The Microbial Flora of the Upper and Lower Respiratory Tracts of Feedlot Calves With Undifferentiated Bovine Respiratory Disease.

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Introduction

Undifferentiated bovine respiratory disease (UBRD) is a syndrome frequently affecting recently weaned calves shortly after their arrival in the feedlot (5). The etiology of the disease is complex and can involve multiple viral and bacterial agents. Previous workers have examined either upper airway samples from live animals (1, 10), or samples of lung tissue from fatal cases (4), to elucidate the bacterial flora of the respiratory tract in naturally occurring UBRD. While much useful information has been obtained there are limitations to both approaches. Firstly, the reliability with which upper airway isolates can be used to correctly predict the presence of organisms actually in the lung is unknown. Secondly, pulmonary isolates from fatal cases, especially those previously treated with antibiotics, are unlikely to be representative of all calves with UBRD (7). To address these problems a study was undertaken in which nasopharyngeal swabs and bronchoalveolar lavage were used to sample the upper and lower respiratory tracts of both sick and clinically normal feedlot calves. This paper describes the bacteriologic culture results of the samples obtained.

Materials and Methods

Study farm and animals

Steer calves (n = 136) were observed during a 28 day period following their arrival at a feedlot research facility. All calves were approximately 6 to 8 months old and had been recently weaned and transported to Ontario from Saskatchewan. The animals were purchased at auction and were from multiple sources none of which advertised any kind of preconditioning or prevaccination program. The calves were housed in pens of 4 in a large slatted floor barn with water and a mixture of corn silage, high moisture grain corn and haylage available *ad libitum*.

Definition of cases and controls

All calves were observed twice daily for signs of UBRD. Sick cattle were detected based on criteria commonly used by the feedlot industry, such as depression, lack of rumen fill, anorexia, nasal discharge or elevated respiratory rate. Depending on these signs cattle were given a clinical score between 0 and 10. Any animal with a clinical score ≥ 2 and with a rectal temperature $\geq 40°C$ was designated as a case. Animals with a score ≥ 6 became cases irrespective of body temperature in order that obviously very sick animals would be treated in the event that they had a normal temperature. For each case detected, a clinically healthy calf was randomly selected as a control. Inevitably, some control calves later became sick. These were discarded from the control group and were included with the cases. In order to keep the case and control groups approximately equal in number, four controls were selected for every three cases.

Treatment, sampling and microbiological procedures

For the first 2 days following detection, cases were treated with 45,000 i.u./kg body weight of procaine penicillin given subcutaneously. On the third day (48 hours after treatment commenced) the animal's response to therapy was assessed. Those with a rectal temperature less than 40°C were deemed responsive to therapy and received two additional days of penicillian therapy. Calves with rectal temperatures $\geq 40°C$ were deemed "non-responders" and a four day course of intramuscular trimethorprim-sulfadoxine (TMS) (Trivetrin, Coopers Agropharm, Ajax, Ontario, Canada) at a dose of 16mg (combined)/kg body weight was initiated. Cases that relapsed following a prior course of treatment were given TMS at the same dose for four days.

Samples for microbiological evaluation were obtained from the upper airway using a guarded nasopharyngeal swab (NS) and from the lung by bronchoalveolar lavage

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(BAL) (11). The BAL procedure was performed standing, under chute restraint without sedation. A flexible fiberoptic endoscope was passed transnasally and wedged in the right apical lobe bronchus where lavage was performed with two aliquots of 120 mls of phosphate buffered saline. In order to minimize bacterial contamination of the encoscope in the upper airway, it was covered by a sterile plastic sheath which could be removed once it was positioned in the trachea.

Nasopharyngeal swabs and BAL fluid sediment were plated on to heart brain infusion blood agar and incubated at 37°C in 5% CO_2 for 48 hours, and on to MacConkey's agar and a selective medium for *Haemophilus somnus* which were incubated at 37°C in room air. Bacterial colonies were identified using standard bacteriological procedures and quantified as scant, (1-9 colonies) moderate, (10-30) and large (>30 colonies) numbers. *Pasteurella* spp. isolates underwent antibiotic sensitivity testing by the disk diffusion method. The same samples were cultured for mycoplasmas and colonies were identified by immunofluorescence.

Sampling protocol

On the first day that a calf was determined to be a case (day 1) a NS and BAL were performed. Controls were sampled in the same manner. Cases that responded to penicillin treatment were subjected to the same sampling procedures on days 6 and 12 (ie. 2 and 7 days after finishing the course of antibiotics). Calves deemed "non-responders" on day 3 were resampled at that time and again on days 8 and 13 (ie. 2 and 7 days after finishing TMS therapy). For sampling purposes any animal that relapsed was regarded as a new case and re-entered the sampling protocol.

Results

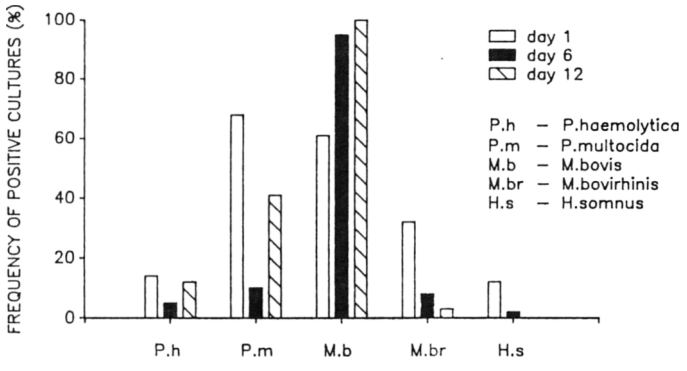
The observed morbidity and mortality rates in this group of calves were 43% and 0% respectively. Samples were obtained from 59 cases and 60 controls. The bacteriologic culture results of all day 1 samples are shown in Table 1. Differences between the isolation rates of various pathogens from cases and controls were evaluated by contingency table chi square analysis. *Pasteurella multocida* was isolated more frequently from the nasopharynx ($p < 0.05$) and the lung ($p < 0.01$) in cases than controls. *Pasteurella haemolytica* was more prevalent in nasopharyngeal swabs from controls than cases ($p < 0.05$). Using the Mantel-Haensel procedure, differences in isolation rates from cases and controls were tested for each organism while controlling for the presence of the others. *P. multocida* was present in BAL fluid from cases more often than controls ($p < 0.05$). No other significant ($p < 0.05$) differences were found. The results of BAL cultures from cases before (day 1), and after (days 6 and 12), treatment (Fig. 1) show the effects of therapy on pulmonary flora.

TABLE 1 Bacteriologic Culture Results in Cases (n = 59) and Controls (n = 60) on Day 1 (Pretreatment)

Organism	Number of Positive Isolations			
	Nasopharyngeal Swab		Bronchoalveolar Lavage	
	Control	Case	Control	Case
P. haemolytica	19 (6)*	9 (6)*	7 (6)*	8 (6)*
P. multocida	28 (21)	41 (35)	26 (21)	40 (35)
M. bovis	26 (21)	27 (24)	31 (21)	36 (24)
M bovirhinis	21 (13)	19 (14)	20 (13)	19 (14)
H. somnus	4 (3)	7 (3)	3 (3)	7 (3)
Streptomyces spp.	2 (2)	0	14 (2)	7
Neisseria spp.	13 (2)	11	2 (2)	0
Bacillus spp.	0	0	2	1

* numbers in parentheses indicate number of calves in which the organism was isolated from both NS and BAL.

FIGURE 1. FREQUENCY OF POSITIVE CULTURES IN CASES BEFORE (day 1) AND AFTER (days 6 AND 12) TREATMENT FROM BAL FLUID.



Agreement between NS and BAL culture results for *Pasteurella* spp., mycoplasmas and *H. somnus* was determined using the kappa statistic. The summary kappa for all samples on day 1 was 0.53. There was no difference between cases and controls. Kappa values for cases only on days 6 and 12 (after treatment) were 0.43 and 0.46 respectively. Kappa values were also calculated when BAL cultures were only regarded as positive if organisms were isolated in large numbers. In these instances, kappa was 0.38 for all samples on day 1 and 0.27 and 0.18 for cases on days 6 and 12. When isolation rates of different organisms from NS and BAL samples were compared at the group level using McNemar's chi square, no significant differences were identified.

Antimicrobial resistance was encountered only among *P. haemolytica* isolates, and when present was always to a

combination of penicillin, ampicillin and tetracycline. Resistant strains were only isolated from cases and were more prevalent after (12/13) than before (2/8) treatment. Antimicrobial sensitivity patterns were the same for NS and BAL isolates within the same animal. Of the 59 cases treated only 5 did not respond to penicillin. Only *M.bovis* was isolated from BAL fluid in these calves. Eleven cases relapsed (three of which were originally non-responders).*M. bovis* was isolated from BAL fluid obtained at the time of relapse in all instances. *P.multocida* and *P.haemolytica* were isolated from five and two calves respectively.

Discussion

Of the organisms isolated from the upper and lower airways of these calves, *P. haemolytica, P. multocida, M. bovis* and *H. somnus* are recognized bovine respiratory pathogens (12). Previous attempts to demonstrate an etiologic role for these agents in UBRD have utilized upper airway cultures and serologic evidence (6) as a measure of whether an animal was or had been infected with a particular organism. Using the BAL technique to sample sick and clinically normal calves, we were able to evaluate the microbial flora present in the area of lung sampled. The right apical lobe bronchus was chosen as the sampling site as most bacterial pneumonias in calves show an anteroventral distribution.

Pathogens were present in the lungs of both cases and controls indicating that in many instances the changes they cause (if any) are subclinical. *P. multocida* was associated with significantly more cases than controls. Experimentally this organism will cause a fibrinous bronchopneumonia (3), and on occasion it is the only organism isolated from pneumonic lungs at post-mortem (8), but its overall role in UBRD is unclear. Our data suggests that further investigation of the subject is warranted. The prevalence of *M. bovis* in the lung increased steadily with time to a level approaching 100% (Fig. 1). This occurred in both treated and control calves indicating that it may be an effect of exposure to the organism in the feedlot or gradual colonization of the respiratory tract rather than to antibiotic therapy. *H. somnus* was more common in sick than control calves but the low prevalence of the organism meant that no statistically significant differences were found. It is noteworthy that *P. haemolytica* was neither particularly prevalent in these calves nor strongly associated with clinical cases. There is strong evidence in the literature for a causal association of *P. haemolytica* with UBRD and especially fibrinous pneumonia (9, 10). However, it is certainly not present in all cases and in some UBRD outbreaks it does not appear to be of major importance.

Streptomyces spp. and *Bacillus* spp. are common inhabitants of the feedlot environment and are therefore frequently found in the bovine respiratory tract. *Neisseria* spp. are commensals of the nasopharynx and in our study they were isolated from 24 NS samples but were only recovered twice from BAL fluid indicating that there was minimal contamination of BAL samples by organisms from upper airways.

Comparisons of NS and BAL culture results were performed to evaluate the utility of NS cultures in predicting the presence of organisms in the lung. Kappa values were calculated to determine the level of agreement between the two culture techniques at the individual animal level. Kappas of <0.4, 0.4-0.6 and >0.6 reflect poor, moderate and good agreement respectively, thus NS and BAL samples show only moderate agreement at best and it decreases once animals have been treated. When NS and BAL results were compared at the group rather than the individual animal level the results were not significantly different, indicating that NS samples can be reliably used for diagnostic or research purposes to predict the pulmonary microbial flora in a group of calves.

Figure 1 shows that bacteria but not mycoplasmas are largely cleared from the lung following treatment (day 6) but later some recolonization by bacteria occurs. Antimicrobial resistance to *P. haemolytica* occurred frequently following treatment and the resistance pattern found has been reported in previous work (2). In the field, lack of response to treatment and relapses are frequently blamed on the presence of resistant strains of organism and on failure of the antibiotic to clear organisms from the lung. In this study, however, this was not always the case, suggesting that other factors exist to explain the persistence or recurrence of clinical signs.

Summary

The upper and lower respiratory tracts of feedlot calves with (59 cases) and without (60 controls) signs of undifferentiated bovine respiratory disease were sampled before and after antibiotic treatment (penicillin, trimethoprim-sulfadoxine) using nasopharyngeal swabs (NS) and bronchoalveolar lavage (BAL). Samples were cultured for bacteria and mycoplasmas. Pathogens were present in the lungs of sick and control calves. *P. multiocida* was significantly associated with morbidity. At a group level NS cultures were reliable predictors of BAL culture. *P. haemolytica* isolated after treatment were frequently resistant to a combination of penicillin, ampicillin and tetracyclines. Bacteria but not mycoplasmas were largely cleared from the lungs after therapy but later some recolonization occurred. Treatment failure was seldom associated with antimicrobial resistance.

Acknowledgements

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Abstracts:

Effect of prostaglandin treatment on the fertility of problem cows

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Four autumn-calving dairy herds were selected to investigate the effect of an injection of prostaglandin in the period 14 to 28 days (mean 22 days) after calving on subsequent fertility. The cows were selected on the basis of having a condition likely to affect their fertility, including assisted calving, endometritis, retained fetal membranes, milk fever, cows with five or more lactations, cows having twins, or a combination of any of these conditions. They were assigned to treatment or control grops and paired as closely as possible on the basis of their condition and date of calving. Milk progesterne concentrations were measured on the day of treatment and then three and 10 days later. The trial ran for four months and involved 90 treated and 90 control cows. The combined data from all the animals in the trial failed to show any difference between the calving to conception interval, the first service conception rate or the numbers of services per conception of the treated and control groups. A Student's paired *t* test for groups of cows with a particular condition, both within individual herds and in all herds, failed to show any significant effect of treatment ($P < 0.05$). Milk progesterone data showed that the presence of a corpus luteum did not influence the outcome of prostaglandin treatment. There was no evidenc for excessive failure of luteolysis. It was concluded that there was no benefit ina routine injection of prostaglandin to dairy cows in the period 14 to 28 days after calving when re-breeding commenced more than 70 days after calving.

Winter dysentery in dairy herds: Electron microcopic and serlolgical evidence for an association with coronavirus infection

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Faeces and, or, paired sera were collected from cows in six dairy herds with classical winter dysentery. Similar samples were collected from cows in three other dairy herds experiencing nonhaemorrhagic diarrhoea during the survey period. Coronavirus was the only enteric pathogen identified by immune electron microscopy (IEM) in all six outbreaks, occurring in 26 of 29 (90 per cent) of the affected cows and in one of 11 normal cows from the same herds. Nineteen of 26 affected cows (73 per cent) developed greater than four-fold increases in neutralizing antibody titres to the Mebus strain of bovine coronavirus, compared with two of eight normal cows in the same herds. No cows showed greater than four-fold increases in antibody titres to bovine virus diarrhoea virus. None of the cows from the three herds with non-haemorrhagic diarrhoes shed coronavirus in faeces detectable by IEM or developed greater than two-fold rises in coronavirus antibody titres in paried sera. No enteric pathogens were identified in two of the herds. However, two cows in the third herd shed a group B rotavirus detected by IEM. These findings provide additional evidence for a possible role for bovine coronavirus in the aetiology of winter dysentery. Furthermore, this is the first report of a group B rotavirus associated with diarrhoea in adult cattle.