Aerosol Vaccination of Calves with *Pasteurella haemolytica* Against Experimental Respiratory Disease

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ABSTRACT

Three experiments were conducted on calves in which the efficacy of vaccination with live *Pasteurella haemolytica* in aerosol was tested by challenge with sequential aerosol exposure to bovine herpesvirus 1 and *P. haemolytica*. Neither single nor multiple aerosol vaccinations protected against the experimental disease. Macroscopically recognizable rhinitis, tonsillitis, tracheitis and pneumonia occurred in both controls and vaccinates. In one experiment as many as three aerosol vaccinations with live *P. haemolytica* for up to 20 minutes failed to elicit clinical signs in exposed calves. *Pasteurella haemolytica* was isolated less frequently from tissues of vaccinated calves than from those of nonvaccinated calves. *Pasteurella haemolytica* was isolated from deep nasal swabs of 4/14 vaccinated calves five and six days after viral exposure. It was concluded that although bovine herpesvirus 1 vaccination has been shown previously to prevent the experimental disease produced by bovine herpesvirus 1-*P. haemolytica*, live *P. haemolytica* vaccination by aerosol will not provide the same protection.

RÉSUMÉ

Les trois expériences décrites dans cet article impliquaient des veaux chez qui les auteurs voulaient déterminer l'efficacité de la vaccination avec des aérosols d'une souche vivante de *Pasteurella haemolytica*, en les soumettant à une infection de défi ultérieure, au moyen d'aérosols séquentiels de l'herpèsvirus bovin du type #1 et de *P. haemolytica*. Cette vaccination, réalisée à l'aide d'une ou de plusieurs nébulisations, ne réussit pas à protéger les veaux contre l'infection de défi. En effet, des lésions macroscopiques de rhinite, d'amygdalite, de trachéite et de pneumonie se développèrent, tant chez les sujets vaccinés que chez les témoins. Dans une de ces expériences, autant que trois nébulisations vaccinales qui durèrent jusqu'à 20 minutes, ne réussirent toutefois pas à provoquer l'apparition de signes cliniques chez les veaux ultérieurement soumis à l'infection de défi. On isola moins souvent *P. haemolytica* des tissus des veaux vaccinés que de ceux des témoins. On l'isola aussi des écouvillons nasaux de quatre des 14 veaux vaccinés, de cinq à six jours après leur contact avec le virus. Il semble par conséquent que, même si la vaccination avec l'herpèsvirus bovin du type #1 a déjà réussi à prévenir la maladie expérimentale imputable à l'action conjointe de ce virus et de *P. haemolytica*, la vaccination avec des aérosols d'une souche vivante de cette bactérie ne confère pas la même protection.

INTRODUCTION

Pasteurella haemolytica has long been recognized as a common respiratory pathogen for cattle. This has led to the search for effective immunizing bacterins (2,3,5) and their testing in cattle under field conditions (9,11,12,20,24). A significant reduction of clinical respiratory disease has been reported with the use of formalized *P. haemolytica* grown in chick embryos (20). This partial protection prompted the interpretation that "the pasteurella group of organisms play a role in the respiratory infections of cattle but are only a part of the series of events that lead to pneumonia" (20). In the past bacterins tested in the field involved the use of killed organisms inoculated parenterally into test animals; control animals were left to develop natural respiratory disease, the precise etiology of which remained unknown.

Subcutaneous or aerosol vaccination of calves with live *P. haemolytica* and subsequent inoculation of the homologous organism into the caudal lobe has been reported (5). Despite the massive and unorthodox challenge eight vaccinated calves survived but four of eight controls died within four days.

Respiratory disease produced by sequential exposure to bovine herpesvirus 1 (BHV1) and *P. haemolytica* aerosols has provided a suitable model (18) for testing the prophylactic value of live *P. haemolytica* administered by aerosol. Using this model the prevention of

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TABLE I. Experimental Design

Exp.	Animals (n)		Vaccination Data			Treatments (days)[a]			
	Controls	Vaccinates	No.	Days	Aerosol (min)	Weaning	BHV1 Exposure	*P. haem.* Challenge	Slaughter
A	5	5	×2	0,5	5,5	25	25	31	35
B	4	4	×3	0,6,15	5,5,10	43	43	48	76
C	5	5	×1	0	10	-2	26	30,32	34

[a]Day 0 = day of first vaccination

respiratory disease by prior intranasal vaccination with BHV1 has been reported (15,21). It was therefore thought worthwhile to determine whether single or repeated live *P. haemolytica* vaccination would also prevent the BHV1-*P. haemolytica* experimental disease. Vaccination and challenge with homologous *P. haemolytica* (10) in aerosol were selected to provide the optimum conditions (14) for this study.

MATERIALS AND METHODS

ANIMALS

A total of 28 three to five month old beef calves were used in three controlled experiments [A (n = 10), B (n = 8) and C (n = 10)], which were conducted in three separate years. Experiment A and B calves were derived from a herd on prairie grassland (18), while experiment C calves were born and raised in confinement with their dams. Neither clinical nor pathological evidence of natural respiratory disease has been observed in any of approximately 1000 calves from these herds during the past 15 years.

EXPERIMENTAL DESIGN

Bacterial aerosols given before viral exposure are referred to as vaccinations; those given after the viral exposure are referred to as challenges. The day of the first vaccination is designated as day 0 of the experiment.

Every effort was made to treat calves in each experiment equally, except for the vaccination procedures. There were differences in protocol between experiments. These differences were introduced either (a) to facilitate a vaccination effect (number of vaccinations, duration of vaccination, days between vaccination and viral exposure, Table I), (b) imposed by cattle management considerations (calves from three breeding seasons, weaning times, Table I), (c) to accommodate technical difficulties (first *P. haemolytica* challenge in experiment C had a very low titre, Table II) or (d) because within general guidelines of this model (*P. haemolytica* aerosol must follow the BHV1 aerosol no sooner than four days, and symptoms and lesions of viral-bacterial disease in susceptible calves develop within two days after the bacterial aerosol, Table I). Calves of experiment B were killed 28 days after the bacterial challenge because all but one calf (control B1) failed to show clinical symptoms and therefore animals were utilized for a serological study. Each experiment was treated as an independent study and comparisons are not justifiable because of the differences in protocol between experiments.

On day 0 the control calves in experiments A and B were exposed to an aerosol of brain heart infusion (BHI) broth and 5% fetal calf serum (FCS). As this had no effect on these animals, this procedure was omitted in experiment C in order not to subject further calves to this stressful manipulation. Experiment A and B control calves and their dams were separated from vaccinates and their dams on day 0 until the day of BHV1 exposure (= day 26 experiment A; day 44 experiment B), when all calves were weaned. Experiment C calves were weaned two days before vaccination and controls were also kept separate from the vaccinates until day of BHV1 exposure (day 27). Following BHV1 exposures, experiment A and B calves were reunited in a pasture and those of experiment C in a corral.

AEROSOLS

All bacterial vaccinations and challenges using live *P. haemolytica* and all viral exposures (Tables I and II) were done by aerosol as described in previous experiments in which extensive respiratory disease was produced (18).

MICROBIOLOGICAL STUDIES

Antibody to *P. haemolytica* in serum of all calves was determined on day 0 by a modified complement fixation (CF) test (4), using guinea pig complement supplemented with 5% fresh normal bovine serum. This modification increased the sensitivity of the test, without loss of specificity. Deep nasal swabs were collected from left nostrils just before exposure to all aerosols (except day 0 in experiment C) and at slaughter. All nasal

TABLE II. Concentration of Suspensions Used for the Production of Aerosols

Exp.	*P. haemolytica*/mL		BHV1 Suspension $TCID_{50}$/mL
	Vaccination	Challenge	
A	9.4×10^4 7.5×10^4	10^8	2×10^8
B	2.5×10^7 7.2×10^7 2.2×10^7	10^7	2×10^8
C	1.9×10^7	1.8×10^1 3.9×10^7	10^7

swabs, as well as lung tissue and tracheal swabs collected at postmortem were examined for the presence of *P. haemolytica*, *P. multocida* (18) and *Mycoplasma* spp. (16). *Pasteurella haemolytica* biotype A serotype 1 was used for all vaccinations and challenges. Suspensions of *P. haemolytica* were produced in BHI broth and 5% FCS, as previously described (18). The concentrations of bacterial suspensions used in the three experiments are given in Table II. In all cases, calves were exposed to amounts of *P. haemolytica* shown to be sufficient to produce extensive respiratory disease in synergism with BHV1 (18). The procedures used to isolate BHV1 from nasal swabs and from suspensions of lung tissue in Eagle's medium, and the determination of the neutralizing titer of sera (SN) for BHV1 and the viral titrations of suspensions have been described elsewhere (17). The concentration of BHV1 in suspensions used for aerosolization was 2×10^8 tissue culture infective $dose_{50}$ ($TCID_{50}$) /mL for experiment A and B and 10^7 $TCID_{50}$/mL for experiment C (Table II).

PATHOLOGICAL STUDIES

The postmortem procedures and examination of the respiratory tract have been described elsewhere (18). Macroscopically recognizable pathological changes in the respiratory tracts were recorded. The extent of pneumonia was estimated visually, expressed as a percentage of each lobe and pneumonic tissue was calculated as a percentage of the total lung. For this calculation the lobes of four healthy adult cattle lungs were weighed and the percent of each lobe to total lung mass was calculated. The differences between corresponding lobes among lungs were found to be within 1.5%. The average ratio of each lobe to total lung mass, expressed in percent for the four lungs was as follows: left cranial = 5%, left posterior cranial = 6%, left caudal = 32%, intermediate = 4%, right cranial = 6%, right posterior cranial = 5%, right middle = 7% and right caudal = 35%. Using these figures and the percentage of pneumonic tissue estimated visually for each lobe, the total percentage of pneumonic tissue was calculated for each calf. The values represent only estimates of the visible amount of pneumonia.

RESULTS

MICROBIOLOGICAL STUDIES

Complement fixation antibodies were not detected in any experiment on day 0. Nasal swabs of calves in experiment A and B did not yield *P. haemolytica* on day 0.

On the day of viral exposure *P. haemolytica* was isolated from nasal swabs of only two calves (vaccinates C6, C9). On the day of viral exposure controls and vaccinates of the respective experiments were grouped together. Five to six days later, but before *P. haemolytica* challenge, eight nasal swabs yielded *P. haemolytica* (four vaccinates and four nonvaccinates) (Table III).

The number of isolations of *P. haemolytica* from nose, trachea and lung on the day of slaughter are given in Table III. In experiments A and C, in which the time intervals between challenge and slaughter were equal, *P. haemolytica* was isolated less frequently from vaccinates (14/28, two swabs were not suitable) than from nonvaccinates (29/30) from nose, trachea and lung on day of slaughter.

Pasteurella multocida was isolated from nose, trachea and lung of calves (A6, A9).

Mycoplasma bovirhinis was isolated only in experiment C. Seven nasal swabs were positive on the day of viral exposure, five were positive on the day of bacterial challenge and all nasal swabs, tracheas and lungs, except lungs C1, C2, C7, C9 and C10, yielded *M. bovirhinis* on the day of slaughter.

TABLE III. *Pasteurella haemolytica* Isolations and Extent of Pneumonia

	Calves		*P. haemolytica*				Pneumonia	
			Nasal	Tissues[b]				
Exp.	Controls	Vaccinates	Swabs[a]	N	T	L	% of Lungs	No. of Lobes
A	1		+	+	+	+	5	1
	2		-	+	+	+	3	1
	3		-	+	+	+	3	2
	4		+	+	+	+	10	3
	5		+	+	+	+	10	4
		6	+	+	+	+	3	2
		7	-	+	-	-	3	1
		8	-	+	-	+	1	1
		9	+	ND[c]	+	+	80	8
		10	+	+	-	+	0	
B	1		+	+	ND	+	20	5
	2		-	-	ND	-	0	
	3		-	-	ND	-	0	
	4		-	-	ND	-	0	
		5	-	-	ND	-	0	
		6	-	-	ND	-	0	
		7	-	-	ND	-	0	
		8	+	+	ND	+	0	
C	1		-	+	+	+	50	8
	2		-	+	+	+	15	6
	3		-	+	+	+	70	7
	4		-	+	+	+	40	8
	5		-	-	+	+	15	6
		6	-	+	-	-	15	7
		7	-	+	-	-	15	7
		8	-	+	-	-	3	4
		9	-	ND	+	-	10	7
		10	-	-	-	-	10	6

[a]Nasal swabs just before bacterial challenge (experiment A - day 31, B - day 48, C - day 30)
[b]N = Nasal epithelium; T = Trachea; L = Lung
[c]Not done

In addition 13 isolations of *Acholeplasma laidlawii* were made from experiments A and B combined.

VIROLOGICAL STUDIES

No BHV1 SN antibodies were detected throughout experiments A and C. In experiment B all sera were positive for SN antibodies on day 74 (31 days after viral exposure). Bovine herpesvirus 1 was isolated from all nasal swabs of experiment A calves six days (day of bacterial challenge) and ten days (day of slaughter) after viral exposure, and from lungs and nasal swabs of B1, B4, B6 and B8 on the day of slaughter.

PATHOLOGICAL STUDIES

Rhinitis was evident in one vaccinated (C7) and one control calf (C3) of experiment C. Purulent tonsillitis was present in three vaccinates each of experiments A (A6, A7, A9) and C (C6, C7, C8), and in three and four controls of experiments A (A1, A3, A5) and C (C1, C2, C3, C4) respectively. Purulent tracheitis was seen in only one vaccinate (A9) and in four controls (A3, C1, C3, C4). Experiment B was without any such pathological changes.

In experiment B only one calf (control) had viral-bacterial pneumonia. In experiments A and C all calves but one (A10) had viral-bacterial pneumonia involving one to seven lobes per calf (Table III). Vaccinate A9 had very extensive pneumonia from which both *P. haemolytica* and *P. multocida* were isolated.

The bacterial challenge culture in experiment A and the first challenge culture in experiment C were four to six logs less concentrated than the culture used in experiment B (Table II). One day after the first bacterial challenge (low concentration) in experiment C a marked rise in rectal temperature (42°C) was produced in calves C1, C4 and C7, and a moribund state occurred in two of these calves at the time of the second challenge two days later. Possibly the titre of the suspension used for the first bacterial challenge was higher than indicated by the titration.

Three live *P. haemolytica* aerosol vaccinations with a total exposure time of 20 minutes did not elicit a clinical response from any calves in experiment B.

DISCUSSION

The results indicate that a single or multiple aerosol vaccination with *P. haemolytica* did not significantly reduce the amount of pneumonic tissue produced experimentally, and did not influence the number of pneumonic lobes in vaccinates as compared to controls. In experiment B vaccinates failed to develop disease on challenge but unfortunately insufficient experimental disease in the controls prevented the measurement of protection offered by vaccination. Subcutaneous inoculation of a killed *P. haemolytica* bacterin prepared in chick embryo yolk sac has been reported to significantly reduce the clinical manifestations of respiratory disease in calves (20). An immune response in calves has also been associated with live *P. haemolytica* inoculated by aerosol (8). In lambs a significant protective function was associated with *P. haemolytica* vaccine administered intramuscularly and followed by aerosol challenged with parainfluenza type 3 virus and homologous *P. haemolytica* (10)

In this study vaccination by *P. haemolytica* aerosol was chosen to stimulate a local immune response rather than a serum antibody response. The serum antibody response to aerosol vaccination with live *Proteus mirabilis* does not appear to be related to the degree of immune enhancement of pulmonary antibacterial mechanisms (14). The local application of antigen has been shown to stimulate an immune response in the respiratory tract (1, 11, 13). In the lower respiratory tract of the rabbit, antibodies to *P. haemolytica* have been demonstrated after aerosol and intramuscular exposure to both dead and live *P. haemolytica* (23). However in the dog, antibody-forming cells in bronchoalveolar spaces appeared in response to intravenous inoculation of sheep erythrocytes, but not after direct application of the cells to respiratory tissue (19).

Despite two and three previous aerosol exposures (experiments A and B) to *P. haemolytica*, the organism was not isolated from nasal swabs of the nine vaccinates on the day of viral exposure 20 and 28 days later. However, five or six days after viral exposure (days of bacterial exposure) swabs from four of these calves (A6, A9, A10, B8) became positive for *P. haemolytica*. Control calves A1, A4, A5 and B1 also yielded nasal *P. haemolytica* just before bacterial challenge. This increased incidence of *P. haemolytica* isolations after viral challenge and mixing of experimental groups may be due to recrudescence of *P. haemolytica* following BHV1 infection (7) and/or the result of natural spread among the grouped calves.

The isolations of *M. bovirhinis* from swabs and tissue of experiment C were noteworthy. The calves in this experiment were raised with their dams in confinement. This mycoplasma is regularly associated with calves from this sources but not with prairie-raised calves such as those used in experiments A and B. The role of *M. bovirhinis* in the pathogenesis of experimental disease is not known, although the amount of pneumonic tissue produced in this experiment was considerably more than in experiments A and B. The severity of disease in experiment C may have been an expression of synergism between BHV1, *P. haemolytica* and *M. bovirhinis*. Failure to isolate mycoplasma from calves of experiments A and B (ten calves with macroscopically recognizable pneumonia) indicates that production of the experimental disease was not dependent on mycoplasma, although studies for *M. dispar* were not included.

It is important in experimental studies on respiratory disease to consider the challenge dose. Results of experiments A and C point to lower dosage requirements for effective challenge than

previously reported (18). It is not known how the range of concentrations of bacterial challenges used in these experiments compares to natural exposures to *P. haemolytica*. Although the concentrations of bacterial suspensions used for aerosol vaccinations in this study were high, the number of organisms retained by the healthy respiratory tissue may have been too few for effective vaccination.

It is not known which sites in the upper and lower respiratory tract are most critical, or how much antigenic stimulation is necessary for effective immunization against challenge employed. Aerosol exposure was used here to ensure that all sites in the respiratory tract were exposed to vaccination and challenge. This route of vaccination has been found superior to the parenteral routes for the immune enhancement of pulmonary bactericidal activity to *P. mirabilis* (14). In one study killed *P. haemolytica* bacterin was simultaneously given parenterally and by aerosol twice 14 days apart (8). The immunized calves were then challenged with a large intratracheal inoculum containing 25 mL of live culture and 35 mL of sterile saline. More pneumonic tissue was produced in the vaccinates than in the controls. Yet, in another study, double vaccination by the subcutaneous or aerosol route prevented death in eight calves when challenged by 5 mL of homologous *P. haemolytica* culture into the caudal lobe; four of eight controls succumbed to the challenge (5).

In this study it was determined that single or multiple aerosol vaccination with ive *P. haemolytica* did not protect against the BHV1-*P. haemolytica* challenge. It is noteworthy that studies which included two aerosol vaccinations with *P. haemolytica* have reported slight enhancement of clearance but not elimination of challenge *P. haemolytica* in calves, infected naturally with bovine virus diarrhea (22). It is concluded that although BHV1 vaccination will effectively prevent experimental disease on challenge with BHV1 and *P. haemolytica* (15, 21), vaccination by aerosol with *P. haemolytica* is not protective against a similar challenge. Evidently, future studies on *P. haemolytica* vaccination should employ different antigenic material, amounts and/or methods of vaccination.

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