankful to the Dean Post Graduate Studies, G B Pant University of Agriculture & Technology, Pantnagar and Indian Council of Agricultural Research, New Delhi for providing the necessary facilities/fund to carry out the study.

### References


### Table 3: Number of cattle remains positive for concerned attributes in respective diagnostics after the therapeutic trial

<table>
<thead>
<tr>
<th>Tests</th>
<th>Group A (n=9)</th>
<th>Group B (n=9)</th>
<th>Group C (n=9)</th>
</tr>
</thead>
<tbody>
<tr>
<td>RBPT</td>
<td>6</td>
<td>6</td>
<td>4</td>
</tr>
<tr>
<td>STAT</td>
<td>5</td>
<td>6</td>
<td>3</td>
</tr>
<tr>
<td>i-ELISA (on serum)</td>
<td>7</td>
<td>7</td>
<td>4</td>
</tr>
<tr>
<td>MRT</td>
<td>4</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td>Milk-ELISA</td>
<td>6</td>
<td>5</td>
<td>4</td>
</tr>
<tr>
<td>PCR on milk</td>
<td>4</td>
<td>4</td>
<td>3</td>
</tr>
</tbody>
</table>

n = number of animals. RBPT: Rose Bengal Plate Test; STAT: Standard Tube Agglutination Test; i-ELISA: Indirect-Enzyme Linked Immunosorbent Assay; MRT: Milk Ring Test; ELISA: Enzyme Linked Immunosorbent Assay; PCR: Polymerase Chain Reaction


