*Original Research*

# Clinical and Haemato-biochemical Studies on Respiratory Disease in Buffaloes

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**Abstract**

*The purpose of this study was to determine clinical and haemato-biochemical parameters of buffaloes suffering from respiratory disease. The present study included 39 clinical cases of buffaloes aged 3-8 years affected with respiratory disease brought to VCC, LUVAS, Hisar with the complaint of anorexia, fever, nasal discharge, coughing, dyspnoea and abnormal lung sounds on auscultation of thoracic area. The results were compared with seven healthy control animals. Clinical and haemato-biochemical studies revealed appreciable tachypnea, tachycardia, neutrophilia, hyperproteinemia, hyperglobulinemia, hyperglycemia, increased hepatic enzyme activity level, compromised kidney function, while low A/G ratio, hypoalbuminemia, hyponatremia, hypochloremia and reduced anion gap in affected animals. These findings may be important in the diagnosis and assessment of animals affected with respiratory disease.*

**Key words:** Bovine Respiratory Disease, Buffaloes, Clinical, Haemato-Biochemical Parameters

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## Introduction

Bovine respiratory disease (BRD) is one of the major health problem of buffaloes. BRD is one of the most common cause of morbidity and mortality in cattle (Murray *et al*., 2017) and most economically important disease of feedlot cattle with an estimated total loss of more than 3 billion dollars per year globally (Watts and Sweeney, 2010). Therefore, its timely diagnosis and treatment is very essential. Bovine respiratory disease is a multi-factorial disease involving interaction of infectious agents, compromised host immune system and environmental factors (Grissett *et al*., 2015). BRD encloses pneumonias in cattle resulting in a complex range of pulmonary lesions (Guterbock, 2014). Inflammation associated with BRD can lead to significant pulmonary damage and reduced lung function.

Respiratory and clinical signs seems to be vary with the degree of damage in lung tissue. Disease is characterized by clinical signs like fever, nasal discharge, coughing, tachypnea and dyspnoea along with the severe changes in blood gases (Ozkanlar, 2012). Haematological alterations can indicate whether the infection is bacterial or viral and also indicates about the severity of the disease. Respiratory disease leads to disturbances in basic respiratory functions *i.e.* gaseous exchange. Changes in results of haematology and plasma biochemical analyses could provide information that would help in identification of inflammatory response associated with pneumonia and helps in the early detection of BRD and prediction of disease severity.

## Materials and Methods

### Animals and Clinical Examination

A total of 39 buffaloes exhibiting clinical signs of respiratory disease of various degree aged between 3-8 years brought to VCC, LUVAS were examined thoroughly using standard clinical examination procedures focused on the case history, examination of the general health state (body temperature, food intake, behaviour, pulse rate, body condition) and then with a special focus on the respiratory system. The respiratory system was examined by physical inspection (breathing rate, nasal discharges, type of breathing, dyspnoea, coughing), auscultation of thoracic area for abnormal lung sounds and radiological examination of the study buffaloes manifesting clinical signs of the disease. Seven clinically healthy buffaloes served as control.

### Blood Sample Collection and Analyses

Blood samples were collected from both healthy and diseased animals from jugular vein. Blood for haematological examination (~5 ml) was collected into EDTA coated sterile vials and for plasma biochemistry (~10 ml) into heparinised tubes. The separated plasma was stored at -20°C until analysed. Estimation of haematological parameters included Hb (g/dl), PCV (%), TEC ($10^6$/µl), WBC ($10^3$/µl) and DLC (%) was done by using haematology cell counter (MS4s, Melet Schloesing Laboratories, France). Biochemical parameters viz., activity of AST (U/l), ALT (U/l), ALP (U/l), direct and total bilirubin (mg/dl), total proteins (g/dl), albumin (g/dl), globulin (g/dl), glucose (mg/dl), creatinine (mg/dl), urea (mg/dl) were estimated in plasma using fully automated random access clinical chemistry analyzer (EM 180™ Erba Mannheim, Germany) with kits procured from Transasia Bio-medical Limited (Mumbai). Plasma concentrations of $Na^+$ (mmol/l), $K^+$ (mmol/l) and $Cl^-$ (mmol/l) were analysed by fully automated Easylyte® expand Na/K/Cl/Ca/Li analyser with kits procured from Transasia Bio-medical Limited (Mumbai). Plasma Bicarbonate (mmol/l) was estimated manually by Van Slyke titration method.





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**Statistical Analysis**

The data was analyzed statistically by DMRT using SPSS v20.0. Independent sample t-test was performed to compare healthy with diseased animals.

**Result and Discussion**

The results of haemato-biochemical alterations of diseased buffaloes and comparison with healthy animals are presented in Tables 1-2 including the means, standard errors (S.E.) and significance of differences. In this study, affected animals were showing high rectal temperature, significant ($p<0.05$) increase in respiration and pulse rate. All the animals were showing inappetance/anorexia, abnormal lung sounds (crackles, wheezes or plueritic frictional rubs) on auscultation of thoracic area while approximate 71 percent animals were showing severe nasal discharge (serous to muco-purulent) and difficulty in respiration. Sixty one percent animals were showing severe coughing and 75 percent had severe congestion of mucus membrane. Similar types of findings were consistently observed by various other researchers (Collie, 1992; Griffin *et al.*, 2010; Ragbetli *et al*., 2010; Urban-Chmiel and Grooms, 2012 and Love *et al.*, 2014). All cases selected for study were found positive for pneumonic changes in the radiographic examination.

In haematological profile (Table 1) neutrophils showed significantly ($p<0.05$) higher mean values in diseased animals. The increase of WBC, mainly neutrophils, is a frequent finding in many diseases as a consequence of inflammatory processes like in respiratory diseases.

**Table 1:** Changes in clinical and haematological parameters (mean ± S.E.) in buffaloes affected with respiratory disease

| Haematological Parameters | Healthy Control (n=7) | Diseased Animals (n=39) |
|---|---|---|
| Temperature (°F) | 101.45 ± 0.23 | 102.46 ± 0.21 |
| Respiratory rate (per min) | $19.28^{a}$ ± 2.35 | $35.21^{b}$ ± 2.62 |
| Pulse rate (per min) | $56.57^{a}$ ± 3.57 | $71.36^{b}$ ± 1.71 |
| Hb (g/dl) | 11.75 ± 0.92 | 10.57 ± 0.39 |
| PCV (%) | 36.00 ± 2.54 | 36.31 ± 1.19 |
| TEC ($10^{6}$/µl) | 7.71 ± 1.08 | 7.43 ± 0.48 |
| TLC ($10^{3}$/µl) | 8.55 ± 0.79 | 9.75 ± 0.64 |
| N (%) | $37.71^{a}$ ± 2.26 | $59.05^{b}$ ± 2.56 |
| L (%) | $57.28^{b}$ ± 2.74 | $38.07^{a}$ ± 2.54 |
| M (%) | $3.14^{b}$ ± 0.73 | $1.77^{a}$ ± 0.22 |
| E (%) | $1.85^{b}$ ± 0.55 | $0.59^{a}$ ± 0.11 |

*Means bearing different superscripts (a, b) differ significantly ($p<0.05$) in row for each parameter*

Compared to healthy animals there was increase in total leukocyte count and significantly ($p<0.05$) high neutrophil count which was also found by Vestweber *et al.* (1990); Soltesova *et al.* (2015); Venkatesakumar *et al*. (2016). El-Sebaie *et al.* (1987) found that the hematological response is biphasic in which there was leukopenia at the early stage of diseases; however, there was leukocytosis on the late stage of respiratory





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diseases. Caswell (2014) suggested that stress and viral infections may prevent the recruitment of neutrophils to the lungs leaving a higher number in the peripheral blood.

In the current study, significant ($p<0.05$) lymphopenia was observed in buffaloes affected with respiratory disease as compared to healthy control animals. Similar results were also found by Youssef *et al*. (2015). Abdullah *et al.* (2013) also reported that high concentration of endotoxin can cause lysis of lymphocytes leading to significant ($p<0.05$) lymphopenia in calves suffering from respiratory infection. Viruses can also cause modification of the innate and adaptive immune systems through altered alveolar macrophage function, suppression of lymphocyte proliferation and induced apoptosis and modified cytokine and other inflammatory mediator release (Srikumaran *et al*., 2007). In this study, monocyte and eosinophil counts decreased significantly ($p<0.05$) in the diseased buffaloes as documented by Smedegard *et al.* (1989) who stated that endotoxin can cause reduction in monocyte number. Similar finding was also reported by Abdullah *et al.* (2013). Richeson *et al*. (2013) reported that calves with low or intermediate eosinophil counts had a significantly higher risk for having a diagnosis of BRD which might be due to chronic stress or inflammation among cattle where the hypothalamic-pituitary adrenal axis is stimulated to a greater extent, resulting in endocrine-induced effects on hematopoiesis, which in turn results in a reduction in the eosinophil count. No significant difference in the means were evident for concentration of PCV and TEC.

**Table 2:** Changes in biochemical parameters (mean ± S.E.) in plasma of buffaloes affected with respiratory disease

| Biochemical Parameters | Healthy Control (n=7) | Diseased Animals (n=39) |
|---|---|---|
| AST (U/L) | $92.71^{a} \pm 7.46$ | $184.41^{b} \pm 9.34$ |
| ALT (U/L) | 31.28 ± 3.36 | 46.39 ± 4.26 |
| ALP (U/L) | 107.00 ± 17.54 | 88.48 ± 8.19 |
| Direct bilirubin (mg/dl) | 0.21 ± 0.05 | 0.18 ± 0.02 |
| Total bilirubin (mg/dl) | 0.29 ± 0.06 | 1.41 ± 0.27 |
| Total Protein (g/dl) | $7.01^{a} \pm 0.15$ | $8.27^{b} \pm 0.16$ |
| Albumin (g/dl) | $3.16^{b} \pm 0.17$ | $2.62^{a} \pm 0.06$ |
| Globulin (g/dl) | $3.46^{a} \pm 0.10$ | $5.64^{b} \pm 0.17$ |
| A/G ratio | $0.90^{b} \pm 0.01$ | $0.02^{a} \pm 0.16$ |
| Glucose (mg/dl) | $61.01^{a} \pm 4.32$ | $116.06^{b} \pm 5.79$ |
| Creatinine (mg/dl) | 1.54 ± 0.13 | 2.37 ± 0.19 |
| Urea (mg/dl) | $24.71^{a} \pm 2.87$ | $68.18^{b} \pm 6.84$ |
| Sodium (mmol/l) | $139.34^{b} \pm 2.85$ | $128.36^{a} \pm 0.64$ |
| Potassium (mmol/l) | $4.74^{b} \pm 0.29$ | $3.82^{a} \pm 0.14$ |
| Chloride (mmol/l) | $104.27^{b} \pm 1.72$ | $97.92^{a} \pm 0.99$ |
| Bicarbonate (mmol/l) | 23.00 ± 1.95 | 23.26 ± 0.61 |
| Anion Gap (mmol/L) | $19.40^{b} \pm 1.41$ | $11.00^{a} \pm 0.38$ |

*Means bearing different superscripts (a, b) differ significantly ($p<0.05$) in row for each parameter.*







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Among the evaluated plasma biochemical parameters (Table 2) AST, total protein, globulin, glucose and urea were having significantly higher while albumin, A/G ratio, $Na^+$, $K^+$, $Cl^-$ and anion gap were having significantly lower mean values as compared to healthy control values ($p<0.05$). Non-significantly higher means of ALT, total bilirubin, creatinine and bicarbonate while lower means of direct bilirubin, ALP was found.

Significant ($p<0.05$) elevation of the aspartate aminotransferase (AST) activity was also observed by Abdullah *et al.* (2013) and Šoltésová *et al.* (2015). AST originates mainly from muscles and liver. Higher activity of AST was found probably as a result of increased respiration rate and muscle work during prolonged duration or severe cases of respiratory disease. Plasma total bilirubin was mildly increased in the present study in comparison to healthy animals which might be due to hepatic damage. Alteration in protein profile corresponds to changes occurs during acute phase response. High level of the total protein in the blood is usually associated with the inflammatory processes, when the synthesis of acute phase proteins and the production of immunoglobulins increases (Evans, 2003). Plasma albumin is the major negatively reacting acute phase protein *i.e.*, its concentration decreases in case of inflammation and infection (Eckersall and Bell, 2010). Significant ($p<0.05$) hyperglobulinemia is usually related to infection and inflammation, due to increased synthesis of acute phase proteins, complement proteins and immunoglobulins (Evans, 2003). The significant ($p<0.05$) decrease in A/G values occurs mainly due to the increased immunoglobulin synthesis following antigenic stimulation (Evans, 2003). Alterations in protein profile indicate significant changes in protein synthesis in the liver, which likely modifies amino acid requirements for the animals.

Marked hyperglycemia shown in this study might have been caused by responses of animals to stress and fasting which is likely related to factors like adrenal gland activity, rate of glycogenolysis, lipolysis, or both, quantity and source of nutrients being absorbed from the gastrointestinal tract and rate of tissue utilization of nutrients (Cole *et al*., 1988). Montgomery *et al.* (2009) also observed that plasma glucose concentrations, being greatest for heifers never treated for apparent BRD and decreased for heifers that were treated. Significant ($p<0.05$) rise of blood urea might be due to increased catabolism of body protein (Orr *et al*., 1988) and pre-renal causes *i.e.* reduced renal blood flow and GFR.

Although, all of the nutrient levels were dependent at least to some degree on dietary intake**.** Ragbetli *et al.* (2010) and Basoglu *et al.* (2016) also reported hyponatremia and hypokalemia in calves with acute bronchopneumonia. Inappetance and change in feed might be explain the lower plasma concentrations of estimated minerals. The plasma bicarbonate concentrations were enhanced (Hanzlicek *et al*., 2010), might be due to compensatory response and insufficient $CO_2$ elimination. Significant hypochloremia suggests that the renal reabsorption of bicarbonates exchanged against chloride in nephrocytes was amplified (Carraro-Lacroix and Malnic, 2010). Anion gap provide an approximation of unmeasured anions which consists of





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negatively charged plasma proteins. Although the anion gap is used in the interpretation of acid-base disorders. Decrease in anion gap resulting from decrease in unmeasured anion occurs most commonly with hypoalbuminemia.

## Conclusion

Respiratory disease in buffaloes not only causes clinical discomfort but also has an impact on several blood constituents. These findings may be important in the diagnosis and assessment of diseased animals. More research in future should be done to know the sickness response of individual animals and to determine the metabolic changes occurs during the course of respiratory disease.

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## References

1. Abdullah FFJ, Osman AY, Adamu L, Zakaria Z, Abdullah R, Zamri-Saad, M and Saharee AA. 2013. Haematological and biochemical alterations in calves following infection with *Pasteurella multocida* type B: 2, bacterial lipopolysacchride and outer membrane protein immunogens (OMP). Asian Journal of Animal and Veterinary Advances. 8(6): 806-813.
2. Basoglu A, Baspinar N, Tenori L, Vignoli A and Yildiz R. 2016. Plasma metabolomics in calves with acute bronchopneumonia. Metabolomics. 12: 128.
3. Carraro-Lacroix LR and Malnic G. 2010. Acid-base transport by the renal distal nephron. Journal of Nephrology 23(16): 19-27.
4. Caswell JL. 2014. Failure of Respiratory Defenses in the Pathogenesis of Bacterial Pneumonia of Cattle. Veterinary Pathology. 51(2): 393-409.
5. Cole NA, Camp TH, Rowe Jr LD, Stevens DG and Hutcheson DP. 1988. Effect of transport in feeder calves. American Journal of Veterinary Research. 49: 178-183.
6. Collie DDS. 1992. Pulmonary function changes and clinical findings associated with chronic respiratory disease in calves. The British Veterinary Journal. 148: 33.
7. Eckersall PD and Bell R. 2010. Acute phase proteins: Biomarkers of infection and inflammation in veterinary medicine. The Veterinary Journal. 185: 23-27.
8. El-Sebaie A, Amer A, Nafie E, Abd el-all TH and Sadik AH. 1987. Clinical hematological and acid-base changes accompanying some respiratory and alimentary manifestations among fattening buffalo calves. Assiut Veterinary Medical Journal. 37:148-154.
9. Evans EW. 2003. Proteins, lipids and carbohydrates. In: Textbook of Duncan and Prasse's Veterinary Laboratory Medicine: Clinical Pathology. Wiley-Blackwell, fourth edition. Iowa State Press, 450 pp.
10. Griffin D, Chengappa MM, Kuszak J and McVey DS. 2010. Bacterial pathogens of the bovine respiratory disease complex. Veterinary Clinics North America: Food Animal Practice. 26: 381-394.
11. Grissett GP, White BJ and Larson RL. 2015. Structured Literature Review of Responses of Cattle to Viral and Bacterial Pathogens Causing Bovine Respiratory Disease Complex. Journal of Veterinary Internal Medicine. 29: 770- 780.
12. Guterbock WM. 2014. The impact of BRD: the current dairy experience. Animal Health Research Reviews. 15: 130–134.





 DOI 10.5455/ijlr.20171210043959

13. Hanzlicek GA, White BJ, Mosier D, Renter DG and Anderson DE. 2010. Serial evaluation of physiologic, pathological and behavioral changes related to disease progression of experimentally induced *Mannheimia haemolytica* pneumonia in postweaned calves. American Journal of Veterinary Research. 71: 359-369.
14. Love WJ, Lehenbauer TW, Kass PH, Van Eenennaam AL and Aly SS. 2014. Development of a novel clinical scoring system for on-farm diagnosis of bovine respiratory disease in pre-weaned dairy calves. Peer Journal. 238: 1-25.
15. Montgomery SP, Sindt JJ, Greenquist MA, Miller WF, Pike JN, Loe ER, Sulpizio MJ and Drouillard JS. 2009. Plasma metabolites of receiving heifers and the relationship between apparent bovine respiratory disease, body weight gain and carcass characteristics. Journal of Animal Science. 87: 328-333.
16. Murray GM, More SJ, Sammin D, Casey MJ, McElroy MC, O Neill RG, Byrne WJ, Earley B, Clegg TA, Ball H, Bell, CJ and Cassidy, JP. 2017. Pathogens, patterns of pneumonia and epidemiologic risk factors associated with respiratory disease in recently weaned cattle. Journal of Veterinary Diagnostic Investigation. 29(1): 20-34.
17. Orr C, Hutcheson DP, Cummins JM and Thompson GB. 1988. Nitrogen kinetics of infectious bovine rhinotracheitis-stressed calves. Journal of Animal Science. 66: 1982-1989.
18. Ozkanlar Y., Aktas MS, Kaynar O, Ozkanlar S, Kirecci E and Yildiz L. 2012. Bovine respiratory disease in naturally infected calves: clinical signs, blood gases and cytokine response. *Rev. Méd. Vét.* 163(3): 123-130.
19. Ragbetli C, Ceylan E and Tanritanir P. 2010. The effect of tulathromycin treatment on biochemical parameters in montofon calves with pneumonia. Asian Journal of Animal and Veterinary Advances. 5(2): 169-174.
20. Richeson JT, Pinedo PJ, Kegley EB, Powell JG, Gadberry MS, Beck PA and Falkenberg SM. 2013. Association of hematologic variables and castration status at the time of arrival at a research facility with the risk of bovine respiratory disease in beef calves. American Veterinary Medical Association. 243: 1035-1041.
21. Smedegard G, Lui L and Hugli TE. 1989. Endotoxin-Induced shock in the rat: A role for C5a. American Journal of Pathology. 135: 489-497.
22. Šoltésová H, Nagyová V, Tóthová C and Nagy O. 2015. Haematological and blood biochemical alterations associated with respiratory disease in calves. Acta Veterinaria Brno. 84: 249-256.
23. Srikumaran S, Kelling CL and Ambagala A. 2007. Immune evasion by pathogens of bovine respiratory disease complex. Animal Health Research Reviews. 8: 215-229.
24. Urban - Chmiel R and Grooms DL. 2012. Prevention and control of bovine respiratory disease. Journal of Livestock Science. 3: 27-36.
25. Venkatesakumar E, Vijayakumar G, Subramanianm M, Balasubramaniam GA and Rajeswar JJ. 2016. Demographic study of pneumonia in cattle in and around namakkal, Tamilnadu. The Indian Veterinary Journal. 93(5): 37-40.
26. Vestweber JG, Klemm RD, Leipold HW, Johnson DE and Bailie WE. 1990. Clinical and pathologic studies of experimentally induced *Pasteurella haemolytica* pneumonia in calves. American Journal of Veterinary Research. 51: 1792-1798.
27. Watts JL and Sweeney MT. 2010. Antimicrobial resistance in bovine respiratory disease pathogens: measures, trends, and impact on efficacy. Veterinary Clinics North America: Food Animal Practice. 26: 79-88.
28. Youssef MA, El-khodery SA and Abdo M. 2015. A comparative study on selected acute-phase proteins (APPs) and immunoglobulins in buffalo and bovine calves with respiratory disease. Comparative Clinical Pathology. 24(3): 515-520.







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