

# Therapeutic study on Bacterial Respiratory Disease in Buffalo Calves

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## ABSTRACT

The cultural isolation and identification of nasal discharges from calves with respiratory disease revealed *Pasteurella* spp., *E. coli*, *Streptococci* spp., *Klebsiella* spp., *Staphylococci* spp. and *Pseudomonas* spp. The *Pasteurella* spp. was also identified from blood smears of clinical cases. The most predominant clinical signs observed were anorexia, pyrexia, depression, nasal and ocular discharges, congested conjunctival mucous membrane, cough and open mouth breathing. The prominent physical examination findings were tachypnoea, tachycardia, crackles, wheezes and dyspnoea. Haematological study showed anaemia, leukocytosis with neutrophilia and lymphopenia. Serum biochemical parameters showed hike in ALT, ALP, TP and fall in albumin. Thoracic radiography revealed consolidation of lungs. Therapeutic efficacy was proved to be superior in buffalo calves treated with Ceftiofur sodium when compared to Cefoperazone. Combination of Ceftiofur sodium, Meloxicam and Chlorpheniramine maleate was found to be effective for the treatment of bacterial respiratory disease in buffalo calves caused by *Pasteurella* spp.

**Key words:** Buffalo calf, Ceftiofur sodium, Lymphopenia, *Pasteurella* spp., Respiratory disease

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## INTRODUCTION

Bovine respiratory disease is common in calves and causing gigantic economic losses due to reduced live weight gain and feed conversion efficiency, poor performance and cost of treatment. It is a syndrome of diverse etiologies that is caused by one or more of wide range of microorganisms including *Pasteurella multocida*, *Manheimia haemolytica*, *Histophilus somnei* and *Mycoplasma bovis*, which are most commonly reported (Godinho *et al.*, 2007). Bovine respiratory disease affected calves exhibit signs of fever, nasal discharges, depression, cough and separate themselves from the rest of the pen along with severe alteration in blood gases (Zeineldin *et al.*, 2017). This generally occurs within the first 2 weeks of calves transported to the feedlot farms. Auscultation of the lung in BRD affected calves exhibit abnormal lung sounds including loud wheezing, crackling sound and moist rales due to the exudates produced by inflammatory cells and goblet cells as a result of pneumonia. Haematological picture of bovine respiratory disease is affected calves characterized by change in blood Hb, RBCs, PCV, Total WBCs and DLC count. Severe biochemical alterations in bovine respiratory disease affected calves were also common and might display reasonably predictable changes in response to inflammation and resulted in significant difference in protein profile. The inflammation associated with bovine bacterial respiratory disease could lead to significant pulmonary damage and reduced lung function. Treatment for bovine respiratory disease in calves frequently involved antimicrobial administration and non-steroidal anti-inflammatory drugs. Less systematic work has been done on bacterial respiratory disease in buffalo calves in Andhra Pradesh particularly on

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etiological, diagnosis and treatment aspects, hence this study was planned and executed.

## MATERIALS AND METHODS

The study was conducted on 54 clinical cases brought to the large animal outpatient ward, Veterinary Clinical Complex (VCC), NTR College of Veterinary Science, Gannavaram, Veterinary Hospitals and farms nearby Vijayawada (Andhra Pradesh, India) with clinical signs suggestive of bovine respiratory disease. These animals were screened by using specially designed data sheet and subjected to detailed clinical examination, haematology (Jain, 1986), serum biochemical profile (Thermo Scientific Instrument using standard kits manufactured by M/S Erba- Mannheim Pvt. Ltd., Mumbai), cardiac biomarkers, thoracic radiography (Masseau

*et al.* (2008), electrocardiography and echocardiography, cultural studies of nasal discharges and blood to confirm the bovine bacterial respiratory disorders. Ten apparently healthy buffalo calves were selected as control group for obtaining normal data for comparison of parameters under study.

### Cultural Isolation and Antibiotic Sensitivity

Clinical samples collected for the present study included nasal discharges, whole blood and serum. The samples were subjected to cultural isolation of bacterial pathogens and their antibiotic sensitivity test (Quinn *et al.*, 1999).

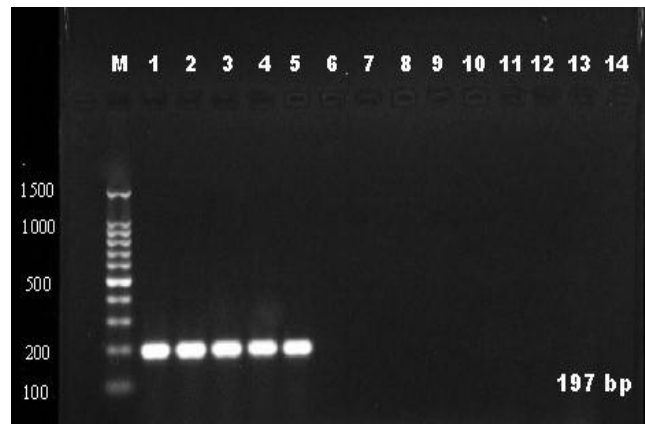
### Treatment Trials

Out of 54 calves diagnosed as suffering from respiratory disorders, sixteen (16) confirmed cases of bacterial respiratory disease caused by *Pasteurella* spp. were randomly divided in two groups of eight (8) animals each. The buffalo calves in group I and II were treated with Ceftiofur sodium @ 2.2 mg/kg b.wt. and Cefoperazone @ 20 mg/kg b.wt., respectively, intra muscularly daily for a period of seven days (Hornish and Kotarski, 2002). In both the groups, Meloxicam @ 0.5 mg/kg b.wt. and Chlorpheniramine maleate @ 0.5 mg/kg b.wt. were used once in a day intra muscularly for three days

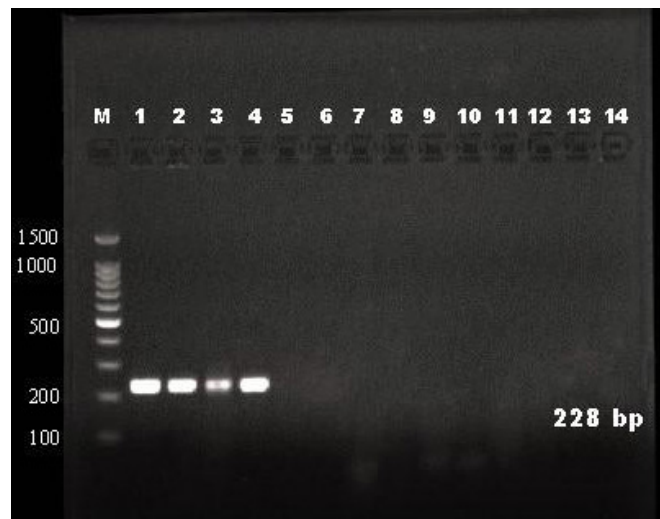
Bovine respiratory disease caused by other bacteria, viz., *E.coli*, *Streptococci* spp., *Klebsiella* spp., *Staphylococci* spp. and *Pseudomonas* spp., were also treated with sensitive antibiotics. The calves under the therapeutic trials were monitored for a period of five days and two treatment regimens were evaluated at seven days period based on the improvement in clinical signs, haematology, serum biochemical profile and bacterial load. Data collected were subjected to statistically analysis as described by Snedecor and Cochran (1994).

## RESULTS AND DISCUSSION

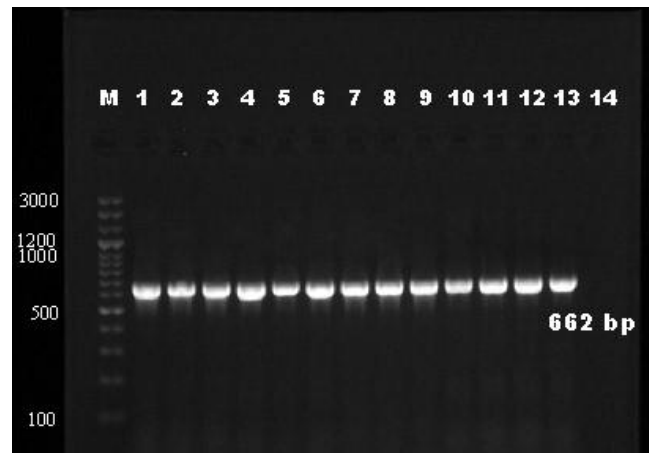
The result revealed the involvement of many bacterial species in buffalo calves with bacterial respiratory disease. The *Pasteurella* spp. were the most predominant bacteria isolated at 29.62 % followed by *E. coli* (25.92%), *Streptococcus* spp. (18.51%), *Klebsiella* spp. (11.11%), *Staphylococcus* spp. and *Pseudomonas* spp. (7.40 % each). Van Driessche *et al.* (2017) also reported that *Pasteurella multocida* was most predominant bacteria isolated. Various isolates were confirmed by PCR. The amplification of DNA of *Streptococcus* spp. was at 197 bp (Fig. 1), which was in agreement with earlier report of Picard *et al.* (2004). The amplification of DNA of *Staphylococcus* was at 228 bp (Fig. 2), which agreed with previous finding of Riffon *et al.* (2001). *E. coli* isolates were also confirmed by PCR using Eco (Eco 2083 and Eco 2745) primers. The amplification of DNA of *E.coli* (Fig. 3) was carried out at 662 bp (Anand Kumar, 2009).



**Fig. 1:** PCR amplification of *Streptococcus* species. M- Molecular weight marker, Samples 1, 2, 3, 4, 5 +ve; Samples 6, 7, 8, 9, 10, 11,12, 13 -ve; 14-Negative control



**Fig. 2:** PCR amplification of *Staphylococcus* species. M- Molecular weight marker, Samples 1, 2, 3, 4 +ve; Samples 5, 6, 7, 8, 9, 10, 11,12, 13 -ve; 14-Negative control



**Fig. 3:** PCR amplification of *E.coli*. M- Molecular weight marker, Samples 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11,12, 13 +ve ; 14-Negative control

The most prominent clinical signs observed in buffalo calves with bacterial respiratory disease were anorexia (100%), pyrexia (100%), depression (85.18%), nasal and ocular discharges (70.37%) congested conjunctival mucous membrane (66.66%), cough (14.81%) and open mouth breathing (11.11%). The present findings are in agreement with earlier studies of McDanel *et al.* (2018). Fever is an indication of microbial infection. It is one of the mechanism that immune system utilizes to get rid the body from invading pathogens and as a normal response to immune stimulus. The change in attitude, appetite, rectal temperature and mucus membrane congestion of the buffalo calves might be attributed to toxemia which was associated with the release of bacterial endotoxins (Attia *et al.*, 2016). Marked dyspnea with open mouth breathing was observed in 11.11 % of cases, which might be due to advanced pulmonary parenchymal destruction. The observed nasal discharges and ocular discharges were also attributed to inflammation changes in nasal and ocular mucous membrane as reported by Ramadan *et al.* (2019).

The prominent physical examination findings were dyspnea, tachycardia, tachypnea, crackles and wheezes. Bovine respiratory disease affected calves with shallow rapid respiration due to hypoxia and dyspnea in some cases might be attributed to severe inflammation in bronchioles and alveoli that interfere with gas exchange and respiration (Ramadan *et al.*, 2019). Auscultation of the lung in BRD affected calves showed abnormal lung sounds including loud wheezing, crackling sound and moist rales due to the exudates produced by inflammatory cells and goblet cells as a result of pneumonia (Kumar *et al.*, 2018).

The haematological examination of the bacterial respiratory disease affected calves revealed a significant decrease in RBC count, PCV and Hb compared to healthy calves (Table 1), which were similar to previous studies (Ismael *et al.*, 2017; Ramadan *et al.*, 2019). These results in diseased calves might be attributed to micro-haemorrhages and sequestration of red blood cells during disseminated micro-thrombosis (Gutierrez *et al.*, 1999). It could also have resulted from destruction of red blood cells by micro-organisms secretions (Mondal *et al.*, 2004). Anaemia could be attributed to reduction of energy and protein intake or sequestration of iron in bone marrow macrophages and hepatocytes during infection, thus become unavailable for utilization in haemoglobin synthesis leading to inhibition of erythropoiesis (Aytekin *et al.*, 2011). In bovine respiratory disease affected calves when compared to control calves, there was a significant increase in both WBCs count and neutrophils, whereas significant decrease in lymphocytes (Table 1). These results gained support from the findings of Ismael *et al.* (2017) and Kumar *et al.* (2018). The increase in WBC count, mainly neutrophils, is a frequent finding in many diseases as a sequence of inflammatory process in

respiratory disease. The high concentration of endotoxin causes lysis of lymphocyte leading to significant lymphopenia in calves suffering from bacterial respiratory disease (Kumar *et al.*, 2018). The significant increase in WBCs and neutrophils occurred in pneumonia might be attributed to inflammatory lesions and presence of bacterial infection (Smith, 2014). No significant difference ( $p > 0.05$ ) was observed among eosinophil concentration in infected calves when compared with control group.

The study revealed significant elevation in total protein, ALP and AST values in bovine respiratory disease affected calves when compared to control group. Though there was significant elevation in total bilirubin and creatinine values in calves with bovine respiratory disease, the values were within normal range (Table 1). The present findings were in agreement with earlier reports (El-Bahr and El-Deeb, 2013; Kumar *et al.*, 2018; Ramadan *et al.*, 2019). The result also revealed that there was a significant increase in serum total protein and a significant decrease in serum albumin. High level of total protein in blood of the diseased calves and increase in the production of immunoglobulin are usually associated with the inflammatory processes. Hyperproteinemia is usually related to infection and inflammation due to increased synthesis of acute phase proteins, complement proteins and immunoglobulins (Ramadan *et al.*, 2019). Hypoalbuminemia could be due to anorexia and inability of liver to synthesize protein (Kumar *et al.*, 2018). Others suggested that certain bacterial toxins increase capillary permeability and permit escape of plasma proteins in tissues, so osmotic pressure of proteins is increased in tissue fluids and at the same time decreased in blood (Omran *et al.*, 2005). Albumin is also considered a negative acute phase proteins and its value frequently and markedly declines during inflammation (Georgievia *et al.*, 2011). The significant elevation in AST, ALP levels in this investigation could be attributed to dysfunction of liver due to hepatic degenerative and necrotic changes caused by bacterial infection and toxins (Aytekin *et al.*, 2011).

The prominent thoracic radiographic findings were consolidation, pulmonary infiltration, pulmonary edema of the lung. Masseur *et al.* (2008) reported similar clinical findings in bacterial respiratory disease in buffalo calves. In the present study the thoracic radiographic appearance of pulmonary parenchyma was interstitial pattern, bronchial pattern and alveolar pattern. Thoracic radiography was a sensitive in identifying interstitial pattern in buffalo calves having interstitial pneumonia, embolic pneumonia and chronic pneumonia.

In the present study 29.62 % of *Pasteurella* spp., 25.92 % of *E. coli*, 18.51 % of *Streptococcus* spp., 11.11 % of *Klebsiella* spp., 7.40 % of *Staphylococcus* spp., and 7.40 % of *Pseudomonas* spp., isolates were sensitive to Ceftiofur (62.96%), while the same isolates were also sensitive to Cefoperazone (61.11%) (Table 2) and no resistance was observed.

In the present study clinical cases showed signs of improvement after 5<sup>th</sup> day of treatment in Group I and 7<sup>th</sup> day after treatment in Group II. Nasal discharge, cough and abnormal lung sounds showed marked reduction 5<sup>th</sup> day post-treatment in Group I and 7<sup>th</sup> day post-treatment in Group II. Post-treatment mean values of RBCs, Hb, PCV and albumin were significantly increased, whereas mean values of TLC, neutrophil and total protein were significantly reduced. It was noticed that *Pasteurella* spp., microorganisms disappeared from the nasal discharges and blood after 7<sup>th</sup> day of post treatment. This was in accordance with previous studies (Kurcubic *et al.*, 2013). The present study demonstrated that one diseased buffalo calf in Group I and two diseased calves in Group II died between 3-4 days of treatment. In the present study the complete cure of 7 out of

8 buffalo calves with Ceftiofur sodium and 6 out of 8 buffalo calves with Cefoperazone was recorded.

Based on the result it is noted that Ceftiofur sodium could be effectively used in the treatment of bacterial respiratory disease in buffalo calves. Ceftiofur sodium sensitivity was observed in all microorganisms isolated from nasal swabs. In this study Ceftiofur sodium was superior antimicrobial as compared to Cefoperazone (Table 3) for the treatment of bacterial respiratory disease in buffalo calves. It is evident from the present study, that the findings recorded about the antimicrobial sensitivity of *Pasteurella* spp. are in accordance with results demonstrated by previous studies (Hornish and Kotarski, 2002). Ceftiofur has demonstrated high *in vitro* activity against bacterial pathogens associated with respiratory disease such as *Pasteurella* spp., *Manhemia*

**Table 1:** Haemato-biochemical findings in treatment groups

Parameter	Control group (n=10)	Treatment Group I (n=8)		Treatment Group II (n=8)	
		Pre	Post	Pre	Post
Hb (g/dL)	11.00±0.46	7.31 ± 0.24	11.12 ± 0.5**	5.93 ± 0.61	8.47±1.94**
PCV (%)	31.50±1.70	28.28±1.90	33.85±2.37*	29.0 ± 4.30	34.42± 5.53*
TEC (10 <sup>6</sup> /cmm)	7.42±0.55	5.64±0.42	8.32±0.54**	4.69± 0.40	7.90±1.50*
TLC (10 <sup>3</sup> /cmm)	8.03±0.66	12.82±0.71	7.79±0.66**	11.8 ± 1.20	6.54±1.23**
Neutrophils (%)	40.60±2.00	75.85±2.32	43.85±2.40**	66.71±4.01	39.28±7.05*
Lymphocytes (%)	58.60±1.90	22.71±2.60	55.28±2.23**	30.85±3.80	46 ± 8.02**
Eosinophils (%)	1.40±0.58	1.14±0.34	0.85±0.34	2.20 ± 0.64	0.42± 0.29*
Temperature (°F)	100.20±0.25	103.11±0.17	100.28±0.39**	103.08± 0.23	86.14 ±14.36
BUN (mg/dL)	32.90±1.70	33.85±4.25	35.14±3.34	38.42± 6.90	32.7 ±6.50
Creatinine (mg/dL)	0.9±0.40	0.95±0.08	0.81±0.06	0.99 ± 0.06	0.65 ± 0.12 *
AST(IU/L)	64.80±3.05	226.28±18.36	68.0±3.10**	117.28± 27.34	64.57± 12.38*
ALP(IU/L)	91.20±3.60	145.71±12.57	79.28±6.84**	125 ± 4.49	59.0 ±12.68**
Albumin (g/dL)	3.26±0.27	3.27±0.44	4.80±0.36*	3.0 ± 0.15	3.27 ± 0.5
Total protein (g/dL)	5.9±0.52	8.11±0.85	5.21±0.44**	7.5 ± 0.47	4.67 ±0.92*
Total bilirubin (mg/dL)	0.45±0.15	0.80±0.09	0.45 ± 0.04	0.4 ± 0.06	0.44 ± 0.08

\*\*P≤ 0.01 Statistically highly significant, \*P≤0.05 Statistically significant

**Table 2:** Antibiogram of bacterial isolates from nasal discharges of buffalo calves with respiratory diseases (n=54)

Drug	Sensitive		Intermediate		Resistant	
	No	%	No	%	No	%
Amoxicillin-Clavulanate	27	50.00	9	16.66	18	33.33
Amikacin	6	11.11	5	9.25	33	61.11
Cefoperazone	33	61.11	21	38.88	0	0
Ceftiofur	34	62.96	19	37.03	0	0
Ceftriaxone	27	50.00	21	38.88	6	11.11
Ciprofloxacin	18	33.33	24	44.44	6	11.11
Co-Trimoxazole	28	51.85	8	14.81	18	33.33
Enrofloxacin	45	83.33	4	7.40	5	9.25
Norfloxacin	27	50.00	12	22.22	15	27.77



**Table 3:** Survival rate between treatment groups

Group	Total no of cases	Cases survived	Percent recovery	Cases died	Percent mortality
I (Ceftiofur Na)	8	7	87.50	1	12.50
II (Cefoperazone)	8	6	75.00	2	25.00

spp., *Actinobacillus* spp., *Streptococcus* spp., *Haemophilus* spp., *Salmonella* spp. and *Cholera suis*. The improvement in clinical condition, reduction in TLC, neutrophil count and disappearance of *Pasteurella* spp. microorganisms from blood and nasal discharges in Group I might be attributed to bactericidal action of Ceftiofur sodium (Inhibition of cell wall synthesis) as reported by Hornish and Kotarski (2002) and Timsit *et al.* (2017).

## CONCLUSION

The findings of this study concluded that the occurrence of bacterial respiratory disease in buffalo calves was 22.50 %. The *Pasteurella* spp., *E. coli*, *Streptococci* spp., *Klebsiella* spp., *Staphylococci* spp. and *Pseudomonas* spp. were identified from nasal discharge and *Pasteurella* spp. from blood samples of buffalo calves with bacterial respiratory disease. The combination of Ceftiofur sodium, Meloxicam and Chlorpheniramine maleate was found to be effective in the treatment of respiratory disease caused by *Pasteurella* spp. in buffalo calves.

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