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BACTERIAL ISOLATES FROM BOVINE RESPIRATORY TRACT INFECTION AND THEIR ANTIBIOGRAM*

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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. Perusal of literature indicated that there was 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 involves isolation and identification of the causative organisms and determination of the antibiotic sensitivity of these isolates. The present study deals with the isolation of bacteria from the respiratory infections of bovines and their antibiogram.

Materials and methods

Bovines, which were presented, at the University and Government Veterinary Hospitals, Kerala, with clinical signs of respiratory disease during November 2001 to June 2002, formed the animals for the study. Nasal swabs and deep nasal washings for isolating bacteria 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 sterile syringe and a knot was tied to the flutter valve apparatus immediately after collection. They were directly streaked into blood agar and brain heart infusion agar and incubated in candle jar at 37°C for 24h. Selective media like Mannitol Salt Agar, Eosin Methylene Blue Agar and Mac conkey Agar were used. Isolation and identification of bacteria were done as per Barrow and Feltham (1993). *In vitro* antibiotic sensitivity test was done using disc diffusion technique (Baur *et al.*, 1966). The antibiotic discs used were that of amoxycillin, ciprofloxacin, chloramphenicol, enrofloxacin, gentamicin, oxytetracycline, streptomycin and trimethoprim (Hi-media).

Results and discussion

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 (48.72%) were Gram positive organisms and 20 (51.28%) were Gram negative organisms. The different bacterial isolates were *Staphylococcus*

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aureus (15.38%), *Staphylococcus epidermidis* (12.82%), *Streptococcus pyogenes* (20.51%), *Escherichia coli* (17.95%), *Klebsiella pneumoniae* (12.82%), *Pseudomonas aeruginosa* (10.26%), *Mannheimia haemolytica* (7.69%) and *Proteus vulgaris* (2.56%) (Table). All of these organisms except *Proteus vulgaris* were isolated from bovines with respiratory disease by earlier workers (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 chlamydiae from bovine respiratory tract infections.

Majority of the clinical samples yielded 2 bacterial isolates (61.11%), followed by 3 isolates (27.75%). From two cases (11.11%) the single isolate obtained was *Streptococcus pyogenes*. In 88.89 per cent of animals more than one bacterium could be isolated.

Many research workers have reported isolation of staphylococci from respiratory tract infections in bovines (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%). *Escherichia coli* has been isolated from respiratory disease complex of cattle, sheep and goat (Sahin, 1997; Selim *et al.*, 1998; Aslan *et al.*, 2002).

Table Aerobic bacteria isolated from deep nasal washings and nasal swabs 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	27.78
3	*Streptococcus pyogenes*	8	20.52	44.44
	Gram negative			
4	*Escherichia coli*	7	17.95	38.89
5	*Klebsiella pneumoniae*	5	12.82	27.78
6	*Pseudomonas aeruginosa*	4	10.26	22.22
7	*Mannheimia haemolytica*	3	7.69	16.66
8	*Proteus vulgaris*	1	2.56	5.56

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 *Pseudomonas aeruginosa* produce proteolytic enzymes like protease and elastase, which help 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%). Eventhough many doubts have been cast upon the significance of *Pasteurella* spp. as primary pathogens in bovine respiratory disease, these were the sole organisms isolated from the respiratory tract of pneumonic calves of all ages and occasionally from nonpneumonic tissue, by previous workers (Rowe *et al.*, 2001; Aslan *et al.*, 2002; Catry *et al.*, 2002). Although there is a great deal of evidence in the literature for a casual association of *Mannheimea haemolytica* with bovine respiratory disease, present study shows that these are not of major importance in many disease outbreaks. 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

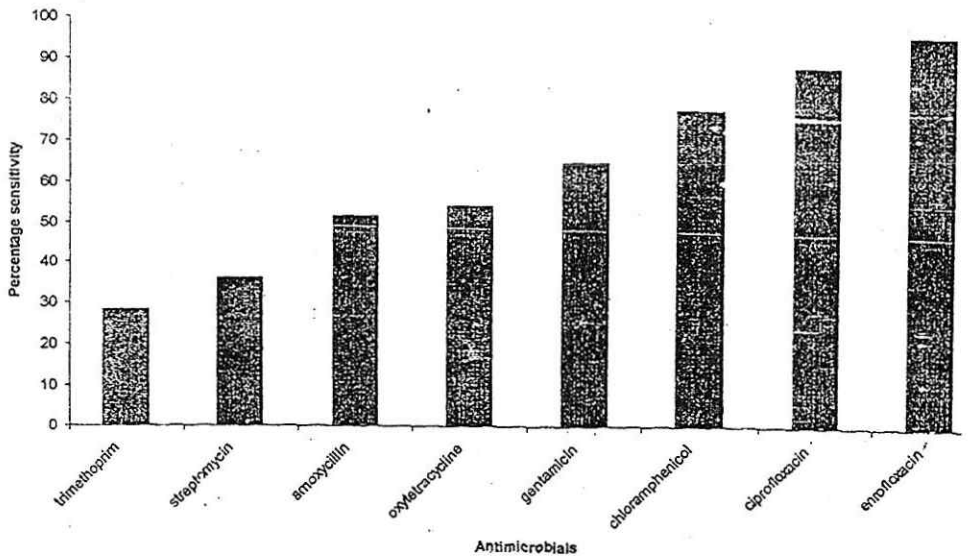

Fig. Percentage Distribution of Antimicrobial sensitivity pattern of 39 isolates from bovine respiratory disease

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 mucous membranes (Merchant and Packer, 1983).

The infrequent and inconsistent variety of bacterial organisms isolated, as is evident from the present clinical study, throws light on the difficulties encountered in developing control measures for bovine respiratory disease of feedlot cattle. Several vaccines have been developed for immunizing animals against *Pasteurella* induced pneumonia, considered as the main cause of respiratory disease, with only limited success. As is evident from the present study the different microbial organisms with other environmental, managemental 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 exist in nature.

The *in vitro* antibiotic sensitivity studies on 39 bacterial isolates from bovine respiratory tract infection revealed that enrofloxacin was the most sensitive antibiotic, followed by ciprofloxacin, chloramphenicol, gentamicin, oxytetracycline and amoxycillin. More than half of the isolates were found to be resistant to trimethoprim and streptomycin (Figure), which are 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 were highly sensitive to enrofloxacin (94.87%) and ciprofloxacin (87.18%). This is in agreement with the report of antibiotic susceptibility test of Jongshli *et al.* (2001). Cent per cent efficacy to enrofloxacin had been reported by Hutt and Goossens (2001), Jongshli *et al.* (2001) and Catry *et al.* (2002).

Present study indicated that 76.92 per cent of isolates were not sensitive to chloramphenicol which is not in agreement with the findings of 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 that of Chakraborty *et al.* (2001)

Out of 19 Gram positive isolates highest sensitivity was shown to enrofloxacin and ciprofloxacin (100%), followed by gentamicin (84.21%) and chloramphenicol (73.68%). Resistance was noted in more than 50 per cent of isolates towards amoxycillin, streptomycin, oxytetracycline and trimethoprim. While the Gram negative organisms of present study were not fully susceptible to any of the antimicrobials used, highest sensitivity was shown for enrofloxacin (90%), followed by chloramphenicol (80%), ciprofloxacin (75%), oxytetracycline (60%), amoxycillin (55%), gentamicin (45%), trimethoprim (30%) and streptomycin (25%). Similar findings were reported by Selim *et al.* (1998), with the exception of the resistance shown to trimethoprim.

The use of preventive products in cattle industry for preventing respiratory disease and the methods used 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 for bovine respiratory disease requires much more care and patience.

Summary

The bacterial organisms present in upper respiratory tract of bovines with respiratory disease were isolated and identified to the species level from the nasal swabs and deep nasal washings collected. Out of the total 39 bacterial isolates, 19 (48.72%) were Gram positive and 20 (51.28%) 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). Overall antibiotic sensitivity pattern of the 39 isolates showed maximum sensitivity to enrofloxacin (94.87%), followed by ciprofloxacin (87.18%), chloramphenicol (76.92%), gentamicin (64.10%), oxytetracycline (53.85%), amoxycillin (51.28%), streptomycin (35.9%) and trimethoprim (28.21%). Gram positive isolates were cent per cent sensitive to enrofloxacin and ciprofloxacin, followed by gentamicin (84.21%) and chloramphenicol 73.68). The Gram negative isolates showed highest sensitivity to enrofloxacin (90%), followed by chloramphenicol (80%), ciprofloxacin (75%) and oxytetracycline (60%). Multiple bacterial organisms present in the bovine respiratory infection questions the use of many available preventive veterinary products.

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