

# Experimental Bovine Pneumonic Pasteurellosis

## II. Genesis and Prevention

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### ABSTRACT

Two experiments were conducted. In the first, 16 crossbred Hereford calves were divided into two equal groups. The first group was vaccinated intranasally with a commercial vaccine against bovid herpesvirus 1 and the second group was unvaccinated. The calves were later exposed to an aerosol of bovid herpesvirus 1 (strain 108) for five minutes. Four calves from each group were subjected to transportation and four calves from each group were kept in an environmental chamber for four days. Four days after viral aerosol all calves were exposed to an aerosol of *Pasteurella haemolytica* and the same subgroups were again transported or held in the chamber for a further four days.

The calves that did not die from pneumonia were necropsied ten days after the final day of transport. Pulmonary lesions were present in both vaccinated and control animals but were less extensive in the vaccinated calves. Six of eight vaccinated but none of the eight control calves survived.

In the second experiment, eight crossbred Hereford calves were divided into two equal groups. One group was vaccinated with bovid herpesvirus 1 (strain 108) and the other acted as controls. Four weeks later all calves were sequentially exposed to aerosols of bovid herpesvirus 1 (strain 108) and *P. haemolytica* four days apart. Three of the four controls but none of the vaccinates died from pneumonia. Every lobe of the lungs in all the controls was affected by pneumonia while no pulmonary lesions were found in the vaccinated calves. The differences in efficacy of the modes of vaccination and the possible role of transport stress are discussed.

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### RÉSUMÉ

Cet article décrit deux expériences. Dans la première, on utilisa deux groupes de huit veaux Hereford croisés. On vaccina les sujets du premier groupe, par la voie intra-nasale, avec un vaccin commercial contre l'herpèsvirus bovin 1; ceux du second groupe servirent de témoins. On soumit ultérieurement ces veaux à une pulvérisation d'une durée de cinq minutes, avec la souche #108 de l'herpèsvirus bovin 1. On soumit ensuite quatre veaux de chacun des deux groupes à un stress de transport; on garda les autres dans une chambre expérimentale, durant quatre jours. Quatre jours après la pulvérisation du virus, tous les veaux en subirent une autre, avec une souche de *Pasteurella haemolytica*; on répéta ensuite, avec les mêmes sujets, le stress du transport et le séjour dans la chambre expérimentale, pour une durée de quatre jours.

On effectua la nécropsie des veaux qui ne moururent pas de pneumonie, dix jours après le dernier stress de transport. On décéla des lésions pulmonaires, tant chez les veaux vaccinés que chez les témoins; elle s'avérèrent toutefois moins graves chez les veaux vaccinés. Six des huit sujets vaccinés survécurent, tandis que les huit témoins moururent.

Pour la deuxième expérience, on utilisa deux groupes de quatre veaux Hereford croisés. On en vaccina un, avec la souche #108 de l'herpèsvirus bovin 1, tandis que l'autre servit de témoin. Quatre semaines plus tard, on soumit tous ces veaux à une pulvérisation avec la souche #108 de l'herpèsvirus bovin 1; quatre jours plus tard, on effectua une autre pulvérisation, mais avec une souche de *P. haemolytica*. Trois des quatre témoins moururent de pneumonie, tandis que les quatre veaux vaccinés survécurent. Tous les lobes pulmonaires de tous les veaux témoins présentaient des lésions de pneumonie, tandis qu'on n'en décéla pas chez les sujets vaccinés. Les auteurs commentent les différences dans l'efficacité des modes de vaccination et le rôle probable du stress du transport.

## INTRODUCTION

It was concluded from previous experiments (2) that the development of experimental bovine pneumonic pasteurellosis could be prevented by vaccination against the viral component of this disease model. In that work there were two vaccinated groups, the first being calves inoculated with the same strain as the subsequent challenge virus, bovid herpesvirus 1 (strain 108) and the second being calves inoculated with a commercial intranasal vaccine (Contravac).<sup>1</sup> No pulmonary lesions were seen in Group I but some small nodules that proved later to have the histological appearance of bovine pasteurellosis were found in Group II.

The experiments described here were designed to attempt reproduction of the disease under more typical field conditions by transporting animals and to investigate prevention of the disease under these circumstances.

Because of the equivocal results of the first phase of this present work, a second experiment was conducted to confirm the results reported earlier (2).

## MATERIALS AND METHODS

### Experiment I

Sixteen three month old Hereford cross-bred calves from the A.D.R.I.(W) herd were divided into two groups of eight animals. The first group (Group I) was vaccinated three times intranasally (2 ml each nostril) with Contravac, a commercial vaccine containing BHV1 and parainfluenza-3 (PI<sub>3</sub>) virus, at 73, 45 and 12 days before the first viral aerosol. On each vaccination day the calves were bled and their nasal passages swabbed both close to the nostril (superficially) and 10-13 cm from the external nares (deeply). The control group (Group II) was treated similarly with Eagle's minimum essential medium (MEM)<sup>2</sup> replacing the vaccine for intranasal inoculation. All calves were still with their dams during this period.

The calves were then weaned and divided into four subgroups and two replicates (R1 and R2) as shown in Figure 1. On day 0 calves of the first replicate were bled, their noses were swabbed and they were exposed to an aerosol of BHV1 (strain 108) containing  $2 \times 10^8$  TCID<sub>50</sub>/ml for five minutes. The animals were then divided into those to be transported and those to remain in the environmental chamber. Four calves of each replicate, two vaccinates and two controls, were loaded and kept outdoors on a three-quarter ton truck for eight days and four calves of the same replicate, two vaccinates and two controls, remained in the environmental chamber (Fig. 1). The temperature of this chamber was controlled at  $-15^\circ\text{C}$  during the night and  $25^\circ\text{C}$  during the day. The calves were transported for four hours on day 0 and eight hours on days 1, 2 and 3. On day 4 they were bled and swabbed again and given a five minute aerosol of *P. haemolytica* (biotype A, serotype 1). The methods of production of aerosols and their application to calves were those of Jericho and Langford (1). In this experiment the bacterial aerosols differed between the two replicates. Due to unforeseen circumstances, calves of the first replicate were given a bacterial aerosol from a culture that had not been previously frozen. Calves of the second replicate were given an aerosol from the same culture but this culture had been thawed after being frozen for three weeks. The bacterial aerosols to which each calf was exposed were sampled as previously described (1).

The animals of different subgroups were then transported for a further four hours on day 4 and eight hours on days 5, 6 and 7 or left in the chamber as before. The mean distances and ranges travelled by the truck in four hours were 200 (180-225) km and

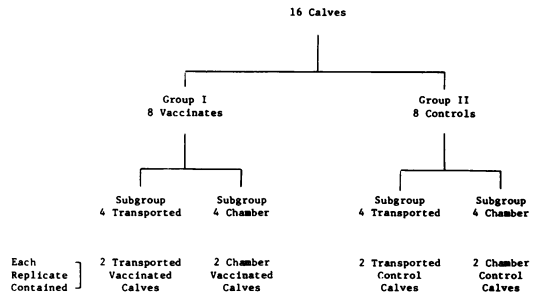


Fig. 1. Design of Experiment I. The experiment was performed in two replicates of eight calves using two from each subgroup to produce a replicate.

<sup>1</sup>Contravac, Connaught Laboratories, Toronto, Ontario.

<sup>2</sup>Gibco, Grand Island, New York.

in eight hours were 360 (250-450) km. The first replicate was transported during the first week of November in clear weather with temperatures varying from  $-4^{\circ}\text{C}$  during the night and up to  $21^{\circ}\text{C}$  during the day. The second replicate was transported in the fourth week of November with a slightly lower range of temperature ( $-4^{\circ}\text{C}$  to  $13^{\circ}\text{C}$ ) and in cloudy weather with flurries of wet snow. On day 8 all surviving animals were placed in a loose stall and swabbed on day 11 and 18, and all surviving calves were killed for necropsy on day 18. Specimens of upper and lower respiratory tracts were taken for isolation of viruses and bacteria and for histopathological examination. The temperatures of the calves were taken twice daily.

#### *Experiment II*

Eight three month old crossbred calves from the same source were divided into two groups. Four calves were vaccinated with 2 ml of BHV1 (strain 108) containing  $2 \times 10^8$  TCID<sub>50</sub>/ml into each nostril. The remaining four calves were given MEM (2 ml) medium into each nostril. All calves remained with their dams until the day of viral aerosol (day 0). Four weeks after inoculation the eight calves were weaned and placed in the environmental chamber which on this occasion remained at ambient temperatures ( $15-19^{\circ}\text{C}$ ). Each calf was exposed to aerosols of BHV1 (strain 108) and *P. haemolytica* sequentially for five minutes as in Experiment I. Thirty liters of air were sampled from each aerosol in 0.1% gelatin saline for *P. haemolytica* and in MEM fluid for BHV1. The culture of *P. haemolytica* was the same as for Experiment I and was frozen for about three weeks.

Clinical examinations were made daily. Nasal samples were taken before each aerosol administration and at necropsy. At necropsy normal and affected lung was examined for the presence of BHV1 and *P. haemolytica*.

Unless stated otherwise, viral and bacterial cultures were kept at  $-80^{\circ}\text{C}$  and thawed immediately prior to use. The methods of titration and isolation of bacteria from specimens were described by Jericho and Langford (1). The BHV1 isolations and the measurement of serum neutralizing antibodies against BHV1 were made as described earlier (3). Attempts to grow PI<sub>3</sub> virus from the commercial vaccine followed the same procedures as for BHV1 except

that tissue cultures were incubated for three days longer (seven days) than for the BHV1 isolations. All swabs and tissues were also examined for other pathogenic bacteria and mycoplasma with negative results.

## RESULTS

### CLINICAL SIGNS

#### *Experiment I*

All control calves had a serous nasal discharge on day 3 after challenge with the viral aerosol. By day 5, one day after the bacterial aerosol, the chamber control calves were dyspnoeic and two calves (R1) died on day 6 and two (R2) died on day 7. The transported control calves showed respiratory distress by day 5 and two calves (R1) died on that day, one calf (R2) died on day 6 and the remaining calf (R2) on day 10.

One vaccinated calf (R2) kept in the chamber became dyspnoeic on day 5 after infection and died on day 9 and one transported vaccinated calf (R1) died on day 14. The two remaining vaccinated R1 calves were treated from day 14 to day 17 with 440 mg pyrrolidinomethyl tetracycline (Reverin)<sup>3</sup> intramuscularly twice daily. This was done as their condition was deteriorating late in the course of the experiment and it was considered important to obtain serum from these calves eighteen days after initial viral aerosol for serological studies.

The mean daily temperatures of the calves are given in Fig. 2. The control calves kept in the chamber developed a high fever,  $40.5^{\circ}\text{C}$  or more, by day 2 after the viral aerosol and their temperatures remained high until the time of death. The temperature rise did not develop as rapidly in the transported control calves and appeared to decrease markedly between days 5 and 7 after infection. This apparent drop in mean temperature was influenced by the R2 calf that died on day 6 when its temperature was  $36.7^{\circ}\text{C}$  and in fact, the temperature of the remaining calf in that subgroup was  $40.8^{\circ}\text{C}$ . The temperatures of the vaccinated calves in both subgroups rose

<sup>3</sup>Reverin, Hoechst Pharmaceuticals, Montreal, Quebec.

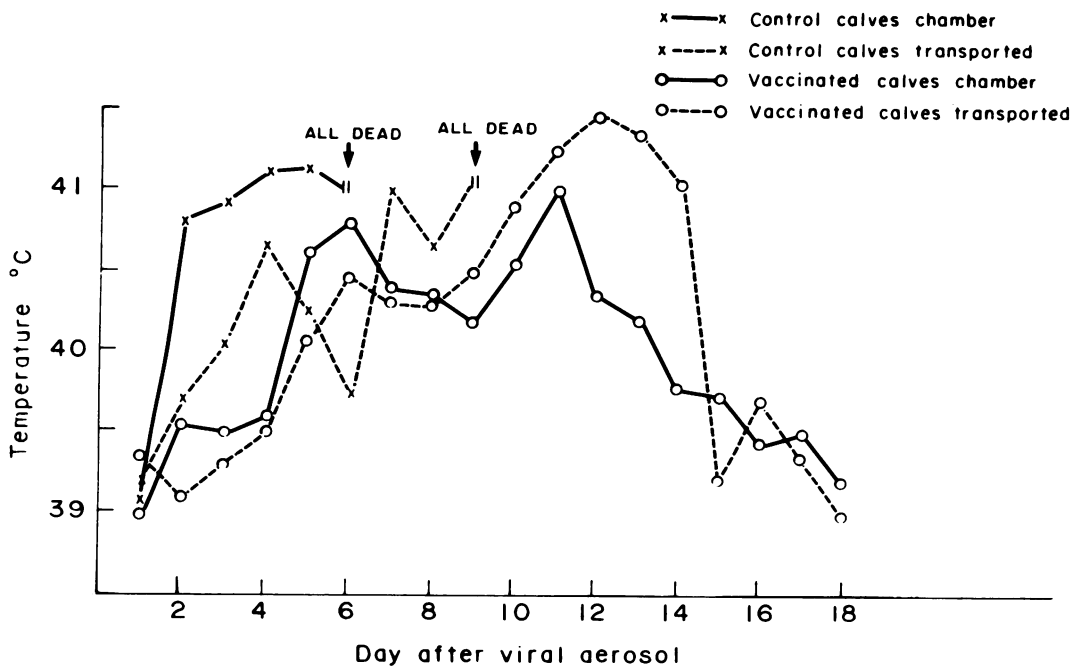


Fig. 2. Mean daily temperatures of calves given BHV1 (strain 108) and *P. haemolytica*, Experiment I.

more slowly to peak at day 6, remained relatively steady or dropped slightly and rose again peaking a second time between days 11 and 13.

### Experiment II

By day 5 one of the control calves was in respiratory distress and two others had a mucopurulent nasal discharge. On day 7 two calves were unable to stand and were dyspnoeic. They were killed and necropsied. The remaining control calf survived until the end of the experiment. The mean daily temperatures of control calves increased on day 2 and reached a peak of 41.7°C two days after the bacterial aerosol (Fig. 3). The mean temperatures of the vaccinated calves remained in the normal range throughout the experiment.

### PATHOLOGY

#### Experiment I

The pulmonary lesions were similar in all control calves. There was mucopurulent floccular material and subepithelial ecchymoses in the nasal meati, larynx and trachea of each control calf.

In all control calves more than 80% of

the anterior and posterior apical lobe of the right lung, apical lobe of the left lung, cardiac lobes of right and left lungs and intermediate lobe of the right lung were consolidated. Up to 40% of the anteroventral region of the diaphragmatic lobes was also consolidated. The pulmonary lesions varied according to the length of time that elapsed between receiving the bacterial aerosol and death. Animals that died on day 5 (36 hours after bacterial aerosol) had different lesions from those dying on day 6 and later. Their lungs did not collapse when the thoracic cavity was opened. The general color of the lungs was normal but there were many reddish black areas up to two or three lobules in diameter. The interlobular septa throughout the apical, cardiac and anteroventral diaphragmatic lobes were distended with clear gelatinous fluid.

The pulmonary lesions in calves dying on day 6 or later were typical of bovine pneumonic pasteurellosis. The affected lobules were reddish black, hard and raised above the normal surface of the lung, the septa were distended and a dull fibrinous exudate was usually seen on the visceral pleura. There were two to three liters of straw-colored thoracic fluid in the calves that died

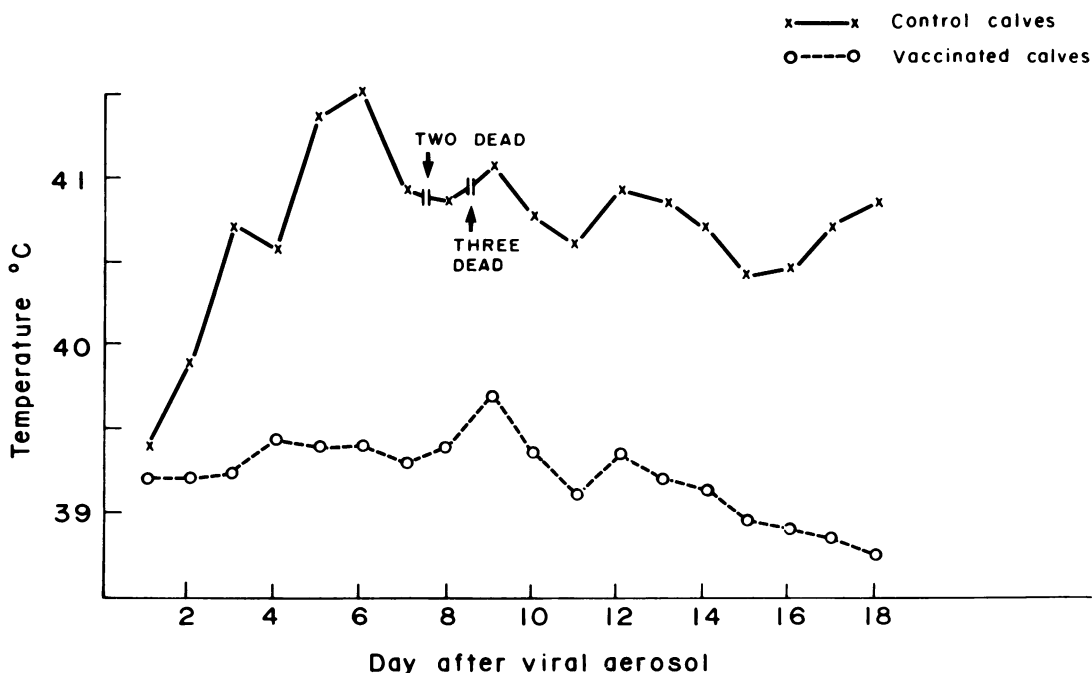


Fig. 3. Mean daily temperatures of calves given BHV1 (strain 108) and *P. haemolytica*, Experiment II.

on days 7 and 10. In some affected lungs there was emphysema with the formation of bullae.

The pulmonary lesions of the two vaccinated calves that died on days 9 and 14 were typical of severe bovine pneumonic pasteurellosis. In animals that survived until day 18 after the viral aerosol the pulmonary lesions differed in that the lesions were distributed in the apical, cardiac and anteroventral diaphragmatic lobes but the consolidation was not complete and usually involved only a few lobules in any one area. The lesions were sunk beneath the general level of the lung and resembled atelectasis. In some areas there were numerous small abscesses within the lung parenchyma.

#### Experiment II

The vaccinated calves had no pulmonary lesions at necropsy. The control calves had purulent rhinitis, laryngitis and tracheitis. All of these calves had at least seven lobes affected with complete or partial consolidation of the lungs similar to that described for the controls of Experiment I.

#### VIROLOGY

The virus isolation data are given in Table I. As calves died at varying times

during the experiment or were killed on day 18, the final column of data for both experiments is recorded as the day of post-mortem examination. There is close correlation between virus isolation and the vaccination or control status of the calves.

The antibody titers of sera to BHV1 are given in Table II. The antibody titer of vaccinated animals increased during the experiments while it changed from negative to positive in a proportion of the control calves. In Experiment I the commercial vaccine used for the first two vaccinations contained  $10^{2.0}$  TCID<sub>50</sub>/ml and the third vaccination contained  $10^{4.5}$  TCID<sub>50</sub>/ml of BHV1. Attempts to isolate PI<sub>3</sub> from the commercial vaccine used in this trial were not successful.

#### BACTERIOLOGY

From Table III it can be seen that *P. haemolytica* became established in the nasal cavities, trachea and all lobes of the lung in all calves of Experiment I. In those of Experiment II it became established in the controls but in only one of the vaccinates. As the lungs of the vaccinated animals of Experiment II appeared normal, isolation was attempted from only one specimen of lung from each calf.

**TABLE I. Isolation of BHV1 from Calves**

Experiment		Day							
		0 <sup>a</sup>	4	P.M. Day					
		NS	NS	NS	TT	LT	L <sub>1</sub>	L <sub>2</sub>	L <sub>3</sub>
I	Vaccinates (Contravac)	1/8 <sup>b</sup>	1/8	1/8	0/8	0/8	0/8	0/8	0/8
	Controls	0/8	3/8	8/8	8/8	8/8	8/8	8/8	8/8
					T			L	
II	Vaccinates (BHV1, 108)	0/4	0/4	0/4	0/4			0/4	
	Controls	0/4	4/4	3/4	1/2 <sup>c</sup>			3/4	

NS: nasal swab, TT: upper trachea, LT: lower trachea, T: trachea, L, L<sub>1</sub>, L<sub>2</sub>, L<sub>3</sub>: lung, apical, cardiac, diaphragmatic lobes, respectively

<sup>a</sup>Day of viral aerosol

<sup>b</sup>Isolated from one of eight calves

<sup>c</sup>Two tracheas not examined

**TABLE II. Titers of Antibodies to BHV1 in Sera**

Experiment		Day		
		0	4	P.M. Day
I	Vaccinates (Contravac)	8/8(1/4-1/32) <sup>a</sup>	8/8(1/4-1/64)	8/8(1/128)
	Controls	0/8	0/8	3/8(1/4-1/16)
II	Vaccinates (BHV1, 108)	4/4(1/4-1/32)	4/4(1/4-1/16)	4/4(1/16-1/64)
	Controls	0/4	0/4	1/4(1/32)

<sup>a</sup>Proportion with positive antisera, range of titers in parenthesis

**TABLE III. Isolation of *P. haemolytica* from Calves at Necropsy**

Experiment		Day						
		SNS	DNS	TT	LT	L <sub>1</sub>	L <sub>2</sub>	L <sub>3</sub>
I	Vaccinates (Contravac)	6/8(+) <sup>a</sup>	7/8(+)	5/8(2+)	5/8(3+)	3/8(3+)	5/8(3+)	4/8(3+)
	Controls	8/8(+)	7/8(+)	8/8(2+)	8/8(3+)	8/8(4+)	8/8(4+)	8/8(4+)
					Trachea	Affected Lung	Not Affected Lung	
II	Vaccinates (BHV1, 108)	ND	1/4(+)	1/4(3+)		ND	0/4	
	Controls	ND	4/4(3+)	4/4(3+)		4/4(4+)	2/3(3+)	

SNS, DNS: superficial and deep nasal swabs, TT, LT: upper and lower trachea; L<sub>1</sub>, L<sub>2</sub>, L<sub>3</sub>: apical, cardiac, diaphragmatic lobes of the lung, ND: not done

<sup>a</sup>Number affected with an arbitrary indication of numbers of colonies per plate (Scale 1-4)

**TABLE IV. Numbers of *P. haemolytica* in Suspension of Culture and in Impinger Fluids**

Experiment	Bacterial Suspension	Impinger <sup>a</sup> Fluids
I	Replicate I	1.5 × 10 <sup>7b</sup>
	Replicate II	5.8 × 10 <sup>3</sup> - 1.3 × 10 <sup>5</sup>
II		1.5 × 10 <sup>2</sup> - 6.0 × 10 <sup>2</sup>
		4.1 × 10 <sup>6c</sup>
		0.2 × 10 <sup>1</sup> - 8.0 × 10 <sup>1</sup>

<sup>a</sup>Range

<sup>b</sup>Fresh culture (24 hours old, never frozen) numbers per milliliter

<sup>c</sup>Cultures deep frozen for three or four weeks

The numbers of bacteria in the bacterial suspensions used as a source of aerosol and in the impingers after the aerosols had been produced are given in Table IV.

## DISCUSSION

Experimental bovine pneumonic pasteurellosis was produced both in transported control calves and in control calves kept in the environmental chamber. The results indicated that vaccination with the commercial vaccine provided some protection against the subsequent development of the disease in both of the above circumstances. All eight control calves died of acute pneumonia (two on day 5, three on day 6, two on day 7 and one on day 10), whereas only two of the vaccinated calves died (day 9 and day 14). Two other vaccinates which were sick recovered following antibiotic treatment on days 14-17. This might be explained by the difference in bacterial counts in the cultures and impingers used, since all the vaccinated calves that died and were treated were in the first replicate which received a bacterial aerosol from a fresh culture with more organisms (Table IV).

The transported control calves died after a shorter period of time than the chamber control calves. Three of the former died at least 24 hours before any of the latter. Thus, it appears that transportation, under the conditions of this experiment, may well be more stressful than the environmental chamber. Mihajlovic *et al* (5) reported no difference in respiratory syndromes between transported and nontransported calves. However, they inoculated the animals with BHV1 after transportation and thus comparisons with our work are of limited value.

It can be seen from Figure 2 that the vaccinated calves also developed high fevers as well as lobar fibrinous pneumonia. This differs from a similar group in earlier work (2) which did not develop clinical disease and in which small (4-6 mm) foci of pneumonic pasteurellosis were found. However, the calves vaccinated with BHV1 (strain 108) in Experiment II developed neither fever nor lesions (Fig. 3).

Intranasal vaccination with commercial intranasal vaccine conferred some, albeit incomplete, protection against challenge with BHV1 (strain 108) and *P. haemolytica*

under the conditions described here. Complete protection occurred in Experiment II where BHV1 (strain 108) was used to vaccinate and challenge. However, because of the differences between Experiments I and II the degree of protection obtained in the two experiments cannot be compared with any degree of confidence. Thus, several questions remain: 1) was the difference in degree of resistance between groups vaccinated with commercial vaccine compared to BHV1 (strain 108) due to the greater challenge with *P. haemolytica* 2) was the difference in resistance due to difference in degrees of stress between calves of the first and second experiments 3) was the difference in resistance between the groups of calves due to vaccination with different strains of BHV1 or 4) was the difference in resistance between the two groups of vaccinates due to the difference in numbers of virus units used for vaccination.

In earlier work (6) the difference in degree of resistance between calves vaccinated with the different strains of virus was far less marked than that reported here. In that work, the degrees of challenge with *P. haemolytica* and stress were directly comparable between the groups of calves. From our results obtained here and elsewhere (1,6) we conclude that increased resistance to experimental bovine pneumonic pasteurellosis is conferred by prior vaccination with BHV1 but that the degree of resistance is probably influenced by many factors such as the strain and numbers of BHV1 used to vaccinate, the degree and type of stress to which the animals are exposed and the numbers and condition (frozen vs. unfrozen) of *P. haemolytica* with which the calves are subsequently challenged.

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