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Study on diagnosis and prognosis of bacterial respiratory disease in buffalo calves

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Abstract

The study revealed that occurrence of Bacterial respiratory disease in buffalo calves was 21.25% per cent. Cultural isolation and identification of nasal discharges from diseased calves 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 anemia, leukocytosis with neutrophilia and lymphopenia. Serum biochemical parameters showed hike in ALT, ALP, TP and fall in albumin. Thoracic radiography revealed consolidation of lungs. The electrocardiographic, echocardiographic examination and cardiac biomarkers revealed cardiac function was normal in buffalo calves with bacterial respiratory disease. Prognosis of bacterial respiratory disease was satisfactory.

Keywords: Bacterial respiratory disease, diagnosis, prognosis and buffalo calves

Introduction

Bovine respiratory disease constitutes one of the key health issues and most costly disease occurring in calves in all parts of the world. 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. The success of any dairy enterprise depends upon the survival of the calf produced. Bacterial respiratory disease in calves is a multifactorial disease complex involving interaction between a wide range of pathogens, immune status and environmental stress. 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* are most commonly reported (Godinho *et al.*, 2007). Current data on the prevalence and relative importance of different bovine respiratory pathogens in preweaned dairy calves was scant and available data usually from large dairy farms. The diseased calves usually exhibit clinical signs of acute respiration distress, although, in some cases sudden death appear before the development of significant pulmonary lesions. Some of the pathogens are normal inhabitant of respiratory tract of buffalo calves. However, any activity that is precipitating stress leads to clinical disease conditions. The diagnosis of bovine respiratory disease in calves poses significant challenges to the clinicians. 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 (Metwally *et al.*, 2017). Haematological picture of Bovine respiratory disease affected calves characterized by change in blood Hb, RBCs, PCV, Total WBCs and DLC count. Severe Biochemical alteration 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. Although various forms of calf respiratory diseases are known, their clinical manifestation is frequently less differentiated. Despite the great variety of combination of pathogens that have been implicated as the causes of this disease, the clinical entity of most manifestation is bronchopneumonia. Calves typically face two primary problems that contribute a high incidence of bacterial respiratory disease. First, stress associated with weaning and transportation has a negative effect on calf immune system. Second, this stress typically occurs when the animal is exposed to a variety of infectious agents as a result of marketing management producers. Nutrition can also interact with these two factors, most likely as a result of preweaned nutritional deficiencies or through decreased feed intake associated with stress. Less systematic work has been done on Bacterial respiratory disease in buffalo calves in Andhra Pradesh particularly on etiological, diagnosis and prognosis aspects. Hence the present research work was undertaken with the following objectives

- To identify the bacterial etiology of respiratory disease in buffalo calves.
- To study the occurrence of bacterial respiratory disease in buffalo calves
- To record the clinical signs, haemato-biochemical changes, and thoracic radiographic findings in buffalo calves with bacterial respiratory disease.
- Prognostic evaluation of bacterial respiratory disease in buffalo calves with thoracic ultrasonography
- To diagnose cardiac insufficiency in buffalo calves with bacterial respiratory diseases using electrocardiography, thoracic radiography, echocardiography, and cardiac biomarkers.

Materials and methods

The study comprised apparently healthy buffalo calves and clinical cases. Ten apparently healthy buffalo calves were selected as control group for obtaining normal data for comparison of parameters under study. The study was conducted in 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 with clinical signs suggestive of bovine respiratory disease were screened by using specially designed data sheet and subjected to detailed clinical examination, haematology, serum biochemical profile, radiography, cultural studies of nasal discharges and blood to confirm the bovine bacterial respiratory disorders.

Clinical material

Clinical samples collected for the present study included nasal discharges, whole blood and serum.

Laboratory material

All the dehydrated media used in the present study were obtained from Hi-media Laboratories Pvt. Ltd., Mumbai, chemicals from S D Fine Chemicals Ltd, Mumbai.

Methods

History and clinical examination The buffalo calves that were presented with the symptoms suggestive of respiratory disease viz anorexia, pyrexia, cough, nasal and ocular

discharges, congested mucous membranes, depression and open mouth breathing were subjected for detailed clinical examination. The history related to age, gender and breed was recorded on a specially designed case sheet (Appendix I). The blood, serum and nasal swabs were collected from the suspected calves and were subjected to further detailed laboratory examination.

Clinical illness scoring in the present study, the clinical symptoms in buffalo calves affected with respiratory diseases were scored as per the method suggested by Love *et al.* (2014) as follows.

Clinical sign	Score
Coughing (induced or spontaneous coughing)	2 points
Nasal discharge (any discharge)	3 points
Ocular discharge (any discharge)	2 points
Ear and head carriage (ear droop or head tilt)	5 points
Fever (≥ 39.2 °C or 102.5 °F)	2 points
Respiratory quality (abnormal respiration).	2 points

Calves were categorized "BRD positive" if their total score is ≥ 4 .

Collection of blood samples Blood and serum samples were collected aseptically from healthy animals and clinical cases for carrying out different laboratory and haemato-biochemical examination by standard tests. Blood samples were collected aseptically using sterile EDTA vacuutainers from jugular vein and transported on ice to the laboratory. Blood smears were prepared and fixed with methanol.

Five ml of blood was collected into a sterilized test tube for bacterial isolation

Separation of serum Approximately 5 ml of blood was collected in safe lab clot accelerator for biochemical analysis. Collected serum samples were kept in a slant position with an angle of 20-30 degrees and keeping it undisturbed for 2-3 hrs. Then they were subjected to centrifugation @ 2000 rpm for 15 minutes and serum was separated and stored in Eppendorf tubes at -20 °C until further assay.

Collection of nasal discharges

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

Preparation of glassware For 24 hours, all the glassware used in this analysis was dipped in 1 per cent hydrochloric acid, then washed under running tap water, soaked again for 24 hours in teepol solution, cleaned with brush and washed under running water. The glassware was then rinsed in glass distilled water and dried. Then carefully packed and sterilized at 160°C for one and half hour. The sterilized glassware were used for cultural and biochemical analysis.

Preparation of media

All media obtained as dehydrated powders were rehydrated by adding distilled water to dissolve as directed by the manufacturer. The media were then sterilized at 15 lb of pressure for 15 minutes by autoclaving. The blood agar was prepared in the laboratory as per the procedure described by Quinn *et al.* (1999).

Isolation of bacteria

Nasal swabs collected from calves affected with Bovine respiratory diseases were inoculated into TSB broth and incubated at 37°C for 24 hours aerobically. Then a loopful 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, Nutrient agar, Blood agar, Eosine-Methylene-Blue agar. For isolation of *Streptococcus spp* the nasal swabs were inoculated into the Streptococcus selective broth and incubated at 37°C in an anaerobic jar for 24 hours. After incubation in SSB, the broth was examined for the presence of *Streptococcus spp* by gram staining and further inoculated onto Streptococcus selection agar.

Identification of bacteria

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

Gram's staining

On a clean microscopic slide bacterial smears were prepared by mixing 24 hour old cultures with sterile saline. The smear was then air dried, heat fixed over flame and then stained with Gram's stain and examined under oil immersion. The bacteria were identified as Gram positive or Gram negative, cocci, bacilli or coccobacilli.

Motility test by hanging drop technique

A drop of 6-8 hour old broth cultures was placed on a cover slip and the cover slip was then placed upside down on a microscopic slide containing a circular depression. Then the motility of bacteria was examined under high power. This technique is known as hanging drop technique.

Catalase activity

This was tested by placing a loopful of bacterial colony on microscopic slide and then mixed with a drop of three percent hydrogen peroxide solution. Production of gas bubbles after few seconds indicated the bacteria as catalase positive whereas absence of gas bubbles was taken as negative.

Cultural characters on blood agar

Blood agar plates containing 5-10 per cent of defibrinated sheep blood were used for this test. The bacteria were grown on these plates and the type of haemolysis was recorded after 24 hours of incubation at 37°C. The appearance of zones of clear circumscribed areas around the colonies was taken as indication of occurrence of haemolysis.

Indole test

The medium was inoculated with the culture and incubate for 48 hours at 37°C. After incubation 0.5 ml of Kovacs reagent was added and shaken gently. A red colour indicated a positive reaction

Methyl Red (Mr) Test

The medium was inoculated from a young agar slope culture and incubated at 37°C for 48 hr. Five drops of the methyl red reagent was added, mixed and read the results immediately. Positive results are bright red and negative results are yellow.

Voges -Proskauer (VP) test

The test was done to detect the acetone production of the organisms by growing in MR-VP medium incubated at 37°C for 48 hrs add 1 ml of 40 per cent potassium hydroxide and 3 ml of 5 per cent solution of alpha Naphthol in absolute

Ethanol. A positive reaction is indicated by the development of pink colour in 2-5 minutes, becoming Crimson in 30 minutes. The tube can be shaken at intervals to ensure maximal aeration. A uniform dark brownish colour was considered as negative.

Citrate utilization test

The bacterial use of citrate was tested on simon's citrate agar slants. A light suspension of the test organisms in sterile saline was inoculated in linear streaks in citrate agar slants. The slants were then incubated at 37°C for 48 hours. Growth associated with a colour change to Prussian blue from green colour was regarded as positive where as a green colour media was considered as negative.

Nasal discharges culture

For isolation of bacteria, the nasal discharges were streaked on blood agar, nutrient agar, streptococcus selection broth and MacConkey agar. As per standard methods, culture plates were incubated. Presumptive and definitive identification of pathogens were done by staining characteristics, colony morphology and standard biochemical tests (Barrow and Feltham 1993 and Quinn *et al.*, 1999) [17].

Haematology

Five ml of venous blood was collected in two vacutainer tubes containing K3 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)

Molecular characterization of bacterial isolates

The TSB medium was inoculated with the isolates and incubated at 37°C for 16 to 18 hr and overnight cultures adjusted to 0.5 McFarland standards were used for DNA isolation. Deoxyribonucleic acid (DNA) from bacterial cell mass was extracted by the high salt method of DNA extraction (Aravindakshan *et al.*, 1997) [8] with suitable modifications (Anand Kumar, 2009) [6]

Quality and purity of extracted DNA was checked by agarose gel electrophoresis. The concentration of the DNA obtained was measured on ethidium bromide stained agarose gel by comparing with different concentrations of DNA marker and the DNA extracted was used as template in PCR (Anand Kumar, 2009) [6].

PCR and Detection of PCR Products

The PCR tests were carried out in Proflex PCR system, Applied Biosystems. All the reactions were carried out in a volume of 25 µl in 0.2 ml PCR tubes. The composition of Master Mix (2X) is shown in Table 6. The PCR amplicons were analysed by electrophoresis on 1.7 percent agarose gel stained with 0.5 µg of ethidium bromide/1 ml in Tris-Borate EDTA (TBE) buffer. Electrophoresis was carried out at 70V for 50 min in submarine gel electrophoresis unit (BIORAD, UK) and the PCR products were visualized in BIORAD molecular imager XR+. The sizes of PCR products were verified by comparison with quantitative DNA ladder (SRL, Mumbai). Negative control with sterile distilled water was maintained in each PCR. *Staphylococcus* species and *Streptococcus* species were detected using genus specific oligonucleotide primers and *E. coli* was detected using species specific oligonucleotide primers in PCR. Oligonucleotide primers and Thermal cycling conditions

required for detecting the genes responsible for molecular characterization of *Staphylococcus spp*, *Streptococcus spp* and *E. coli*

Table 1; Composition of the PCR Reaction Mixture and Final Concentrations of Reagents

S. No	Reagents	Quantity (µl)	Final concentration
1	2X master mix (Promega)	12.5	2x
2	Forward primer	1.25	20 pmole/ µl
3	Reverse primer	1.25	20 pmole/ µl
4	DNA template	3	150 ng
5	Distilled water	7	---
	Total	25.0	---

Table 2: Oligonucleotide primers and thermal cycler conditions of PCR for detection of *Staphylococcus*, *Streptococcus* and *E. coli*.

Primer	Sequence	Product size	Initial denaturation		Denaturation		Annealing		Extension	
			Temp	Time	Temp	Time	Temp	Time	Temp	Time
Str I&II	F- GTA CAG TTG CTT CAG GAC GTA TC R- ACG TTC GAT TTC ATC ACG TTG (Picard <i>et al.</i> , 2004)	197 bp	94 °C	3 min	94 °C	30 sec	60 °C	30 sec	72 °C	30 sec
16SrRNA f 16SrRNA r	F-GTA GGT GGC AAG CGT TAT CC R-CGC ACA TCA GCG TCA G (Riffon <i>et al.</i> , 2001)	224 bp	94 °C	2 min	94 °C	45 sec	50.2 °C	1 min	72 °C	2 min
Eco (Eco 2083 & Eco 2745)	F- GCT TGA CAC TGA ACA TTG AG R- GCA CTT ATC TCT TCC GCA TT (Riffon <i>et al.</i> , 2001)	662 bp	94 °C	2 min	94 °C	45 sec	57 °C	1 min	72 °C	2 min

Serum biochemistry

Serum biochemical parameters like serum total protein (g/dl), AST(U/L), ALP (IU/L), albumin (g/dl), total bilirubin (mg/dl), BUN (mg/dl) and creatinine (mg/dl) were estimated by using a Thermo Scientific Instrument (Multiskan GO Analyser) using standard kits manufactured by M/S Erba-Mannheim Pvt. Ltd., Mumbai.

Cardiac biomarkers

Qualitative measurement of cardiac troponinI and estimation Creatin kinase MB were done as per the standard methods.

Radiography

Thoracic radiography in affected calves was performed as described by Masseau *et al.* (2008).

Electrocardiography

Electrocardiography in affected calves' war performed by standard method.

Echocardiography

Echocariography in affected calves was performed by standard method.

Statistical analysis

Data collected were subjected to statistically analysis as per described by Snedecor and Cochran (1994)

Results and discussion

The occurrence of respiratory disease in buffalo calves was observed to be 21.25 per cent. Investigation on age wise occurrence of bacterial respiratory disease revealed that buffalo calves of 0-2 weeks (55.88%) of age group were most commonly affected. Suckling calves had an increased risk of death during the first week of life. Although the presence of dam has been found to increase the amount of IgG absorbed, calves left to suckle their dams often failed to ingest adequate volume of colostrums and were prone to increased risk of diarrhea and respiratory disease (Fanklin *et al.*, 2003 and

Svensson *et al.*, 2003). In the present study Graded murrah buffalo calves (72.54%) were most commonly affected with bacterial respiratory disease followed by non-descript calves (27.45%). The percentage of occurrence in Graded murrah buffalo calves might be related to their number in hospital attendance and probably rearing Graded murrah buffloes for milk production in large numbers. The present study revealed that female buffalo calves (85.29%) were most commonly affected than male buffalo calves (14.7%). Hence it was opined that female predominance in present study due to rearing of female buffalo calves for milk production in large numbers. 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 the percentage of 29.62 per cent followed by *E.coli* (25.92%), *Streptococcus spp* (18.51%), *Klebsiella spp* (11.11%), *Staphylococcus spp* and *Pseudomonas spp* each (7.40%). Mohammadi *et al.* (2006) and Van Driessche *et al.* (2017) also reported that *Pasteurella multocida* was most predominant bacteria isolated. The findings were in agreement with previous studies (Mohammadi *et al.*, 2006; Svensson *et al.*, 2006 and Enany *et al.*, 2012). *Streptococcus* isolates were confirmed by PCR. The amplification of DNA of *Streptococcus spp* was at 197 bp. The findings were in agreement with earlier reports of Picard *et al.* (2004). *Staphylococcus* isolates were confirmed by PCR. The amplification of DNA of *Staphylococcus* was at 228 bp. The findings were in agreement with previous findings 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* was at 662 bp (Riffon *et al.*, 2001). 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 were in agreement with earlier studies of Aslan *et al.* (2002)^[9], McDanel *et al.* (2018), Sharma *et al.* (2011), Currin and Whittier (2000)^[26],

Griffin (2010), Richeson *et al.* (2013) and Allen *et al.* (1991) [2]. 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) [10]. Marked dyspnea with open mouth breathing was observed in 11.11 per cent of cases might be due to advanced pulmonary parenchymal destruction. The observed nasal discharges and ocular discharges was 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. These findings were similar to the observation made by previous researchers (Aslan *et al.*, 2002; Radostits *et al.*, 2007 and Griffin, 2010) [9]. 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). The observed nasal discharges might be also attributed to inflammatory changes in the nasal mucus membrane. In bacterial respiratory disease affected calves, heart rate was increased to compensate the hypoxia (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 (Metwally *et al.*, 2017 and Kumar *et al.*, 2018). The clinical signs and physical examination findings in buffalo calves with respiratory disease due to *Pasteurella multocida*, *E.coli* spp, *Streptococcus* spp, *Klebsiella* spp, *Staphylococcus* spp and *Pseudomonas* spp observed in present study were in agreement with the reports of previous authors (McDanel *et al.*, 2018; Autio *et al.*, 2007 [11]; Griffin 2010; Sharma *et al.*, 2011; Shakespeare 2012; Richeson *et al.*, 2013; Allen *et al.*, 1991; Joshi *et al.*, 2016 and Reddy *et al.*, 2018) [2,10]. The mean $\pm$ SE values of haematological parameters (Hb, PCV, TEC, TLC, DLC) in apparently healthy buffalo calves were within normal range as reported by Krahwinkel (1988). Similar to previous studies the haematological examination of the bacterial respiratory disease affected calves revealed significant decrease in RBC count, PCV and Hb (Sadeghiana *et al.*, 2011; Ismael *et al.*, 2017 and Ramadan *et al.*, 2019). These results in diseased calves might be attributed to micro- hemorrhages 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). Anemia 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 hemoglobin synthesis leading to inhibition of erythropoiesis (Aytekin *et al.*, 2011) [15]. 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. These results gained support from the findings of Bednarek *et al.* (2003) [20], Thirunavakkarasu *et al.* (2005), 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 and Akgul *et al.*, 2019) [12]. The significant increase in WBCs and neutrophils occurred in pneumonia might be attributed to inflammatory lesions and presence of bacterial infection (Sayed *et al.*, 2002 and Smith., 2014). On the other hand, the significant decrease of lymphocytes might be attributed to stimulation of adrenal gland with the tissue invaded by bacterial toxins or might be resulted from immune suppression as a result of various stress conditions (El-Naser and Khamis (2009) and Aytekin *et al.*, (2011). [14]. No significant difference ($P>0.05$) was observed among eosinophil concentration in infected calves when compared with control group. The mean $\pm$ SE values of serum biochemical profile in apparently healthy buffalo calves were within normal range as reported by Kaneko *et al.* (2008). The present study revealed that significant elevation in total protein, ALP and AST values in bovine respiratory disease when compared to control groups. Though there was significant elevation in total bilirubin and creatinine values of bovine respiratory disease, the values were within normal range. The present findings were in agreement with earlier reports (El-Bahr and El-Deeb 2013; Almujaalli *et al.*, 2015 [14]; Soltesova *et al.*, 2015; Kumar *et al.*, 2018; Kumar *et al.*, 2018 and Ramadan *et al.*, 2019). The result of the present investigation revealed that there was a significant increase in serum values of total protein and a significant decrease in serum concentration of albumin. High level of total protein in blood of the diseased calves and increase 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 (EI seidy *et al.*, 2003; Evans *et al.*, 2003; Kovacic *et al.*, 2017; and Ramadan *et al.*, 2019). Hypoalbuminemia could be due to anorexia and inability of liver to synthesize protein (Vestweber *et al.*, 1990; EI seidy *et al.*, 2003; Sadeghian *et al.*, 2011; and 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. (Ceron *et al.*, 2005 [25]; Eckersall and Bell, 2010 and Georgieva *et al.*, 2011) [24]. 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 (Raghib *et al.*, 2004 and Aytekin *et al.*, 2011) [15]. The prominent thoracic radiographic findings were consolidation, pulmonary infiltration, pulmonary edema of the lung Masseau *et al.* (2008) reported similar clinical findings in bacterial respiratory disease in buffalo calves. In the present study the radiographic appearance of pulmonary parenchyma was interstitial pattern, bronchial pattern and alveolar pattern had been identified on thoracic radiography. Thoracic radiography was a sensitive in identifying interstitial pattern in buffalo calves having interstitial pneumonia, embolic pneumonia and chronic pneumonia. Identification of bronchial pattern on radiography was probably due to age related changes rather than a sign of disease (Masseau *et al.*, 2008). In the present study 29.62 per cent of *Pasteurella* spp, 25.92 per cent of *E.coli*, 18.51 per cent of *Streptococcus* spp, 11.11 per cent of *Klebsiella* spp, 7.40 per cent of *Staphylococcus* spp and 7.40 per cent of *Pseudomonas* spp isolates.

Table 3: Occurrence of bacterial respiratory disease in buffalo calves with relation to breed, gender and age. (n= 102)

Breed	No. of cases	Per centage
Graded murrah	74	72.54
Non descriptive	28	27.45
7Gender	No. of cases	Per centage
Male	15	14.70
Female	87	85.29
Age	No. of cases	Per centage
0-2 weeks	62	55.88
2weeks-2 months	24	19.60
2-3 months	14	12.74

Table 4 : Etiology of Bacterial Respiratory Disease In Buffalo Calves N=102

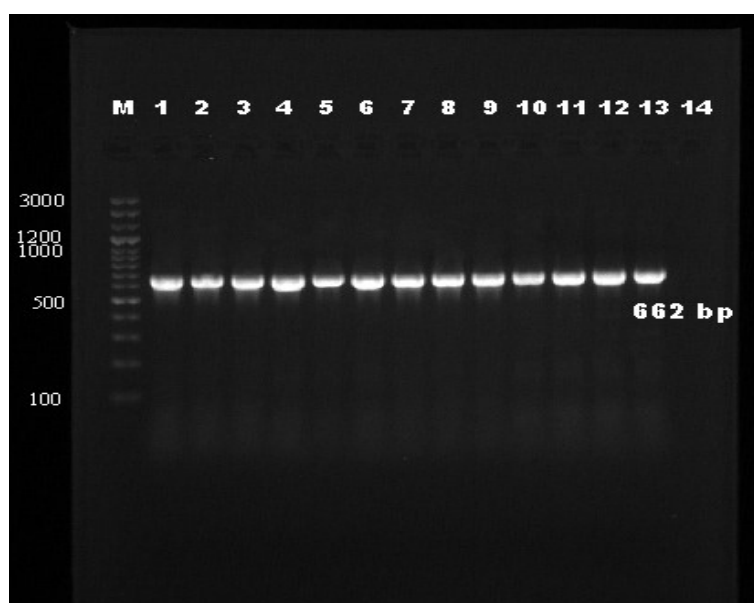
Name of the organism	No. of isolates	Percentage
<i>Pasteurella spp</i>	18	17.77
<i>E.coli</i>	20	20.00
<i>Streptococci spp</i>	18	17.77
<i>Klebsiella spp</i>	13	12.22
<i>Staphylococci spp</i>	19	18.88
<i>Pseudomonas spp</i>	14	13.33

Table 5: Clinical signs in bovine respiratory disease with bacterial involvement in buffalo calves (n=54)

Clinical signs	No. of cases	Percentage
Anorexia	54	100.00
Pyrexia	54	100.00
Depression	46	85.18
Nasal and ocular discharges	38	70.37
Congested conjunctival mucus membrane	36	66.66
Cough	8	14.81
Open mouth breathing	6	11.11

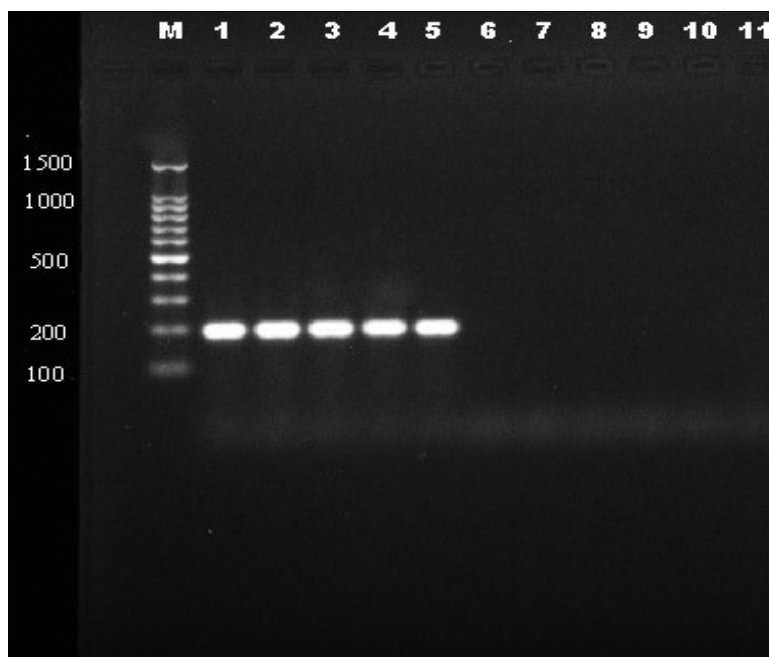
Table 6: Physical examination findings in bovine respiratory disease with bacterial involvement (n=54)

Physical examination findings	No. of cases	Percentage
Tachypnoea	49	90.74
Tachycardia	46	85.18
Crackles	42	77.77
Wheezes	26	48.14
Dyspnoea	8	14.81



M- Molecular weight marker
 1- Sample +ve 8- Sample -ve
 2- Sample +ve 9-Sample -ve
 3- Sample +ve 10- Sample -ve
 4- Sample +ve 11-Sample -ve
 5- Sample +ve 12-Sample -ve
 6-Sample -ve 13-Sample -ve
 7-Sample -ve 14-Negative control

Fig 1: PCR amplification of Streptococcus species



M- Molecular weight marker
 1- Sample +ve 8- Sample +ve
 2- Sample +ve 9-Sample +ve
 3- Sample +ve 10- Sample +ve
 4- Sample +ve 11-Sample +ve
 5- Sample +ve 12-Sample +ve
 6-Sample +ve 13-Sample +ve
 7-Sample +ve 14-Negative control

Fig 2: PCR amplification of E.col



M- Molecular weight marker
 1- Sample +ve 8- Sample -ve
 2- Sample +ve 9-Sample -ve
 3- Sample +ve 10- Sample -ve
 4- Sample +ve 11-Sample -ve
 5- Sample -ve 12-Sample -ve
 6-Sample -ve 13-Sample -ve
 7-Sample -ve 14-Negative contro

Fig 3: PCR amplification of *Staphylococcus species*

Table 7: Hematobiochemical findings in control and calves affected with Bovine respiratory disease.(n=102)

S.no	Parameter	Healthy animals (n=10)	Diseased animals (n=102)
1	Hb (g/dl)	11.0±0.46**	7.65±0.21
2	PCV (%)	31.50±1.70**	25.57±2.01
3	TEC(10 ⁶ /cmm)	7.42±0.55*	3.93±.65
4	TLC(10 ³ /cmm)	8.03±0.66**	12.89±0.89
5	Neutrophils (%)	40.60±2.00**	68.78±1.35
6	Lymphocytes (%)	58.60±1.90**	32.54±1.41
7	Eosinophils (%)	1.40±0.58	1.58±0.92
8	BUN (mg/dl)	32.90±1.7	38.94±2.08
9	Creatinine (mg/dl)	0.90±0.4*	1.02±.030
10	AST(IU/L)	64.80±3.05**	148±23
11	ALP(IU/L)	91.20±3.6**	125±8.4
12	Albumin (g/dl)	3.26±0.27**	2.89±1.02
13	Total protein (g/dl)	5.90±0.52**	7.30±1.74
14	Total bilirubin (mg/dl)	0.45±0.15*	0.68±0.31

P<0.01 Statistically highly significant

P<0.05 Statistically significant

Table 8: Thoracic radiographic findings in buffalo calves with bacterial respiratory disease

Radiographic findings	No of cases	Percentage
Interstitial pattern	16	66.66
Bronchial pattern	4	16.66
Alveolar pattern	4	16.66

Conclusion

- The *Pasteurella spp* were the most predominant bacteria isolated at the percentage of 17.77% per cent followed by *E.coli* (20%), *Streptococcus spp* (17.77%), *Klebsiella spp* (12.22%), *Staphylococcus spp* 18.88% and *Pseudomonas spp* (13.33%). with respiratory bacterial diseases.
- Echocardiographic and electrocardiographic examination revealed cardiac function is normal in buffalo calves with respiratory bacterial disease.
- Cardiac biomarkers cardiac troponin I and cardiac troponin T were found to be normal upon qualitative estimation
- Prognosis of bacterial respiratory diseases was satisfactory if identified early and treated accordingly

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