THERAPEUTIC STUDIES ON BACTERIAL PNEUMONIA IN BUFFALOES

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ABSTRACT

The aim of study is to identify the etiological agents in bovine respiratory disease associated with bacterial involvement, to study the clinical signs, haemato-biochemical and radiographic changes in bovine respiratory disease associated with bacterial involvement and to assess the efficacy of adopted therapeutic regimen for bovine respiratory disease associated with bacterial involvement. The study revealed that occurrence of respiratory disease with bacterial involvement was 23.05%. In the present study, buffaloes were more affected than cattle. Cultural isolation and identification of nasal discharges from the clinical cases revealed *P. multocida*, *Pseudomonas* spp., *Klebsiella* spp. and *E. Coli*. xviii The *P. multocida* was also identified from the blood samples of the clinical cases by PCR. Twenty five buffaloes were found positive for malignant catarrhal fever with PCR. The most prominent clinical signs observed in bovine respiratory disease with bacterial involvement were respiratory distress, anorexia, nasal discharges, congested conjunctival mucous membrane, muzzle dryness, cough, oral breathing and sneezing. The prominent physical findings were dyspnoea, tachycardia, tachypnoea, pyrexia, crackles and wheezes. Haematological study showed normal haemogram and leukocytosis with neutrophilia. Serum biochemical profile showed hike in total protein and AST levels and fall in albumin levels. However eosinophils, lymphocytes and monocytes values were with in normal range. Thoracic radiography revealed consolidation of lung. Therapeutic efficacy was found to be superior in Group II. Combination of Ceftiofur sodium, Flunixin meglumine and Chlorpheneraminemaleate was found to be effective for the treatment of bovine respiratory disease caused by *Pasteurella multocida.*

Keyword: *Bubalus bubalis*, buffaloes, bacterial, therapeutics, haemato-biochemical

INTRODUCTION

Bacterial pneumonia is one of the most common cause for the production loss and mortality in bovines. The anatomical and physiological features of the respiratory system of bovines may predispose them to the development of pulmonary lesions much more than other farm animal species. Bovines have a small physiological gaseous exchange capacity and greater resultant basal ventilator activity. Some of the pathogens are normal inhabitant of the respiratory tract of bovines. However, any activity that is precipitating

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stress leads to clinical disease condition. Common commensal bacterial organisms associated with bovine bacterial pneumonia are *Mannheimia haemolytica*, *Pasteurella multocida*, *Histophilus somni*, and *Mycoplasma bovis*. Inflammation associated with bovine bacterial. Bacterial pneumonia can lead to significant pulmonary damage and reduced lung function. The diagnosis of bovine bacterial bacterial pneumonia poses significant challenge to the clinician. The clinical signs alone may not be diagnostic and therefore diagnostic laboratory is often used to assist the clinician. Treatment for bovine bacterial bacterial pneumonia frequently involved antimicrobial administration and non-steroidal anti inflammatory drugs. Unless specific therapeutic regimen against bovine bacterial bacterial pneumonia is initiated at an early stage, the affected bovines become unproductive and are to be disposed off. Perusal of available literature revealed that not much attention has been paid towards bovine respiratory disorders in India. Keeping in view of above facts, the present study was designed to study the therapy of of bacterial pneumonia in buffaloes.

MATERIALS AND METHODS

The study comprised apparently healthy bovines and clinical cases. Ten apparently healthy bovines were selected as control group for obtaining normal data for comparison of parameters under study. The study was conducted in 72 clinical cases brought to the large animal outpatient medicine ward of Teaching Veterinary Clinical Complex (TVCC), College of Veterinary Science, Gannavaram, surrounding Veterinary Dispensaries and Rural Livestock Units (RLU) with clinical signs suggestive of bovine bacterial pneumonia were screened by using specially designed data sheet and subjected to detailed clinical examination, haematology, serum biochemical profile, radiography, cultural isolation of nasal discharges and blood to confirm the bovine bacterial respiratory disorders.

Nasal discharges

Deep nasal discharges were collected from animals with clinical signs suggestive of bacterial respiratory disease.

Whole blood

Five ml of blood was collected into a sterilized test tube for bacterial isolation. For *in vitro* antibacterial sensitivity testing, the following standard bio-discs (Hi-Media Laboratory Ltd.) were used:

Amoxycillin - Clavulanic acid	30 mcg
Enrofloxacin	30 mcg
Gentamicin	5 mcg
Moxifloxacin	30 mcg
Ceftiofur sodium	30 mcg

Preparation of glassware

All the glassware used in this study were dipped into 1% hydrochloric acid for 24 h, then washed under running tap water, again soaked in teepol solution for 24 h, cleaned with brush and washed under running water. The glassware was then rinsed in glass distilled water and dried at 50^{o}C carefully packed and sterilized at 60^{o}C for one and half hour in hot air oven. The sterilized glassware used for cultural and biochemical analysis.

Preparation of media

All the media obtained as dehydrated powders were rehydrated as per the manufacturer's instructions by adding distilled water to dissolve.

Then the media were sterilized by autoclaving at 15 lb pressure for 15 minutes. The blood agar and Edwards medium were prepared in the laboratory as per procedure given by Quinn *et al*. (1994).

Isolation of bacteria

Swabs collected from bovines affected with respiratory disorders were inoculated into BHI broth and incubated at 37°C for 24 h aerobically then a loop ful of broth culture was streaked on BHI agar plates. Based on morphology and Gram's staining properties, cultures were inoculated into specific or selective media like MacConkey agar, Mannitol salt agar, Eosine-Methylene-Blue agar, Edwards medium and *Pseudomonas* isolation agar. Pathogenicity was tested by inoculating on blood agar.

Identification of bacteria

Primary identification of bacteria was done on Gram's stainin, colony morphology and type of haemolysis on blood agar. Pure cultures were identified upto genus level as per the Bergey's Manual of Determinative Bacteriology (Holt *et al*., 1994).

The following tests were done on 24 to 48 h old pure cultures for identification of bacteria.

Gram's staining, Motility test, Catalase activity, Indole test,

Methyl red (MR) test, Voges-Proskauer (VP) test, and Citrate utilization test

Antibiotic sensitivity test

The antibiotic sensitivity of the whole swabs as well as individual isolates was done *in vitro* by disc diffusion method on MHA plates. The MHA plates were inoculated with 6 to 8 h pure culture grown in nutrient broth to test the sensitivity. The culture was applied on to the surface of the MHA agar plates using a sterile swab. The plates then were kept covered for 15 minutes at room temperature for drying the inoculum. The individual colonies for different organisms were suspended in the BHI broth and incubated for 2 to 4 h before being placed on the Mueller and Hinton agar (MHA) to evaluate the antibiotic sensitivity using the antibiotic discs namely Amoxiclav, Ceftiofur sodium, Enrofloxacin, Moxifloxacin and Gentamicin The zone of inhibition was compared with standards of NCCLS after 24 h of incubation in the incubator at 35°C (Bauer *et al*., 1966; Quinn *et al*., 1994).

Reading sensitivity

The inoculated MHA plates were incubated at 37°C for 18 to 24 h. The sensitivity patterns of isolates to different antibiotic discs were read by measuring the diameter of inhibition in millimetre as per the chart provided by manufacturer.

Nasal discharges culture

The nasal discharges were streaked on blood agar, nutrient agar, brain heart infusion (BHI) agar, cetrimide agar, chocolate agar and MacConkey agar for isolation of bacteria. Culture plates were incubated as per standard methods. Presumptive and definitive identification of pathogens were done by staining characteristics, colony morphology and standard biochemical tests (Barrow and Feltham 1993; Quinn *et al*., 1994)

Detection of *Pasteurella multocida* by PCR assay

The collected swabs were kept in Brain heart infusion (BHI) broth and incubated over night at 37°C. After incubation 1 ml of bacterial suspension was centrifuged in eppendorf tube at 13,000 rpm for 90 seconds at 4°C. The supernatant was discarded and the bacterial pellet was suspended

in 500 µl. of PBS (pH 7.4) and centrifuged again at 10,000 rpm for 90 seconds at 4°C. The bacterial pellet was suspended in 500 µl of milli Q water. This bacterial suspension was boiled for 10 minutes and chilled on ice for 5 minutes. After chilling the suspension was centrifuged and 0.5 ml/ 500 µl of supernatant containing bacterial DNA was used as template DNA for PCR. (Ewers *et al.*, 2006; Varte *et al*., 2014).

Primers used in PCR

KMT 1 Forward primers = ATCCGCTATTTACCCAGTGG

Reverse primers = GCTGTAAACGAACTCGCCACR

Reaction mixture: (for 10 µ lit)

1. Gotaq green promega 2x master mix : 5 µl
2. Forpri (20 pm/µ lit) : 0.125 µl
3. RP (20 pm/µ lit) : 0.125 µl
4. 25 m M concentration reaction ($MgCl_{2)}$: 0.4 µl
5. DNA template : 0.5 µl
6. Water : to makeup 10 µl

Programme

Denaturation	: 94°C for 30 seconds
Annealing	: 57°C for 40 seconds
Elongation	: 72°C for 60 seconds

30 cycles of programme followed by final extension at 72°C for 10 minutes.

Gel electrophoresis

PCR amplification products were analysed by gel electrophoresis on a 1% agarose gel stained with ethidium bromide, and photographed by UV exposure.

Pasteurella multocida

DNA was extracted from the *Pasteurella multocida* using DNA isolation kit. PCR identification of KMT1 clone of *Pasteurella multocida* was done at 460 bp as per Townsend *et al.* (1998).

Haematology

Five ml of venous blood was collected in two vacationer tubes containing K_3 EDTA as anticoagulant for haematological investigations. Haematological parameters including packed cell volume (PCV), haemoglobin (Hb), red blood cell (RBC), white blood cell (WBC) and differential counts (DC) were done as per standard methods (Jain, 1986).

Serum biochemistry

Five ml of venous blood was collected in vacationer tubes without anticoagulant taking all precautions for avoiding haemolysis as suggested by Alleman (1990). Serum was separated and used for the estimation of total protein by Modified biuret method, albumin by Dumas method (Varley *et al*., 1980), Aspartate amino transferase (AST) by Modified IFCC method (Bergymer, 1986).

Radiography

All the healthy and diseased animals were subjected to radiographic examination as described by Masseau *et al*. (2008).

Selection criteria for inclusion in the treatment trials

Out of 72 bovines diagnosed as suffering from respiratory disorders, 24 buffaloes were randomly selected and divided into two groups for the therapeutic trails. The criteria for selection of therapeutic trials were

1. History and clinical signs suggestive of respiratory involvement

2. Clinical case samples that were positive for bipolar organisms of *Pasteurella multocida*

3. Animals which were free from other respiratory disorders.

Treatment trials

Twenty four (24) confirmed cases of *Pasteurella multocida* respiratory infection were randomly selected in two groups of twelve (12) animals each for clinical trials using different therapeutic regiment.

Group I

The buffaloes in Group I were treated with Moxifloxacin 5 mg/Kg b.wt intra muscularly daily for a period of five days.

Group II

The buffaloes in Group II were treated with Ceftiofur sodium 2 mg/Kg b.wt intra muscularly daily for a period of five days.

In both the groups the following drugs were used for three days

1. Flunixin meglumine 1.1 mg/Kg b.wt once in a day intra muscularly

2. Chlorpheneramine maleate 0.5 mg/Kg b.wt once in a day intra muscularly

Bovine bacterial pneumoniaassociated with *Pseudomonas* spp., *Klebsiella* spp. and *E. coli* spp. animals were treated with sensitive antibiotics.

Evaluation of the treatment

The buffaloes 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, thorasic radiography and bacterial load. The data collected were subjected to statistical analysis as per Snedecor and Cochran (1994) and critically discussed.

RESULTS AND DISCUSSION

Therapeutic studies on bacterial pneumonia in buffaloes was carried out with total number of 82 bovines, among them 10 were apparently healthy bovines and 72 were clinical cases of respiratory disease. The occurrence of bacterial pneumonia was observed to be 23.05%. Investigation on age wise occurrence of bacterial pneumonia revealed that the bovine of 3 to 7 years of age group (34.72%) were more commonly affected (Table 1). Productive stress might be reason for the higher incidence of bacterial pneumonia in adult lactating buffaloes. In the present study Graded Murrah buffaloes (75%) were most commonly affected with bacterial pneumonia followed by non descriptive buffaloes (16.66%) and cross breed cows (8.33%). The findings were in agreement with previous study (Sindhu *et al.*, 2008). The percentage of occurrence in Graded Murrah buffaloes might be related to their numbers in hospital attendance and probably rearing Graded Murrah buffaloes for milk production in large numbers. The present study revealed that female bovine (87.53%) were more commonly affected than male bovine (12.5%). A female predominance in the present study concur with earlier reports (Thirunavukkarasu *et al*., 2005).

Hence it is opined that the female predominance in the present study due to rearing female bovine for milk production in large numbers. The result revealed the presence of many bacterial

species as single or mixed isolates. *Pasteurella multocida* was the most predominant bacteria isolated at a percentage of 43.05%, followed by *Pseudomonas* spp., at a percentage of 16.66%, *Klebsiella* spp. at a percentage of 9.72%, *E. coli* at a percentage of 8.33% and mixed infection at a percentage of 22.22% (Table 2). Similar reports were reported by previous studies. Sura *et al*. (2007); Kumar *et al.* (2009); Shayegh *et al*. (2009); Yenner *et al*. (2009); Griffin *et al*. (2010); Sayyari and Sharma (2011); Durrani *et al*. (2013). The difference between the results were mainly due to the geographical distribution at which the investigator was adopted. Townsend *et al*. (1998); Durani *et al*. (2013) confirmed *Pasteurella multocida* with PCR using KMT1T7 and KMT1SP6 primers to amply the DNA of *Pasteurella multocida* at 460 bp. The most prominent clinical signs observed in bovines with bacterial pneumonia were respiratory distress (93.05%), anorexia (90.27%), nasal discharges (81.94%), congested conjunctival mucous membrane (75%), muzzle dryness (58.33%), cough (56.%), oral breathing (52.77%) and sneezing (34.72%) (Table 3).

The present findings concur with earlier studies (Thomas *et al.* (2002); Panousis (2009); Kumar *et al.* (2011); Ozkanlar *et al.* (2012); Shakespeare (2012); Scott (2013). In the present study respiratory distress, nasal discharge and cough were the most common clinical signs and were noticed in 93.05%, 81.94% and 56.94% of cases respectively. Thomas (2002) who found the respiratory distress, nasal discharges, sneezing and cough were the predominantly reported in bovines with bacterial pneumonia and it may be attributed to the irritation of air ways because of infiltration of lungs. Congested conjunctival mucous membrane observed in 75% of cases which may be due to systemic illness (Rosenberger, 1979). In the present study anorexia was recorded in 90.27% of the bovine respiratory disease. Anorexia may be due to dehydration and increased levels of endotoxins due to bacterial involvement (Radostitis *et al*., 2007).

Oral breathing was observed in 52.77% cases which may be due to advanced pulmonary parenchymal disease. The prominent physical examination findings were dyspnoea, tachycardia, tachypnoea, pyrexia, crackles, wheezes. These findings were similar to observations made by earlier workers. (Thomas *et al*. (2002); Panousis (2009); Ozkanlar *et al*. (2012); Shakespeare (2012). Dyspnoea was noticed in 94.44% (Table 4) cases Thomas *et al*. (2002) observed dyspnoea in bovine respiratory diseases which may be due to anoxia and hyperapnoea. Adventitious lung sounds were observed in 70.83% cases out of which 47.05% had wheezes and 52.95% had crackles. Thayer and Robinson (1984) observed abnormal lung sounds such as crackles wheezes and increased breath sounds as the most consistent clinical findings in pneumonia cases.

They also opined that increased breath sounds generated as a result of sudden removal of barrier separating two components containing gas, and wheezes were generated by collapsed air way under tension. Thompson *et al.* (2001) observed loudest strider during inspiration phase in partial upper respiratory tract infection. The clinical signs in bovines with Bacterial pneumonia due to *Pasteurella multocida*, *Pseudomonas* spp., *Klebsiella* spp. and *E. coli* observed in the present study were in agreement with above authors.

The mean ± SE values of haematological parameters (Hb, PCV, TEC, TLC, DLC) in apparently healthy bovine were within normal range as reported by Krahiwinkel (1988). The present study revealed the values of haemogram were within normal range and highly significant

elevation in total leukocyte count and the values of neutrophils (Table 5). Though there was significant elevation observed in leukocyte count of bovine bacterial pneumonia the values were within normal range. The present findings were in agreement with Martin and Lumsden (1987); Thirunavukkarasu *et al.* (2005); Sadeghiana *et al.* (2011); Shakespeare (2012); Nittin *et al.* (2013). No significant difference was observed among monocytes, eosinophils and basophil count in infected cases when compared with control animals. The elevation in lymphocyte count observed was statistically non significant as reported by Sindhu *et al.* (2008).

The elevation in mean total leukocytes and neutrophils count with bacterial pneumoniais due to inflammation of the pulmonary parenchyma with bacterial involvement Thirunavukkarasu *et al.* (2005). In regard to the white blood cell parameters, results of present study showed significant increase in total leukocytic count which may be explained by absolute neutrophilia. Total leukocyte count was reported to increase in acute inflammatory disease particularly those due to bacterial infection. This could be attributed to that infectious agents and products of tissue injury stimulate a variety of cells to release growth factors, cytokines and other mediators of inflammation that act as prompt stimuli and or all inter related in causing the increasing in total white blood cells count and more production, proliferation, maturation and bone marrow release of mature and immature neutrophils. (Sayad *et al*., 2002). Polymorphonuclear leukocytosis represented by a neutrophilia is the stress to which the animal exposed during the course of respiratory illness that result in endogenous release of cortico steroids which have major role in regulating circulating concentration of leukocytes in moderate and severe pneumonia (EI Naser and Khamis, 2009).

The mean ± SE valuesof serum biochemical profile in apparently healthy bovine were within normal range as reported by Coles (1986); Kaneko *et al.* (2008). The present study revealed that significant elevation in total protein and AST values in bovine Bacterial pneumonia when compared to control groups. Though there was significant elevation was observed in total proteins and AST values of bovine respiratory disease, the values were within normal range. The significant reduction in albumin level observed was within normal range.

The present findings were in agreement with earlier reports. (Ezhilpraveena *et al*., 2007). Serum biochemical alterations in Bacterial pneumonia were common and might display reasonable predictable changes in response to inflammation. In this regard, the result of the present investigation revealed that there was a significant increase in serum values of total protein, globulin and a significant decrease in serum concentration of albumin. Hypoalbuminia could be due to anorexia and inability of liver to synthesise protein. (EI Seidy *et al*., 2003). Others suggested that certain bacterial toxins increase capillary permeability and permit escape of plasma proteins in tissues, so osmatic 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. (Ceron *et al*., 2005; Georgieva *et al*., 2011).

The hyperproteinimia seen in this investigation may be attributed to hyper globulinimia resulting from increased γ globulins as declared by the result of serum protein electrophoresis (EI Seidy *et al*., 2003). The significant elevation in AST levels in this investigation could be attributed to dysfunction of liver due to hepatic degenerative

and necrotic changes caused by bacterial infection and toxins (Raghib *et al*., 2004; Talkhan *et al*., 2009; Aytekin *et al*., 2011).

The prominent thoracic radiography finding of the study were consolidation and pulmonary infiltration of the lung. Thirunavukkarasu *et al*. (2006); Masseau *et al*. (2008) reported similar findings in bovine bacterial respiratory disease. In present study the radiographic appearance of the pulmonary parenchyma was interstitial pattern, bronchial pattern, alveolar pattern had been identified on thoracic radiography (Table 6).

Thoracic radiography was sensitive in identifying interstitial pattern in bovines having interstitial pneumonia, embolic pneumonia and chronic pneumonia. Identification of a bronchial pattern on radiographs is probably due to an age related change rather than a sign of bronchial disease (Masseau *et al*., 2008). In the present study, 92.5% of *Pasteurella multocida* isolates were highly (Table 7) sensitive to Ceftiofur sodium, while 87.65% to Moxifloxacin and no resistant was observed. (Kehrenberg *et al*., (2001). In the present study, the clinical cases show signs of improvement after third day of treatment in the both the groups. After fifth day of treatment clinical signs resolved and improved in physical activity in both the groups. Nasal discharges, cough, lung sounds showed marked reduction.

The animal returned to feeding and rumination. Post treatment mean values of total leukocyte count, neutrophil and total protein significantly reduced. Disappearance of *Pasteurella multocida* organisams from the blood (Table 8) after fifth day of post treatment. These findings were inaccordance with previous studies of Watts (1994); Hornish and kotarski (2002); Veena and Sumathi (2011); Kurcubic *et al*. (2013). The present study demonstrated that four diseased buffaloes in Group I and one diseased buffalo in Group II were died between 4 to 5 days of post treatment. In the study, the complete healing of eleven out of twelve buffaloes treated with Ceftiofur sodium. Based on this result, it conclude that Ceftiofur sodium can be effectively used in the treatment of bovine bacterial pneumonia. Ceftiofur sodium sensitivity was observed to all micro organisms isolated from nasal discharges samples. In the study, Ceftiofur was superior antimicrobial as compared to Moxifloxacin for the treatment of bovine bacterial pneumonia. It is very clear from the present study that the findings recorded about the antimicrobial sensitivity of *Pasteurella multocida* were in accordance to result demonstrated by previous workers (Jim *et al*., 1992; Steven *et al*., 1992; Watts *et al*., 1994; Kesler and Bechtol, 1999; Hornish and Kotarski, 2002). The improvement in clinical condition, reduction in total leukocyte count and disappearance of *Pasteurella multocida* organisams from blood in Group I animals might be attributed to bactericidal action of Moxifloxacin as reported by Patmore *et al*. (2000); Lion *et al.* (2006); Ledbetter *et al*. (2007); Govendir *et al*. (2011); Malik *et al*. (2013).

The present study demonstrated that four diseased buffaloes in Group I and only one buffalo in Group II were died between 4 to 5 days of post treatment (Table 9 and Figure 1). Response to the treatment was studied based on the clinical improvement, haematobiochemical findings, bacterial cultural and survival rate. The present study revealed improvement of clinical condition, significant decrease in total leukocyte count and neutrophil count in both treatment groups in five days post treatment when compared with pre treatment values. There was disappearance of *Pasteurella multocida* in both treatment groups of five days treatment. The present study demonstrated

Table 1. Occurrence of bovine bacterial pneumonia with relation to age, breed and gender.

Age	**No. of cases**	**Percentage**
0-6 m	17	23.61%
6-12 m	8	11.11%
1-3 years	9	12.5%
3-7 years	25	34.72%
7 years above	13	18.05%
Breed	**No. of cases**	**Percentage**
Graded Murrah	54	81.81%
Non descriptive	12	18.18%
Cross breed cows	6	8.335
Gender	**No. of cases**	**Percentage**
Male	9	12.5%
Female	63	87.53%

Table 2. Etiology of bovine bacterial pneumonia.

Name of the organism	**No. of isolates**	**Percentage**
Pasteurella multocida	31	43.05%
Pseudomonas spp.	12	16.66%
Klebsiella spp.	7	9.72%
E. coli	6	8.33%
Mixed infections	16	22.22%

Table 3. Clinical signs in bovine bacterial pneumonia.

Clinical signs	**No. of cases**	**Percentage**
Respiratory distress	67	93.05
Anorexia	65	90.27%
Nasal discharges	59	81.94%
Congestedconjenctival mucous membrane	54	75%
Muzzle dryness	42	58.33%
Cough	41	56.94%
Oral breathing	38	52.77%
Sneezing	25	34.72%
Corneal opacity	24	33.33%

Table 4. Physical examination findings in bovine bacterial pneumonia.

Physical examination findings	No. of cases	Percentage
Dyspnoea	68	94.44%
Tachycardia	59	81.94%
Tachypnoea	59	81.94%
Pyrexia	56	77.77%
Crackles	27	37.50%
Wheezes	24	33.33%

Table 5. Haemato-biochemical findings in control and bovines with bovine bacterial pneumonia.

S. No	Parameter	Diseased animals	Healthy animals
1	Hb (g/dl)	10.25±0.18	10.40±0.16
2	PCV (%)	32.5± 0.42	32.2± 0.57
3	RBC (10^6/cmm)	5.67± 0.42	5.24± 0.66
4	Total leukocyte count (10^3/cmm)	10.87± 0.27**	5.5± 0.26
5	Neutrophils (10^3/cmm)	4.29±0.16**	1.80±0.13
6	Lymphocytes (10^3/cmm)	4.5±0.18*	3.2±0.20
7	Eosinophils (10^3/cmm)	0.06±0.02*	0.02±0.01
8	Monocytes (10^3/cmm)	0.12±0.03*	0.07±0.01
9	Totalprotein (g/dl)	7.58±0.15*	7.2±0.13
10	Albumin (g/dl)	3.38±0.71	4.03±0.91
11	AST (IU/L)	71.16±0.69*	68.1±0.75

**P≤0.01 = Statistically highly significant, *P≤0.05 = Statistically significant.

Table 6. Thoracic radiographic findings in bovine bacterial pneumonia.

Radiography findings	No. of cases	Percentage
Interstitial pattern	24	46.15%
Bronchial pattern	13	25%
Alveolar pattern	7	13.46%
Pulmonary edema	8	15.38%

Table 7. Antibiogram.

S. No.	Name of the organism	No. of isolates	Antibiotic	Percentage of sensitivity
1	*Pasteurella multocida*	31	Amoxiclav	78
			Moxifloxacin	87.65
			Ceftiofursodium	92.5
			Enofloxacin	75
			Gentamicin	73
2	*Pseudomonas* spp.	12	Amoxiclav	68
			Moxifloxacin	89.5
			Ceftiofursodium	87.56
			Enofloxacin	82
			Gentamicin	76
3	*Klebsiella* spp.	07	Amoxiclav	72
			Moxifloxacin	91
			Ceftiofursodium	89.25
			Enofloxacin	85
			Gentamicin	78.26
4	*E. coli*	06	Amoxiclav	75.56
			Moxifloxacin	86.90
			Ceftiofursodium	89.25
			Enofloxacin	72.5
			Gentamicin	62.78

Table 8. Haemato-biochemical findings in treatment groups.

S. No	Parameter	Control group	Treatment Group I (moxi)		Treatment Group II (cef)	
			Pre treat	Post treat	Pre treat	Post treat
1	Hb (g/dl)	10.40±0.16	10.33±0.28	10.5±0.19	10.16±0.24	10.58±0.14
2	PCV (%)	32.2±0.57	32.75±0.57	31.25±0.57	32.25±0.62	31.1±0.56
3	Total erythrocyte count (10^6/cmm)	5.24±0.66	5.75±0.27	5.64±0.25	5.60±0.11	5.31±0.71
4	Total leukocyte count (10^3/cmm)	5.5±0.26	11.0±0.44*	5.75±0.27	10.75±0.32*	5.5±0.35
5	Neutrophils (10^3/cmm)	1.80±0.13	4.33±0.28*	2.41±0.22	4.25±0.17*	2.16±0.20
6	Lymphocytes (10^3/cmm)	3.2±0.20	4.25±0.27*	3.41±0.14	4.75±0.21*	3.25±0.17
7	Eosinophils (10^3/cmm)	0.02±0.01	0.06±0.02	0.04±0.01	0.05±0.01	0.04±0.01
8	monocytes (10^3/cmm)	0.07±0.01	0.10±0.02	0.9±0.01	0.08±0.01	0.07±0.01
9	Total proten (g/dl)	7.2±0.13	7.66±0.18*	6.83±0.24	7.5±0.26*	6.08±0.19
10	Albumin (g/dl)	3.59 ±0.91	3.66±0.84	3.72±0.99	3.27±0.1	3.45±0.1
11	AST (IU/L)	68.1±0.75	72.25±0.35*	68.3±0.51	74±0.56*	68±0.71

**P≤0.01 = Statistically highly significant, *P≤0.05 = Statistically significant.

Table 9. Survival rate between treatment groups.

Group	Total No. of cases	No. of cases survived	Percentage	No. of cases died	Percentage
I (M)	12	8	66%	4	34%
II (C)	12	11	91%	1	9%

Figure 1. Genus Specific PCR for the identification of *Pasteurella* spp by KMT1 gene ampliflication

M - Molecular weight marker
1 - Sample No.1
2 - Control
3 - Sample No.23
4 - Sample No.2
5 - Sample No.15
6 - Sample No.21

that four diseased buffaloes in Group I and only one buffalo in Group II were died between 4 to 5 days of post treatment. Improvement in clinical condition, disappearance of *Pasteurella multocida* from the blood and survival rate were used to demonstrate the therapeutic efficacy of two treatment groups.

Bovine bacterial pnemonia associated with *Pseudomonas* spp., *Klebsiella* spp., and *E. coli* treated with sensitive antibiotics gave satisfactory results.

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