Vaccination Studies Against Experimental Bovine *Pasteurella* Pneumonia

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ABSTRACT

Vaccination-challenge experiments were conducted in colostrum-deprived calves to evaluate the efficacy of *Pasteurella* bacterins and vaccines against experimental pneumonic pasteurellosis. Calves were vaccinated with formalin-killed bacterins and live vaccines, then challenge exposed intratracheally with *P. haemolytica* or *P. multocida*. Infectious bovine rhinotracheitis virus was inoculated intranasally three to four days prior to *P. haemolytica* challenge-exposure. All calves were examined for macroscopic and microscopic lesions after being found dead or following euthanasia four to seven days after challenge exposure with the bacterial pathogen. Clinical, hematological, and pathological responses to challenge exposure in aluminum hydroxide absorbed *P. haemolytica* and *P. multocida* bacterin-treated calves were consistent with the pneumonic lesions of pulmonary pasteurellosis in the control calves. An oil-adjuvanted *P. haemolytica* bacterin limited clinical and pathological responses in the affected calves whereas a *P. multocida* oil-adjuvanted bacterin did not. Both clinical and pathological responses to challenge exposure in calves vaccinated with live *Pasteurella* vaccines were less severe than those of the control calves. Vaccine effectiveness appeared to be dose dependent.

Key words: *Pasteurella haemolytica*, *Pasteurella multocida*, bovine pneumonic pasteurellosis, immunization.

RÉSUMÉ

Cette expérience portait sur la vaccination et l'infection de défi de veaux privés de colostrum; elle visait à évaluer l'efficacité de vaccins tués ou atténués, préparés avec *Pasteurella haemolytica* et *Pasteurella multocida*, contre la pasteurellose pulmonaire. Les auteurs vaccinèrent les veaux avec des vaccins tués par la formaline ou atténués et ils les soumirent ensuite à une infection de défi intratrachéale, avec *P. haemolytica* et *P. multocida*. Trois à quatre jours avant l'infection avec *P. haemolytica*, les veaux reçurent le virus de rhino-trachéite infectieuse bovine, en injection intranasale. Les auteurs recherchèrent des lésions macroscopiques et microscopiques, tant chez les veaux qui moururent que chez ceux qu'ils sacrifièrent au bout de quatre à sept jours après l'infection de défi. Les observations cliniques, hématologiques et pathologiques consécutives à l'infection de défi, chez les veaux vaccinés avec une bactérine adsorbée à l'hydroxyde d'aluminium, qui contenait *P. haemolytica* et *P. multocida*, s'avérèrent semblables à celles des témoins. Une bactérine de *P. hamolytica*, enrichie d'un adjuvant huileux, amenuisa les réactions cliniques et pathologiques, chez les veaux affectés, contrairement à une bactérine de *P. multocida*, également enrichie d'un adjuvant huileux. Les réactions cliniques et pathologiques, chez les veaux soumis à une infection de défi après l'administration des vaccins atténués, se révélèrent moins sévères que celles des témoins. L'efficacité des vaccins sembla proportionnelle à la dose.

Mots clés: *Pasteurella haemolytica*, *Pasteurella multocida*, pasteurellose pulmonaire bovine, immunisation.

INTRODUCTION

Pneumonic pasteurellosis is a major cause of economic loss and mortality in feedlot cattle (1,2,3,4). *Pasteurella haemolytica* biotype A serotype 1 and, to a lesser extent, *P. multocida* Heddleston type 3 are the serotypes most commonly isolated from pneumonic bovine lungs (5,6). Commercial bacterins containing one or more strains of chemically inactivated *Pasteurella* have been used for more than 50 years in attempts at preventing this disease (7).

Conflicting reports appear in the literature as to the efficacy of the *Pasteurella* bacterins (7,8,9,10). *Pasteurella* bacterins also have been reported to cause transient endotoxic shock (11). In addition, parenteral immunization of cattle with an experimental oil-adjuvanted *P. haemolytica* bacterin resulted in a more severe pneumonia than occurred in nonvaccinated cattle (12.). This detrimental effect was explained by the fact that lung antibody induced by parenteral immunization with the oil-adjuvanted

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bacterin opsonizes with *P. haemolytica*, leading to its phagocytosis, which results in death of the alveolar macrophages (13).

Aerosol and subcutaneous immunization with experimental live *P. haemolytica* and *P. multocida* vaccines have been reported to protect cattle against transthoracic homologous challenge exposure (14). Recently, live *P. haemolytica* and *P. multocida* vaccines have been introduced to prevent pneumonic pasteurellosis (15,16,17). The experiments which are the subject of the present report were designed to evaluate the efficacy of *Pasteurella* bacterins and vaccines in colostrum-deprived calves against experimental pneumonic pasteurellosis.

MATERIALS AND METHODS

CALVES

Colostrum-deprived calves (n = 78), of varying sex and breed, that included angus, charolais, jersey, hereford, and holsteins, weighing 70 to 100 kg were used. Groups of 12 to 16 calves with no detectable antibodies to *P. haemolytica* by indirect hemagglutination, to infectious bovine rhinotracheitis (IBR), bovine diarrhea (BVD), or parainfluenza -3 (PI-3) viruses by standard virus neutralization assay, were obtained at eight to ten weeks of age. Calves were acclimated three to four weeks prior to use and maintained in pens on elevated vinyl-clad steel mesh and housed indoors under controlled environmental conditions at 68° to 78° F. Calves were vaccinated and later exposed to challenge infection with IBR virus and *P. haemolytica* or with *P. multocida* alone. Each series of experiments consisted of three vaccinated groups and one sham vaccinated control group.

BACTERINS, VACCINES, AND VACCINATION PROCEDURES

The bacterins, live bacterial vaccines, and vaccination procedures used in these studies are shown in Table I. Bacterins and live bacterial vaccines were prepared by commercial manufacturers and met established standards of potency. Two Pasteurella Haemolytica — Multocida Bacterins designated HM-1 and HM-2, one Escherichia Coli — Pasteurella Haemolytica — Multocida — Salmonella Typhimurium Bacterin designated HM-3, each contained formalin inactivated *P. haemolytica* serotype 1, and *P. multocida*, serotype A-3. The fourth bacterin, designated M-1, contained only formalin-inactivated *P. multocida* serotype A-3. Bacterins HM-1 HM-2, and M-1 were adsorbed to aluminum hydroxide [$Al(OH)_3$]. Bacterin HM-3 was incorporated into a mineral oil adjuvant. Bacterins contained 4.0×10^9 to 1.6×10^{10} organisms per dose and were administered according to the manufacturers recommendations in two — 2.0 mL doses spaced two to three weeks apart. Bacterin HM-3 was administered by the subcutaneous (SC) route; all other bacterins were administered by the intramuscular (IM) route.

One lyophylized *P. haemolytica* serotype A-1 vaccine (HV) was used in two-treatment groups in these experiments. Group HV-1A represented calves given vaccine containing less than 2×10^6 CFU/dose; group HV-1B represented calves given vaccine containing more than 2×10^6 CFU/dose. The vaccine was administered as a single 0.5 mL dose by the intradermal (ID) route.

Two *P. multocida* serotype A-3 vaccines, MV-1 and MV-2, were used. Vaccine MV-1 contained 7.4×10^9 CFU/dose and was administered in two — 2.0 mL doses spaced four weeks apart by the IM route. Vaccine MV-2 contained 3.2×10^7 CFU/dose and was administered as a single 0.5 mL dose by the ID route.

CHALLENGE EXPOSURE

Two to four weeks after the final dose of bacterin or vaccine, both vaccinated and control calves were challenge inoculated. Challenge exposure with *P. haemolytica* was carried out by a combination of selected methods described by Jericho and Langford (18) and Wilkie *et al* (19). Calves were infected with IBR virus by intranasal (IN) inoculation. The Cooper strain of IBR virus (obtained from D.L. Croghan, National Veterinary Services Laboratories, Ames, Iowa) was used. A 4.0 mL dose of a thawed frozen stock tissue culture virus preparation was introduced IN by nebulizer (20), 2.0 mL per nostril. The inoculum contained approximately 6×10^8 $TCID_{50}$ per dose.

A bovine isolate of *P. haemolytica*, (A-1, obtained from B.N. Wilkie, University of Guelph, Guelph, Ontario, Canada) biotype A, serotype 1 was used as a challenge exposure strain. For the preparation of challenge exposure inoculum, brain heart infusion (BHI) (Difco, Detroit, Michigan) bovine blood (5%) agar plates were inoculated with a frozen culture of *P. haemolytica* strain A-1 and incubated for 18 hours at 37°C. The growth from two plates was collected in 20 mL of BHI broth which was mixed into a 500 mL volume of BHI broth and incubated for 18-20 hours on a gyrorotary shaker at 37°C. Bacterial cells were collected by centrifugation, washed once, resuspended in potassium phosphate buffer (pH 7.4, 0.1 M) to OD of 0.75 (Spectronic 20, Bausch and Lomb, Inc., Rochester, NY) reading at 525 nm to contain approximately 4×10^7 CFU/mL, and used within 15 to 30 minutes.

TABLE I. Bacterins, Live Bacterial Vaccines and Procedures Used for Vaccination in Experimental Colostrum-deprived Calves

Materials		Procedures		
Designation	Adjuvant	Dose	Route	Schedule
Bacterins:				
H[a]M[b]-1	$Al(OH)_3$	2.0 mL	IM[c]	Days 0 & 20
HM-2	$Al(OH)_3$	2.0 mL	IM	Days 0 & 21
HM-3	Mineral Oil	2.0 mL	SC[d]	Days 0 & 21
M-1	$Al(OH)_3$	2.0 mL	IM	Days 0 & 16
Live Bacterial Vaccines:				
HV[e]-1A (low dose)	None	0.5 mL	ID[f]	Day 21
HV-1B (high dose)	None	0.5 mL	ID	Day 21
MV-1	None	2.0 mL	IM	Days 0 & 14
MV-2	None	0.5 mL	ID	Day 20

[a] *Pasteurella haemolytica* [c] Intramuscular [e] Vaccine
[b] *Pasteurella multocida* [d] Subcutaneous [f] Intradermal

Four to five days after IBR virus inoculation, calves were infected endobronchially (EB) with a 25 mL volume of the washed *P. haemolytica* strain A-1 inoculum. Each dose contained approximately 1.7×10^9 CFU. A 30 mL volume of the phosphate buffer was administered EB to each calf immediately after challenge exposure.

Inoculum and buffer were introduced through a cannula (outer diameter 1.5 mm, length 54 cm) passed through a 12 gauge needle inserted into the trachea. Calves were mildly sedated with Rompun, and a local anesthetic (Rapicaine, Haver-Lockhart Laboratories, Shawnee Mission, Kansas) was administered at the tracheal site.

A bovine isolate of *P. multocida* P-1062 (obtained from K.R. Rhoades, National Animal Disease Center, Ames, Iowa) serotype A-3, was used as the challenge exposure strain. The challenge exposure inoculum was prepared in a manner similar to that used for preparation of the *P. haemolytica* inoculum. Tryptic soy agar and broth media (Difco, Detroit, Michigan) were used to propagate the *P. multocida* challenge exposure strain. Calves were challenge exposed EB to 6.8×10^9 CFU per 25 mL dose of *P. multocida* followed with 30 mL of broth EB. Exposure was not preceded by IBR inoculation, nor were the bacterial cells washed prior to challenge.

CLINICAL AND PATHOLOGICAL ASSESSMENT

The progression of clinical signs was consistent with respiratory system infection. These subjective observations were not scored on a daily basis. Rectal temperature of each calf was monitored daily for four to ten days after challenge exposure. Untreated and ethylene diamine tetra acetate (EDTA)-treated blood samples were collected a minimum of three times during the acclimation and postvaccination periods and three to four times during the four to ten day postchallenge exposure period. Total white blood cell count (TWBC), differential count, fibrinogen (FIB), hemoglobin, packed cell volume, and total protein (TP) determinations were made on the blood samples collected with EDTA.

Abbot Laboratories, ABA-100 (Abbot Laboratories, S. Pasadena, California) procedures were used for the determination of bilirubin, blood urea nitrogen, serum glutamic oxalacetic transaminase (SGOT), and lactic dehydrogenase (LDH) on the serum samples.

Surviving calves were sedated with Rompun and euthanized with Surital (Parke, Davis and Co. Detroit, Michigan) at the termination of each experiment, four to five days after the *P. haemolytica* strain A-1 challenge exposure and seven to ten days after the *P. multocida* strain 1062 challenge exposure.

Specimens from heart, lung, bronchial lymph nodes, two tracheal sites, liver, kidney, spleen, multiple intestinal segments, and adrenal gland were taken for histopathological examination. The lungs were removed for evaluation.

Two scoring systems were used to estimate the extent of pneumonia (Table II). In initial experiments extent of pneumonia was estimated visually and scored numerically from zero (0) for no involvement to +3 for diffuse, or multifocal with varying degrees of lung mass. The extent of pneumonia was determined by palpation in later experiments and scored according to the system described by Jericho and Langford (21). The system was based on the percentage of consolidation per lung lobe, and pneumonic tissue was calculated as the percentage of the total lung mass involved.

Multiple sets of tissue from lung lesion sites and bronchial lymph nodes were excised and cooled. Specimens for bacteriological and mycoplasmal surveys were submitted in the fresh state for culture. Standardized methods were used for identifying the aerobic bacteria and the viruses which were isolated.

TABLE II. Numerical Scoring System Used to Assess Extent of Pneumonia in Calves following Challenge-exposure

Extent of pneumonia	Observed score	Palpated score
None	0	0
Mild (focal)	1+	1-5
Moderate	2+	6-14
Severe (diffuse)	3+	15-100

[a] Score = Percentage of total lung mass that is consolidated

STATISTICAL ANALYSIS

A separate statistical analysis was made for each measured animal parameter. The data analyzed represented the net change after challenge exposure for each calf. A two-way analysis of variance with unequal subclass frequencies was used in the analyses. Parameter treatment means were compared by the least significant difference method to determine if there were significant differences ($p<0.05$) between treatment groups following challenge exposure.

RESULTS

CLINICAL RESPONSE TO CHALLENGE

Intranasal inoculation of IBR virus caused elevated rectal temperatures of 40.0° to 41.1°C and clinical signs typical of IBR virus infection within 72 to 96 hours. Depression, rhinitis, deep-rapid breathing, and the elevated temperatures persisted or decreased slightly to the time of the *P. haemolytica* serotype A-1 EB challenge exposure.

The additional inoculation of viable *P. haemolytica* serotype A-1 caused elevated rectal temperatures of 41.1 to 41.7°C and increased breathing rates. Depression, ataxia, anorexia, dyspnea, and recumbency occurred in the more severely affected calves. Wheezing, coughing, and thick mucopurulent nasal discharges were observed frequently. Lung sounds, dry or moist rales, increased in intensity within several days after the *P. haemolytica* inoculation, and friction sounds occurred on occasion in the most severely affected calves.

Clinical responses varied among individual calves and between groups of calves. Clinical responses were more severe in the nontreated control and in the bacterin-treated calves and were less severe in the vaccine-treated calves.

Of 11 control calves, two died within 48 hours after challenge exposure. One of six calves vaccinated with the HM-2 bacterin died within 48 hours after challenge exposure.

Pasteurella multocida serotype A-3 challenge inoculation caused temperature elevations, depression, anorexia, and malaise within 24 hours, and the duration varied among groups of calves. Elevated temperatures persisted in the control group for eight days, for six to seven days in the bacterin-treated groups, and for three days in the vaccine-treated groups. Calves remained anorectic for three to five days and lethargic for two to four days postchallenge exposure (PCE). Often control calves, two died within 96 to 120 hours after *P. multocida* challenge exposure. No vaccinates died.

The extent of lung lesions in challenged calves was used to evaluate the effectiveness of the bacterins and vaccines studied. Effectiveness was based upon the ability of a bacterin or vaccine to limit diffuse lung lesions to moderate or 2+ as observed or to no greater than 14% as determined by palpation at necropsy. Efficacy estimates (proportion of calves without lung lesions) of bacterins and vaccines were adjusted for the control response by Abbot's Relationship (22):

$P = \frac{Pv - Pc}{1\text{-}Pc}$ where P = proportion of susceptible vaccinates without diffuse lung lesions, Pv = proportion of vaccinates without diffuse lung lesions, and Pc = proportion of controls without lung lesions.

The effectiveness of *P. haemolytica* containing bacterins and vaccines in limiting clinical responses of increased rectal temperatures, diffuse or multifocal lung lesions, and resulting efficacy estimates against an IBR virus and *P. haemolytica* challenge exposure are summarized in Table III.

The 11 control calves had clinical disease, and seven developed diffuse lung lesions after challenge exposure. Lesions occurred in the control, bacterin, and low dose vaccine-treated groups. In these three groups, lung lesions were multilobular and often extensively coalescent. Extensive consolidation with coagulative necrosis was accompanied by fibrinopurulent pleuritis with adhesions. Microscopic lesions were mostly in the acute or subacute stages of inflammation and were consistent with the pattern described for bovine pneumonic pasteurellosis, synonymously called fibrinous lobar pneumonia (23). Characteristic microscopically detectable changes included edema with abundant fibrin, hemorrhage, abscessation, fibrosis, and variable inflammatory cell response that was often fibrinopurulent in parenchyma, interstices, and airways.

Bacterins HM-1 and HM-2, containing an $Al(OH)_3$ adjuvant, were the least effective in limiting both clinical responses and diffuse lung lesions and showed the lowest efficacy values (Table III). Bacterin HM-3, which tained a mineral oil adjuvant, showed a high degree of effectiveness in limiting both clinical responses and severe lung lesions, and also showed a high efficacy value. The *P. haemolytica* HV-1A (low dose) vaccine was less effective in limiting clinical and lesion responses and showed a lower efficacy value (0.37) than the HV-1B (high dose) vaccine (efficacy value of 1.00). No significant clinical responses were observed in the HV-1B group. Lung lesions were limited to small focal areas of consolidation generally confined to one lung lobe.

Isolations of IBR virus and *P. haemolytica* were made from 40 to 75 percent of lung lesion sites and bronchial lymph node tissues cultured at necropsy. A higher percentage of isolations was made from the more severely affected calves. Specimens for bacterial and mycoplasma surveys were cooled and submitted for culture in the fresh state. No other pathogenic organism including *Haemophilus somnus*, *Mycoplasma* sp., parainfluenza-3 virus, or bovine respiratory syncytial virus was isolated.

The effectiveness of *P. multocida* containing bacterins and vaccines in limiting clinical and pathological responses and efficacy estimates against *P. multocida* challenge exposure are summarized in Table IV. Only four of six calves vaccinated with the MV-1 vaccine showed limited clinical response. Diffuse lung lesions occurred in nine of ten control calves, in nine of ten calves vaccinated with the M-1 aluminum hydroxide adjuvanted bacterin, and in three of four calves vaccinated with the mineral oil adjuvanted bacterin. The live vaccines were more effective in that diffuse lung lesions occurred in two of six and zero of four calves vaccinated with the MV-1 and MV-2 vaccines respectively. Efficacy values for the live vaccines were higher than those for the $Al(OH)_3$ or oil adjuvanted bacterins tested. In the most severely affected calves, consolidation and necrosis were accompanied by abundant fibrinopurulent exudates, edema, and adhesions. Lung lesions were distributed multilobularly and in

TABLE III. Response of *P. haemolytica* Vaccinated and Nonvaccinated Colostrum-deprived Calves and Efficacy of Vaccination after Challenge-exposure with IBR Virus and *P. haemolytica* Strain A-1

Vaccination group	Clinical response[a] No. positive/No. tested	Diffuse lung lesions[b] No. positive/No. tested	Efficacy[c]
Bacterins:			
H[d]M[e]-1	4/4	4/4	-0.57
HM-2	6/6	3[g]/6	0.21
HM-3	3/6	1/6	0.74
Live Bacterial Vaccines:			
HV[f]-1A (low dose)	4/5	2/5	0.37
HV-1B (high dose)	0/3	0/3	1.00
Control (Nonvaccinated)	11/11	7[h]/11	0.00
Control (Nonvaccinated, Nonchallenged)	0/6	0/6	NA[i]

[a] Increased rectal temperatures
[b] Lesions scores of 3+ or > 14%
[c] Proportion of susceptible vaccinates without diffuse lung lesions
[d] *Pasteurella haemolytica*
[e] *Pasteurella multocida*
[f] Vaccine
[g] One death 2 d post-challenge exposure
[h] Two deaths 2 d post-challenge exposure
[i] Not applicable

some instances all lung lobes were affected.

Microscopic lesions were characterized by extensive fibrosis with abscesses and necrosis, edema, emphysema, and/or atelectasis. Interlobular septae and pleura were moderately to markedly thickened by edema with fibroplasia or fibrosis and had organizing lymph thrombi. This lesion pattern includes the morphological changes in lungs of calves challenge-exposed to *P. haemolytica* and is consistent with bovine pneumonic pasteurellosis.

Pasteurella multocida was isolated from 97 percent of the lung lesion sites and 82 percent of bronchial lymph nodes cultured at necropsy. Isolation percentages tended to be higher than those observed in the *P. haemolytica* vaccination-challenge studies.

Hematological, blood chemistry, and temperature data were examined possible significant changes following each challenge exposure. A separate analysis was made for each parameter. The data represent the net change after challenge for each calf in each treatment group. Group means were analyzed for significant differences as described in Materials and Methods. Due to extreme animal-to-animal (within group) variation, few parameters produced treatment means that differed significantly ($p < 0.05$). Table V summarizes the parameter data analysis (p values) among treatment groups obtained in the *P. haemolytica* vaccination-challenge studies. Significance levels following both the IBR virus and *P. haemolytica* challenge exposure are shown. Group means differing significantly following the IBR virus challenge included the elevated band neutrophil counts in the live vaccine low dose HV-1A treatment group relative to all the additional treatment groups and a lesser temperature elevation in the bacterin HM-1 treatment group relative to only the bacterin HM-2 treatment group. It should be mentioned that lesion severity following IBR virus challenge was not assessed throughout these studies, though typical ulcerative nasal lesions with overlying adherent fibrinonecrotic masses were usually found at necropsy. In preliminary experiments, where IBR virus was given alone, lesions resembling those attributed to *P. haemolytica* alone or to *P. haemolytica* following IBR virus were not observed. Such lesions are readily distinguished from those attributed to IBR virus given alone.

Lesion severity was the only parameter of the eleven analyzed that showed-significant group differences following *P. haemolytica* challenge exposure. Lung lesion severity was significantly less in the live vaccine high dose HV-1B and bacterin HM-3 groups than in the bacterin groups HM-1, HM-2, live vaccine low dose HV-1A, and challenge control groups.

Table VI summarizes the parameter data analysis (p values) among treatment groups obtained in the *P. multocida* vaccination-challenge studies. Significance levels following the *P. multocida* challenge exposure are shown. Group means differing significantly included the lowered monocyte

TABLE IV. Response of *P. multocida* Vaccinated and Nonvaccinated Colostrum-deprived Calves and Efficacy of Vaccination after Challenge Exposure with *P. multocida* Serotype A-3

Vaccination group	Clinical response[a] No. positive/No. tested	Diffuse lung lesions[b] No. positive/No. tested	Efficacy[c]
Bacterins:			
M-1	10/10	9/10	0.00
HM-3	4/4	3/4	0.17
Live bacterial vaccines:			
MV-1	4/6	2/6	0.63
MV-2	4/4	0/4	1.00
Control (Nonvaccinated)	10/10	9[d]/10	0.00
Control (Nonvaccinated, Nonchallenged)	0/4	0/4	NA[e]

[a] Increased rectal temperatures
[b] Lung lesion scores of 3+ or > 14%
[c] Proportion of susceptible vaccinates without diffuse lung lesions
[d] Two deaths at d 4 and 5 postchallenge exposure
[e] Not applicable

TABLE V. Statistical-Evaluation of *P. haemolytica* Vaccinated and Nonvaccinated Colostrum-deprived Calves after Challenge-exposure with IBR Virus and *P. haemolytica*. Parameter Analysis p Values

Parameter	Significance levels (p) following challenge exposure: Post IBR Virus	Post *P. haemolytica*
Lactic Dehydrogenase	0.42	0.86
SGOT[a]	0.16	0.87
Total protein	0.48	0.97
Fibrinogen	0.41	0.16
TWBC[b]	0.76	0.79
Monocytes	0.47	0.46
Lymphocytes	0.41	0.82
Neutrophils		
segmented	0.56	0.47
banded	0.04[c](1)	0.33
Temperature	0.03[c](2)	0.10
Lung Lesion Severity	NA	0.002[c](4)

[a] Serum glutamic oxalacetic transaminase
[b] Total white blood cell count
[c] Treatment group means differing significantly ($p < 0.05$)
(1) Live Vaccine low dose HV-1A > Bacterins HM-1, HM-2, HM-4, Live Vaccine High dose HV-1B and challenge controls
(2) Bacterin HM-1 < Bacterin HM-2
(3) Not assessed
(4) Live Vaccine (High Dose) HV-1B and Bacterin HM-3 < Bacterins HM-1, HM-2, Live Vaccine HV-1A and challenge controls

TABLE VI. Statistical Evaluation of *P. multocida* Vaccinated and Nonvaccinated Colostrum-deprived Calves after Challenge Exposure with *P. multocida*: Parameter Analysis, p Values

Parameter	Significance level (p) following challenge	Group[+] means differing significantly
Lactic Dehydrogenase	0.15	
SGOT[a]	0.09	
Total Protein	0.11	
Fibrinogen	0.75	
TWBC[b]	0.07	
Monocytes	0.02++	+K+++ < MV-1 and MV-2
Lymphocytes	0.02++	MV-2 > M-1 and HM-3
Neutrophils		
segmented	0.64	
banded	0.21	
Temperature	0.002++	MV-2 < M-1 and + K MV-1 < +K
Lung Lesion Severity	0.0003+++	MV-2 < M-1, HM-3, MV-1, & + K MV-1 < M-1 and + K

[a]Serum glutamic oxalacetic transaminase

[b]Total white blood cell count

\+ Bacterin and live bacterial vaccine vaccination groups

++ $p < 0.05$

+++ Challenge controls

counts in the positive control group relative to the vaccine-treated groups MV-1 and MV-2 and elevated lymphocyte counts in the vaccine-treated group MV-2 relative to the bacterin-treated groups M-1 and HM-3. Body temperature elevations of the vaccine-treated groups MV-1 and MV-2 were significantly smaller than the positive control group, with the MV-2 elevation being smaller than that of the M-1 bacterin group. Lesion severity in the MV-2 vaccine group was significantly less than that in all other treatment groups including the positive control group. Additionally, similar results were shown in the MV-1 vaccine group relative to the M-1 bacterin and the positive control group.

DISCUSSION

The purpose of the present study was to investigate the efficacy of several *Pasteurella* bacterins and vaccines against experimentally produced pasteurellosis with pneumonia as a potential adjunct to development of test methods suitable for the evaluation of products intended for use in cattle. The respiratory diseases experimentally produced by sequential exposure to IBR virus IN and *P. haemolytica* EB and by *P. multocida* alone EB provided workable models for testing the efficacy. Commercially prepared combined bacterins containing formalinized *P. haemolytica* in an $Al(OH)_3$ adjuvant were of limited effectiveness against experimental bacterial pneumonia. A bacterin containing a mineral oil adjuvant given SC and a live vaccine given ID were more effective. The live *P. haemolytica* vaccine, given at a dose of $>2 \times 10^6$ viable organisms, was the most effective in limiting both clinical responses and lung lesions with challenge-exposed vaccinated calves remaining essentially clinically normal and lung lesions limited to focal areas of consolidation. A dose dependency of the live *P. haemolytica* vaccine was indicated since at a dose of $<2 \times 10^6$ CFU the vaccine was less effective against the challenge.

Pasteurella multocida containing bacterins in either an $Al(OH)_3$ given IM or mineral oil adjuvant given SC and live vaccines given IM or ID were generally ineffective in limiting clinical responses to EB challenge exposure with cultures of *P. multocida*. Bacterins also were ineffective in limiting diffuse lung lesions after challenge exposure. Live vaccines, however, were effective and lung lesions were generally limited to focal areas of consolidation as was observed with the *P. haemolytica* vaccine.

These results are not in agreement with those previously reported (24) concerning the adverse affects of vaccination of calves subcutaneously with formalin killed bacterial cells in Freund's complete adjuvant against experimental *Pasteurella*-induced fibrinous pneumonia. Immunization with formalin-killed $Al(OH)_3$ adjuvanted bacterial bacterial cells by the parenteral route was not associated with adverse pulmonic response to challenge though responses observed were no less than those observed in nonvaccinated control calves following challenge-exposure.

Ineffectiveness of the *P. multocida* component of the mineral oil adjuvanted bacterin in limiting severity of lesions following *P. multocida* EB further supports earlier findings on the ineffectiveness of *Pasteurella* bacterins against infection under field conditions (25,26,27).

Our results on the *P. haemolytica* mineral oil adjuvanted bacterin differ from those earlier reported (12,19) in which *P. haemolytica* bacterins prepared in Freund's incomplete adjuvant resulted in enhanced disease following EB challenge exposure. Analysis of preinoculation and postinoculation mean hematological, blood chemistry, temperature, and lesion data from 40 calves in the present *P. haemolytica* studies indicated that lesion severity was the only parameter of the 11 analyzed that showed significant differences. The extreme within group variation observed accounted for few treatment means differing significantly. This is in contrast to those results reported by Schmitz *et al* (28) who found significant direct correlations between the severity of pneumonia and postchallenge inoculaton mean values of bilirubin, LDH, SGOT, fibrinogen, and body temperature. The fact that our data were separated for analysis between effects after IBR virus followed in four days by *P. haemolytica* could account for this difference.

Group means differing significantly in the *P. multocida* studies included lowered monocyte counts in the control group relative to the vaccine-treated groups and the elevated lymphocyte counts in one of the vaccine treatment groups relative to the bacterin-treated groups. Both a lower temperature response and lower lung lesion severity were evident in the vaccine-treated group relative to the control and bacterin-treated gorups following challenge exposure.

A number of factors that could account for these differences have been described (19,29) and make a direct comparison of results difficult. Evident, however, are the accumulating data reported in experimental studies (24,29) that support field observations (7,8) concerning the ineffectiveness of killed bacterins in controlling *Pasteurella* pneumonia and the need for development of efficacious biologicals.

The enhanced resistance observed in our studies to experimental challenge exposure by immunization with live *P. haemolytica* or live *P. multocida* is in agreement with results reported by other investigators (12, 14,15,16,17,29,30).

The resistance provided by the live *P. haemolytica* vaccine appeared dose dependent and may provide an essential correlate for potency testing of these vaccines.

Studies to gain a better understanding of the nature and mechanisms involved in development of acceptable levels of protection against laboratory challenge are being explored. Potassium thiocyanate extracts (31) and encapsulated young cultures (32) of *P. haemolytica* have been evaluated in vaccination studies in calves and were shown to provide varying degrees of protection. Some protection was shown in calves vaccinated with cell free culture supernatants of *P. haemolytica* containing cytotoxin, whereas a fuller protection appeared to require an immune stimulation to both the cytotoxin and surface antigens of *P. haemolytica* (33).

The cytotoxin neutralizing activity of sera may yield assay systems to provide an indicator of protection against pneumonic pasteurellosis (33). The modified direct complement fixation test that detects bovine serum antibody against the sonicated somatic antigen of *P. haemolytica* has been suggested as an assay system for vaccination effectiveness (34).

The nature of the apparent antigen(s) produced by live *P. multocida* vaccines remains to be elucidated.

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