

## ANTIBIOTIC SENSITIVITY OF *PASTEURELLA HAEMOLYTICA* ISOLATED BY MEANS OF A FIBREOPTIC ENDOSCOPE FROM CASES OF PNEUMONIC PASTERELLOSIS IN CATTLE

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### ABSTRACT

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Bacterial isolations from tracheal and bronchial washes obtained with the aid of a fibreoptic endoscope were carried out over a 7 month period in a feedlot on calves suffering from acute pneumonic pasteurellosis. *Pasteurella haemolytica* and *Pseudomonas aeruginosa* represented the majority of isolates. Antibiotic sensitivities of the *Pasteurella* isolates are reported on.

### INTRODUCTION

*Pasteurella haemolytica* has been widely implicated as the most important bacterial pathogen associated with pneumonic pasteurellosis (Hjerpe, 1975; Houghton & Gourlay, 1984; Jensen, Pierson, Braddy, Saari, Lauer-mann, England, Keyvanfar, Collier, Horton, McChes-ney, Benitez & Christia, 1976).

A study was undertaken in a large South African feedlot with the objectives of establishing what bacterial pathogens are associated with pneumonic pasteurellosis and monitoring the antibiotic sensitivities of these bacteria.

### MATERIALS AND METHODS

#### Experimental animals

Groups of weaner calves each with a body mass of 200-250 kg were used in a commercial feedlot with a population of  $\pm 50\ 000$  head. The study extended over a 7 month period in which twice monthly visits were conducted during July 1984 and March-August 1985. During this time, the 130 tracheal and bronchial washes carried out represented about 4 % of new cattle suffering from suspected pneumonic pasteurellosis.

#### Clinical procedures

Calves with suspected acute pneumonic pasteurellosis were selected. The diagnosis was based on clinical signs (pyrexia, listlessness, nasal discharge and coughing) and endoscopic examination (presence of tracheitis and visible pus in the lower trachea). The latter, however, was not necessarily a prerequisite.

Tracheal washes were obtained from each of the selected calves showing tracheitis with pus in the lower trachea, while bronchial washes were carried out when no tracheitis or pus was observed.

Both these procedures were carried out with the aid of a fibreoptic endoscope. When a tracheal wash was performed, the endoscope was positioned low down in the trachea. A sterile polyethylene catheter was fed through a channel in the endoscope and visibly brought into contact with the pus, if present. With the aid of a syringe containing sterile normal saline connected to the catheter, a sample was collected and stored on ice for bacteriological examination.

Bronchial washes were performed by guiding the

catheter for some distance into the bronchus of the right apical lobe. The bronchus was then flushed with 10-20 ml of sterile normal saline.

#### Bacteriological procedures

The method for bacterial isolations done on tracheal and bronchial washes carried out during July 1984 and March 1985 (Table 1) was as follows: Samples were centrifuged in a refrigerated centrifuge at 2 000 g for 30 min at 4 °C. The supernatant was removed and the sediment cultured on both MacConkey's<sup>1</sup> and 5 % Columbia base blood agar plates. The MacConkey's plates were incubated in an atmosphere containing 5 % CO<sub>2</sub> at 37 °C and the blood agar plates at 37 °C.

After a 24 h incubation period, all the cultures containing colonies suspected of being *Pasteurella* were subcultured onto both MacConkey's and blood agar plates and incubated as described above. Plates showing no growth of *Pasteurella* type colonies were incubated for a further 24 h.

Organisms from subcultures were stained using the Gram's method and tested for the presence of oxidase. Gram negative, oxidase positive bacilli were then identified using the API 20 NE system. No serotyping was performed.

The method employed for bacterial isolations carried out on washes obtained between April and August 1985 (Table 1) was as follows: The sample was inoculated within 2 h of receipt onto blood tryptose agar (BTA) prepared with bovine blood; MacConkey agar; chocolate agar and serum broth. Two BTA plates were inoculated in each case, and 1 of them was cross-inoculated with a feeder *Staphylococcus aureus* culture. This cross inoculated BTA plate and the chocolate agar plate were incubated in an atmosphere of 10 % CO<sub>2</sub> for the possible isolation of *Haemophilus*, and the others in a normal atmospheric incubator. All cultures were discarded as negative after 4 days. All isolates were identified according to standard criteria, as described by Buchanan & Gibbons (1974). Serotyping of *Pasteurella* was carried out, as described by Aarsleff & Biberstein (1970).

### RESULTS AND DISCUSSION

The results of the bacterial isolations are shown in Table 1.

The results of the antibiotic sensitivities are shown in Table 2.

Sample collection for microbiological examination with the aid of a fibreoptic endoscope in clinical cases of acute pneumonic pasteurellosis is a quick and easy technique and, in this study, resulted in a 47 % positive

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TABLE 1 Results of bacterial isolations from calves with acute pneumonic pasteurellosis

Time of bacterial isolations	Number of tracheal and bronchial washes	Number of isolates	Bacterial identification and number of isolates
July 1984	46	20	<i>P. haemolytica</i> (20)* <i>Ps. aeruginosa</i> (2) <i>Ps. fluorescens</i> (1) <i>Ps. testosteroni</i> (1) <i>E. coli</i> (1)
March–August 1985	84	42	<i>P. haemolytica</i> type 1 (11) <i>P. haemolytica</i> type 9 (1) <i>P. haemolytica</i> (cross reacts with types 1 +6) (1) <i>P. multocida</i> type A (1) <i>P. multocida</i> untypable (1) <i>Ps. aeruginosa</i> (14) <i>Ps. alcaligenes</i> (6) <i>Ps. fluorescens</i> (1) <i>E. coli</i> (1) <i>Ps. cepacea</i> (1) <i>Klebsiella</i> 64 (1) <i>Serratia marcescens</i> (1) <i>Neisseria</i> spp. (1) <i>Staph. aureus</i> (1)

\* Indicates number of isolates

TABLE 2 Results of the antibiotic sensitivities against bacterial isolates from calves with acute pneumonic pasteurellosis

Time of bacterial isolations	Isolates	Number of isolates tested	Resistance %			
			Penicillin	Oxytetracycline	Sulphonamide	Kanamycin
July 1984	<i>P. haemolytica</i>	15	20	70	10	10
	<i>Ps. aeruginosa</i>	2	100	100	0	100
March–August 1985	<i>P. haemolytica</i>	12	41,7	0	33	15
	<i>Ps. aeruginosa</i>	8	100	100	87,5	0

bacterial isolation rate. Of these *Pasteurella haemolytica* represented 45 % and *Pseudomonas aeruginosa* 25 % of the clinical cases. The latter is an uncommon isolate from pneumonic cases (M. Henton, unpublished data, 1985). The importance of the high incidence is not known, but the fact that it was almost always isolated in pure culture seems significant. *Pseudomonas aeruginosa* could act as an opportunist and, thus complicate *Pasteurella* pneumonia cases.

Oxytetracycline was used extensively during previous treatment regimes in this feedlot but during July 1984, 70 % of *Pasteurella haemolytica* isolates proved to be resistant to oxytetracycline on *in vitro* sensitivity testing. All the *Pasteurella haemolytica* isolates obtained during the March–August 1985 period proved to be susceptible to oxytetracycline on *in vitro* sensitivity testing. The use of tetracyclines was suspended in this feedlot during the period July 1984–August 1985. During the same period there was a 20 % increase in resistance of the *Pasteurella haemolytica* isolates to both penicillin and sulphonamides.

These 2 drugs were used in treatment regimes for pneumonic pasteurellosis during that time.

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