CLINICO - THERAPEUTIC STUDIES ON BACTERIAL INFECTIONS OF RESPIRATORY TRACT IN BOVINES

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Thesis submitted in partial fulfillment of the requirement for the degree of

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DECLARATION

I hereby declare that this thesis entitled **"CLINICO-THERAPEUTIC STUDIES ON BACTERIAL INFECTIONS OF RESPIRATORY TRACT IN BOVINES"** is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title, of any other University or Society.

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Certified that the thesis entitled **"CLINICO-THERAPEUTIC STUDIES ON BACTERIAL INFECTIONS OF RESPIRATORY TRACT IN BOVINES"** is a record of research work done independently by **Dr. Siji. P.C.**, under my guidance and supervision and that it has not previously formed the basis for the award of any degree, fellowship or associateship to her.

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Introduction

1. INTRODUCTION

Respiratory tract infection has been extensively documented as the most economically important health problem in humid tropics. Ordinarily majority of the cattle diseases (40-80 per cent) involve the respiratory system. The high morbidity rate of respiratory disease causes tremendous economic losses to the marginal farmers in India. Bacterial infections of respiratory tract contribute a major fraction of the bovine respiratory disease complex, a syndrome of complex etiology and are usually the result of a combination of environmental and managemental factors along with infectious agents.

The bovine respiratory disease complex consists of three important clinical entities viz. (1) enzootic pneumonia, (2) shipping fever complex, (3) atypical or hypersensitivity pulmonary diseases. The enzootic pneumonia occurs in young calves of less than six months of age in enclosed crowded condition, high humidity and with inadequate ventilation. Shipping fever complex are the acute diseases of adult life in dairy, feedlot or cow-calf operations. Atypical or hypersensitivity pulmonary diseases have also found to play a significant role in causing mortality in bovines.

The increase in frequency and economic impact of bovine respiratory disease complex can be correlated with the escalating industrialization of cattle production. In intensive cattle operations animals from multiple sources, exposure to many organisms, stress and management practices are the risk factors that can lead to disease. Beef cattle with low functional pulmonary hardiness are more prone to such infections. Many workers have evaluated the bacterial pathogens of bovine respiratory disease worldwide and isolated a number of bacterial organisms like pasteurella, haemophilus, streptomyces, bacillus, neisseria, pseudomonas, streptococcus, staphylococcus, salmonella, moraxella, actinomyces, corynebacterium, klebsiella and *Escherichia coli* as etiological agents. However perusal of literature indicated that there is a paucity of sufficient information on the possible bacterial etiology and the effect of antimicrobials and

antibiotic sensitivity test on bacteria present in the airways of bovines suffering from respiratory disease.

The bacterial organisms present in respiratory disease either as primary or secondary infections necessitate an effective antimicrobial to be selected and administered as rapidly as possible. The most common and precise technique required for this judicious treatment requires the identification of bacterial etiology and determination of the antibiotic sensitivity of these isolates. However the random selection and indiscriminate use of antibiotics in the treatment of respiratory tract infection has lead to the development of resistance to most of the present day antibiotics, which warrants the use of newer therapeutic agents for effective treatment.

The present study envisages the use of two antibiotics viz. enrofloxacin and florfenicol in the treatment of upper respiratory tract infection. Enrofloxacin, a flouroquinolone is a synthetic broad-spectrum bactericide which acts by irreversible inhibition of bacterial enzyme DNA-gyrase (Topoisomerase) which is important in the supercoiling and in spatial arrangement of DNA. Florfenicol, fluoroanalogue of thiamphenicol is recommended as a drug of choice in respiratory tract infection in bovines. It is a synthetic broad-spectrum bacteriostat which acts by binding to the 50S ribosomal subunit and inhibiting bacterial protein synthesis. Taking into consideration the importance of upper respiratory infections this study was undertaken with the following objectives

1. The assessment of the comparative efficacy of enrofloxacin (Gyroflox) and florfenicol (Nuflor®) in bovine respiratory tract infections.

2. To study the clinical and haematological parameters in respiratory tract infections.

3. To isolate different bacterial species from respiratory tract infection of bovine.

4. To determine the *in vitro* antibiotic sensitivity tests of the bacterial isolates.

Review of Literature

2. REVIEW OF LITERATURE

2.1 ETIOLOGY

Rosenbusch and Merchant (1939) named the typical organism responsible for haemorrhagic septicaemia as *Pasteurella multocida* after comparing all the earlier names assigned to this bacterium.

Davies (1955) reported that acute bronchopneumonia is usually caused by bacterial species like pasteurella, streptococcus, haemophilus and corynebacterium.

Shipping fever caused by *Pasteurella multocida* is primarily a respiratory pathogen and stress factors act as an important predisposing factor causing disease varying from a mild to rapidly fatal pneumonia (Galloway, 1972).

Allan (1977) isolated 52 bacterial spp. from 92 pneumonic lungs, which were *Mannheimia haemolytica* (17.40 per cent), *Pasteurella multocida* (11.50 per cent), *Corynebacterium pyogenes* (9.60 per cent) and *Staphylococcus aureus* (7.60 per cent).

Gilmour (1977) postulated that pasteurella organisms being a normal inhabitant in the upper respiratory tract of clinically normal animals and the interference in the clearance of these organisms from lung predisposes the animals to pasteurella pneumonia.

Mannheimia haemolytica and *Pasteurella multocida* are widely accepted as primary lung pathogens causing pneumonia in calves (Selman, 1981).

Srivastava and Uppal (1985) first isolated *Mycoplasma bovigenitalium* from respiratory tract of buffaloes in India.

Charan *et al.* (1986) isolated chlamydia from 109 clinical cases of respiratory disorders in young calves.

Mannheimia haemolytica biotype A serotype 1 (AST 1) was the serotype most frequently isolated from pneumonic lungs and the nasal passage of cattle with acute respiratory disease (Frank, 1986).

Frank (1988) studied the colonization of nasopharynx with *Mannheimia haemolytica* ST 1 in conditions of stress, respiratory disease induced by viruses and during change in climate leading to pneumonic pasteurellosis.

Mannheimia haemolytica plays pivotal role in the pathogenesis of shipping fever by inducing pneumonia and is mainly attributed to the production of ruminant specific leukotoxin (Wilkie and Shewen, 1988).

Allen *et al.* (1991) isolated *Pasteurella multocida* (40), *Mycoplasma bovis* (36), *Mycoplasma bovirhinis* (19), *Mannheimia haemolytica* (4), *Haemophilus somnus* (7), streptomyces (7), bacillus (1) from broncho-alveolar lavage cultures and *Pasteurella multocida* (41), *Mycoplasma bovis* (27), *Mycoplasma bovirhinis* (14), neisseria (11), *Mannheimia haemolytica* (9), and *Haemophilus somnus* (7) from nasopharyngeal swabs of 59 calves with respiratory disease.

Mycoplasma and acholeplasma were isolated from 69 bovines with respiratory tract infection and found that the occurrence of infection in normal animals was quite low (Pal and Singh, 1994).

Katoh *et al.* (1996) isolated 140 *Pasteurella multocida*, 23 *Mannheimia haemolytica*, 137 *Mycoplasma bovis*, 76 *Mycoplasma bovirhinis* and 104 *Ureaplasma diversum* from 271 of 329 nasal swabs of bovine pneumonic cases. Among the 271 cases 38.7 per cent were of mixed infection with pasteurella and mycoplasma and / or *Ureaplasma diversum*.

Presence of *Mannheimia haemolytica, Pasteurella multocida, Haemophilus somnus, Pseudomonas aeruginosa, Escherichia coli*, streptococci, staphylococcus, salmonella and moraxella spp. were reported in the respiratory disease complex of cattle, sheep and goat (Wikse and Baker, 1996).

Nasser and El-Sayed (1997) made 14 isolations of pasteurella, 13 isolations of haemolytic staphylococci, 14 haemolytic streptococci and 9 *Escherichia coli* from 20 calves exhibiting acute signs of bovine respiratory disease from Italy.

Roberson *et al.* (1997) reported that apart from bacterial pneumonia, manifestations of respiratory distress was also noticed in L-tryptophan toxicosis, hypersensitivity pneumonitis, fusarium pneumotoxicosis, perillament toxicosis, bovine respiratory syncytial virus infection and 4-ipomeanol toxicosis (mouldy sweet potato poisoning).

Sahin (1997) isolated 12 mycoplasma, 15 *Escherichia coli*, 11 *Mannheimia haemolytica*, 8 *Pasteurella multocida*, 8 staphylococci, 7 streptococci, 3 bacilli and 1 actinomyces from 109 pneumonic lungs of cattle.

Gunduz and Erganis (1998) isolated 48 *Mannheimia haemolytica* and 52 *Pasteurella multocida* from 340 pneumonic lung samples in Turkey.

Mannheimia haemolytica, Pasteurella multocida, Haemophilus somnus, Corynebacterium pyogenes, salmonella spp. and *Escherichia coli* were isolated in an outbreak of respiratory disease among feedlot calves in Egypt (Selim *et al.*, 1998).

Aguade and Romero (2000) isolated 34 strains of *Pastuerella multocida,* 31 *Mannheimia haemolytica* and 11 *Haemophilus somnus* from pneumonic lungs of dairy calves in California.

Evan's multifactorial nature of shipping fever pneumonia was elucidated by isolating pastuerella spp., and respiratory bovine coronavirus from 26 cattle that died during two severe epizootics of pneumonia in USA (Storz *et al.*, 2000).

Pasteurella multocida (45), *Mannheimia haemolytica* (37), *Arcanobacterium pyogenes* (6), *Pasteurella aerogenes* (1) and *Haemophilus*

somnus (1) were isolated from nasal swabs taken from cattle with clinical signs of bronchopneumonia in Germany (Hutt and Goossens, 2001).

Lonaragan *et al.* (2001) isolated 2 and 7 *Mannheimia haemolytica,* 12 and 13 *Pasteurella multocida* and 2 and none *Haemophilus somnus* respectively from lungs of 108 cases of acute interstitial pneumonia and 50 cases of bronchopneumonia from 14 feedlot cattle in the Western United States.

Rowe *et al.* (2001) reported that *Mannheimia haemolytica* serotype A1, and A2 survived 156 days in bovine tracheo-bronchial washings and the survival mechanisms had direct implications on pathogenesis.

Aslan *et al.* (2002) isolated 25 per cent *Mannheimia haemolytica,* 20 per cent *Klebsiella pneumonia,* 15 per cent *Arcanobacterium pyogenes,* 10 per cent haemolytic streptococci, 5 per cent staphylococci, 5 per cent *Escherichia coli,* 5 per cent penicillium spp., 5 per cent aspergillus spp. and 10 per cent yeast from 27 calves suffering from respiratory tract infection in Turkey.

Pasteurella multocida (68 per cent), *Mycoplasma bovirhinis* (14 per cent), *Mycoplasma bovis* (11 per cent), *Haemophilus somnus* (3 per cent) and alpha haemolytic streptococci (3 per cent) were isolated from 35 samples of 80 tracheal washings of calves suffering from acute respiratory distress. In 14 per cent of the positive samples more than one pathogen were isolated (Catry *et al.,* 2002).

2.2 EPIDEMIOLOGY

2.2.1 Occurrence

Bain *et al.* (1982) opined that apart from flash epidemics, which occur irregularly, losses in most Asian countries due to haemorrhagic septicaemia are fairly consistent year after year, suggesting that by the present methods of control, a balance has been struck which could only be improved by improvement in prophylaxis.

Ribble *et al.* (1995) found that there was a large annual variation of 10-57 per cent in the proportion of mortality in cattle due to fibrinous pneumonia in South Western Alberta.

In an outbreak of respiratory disease among feedlot calves in Egypt, a high morbidity rate of 69.34 per cent and a low mortality of 4.1 per cent were reported (Selim *et al.*, 1998).

A high occurrence of haemorrhagic septicaemia was reported in buffaloes in various districts of Haryana, India from July 1995 to June 1998 with the morbidity, cumulative mortality and case fatality rates as 2.44, 0.68 and 27- 75 per cent respectively (Jindal *et al.*, 2002).

2.3 CLINICAL PATHOLOGY

Hyperthermia (39.6 to 40.5°C), tachypnoea (44 ± breaths/min), depression, cough, nasal discharge, lack of appetite and moderate rales on auscultation were reported as the typical signs of shipping fever (Lekeux and Art, 1988).

Roth (1988) identified that the factors responsible for protecting cattle from *Mannheimia haemolytica* are the action of alveolar macrophages and neutrophils, the presence of leukotoxin-neutralising antibody directed against a carbohydrate protein surface component and the cell mediated immune response, which enhances phagocytosis.

Clinical signs such as depression, anorexia, presence of copious nasal discharge, presence of cough noted more than once during an observation period, respiratory rate greater than 40 breaths/min and temperature greater than 40°C were reported in natural respiratory disease (Allen *et al.*, 1991).

Nasser and El-Sayed (1997) reported clinical parameters in acute bovine respiratory disease as increased rectal temperature, tachypnoea, depression, nasal discharge, change in character of respiration and biochemical variations like

marked hypoalbuminaemia, hyperglobulinaemia with significant rise in alpha 1 and alpha 2 globulins.

Yilmaz *et al.* (2000) reported an increase in white blood cells and reduction in neutrophil count after treatment with antibiotic in 40 calves with bronchopneumonia.

2.4 DIAGNOSIS

Exfoliative cytopathological examination of nasal epithelial cells could be used as a preliminary tool for the diagnosis of respiratory tract infection in calves (Charan *et al.*, 1986).

Martin *et al.* (1989) detailed the case definition of respiratory disease as calves with depression, anorexia, increased respiratory rate, cough and nasal discharge.

Transtracheal aspiration could be effectively used for the examination of bronchial mucus smear for the field diagnosis of pulmonary tuberculosis (Prathaban *et al.*, 1989).

Nasopharyngeal swabs could be reliably used for research or diagnostic purposes in order to obtain a precise estimate of the pulmonary microbial flora in large groups of feedlot calves (Allen *et al.*, 1991).

Espinasse *et al.* (1991) demonstrated transtracheal aspiration as a simple innocuous technique to perform in the field condition in calves to have a ready access to the pathogens of lower respiratory tract.

Paulsen *et al.* (1992) reported the use of an indwelling bronchial catheter model, which aid in the repeated lavage and quantitative bacteriologic examination of the primary lesion site in cases of pneumonic pasteurellosis.

Six *et al.* (1996) obtained 72 bacterial isolates by transtracheal aspiration in 93 veal calves with respiratory disease from 45 farms in Western France of

which 80 per cent contained pasteurella and 65 per cent contained mycoplasma and half of the infections were of mixed nature.

For the diagnosis of bovine respiratory disease a simple and sensitive technique was devised for collection of clinical materials from lower respiratory tract by passing guarded culture equipment through an opened mouth and exposing the larynx for the diagnosis of bovine respiratory disease (Walker, 1996).

Nasal swabs could be used as an ideal clinical material to predict the bacterial pathogens within the lung in acutely ill animals (De Rosa *et al.*, 2000).

Caldow (2001) described the technique of performing broncho-alveolar lavage in the investigation of bovine respiratory disease.

2. 5 ANTIBIOGRAM

Sharma and Joshi (1984) isolated *Pasteurella multocida* strains from different species of animals and were sensitive to furadantin and chloramphenicol (100 per cent), chlortetracycline (93.75 per cent) oxytetracycline (90.62 per cent), penicillin (75 per cent), ampiclox (71.87 per cent) and triple sulpha (18.75 per cent).

Allan *et al.* (1985) found that *Mannheimia haemolytica* A1 isolates from 80 cases of bovine pneumonic pasteurellosis were sensitive to chloramphenicol (100 per cent), sulphamethoxazole-trimethoprim (98 per cent), oxytetracycline (80 per cent), ampicillin(85 per cent), penicillin (82 per cent), streptomycin (3 per cent) and lincomycin (1 per cent).

Shoo (1988) reported no difference in antibiotic sensitivity pattern of *Mannheimia haemolytica* isolates from respiratory tract of healthy and diseased calves.

Kulkarni *et al.* (1990) conducted *in vitro* drug sensitivity tests on 22 *Pasteurella multocida* isolates from outbreaks of pasteurellosis in Maharashtra

and reported that the organisms were sensitive to tetracycline (95.2 per cent), chloramphenicol (90.0 per cent), neomycin (84.6 per cent), penicillin (66.6 per cent), erythromycin (57.1 per cent), gentamicin (50 per cent), septran (26.3 per cent), streptomycin (25 per cent), ampicillin (22.2 per cent), triple sulpha (17.6 per cent) and sulfadiazine (4.5 per cent).

In vitro antibiotic susceptibility test for *Mannheimia haemolytica*, *Pasteurella multocida*, *Streptococcus equi*, *Arcanobacterium pyogenes*, *Corynebacterium pseudotuberculosis* to ciprofloxacin, enrofloxacin and norfloxacin revealed that all the drugs were equally effective against the Gram negative bacteria tested (Prescott and Yielding, 1990).

Antimicrobial susceptibility of 27 *Pasteurella multocida* isolates employing serial broth dilution technique were conducted and considerable number of isolates were found to be resistant to fucidin, sulfamethoxazole, spiramycin and clindamycin and suggested limited use of sulfadimidine in field practice (Abeynayake *et al.*, 1993).

Erdag *et al.* (1993) isolated 56 *Pasteurella multocida*, 34 *Streptococcus pneumonia*, 24 *Arcanobacterium pyogenes*, 24 *Escherichia . coli*, 9 *Staphylococcus aureus*, and 6 *Mycoplasma bovis* from 160 bovine pneumonic lungs and most of the isolates were sensitive to gentamicin, danofloxacin, erythromycin, lincospectin, enrofloxacin and oxytetracycline

Antibiotic susceptibility test of *Mycoplasma bovis* strains isolated from respiratory tract of bovines in Northern Ireland with enrofloxacin, lincomycin, spectinomycin and tilmicosin concluded that enrofloxacin was the only antibiotic which exhibited any measurable mycoplasmacidal activity (Ball *et al.*, 1995).

Ceftiofur was found to be highly effective *in vitro* against Australian bovine and porcine isolates of *Actinobacillus pleuropneumoniae* from respiratory tract (Blackall *et al.*, 1996).

Gupta *et al.* (1996) found that the 39 field isolates of *Pasteurella multocida* from cases of pneumonia in calves were sensitive to gentamicin and chloramphenicol (90 per cent), polymixin B (87 per cent), nitrofurantoin (72 per cent), chlortetracycline (71 per cent), nalidixic acid (62 per cent), oxytetracycline (62 per cent), erythromycin (54 per cent) and neomycin (51 per cent).

Hormansdorfer and Bauer (1996) reported resistance of 375 bovine pasteurella isolates to oxacillin (41.18 per cent), tylosin (79.14 per cent), streptomycin (53.21 per cent), sulfonamides (57.33 per cent), tetracycline (47.2 per cent), cephalothin (5.07 per cent), polymixin B (0.27 per cent), enrofloxacin (0.53 per cent) and chloramphenicol (16.27 per cent). But all the strains were sensitive to its structural analogue florfenicol at a minimum inhibitory concentration of 0.25µg/ml.

Katoh *et al.* (1996) opined that the susceptibility of pasteurella and mycoplasma to thiamphenicol and oxytetracycline were extensively variable suggesting common distribution of the resistant strains to these drugs.

In vitro antibiotic susceptibility test was found to be the most ideal technique for the selection of effective antimicrobial agents in the treatment of bovine respiratory disease (Martel, 1996).

Pasteurella multocida isolated from a Holstein heifer affected with concurrent theileriosis and haemorrhagic septicaemia was highly sensitive to tetracycline, norfloxacin, ciprofloxacin, pefloxacin and moderately sensitive to gentamicin (Mukhopadhyay *et al.*, 1997).

All the mycoplasmas isolated from 109 pneumonic cattle were sensitive to enrofloxacin, danofloxacin, tilmicosin, tetracycline and oxytetracycline, less sensitive to erythromycin, gentamicin and resistant to streptomycin (Sahin, 1997).

Almajano *et al.* (1998) reported that all the pasteurella and moraxella isolates from calves with respiratory disease was sensitive to florfenicol.

Gunduz and Erganis (1998) reported sensitivity pattern of 48 *Mannheimia haemolytica* isolates from 340 pneumonic lung samples from bovine of which 44 were sensitive to amoxycillin, 35 to oxytetracycline, 34 to gentamicin while 36 strains were resistant to penicillin and 22 to ampicillin.

In vitro antibiotic sensitivity test of 89 bacterial isolates from cattle revealed that *Mannheimia haemolytica* were more resistant than *Pasteurella multocida* (Hormansdorfer and Bauer, 1998).

Antibiogram of the bovine isolates obtained in an outbreak of respiratory disease in Egypt revealed that all Gram positive organisms were sensitive to penicillin, enrofloxacin, ampicillin, flumequine and trimethoprim-sulphamethoxazole and Gram negative isolates were sensitive to enrofloxacin, chloramphenicol, gentamicin, oxytetracycline, erythromycin, cephalomycin and trimethoprim-sulphamethoxazole (Selim *et al.*, 1998).

El-Shabiny *et al.* (1999) isolated 30 mycoplasma strains from 170 lung samples from abattoir and were sensitive to trospectinomycin sulfate, enrofloxacin, norfloxacin, tiamulin and ciprofloxacin.

Robert and Nicholas (1999) conducted antibiotic susceptibility of *Mycoplasma mycoides* isolated from bovine respiratory infections and tilmicosin was found to have superior inhibitory and mycoplasmacidal activity compared to ampicillin.

A study on the antimicrobial susceptibility and plasmid DNA profiles in field strains of 49 *Mannheimia haemolytica* isolates obtained from cattle with respiratory disease revealed that all the isolates were sensitive to florfenicol and resistant to flumequine. Multiple drug resistance was found in all strains with or without plasmids (Wernicki *et al.*, 1999).

Aguade and Romero (2000) reported >90 per cent resistance in pasteurella spp. for kanamycin and lincomycin, >80 per cent resistance for penicillin, streptomycin and >20 per cent resistance for tilmicosin and florfenicol.

Highest sensitivity was recorded with cefotaxime and >87 per cent of isolates were sensitive to mezlocelin or cefalexime.

Ayling *et al.* (2000) compared *in vitro* activity of danofloxacin, florfenicol, oxytetracycline, spectinomycin and tilmicosin against 62 field isolates of *Mycoplasma bovis* from British cattle with respiratory disease and found highest susceptibility to danofloxacin followed by florfenicol.

Cloeckaert *et al.* (2000) reported the presence of plasmid-mediated florfenicol resistance encoded by the Flo-R gene in *Escherichia coli* isolated from cattle, which is identical to Flo-R of *Salmonella enterica* serovar *typhimurium* DT 104.

Antibiotic sensitivity test of *Mannheimia haemolytica* and *Pasteurella multocida* isolates from Dutch calves did not detect resistance to ceftiofur and florfenicol (Mevius and Hartman, 2000)

Multiple resistance to antimicrobial agents was reported for *Pasteurella multocida* serotype A and *Haemophilus somnus* strains isolated from lungs of pneumonic calves (Nakaya *et al.*, 2000).

Mycoplasma bovis was incriminated as the sole pathogen in respiratory disease of cattle with resistance to tetracyclines, tilmicosin, florfenicol, spectinomycin and only danofloxacin showed significant antimycoplasma activity (Nicholas *et al.*, 2000).

Popova and Dimitrov (2000) conducted *in vitro* testing of antibacterial activity of amphenicols and combination of these drugs to oxytetracycline against 20 Gram positive and 17 Gram negative pathogenic bacteria and found that florfenicol had better antibacterial activity compared to thiamphenicol, oxytetracycline and thiamphenicol-oxytetracycline combination.

Pseudomonas aeruginosa isolated from bovine respiratory tract was fully susceptible to ciprofloxacin, ofloxacin and norfloxacin, azithromycin (85.7 per

cent), gentamicin (85.72 per cent), enrofloxacin (57.14 per cent) and was fully resistant to chloramphenicol, kanamycin, streptomycin, bacitracin, amikacin, erythromycin, nalidixic acid, nitrofurantoin and penicillin G (Chakraborty *et al.*, 2001).

Hutt and Goossens (2001) reported 100 per cent susceptibility to florfenicol, ceftiofur, enrofloxacin, 98 per cent to amoxycillin and tetracycline and 13 per cent to sulfamethoxazole-trimethoprim in 45 *Pasteurella multocida* isolates from 94 nasal swabs of bovines with bronchopneumonia.

Pasteurella organisms were isolated from 158 pneumonic lungs and the isolates obtained were susceptible to enrofloxacin, ciprofloxacin, amoxycillin, norfloxacin, cephalexin, cephazoline, and orbifloxacin (Jongshli *et al.*, 2001).

Aminoglycocides were found to be less active against *Pasteurella multocida* isolated from bovines with respiratory diseases (Yoshimura *et al.*, 2001).

Catry *et al.* (2002) reported the antimicrobial resistance to 24 *Pasteurella multocida* strains isolated from tracheal washings of bovines suffering from acute respiratory distress and found 100 per cent susceptibility to amoxycillin and clavulanate, enrofloxacin, ceftiofur and florfenicol, 96 per cent susceptible to tetracycline and ampicillin and 92 per cent to the sulpha-trimethoprim.

2 .6 TREATMENT

2.6.1 Enrofloxacin

Lekeux and Art (1988) reported the successful treatment of shipping fever in feedlot cattle using enrofloxacin at 5 mg/kilogram body weight intramuscular for three consecutive days.

Enrofloxacin was found to be effective in treating natural infections of respiratory system by *Pastuerella multocida* and *Mannheimia haemolytica* with doses of 2.5 to 7.5 mg/kg body weight after 5 to 8 daily administrations (Vancutsem *et al.*, 1989).

A clinical trial on the oral prophylactic use of enrofloxacin (Baytril solution) against respiratory diseases of calves concluded that 10 days of treatment were required for the prophylactic effect (Baumgartner and Pangerl 1990).

Hunkenmoller (1991) compared the efficacy of enrofloxacin orally at 2.5 mg/kg body weight and gentamicin intramuscularly at 4 mg/kg body weight in 149 cattle with pneumonia and found 72 per cent recovery after enrofloxacin therapy compared to 63 per cent after gentamicin therapy.

Boon *et al.* (1992) compared the clinical efficacy of enrofloxacin (Baytril) and a combination of neomycin for treating respiratory tract infection of veal calves and found that both preparations were equally effective when injected daily for five days.

Belli *et al.* (1993) evaluated the *in vivo* activity of enrofloxacin in experimental respiratory infection of calves caused by *Mycoplasma bovis* at 5 mg/kg body weight intramuscularly for 3 days and it was not found to be effective.

Tras *et al.* (1993) conducted experimental studies on the withdrawal time of enrofloxacin after intramuscular injection at 2.5 mg/kg body weight and no antibiotic residue was detected in milk at 96 and 120 h. after administration by high performance liquid chromatography.

No significant difference was reported in the efficacy of enrofloxacin and ceftiofur in the treatment of infectious enzootic bronchopneumonia in beef cattle (Espinasse *et al.*, 1994).

The efficacy and safety regimen of enrofloxacin was ascertained for the treatment of bacterial respiratory disease in cattle and was found to be 2.5–5 mg/kg body weight administered subcutaneously (Highland *et al.*, 1994).

Schakel and Trenti (1994) conducted a clinical trial with injections of enrofloxacin at 5 mg/kg/24 h, tetracycline LA at 1 ml /10 kg /24 h, trimethoprim-sulphadimidine at 1 ml/10 kg/24 h, neomycin sulphate at 1.45 mg/ml with procaine penicillin at 1 ml/10 kg body weight/24 h until recovery in clinical shipping fever of calves and found enrofloxacin to be the most effective drug.

Pyorala *et al.* (1994) reported local tissue damage in cows after intramuscular injections of antimicrobial agent and found serious clinical reactions with enrofloxacin, spiramycin and terramycin. Among the vehicles used glycerol formal and propylene glycol induced maximum tissue irritation.

Winter and Hofmann (1994) reported that the single subcutaneous injection of tilmicosin at 10 mg/kg body weight was almost as effective as the 5 day treatment with enrofloxacin (Baytril) or procaine-dihydrostreptomycin (pemycin) in the therapy of chronic bronchopneumonia in calves.

Different treatment regimens of enrofloxacin were compared in the clinical efficacy against bovine respiratory disease and found that the single dose regimen of 7.5–12.5 mg/kg body weight intramuscularly had equally comparable results with the established dose regimen of 2.5 to 5 mg/kg body weight intramuscularly for 3-5 days (Hamm *et al.*, 1998).

Similar therapeutic efficacy was reported with tilmicosin and enrofloxacin in the treatment of endemic pasteurellosis of milk fed calves in Hungary (Fodor *et al.*, 2000).

Enrofloxacin at 5 mg/kg body weight and difloxacin at 2.5 to 5 mg/kg body weight for 5 days were found to be equally effective for treatment of calves with experimentally induced pneumonic pasteurellosis (Olchowy *et al.*, 2000).

2.6.2 Florfenicol

Florfenicol was reported to have excellent penetration in both bronchial secretion and tissue cage fluid and the concentration in bronchial secretion were higher than those in serum showing the affinity for lung tissues (Varma, 1994).

The sensitivity of florfenicol over chloramphenicol and thiamphenicol resistant organisms was explained by the presence of fluorine atom, which prevents acetylation of drug by chloramphenicol acetyl transferase (CAT) enzyme of the organisms allowing interaction with bacterial ribosomes. (Sams, 1994). He also reported that florfenicol do not bear a nitro group in the structure unlike chloamphenicol and hence there is no development of aplastic anaemia.

Bordes *et al.* (1995) reported that florfenicol at 20 mg/kg body weight is superior to amoxycillin at 15 mg/kg intramuscularly, 2 doses given at 48 h interval in the treatment of bovine respiratory disease.

A field trial on the efficacy of florfenicol and amoxycillin in 66 beef cattle affected with respiratory disease revealed greater effectiveness to florfenicol than amoxycillin (Libersa *et al.*, 1995).

Florfenicol at 20mg/kg body weight twice intramuscularly at 48 h interval and ceftiofur at 1 mg/kg bodyweight once daily for 5 consecutive days yielded comparable results in the therapy of acute pneumonia in 100 housed beef calves in France (Navetat *et al.*, 1995).

Florfenicol was found to be the most effective antibiotic for the treatment of acute bovine respiratory diseases caused by multiple bacterial pathogens compared to spiramycin, long acting oxytetracycline and enrofloxacin (Lockwood *et al.*, 1996).

Madelenat *et al.* (1997) compared the efficacy of florfenicol at 20 mg/kg body weight with that of long acting spiramycin at 100000 IU/kg twice with 48 h interval both combined with flunixine meglumine (Finadyne) at 2 mg/kg body

weight for treating bovine respiratory disease in 90 veal calves and concluded that the treatment involving florfenicol was better than that of spiramycin.

Antibiotic susceptibility test of bacterial isolates from calves showing acute clinical signs of respiratory disease showed sensitivity to florfenicol, chloramphenicol, gentamicin and oxytetracycline (Nasser and El-Sayed, 1997).

Quintavalla *et al.* (1997) conducted a study on the clinical efficacy and safety of florfenicol at 20 mg/kg body weight at 48 h interval in respiratory diseases in young beef cattle and was found to be more effective than tilmicosin given at 10 mg/kg body weight subcutaneously.

Efficacy of florfenicol was compared to a penicillin-streptomycin/ dexamethasone formulation in the treatment of bovine respiratory syndrome and found that even though penicillin – streptomycin/dexamethasone initially had a greater effect than florfenicol, relapses were noticed after treatment (Almajano *et al.*, 1998).

Hoar *et al.* (1998) compared the clinical efficacy, field efficacy and safety of florfenicol and tilmicosin for the treatment of undifferentiated bovine respiratory disease of cattle in Western Canada and found that the results were comparable to each other.

A comparative clinical trial with florfenicol and tilmicosin in the treatment of an outbreak of respiratory disease in 203 calves confirmed the efficacy of florfenicol in terms of the statistically greater reduction of fever, greater improvement of clinical scores and greater treatment success rate than tilmicosin (Smitherman *et al.*, 1998).

The clinical efficacy of a long-acting drug florfenicol at 20 mg/kg body weight intramuscularly at 48 h interval was compared with that of non-long-acting agents like lincomycin at 5 mg/kg body weight and spectinomycin at 10 mg/kg body weight every 12 h over 4 days and better efficacy of florfenicol was confirmed than other two non-long-acting agents (Pagani *et al.*, 1999).

A study to compare the efficacy of tilmicosin and florfenicol for the initial treatment of bovine respiratory disease at the University of Arkansas beef research unit revealed that most of the pasteurella spp. were resistant to tilmicosin, but all the isolates were susceptible to florfenicol (Copeland *et al.*, 2000).

A controlled clinical study in 100 calves suffering from acute bronchopneumonia under field conditions in Germany with either florfenicol (Nuflor®) at 40 mg/kg body weight subcutaneously or with tilmicosin at 10 mg/kg body weight subcutaneously showed a success rate of 95 per cent with florfenicol compared to only 78 per cent in the tilmicosin group (Goossens, 2000).

2.6.3 Other Antimicrobials

Penicillin, streptomycin and tetracycline are widely used against *Pasteurella multocida* isolates rather than the traditional drug sulfonamide and chloramphenicol, which are used as substitute (Carter 1986).

Hjerpe (1986) reported that the best available therapy for pneumonic cattle was the parenteral administration of an effective antibiotic for a period of three to seven days.

Tilmicosin at 10 mg/kg body weight single subcutaneous injection was found to be more effective than daily intramuscular administration of 5 mg lincomycin and 10 mg spectinomycin per kg body weight for three days in the treatment of respiratory infections in calves (Picavet *et al.*, 1991).

Giles *et al.* (1991) compared the efficacy of danofloxacin and oxytetracycline in respiratory tract infection in beef cattle and concluded that danofloxacin therapy was characterized by significantly fewer treatment days, a higher response rate, significantly better reduction of pyrexia and fewer cattle requiring re-treatment.

Long acting antibiotic tilmicosin at 10 mg/kg body weight subcutaneously was found to be more effective than oxytetracycline dihydrate at 20 mg/kg body weight intramuscularly in the treatment of an outbreak of acute pneumonia (Laven and Andrews, 1991).

Treatment with ceftiofur sodium was more effective than trimethoprim-sulphadoxine combination in 555 beef calves with respiratory disease (Jim *et al.*, 1992).

Secondary bacterial invasion after bovine respiratory syncytial virus infection can be controlled effectively with single subcutaneous injection of tilmicosin at 10 mg/kg body weight (Scott *et al.*, 1996).

The combination of the mucolytic agent bromhexine (Bisolvon) at 0.5 mg/kg intramuscularly on the first day followed by oral treatment at the same dosage twice daily for 3 days coupled with enrofloxacin or cefquinone or ceftiofur or florfenicol had a better response than the single antibiotic therapy in acute respiratory disease of cattle (Schmidt *et al.*, 1998).

Amoxycillin at the dose of 10 mg/kg body weight was more effective than sulphadoxine–trimethoprim at 15 mg/kg body weight in the treatment of bovine respiratory disease complex (Kennerman *et al.*, 2000).

Combinations of antimicrobials are more potent than the activity of individual drugs in respiratory diseases, especially against resistant microorganisms (Popova and Dimitrov, 2000)

Yilmaz *et al.* (2000) opined that combination of enrofloxacin and single dose of nonsteroidal anti-inflammatory drug, flunixine meglumine was more effective with shorter duration of therapy in the treatment of enzootic calf pneumonia than enrofloxacin alone.

The combination therapy of enrofloxacin and Parapox virus D 1701, which is an immunomodulator was found to be more effective than the single

therapy with enrofloxacin in calves with enzootic pneumonia (Kaymaz *et al.*, 2001).

2.7 PROPHYLAXIS AND CONTROL

Rau and Govil (1950) used suspension of *Pasteurella multocida* organisms lysed by saponin as vaccine in cattle and buffaloes for the control of haemorrhagic septicaemia.

Comparative efficacy of the oil-adjuvant and multiemulsion oil adjuvant vaccine against haemorrhagic septicaemia in cattle was assayed in calves, rabbits and mice. Both vaccines were shown to be safe and immunogenic as assessed by passive mouse protection test and rabbit challenge test (Mittal *et al.*, 1979).

Morisse (1979) compared the efficacy of three vaccines, namely (1) a preparation of ribosomes and cell wall extracts of *Klebsiella pneumoniae* (2) an inactivated, emulsified *Pasteurella multocida* and *Haemophilus influenza* (3) inactivated trivalent *Pasteurella multocida, Bordetella bronchiseptica* and *Haemophilus influenza* vaccine. The inactivated *Pasteurella multocida* and *Haemophilus influenzae* were found effective.

Some measures like avoiding over crowding, isolation of sick animals, avoiding immunosuppression by reducing stress and vaccinating cattle against common viral respiratory pathogens, development of *Pasteurella haemolytica* vaccines and immunomodulators are ideal management and prophylactic measures to reduce *Pasteurella haemolytica* infections (Roth, 1988).

Conlon *et al.* (1995) conducted an efficacy trial with single and double dose of "Presponse" (*Pasteurella haemolytica* bacterial extract) vaccine at 21 days interval to calves, which yielded similar protective immune response and concluded the presence of *Pasteurella haemolytica* as a natural commensal of the upper respiratory tract of calf.

Lee *et al.* (2001) investigated the feasibility of expressing a fusion protein of *Mannheimia haemolytica* A, leukotoxin in a forage plant like transgenic white clover for use as an edible vaccine against bovine pneumonic pasteurellosis.

Chimaeric protein of *Bordetella bronchiseptica* fimbrial protein enhanced the immunogenicity of *Mannheimia haemolytica* leukotoxin and hence can be included in the new generation vaccines against shipping fever pneumonia (Rajeev *et al.*, 2001).

A study on the effect of vaccination prior to transit and administration of florfenicol at time of arrival in a feedlot on the health of transported calves concluded that prophylactic use of florfenicol will reduce the incidence of respiratory tract disease in calves (Frank *et al.*, 2002).

Kato *et al.* (2003) compared the prophylactic efficacy of enrofloxacin and tilmicosin with that of vaccine on bovine respiratory disease in beef cattle caused by *Pasteurella multocida* and mycoplasma and found that occurrence of respiratory disease in the enrofloxacin and tilmicosin treated groups were significantly lower than in the vaccinated group.

Materials and Methods

3. MATERIALS AND METHODS

The present study was carried out in the Department of Veterinary Epidemiology and Preventive Medicine with the clinical cases presented at the University Veterinary Hospitals, Kerala Agricultural University Farms and State Animal Husbandry Veterinary Hospitals during the period of November 2001 to June 2003.

The bovines, which were presented with clinical signs of respiratory disease, formed the material for the study. A group of apparently healthy animals in the University Livestock Farm, Mannuthy were selected as the control group. Signalment, anamnesis and symptoms of each case were recorded as per the proforma (Appendix I). Clinical materials were collected from these animals and processed for the laboratory investigations.

3.1 CLINICAL FINDINGS

A detailed preliminary clinical examination was conducted in the experimental and control groups together with any observations of concurrent diseases. In addition the respiratory system was examined and the rate and character of respiration assessed. Based on the clinical data and symptoms each animal was assigned a clinical illness index score ranging from 0 (Normal) to 3 (Severely ill). Details of these scores are given in Table 1.

3.2 COLLECTION OF CLINICAL MATERIALS

Deep nasal washings, nasal swabs and blood samples taken from the bovines with respiratory symptoms formed the material for laboratory examination and diagnostic study.

Table 1. Definition of clinical illness index score for cattle with respiratory disease

Clinical illness index score	Definition	Clinical description
0	Normal	No abnormal clinical signs
1	Slightly ill	Animal shows abnormal respiration, with a detectable tachypnoea and hyperpnoea, mild rales on auscultation
2	Moderately ill	Animal shows abnormal respiration, with a mild to moderate dyspnoea, clear rales on auscultation usually combined with depression and partial anorexia
3	Severely ill	Animal shows severely abnormal respiration with marked dyspnoea combined with depression and partial or complete anorexia

3.2.1 Collection of Blood

3.2.1.1 Blood for Culture

About five milliliter of blood was collected from jugular vein after sterilizing the skin with 70 per cent alcohol using a cotton swab into a sterile 30 ml vial with glass beads. The blood was defibrinated by steady and uniform rotation of the vial.

3.2.1.2 Blood for Haematology

About three milliliter of blood was collected from jugular vein using sterile disposable needle and transferred to a sterile vial containing EDTA as anticoagulant at the rate of 2 mg/ml of blood.

3.2.2 Collection of Nasal Swab/Deep Nasal Washings

Sterile cotton swab was introduced into the nasal cavity after sterilizing the external nares and nasal inlet with 70 per cent alcohol. Deep nasal washings were collected by introducing a sterile flutter valve apparatus connected to a sterile 20 ml syringe having 10 ml of normal saline. The washings were collected into the same syringe and a knot was tied to the flutter valve apparatus immediately after collection.

3.3 ISOLATION AND IDENTIFICATION OF THE BACTERIAL ORGANISM

3.3.1 Glasswares and Reagents

Borosil brand of glasswares, analytical or guaranteed grade of reagents, chemicals and culture media (Hi-media) were used for the study.

3.3.1.2 Preparation of Glassware and Culture Media

The petriplates and test tubes were kept in 0.1 per cent hydrochloric acid overnight. They were washed in running tap water and immersed in detergent

solution for one day. The petriplates and test tubes were washed thoroughly in running tap water. The glasswares were then washed in distilled water. Then they were dried and sterilized in hot air oven at 160°C for one hour.

The culture media was reconstituted in double glass distilled water according to manufacturer's (Hi-media) instructions. It was then sterilized by autoclaving at 121°C and 15 lbs of pressure for 15 min. It was cooled to 45°C, poured in to sterile petriplates and test tubes and incubated at 37°C for 24 h to test the sterility.

3.3.2 Collection of Sheep Blood

The sheep blood for the preparation of blood agar was collected from the animals maintained in the University Goat and Sheep Farm, Mannuthy. Blood collection was carried out under sterile conditions in round bottom flasks containing glass beads. The defibrinated blood was stored at 4°C in 10 ml aliquots until use.

3.3.3 Preparation of Blood Agar

The dehydrated blood agar base (Hi-media) was prepared as per the manufacturer's instructions, to which sterile blood was added at five per cent level to make blood agar.

3.3.4 Isolation of Bacteria

Isolation of the bacteria was attempted from defibrinated blood, nasal swab and deep nasal washings.

Defibrinated blood was inoculated directly into blood agar and brain heart infusion agar (BHIA) and incubated aerobically at 37°C for 24 h.

Nasal swabs collected were introduced into brain heart infusion broth (BHIB) and incubated aerobically at 37°C for six hours. Blood agar and brain

heart infusion agar were streaked with broth culture at the end of six hours and incubated aerobically at 37°C for 24 h.

Deep nasal washings collected were directly streaked into blood agar and brain heart infusion agar and incubated aerobically at 37°C for 24 h.

Plates were examined after 24-48 h. Isolated colonies were selected and a representative sample was streaked on nutrient agar slants for further identification. Slants were preserved by storing in refrigerator at 4° C.

3.3.5 Identification of Bacteria

The isolates were stained by Gram's method and depending on the preliminary characters selective media were used. The morphological, cultural, biochemical and sugar fermentation of the isolates belonging to different species were determined as per the methods described by Barrow and Feltham (1993). Selective media used in the present study were

1. Mannitol salt Agar
2. Edward's medium
3. Mac Conkey Agar
4. Eosin methylene blue Agar

Staphylococcus aureus was grown on Mannitol salt agar. *Streptococcus pyogenes* was grown on Edward's media. Mac Conkey agar was used to differentiate lactose fermenting and non-fermenting bacteria. *Escherichia coli* were grown on eosin methylene blue agar.

A rapid biochemical test kit (Hi-media) was employed for the identification of bacteria. The biochemical tests employed for the identification of isolates were

1. Catalase test
2. Oxidase test

3. Oxidation – fermentation test
4. Carbohydrate utilization test
5. Citrate utilization test
6. Coagulase test
7. Decarboxylase test
   Lysine and Ornithine
8. Indole production
9. Methyl red test
10. Nitrate reduction test
11. TDA test
12. TSI agar
13. ONPG reduction
14. Urease test
15. Voges – Proskauer test

3.4 ANTIBIOGRAM

3.4.1 Procedure

In vitro antibiotic sensitivity of the organisms were studied using disc diffusion technique (Bauer *et al.*, 1966).

Five colonies of each pure culture were picked up with sterile platinum loop and were used as the inoculum in four milliliter of peptone broth. Inoculum was applied on the surface of a Mueller Hinton agar (MHA) using a sterile cotton swab and the plate was kept covered for 15 min at room temperature for drying the inoculum. The antibiotic discs were then placed 20 mm apart and they were gently pressed on to the surface of the agar to ensure contact. The plates were incubated at 37°C for 18-24 h.

3.4.2 Antibiotic Discs

The antibiotic discs used in the present study were

1. Antibiotic discs (Hi-media)

a.	Amoxycillin	(Am)	-	30 mcg/disc
b.	Chloramphenicol	(C)	-	30 mcg/disc
c.	Ciprofloxacin	(RC)	-	5 mcg/disc
d.	Enrofloxacin	(Ex)	-	5 mcg/disc
e.	Gentamicin	(GM)	-	10 mcg/disc
f.	Oxytetracycline	(OX)	-	30 mcg/disc
g.	Streptomycin	(SM)	-	10 mcg/disc
h.	Trimethoprim	(Tr)	-	25 mcg/disc

3.4.3 Interpretation

The zone of inhibition of bacterial growth around each disc was measured and interpreted as sensitive, moderately sensitive or resistant by comparing the ranges given by the manufacturer.

3.5 HAEMATOLOGICAL PARAMETERS

The following haematological parameters were estimated.

3.5.1 Haemogram

3.5.1.1 Erythrocyte Sedimentation Rate (ESR)

It was estimated using Wintrobe's method by keeping the blood for one hour (Benjamin, 1985).

3.5.1.2 Packed Cell Volume (PCV)

Estimated by Wintrobe's method as per Coles (1986) and expressed as per cent.

3.5.1.3 Haemoglobin (Hb)

It was estimated by acid-haematin method using Sahli's haemoglobinometer and was expressed as gram per deciliter (Coles, 1986).

3.5.1.4 Total Erythrocyte Count

Total RBC count was estimated using Haem's fluid as per Coles (1986) and value expressed as $x10^6$ cells/mm^3 of blood.

3.5.1.5 Erythrocyte Indices

The erythrocyte indices were calculated to identify the type of anaemia using the following formulae (Coles, 1986).

a. Mean Corpuscular Volume (MCV) = $\frac{\text{PCV/1000 ml of blood}}{\text{Total erythrocyte count in millions/mm}^3}$

Value expressed as fentoliters

b. Mean Corpuscular Haemoglobin (MCH) = $\frac{\text{Haemoglobin in grams/1000 ml}}{\text{Total erythrocyte count in millions/mm}^3}$

Value expressed as picogram

c. Mean Corpuscular Haemoglobin Concentration (MCHC) = $\frac{\text{Haemoglobin in grams/10000 ml of blood}}{\text{PCV/100 ml of blood}}$

Value expressed as grams /deciliters

3.5.2 Leukogram

3.5.2.1 Total Leukocyte Count (TLC)

Total WBC count was estimated using Thoma's fluid as per Coles (1986) and value expressed as x 10^3 cells/mm^3 of blood.

3.5.2.2 Differential Leukocyte Count (DLC)

Blood smear was stained by Giemsa's stain and 100 leukocytes were counted under oil immersion objective and differential counts were expressed as percentage (Benjamin, 1985).

3.6 TREATMENT TRIAL WITH ENROFLOXACIN AND FLORFENICOL

Treatment was initiated in cases wherein there were clinical signs of respiratory disease. The animals were divided into two treatment groups. Two broad-spectrum chemotherapeutic agents were tested for their efficacy in treating clinical cases.

Group I – comprised of nine animals and were treated with enrofloxacin (Gyroflox)* at 5 mg/kg body weight intramuscularly once daily for three to five days.

Group II – comprised of nine animals and were treated with *florfenicol* (Nuflor®)** at 20 mg/kg body weight deep intramuscular in the neck two doses at 48 h interval.

Animals were allotted into each group randomly on the day of presentation. Samples were collected aseptically and subjected to culture and sensitivity test and haematological estimations. Efficacy of drugs was assessed in terms of clinical response. Blood was collected on the first day and on the 4^{th} or on the 6^{th} day in case of enrofloxacin group and on the 4^{th} day in the case of florfenicol group. Animals were kept under observation for a minimum of 10 days. The data obtained from various parameters were statistically compared between pre and post-treatment groups.

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*Gyroflox - Indian Immunologicals, Hyderabad

**Nuflor® - Schering -Plough Animal Health Care, New Jersey

3.7 RESPONSE TO TREATMENT

Response to treatment was assessed based on the clinical findings and haematological parameters and cases were classified as successful or failed in the treatment. After successful treatment, if the animal again manifested respiratory symptoms during the observation period, the cases were designated as recurrence of infection. Clinical data and haematological parameters of post-treatment groups (I and II) were statistically compared to healthy animals (group III).

3.8 EVALUATION OF EFFICACY OF DRUGS

Efficacy of drugs was evaluated by comparing clinical data and haematological parameters between the data collected from two pre-treatment groups and post-treatment groups.

3.9 STATISTICAL ANALYSIS

The data obtained were analysed statistically as per the procedure described by Snedecor and Cochran (1980).

Results

4. RESULTS

The bovines, which were presented at the University Veterinary Hospitals, Kerala Agricultural University Farms and State Animal Husbandry Veterinary Hospitals during the period of November 2001 to June 2003 with clinical signs of respiratory disease, formed the experimental group. The control group (group III) consisted of six apparently normal healthy bovines, which were maintained in the University Livestock Farm, Mannuthy.

On the day of presentation, blood, nasal swabs and deep nasal washings were collected aseptically from diseased animals and was subjected to culture and sensitivity test. Haematological parameters and clinical data were recorded on the first day of presentation of animals and on the fourth or on the sixth day in group I and on the fourth day in group II were compared statistically with that of control animals (group III).

4.1 CLINICAL FINDINGS

4.1.1 Clinical Data of Diseased and Control Animals

The temperature, pulse and respiratory rate per minute were recorded in bovines with respiratory disease (group I and II) and in healthy bovines (group III). The mean temperature (°F), pulse and respiratory rate per minute in bovines affected with respiratory disease (103.05 ± 1.08, 81.31 ± 1.35 and 55.13 ± 11) were compared with that of control (group III) (101.9 ± 0.37, 81 ± 1.32 and 40.25 ± 7.26) and was found to have a highly significant rise ($P<0.01$) in temperature and respiration rate in affected group when compared to that of healthy animals. But the rise in pulse rate observed in affected group was not statistically significant with that of control animals (Table 2).

Table 2. Clinical data of diseased and healthy bovines

Sl. No.	Group	Temperature (°F)	Pulse / min	Respiration / min
1	Control (n=6)	101.9 ± 0.37	81 ± 1.32	40.25 ± 7.26
2	Diseased (n=18)	103.05 ± 1.08**	81.31 ± 1.35^{NS}	55.13 ± 11**

NS - Nonsignificant ($P \geq 0.05$)
** - Highly significant ($P < 0.01$)

4.1.2 Clinical Symptoms

The different clinical signs observed in bovines with respiratory disease were cough (38.88 per cent), rhinorrhoea (94.44 per cent), dry muzzle (61.11 per cent), inspiratory dyspnoea (83.33 per cent), expiratory dyspnoea (11.11 per cent), crackles on tracheal auscultation (100 per cent), crackles on lung auscultation (11.11 per cent), congested mucous membrane (33.33 per cent), lacrymation (22.22 per cent), diarrhoea (44.44 per cent), inappetance (66.66 per cent), lymphadenomegaly (77.77 per cent), pyrexia (61.11 per cent) reduction in milk production (27.77 per cent) out of the 7 lactating animals. Based on the clinical signs observed in diseased animals they were classified into three groups as slightly ill (27.77 per cent), moderately ill (66.66 per cent) and severely ill (5.55 per cent) with the clinical illness index score as 1, 2 and 3 respectively (Table 3).

4.2 EXAMINATION OF CLINICAL MATERIALS

No bacterial organisms could be isolated from defibrinated blood collected from diseased animals. All nasal swabs/deep nasal washings were positive for bacterial organisms.Blood collected using anticoagulants from pre and post-treatment groups and control group were used for haematology.

4.3 BACTERIAL ISOLATES FROM THE BOVINES WITH RESPIRATORY DISEASE

Nasal washings and nasal swabs from 18 animals with respiratory disease were subjected to cultural examination. All the samples yielded bacteria. Out of the 39 bacterial isolates obtained 19 (48.71%) were Gram positive organisms and 20 (51.29%) were Gram negative organisms (Fig. 1). The different bacterial isolates were *Staphylococcus aureus* (15.38 per cent), *Staphylococcus epidermidis* (12.82 per cent), *Streptococcus pyogenes* (20.51 per cent), *Escherichia coli* (17.95 per cent), *Klebsiella pneumoniae* (12.82 per cent),

Table 3. Clinical signs observed in bovines with respiratory disease

-cases exhibiting the symptoms (n = 18)

Sl. No.	Clinical signs		Number	Per cent
1	Cough		7	38.88
2	Presence of Rhinorrhoea		17	94.44
3	Dry muzzle		11	61.11
4	Depth of respiration	Inspiratory dyspnoea	15	83.33
		Expiratory dyspnoea	2	11.11
5	Crackles on auscultation	Trachea	18	100
		Lung	2	11.11
6	Congested mucous membrane		6	33.33
7	Lacrymation		4	22.22
8	Diarrhoea		8	44.44
9	Inappettance		12	66.66
10	Lymphadenomegaly		14	77.77
11	Pyrexia		11	61.11
12	Reduction in milk production (milch cow – 7)		5	27.77
13	Clinical Illness index score	Slightly ill (1)	5	27.77
		Moderately ill (2)	12	66.66
		Severely ill (3)	1	5.55

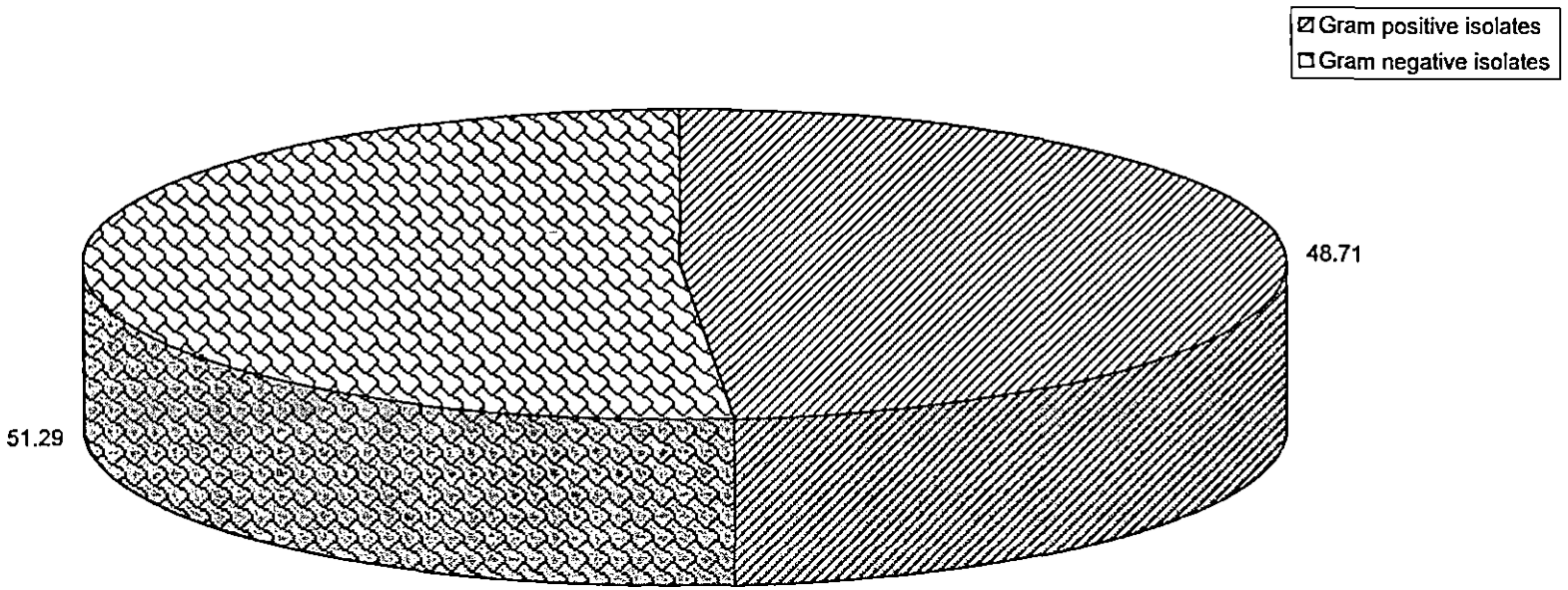


Fig.1 Percentage distribution of Gram positive and Gram negative bacterial isolates from bovine respiratory disease

Pseudomonas aeruginosa (10.26 per cent), *Pasteurella haemolytica* (7.69 per cent) and *Proteus vulgaris* (2.56 per cent) (Table 4 and Fig. 2).

The isolates obtained in mixed infections are depicted in Table 5. Majority of the clinical samples yielded 2 bacterial isolates (61.11 per cent) followed by 3 isolates (27.75 per cent). From two cases (11.11 per cent) the single isolate obtained was *Streptococcus pyogenes*.

Escherichia coli could be isolated from five cases in combination with other bacteria. From 4 cases *Streptococcus pyogenes* and *Staphylococcus epidermidis* could be isolated. *Staphylococcus aureus* was isolated from three cases. *Pasteurella haemolytica, Klebsiella pneumoniae* and *Pseudomonas aeruginosa* could be isolated from two cases in combination with another bacteria.

Three species of bacteria could be isolated as the etiological agents of respiratory tract infection in five cases, of which *Klebsiella pneumoniae* and *Staphylococcus aureus* were the most common. *Streptococcus pyogenes, Escherichia coli* and *Pseudomonas aeruginosa* were isolated from two cases.

Staphylococcus aureus was grown on Mannitol salt agar (Plate.1) and it yielded yellow coloured colonies and the medium changed from pink to yellow. The colonies of *Streptococcus pyogenes* grown on Edward's medium (Plate 2) revealed small transparent haemolytic colonies. Mac Conkey agar was used for differentiating lactose fermenting and lactose non-fermenting colonies of *Escherichia coli* and *Pseudomonas aeruginosa* (Plate 3). The rapid biochemical test kit (Hi-media) was used for the identification of bacteria (Plate 4). The battery of biochemical tests for the identification of *Escherichia coli* and *Klebsiella pnuemoniae* are shown in (Plate 5).

Table 4. Aerobic bacteria isolated from deep nasal washings of 18 bovines with respiratory disease

Sl. No.	Microorganism	No. of bovines positive	Occurrence of isolate (%)	Percentage of bovines positive
	Gram positive			
1.	*Staphylococcus aureus*	6	15.38	33.33
2.	*Staphylococcus epidermidis*	5	12.82	27.77
3.	*Streptococcus pyogenes*	8	20.51	44.44
	Gram negative			
4.	*Escherichia coli*	7	17.95	38.88
5.	*Klebsiella pneumoniae*	5	12.82	27.77
6.	*Pseudomonas aeruginosa*	4	10.26	22.22
7.	*Pasteurella haemolytica*	3	7.69	16.66
8.	*Proteus vulgaris*	1	2.56	5.55

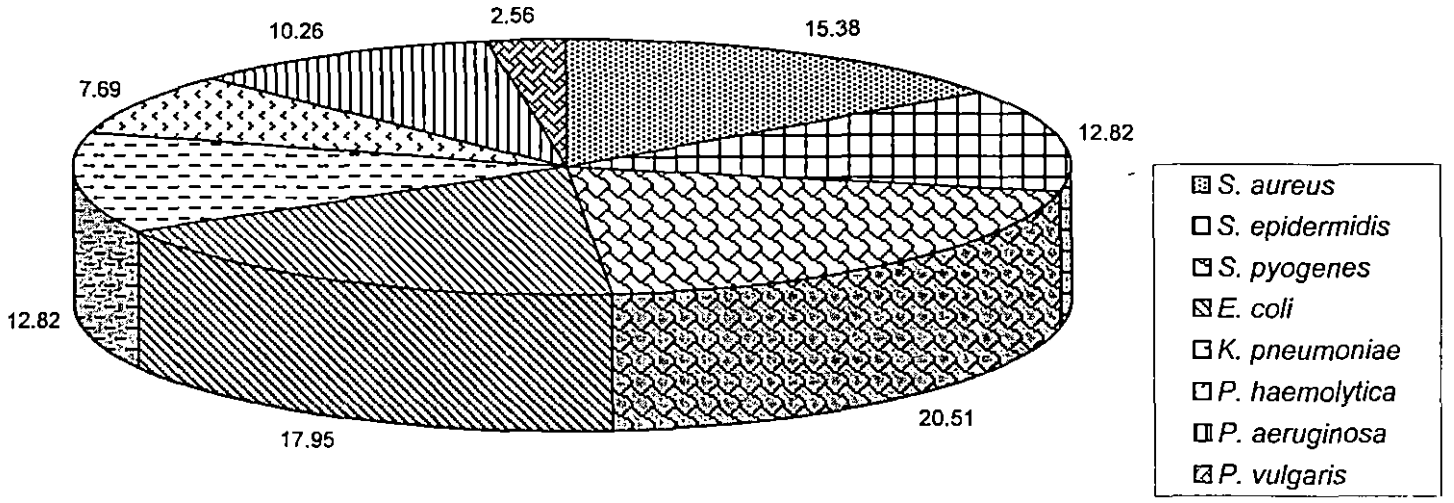


Fig. 2 Bacterial isolates from bovine respiratory tract disease

Table 5. Distribution of single and mixed isolates in 18 clinical cases of respiratory disease in bovines

Sl. No.	Type of bacterial isolate	No. of bovines positive
	Single isolates	
1.	*Streptococcus pyogenes*	2 (11.11)
	Mixed isolates	
(a)	**Two isolates**	
1.	*Staphylococcus aureus, Escherichia coli*	2 (11.11)
2.	*Staphylococcus aureus, Pasteurella haemolytica*	1 (5.55)
3.	*Staphylococcus epidermidis, Escherichia coli*	1 (5.55)
4.	*Streptococcus pyogenes, Pasteurella haemolytica*	1 (5.55)
5.	*Streptococcus pyogenes, Staphylococcus epidermidis*	3 (16.66)
6.	*Escherichia coli, Klebsiella pneumoniae*	1 (5.55)
7.	*Escherichia coli, Pseudomonas aeruginosa*	1 (5.55)
8.	*Klebsiella pneumoniae, Pseudomonas aeruginosa*	1 (5.55)
	Total	11 (61.11)
(b)	**Three isolates**	
1.	*Staphylococcus aureus, Klebsiella pneumoniae, Escherichia coli*	1(5.55)
2.	*Staphylococcus aureus, Klebsiella pneumoniae, Pasteurella haemolytica*	1(5.55)
3.	*Staphylococcus aureus, Streptococcus pyogenes, Pseudomonas aeruginosa*	1(5.55)
4.	*Staphylococcus epidermidis, Klebsiella pneumoniae, Pseudomonas aeruginosa*	1(5.55)
5.	*Streptococcus pyogenes, Escherichia coli, Proteus vulgaris*	1(5.55)
	Total	5(27.75)

Figures in parenthesis indicate per cent.

Plate 1. *Staphylococcus aureus* grown on Mannitol salt agar yielding yellow colonies and medium changing from pink to yellow.

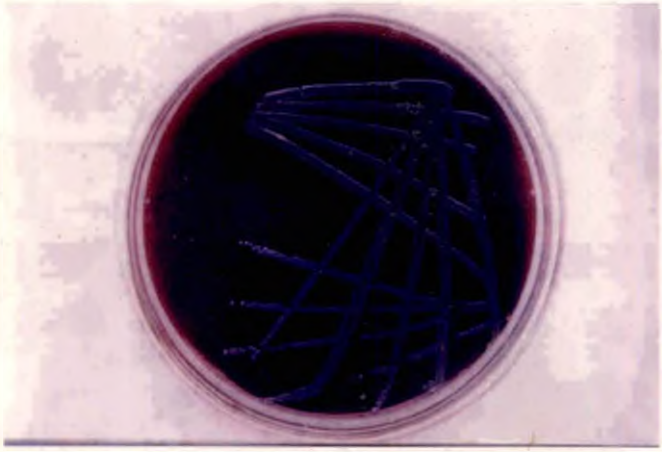


Plate 2. *Streptococcus pyogenes* grown on Edward's medium revealing transparent haemolytic colonies

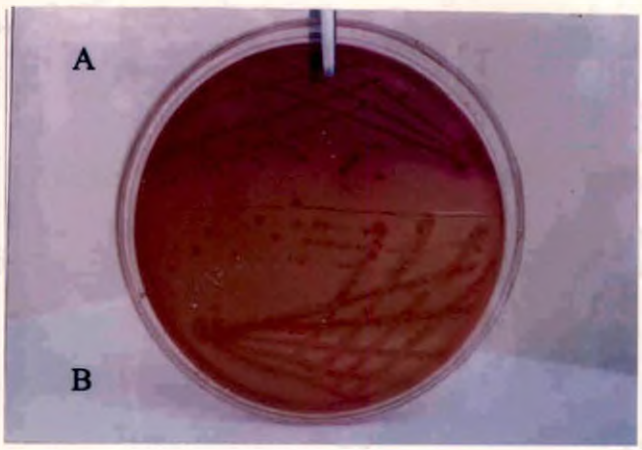


Plate 3. Mac Conkey agar differentiating (A) lactose fermenting colonies (B) lactose non-fermenting colonies

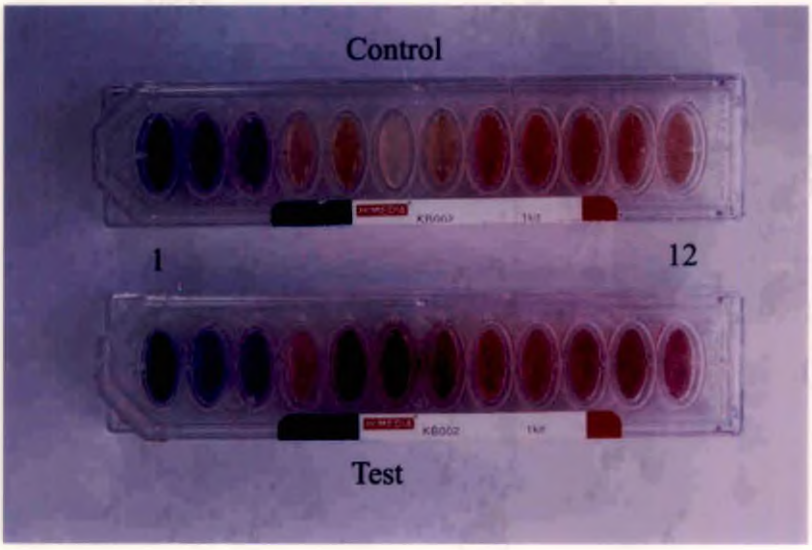
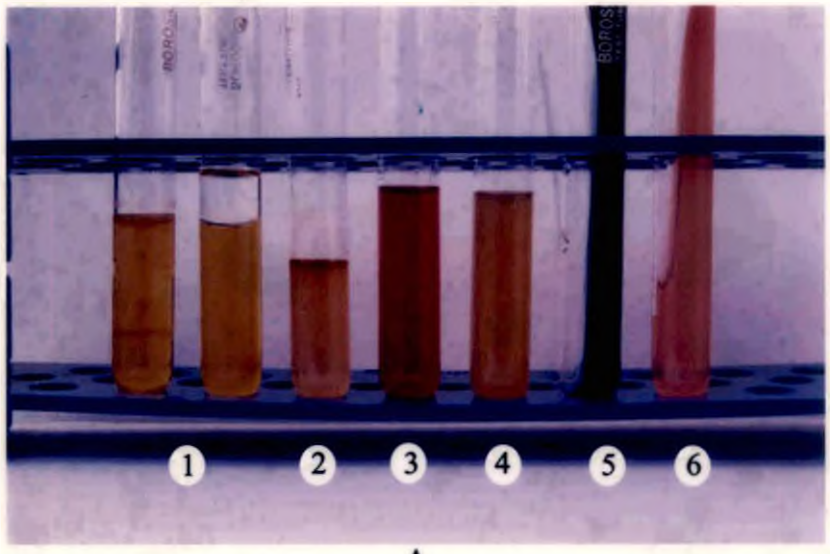


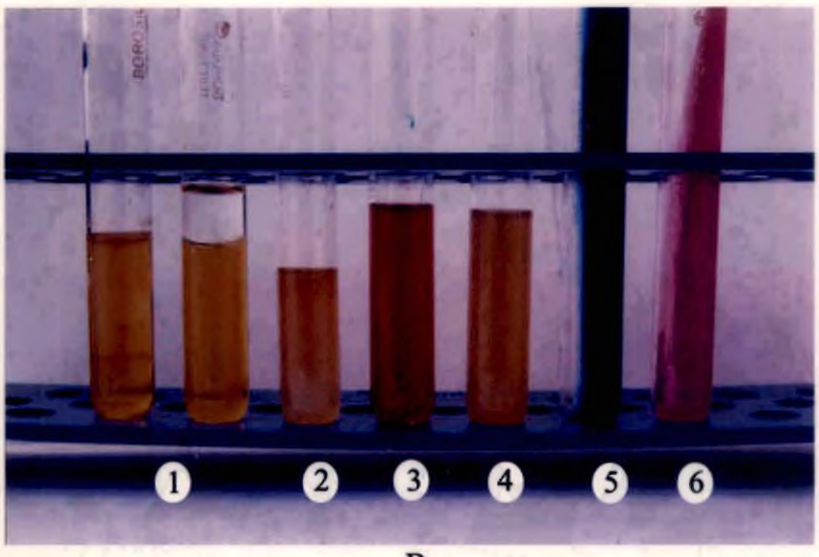
Plate 4. Hi assorted biochemical test kit

Tests

1. Citrate	+	7. H_2S	+
2. Lysine decarboxylation	+	8. Glucose	+
3. Ornithine decarboxylation	-	9. Adonitol	+
4. Urease	+	10. Lactose	+
5. TDA	+	11. Arabinose	+
6. Nitrate	+	12. Sorbitol	+



A



B

Plate 5. Battery of biochemical tests (A) *Escherichia coli* (B) *Klebsiella pneumoniae.*

	Tests	A	B
1	Oxidation-fermentation	Fermentation	Fermentation
2	Indole	Positive	Negative
3	Methyl red	Positive	Positive
4	Voges -proskauer	Negative	Negative
5	Citrate utilization	Negative	positive
6	Urease	Negative	Positive

4.4 ANTIBIOGRAM

In vitro antibiotic sensitivity studies of different isolates from respiratory tract infections showed that *Staphylococcus aureus* was sensitive to enrofloxacin and ciprofloxacin (100 per cent), gentamicin (83.30 per cent), chloramphenicol (66.66 per cent), amoxycillin, streptomycin and oxytetracycline (50 per cent) and trimethoprim (16.66 per cent) (Table 6).

The *Staphylococcus epidermidis* isolated was sensitive to enrofloxacin, gentamicin, ciprofloxacin (100 per cent) and to chloramphenicol (80 per cent) followed by amoxycillin (60 per cent). Only 40 per cent of the isolates were found to be sensitive to streptomycin, trimethoprim and oxytetracycline (Table 6).

Streptococcus pyogenes isolates were cent per cent sensitive to enrofloxacin and ciprofloxacin, 75 per cent to chloramphenicol and gentamicin, 50 per cent to streptomycin and oxytetracycline, 37.5 per cent to amoxycillin and 25 per cent to trimethoprim (Table 6).

None of the antibiotics tested showed cent per cent sensitivity to the *Escherichia coli* isolates. Maximum sensitivity was shown to chloramphenicol, enrofloxacin and ciprofloxacin (85.71 per cent). Isolates had a sensitivity of (71.43 per cent) to gentamicin and (42.86 per cent) to amoxycillin, streptomycin and oxytetracycline. Least sensitivity was shown to trimethoprim with 14.29 per cent (Table 6).

The *Klebsiella pneumoniae* isolated from respiratory tract infection was susceptible to enrofloxacin (100 per cent), to chloramphenicol and ciprofloxacin (80 per cent) and to amoxycillin, trimethoprim and oxytetracycline (60 per cent). Only (20 per cent) sensitivity was shown to streptomycin (Table 6).

Three *Pasteurella haemolytica* isolates obtained were susceptible to oxytetracycline and 2 of the isolates were susceptible to amoxycillin,

enrofloxacin, and trimethoprim. One of the isolates was sensitive to chloramphenicol, gentamicin, ciprofloxacin and none of the isolates were sensitive to streptomycin (Table 6).

All the four *Pseudomonas aerugenosa* isolated were susceptible to chloramphenicol, enrofloxacin, three to ciprofloxacin, oxytetracycline and two to amoxycillin and gentamicin (Table 6). Antibiogram of *Pseudomonas aerugenosa* is shown in (Plate 6).

Single bacterial isolate *Proteus vulgaris* obtained was found to be sensitive to amoxycillin, chloramphenicol, enrofloxacin, ciprofloxacin, gentamicin and streptomycin (Table 6). The isolate was not sensitive to streptomycin and trimethoprim.

Out of 19 Gram positive isolates, highest sensitivity was shown to enrofloxacin and ciprofloxacin (100 per cent) followed by gentamicin (84.21 per cent) and chloramphenicol (73.68 per cent). Resistance was noted in more than 50 per cent of isolates towards amoxycillin, streptomycin, oxytetracycline and trimethoprim. Whereas the Gram negative organisms of present study was not fully susceptible to any of the antimicrobial used. The order of sensitivity was to enrofloxacin (90 per cent), chloramphenicol (80 per cent), ciprofloxacin (75 per cent), oxytetracycline (60 per cent), amoxycillin (55 per cent), gentamicin (45 per cent), trimethoprim (30 per cent) and streptomycin (25 per cent) (Table 7).

Results of the *in vitro* antibiotic sensitivity tests in terms of sensitive, moderately sensitive and resistant are depicted in (Appendix 2).

The *in vitro* antibiotic sensitivity studies on 39 bacerial isolates from bovine respiratory tract infection revealed that enrofloxacin was the most sensitive antibiotic followed by ciprofloxacin, chloramphenicol, gentamicin, oxytetracycline, amoxycillin. More than half of the isolates were found to be resistant to trimethoprim and streptomycin (Table 8and Fig.3).

Table 6. ***In vitro*** **antimicrobial sensitivity pattern of 39 bacterial isolates associated with respiratory disease in bovines.**

Sl. No.	Microorganism	Total no. of isolates	Number of isolates sensitive							
			Am	C	Ex	GM	RC	SM	Tr	OX
1.	*Staphylococcus aureus*	6	3 (50)	4 (66.66)	6 (100)	5 (83.30)	6 (100)	3 (50)	1 (16.66)	3 (50)
2.	*Staphylococcus epidermidis*	5	3 (60)	4 (80)	5 (100)	5 (100)	5 (100)	2 (40)	2 (40)	2 (40)
3.	*Streptococcus pyogenes*	8	3 (37.5)	6 (75)	8 (100)	6 (75)	8 (100)	4 (50)	2 (25)	4 (50)
4.	*Escherichia coli*	7	3 (42.86)	6 (85.71)	6 (85.71)	5 (71.43)	6 (85.71)	3 (42.86)	1 (14.29)	3 (42.86)
5.	*Klebsiella pneumoniae*	5	3 (60)	4 (80)	5 (100)	0	4 (80)	1 (20)	3 (60)	3 (60)
6.	*Pasteurella haemolytica*	3	2 (66.66)	1 (33.33)	2 (66.66)	1 (33.33)	1 (33.33)	0	2 (66.66)	3 (100)
7.	*Pseudomonas aeruginosa*	4	2 (50)	4 (100)	4 (100)	2 (50)	3 (75)	0	0	3 (75)
8.	*Proteus vulgaris*	1	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	0	0

Am-Amoxycillin RC -Ciprofloxacin OX -Oxytetracycline Tr-Trimethoprim

C-Chloramphenicol GM -Gentamicin Ex-Enrofloxacin SM-Streptomycin

Plate 6. Antibiogram of *Pseudomonas aeruginosa*

Am- amoxycillin-sensitive	GM - gentamicin- sensitive
C - chloramphenicol-sensitive	OX - oxytetracycline-sensitive
RC- ciprofloxacin-sensitive	SM - streptomycin-moderately sensitive
Ex - enrofloxacin-sensitive	Tr - trimethoprim -resistant

Table 7. Antibiotic sensitivity tests of Gram positive and Gram negative isolates of bovine respiratory tract infection

Sl. No.	Antibiotic discs	Number of isolates sensitive	
		Gram positive	Gram negative
1	Amoxycillin (Am)	9 (47.37)	11 (55)
2	Chloramphenicol (C)	14 (73.68)	16 (80)
3	Enrofloxacin (Ex)	19 (100)	18 (90)
4	Gentamicin (GM)	16 (84.21)	9 (45)
5	Ciprofloxacin (Rc)	19 (100)	15 (75)
6	Streptomycin (SM)	9 (47.37)	5 (25)
7	Trimethoprim (Tr)	5 (26.32)	6 (30)
8	Oxytetracycline (OX)	9 (47.37)	12 (60)

Total number of Gram positive isolates = 19
Total number of Gram negative isolates = 20
Figures in parenthesis indicate percentage

Table 8. Percentage distribution of antimicrobial sensitivity pattern of 39 bacterial isolates from clinical cases of respiratory disease in bovines

Sl. No.	Antimicrobial agents		Sensitive		Moderately sensitive		Resistant	
			Number	Per cent	Number	Per cent	Number	Per cent
1.	Amoxycillin	(Am)	20	51.28	6	15.38	13	33.33
2.	Chloramphenicol	(C)	30	76.92	3	7.69	6	15.38
3.	Enrofloxacin	(Ex)	37	94.87	1	2.56	1	2.56
4.	Gentamicin	(GM)	25	64.10	11	28.21	3	7.69
5.	Ciprofloxacin	(Rc)	34	87.18	5	12.82	0	-
6.	Streptomycin	(SM)	14	35.90	11	28.21	14	35.90
7.	Trimethoprim	(Tr)	11	28.21	5	12.82	23	58.97
8.	Oxytetracycline	(OX)	21	53.85	6	15.38	12	30.77

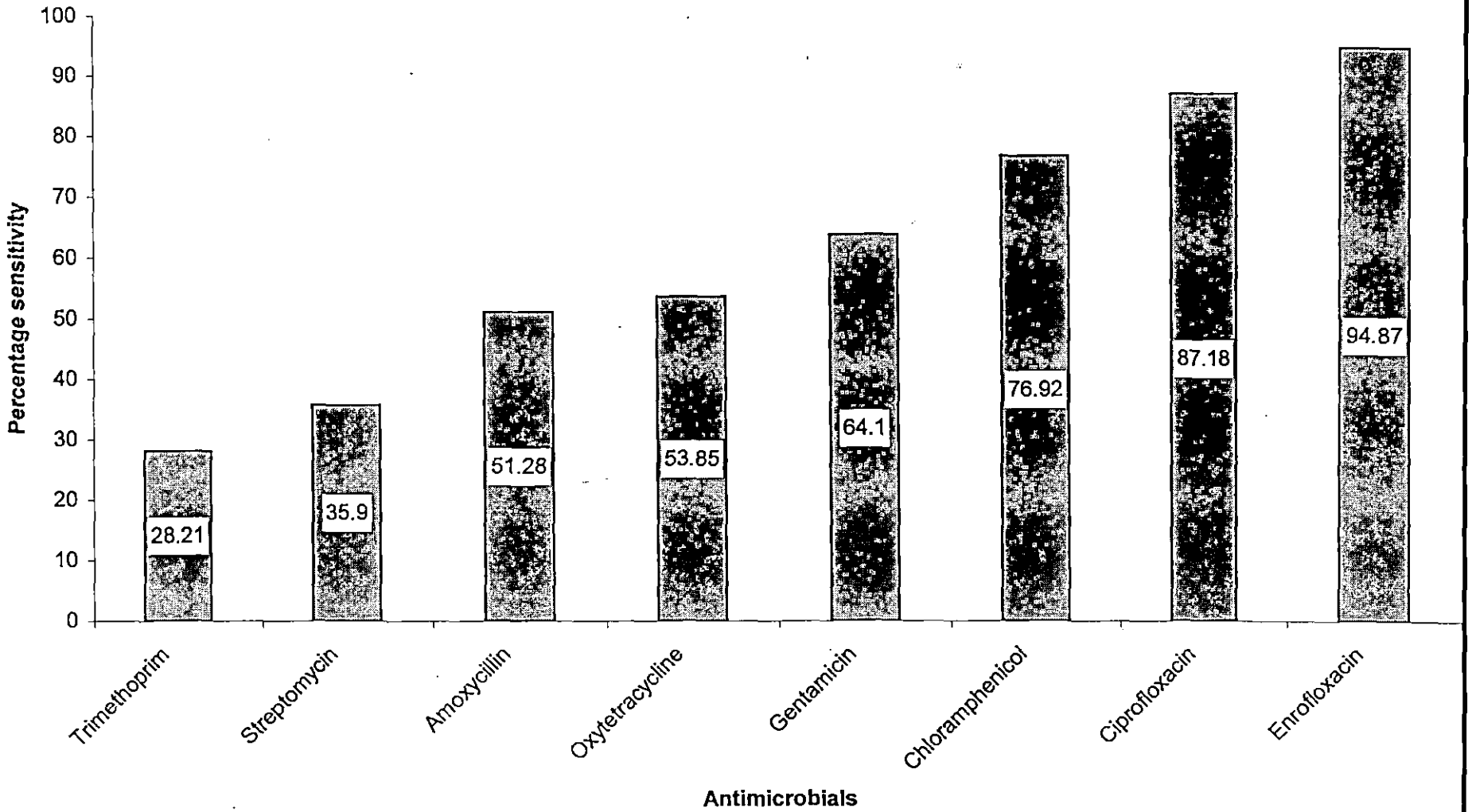


Fig. 3 Percentage Distribution of Antimicrobial sensitivity pattern of 39 isolates from bovine respiratory disease

4.5 EVALUATION OF ANIMALS WITH RESPIRATORY DISEASE

The bovines with clinical signs of respiratory disease were divided into two groups at random for the treatment trial. The haematological values of (group I and II) were compared with that of control animals (group III).

4.5.1 Haematological Parameters - Pre-Treatment Groups (I and II) and Group III

The following haematological parameters were estimated in bovines with respiratory tract infection (pre-treatment groups I and II) were compared statistically with the healthy bovines (group III).

4.5.1.1 Haemogram

4.5.1.1.1 Erythrocyte Sedimentation Rate (ESR)

The control animals had a mean ESR value of (3.67 ± 0.82 mm/24 h) whereas pre-treatment groups I and II had a mean ESR value of (14.25 ± 5.60mm/24 h and 11.88 ± 2.03mm/24 h) respectively. Highly significant difference ($P<0.01$) was noted in the mean values of ESR in both the pre-treatment groups with that of control (Table 9).

4.5.1.1.2 Packed Cell Volume (PCV)

Statistical analysis of PCV values revealed a significant reduction ($P<0.05$) in the mean values of pre-treatment groups I and II (31.38 ± 4.17 per cent and 32.5 ± 4.87 per cent) compared to the mean value of control animals (35.67 ± 1.63 per cent) (Table 9).

4.5.1.1.3 Haemoglobin (Hb)

The haemoglobin values were significantly lowered ($P<0.01$) in the diseased bovines (group I) compared to the group III (control). The mean values for group I and control were (10.73 ± 1.37 g/dl and 12.67 ± 0.82 g/dl)

respectively. But the reduction in the haemoglobin concentration in the group II was not statistically significant with the control. The mean value of haemoglobin in group II was (11.43 ± 1.62 g/dl) (Table 9).

4.5.1.1.4 *Total Erythrocyte Count*

There was a significant reduction ($p<0.05$) in the mean erythrocyte count in the pre-treatment groups I and II with control group and the mean values were (6.55 ± 0.92 x 10^6/mm^3, 6.78 ± 0.67 x 10^6/mm^3 and 7.68 ± 1.51 x 10^6/mm^3) respectively (Table 9).

4.5.1.1.5 Erythrocyte Indices

Mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were calculated for different groups and subjected to statistical analysis.

Mean Corpuscular Volume (MCV)

Statistical analysis revealed no significant difference in the mean values for MCV between pre-treatment groups I and II with group III (control). The mean values were (49.14 ± 3.86 fl, 48.55 ± 3.41 fl and 46.52 ± 1.31 fl) respectively (Table 9).

Mean Corpuscular Haemoglobin (MCH)

Statistical analysis revealed no significant difference in the mean MCH values between groups I and II with control. The mean values were (16.60 ± 1.18 pg, 16.80 ± 1.18 pg and 16.51 ± 0.49 pg) respectively (Table 9).

Mean Corpuscular Haemoglobin Concentration (MCHC)

No significant difference was observed between the mean MCHC values of pre-treatment groups I, II and control group III (33.90 ± 2.78 g/dl, 34.64 ± 1.33 g/dl and 35.49 ± 0.88 g/dl) by statistical analysis (Table 9).

4.5.1.2 Leukogram

4.5.1.2.1 Total Leukocyte Count (TLC)

Statistical analysis showed no significant difference in the mean total leukocyte count of pre-treatment groups I and II (10.81 ± 0.88 x $10^3/mm^3$ and 11.18 ± 0.42 x $10^3/mm^3$) from the control group, which was (10.44 ± 0.91 x $10^3/mm^3$) (Table 9).

4.5.1.2.2 Differential Leukocyte Count

Neutrophil Count

There was no significant difference between the mean values of control group (22.50 ± 1.38 per cent) and the pre-treatment groups I and II (23.25 ± 6.52 per cent and 23.90 ± 6.20 per cent) (Table 9).

Lymphocyte Count

The mean value of lymphocyte count for pre-treatment group I and control (73.38 ± 7.67 per cent and 71 ± 1.79 per cent) did not differ significantly. There was no significant difference between the mean values of pre-treatment group II (72 .50 ± 7.50 per cent) and control (71 ± 1.79 per cent) (Table 9).

Monocyte Count

The monocyte counts were not found statistically significant for the pre-treatment groups I and II (1.38 ± 1.51 per cent and 1.50 ± 1.40 per cent) with the control (2.83 ± 0.98 per cent) (Table 9).

Eosinophil Count

There was no significant difference in the mean value of pre-treatment groups I and II (1.75 ± 1.58 per cent and 1.90 ± 1.50) with that of control (3.33 ± 1.63 per cent) (Table 9).

Table 9. Haematological parameters – pre-treatment groups (I and II) and group III

Sl. No.	Parameters	Mean ± standard deviation		
		Group I (enrofloxacin) (n = 9)	Group III (control) (n = 6)	Group II (florfenicol) (n = 9)
	Haemogram			
1.	ESR mm/24 h	14.25 ± 5.60**	3.67 ± 0.82	11.88 ± 2.03**
2.	PCV per cent	31.38 ± 4.17*	35.67 ± 1.63	32.50 ± 4.87*
3.	Haemoglobin g/dl	10.73 ± 1.37**	12.67 ± 0.82	11.43 ± 1.62^{NS}
4.	RBC x 10^6/mm^3	6.55 ± 0.92*	7.68 ± 1.51	6.78 ± 0.67*
5.	MCV (fl)	49.14 ± 3.86^{NS}	46.52 ± 1.31	48.55 ± 3.41^{NS}
6.	MCH (pg)	16.60 ± 1.18^{NS}	16.51 ± 0.49	16.80 ± 1.18^{NS}
7.	MCHC (g/dl)	33.90 ± 2.78^{NS}	35.49 ± 0.88	34.64 ± 1.33^{NS}
	Leukogram			
1.	Total leukocyte count x 10^3/mm^3	10.81 ± 0.88^{NS}	10.44 ± 0.91	11.18 ± 0.42^{NS}
2.	Differential leukocyte count			
1.	Neutrophil (per cent)	23.25 ± 6.52^{NS}	22.50 ± 1.38	23.90 ± 6.20^{NS}
2.	Lymphocyte (per cent)	73.38 ± 7.67^{NS}	71 ± 1.79	72.50 ± 7.50^{NS}
3.	Monocyte (per cent)	1.38 ± 1.51^{NS}	2.83 ± 0.98	1.50 ± 1.40^{NS}
4.	Eosinophil (per cent)	1.75 ± 1.58^{NS}	3.33 ± 1.63	1.90 ± 1.50^{NS}

NS - Nonsignificant ($P \geq 0.05$)
* - Significant ($P < 0.05$)
** - Highly significant ($P < 0.01$)

4.6 TREATMENT TRIAL WITH ENROFLOXACIN AND FLORFENICOL

Eighteen animals with respiratory diseases were allotted at random into two groups and treated with enrofloxacin and florfenicol. They were subjected to treatment trial for three to five days with enrofloxacin (group I) depending on the severity of infection and three days with florfenicol (group II). In both groups there was a complete clinical cure in six cases and recurrence was noticed in two cases in both the groups and there was a failure of treatment in one case in each group. Various clinical data and haematological parameters of these animals before and after therapy were compared statistically by paired t-test.

4.6.1. Clinical Data of Pre and Post-Treatment Groups (I and II)

The mean temperature (°F), pulse and respiration per minute obtained in the group 1 before and after treatment were (103.05 ± 1.48, 80.63 ± 0.92, 54.5 ± 9.72, 101.75 ± 0.46, 80.38 ± 0.74 and 40.50 ± 8.60) respectively. The difference observed in average body temperature before and after treatment with enrofloxacin was highly significant ($P<0.01$). The difference observed in the average rate of respiration was highly significant ($P<0.01$) in the affected animals of group 1 compared to the animals recovered from the disease. No significant difference was noted in the pulse rate of affected and recovered animals eventhough a slightly elevated value was noticed in diseased animals. (Table 10).

The mean body temperature (°F), pulse and respiration per minute obtained in animals treated with florfenicol before and after treatment were (103.05 ± 0.55, 82 ± 1.41, 55.75 ± 12.80, 102.05 ± 0.14, 81.63 ± 1.51 and 40 ± 6.23) respectively. The difference observed in average body temperature was significantly higher in diseased animals than recovered animals ($P<0.05$). The difference observed in average respiration rate was highly significant ($P<0.01$) in the affected animals of group II compared to the animals recovered from the disease. No significant difference was noted in the pulse rate of affected and

recovered animals even though a slightly elevated value was noticed in diseased animals (Table 10).

4.6.2 Haematological Parameters of Pre and Post-Treatment Groups (I and II)

4.6.2.1 Haemogram

4.6.2.1.1 Erythrocyte Sedimentation Rate (ESR)

A highly significant reduction ($P<0.01$) in the mean ESR value was observed after treatment in both group I and group II (8.0 ± 2.20 mm/24 h and 6.88 ± 1.96 mm/24 h) compared to pre-treatment mean values (14.25 ± 5.60 mm/24 h and 11.88 ± 2.03 mm/24 h) (Table 11).

4.6.2.1.2 Packed Cell Volume (PCV)

The mean PCV values that recorded before and after treatment in group I were (31.38 ± 4.17 per cent and 32.38 ± 4.21 per cent) and in group II were (32.50 ± 4.87 per cent and 33.38 ± 4.93 per cent) respectively. A significant increase ($P<0.05$) could be observed after therapy in both groups (Table 11).

4.6.2.1.3 Haemoglobin (Hb)

A significant increase ($P<0.05$) in the mean value of haemoglobin value (11.18 ± 1.38 g/dl) was observed after treatment compared to the mean pre-treatment value of (10.73 ± 1.37 g/dl) in group I (Table 11).

No significant difference was noted between the pre-treatment mean value (11.43 ± 1.62 g/dl) and post-treatment mean value of (11.75 ± 1.37 g/dl) in group II even though a slight increase was noticed (Table 11).

Table 10. Clinical data of pre and post-treatment groups (I and II) (n=8)

Sl. No.	Parameter	Group I (treated with enrofloxacin)		Group II (treated with florfenicol)	
		Before treatment	After treatment	Before treatment	After treatment
1.	Temperature (°F)	103.05 ± 1.48	101.75 ± 0.46*	103.05 ± 0.55	102.05 ± 0.14**
2.	Pulse/min	80.63 ± 0.92	80.38 ± 0.74^{NS}	82 ± 1.41	81.63 ± 1.51^{NS}
3.	Respiration/min	54.50 ± 9.72	40.50 ± 8.60**	55.75 ± 12.80	40 ± 6.23**

NS - Nonsignificant (P≥0.05)
** - Highly significant (P<0.01)
* -Significant (p<0.05)

4.6.2.1.4 Total Erythrocyte Count

The mean values for RBC count before and after treatment in group I and group II were ($6.55 \pm 0.92 \times 10^6/mm^3$ and $6.69 \pm 0.94 \times 10^6/mm^3$, $6.78 \pm 0.67 \times 10^6/mm^3$ and $6.89 \pm 0.86 \times 10^6 / mm^3$). No significant difference was observed in the values of erythrocyte count after treatment though a slight increase was observed from pre-treatment values (Table 11).

4.6.2.1.5 Erythrocyte Indices

Statistical analysis showed significant difference between the pre and post-treatment mean values of MCV (49.14 ± 3.86 fl and 46.84 ± 4.75 fl) of group I. No significant difference was observed in the pre and post-treatment mean values of MCV (48.55 ± 3.41 fl and 48.74 ± 5.27 fl) of group II (Table 11).

No significant difference was observed in the mean values of MCH and MCHC between pre and post-treatment values of group I and II (16.50 ± 1.18 pg and 16.33 ± 1.46 pg, 16.80 ± 1.18 pg and 16.89 ± 1.33 pg, 33.90 ± 2.78 g/dl and 35.09 ± 3.78 g/dl and 34.64 ± 1.33 g/dl and 35.08 ± 2.89 g/dl) (Table 11).

4.6.2.2 Leukogram

4.6.2.2.1 Total Leukocyte Count (TLC)

Mean values for TLC before and after treatment in group I and group II were ($10.81 \pm 5.60 \times 10^3/mm^3$ and $10.47 \pm 0.82 \times 10^3/mm^3$ and $11.18 \pm 0.42 \times 10^3/mm^3$ and $10.73 \pm 0.52 \times 10^3/mm^3$) respectively. Statistical analysis revealed no significant difference between the TLC values before and after treatment (Table 11).

4.6.2.2.2 Differential Leukocyte Count (DLC)

No significant difference was observed between the pre and post-treatment mean values of differential cell counts in both group I and group II. The mean values for neutrophil, lymphocyte, monocyte and eosinophil in group I

before treatment were (23.25 ± 6.52 per cent, 73.38 ± 7.67 per cent, 1.38 ± 1.51 per cent, 1.75 ± 1.58 per cent) and after treatment were (23.88 ± 7.45 per cent, 72.88 ± 7.24 per cent, 1.50± 1.31 per cent and 1.88 ± 1.25 per cent) respectively. The values for group II before treatment were (23.90 ± 6.20 per cent, 72.50 ± 7.50 per cent, 1.50 ± 1.40 per cent and 1.90 ± 1.50 per cent) and after treatment were (24.10 ± 7.50 per cent, 72.40 ± 7.40 per cent, 1.60 ± 1.20 per cent and 2 ± 1.13 per cent) respectively (Table 11).

4.7 RESPONSE TO TREATMENT

Recovery was noticed in 8 out of nine animals with respiratory disease that were subjected to treatment trial with enrofloxacin in group I and with florfenicol in group II. In both groups there was recurrence of the disease in two cases. There was a failure in treatment in one case each in both groups, one succumbed to death and the other was disposed off by the owner. Haematological parameters obtained after treatment were compared with the apparently healthy animals (group III) to assess the response to treatment.

4.7.1 Clinical Assessment

Detailed clinical observations were recorded from nine animals of group I and group II before and after treatment. Depending on the degree of the severity of infection, duration of therapy varied between 3 to 5 days in group I, but no cattle required treatment with florfenicol beyond three days. Clinical condition improved rapidly in majority of animals along with the reduction in pyrexia and respiratory rate. The animals became more alert, their appetite improved and the clinical signs of respiratory dysfunction waned. The clinical improvement was more noticeable in the florfenicol treated cattle after 24 h of treatment. One animal in the enrofloxacin treated group required treatment for 5 days.

During the observation period after the completion of therapy two animals in both groups had recurrence of the symptoms of respiratory tract

Table 11. Haematological parameters of pre and post-treatment groups (I and II) (n=8)

Sl. No.	Parameters	Group I (enrofloxacin)		Group II(florfenicol)	
		before treatment	after treatment	before treatment	after treatment
	Haemogram				
1.	ESR mm/24 h	14.25 ± 5.60	8.00 ± 2.20**	11.88 ± 2.03	6.88 ± 1.96**
2.	PCV (per cent)	31.38 ± 4.17	32.38 ± 4.21*	32.50 ± 4.87	33.38 ± 4.93*
3.	Haemoglobin (g/dl)	10.73 ± 1.37	11.18 ± 1.38*	11.43 ± 1.62	11.75 ± 1.37^{NS}
4.	RBC x 10^6/mm^3	6.55 ± 0.92	6.69 ± 0.94^{NS}	6.78 ± 0.67	6.89 ± 0.86^{NS}
	Erythrocyte indices				
1.	MCV (fl)	49.14 ± 3.86	46.84 ± 4.75*	48.55 ± 3.41	48.74 ± 5.27^{NS}
2.	MCH (pg)	16.50 ± 1.18	16.33 ± 1.46^{NS}	16.80 ± 1.18	16.89 ± 1.33^{NS}
3.	MCHC (g/dl)	33.90 ± 2.78	35.09 ± 3.78^{NS}	34.64 ± 1.33	35.08 ± 2.89^{NS}
	Leukogram				
1.	Total leukocyte count x10^3/mm^3	10.81 ± 5.60	10.47 ± 0.82^{NS}	11.18 ± 0.42	10.73 ± 0.52^{NS}
2.	Differential leukocyte count				
	Neutrophil (per cent)	23.25 ± 6.52	23.88 ± 7.45^{NS}	23.90 ± 6.20	24.10 ± 7.50^{NS}
	Lymphocyte (per cent)	73.38 ± 7.67	72.88 ± 7.24^{NS}	72.50 ± 7.50	72.40 ± 7.40^{NS}
	Monocyte (per cent)	1.38 ± 1.51	1.50 ± 1.31^{NS}	1.50 ± 1.40	1.60 ± 1.20^{NS}
	Eosinophil (per cent)	1.75 ± 1.58	1.88 ± 1.25^{NS}	1.90 ± 1.50	2 ± 1.10^{NS}

NS - Nonsignificant ($P \geq 0.05$)
* - Significant ($P < 0.05$)
** - Highly significant ($P < 0.01$)

infection. In order that the welfare of the animals was not compromised they were given additional therapy and were considered as recurrence of infection.

4.7.2 Haematological Parameters - Post-Treatment Groups (I and II) and Group III

The following haematological parameters estimated after treatment in bovines with respiratory tract infection (post-treatment groups I and II) were compared with the healthy bovines (group III).

4.7.2.1 Haemogram

4.7.2.1.1 Erythrocyte Sedimentation Rate (ESR)

The mean ESR values of animals recovered from the disease after treatment of groups I and II (8 ± 2.20 mm/24 h and 5.88 ± 1.96mm/24 h) were found significantly different ($P<0.05$) from that of control group (3.97 ± 0.82mm/24h)(Table 12).

4.7.2.1.2. Packed Cell Volume (PCV)

There was no significant difference in the PCV between the post-treatment groups (I and II) and the healthy animals (group III). The mean value of PCV in post-treatment groups I and II were (32.38 ± 4.21per cent and 33.38 ± 4.93per cent) and that of group III was (35.67±1.63 per cent) (Table 12).

4.7.2.1.3 Haemoglobin (Hb)

A significant reduction of haemoglobin ($p<0.05$) was observed in post-treatment group I with the control. There was no significant difference in the post-treatment group II with that of control. The mean haemoglobin values of post-treatment groups I and II for haemoglobin were (11.18 ± 1.34 g/dl and 11.75 ± 1.37 g/dl) and for control was (12.67 ± 0.82 g/dl) (Table 12).

4.7.2.1.4. Total Erythrocyte Count

There existed a significant reduction ($P < 0.05$) in the mean value of total erythrocyte count of group I animals treated with enrofloxacin (6.69 ± 0.94 x $10^6/mm^3$) with the healthy animals (7.68 ± 1.51 x $10^6/mm^3$). No significant difference was noted between the mean values of total erythrocyte count of group II animals treated with florfenicol (6.89 ± 0.86 x $10^6/mm^3$) with the healthy animals (Table 12).

4.7.2.1.5 Erythrocyte Indices

Mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration were calculated for post-treatment groups and subjected to statistical analysis.

Mean Corpuscular Volume (MCV)

Mean value of MCV of recovered animals of groups I, II and control were (46.84 ± 4.75 fl, 48.74 ± 5.27 fl and 46.52 ± 1.31 fl) respectively. No significant difference existed between recovered animals of groups I and II with the control (Table 12).

Mean Corpuscular Haemoglobin (MCH)

The mean MCH values for post-treatment groups I, II and control (16.33 ± 1.46 pg, 16.88 ± 1.33 pg and 16.51 ± 0.49 pg) did not differ significantly from one another (Table 12).

Mean Corpuscular Haemoglobin Concentration (MCHC)

The mean MCHC values for post-treatment groups I, II and control (35.09 ± 3.78 g/dl, 35.08 ± 2.89 g/dl and 35.49 ± 0.88 g/dl) did not differ significantly from one another (Table 12).

4.7.2.2 Leukogram

4.7.2.2.1 Total Leukocyte Count (TLC)

There was no significant difference between the mean values of TLC of control group (10.44 ± 0.91 x $10^3/mm^3$) and the post treatment groups I and II (10.47 ± 0.82 x $10^3/mm^3$ and 10.73 ± 0.52 x $10^3/mm^3$) (Table 12).

4.7.2.2.2 Differential Leukocyte Count (DLC)

Neutrophil Count

The mean values of neutrophil count for post-treatment groups I and II (23.88 ± 7.45 per cent and 24.10 ± 7.50 per cent) did not differ significantly from the control (22.50 ± 1.38) (Table 12).

Lymphocyte Count

The mean values of lymphocyte count for post-treatment groups I and II were (72.88 ± 7.24 per cent and 72.40 ± 7.40 per cent) did not differ significantly from that of control (71 ± 1.79 per cent) (Table 12).

Monocyte Count

No significant difference existed between the mean values of post-treatment groups I and II (1.50 ± 1.30 per cent and 1.60 ± 1.20 per cent) from the control (2.83 ± 0.98 per cent) (Table 12).

Eosinophil count

The mean values of eosinophil count for post-treatment groups I and II (1.88 ± 1.25 per cent and 2 ± 1.10 per cent) did not differ significantly from that of control (3.33 ± 1.63 per cent) (Table 12).

Table 12. Haematological parameters - post-treatment groups (I and II) and group III

Sl. No.	Parameters	Mean ± standard deviation		
		Group I (enrofloxacin) (n = 8)	Group III (control) (n = 6)	Group II (florfenicol) (n = 8)
	Haemogram			
1.	ESR mm/24 h	8 ± 2.20*	3.97 ± 0.82	5.88 ± 1.96*
2.	PCV per cent	32.38 ± 4.21^{NS}	35.67 ± 1.63	33.38 ± 4.93^{NS}
3.	Haemoglobin g/dl	11.18 ± 1.34*	12.67 ± 0.82	11.75 ± 1.37^{NS}
4.	RBC x 10^6/mm^3	6.69 ± 0.94*	7.68 ± 1.51	6.89 ± 0.86^{NS}
	Erythrocyte indices			
5.	MCV (fl)	46.84 ± 4.75^{NS}	46.52 ± 1.31	48.74 ± 5.27^{NS}
6.	MCH (pg)	16.33 ± 1.46^{NS}	16.51 ± 0.49	16.88 ± 1.33^{NS}
7.	MCHC (g/dl)	35.09 ± 3.78^{NS}	35.49 ± 0.88	35.08 ± 2.89^{NS}
	Leukogram			
1.	Total leukocyte count x 10^3/mm^3	10.47 ± 0.82^{NS}	10.44 ± 0.91	10.73 ± 0.52^{NS}
2.	Differential leukocyte count			
1.	Neutrophil (per cent)	23.88 ± 7.45^{NS}	22.50 ± 1.38	24.10 ± 7.50^{NS}
2.	Lymphocyte (per cent)	72.88 ± 7.24^{NS}	71 ± 1.79	72.40 ± 7.40^{NS}
3.	Monocyte (per cent)	1.50 ± 1.30^{NS}	2.83 ± 0.98	1.60 ± 1.20^{NS}
4.	Eosinophil (per cent)	1.88 ± 1.25^{NS}	3.33 ± 1.63	2 ± 1.10^{NS}

NS - Nonsignificant ($P \geq 0.05$)
* - Significant ($P < 0.05$)

Table 13. Clinical data of pre-treatment groups I and II (n=8)

Sl. No.	Parameter	Pre-treatment	
		Group I	Group II
1	Temperature (°F)	103.05 ± 1.48	103.05 ± 0.55^{NS}
2	Pulse/min	80.63 ± 0.92	82 ± 1.41*
3	Respiration/min	54.50 ± 9.72	55.75 ± 12.80^{NS}

NS - Nonsignificant (P≥0.05)
* - Significant (P<0.05)

Table 14. Clinical data of post-treatment groups I and II (n=8)

Sl. No.	Parameter	Post-treatment	
		Group I	Group II
1	Temperature (°F)	101.75 ± 0.46	102.05 ± 0.14^{NS}
2	Pulse/min	80.38 ± 0.74	81.63 ± 1.51*
3	Respiration/min	40.50 ± 8.60	40.00 ± 6.23^{NS}

NS - Nonsignificant (P≥0.05)
* - Significant (P<0.05)

Table 15. Haematological parameters of pre-treatment groups I and II (n=8)

Sl. No.	Parameters	Mean ± standard deviation	
		Group I after treatment (enrofloxacin)	Group II after treatment (florfenicol)
	Haemogram		
1.	ESR mm/24 hr	14.25 ± 5.6	11.88 ± 2.03^{NS}
2.	PCV per cent	31.38 ± 4.17	32.50 ± 4.87^{NS}
3.	Haemoglobin g/dl	10.73 ± 1.37	11.43 ± 1.62^{NS}
4.	RBC x 10^6/mm^3	6.55 ± 0.92	6.78 ± 0.67^{NS}
	Erythrocyte indices		
5.	MCV (fl)	49.14 ± 3.86	48.55 ± 3.41^{NS}
6.	MCH (pg)	16.50 ± 1.18	16.80 ± 1.18^{NS}
7.	MCHC (g/dl)	33.90 ± 2.78	34.64 ± 1.33^{NS}
	Leukogram		
1.	Total leukocyte count x 10^3/mm^3	11.18 ± 0.42	10.81 ± 0.88^{NS}
2.	Differential leukocyte count		
1.	Neutrophil (per cent)	21.88 ± 9.02	23.25 ± 6.52^{NS}
2.	Lymphocyte (per cent)	75 ± 8.90	73.38 ± 7.67^{NS}
3.	Monocyte (per cent)	2 ± 0.76	1.38 ± 1.51^{NS}
4.	Eosinophil (per cent)	1 ± 0.93	1.75 ± 1.59^{NS}

NS – Nonsignificant ($P \geq 0.05$)

Table 16. Haematological parameters of recovered animals of 2 post-treatment groups (I and II) (n=8)

Sl. No.	Parameters	Mean ± standard deviation	
		Group I after treatment (enrofloxacin)	Group II after treatment (florfenicol)
	Haemogram		
1.	ESR mm/24 h	8 ± 2.20	6.88 ± 1.96^{NS}
2.	PCV per cent	32.38 ± 4.21	33.38 ± 4.93^{NS}
3.	Haemoglobin g/dl	11.18 ± 1.38	11.75 ± 1.37^{NS}
4.	RBC x $10^6/mm^3$	6.69 ± 0.94	6.89 ± 0.86^{NS}
	Erythrocyte indices		
5.	MCV (fl)	46.84 ± 4.75	48.74 ± 5.27^{NS}
6.	MCH (pg)	16.33 ± 1.46	16.89 ± 1.33^{NS}
7.	MCHC (g/dl)	35.09 ± 3.78	35.08 ± 2.90^{NS}
	Leukogram		
1.	Total leukocyte count x $10^3/mm^3$	10.47 ± 0.82	10.73 ± 0.52^{NS}
2.	Differential leukocyte count		
1.	Neutrophil (per cent)	23.88 ± 7.45	24.10 ± 7.50^{NS}
2.	Lymphocyte (per cent)	72.88 ± 7.24	72.40 ± 7.40^{NS}
3.	Monocyte (per cent)	1.50± 1.31	1.60 ± 1.20^{NS}
4.	Eosinophil (per cent)	1.88 ± 1.25	2 ± 1.10^{NS}

NS - Nonsignificant ($P \geq 0.05$)

4.8 EVALUATION OF THE EFFICACY OF DRUGS

In order to evaluate the efficacy of enrofloxacin and florfenicol, clinical data and haematological parameters of pre-treatment groups I and II and post-treatment groups (I and II) were compared statistically.

4.8.1 Clinical Data - Pre-Treatment Groups (I and II) and Post-Treatment Groups (I and II)

There was no significant difference in the mean values of body temperature (°F) and respiration per minute between the pre-treatment groups I and II .The mean values were (103.05 ± 1.48, 54.50 ± 9.72, 103.05 ±0.55 and 55.75 ± 12.80) respectively (Table 13).

After therapy with two antimicrobials, the recovered animals recorded a mean temperature (° F) of (101.75 ± 0.46 and 102.05 ± 0.14) and a mean respiratory rate of (40.50 ± 8.60 and 40 ± 6.23) in groups I and II. No significant difference was noted in the temperature and respiration rate between the two post-treatment groups (Table 14).

4.8.2 Haematological Parameters between Two Pre-Treatment Groups and Two Post-Treatment Groups

The pre-treatment haematological parameters of groups I and II were compared. Results indicated that there was no significant difference in the pre-treatment groups I and II with respect to haemogram, erythrocyte indices and leukogram (Table 15).

Post-treatment haematological parameters were compared to assess the efficacy of antimicrobials used. The results indicated that no significant difference existed between different haematological parameters between the two groups of antimicrobial therapy (Table 16).

Discussion

5. DISCUSSION

Bacterial infections of respiratory tract in bovines are a baffling problem in the developing countries and have attracted the all time attention of veterinary scientists. The therapeutic management of such infections needs updating of the antibiotic sensitivity test of various bacterial isolates having predilection to the respiratory system. The clinical trial to evaluate newer chemotherapeutic agents in the treatment of respiratory infection is of paramount importance for the clinician to opt for a better antimicrobial in a world of antibiotic resistance of the bacterial organism. In these circumstances the present study gives an insight into the judicious selection of an ideal antimicrobial for the therapeutic management of bacterial infections of respiratory tract in bovines. The present study envisages the clinical trial to evaluate the comparative efficacy of two antibiotics, namely enrofloxacin and florfenicol in the treatment of bovine respiratory disease.

5.1 CLINICAL FINDINGS

5.1.1 Clinical Data

The high statistical differences noted in the rectal temperature of diseased animals from that of control emphasizes the infectious etiology of respiratory tract with no involvement of the other systems of body which is evident from the history and clinical examination. Pyrexia manifested by 11 animals (61.11 per cent) may be attributed to the toxaemic conditions whereas in rest of the animals infection may be suggestive of less severe form of infection. A high statistical significance in the respiratory rate was also noted between diseased and control animals.

5.1.2 Clinical Symptoms

The common complaints exhibited by the animals included in this study were inappetance, nasal discharge, reduction in milk production and cough. On clinical examination there was exaggerated sounds on auscultation of trachea in

all the cases that is indicative of upper respiratory tract infection whereas crackles on auscultation of the lung was evident only in two cases. Nasal discharge of varying nature was present in most of the cases (94.44 per cent). Inspiratory dyspnoea was evident in 15 (83.33 per cent) cases. In 11 animals (61.11 per cent) there was pyrexia. Lymphadenomegaly was noticed in 14 (77.77 per cent) cases. Cough was present in 7 cases (38.88 per cent), six cases (33.33 per cent) showed congestion of mucous membrane. Reduction in milk production was noticed in five out of seven lactating animals. Based on the manifestation of clinical signs animals were given clinical illness index score of slightly ill (1), moderately ill (2) and severely ill (3) in 5 (27.77 per cent), 12 (66.66 per cent) and 1 (5.55 per cent) of the diseased animals.

There are varying opinions regarding the most useful case definition in respiratory disease in bovines. Many protocols have been used to select animals that require antimicrobial therapy. Clinical signs exhibited by the animals selected for the therapeutic trial was in accordance with many workers (Lekeux and Art, 1988; Martin *et al.*, 1989; Allen *et al.*, 1991; Nasser and El-Sayed, 1997). An obvious limitation observed in this clinical trial was the diverse nature of clinical signs which has made the clinical assessment of cases quite subjective. This is important in the study where the relevance of the differences found between the cases and controls depends on the criteria used to define the case. It is due to this reason, we have adopted a simple clinical illness index score (Table 1) in order to evaluate the clinical cases included in the study. This makes the assessment more objective and helps to record the nature and prevalence of the clinical signs exhibited in different cases and it was recorded as per the proforma (Appendix I).

5.2 EXAMINATION OF CLINICAL MATERIALS

No bacteria could be isolated from defibrinated blood.All the nasal swabs/ deep nasal washings were positive for bacteria.

5.3 BACTERIAL ISOLATES FROM RESPIRATORY TRACT INFECTION

Different aerobic bacteria isolated from deep nasal washings of bovines with respiratory disease were *Staphylococcus aureus* (15.38 per cent), *Staphylococcus epidermidis* (12.82 per cent), *Streptococcus pyogenes* (20.51 per cent), *Escherichia coli* (17.95 per cent), *Klebsiella pneumoniae* (12.82 per cent), *Pasteurella haemolytica* (7.69 per cent), *Pseudomonas aeruginosa* (10.26 per cent) and *Proteus vulgaris* (2.56 per cent). All of these organisms except *Proteus vulgaris* were isolated from bovines with respiratory disease by earlier workers (Wilkie and Shewen, 1988; Allen *et al.*, 1991; Katoh *et al.*, 1996; Wikse and Baker, 1996; Nasser and El-Sayed, 1997; Sahin, 1997; Gunduz and Erganis, 1998; Selim *et al.*, 1998; Aguade and Romero, 2000; Hutt and Goossens, 2001; Lonaragan *et al.*, 2001; Aslan *et al.*, 2002; Catry *et al.*, 2002). The same workers have isolated mycoplasmas, acholeplasmas, ureaplasmas and chlamydias from bovine respiratory tract infections.

In the present study out of the 39 bacterial isolates 19 (48.71 per cent) were Gram positive organisms and 20 (51.29 per cent) were Gram negative organisms. In 88.89 per cent of animals more than one bacterium could be isolated. But this is not in accordance with the work of Catry *et al.* (2002) who isolated more than one bacterium in only 14 per cent of cases from 80 calves suffering from acute respiratory distress.

Streptococcus pyogenes was obtained from 20.51 per cent of cases. This bacteria was isolated singly only in two cases. Many workers have isolated this organism from respiratory tract infection of bovine (Wikse and Baker, 1996; Nasser and El-Sayed, 1997; Sahin, 1997; Aslan *et al.*, 2002; Catry *et al.*, 2002). Synergy of these organisms with *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Pasteurella haemolytica* and *Pseudomonas aeruginosa* might have complicated the disease manifestation of the cases in the present study.

Previous research workers have reported isolation of staphylococci from respiratory tract infections in bovines (Wikse and Baker, 1996; Nasser and El-Sayed, 1997; Sahin, 1997; Aslan *et al.*, 2002). Staphylococci are considered to be opportunistic pathogens found on the skin and capable of invading and localizing in any part of the tissue under suitable conditions. They produce a variety of enzymes and toxins that are believed to play a role in initiating the infection (Gillespie and Timoney, 1981). These organisms can invade the lungs under environmental stress, causing pneumonia, abscess in the lung parenchyma and death.

Out of the Gram negative organisms *Escherichia coli* was isolated from seven cases (17.95 per cent). *Escherichia coli* has been isolated from respiratory disease complex of cattle, sheep and goat (Wikse and Baker, 1996; Nasser and El-Sayed, 1997; Sahin, 1997; Selim *et al.*, 1998; Aslan *et al.*, 2002).

Klebsiella pneumoniae contributed 12.82 per cent of total isolates in the present study. These organisms were isolated from the two cases where infection of lungs was evident by auscultation. Klebsiella organism with its capsular factor is capable of producing acute bronchopneumonia and more chronic lesions with multiple abscess formation in the lungs (Limson *et al.*, 1956). In the present work isolation of this organism from infected cases correlates with the findings of Aslan *et al.* (2002).

Four isolates of *Pseudomonas aeruginosa* obtained from nasal washings of bovines constituted about 10.26 per cent of cases in the experimental group. Most of the *Pseudomonas aeruginosa* produce proteolytic enzymes like protease, alkaline protease and an elastase, which helps in the degradation of a wide range of substrates including casein, elastin, gelatin, collagen and fibrin (Morihara, 1964). Isolation of these organisms from respiratory infections of bovines has been reported by Wikse and Baker (1996).

In the present study *Mannheimia haemolytica* was isolated only from 3 cases (7.69 per cent). Eventhough many doubts have been cast upon the significance of pasteurella spp. as primary pathogens in bovine respiratory disease, these are the sole organisms isolated from the respiratory tract of pneumonic calves of all ages and occasionally from nonpneumonic tissue by previous workers (Frank, 1986; Wilkie and Shewen, 1988; Allen *et al.,* 1991; Katoh *et al.,* 1996; Wikse and Baker, 1996; Nasser and El-Sayed, 1997; Sahin, 1997; Gunduz and Erganis, 1998; Selim *et al.*, 1998; Aguade and Romero, 2000; Hutt and Goossens, 2001; Lonaragan *et al.*, 2001; Rowe *et al.*,2001; Aslan *et al.*, 2002 ; Catry *et al.*, 2002). It is noteworthy that *Mannheimia haemolytica* or *Pasteurella multocida* was not significantly associated with clinical cases. Although there is a great deal of evidence in the literature for a causal association of *Mannheimea haemolytica* with bovine respiratory disease and in particular with fibrinous pneumonia, present study shows that not all cases involve *Mannheimia haemolytica* and that it may not be of major importance in many disease outbreaks. This may be particularly true of the cases such as the one studied here, which was not associated with high mortality. *Mannheimia haemolytica* has been more associated with mortality, presumably due to the ability to cause severe pulmonary lesions and to develop resistance to certain commonly used antimicrobials.

Although *Pasteurella multocida* are often implicated as the major causative agent of respiratory infection in cattle, no organism could be isolated from live clinical cases. It is reported that *Pasteurella multocida* is usually detectable in blood cultures only in the terminal stages (12-20 h) prior to death. It may be because no such animals were presented at this stage (Carter and De Alwis, 1989).

Only one isolate of *Proteus vulgaris* was recovered in the present study. Perusal of literature did not indicate the association of these organisms in respiratory diseases of bovines. These organisms are always considered as

secondary invaders in wound infection and diseases of mucuous membranes (Merchant and Packer, 1983).

The infrequent and inconsistent variety of bacterial organisms as is evident from the current clinical study paves light on the difficulties encountered in developing control measures for bovine respiratory disease of feedlot cattle. Eventhough control methods like many immunizing products for pasteurella pneumonia which is considered as the main cause of respiratory disease have been developed they show only some degree of success. As it is evident from the present study the different microbial organisms with other environmental, mangemental and host factors precipitates the disease in the field and no matter how well designed and implemented be the laboratory trials of immunizing agents, they exclude many contributing factors that exists in nature.

The cattle industry's use of preventive products and methods has led to conflicting opinions as to their true value. There are differences in the ways each method is used, difference in the health and immune status and the genetics of herds of cattle, different environmental factors and different underlying causes of an outbreak of respiratory disease. Hence formulation of a thorough prophylactic tool of bovine respiratory disease requires much more care and patience.

5.4 ANTIBIOGRAM

As many bacterial species have become highly resistant to many antimicrobials through the inappropriate and indiscriminate use of many antimicorbials, in terms of dosage, duration of therapy and route of administration in outbreaks of respiratory diseases it is of paramount importance to conduct *in vitro* antibiotic sensitivity test to help the clinician for relieving the sufferings of diseased animals. Recently many organisms have started showing multiple drug resistance. The results of *in vitro* antibiotic sensitivity tests are suggestive of the internal genetic structure of each organism. Some of the antibiotic sensitivity test results of bacterial isolates suggest the inadequacy of

attention given in terms of the dose and course for the administration of antimicrobial.

There are variations in the antibiotic sensitivity of different species of organisms from place to place. Similarly the resistance pattern may sometimes be indicative of the transmission of the resistant strains of organisms by means of transportation to nearby locations.

The mechanism behind the development of resistance can be by the change in a bacterial gene, due to the presence of an additional gene or by the extensive propagation of resistant bacteria following antimicrobial therapy, which inhibits or kills susceptible bacteria. The transmission of resistance can also occur by conjugation or by transfer of R-plasmids between the bacteria. The concept of multiple drug resistance is acquiring significance recently and has been reported by many previous workers (Wernicki *et al.*, 1999, Cloeckaert *et al.*, 2000 and Nakaya *et al.*, 2000). Most of the bacteria isolated in the present study also show resistance to many of the antibiotics.

The overall susceptibility of 39 bacterial isolates are enrofloxacin (94.87 per cent), ciprofloxacin (87.18 per cent), chloramphenicol (76.92 per cent), gentamicin (64.1 per cent), oxytetracycline (53.85 per cent), amoxycillin (51.28 per cent). More than 50 per cent of the isolates showed resistance to streptomycin (64.10 per cent) and trimethoprim (71.79 per cent). It is pertinent to note that streptomycin and trimethoprim were the most commonly used antimicrobials for buiatric practice in Kerala, particularly in the treatment of infectious pneumonia.

The present study reveals that almost all the bacteria (94.87 per cent) were highly sensitive to enrofloxacin and ciprofloxacin (87.18 per cent). This is in agreement with the report of antibiotic susceptibility test of Jongshli *et al.* (2001). Cent per cent efficacy to enrofloxacin have been reported by Sahin (1997), Selim

et al. (1998), El-Shabiny *et al.* (1999), Hutt and Goossens (2001), Jongshli *et al.* (2001) and Catry *et al.* (2002).

Since the antibiotic disc for florfenicol was not available, the *in vitro* sensitivity test of these bacterial isolates could not be done. Clinical trial of florfenicol is a pioneering work in India and the drug florfenicol (Nuflor®), which is currently not available in India, was kindly provided by Schering-Plough Animal Health, USA for research purpose. Available literature indicate that the bacterial isolates of respiratory tract infection had cent per cent sensitivity to florfericol (Hormansdorfer and Bauer, 1996; Almajano *et al.,* 1998; Wernicki *et al.,* 1999; Mevius and Hartman, 2000; Hutt and Goossens, 2001; Catry *et al.*, 2002). A low level of resistance was reported by earlier workers (Hunkenmoller, 1991; Hormansdorfer and Bauer, 1996).

Resistance of bacteria to florfenicol was also reported by previous workers (Aguade and Romero, 2000; Ayling *et al.*, 2000; Cloeckaert *et al.*, 2000). A florfenicol resistant gene almost identical to Flo-R of *Salmonella enterica* serovar *Typhimurium* DT104 was detected on 110 to 125 –kb plasmids in 44 *Escherichia coli* isolates of animal origin in France and has been deposited in genebank under accession number AF231986 (Cloeckaert *et al.*, 2000).

Present study indicated that 76.92 per cent of isolates were resistant to chloramphenicol which is not in agreement with the findings of Sharma and Joshi (1984), Allan *et al.* (1985), Gupta *et al.* (1996) and Hormansdorfer and Bauer (1996) who reported more than 80 per cent of sensitivity for chloramphenicol to the bacterial isolates of bovine respiratory tract infection. *Pseudonomonas aeruginosa* isolated from bovine was fully susceptible to ciprofloxacin. This finding is not in agreement with the finding of Chakraborty *et al.* (2001)

Out of 19 Gram positive isolates highest sensitivity was shown to enrofloxacin, ciprofloxacin (100 per cent) followed by gentamicin (84.21 per cent), chloramphenicol (73.68 per cent). Resistance was noted in more than 50

per cent of isolates towards amoxycillin, streptomycin, oxytetracycline and trimethoprim. Whereas the Gram negative organisms of present study was not fully susceptible to any of the antimicrobials used. Highest sensitivity was shown by enrofloxacin (90 per cent), chloramphenicol (80 per cent), ciprofloxacin (75 per cent), oxytetracycline (60 per cent), amoxycillin (55 per cent), gentamicin (45 per cent), trimethoprim (30 per cent) and streptomycin (25 per cent). Similar finding was reported by Selim *et al.* (1998), but disagrees with the resistance shown to trimethoprim.

5.5 HAEMATOLOGICAL ESTIMATION OF PRE-TREATMENT GROUPS (I AND II) AND GROUP III

5.5.1 Haemogram

Different haematological parameters of diseased animals of group I were compared with the healthy bovines indicated a highly significant difference in ESR and haemoglobin in group I. The mean values of ESR and Hb were (14.25 ± 5.60 mm/24 h and 10.73 ± 1.37 g/dl) and that of control was (3.67 ± 0.82 mm/24 h and 12.67 ± 0.82 g/dl). In group II eventhough there was a highly significant ESR (11.88 ± 2.03 mm/24 h) the reduction in the value for haemoglobin (11.43 ± 1.62 g/dl) was not statistically significant.

Statistically significant lowering in the mean values for PCV and total erythrocyte count were detected in groups I and II (31.38 ± 4.17 per cent and 6.55 ± 0.92 x 10^6/mm^3 and 32.50 ± 4.87 per cent and 6.78 ± 0.67 x 10^6/mm^3) when compared with group III where the mean values were (35.67 ± 1.63 per cent and 7.68 ± 1.51 x 10^6/mm^3) respectively.

An overall reduction in the PCV, haemoglobin and total erythrocyte count was observed in bovines with respiratory tract infection with a concomitant rise of ESR. But this finding is not in accordance with Benjamin (1985) who reported almost a steady haematology in bovines with respiratory disease.

The significant increase of ESR in the infected group may be either arising from anaemia in which ESR is increased due to small number of cells that can settle more easily in large volume of the fluid or due to alterations in plasma proteins. Increased globulin level increases ESR whereas ESR is inversely related to albumin level (Benjamin, 1985).

Only few literatures are available regarding the haematological findings of respiratory disease in bovines presents difficulty to correlate. But hypoalbuminaemia and hyperglobulinaemia with significant rise in alpha I and alpha 2 globulins reported in acute bovine respiratory disease (Nasser and El-Sayed, 1997) can be attributed to the reason for elevation in ESR.

No significant difference was noticed in erythrocyte indices of MCV, MCH and MCHC of pre-treatment groups of I and II with that of control.

5.5.2 Leukogram

No significant difference was noted in the TLC or DLC between the pre-treatment group I and group III. Eventhough a slight increase in the values of TLC and neutrophil count of group I ($10.81 \pm 0.88 \times 10^3/mm^3$ and 23.25 ± 6.52 per cent) from the group III ($10.44 \pm 0.91 \times 10^3/mm^3$ and 22.50 ± 1.38 per cent) are suggestive of bacterial infection (Jain, 1986).

In group II also a slight elevation in the mean value of TLC and neutrophil was noted ($11.18 \pm 0.42 \times 10^3/mm^3$ and 23.90 ± 6.20 per cent). There was no significant difference in the values of TLC and DLC of pre-treatment group II and group III.

5.6 TREATMENT TRIAL WITH ENROFLOXACIN AND FLORFENICOL

A total of 18 animals presented with clinical respiratory diseases were allotted into two treatment groups. Group I was treated with enrofloxacin and group II was treated with florfenicol. Detailed clinical data before, during and after therapy were noted. Duration of therapy varied between 3-5 days in group I

depending upon the waning of clinical signs of respiratory tract infection. If complete cure was not noticed even after 3 days of treatment with enrofloxacin at 5 mg/kg body weight intramuscularly the treatment was prolonged for 2 more days. In group II all the animals were treated with florfenicol at 20 mg/kg body weight, two doses intramuscularly at 48 h interval. All the animals in group II required only two injections of antibiotic but in one case of enrofloxacin treated group needed two more days of intramuscular administration of the same antibiotic for a complete clinical cure.

5.6.1 Clinical Data

In response to treatment with both enrofloxacin and florfenicol there was significant fall in the group mean rectal temperature during the first 24 h after therapy in those cases which responded favourably (103.05 ± 1.48 °F, 101.75 ± 0.46 °F) in group I and (103.05 ± 0.55 °F and 102.05 ± 0.14 °F) in group II animals. A significant reduction in respiratory rate of animals after therapy was also evident in the recovered groups in both trials with the mean values of (54.50 ± 9.72/min and 40.50 ± 8.60/min) in group I and (55.75 ± 12.80/min and 40 ± 6.23/min) in group II.

5.6.2 Haematological Parameters of Pre and Post Treatment Groups

The highly significant lowering in the mean values of ESR in the recovered animals were noticed with all other variables being constant during the treatment period in groups I and II suggest that the therapy was beneficial and helps the physiological parameters to regain normally. A significant increase in the packed cell volume also suggests the same in both groups. There was a better response of haemoglobin in group I which was statistically significant. Even though an apparent increase in the mean value of haemoglobin was noted after treatment in group II, it was statistically nonsignificant.

Statistically elevated haemoglobin level of group I may be correlated with the statistically significant lowering of MCV in the group and can be attributed to

the high level of condensation of erythrocyte. This change in haemoglobin and MCV was not observed in group II.

No significant difference was noted in the values of other erythrocyte indices like MCH and MCHC and in the leukogram between pre and post-treatment groups I and II.

5.7 RESPONSE TO THERAPY

After the treatment eight animals in group I and group II improved rapidly in parallel with the reduction in pyrexia. The animals generally became more alert, their appetite improved and the clinical signs of respiratory dysfunction waned. During the observation period after treatment two among the eight cattle in both groups again met the original criteria for treatment. In order that the welfare of these animals were not compromised they were given additional therapy. One animal in the group II succumbed to death. One animal in group I was disposed off by the owner. The differential eosinophil count of this particular animal was very high (20 per cent), suggesting atypical or hypersensitivity pneumonia irresponsive to the treatment regimens. The persistence of high neutrophil count in the relapsers of treatment is responsible for the increase in reactivity of air passages to allergens and cause mucus hypersecretion. A positive association was noticed between the increased proportion of neutrophils and isolations of *Pasteurella multocida* and *Mycoplasma bovis* from broncho-alveolar lavage fluid (Allen *et al.*, 1991). In previous studies a larger proportion of neutrophils have been observed in broncho-alveolar lavage fluid in cases where recurrence was noticed. The presence of inflammatory cells in the airways may have contributed to persistence or recurrence of clinical signs with release of inflammatory mediators which affect smooth muscle contractility and increase the reactivity of airways to allergens, as well as causing edema of the respiratory mucosa and mucus hypersecretion (Larsen *et al.*, 1987).

From these cases where relapses were observed in the present study, the organisms isolated were *Streptococcus pyogenes, Mannheimia haemolytica, Klebsiella pneumoniae, Pseudomonas aeruginosa* and *Staphylococcus aureus and Staphylococcus epidermidis*

Mannheimia haemolytica was isolated from two cases of group II, which had recovery followed by recurrence in one of the case. The case in which relapse was noticed was a 4 month old calf presented with a history of taking rubber latex and treated with liquid paraffin, stomachic and chlorpheniramine malleate. The severe respiratory distress manifested has ceased after the first dose of florfenicol itself leading to healthy condition. But after four days of initial presentation animal showed discomfort of digestive system and respiratory system. Animal was subjected to rumenotomy and abomasotomy. Then florfenicol was advocated for the second time for the recurrence but was not found to be effective prompting to change the antibiotic into sulpha-trimethoprim (Inj. Biotrim) at 30 mg/kg intramuscularly for five days for saving the animal. This finding is not in accordance with the previous finding of Marcal *et al.* (2002) wherein florfenicol was reported as an effective antibiotic in post-surgery management with a wide spectrum of action and decreased handling time of sick animals

Relapse was also noticed in one animal in group I from which *Streptococcus pyogenes* and *Staphylococcus epidermidis* was isolated. In group II *Klebsiella pneumoniae, Escherichia coli* and *Staphylococcus epidermidis* were isolated from one case of recurrence.

In the present study two cases from the same locality from which *Streptococcus pyogenes* and *Staphylococcus epidermidis* isolated were allotted into two groups. Both the animals recovered after therapy with either antimicrobial. Both the organism had similar type of antimicrobial susceptibility. The close contact of these two animals in the same barn might have caused the spread of potentially pathogenic agent between the animals.

The present study isolated *Streptococcus pyogenes* as the single infectious bacteria from two of the total cases that is capable of manifesting the disease alone. One animal from the two was allotted to each group. Complete cure was noticed in both the groups.

In the present study clinical data and haematological parameters of eight animals recovered in each group were compared to group III animals.

5.7.1 Clinical Data

No statistical difference was noted between the recovered animals of both groups I and II and healthy animals with respect to temperature, pulse and respiration.

5.7.2 Haematological Parameters - Post-Treatment Groups (I and II) and Control

Though haematological parameters of recovered animals of group I indicated a significant difference in ESR, haemoglobin and total erythrocyte count from that of group III, the difference noted between pre and post-treatment group I was statistically significant with the response to treatment. These may be explained by the fact that a time lag is required for the recovered animals to reach the healthy parameters of group III. Variation in the haematological parameters may also be due to the variation in the age group of animals presented for treatment. There was no significant difference among the different erythrocyte indices and leukogram between the post-treatment group I and control.

There was no significant difference in the values of haemogram and erythrocyte indices between the post-treatment group II and control except for ESR. The mean values of post-treatment group II were more nearing to healthy bovines than that of group I. This can be attributed to the ability of florfenicol to reduce blood dyscrasias (Sams, 1994).

5.8 EVALUATION OF EFFICACY OF DRUGS

The criterion for the determination of comparative efficacy of enrofloxacin and florfenicol were the clinical data, haematological parameters and recovery from clinical signs. Clinical signs appeared to resolve more rapidly in association with the treatment were difficult to quantify in a meaningful way. Clinical illness index score system indicated in the present study helped for the better response analysis. Much difference was not noted between the two groups, with the scoring system.

Rectal temperature and respiration rate varied significantly before and after treatment in recovered animals in both the groups. But no significant difference existed between the post-treatment groups I and II.

No significant difference existed among the different haematological parameters between the recovered animals of both groups I and II.

All the above findings conclusively summerises that the two antibiotics enrofloxacin and florfenicol are equally effective in controlling bacterial infections of respiratory tract. It is evident from both *in vivo* and *in vitro* studies that enrofloxacin is an appropriate therapy for respiratory tract infection in cattle. Even though there is lack of information on the sensitivity analysis on florfenicol, this drug being a congenor of chloramphenicol with an *in vitro* antibiotic sensitivity of (76.92 per cent) and the results of *in vivo* studies prove florfenicol to be potentially effective in treatment of bacterial infections of respiratory tract. This has the added advantage that in bovines it may not lead to aplastic anemia. Further there will be increased susceptibility to the organisms that are resistant to chloramphenicol by inhibition of acetylation of these drugs with chloramphenicol acetyl transferase (CAT) enzyme due to the nitro group in the structure (Sams, 1994).

Florfenicol, being long acting antibiotic is advantageous in that it results in less distress to the animals by reducing the number of repeat injections and the

amount of handling of sick animals. But a controversy still exist that good results in any acute respiratory disease needs frequent re-evaluation of the animals and further treatment when necessary and in the present study, in one post-operative condition case florfenicol was not found to be effective.

Better success rate with the same antimicrobials may be achievable if the treatment aimed not only on eradicating the pathogen alone but also to reduce the associated inflammatory or hypersensitivity reaction aiding in the control of pyrexia and depression as observed by Schmidt *et al.* (1998), Yilmaz *et al.* (2000) and Kaymaz *et al.* (2001).

This pioneering study on florfenicol though was preliminary in nature and limited in size yet significant changes in clinical data, haematologial parameters, clinical signs and clinical illness index score of the animal before and after therapy which could be logically linked to efficacy and comparison between drugs were achieved.

Summary

6. SUMMARY

The present study of " Clinico-therapeutic studies on bacterial infections of respiratory tract in bovines" was envisaged to know the bacterial etiology, antibiogram and to assess the comparative efficacy of two antibiotics namely enrofloxacin (Gyroflox) and florfenicol (Nuflor®) in the treatment of bovine respiratory tract infection. The bovines with respiratory illness presented at the University Veterinary Hospitals, Kerala Agricultural University Farms and State Animal Husbandry Veterinary Hospitals during the period of November 2001 to June 2003 formed the material for study.

Out of the 18 diseased animals, nine were allotted in group I, treated with enrofloxacin at 5 mg/kg body weight intramuscularly for three to five days depending on the severity of the condition, and nine were allotted in group II, treated with florfenicol at 20 mg/kg body weight intramuscularly two doses at 48 h interval. Six apparently healthy bovines constituted group III (control).

The detailed clinical examination, signalment, anamnesis and symptoms of each case were recorded before, during and after therapy. The clinical assessment was based on a clinical illness index score system. Clinical illness index scoring of diseased animals were 1 (slightly ill) in 27.77 per cent, 2 (moderately ill) in 66.66 per cent and 3 (severely ill) in 5.55 per cent. The bacterial organisms present in upper respiratory tract of bovines with respiratory disease was isolated and identified to the species level from the deep nasal washings collected using sterile flutter valve apparatus and normal saline. Out of the total 39 bacterial isolates, 19 (48.71 per cent) were Gram positive and 20 (51.29 per cent) were Gram negative. Different bacterial isolates obtained were *Staphylococcus aureus* (6), *Staphylococcus epidermidis* (5), *Streptococcus pyogenes* (8), *Escherichia coli* (7), *Klebsiella pneumoniae* (5), *Pseudomonas aeruginosa* (4), *Mannheimea haemolytica* (3) and *Proteus vulgaris* (1).

Single bacteria could be isolated only from two cases (11.11 per cent). Mixed bacteria were isolated from 16 cases (88.88 per cent).

Gram positive isolates were cent per cent sensitive to enrofloxacin and ciprofloxacin followed by gentamicin (84.21 per cent) and chloramphenicol (73.68) whereas Gram negative isolates showed highest sensitivity to enrofloxacin (90 per cent) followed by chloramphenicol (80 per cent), ciprofloxacin (75 per cent) and oxytetracycline (60 per cent).

Overall antibiotic sensitivity pattern of the 39 isolates showed maximum sensitivity to enrofloxacin (94.87 per cent) followed by ciprofloxacin (87.18 per cent), chloramphenicol (76.92 per cent), gentamicin (64.10 per cent), oxytetracycline (53.85 per cent), amoxycillin (51.28 per cent), streptomycin (35.9 per cent) and trimethoprim (28.21 per cent).

Diseased animals had a significantly high temperature and respiratory rate from that of control. There was a statistically significant elevation in the ESR of diseased animals. The low values of packed cell volume, haemoglobin, total erythrocyte count in bovines with respiratory tract infection was statistically significant from that of healthy animals. However no significant difference was noted in the values of erythrocyte indices and leukogram.

After treatment the elevation of packed cell volume and lowering of ESR was statistically significant among the recovered animals in both groups. But statistically significant increase in the haemoglobin was present only in the recovered animals of group I. No significant difference was noted in the leukogram of both groups before and after therapy.

Recovery of animals in both groups was assessed on the basis of statistically significant reduction in pyrexia, respiratory rate and overall improvement of clinical signs and illness index score.

The clinical improvement was more noticeable in the florfenicol treated group after 24 h of treatment. One animal in the enrofloxacin treated group required treatment for 5 days.

Recovery was noticed in eight out of nine animals with respiratory disease subjected to treatment trial in both groups I and II. During the observation period after the completion of treatment two animals in both groups had recurrence of the symptoms of respiratory tract infection. One animal died during therapy in group II and one animal in group I was disposed off by the owner.

Clinical illness index score, temperature, respiratory rate and various haematological parameters between the post-treatment groups I and II were not statistically significant indicating comparable efficacy of both enrofloxacin and florfenicol in treatment of bacterial bovine respiratory tract infections.

Both *in vivo* and *in vitro* studies provide good evidence that enrofloxacin is an appropriate therapy for respiratory tract infection and the drug is readily available in India.

Though there is lack of information on the sensitivity test on florfenicol, *in vivo* studies and being a congenor of chloramphenicol with a sensitivity of 76.92 per cent is also potentially effective in counteracting bacterial infections of respiratory tract in bovines.

Florfenicol is having an added advantage of causing less distress to the animals by reducing the number of repeat injections and the amount of handling of sick animals. But in one post-operative case florfenicol was not found to be effective

This pioneering study on the use of florfenicol in treatment of upper respiratory tract infection was found to be equally effective to enrofloxacin in counteracting the bovine respiratory tract infections.

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Appendices

APPENDIX .1

CLINICO-THERAPEUTIC STUDIES ON BACTERIAL INFECTIONS OF RESPIRATORY TRACT IN BOVINES

1. Sl. No. Case No. Date

2. Name and address of the owner Phone Number

3. Details of the animal
   Breed Colour Age Sex Parity

4. **Production details**
   a. Milk yield Morning Evening
   b. Stage of lactation Periparturient Early Mid Late
   c. Number of times milked 1 2 3 More than 3
   d. Type of milking Full hand Thump Stripping
   e. Milker Owner Professional Milking machine

6. **Reproduction details**
   a. Age of puberty b. Age at first calving c. Month of calving d. Sex of calf M F
   e. Parturition Normal Assisted

7. **Management details**
   a. *Herd size* a. single b. farm unit
   b. Origin of calf a. home grown b. purchased
   c. Supply of colostrum Yes No
   d. Weaning practiced Yes No
   e. Age of weaning
   f. Mixing with other new groups Yes No
   g. *Exposure to inclement weather* Yes No
   h. History of transport
   i. Contact with other cattle Yes No
   j. Change of holding / new holding Yes No
   k. Castration Yes No
   l. Dehorning Yes No
   m. Practice of deworming Yes No
   n. Frequency of bathing Daily Once in 2 days Once in a week Once in a fortnight ½M Nil

8. **Housing details**
   a. Type of housing - intensive Semi-intensive : indoor % outdoor % loose housing
   b. Type of bedding - Concrete Wood Sand Straw
   c. Type of roof - Thatched Asbestose Tiles *Concrete*
   d. Side of house - Fully opened Partially opened Closed
   e. Type of shed - Individual lean to none
   f. Spacing - Deficit Adequate Excess
   g. Lighting - Poor Satisfactory over
   h. Sanitation, hygiene Unsatisfactory Ideal Satisfactory
   i. Mode of disinfection Just washing Chemical Physical

9. **Feeding details**
   a. Roughage - hay, straw, silage, grass
   b. Concentrate - pellet, mash, bran
   c. Change in diet - Yes No
   d. Regimen of diet - Morning a b Afternoon a b Evening a b

10. **Health details**
    Previous occurrence of same disease Yes No
    Other disease Yes No

11. Environmental history

a. Exposure to infection, trauma, poison - Yes No
b. Agro-climatic condition of area
c. Season
d. Topography and soil
e. Source of water and feed
f. Waste disposal
g. Sudden drop in environmental temperature within 24-72 hours
h. Nearby respiratory disease infection

12. Examination of patient

a. General appearance - bright moderate dull severely dull
b. Behaviour - lively, restless, aggressive, stubborn, intolerant, anxious
c. Expression
d. Body condition - prime good poor/thin
e. Condition of skin and coat - soft pliable, dull lack of texture, hard leathery
f. Length of hair
g. Degree of depilation of hair maximum moderate minimum

13. Examination of respiratory system

1. Muzzle moist dry
2. Rhinorrhoea Present Absent
3. Nature of discharge serous mucous mixed
4. Cough present / absent
5. Frequency of cough
6. Respiratory rate normal, polypnoea, hyperpnoea, oligopnoea
7. Rhythm normal, abnormal
8. Type of respiration Thoracoabdominal, abdominal, Thoracic
9. Depth of respiration Inspiratory dyspnoea expiratory dyspnoea intermediate dyspnoea
10. Auscultation

14. Conjunctivitis Yes No
15. Lacrymation Yes No
16. Defecation loose intermediate dry
17. Urination Normal Abnormal
18. Appetance normal moderate anorectic
19. Reduction in milk production Yes No
20. Palpation of lymphnode Normal Enlarged
21. Illness index score 0 – normal I – Slightly ill II - Moderately ill III – Very ill IV - Moribund
22. Date of onset of symptoms
23. *Clinical data* - R P T *mm* Rm
24. Treatment
    Antibiotic Dose Route
    Duration Interval
25. Cost of treatment medicine loss of yield Others
26. Post treatment observation period
    4-10 days – recurrent respiratory disease Yes No
    11-28 days – reinfection Yes No

APPENDIX 2

In vitro **antibiotic sensitivity pattern of different bacterial isolates obtained from respiratory tract infection**

Sensitive

Sl. No.	Antibiotic	*S. aureus*	*S. epidermidis*	*S. pyogenes*	*E. coli*	*K. pneumoniae*	*P. haemolytica*	*P. aeruginosa*	*P. vulgaris*
1.	Amoxycillin	3 (50)	3 (60)	3 (37.5)	3 (42.86)	3 (60)	2 (66.66)	2 (50)	1 (100)
2.	Chloramphenicol	4 (66.66)	4 (80)	6 (75)	6 (85.71)	4 (80)	1 (33.33)	4 (100)	1 (100)
3.	Enrofloxacin	6 (100)	5 (100)	8 (100)	6 (85.71)	5 (100)	2 (66.66)	4 (100)	1 (100)
4.	Gentamicin	5 (83.3)	5 (100)	6 (75)	5 (71.43)		1 (33.33)	2 (50)	1 (100)
5.	Ciprofloxacin	6 (100)	5 (100)	8 (100)	6 (85.71)	4 (80)	1 (33.33)	3 (75)	1 (100)
6.	Streptomycin	3 (50)	2 (40)	4 (50)	3 (42.86)	1 (20)	0	0	1 (100)
7.	Trimethoprim	1 (16.66)	2 (40)	2 (25)	1 (14.29)	3 (60)	2 (66.66)	0	-
8.	Oxytetracycline	3 (50)	2 (40)	4 (50)	3 (42.86)	3 (60)	3 (100)	3 (75)	-

Figures in parenthesis indicate percentage

Appendix 2 continued

Moderately Sensitive

Sl. No.	Antibiotic	*S. aureus*	*S. epidermidis*	*S. pyogenes*	*E. coli*	*K. pneumoniae*	*P. haemolytica*	*P. aeruginosa*	*P. vulgaris*
1.	Amoxycillin	1 (16.66)	-	2 (25)	1 (14.29)	1 (20)	-	1 (25)	-
2.	Chloramphenicol	1 (16.66)	1 (20)	-	-	-	1 (33.33)	-	-
3.	Enrofloxacin	-	-	-	-	-	1 (33.33)	-	-
4.	Gentamicin	1 (16.66)	-	-	1 (14.29)	5 (100)	2 (66.66)	2 (50)	-
5.	Ciprofloxacin	-	-	-	1 (14.29)	1 (20)	2 (66.66)	1 (25)	-
6.	Streptomycin	1 (16.66)	1 (20)	1 (12.5)	-	3 (60)	1 (33.33)	4 (100)	-
7.	Trimethoprim	1 (16.66)	-	1 (12.5)	1 (14.29)	-	-	1 (25)	1 (100)
8.	Oxytetracycline	1 (16.66)	1 (20)	1 (12.5)	1 (14.29)	1 (20)	-	1 (25)	-

Figures in parenthesis indicate percentage

Appendix 2 continued

Resistant

Sl. No.	Antibiotic	*S. aureus*	*S. epidermidis*	*S. pyogenes*	*E. coli*	*K. pneumoniae*	*P. haemolytica*	*P. aeruginosa*	*P. vulgaris*
1.	Amoxycillin	2 (23.33)	2 (40)	3 (37.5)	3 (42.86)	1 (20)	1 (33.33)	1 (25)	-
2.	Chloramphenicol	1 (16.66)	-	2 (25)	1 (14.29)	1 (20)	1 (33.33)	-	-
3.	Enrofloxacin	-	-	-	1 (14.29)	-	-	-	-
4.	Gentamicin	-	-	2 (25)	1 (14.29)	-	-	-	-
5.	Ciprofloxacin	-	-	-	-	-	-	-	-
6.	Streptomycin	2 (23.33)	2 (40)	3 (37.5)	4 (57.14)	1 (20)	2 (66.66)	-	-
7.	Trimethoprin	4 (66.66)	3 (60)	5 (62.5)	5 (71.43)	2 (40	1 (33.33)	3 (75)	-
8.	Oxytetracycline	2 (33.33)	2 (40)	3 (37.5)	3 (42.86)	1 (20)	-	-	1 (100)

Figures in parenthesis indicate percentage

CLINICO - THERAPEUTIC STUDIES ON BACTERIAL INFECTIONS OF RESPIRATORY TRACT IN BOVINES

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Abstract of the thesis submitted in partial fulfillment of the requirement for the degree of

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ABSTRACT

The present study was undertaken to know the bacterial etiology, antibiogram and to evaluate efficacy of two antibiotics namely enrofloxacin (Gyroflox) at 5 mg/kg body weight intramuscular once daily for 3 to 5 days and florfenicol (Nuflor®) at 20 mg/kg body weight, deep intramuscular two doses at 48 h interval in the treatment of bovine respiratory tract infection.

The animals in the experimental groups were categorized according to a clinical illness index score system. Clinical data and haematological parameters of diseased animals showed significant difference with regard to temperature, respiratory rate, erythrocyte sedimentation rate, packed cell volume, haemoglobin and total erythrocyte count. No significant difference was noted in the values of erythrocyte indices and leukogram. Bacteria isolated from the respiratory tract of diseased bovines were *Staphylococcus aureus* (6), *Staphylococcus epidermidis* (5), *Streptococcus pyogenes* (8), *Escherichia coli* (7), *Klebsiella pneumoniae* (5), *Pseudomonas aeruginosa* (4), *Mannheimia haemolytica* (3) and *Proteus vulgaris* (1). Out of the 39 bacterial isolates 19 (48.71 per cent) were Gram positive and 20 (51.29 per cent) were Gram negative. Single bacteria could be isolated only from 2 cases (11.11 per cent). Mixed bacteria were isolated from 16 cases (88.88 per cent). Antibiotic sensitivity pattern of the isolates showed maximum sensitivity to enrofloxacin (94.87 per cent) followed by ciprofloxacin (87.18 per cent), chloramphenicol (76.92 per cent), gentamicin (64.10 per cent), oxytetracycline (53.85 per cent), amoxycillin (51.28 per cent), streptomycin (35.90 per cent) and trimethoprim (28.21 per cent).

Recovery of the animals in both groups was assessed on the basis of statistically significant reduction in pyrexia, respiratory rate and overall improvement of clinical signs and illness index score. Recovered animals

recorded a significant difference in the ESR and PCV from diseased animals in both the groups. No significant difference was noted in the temperature, respiratory rate, haematological parameters between the two post-treatment groups. Efficacy of both enrofloxacin and florfenicol was comparable in counteracting bacterial bovine respiratory tract infection.