

# The Pulmonary Clearance of *Pasteurella hemolytica* in Calves Infected with Bovine Parainfluenza-3 Virus

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## ABSTRACT

The purpose of this study was to determine if parainfluenza-3 virus in calves interfered with normal pulmonary bacterial clearance. Three groups (A, B and C) of four calves each were exposed to an aerosol of parainfluenza-3 virus. Three days later the four hour pulmonary clearance of *Pasteurella hemolytica* was determined on the first group (A), seven days later on the second (B) and 11 days later on the third (C). Group A had a mean pulmonary bacterial retention of  $3.6 \pm 3.5\%$ . Group B was  $83.1 \pm 35.9\%$  and Group C was  $41.2 \pm 30.9\%$ .

The results demonstrate that parainfluenza-3 virus interfered with the pulmonary bacterial clearance of *Pasteurella hemolytica* particularly on day 7 and also on day 11 but not on day 3. This inhibition of pulmonary clearance caused by the virus may be a key factor in the pathogenesis of pneumonic pasteurellosis. Histological examination of the lungs did not demonstrate a correlation between pulmonary retention of bacteria and the development of pathological changes.

## RÉSUMÉ

Cette expérience visait à déterminer si le virus para-influenza 3 fait échec à l'élimination pulmonaire normale des bactéries, chez le veau. Trois groupes (A, B et C) reçurent ce virus, au moyen d'aérosols. Trois jours plus tard, on détermina, pour une période de quatre heures, le rythme d'élimination de *Pasteurella hemolytica* des poumons des sujets du groupe A; sept jours plus tard, on répéta le même procédé chez ceux du groupe B et, 11 jours plus

tard, chez ceux du groupe C. La rétention bactérienne pulmonaire moyenne des veaux du groupe A s'établissait à  $3.6 \pm 3.5\%$ , celle des veaux du groupe B, à  $83.1 \pm 35.9\%$ , et celle des veaux du groupe C, à  $41.2 \pm 30.9\%$ .

Les résultats de l'expérience démontrèrent que le virus para-influenza 3 fit échec à l'élimination de *P. hemolytica* des poumons des veaux, surtout le septième jour, également le onzième, mais non le troisième. Cette action du virus pourrait s'avérer le facteur clef de la pathogenèse de la pasteurellose pulmonaire. L'examen histologique des poumons ne révéla pas de corrélation entre la rétention pulmonaire des bactéries et le développement des lésions pulmonaires.

## INTRODUCTION

The normal pulmonary clearance of bacteria in calves was established (24) in order to determine significant factors which might alter normal clearance and allow bacteria to multiply and cause pneumonia (5). A number of factors are known to adversely influence pulmonary clearance in laboratory animals (29). Of particular interest in cattle is the possible influence of previous or concurrent viral infection of the respiratory tract on the pulmonary clearance of bacteria. It has been well documented in laboratory animals that viral infection of the respiratory tract will markedly reduce pulmonary clearance of bacteria about one week after the viral infection (13). The literature contains considerable circumstantial evidence that parainfluenza-3 (PI-3) virus has a role in the pathogenesis of pneumonic pasteurellosis in cattle (1, 25, 27) but the precise role of PI-3 virus has not been determined. The following experiments were conducted in an attempt to determine whether or not PI-3 virus had an influence on the pulmonary clearance of *Pasteurella hemolytica* in calves.

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## MATERIALS AND METHODS

### ANIMALS

Twelve dairy calves, six to eight weeks of age, obtained commercially were selected from a group of 54 similar calves on the basis of having the lowest serum antibody titres to PI-3 virus (Hemagglutination Inhibition Test) (4) and *P. hemolytica* (Indirect Hemagglutination Test) (2, 3). The calves were randomly divided into three groups A, B and C, of four animals each (Table I).

Fifteen random bred male white mice weighing about 30 gm each were obtained commercially.

### AEROSOLIZATION

The aerosol apparatus was a modification of that designed by Laurenzi and Guernieri (23) and Laurenzi *et al.* (21). It was used by Lillie and Thomson (24) and reconstructed so that four calves could be exposed at once (5).

The virus utilized in this experiment was a fourth tissue culture passage of a field isolate of PI-3 virus (4644-9, O.V.C.). A total of 100 ml of viral suspension, which had a 50% cell culture infective dose (C.C.I.D. 50) of  $10^4$  per 0.2 ml, was aerosolized during a 30 minute exposure period for each group (A, B and C) of four calves in three consecutive runs on the same day (day 0). Group A and five mice were exposed to an aerosol with live *P. hemolytica* for 30 minutes three days (day 3) after virus aerosolization with a bacterial suspension of  $5.7 \times 10^{10}$  organisms per ml. Group B and five mice were exposed to an aerosol with a bacterial suspension of  $8.7 \times 10^9$ , seven days (day 7) after PI-3 virus. Group C and five mice were exposed to an aerosol 11 days after PI-3 virus (day 11) with a *P. hemolytica* suspension of  $4.9 \times 10^{10}$ .

The strain of *P. hemolytica* used in this experiment was the same as utilized in previous studies of pulmonary clearance in calves (5, 24). It was a laboratory strain, serotype I, type A of *P. hemolytica* which was originally obtained from E. L. Biberstein, Davis, California. The bacterial suspension was prepared by inoculating two or three colonies into two six litre Erlenmeyer flasks, each containing four litres of brain heart infusion broth. The inoculated flasks were placed in a shaker water bath at 37°C and shaken at 60 oscillations per

minute for ten hours. The culture was centrifuged at 8,000 rpm for 20 minutes, the supernatant fluid was decanted, the pellet was resuspended in 5.0 ml of potassium phosphate buffer solution and then placed in an Erlenmeyer flask. A few minutes before aerosolization 1.0 ml of the bacterial suspension was removed and the Colony Forming Units (CFU) per ml of suspension were determined by serial dilution. A series of eight tubes, each containing 4.5 ml of potassium phosphate buffer solution were prepared. Tenfold serial dilution in phosphate buffer were prepared to a dilution of  $10^{-8}$ . Using a 0.02 ml Eppendorf micropipette each dilution was plated on three plates of 5% bovine blood agar medium with four drops per plate making a total of 12 drops per sample. The plates were incubated at 37°C for 18 hours and the number of colonies were then counted utilizing an electric colony counter. The bacterial concentration of the suspension was then calculated.

### DEPOSITION OF BACTERIA IN LUNGS

Because of time and expense it was decided to use mice rather than calves in order to determine the original deposition of *P. hemolytica* in calves lungs. Gilka compared the deposition of *P. hemolytica* in mice and calves in 15 experiments, one calf and five mice in each experiment, and he concluded that the number of bacteria deposited in calf lung was  $34.3 \pm 5.1\%$  of that deposited in mice exposed to the same aerosol (personal communication, 1974). Therefore, the number of bacteria deposited in calves lungs may be calculated from mice exposed to the same aerosol. For this purpose five mice were exposed simultaneously with each group of calves and the deposition at zero hour was determined as described previously (5, 24).

All groups of calves were euthanized four hours after exposure to *P. hemolytica*. The calves were given barbiturates by intravenous injections and exsanguinated by transection of the brachial arteries. The skin was disinfected and removed and the thorax was opened along the costochondral junctions, leaving the abdominal cavity intact to avoid possible contamination. Once the lungs were exposed two samples of each lung were taken, one anteroventral and the other dorsodiaphragmatic, making a total of four samples per animal. The lung samples were weighed and 10 gm of each were

homogenized in 100 ml of 0.1 M potassium phosphate buffer solution in a blender. Serial dilution and cultures on blood agar media were carried out and the number of bacteria per gm of lung tissue was calculated as reported previously (5, 24).

The four hour pulmonary clearance of *P. hemolytica* by calves was determined from the values obtained at zero hour in mice by simultaneous exposure of mice to the same aerosol as the calves and from the values obtained from calf lungs four hours after exposure to *P. hemolytica*. Both values were compared and the percentage of clearance at four hours by calves was determined by taking the calculated values at zero hour as 100% of bacterial retention. The percent of retention was determined by subtraction of the clearance from 100%.

#### HISTOPATHOLOGICAL EXAMINATION

At necropsy, lung tissues were collected, fixed in 10% buffered formalin solution, embedded in paraffin, sectioned at four to six microns and stained with hematoxylin and eosin.

The histological slides were observed by light microscopy. For purposes of classifying the degree of lesions present the following areas were examined: bronchi, bronchioles, alveoli, alveolar septa and interlobular septa. According to the degree of lesions the histopathological changes were classified as negative (-), mild (+), intermediate (++), severe (+++), and very severe (++++).

TABLE I. Groups of Calves and Serum Antibody Titres Against *P. hemolytica* and PI-3 Virus at the Beginning and End of the Experiments

Calf	PI-3 Virus Antibody Titres		<i>P. hemolytica</i> Antibody Titres	
	Day 0	Day 3	Day 0	Day 3
A1.....	1:16	1:16	1:8	1:8
A2.....	1:16	1:32	1:8	1:8
A3.....	1:8	1:16	1:16	1:16
A4.....	1:16	1:16	1:8	1:8
	Day 0	Day 7	Day 0	Day 7
B1.....	1:16	1:16	1:4	1:8
B2.....	1:8	1:32	0	0
B3.....	1:8	1:16	1:4	1:4
B4.....	1:8	1:16	1:8	1:4
	Day 0	Day 11	Day 0	Day 11
C1.....	1:8	1:16	1:4	1:4
C2.....	1:16	1:16	1:8	1:8
C3.....	1:8	1:16	1:8	1:16
C4.....	1:16	1:16	1:16	1:32

The bronchi and bronchioles were observed for the presence of exudate within the lumen and in the lamina propria for epithelial desquamation and regeneration and for lymphoid hyperplasia. The alveoli were observed for the presence of exudate within lumina as well as for alveolar edema, emphysema and atelectasis. The alveolar septa were observed for inflammation and fibrin thrombi. The interlobular septa were examined for edema and fibrin. The presence or absence of giant cells was also recorded. The 4+ utilized for quantification of exudate indicated that the exudate filled the lumen of bronchioles or alveoli.

## RESULTS

#### CLEARANCE

The four calves of Group A were killed on the third day after the PI-3 viral aerosol and four hours after the bacterial aerosol of *P. hemolytica*. The percentage retention of each calf was 1.2% for A1 and A2, 3.2% for A3 and 8.8% for A4 with a group mean of  $3.6 \pm 3.5\%$ .

The four calves of Group B were killed on the seventh day after the viral aerosol and four hours after the bacterial aerosol. The percentage retention of each calf was 112.5% for B1, 39.5% for B2, 68.6% for B3 and 112.5% for B4 with a group mean of  $83.1 \pm 35.9\%$ .

The four calves of Group C were killed 11 days after the viral aerosol and four hours after the bacterial aerosol. The percentage retention of each calf was 82.4% for C1, 7.1% for C2, 36.5% for C3 and 38.9% for C4 with a group mean of  $41.2 \pm 30.9\%$ .

The details of the inoculum and retention are listed in Table II. The results demonstrate normal clearance of *P. hemolytica* three days after PI-3 virus infection, marked impairment of clearance on day 7 with partial recovery by day 11. There was considerable variation between individual calves within groups B and C.

There were minimal changes in the serum antibody titres against PI-3 virus and *P. hemolytica* for each calf over the experimental period. (Table I).

#### HISTOPATHOLOGY

The details of microscopic lesions for each calf are given in Table III. Whenever

exudate was observed in bronchi, bronchioles or alveoli, it consisted essentially of neutrophils and sometimes macrophages. In calf B1 the bronchioles and alveoli were completely filled with exudate (4+), the remainder of the animals had variable

grades of infiltration by neutrophils.

Animal B2 was the only one that exhibited bronchiolar epithelial desquamation and regeneration (+). Lymphoid hyperplasia was present in seven of the 12 animals. A mild degree of alveolar edema

**TABLE II. Inoculum, Deposition and Retention of *P. hemolytica* in Calves Aerosolized with PI-3 Virus Three Days (A), Seven Days (B) and 11 Days (C) Prior to Bacterial Aerosolization**

Calf	Inoculum	Mouse Deposition	Number of <i>P. hemolytica</i> in Calf Lung <sup>a</sup> at Four Hours				Mean	Retention %
			R1 <sup>b</sup>	R2 <sup>c</sup>	L1 <sup>d</sup>	L2 <sup>e</sup>		
A1			1.2 × 10 <sup>3</sup>	3.3 × 10 <sup>3</sup>	1.7 × 10 <sup>3</sup>	1.8 × 10 <sup>3</sup>	2.0 × 10 <sup>3</sup>	1.2
A2			2.0 × 10 <sup>3</sup>	2.0 × 10 <sup>3</sup>	1.5 × 10 <sup>3</sup>	2.5 × 10 <sup>3</sup>	2.0 × 10 <sup>3</sup>	1.2
A3	5.7 × 10 <sup>10</sup>	1.2 × 10 <sup>5</sup>	5.9 × 10 <sup>3</sup>	2.5 × 10 <sup>3</sup>	5.7 × 10 <sup>3</sup>	5.4 × 10 <sup>3</sup>	4.9 × 10 <sup>3</sup>	3.2
A4			3.1 × 10 <sup>4</sup>	8.7 × 10 <sup>3</sup>	8.8 × 10 <sup>3</sup>	8.8 × 10 <sup>3</sup>	1.4 × 10 <sup>4</sup>	8.8
B1			1.0 × 10 <sup>4</sup>	1.9 × 10 <sup>4</sup>	2.2 × 10 <sup>4</sup>	2.0 × 10 <sup>4</sup>	1.8 × 10 <sup>4</sup>	112.5
B2	8.7 × 10 <sup>9</sup>	1.2 × 10 <sup>4</sup>	1.0 × 10 <sup>4</sup>	2.8 × 10 <sup>3</sup>	8.3 × 10 <sup>3</sup>	4.2 × 10 <sup>3</sup>	6.3 × 10 <sup>3</sup>	39.5
B3			7.5 × 10 <sup>3</sup>	1.0 × 10 <sup>4</sup>	1.8 × 10 <sup>4</sup>	8.7 × 10 <sup>3</sup>	1.1 × 10 <sup>4</sup>	68.8
B4			1.6 × 10 <sup>4</sup>	2.3 × 10 <sup>4</sup>	1.7 × 10 <sup>4</sup>	1.7 × 10 <sup>4</sup>	1.8 × 10 <sup>4</sup>	112.5
C1			2.9 × 10 <sup>5</sup>	1.5 × 10 <sup>5</sup>	3.9 × 10 <sup>4</sup>	9.7 × 10 <sup>4</sup>	1.4 × 10 <sup>5</sup>	82.4
C2	4.9 × 10 <sup>10</sup>	1.3 × 10 <sup>5</sup>	1.2 × 10 <sup>4</sup>	1.6 × 10 <sup>4</sup>	1.3 × 10 <sup>4</sup>	6.2 × 10 <sup>3</sup>	1.2 × 10 <sup>4</sup>	7.1
C3			7.5 × 10 <sup>4</sup>	8.0 × 10 <sup>4</sup>	5.7 × 10 <sup>4</sup>	3.7 × 10 <sup>4</sup>	6.2 × 10 <sup>4</sup>	36.4
C4			5.5 × 10 <sup>4</sup>	4.5 × 10 <sup>4</sup>	5.2 × 10 <sup>4</sup>	1.1 × 10 <sup>5</sup>	6.6 × 10 <sup>4</sup>	38.8

<sup>a</sup>According to the topographical distribution the lung samples were named:

<sup>b</sup>R1 = Right anteroventral

<sup>c</sup>R2 = Right dorsodiaphragmatic

<sup>d</sup>L1 = Left anteroventral

<sup>e</sup>L2 = Left dorsodiaphragmatic

**TABLE III. Classifications of Histopathological Lesions in the Lungs of Calves in Groups A, B and C**

Lesion	Group A (3 days)				Group B (7 days)				Group C (11 days)				
	1	2	3	4	1	2	3	4	1	2	3	4	
Bronchi	Exudate lumen	+	+	+	+	++	+	+	0*	+	-	-	+
	Exudate lam. prop.	+	+	+	+	+	+	-	0	+	-	+	+
	Epith. desquam.	-	-	-	-	-	-	-	0	-	-	-	-
	Epith. regeneration	-	-	-	-	-	-	-	0	-	-	-	-
	Lymphoid hyperpl.	+	+	-	+	-	-	++	0	+	+	-	-
Bronchioles	Exudate lumen	+	+	+	++	++	+	+	0	+	+	+	+
	Exudate lam. prop.	+	+	+	+	++	+	+	0	+	+	+	+
	Epith. desquam.	-	-	-	-	-	+	-	0	-	-	-	-
	Epith. regeneration	-	-	-	-	-	+	-	0	-	-	-	-
	Lymphoid hyperpl.	+	+	-	-	-	-	++	0	+	-	+	-
Alveoli	Exudate	-	-	-	++	++	+	+	0	-	-	-	-
	Edema	-	-	-	+	+	+	+	0	+	-	+	-
	Emphysema	-	-	-	-	-	-	-	0	-	-	-	-
	Atelectasis	+	+	+	+	++	+	+	0	+	+	++	+
Alveolar Septa	Inflammation	+	-	+	+	+	+	+	0	+	+	+	+
	Fibrin thrombi	-	+	-	+	+	-	+	0	-	+	+	-
Interlobular Septa	Edema	+	-	-	+	++	-	+	0	+	-	-	-
	Fibrin thrombi	-	-	-	-	+	-	-	0	-	-	-	-
Giant cells	-	-	-	++	-	-	++	0	-	-	-	-	

\*0 (Not available)

was present in all animals of Group B and calves C1, C3 and A4. Atelectasis was present in all calves, being more prominent in calves B1 and C3 (++) . All calves but A2 had a uniform degree (+) of inflammatory cell infiltration in the alveolar septa. Interlobular septal edema was present in two animals of Group A, two of Group B and one of Group C, being more prominent (++) in calf B1. Giant cell formation was present in calves A4 and B3. No inclusion bodies were seen in any of the tissues examined. No obvious pattern of lesion was found in any of the groups of calves and the grades of lesions were variable among individuals of the same group. However, calves of Group B had a greater prevalence of pneumonic changes than did those of Groups A or C (Table III) and the calf with the highest percentage retention of *P. hemolytica* (B-1) had the highest score of microscopic lesions.

## DISCUSSION

The objective of this investigation was to find out if parainfluenza-3 virus interfered with the pulmonary clearance of *P. hemolytica* in cattle. The results demonstrate that PI-3 virus interfered markedly with the bacterial clearance seven days after viral exposure and to a lesser extent after 11 days but had no effect when animals were exposed to virus three days before the bacterial aerosol (Table II). The normal four hour clearance of *P. hemolytica* in calves in these experiments is within the normal range.

The seven day interval necessary to achieve significant bacterial retention in calves infected with PI-3 virus is similar to the time interval of six to seven days necessary to increase bacterial retention in laboratory animals infected with virus. This was demonstrated by Green (7) in mice inoculated with influenza virus and staphylococcus, by Klein *et al* (20) in mice inoculated with reovirus and *S. aureus*, by Jakab and Green (14) in mice inoculated with Sendai virus and *Proteus mirabilis* and by Jakab (17) in mice inoculated with Sendai virus and *Pasteurella pneumotropica*.

Previous investigations have shown a small variability of bacterial clearance among animals exposed to the same bac-

teria under normal conditions (7, 8, 22, 24). This variability among animals is consistent and similar to that of Group A in which the animals cleared the bacteria rapidly. Group B and to a lesser extent Group C had an increased variability among individuals of the same group in relation to the bacterial clearance. This same variability has also been found in laboratory animals exposed to a combined virus-bacteria infection (7, 13, 15, 19). For instance, the bacterial retention of *S. aureus* in normal untreated murine lung at four hours was  $11.9 \pm 3.2\%$  in a group of 11 mice in contrast to the mean bacterial retention at four hours of  $261 \pm 100\%$  in a group of eight mice infected intranasally seven days before with a suspension of  $32 \times \text{LD}_{50}$  of influenza virus (7).

Immunization via aerosol, either against the virus or bacteria, is known to prevent the synergistic effect in combined virus-bacteria infection on day 7 (6, 15, 18). The extrapolation of these studies in laboratory animals to calves could explain the different results obtained by Gilka *et al* (5) and those obtained in this experiment. Gilka *et al* failed to demonstrate impairment of pulmonary clearance of *P. hemolytica* in calves seven days after aerosolization with PI-3 virus. Some of the calves used by Gilka *et al* in contrast to those used in this experiment had significant serum antibody titres against *P. hemolytica* and this probably prevented the effect of the virus upon the pulmonary clearance of *P. hemolytica*. The calves used in this experiment had low serum antibody titres for both virus and bacteria.

If a serum antibody level to PI-3 virus of 1:20 is considered to be evidence of previous infection (27), then none of the calves had significant titres prior to viral exposure. However, calves A2 and B2 had titres of 1:32 when killed. Calf B2 had the lowest percentage retention in Group B so this titre could possibly have been a factor. Calves A3 and C4 had serum antibody titres to *P. hemolytica* which could be considered significant but clearance (38.8%) was not impaired in calf C4.

Several theories have been postulated in order to explain the role of the virus in combined infection with bacteria. Some authors have suggested that viral induced damage to the lung makes this tissue a favourable site for bacterial growth (10) or that edema favours the implantation and growth of bacteria in the lung (11). Others

have explained the role of the virus as causing destruction of the ciliary epithelium of the air passages, thus preventing the removal of bacteria by these cells and allowing the bacteria to multiply (26). More recent investigations suggested that previous theories were not accurate. Jakab and Green (13, 15) compared the bacterial retention and transport of  $^{32}\text{P}$ -labelled *S. aureus* in the lungs of mice infected with Sendai virus. Seven days after viral infection the lungs had an increased bacterial retention when compared with nonvirus infected controls. However, the radiotracer removal rates of  $^{32}\text{P}$ -labelled *S. aureus* at four and 24 hours after bacterial aerosol indicated no difference between virus infected and untreated mice. These results suggested that bacterial multiplication in the lungs of mice at one week after Sendai virus infection was due to a defect in the *in situ* bactericidal mechanism and not to a defect in transport due to extensive destruction of ciliated epithelium. This defect in bactericidal mechanism was demonstrated to be present in consolidated and nonconsolidated lungs (17).

Detailed investigations have demonstrated a time correlation between inhibition of pulmonary clearance, virus titres in the lung and respiratory lesions (16). The time of maximal inhibition of bacterial clearance in the lungs of mice infected with virus was at the end of the first week and this is the time when the virus titres in the lung are falling rapidly. The peak of virus titres (Sendai virus) in the lung is about day 4 but on day 5 the titres start to decrease. In mice infected with Sendai virus, pneumonia is present about day 6. The presence of the lesions and the sudden inhibition of the bacterial clearance about day 6 or 7 could explain why previous investigations suggested a cause and effect relationship between lesions and defective clearance. The observed lack of correlation of defective bactericidal mechanism and lung lesions is further supported by investigations demonstrating that viruses with low pathogenicity for the lungs of laboratory animals such as encephalomyocarditis (EMC) virus, reovirus and Newcastle disease (ND) virus produced the same delayed clearance or morbidity in mixed virus-bacteria infections as viruses which are highly pathogenic to lung tissue such as influenza or parainfluenza virus (6, 12, 20).

In our work a correlation was not present

between the pulmonary retention of *P. hemolytica* (Table II) and the grade of lesion present in the lungs of the calves (Table III). For instance, calf B1 had a retention of 112.5% and the most marked lesions of all the animals. However, calf C1 had 82.3% bacterial retention and no significant difference in the grade of lesion among animals in the same group or when compared with animals with the lowest retention such as those of Group A. The extrapolation to bovine lungs of some of the previous results in experiments carried out with laboratory animals would support the hypothesis that the decreased clearance of *P. hemolytica* present in calves' lungs seven days after parainfluenza-3 virus exposure is likely due to an interference in the phagocytic activity of alveolar macrophages and not to the extent of lesions present in the lung. Future investigations may demonstrate the precise role of PI-3 virus in the impaired phagocytic mechanism of bovine alveolar macrophages.

Jakab (18) noted that seven days after Sendai virus infection in mice endogenous nasal bacterial flora could be cultured from the lungs of mice. This observation has significant implications in the pathogenesis of pneumonic pasteurellosis in cattle. The rapid quantitative and qualitative changes of the nasal bacterial flora of cattle during shipment (28, 30) results in *P. hemolytica* being present at high incidence and in large numbers in the nasal flora. Those organisms are carried to the lung from the nasal mucosa in inspired air (9). Cattle which develop pneumonic pasteurellosis tend to do so about one to two weeks after the stress of collection and shipment at a time when the lung may be heavily exposed to *P. hemolytica* (9). If a defect in normal pulmonary clearance was present during that time bacterial infection could result in a severe pneumonia. It is known that infection with PI-3 virus is common in young cattle which have been congregated (27) but appears to be present both in those animals which develop pneumonia and those which do not (28, 30). Cattle with the lowest serum antibody titres to *P. hemolytica* and PI-3 virus tend to develop the most extensive pneumonia (28, 30). Therefore, the determining factor in whether or not an animal develops pneumonia could be its immune status to the two agents and the degree of exposure of the lung to bacteria, assuming that PI-3 virus

infection is present.

The results reported in this paper demonstrate that PI-3 virus infection has the capacity to impair bacterial clearance in calves. Given the circumstances of field conditions as mentioned above, infection with PI-3 virus in the proper sequence and relationship to the change in nasal bacterial flora could be highly significant in the pathogenesis of the subsequent bacterial pneumonia in cattle.

Previous aerosol immunization either with the homologous bacteria or virus will eliminate the impairment in clearance in mice caused by the virus (15). Application of these observations to cattle is required and is a promising avenue to investigate for the immunological control of pneumonic pasteurellosis.

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## REFERENCES

- BALDWIN, D. E., R. G. MARSHALL and G. E. WESSMAN. Experimental infection of calves with myxovirus parainfluenza-3 virus and *Pasteurella hemolytica*. *Am. J. vet. Res.* 28: 1773-1782. 1967.
- BIBERSTEIN, E. L., M. GILLS and H. KNIGHT. Serological types of *Pasteurella hemolytica*. *Cornell Vet.* 50: 283-300. 1960.
- CARTER, G. R. A serological study of *Pasteurella hemolytica*. *Can. J. Microbiol.* 2: 483-488. 1956.
- CHANOCK, R. M. and K. M. JOHNSON. Diagnostic procedures for viral and rickettsial disease. 3rd Ed. pp. 470. American Public Health Association Inc. 1964.
- GILKA, F., R. G. THOMSON and M. SAVAN. The effect of edema, hydrocortisone acetate, concurrent viral infection and immunization on the clearance of *Pasteurella hemolytica* from bovine lung. *Can. J. comp. Med.* 38: 251-259. 1974.
- GOLDSTEIN, E., T. AKERS and C. PRATO. Role of immunity in viral induced bacteria superinfection of the lung. *Infection & Immunity* 3: 757-761. 1973.
- GREEN, G. M. Patterns of bacterial clearance in murine influenza. In *Antimicrobial Agents and Chemotherapy*. G. L. Hobby, Editor. Ann Arbor, Michigan: American Society of Microbiology. 1965.
- GREEN, L. H. and G. M. GREEN. Differential suppression of pulmonary antibacterial activity as the mechanism of selection of a pathogen in a mixed bacterial infection of the lung. *Am. Rev. resp. Dis.* 98: 819-824. 1968.
- GREY, C. L. and R. G. THOMSON. *Pasteurella hemolytica* in the tracheal air of calves. *Can. J. comp. Med.* 35: 121-128. 1971.
- HARFORD, C. G., V. LEIDLER and M. HARA. Effect of the lesion due to influenza virus on resistance of mice to inhaled pneumococci. *J. exp. Med.* 98: 53-68. 1949.
- HARFORD, C. G. and M. HARA. Pulmonary edema in influenzal pneumonia of the mouse and the relation of fluid in the lung to the inception of pneumococcal pneumonia. *J. exp. Med.* 91: 245-258. 1950.
- HUGH, R., KUN-YEN HUANG and T. B. ELLIOTT. Enhancement of bacterial infection in mice by Newcastle disease virus. *Infection & Immunity* 3: 448-493. 1971.
- JAKAB, G. J. and G. M. GREEN. The effect of Sendai virus infection on bactericidal and transport mechanism of the murine lung. *J. clin. Invest.* 51: 1989-1998. 1972.
- JAKAB, G. J. and G. M. GREEN. The effect of systemic and aerogenic immunization with *Proteus mirabilis* on bactericidal mechanism of uninfected and Sendai virus infected lungs. *Bact. Proc.* V. 129. 1972.
- JAKAB, G. J. and G. M. GREEN. Immune enhancement of pulmonary bactericidal activity in murine virus pneumonia. *J. clin. Invest.* 52: 2878-2884. 1973.
- JAKAB, G. J. and E. C. DICK. Synergistic effect in viral bacterial infection: combined infection of the murine respiratory tract with Sendai virus and *Pasteurella pneumotropica*. *Infection & Immunity* 8: 762-768. 1973.
- JAKAB, G. J. and G. M. GREEN. Pulmonary defense mechanism in consolidated and nonconsolidated regions of the lung infected with Sendai virus. *J. infect. Dis.* 129: 263-270. 1974.
- JAKAB, G. J. Effect of sequential inoculations of Sendai virus and *Pasteurella pneumotropica* in mice. *J. Am. vet. med. Ass.* 164: 723-728. 1974.
- KASS, E. H., G. M. GREEN and E. GOLDSTEIN. Mechanism of antibacterial action in the respiratory system. *Bact. Rev.* 30: 488-496. 1966.
- KLEIN, J. O., G. M. GREEN, J. G. TILLES, E. H. KASS and M. FINLAND. Effect of intranasal reovirus infection on antibacterial activity of mouse lung. *J. infect. Dis.* 119: 43-50. 1969.
- LAURENZI, G. A., L. BERMAN, M. FIRST and E. H. KASS. A quantitative study of the deposition and clearance of bacteria in the murine lung. *J. clin. Invest.* 43: 759-768. 1964.
- LAURENZI, G. A., J. J. GUARNIERI and R. B. ENDRIGA. Important determinants in pulmonary resistance to bacterial infection. *Med. Thorac.* 22: 48-59. 1965.
- LAURENZI, G. A. and J. J. GUARNIERI. A study of the mechanism of pulmonary resistance to infection. The relationship of bacterial clearance to ciliary and alveolar macrophage function. *Am. Rev. resp. Dis.* 93: 134-141. 1966.
- LILLIE, L. E. and R. G. THOMSON. The pulmonary clearance of bacteria by calves and mice. *Can. J. comp. Med.* 30: 129-137. 1972.
- LILLIE, L. E. The bovine respiratory disease complex. *Can. vet. J.* 15: 233-242. 1974.
- LOOSLI, C. G. Synergism between respiratory viruses and bacteria. *Yale J. Biol. Med.* 40: 522-540. 1968.
- SWEAT, R. L. Epizootiologic studies on bovine myxovirus parainfluenza-3. *J. Am. vet. med. Ass.* 150: 178-183. 1967.
- THOMSON, R. G., M. L. BENSON and M. SAVAN. Pneumonic pasteurellosis of cattle. *Microbiology and immunology*. *Can. J. comp. Med.* 33: 194-206. 1969.
- THOMSON, R. G. and F. GILKA. A brief review of pulmonary clearance of bacterial aerosols emphasizing aspects of particular relevance to veterinary medicine. *Can. vet. J.* 15: 99-106. 1974.
- THOMSON, R. G., S. CHANDER, M. SAVAN and M. L. FOX. Investigation of factors of probable significance in the pathogenesis of pneumonic pasteurellosis in cattle. *Can. J. comp. Med.* 39: 194-207. 1975.