Bacteriological Findings in Nasal and Lower Respiratory Tract Samples of Calves With Acute Respiratory Disease

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Introduction

Infectious calf pneumonia passes off with high morbidity and is frequent in housed dairy animals in the winter. In Sweden the greatest disease problems occur in the "specialized calfrearing units" for meat production. To these farms about 100,000 calves (> 2 weeks old) are now mediated every year from dairy farms. Viral mycoplasmal and bacterial agents are involved in the complex etiology. From emergency slaughtered animals in *Sweden Pasteurella multocida, Pasteurella multocida, Pasteurella hemolytica* and *Actinomyces pyogenes* were the most commonly isolated bacteria. *Mycoplasma dispar* was the most commonly mycoplasmal species isolated.[4] No investigation has been performed in Sweden over bacteria present in the lower respiratory tract at the acute phase of calf pneumonia.

The aim of the present study was to compare the bacteriological flora in the nasal cavity with that of the lower respiratory tract in diseased animals and in healthy animals.

Materials and Methods

In 21 farms samples were taken from 46 calves with clinical symptoms of pneumonia. In 19 farms the calves had been medicated from other herds and in 2 farms the calves were born in the farm. In 16 of the herds, sampling from 18 healthy calves was performed. Diseased animals had a body temperature of >39.5°C and at least one of the following symptoms: elevated respiratory frequency or increased bronchial tones. Moreover they often coughed and had nasal discharge (see Table 1). Healthy calves had a body temperature <39.5°C and no symptoms of respiratory disease. The calves were 2 weeks-3 months old with body weights between 50-125 kg.

Blood sample was collected for fibrinogen determination by heat precepicitation test. Student's t-test was used to analyse the difference between fibrinogen values between diseased and healthy animals.

Table 1. Clinical symptoms of sampled calves

	Diseased	Healthy
Body temperature (°C) $\bar{x}$	40.4	39.0
SD	0.66	0.29
Respiratory frequency/min $\bar{x}$	55	31
SD	19	8
Number of animals with increased bronchial tones	43	1

The nasal samples were taken with cotton swabs (Culturette, Biodisk, Sweden).

Sampling from the lower respiratory tract

The samples from the lower respiratory tract were taken in anesthetised animal (0,25 ml-0, 5 ml per 100 kg body weight Rompun (xylazin), Bayer). When anesthesia was established the lower third of the trachea was fixed with one hand and a cannula-catheter (Vygon hémocath 60, 1,5-2,0 mm, cannula 2,4 mm Ecouén, France) was inserted in the midline downwards in 45° angle to the trachea. After passing the skin and the tracheal wall, the cannula was inserted parallel to the trachea. The catheter was pushed 20-25 cm into the cannula. Phosphate buffer (20 ml of Dulbecco without calcium and magnesium) was infused and immediately aspirated through the catheter. If less than

1.5 ml of fluid was aspirated (the volume of the catheter) another 20 ml of buffer was infused. The sample was put into a test tube. Signs of discomfort were not seen during or after sampling.

Bacteriology

Material from nasal swabs and from the lower respiratory tract was cultured within 2-4 hours aerobically, anaerobically and in 5% CO_2 atmosphere. Identification of isolates was based on standard methods at the National Veterinary Institute, Sweden.

Serotyping of *Pasteurella multocida* somatic antigens was performed by gel diffusion precipitation. Capsular antigen type A was determined by hyaluronidase test and type D by acriflavine.[2] *Pasteurella hemolytica* isolates were serotyped by indirect haemagglutination test.[5]

Mycoplasmology

For the isolation of mycoplasmas, material from the lower respiratory tract was cultivated according to standard methods at the National Veterinary Institute.[1] For a part of the samples a selective broth for cultivation of *Mycoplasma dispar* was used.

Results

The fibrinogen values of diseased and healthy animals (Table 2) differed significantly ($p<0.001$, $t = 4.14$, $df = 62$).

Table 2. Plasma fibrinogen levels of sampled calves

		Diseased	Healthy
Fibrinogen (g/l)	$\bar{x}$	7.2	4.6
	SD	2.4	1.7

In a majority (65.2%) of diseased calves different Pasteurella species was isolated from the lower respiratory tract samples (Table 3), in contrast to healthy calves (33.3%, $p < 0.05, x^2$ - analysis). *P. multocida* was most common and isolated from 45.5% of diseased calves. Of the Pasteurella multocida isolates in diseased calves 78.9% belonged to type 3 capsule A.

Ureplasma was significantly more frequently isolated from diseased than in helathy calves ($p < 0.02 x^2$ analysis).

The results of the bacteriological examinations of nasal and lower tracheal samples agreed in isolated Pasteurella species in 58.7% of those from diseased calves and in 44.5% of those from healthy animals (Table 5). In samples from the lower respiratory tract and in nasal swabs, 2 bacterial species or less were isolated in 92.3% and in 48.7% respectively.

Table 3. Bacteriological findings in samples from the lower respiratory tract of calves.

	Number of diseased animals		Number healthy animals	
P.m.[1] type 3A	12		4	
P.m. type 3D	2		1	
P.h.[2] A2	4			33.3
P.h. NT	3		1	
P.m. 3A × P.h. A1	2			
P.m. 3A × hem. strept.	1			
P.m. 3D × hem. strept.	1			
P.m. 3D × P. spec.	1			
P.m. (not tested)	1			
P.m. (not tested) × Actinomyces pyogenes	1			
P. spec.	2			
Other bacterial species[3]	5	10.9	2	11.1
Mixed bacterial flora	2	4.3	1	5.6
No bacterial growth	9	19.6	9	50
Total number of calves	46		18	

[1]P.m. = Pasteurella multocida
[2]P.h. = Pasteurella hemolytica
[3]Fusobacterium necroforum in one calf

Table 4. Isolated mycoplasmas from the lower respiratory tract.

	Number of diseased animals	%	Number of healthy animals	%
M. dispar	14	30.4	3	16.7
Ureplasma	29	63.0	5	27.8
M. bovirhinis	43	93.5	15	83.3
M. canadense	1	2.2		

Table 5. Prevalence of Pasteurella species in nasal swabs and in samples from the lower respiratory tract in individual animals.

	Diseased animals	%	Healthy animals	%
Identical isolation in nasal and in lower respiratory tract samples	27	58.7	8	44.5
Non identical or no isolation in one of nasal or lower respiratory tract samples	19	41.3	10	55.6

Discussion

Plasma fibrinogen values indicates significant tissue injury in the lungs of diseased animals. Plasma fibrinogen rises as early as 24 hours following the onset of tissue injury.[6]

The isolated bacteria belong to species frequently isolated in calves with respiratory disease,[1,4] but in the contrary to Thomas et al[7] only one anaerobic species was isolated. In comparison with autopsy and emergency slaughter material a low frequency of *Actinomyces pyogenes* was isolated.[1,4] The high frequency of Pasteurella isolates and the lower frequency of other bacterial isolates in nasal and lower respiratory tract samples indicates the importance of Pasteurella species as causative agent of acute respiratory disease in calves. Ureaplasma was more frequently obtained in diseased than in healthy calves. This might indicate a possible role together with bacteria in the etiology of pneumonia.

Since there was only 58.7% agreement between isolated Pasteurella species in nasal and lower respiratory samples from diseased animals, the used sampling method from the lower respiratory tract was more adequate in individual animals. Furthermore, more pure cultures are obtained after cultivation by sampling from the lower respiratory tract. However, nasal sampling might be useful in screening on a herd scale for bacteriological sampling.

Summary

In a Swedish field study nasal and lower respiratory tract samples were taken from 21 farms in 46 diseased and 18 healthy calves. The samples from the lower respiratory tract were taken without contamination of the upper respiratory tract. Diseased calves had a body temperature of > 39,5 C, respiratory symptoms and elevated plasma fibrinogen levels. Different species of Pasteurella was isolated from the lower respiratory tract of 65,2% and 33,3% of diseased and healthy animals respectively. In diseased animals *Pasteurella multocida* (P.m.) was not common. Of the P.m. isolates 78,9% belonged to the same serotype (type 3 capsule A).

In individual diseased animals 58.7% fo the bacteriological examinations of nasal and lower tracheal samples agreed with regard to isolated Pasteurella species. In contrast to cultures from nasal swabs, Pasteurella was mostly isolated in pure culture from lower respiratory tract samples.

References

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