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Antimicrobial susceptibility testing of *Mannheimia haemolytica* and *Pasteurella multocida* isolated from calves with dairy calf pneumonia

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ABSTRACT

This study evaluated the nasopharyngeal microbial flora and antimicrobial susceptibility patterns of the one hundred and thirty Holstein calves with dairy calf pneumonia from dairy farms of Mashhad Suburb between September 2002 and August 2003. The most common micro-organisms isolated were *Pasteurella multocida* 80 (61.54%), *Mannheimia haemolytica* 41 (31.54%), *Bacillus sp.* 15 (11.54%), *Staphylococcus sp.* 3 (2.31%), *Streptococcus sp.* 4 (3.08%), *Pseudomonas sp.* 3 (2.31%), *Proteus sp.* 3 (2.31%) and *E coli* 5 (3.84%). Antimicrobial susceptibility testing was performed on all *M. haemolytica* and *P. multocida* employing the disk diffusion method (Kirby-Bauer). Each strain was tested with 10 antimicrobial agents. With 7 (17.08%), 6 (14.63%), 4 (9.75%) and 1 (2.44%) of *M. haemolytica* were resistant to lincomycin, gentamicin, oxytetracycline and chloramphenicol, respectively. However, resistance to penicillin, lincomycin, amoxicillin, gentamicin and oxytetracycline was observed in 10 (12.50%), 6 (7.50%), 6 (7.50%), 5 (6.25%) and 5 (6.25%) of *P. multocida* isolates, respectively. All *M. haemolytica* and *P. multocida* tested were found susceptible to florfenicol and cephalothin. The results show the need for local veterinarians and producers to be more responsible in the use of antibiotics in the treatment of pneumonia in calves, and growing danger of the dissemination of strains of *M. haemolytica* or *P. multocida* resistant to most antimicrobials which could complicate in the future the treatment of pneumonia in these animals.

Keywords: Antimicrobial resistance, dairy calf *pneumonia*, *Mannheimia haemolytica*, *Pasteurella multocida*

INTRODUCTION

Bovine respiratory disease (BRD) is a major cause of morbidity and mortality in the cattle industry in many part of the world (Lillie 1974, Radostits *et al* 2000, Songer 2005). The BRD complex is made up of several clinical syndromes. The most common syndrome in beef cattle is pneumonic pasteurellosis commonly known as shipping fever (Lillie 1974).

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By contrast, in dairy cattle the most common clinical syndrome is enzootic calf pneumonia commonly known as dairy calf pneumonia (DCP) (Ames *et al* 1994). Dairy calf pneumonia is multifactorial in nature. Stress, housing, ventilation, colostral immunity, and a number of viral agents have been proposed to play an important role in initiating this disease complex. *Mannheimia haemolytica* and *Pasteurella multocida* are frequently isolated from the purulent bronchopneumonic lung associated with this disease. While these organisms are the primary bacterial agents of DCP, they are also part of the normal upper respiratory flora of calves (Apley 1997, DeRosa *et al* 2000, Andrew 2004, Boyce *et al* 2004). Antimicrobial therapy is the most effective method for the prevention and treatment of DCP (Burrows and Ewing 1985, Gibbs 2001). Various antimicrobial agents are licensed and used to treat DCP, including aminopenicillins, cephalosporins, aminoglycosides, tetracyclines, macrolides, lincosamides alone or in combination with spectinomycin, potentiated- sulfonamide, fluoroquinolones, and florfenicol (Apley 1999). However, previous studies have indicated that resistance to these compounds is frequently encountered (Burrows and Ewing 1989, Post *et al* 1991, Apley 1997). As the pattern of bacterial resistance is constantly changing, monitoring of antimicrobial susceptibility is important. It provides information on the pathogenic bacteria isolated from DCP, and assists in choosing the most empirical antimicrobial therapy. The purpose of this study was to determine the antimicrobial susceptibility patterns of *M. haemolytica* and *P. multocida* recovered from calves with dairy calf pneumonia to antimicrobial agents commonly used to treat DCP.

MATERIALS AND METHODS

Experimental Animals. One hundred and thirty Holstein calves from two weeks up to six-month-old in dairy farms of Mashhad Suburb with dairy calf pneumonia were enrolled in the study between September 1, 2002 and August 31, 2003. A case calf one in which pneumonia was diagnosed by the clinician. Clinician diagnosed respiratory tract disease was defined as detection of abnormal clinical signs related to the respiratory tract, such as abnormal sounds on auscultation of the respiratory tract, high rectal temperature (>39.5 ºC), signs of depression, and lack of involvement of other body systems that might explain the fever (Mohammadi *et al* 2004).

Sampling procedures. Samples for microbiological evaluation were obtained from the upper respiratory tracts of calves using nasopharyngeal swabs (NPS) (Allen *et al* 1991). Nasopharyngeal swabs were constructed using sterile cotton roll 1 cm long and 0.5 cm in diameter attached to a 30 cm length of rigid silastic tubing. This swab was enclosed within an outer sleeve of sterile silastic tubing in order to reduce contamination while it was inserted through the nostril of the calf to a depth of approximately 20 cm. At this point the swab was exposed by withdrawing the outer sleeve, and it was moved back and forth several times against the nasopharyngeal mucosa. The swab was then pulled back into the sleeve to protect it during withdrawal. The cotton roll was placed in tryptone broth for subsequent bacterial culture. Samples were refrigerated at 4 ºC until processing, which always occurred within 4 h.

Bacteriological procedures. Nasopharyngeal swabs were plated onto blood agar, MacConkey agar. Blood agar plates were incubated for 48 h at 37 ºC in 5% CO_2, and the MacConkey plates were incubated for 48h at 37 ºC in air. Bacteria grown on blood agar were selected by colonial morphology, followed by subculturing of the selected colonies, using a sterile loop, onto another blood agar plate. Bacteria were identified by standard laboratory procedures (Allen *et al* 1991, Quinn 1994, Songer, 2005). All isolates identified as *M. haemolytica* and *P. multocida* were subcultured onto Mueller-Hinton

agar with antimicrobial sensitivity discs to determine sensitivity patterns to commonly used antimicrobials for treatment of DCP. These antimicrobial were including florfenicol (30 µg), chloramphenicol (30 µg), oxytetracycline (30 µg), amoxicillin (25 µg), gentamicin (10 µg), cephalothin (30 µg), lincomycin (2 µg), enrofloxacin (5 µg), trimethoprim-sulphamethoxazol (25 µg) and penicillin (10 IU).

RESULTS

The bacteriological culture results of samples from the nasopharyngeal swabs are shown in table 1. From the 130 calves enrolled in this study, 154 isolates were obtained. However, there were only three cases (2.31%) that had a sterile culture with no organisms isolated. The most common causal organisms isolated were *P. multocida* 80 (61.54%) and *M. haemolytica* 41 (31.54%). Other organisms distribution of isolates was as follow: *Bacillus spp.* 15 (11.54%), *Staphylococcus spp*. 3 (2.31%), *Streptococcus spp. 4* (3.08%), *Pseudomonas spp*. 3 (2.31%), *Proteus spp.* 3 (2.31%) and *E. coli* 5 (3.84%) (Table1).

Table 1. Bacteriologic culture results in calves with DCP (n=130)

Type of organisms	Number of isolations	% of cases infected
M. haemolytica	41	31.54
P. multocida	80	61.54
Bacillus spp.	15	11.54
Staphylococcus spp.	3	2.31
Streptococcus spp.	4	3.08
Pseudomonas spp.	3	2.31
Proteus spp.	3	2.31
E coli	5	3.84
Mixed flora	44	33.85
None of isolate	3	2.31

The antimicrobial sensitivities of the *M. haemolitica* and *P. multocida* were tested and the result are shown in table 2 and table 3. All *M haemolitica* isolates (n=41) from nasopharyngeal swabs were sensitive to florfenicol, amoxicillin and cephalothin (100%); however, relatively sensitive and bacterial resistance were noted for the same isolates with respect to oxytetracycline (relatively sensitive 12.20%, bacterial resistance 9.75%), gentamicin (relatively sensitive 26. 83%, bacterial resistance 14. 63%), lincomycin (relatively sensitive 14.63%, bacterial resistance 17.0 8%) (Table2).

Table 2. Antimicrobial susceptibility testing results of M. haemolytica strains (n=41) isolated from calves with DCP

Antibiotic	Sensitive(n)	Relatively sensitive(n)	Resistant(n)
Florfenicol	41(100%)	0	0
Chloramphenicol	40(97.56%)	0	1(2.44%)
Oxytetracycline	32(78.05%)	5(12.20%)	4(9.75%)
Amoxicillin	41(100%)	0	0
Penicillin	36(87.80%)	5(12.20%)	0
Gentamicin	24(58.54%)	11(26.83%)	6(14.63%)
Cephalothin	41(100%)	0	0
Lincomycin	28(68.29%)	6(14.63%)	7(17.08%)
Enrofloxacin	34(82.92%)	7(17.08%)	0
Trimethoprim-sulfa methoxazol	37(92.68%)	4(9.76%)	0

Eighty (100%) *P. multocida* isolates were sensitive to florfenicol, chloramphenicol and cephalothin, where as only 71.25% were sensitive to penicillin. Some *P. multocida* isolates were of relatively sensitive to oxytetracycline (6.25% of isolates), amoxicillin (3.75% of isolates), gentamicin (11.25% of isolates), lincomycin (18.75% of isolates), enrofloxacin (16.25% of isolates) and trimethoprim-sulamethoxazol (15% of isolates). Other *P. multocida* isolates were found to be resistant to oxytetracycline (6.25% of isolates), amoxicillin (7.50% of isolates), penicillin (12.50% of isolates), gentamicin (6.25% of isolates) and lincomycin (7.50% of isolates) (Table3).

DISCUSSION

Antimicrobial agents used for the treatment of BRD are selected by the veterinarian on the basis of

perceived efficacy, cost, convenience, availability, toxicity, and residue profile (Mechor *et al* 1988, Gibbs 2001, Songer 2005). A substantial decrease in treatment efficacy, as indicated by increased mortality or number of animals requiring re-treatment, usually prompts the practitioner to submit samples for bacteriologic culturing and antimicrobial susceptibility testing. Thus, monitoring of the antimicrobial susceptibility trends of BRD pathogens is an important aid to veterinarians in selecting the most efficacious and cost- effective therapeutic agents.

Table 3. Antimicrobial susceptibility testing results of *P. multocida* strains (n=80) isolated from calves with DCP

Antibiotic	Sensitive(n)	Relatively sensitive(n)	Resistant(n)
Florfenicol	80 (100%)	0	0
Chloramphenicol	80 (100%)	0	0
Oxytetracycline	70 (87.50%)	5 (6.25%)	5 (6.25%)
Amoxicillin	71 (88.75%)	3 (3.75%)	6 (7.50%)
Penicillin	57 (71.25%)	13 (16.25%)	10 (12.50%)
Gentamicin	66 (82.50%)	9 (11.25%)	5 (6.25%)
Cephalothin	80 (100%)	0	0
Lincomycin	59 (73.75%)	15 (18.75%)	6 (7.50%)
Enrofloxacin	67 (83.75%)	13 (16.25%)	0
Trimethoprim-Sulfamethoxazol	68 (85.00%)	12 (15%)	0

Several factors may influence the level of antimicrobial susceptibility observed for BRD pathogens (Mechor *et al* 1988, Apley 1997). Since *M. haemolytica* and *P. multocida* are normal flora of the bovine respiratory tract, the susceptibilities of isolates in samples collected from the upper respiratory tract may not represent the susceptibility of the causative strain of the pneumonia (Allen *et al* 1991, Jim *et al* 1992, Boyce *et al* 2004). Despite this there is evidence that NPS is and adequate method of obtaining a representative, and uncontaminated, sample of the nasopharyngeal bacteria prevalent in the living animal (Allen *et al* 1991). Also the results of a recent study suggest that a nasal swab culture was genetically identical with the organism causing disease within the lung for 70% of calves (DeRosa *et al* 2000). In contrast, some workers (Martin *et al* 1983, Mechor *et al* 1988, Jim *et al* 1992) consider that the susceptibilities of isolates recovered from pneumonic lungs may overestimate antimicrobial resistance since the pathogen has previously been exposed to the antimicrobial agent. However, one investigator determined that the majority of animals with BRD either were treated with an insufficient dose of the antimicrobial agent or therapy was terminated too early to affect a complete cure (Jim *et al* 1992). Appropriate therapy should expose the pathogen to the antimicrobial agent at a sufficient concentration and duration to minimize the development of resistant population (Gibbs 2001). Thus, the pathogens isolated from pneumonic lungs represent treatment failures, and the resistant populations probably result from inappropriate or subtherapeutic use of antimicrobial agents.

Results of the present study indicate antimicrobial – resistant *Pasteurella spp.* (*M. haemolytica* and *P. multocida*) in the nasopharyngeal swabs is a probable cause for the poor performance of this antibiotic, which is widely used in the treatment of DCP. With 7 (17.08%), 6 (14.63%), 4 (9.75%) and 1 (2.44%) of *M. haemolytica* were resistant to lincomycin, gentamicin, oxytetracycline and chloramphenicol, respectively. However, resistance to penicillin, lincomycin, amoxicillin, gentamicin and oxytetracycline was observed in 10 (12.50%), 6 (7.50%), 6 (7.50%), 5 (6.25%) and 5 (6.25%) of *P. multocida* isolates, respectively. These findings also agree with previously reported data (Fales *et al* 1982, Martin *et al* 1983, Post *et al* 1991, Songer, 2005) indicating that resistance to older antimicrobial agents is frequently observed with *M. haemolytica* and *P. multocida* strains isolated from cattle with BRD. Resistance to these antimicrobial agents is likely to be related to their widespread and unreasonable use. These results show the need for local veterinarians and producers to be more

responsible in the use of antibiotics in the treatment of pneumonia in calves, and growing danger of the dissemination of strains of *M. haemolytica* or *P. multocida* resistant to most antimicrobials which could complicate in the future the treatment of pneumonia in these animals.

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