

# 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 as shedding of *Brucella* in milk had also gone undetectable.

**Keywords:** brucellosis, cattle, haemato-biochemical parameters, oxytetracycline, streptomycin

Volume 1 Issue 2 - 2014

Neelam Kushwaha,<sup>1</sup> Vijendra Singh Rajora,<sup>2</sup>  
Anand Mohan,<sup>3</sup> Jawahar Lal Singh,<sup>2</sup> Santosh  
Kumar Shukla<sup>2</sup>

<sup>1</sup>Division of Medicine, Indian Veterinary Research Institute, India

<sup>2</sup>Department of Veterinary Medicine, GB Pant University of Agriculture & Technology, India

<sup>3</sup>Division of Bacteriology and Bacteriology, Indian Veterinary Research Institute, India

**Correspondence:** Neelam Kushwaha, Department of Veterinary Medicine, College of Veterinary and Animal Science, GB Pant University of Agriculture & Technology, Pantnagar-263145, Uttarakhand, India, Email kushneils@gmail.com

**Received:** April 17, 2014 | **Published:** June 12, 2014

**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; ALT, alanine aminotransferase; AST, aspartate 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.<sup>1</sup> In cattle, it is caused by *B. abortus*, also known as contagious abortion.<sup>2</sup> 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<sup>3-5</sup> and it may lead to temporary or permanent infertility.<sup>6</sup> Diagnosis on the basis of abortion is, however, equivocal since many pathogens can induce abortion; thus laboratory testing is essential.<sup>7</sup> 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.<sup>5</sup> 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.<sup>5</sup> 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.<sup>8</sup> 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.<sup>9</sup> Treatments for brucellosis have been tried previously but none was found successful.<sup>10,11</sup> Streptomycin acts synergistically with penicillin or tetracycline to inhibit the growth of *B. abortus* within bovine cells cultured *in vitro*.<sup>12</sup> 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.<sup>13</sup> 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.<sup>5</sup> 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.<sup>14</sup> 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<sup>5</sup> 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 450nm 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  $\geq 25$  were taken as positive whereas, samples having %P value  $< 25$  were negative. %P of serum was calculated from OD as follow.

$$\%P = \frac{\text{OD of serum sample}}{\text{Average OD of standard strong positive control}} \times 100$$

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

$$\%P = \frac{\text{OD of milk sample}}{\text{Average OD of standard positive control milk}} \times 100$$

### Polymerase chain reaction

Specific DNA sequence of 905 bp, which belong to 16S rRNA of *B. abortus*,<sup>13</sup> was targeted for amplification. Oligonucleotides (F4; 5'-TCGAGCGCCCGCAAGGGG-3' and R2; 5'-AACCATAGTGTCTCCACTAA-3') required for amplification of earlier said sequence were taken from published literature.<sup>15</sup> Methods described for DNA extraction from milk<sup>16</sup> and PCR<sup>15</sup> were followed as mentioned in their respective literatures. Agarose gel (0.8% w/v in 0.5X Tris-borate EDTA) containing 0.5 $\mu$ g/ml ethidium bromide was used to electrophorese the amplified PCR products in 0.5X Tris-borate EDTA at 40 Volt/cm.

### 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.<sup>17</sup> 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 25mg/kg combined with

streptomycin at a dose rate 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 & Cochran<sup>18</sup> 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 trail. 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 (Tables 1 & 2). But, other haemato-biochemical parameters; PCV, TEC, TLC, glucose, total protein, creatinine, albumin, ALT and SD were not significantly altered ( $p > 0.05$ ) after the treatment. RBPT was unable to detect antibody in three cattle (Table 3). Similarly, STAT and ELISA were not able to detect anti-*Brucella* antibodies in serum samples of four and two animals, respectively after the treatment. Furthermore, five cattle reduced the shedding of pathogen to undetectable level in milk, as it was confirmed by PCR (Table 3). Similarly, MRT and ELISA were given negative results respectively, in five and three cattle after the treatment (Table 3). 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. Screening of milk for presence of antibodies were not detected by MRT and ELISA respectively, in five and four cattle (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 1 & 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, significant increased its level ( $p < 0.05$ ) of Hb, TEC, TLC and lymphocytes counts (Table 1). However, alteration in PCV, neutrophils, eosinophils and monocytes count were non-significant ( $p > 0.05$ ). Biochemical parameters; glucose, total protein and albumin showed significant decrease ( $p < 0.05$ ) but, ALT, AST and SD increased significantly ( $p < 0.05$ ) after therapy (Table 2).

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

Parameters	Before treatment				After treatment			
	Control (n=3)	Group A (n=9)	Group B (n=9)	Group C (n=9)	Control (n=3)	Group A (n=9)	Group B (n=9)	Group C (n=9)
Hb (g/L)	135.3±11.00	98±10.48 <sup>a</sup>	99.5±09.78	99.5±10.38 <sup>a</sup>	144±08.70	99.5±10.50 <sup>a</sup>	105.1±10.95	116.5±14.16 <sup>a</sup>
PCV (L/L)	0.38±0.61	0.39±0.02	0.4±0.02	0.39±0.02	0.39±0.02	0.4±0.02	0.41±0.01	0.4±0.02
TEC (X 10 <sup>12</sup> /L)	7.9±0.85	6.41±0.53	6.42±0.61 <sup>a</sup>	6.37±0.69 <sup>a</sup>	7.96±0.75	6.44±0.52	6.51±0.63 <sup>a</sup>	6.45±0.62 <sup>a</sup>
TLC (X 10 <sup>9</sup> /L)	8.2±0.56	3.26±0.24	3.35±0.29	3.85±0.09 <sup>a</sup>	8.26±0.51	3.27±0.25	3.38±0.25	4.16±0.11 <sup>a</sup>
Neutrophil (X 10 <sup>9</sup> /L)	2.96±0.21	2.62±0.52	2.6±0.20	2.73±0.31	3.03±0.31	2.67±.47	2.62±0.19	2.76±0.32
Esinophil (X 10 <sup>9</sup> /L)	1.06±0.15	0.84±0.11	0.88±0.16	0.85±0.12	1.1±0.20	0.85±0.13	0.89±0.15	0.87±0.14
Lymphocyte (X 10 <sup>9</sup> /L)	3.63±0.38	1.97±0.40 <sup>a</sup>	1.96±0.41	1.94±0.39 <sup>a</sup>	3.88±0.42	2.02±0.42 <sup>a</sup>	2±0.41	2.03±0.41 <sup>a</sup>
Monocyte (X 10 <sup>9</sup> /L)	0.3±0.10	0.153±0.03	0.15±0.03	0.158±0.03	0.33±0.06	0.158±0.03	0.16±0.03	0.161±0.03

<sup>a</sup>Figures having same superscripts are significant at 5% level of significance for respective parameters, n, number of animals; Hb, haemoglobin; PCV, packed cell volume; TEC, total erythrocytes count; TLC, total leucocytes count; DLC, differential leucocytes count.

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

Parameters	Before treatment				After treatment			
	Control (n=3)	Group A (n=9)	Group B (n=9)	Group C (n=9)	Control (n=3)	Group A (n=9)	Group B (n=9)	Group C (n=9)
Glucose (mmol/L)	3.33±0.33	5.07±0.16	5.04±0.19	5.027±0.19 <sup>a</sup>	3.3±0.36	5.04±0.17	5.02±0.19	3.94±0.48 <sup>a</sup>
Total Protein (g/L)	74.67±4.93	92.96±6.15	93.33±5.61	92.67±6.29 <sup>a</sup>	74.43±4.89	92.46±6.07	93.05±5.90	64.33±6.59 <sup>a</sup>
Albumin (g/L)	29±2.65	37.77±1.07	38.53±0.80	39.03±0.98 <sup>a</sup>	28.78±2.55	37.31±1.32	37.99±0.84	28±3.25 <sup>a</sup>
Creatinine (µmol/L)	102.67±35.22	179.24±19.47	179.53±20.25	180.65±20.36	102.74±34.26	178.5±19.41	178.65±19.41	180.24±20.11
ALT (units/L)	15.26±3.24	7±2.10	6.72±1.84	6.35±2.04 <sup>a</sup>	15.9±2.82	7.16±1.06	6.78±1.57	14.98±2.42 <sup>a</sup>
AST (units/L)	91.3±4.53	61.53±7.73 <sup>a</sup>	60.88±8.84	59.73±8.50 <sup>a</sup>	91.99±4.57	62.04±7.86 <sup>a</sup>	61.49±8.84	101.18±18.02 <sup>a</sup>
SD (units/L)	9.9±1.25	2.92±0.33	2.82±0.27	2.75±0.32 <sup>a</sup>	9.93±1.10	2.95±0.30	2.85±0.28	6.47±0.50 <sup>a</sup>

<sup>a</sup>Figures having same superscripts are significant at 5% level of significance for respective parameters, n, number of animals; ALT, alanine aminotransferase; AST, aspartate aminotransferase; SD, sorbitol dehydrogenase.

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

Tests	Group A (n=9)	Group B (n=9)	Group C (n=9)
RBPT	6	6	4
STAT	5	6	3
i-ELISA (on serum)	7	7	4
MRT	4	4	3
Milk- ELISA	6	5	4
PCR on milk	4	4	3

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.

## Discussion

Brucellosis has wide socio-economic impact, especially in countries in which rural income relies largely on animal husbandry.<sup>19,20</sup> In India, it causes approximately Rs. 350 million economic losses.<sup>21</sup> Several studies have confirmed widespread prevalence in different States of India.<sup>22-25</sup> High seroprevalence has been reported in Indian dairy herds<sup>26,27</sup> Long-term serological studies at national level

indicated, 5% cattle infected with brucellosis.<sup>28</sup> Despite the advances made in the diagnosis, vaccination and therapy to control the disease, it is still wide spread 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<sup>29</sup> 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.<sup>30</sup> Animals such as moose, infected experimentally with *B. abortus* showed slight lower Hb concentration.<sup>31</sup> or within the range of reference value.<sup>30</sup> Some worker has also reported higher Hb concentration in *Brucella* infected animals.<sup>32</sup> Lymphocyte count was also lowered in *Brucella* infected animals.<sup>32,33</sup> However, Sikder et al.,<sup>30</sup> 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 could give lymphopenic condition.<sup>33</sup> 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.,<sup>32</sup> 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.,<sup>31</sup> 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 bacterium. 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.<sup>34</sup> Combination of both has demonstrated synergistic effect *in vitro*<sup>12</sup> Long term treatment with high doses of oxytetracycline and streptomycin combination in *Brucella* infected Neumann's gazelles resulted in eradication of the infection.<sup>35</sup> But, relapse and abortion were noticed frequently in animals which were received antibiotic treatments.<sup>36,37</sup> Streptomycin-tetracycline could be the choice of therapy for brucellosis, particularly in severe cases.<sup>38</sup> 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.,<sup>34</sup> 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 monitor 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.

## Acknowledgments

The authors are thankful to the Dean Post Graduate Studies, GB 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.

## Conflicts of interest

The authors declared there is no conflict of interest.

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