The Use of Live Cultures of *Pasteurella Haemolytica* or *Pasteurella Multocida* to Immunize Cattle Against Bovine Respiratory Disease

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Introduction

Bovine respiratory disease is the leading cause of loss to the North American cattle industry. The disease is characterized by a severe fibrinous pneumonia or a suppurative bronchitis and bronchopneumonia. The bacteria most commonly associated with these cases of pneumonia and deaths are *Pasteurella haemolytica* (PH) and *Pasteurella multocida* (PM). Attempts to immunize calves with killed bacterins or extracts of these bacteria have been ineffective.[1,14] Recently several investigators have used live cultures of PH and PM to immunize calves against respiratory disease.[5,6,9,11,12,13] The purpose of these investigations was to develop vaccines that effectively immunize calves against pneumonic pasteurellosis. Two vaccines have been developed that contain viable *Pasteurella haemolytica* (PRECON-PH) or *Pasteurella multocida* (BOVICON-PM) (A.H. Robins Corporation, Richmond, Va.).

Materials and Methods

Experimental animals and their management

Twenty-three unweaned cross-bred calves, 5-6 months of age were selected for the evaluation of the *Pasteurella haemolytica* (PH) vaccine. Thirty unweaned cross-bred beef calves and 24 weaned dairy calves 3 to 6 month of age were used to evaluate the *Pasteurella multocida* (PM) vaccine. Fifty-five calves 5-6 month of age were used to study the development of PH cytotoxin neutralizing antibody and the effects of simultaneous vaccination with PH and PM vaccines. All of the calves had been reared on research farms and were selected on a serial basis for the vaccination groups or control group according to their age. The beef calves were housed in loose housing on the farm of origin and at the research center during the period of the trial. The dairy calves were held in loose housing and confinement stanchions. All animals had feed and water available on a daily basis.

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Vaccination Procedure

The vaccines used in these trials were produced by the A.H. Robins Corporation (Richmond, Va.). The vaccines were viable cultures of *Pasteurella haemolytica* A-1 (PRECON-PH) or *Pasteurella multocida* A-3, 4, 12 (BOVICON-PM). The calves were vaccinated with 0.5 cc of PH vaccine or PM vaccine intradermally in the lateral cervical area two weeks prior to stressing and challenge. Eighteen calves were vaccinated to test the safety and efficacy of the PH vaccine. The calves were allotted to three groups of six calves and vaccinated with either (A) 4.5×10^4; (B) 4.5×10^5; or (C) 4.5×10^6PH. Thirty five calves were vaccinated to evaluate the safety and efficacy of the PM vaccine. Fifteen calves were vaccinated with $1.6 - 2.2 \times 10^6$ PM and twenty calves were vaccinated with 1.0×10 PM. All nonvaccinated calves received 0.5 cc intradermal injection of brain heart infusion as a placebo.

Clinical procedures

All calves were observed daily for clinical signs of respiratory disease. Rectal temperature, anorexia, malaise, increased respiratory rate, nasal or ocular discharge and diarrhea were noted. An animal was considered to be a clinical case of respiratory disease if the rectal temperature exceeded 39.4° C and the animal exhibited anorexia, malaise and/or an increased respiratory rate.

Stressing and infection

Forty-eight hours prior to challenge the calves were stressed to simulate the normal stress conditions associated with weaning and sale procedures for calves processed through commercial sale channels. The calves were fasted and each calf received an injection of 0.5 cc of 5% acetic acid into the anterior of the trachea. The calves were then subject to a spray of cold water to produce a mild chill. This stress procedure was repeated after an 18 hour interval. Six hours following the second stressing each calf received an intratracheal injection of a viable culture of PH or PM. The calves in the PH vaccine trial received 15 ml of a PH culture that contained 3.1×10^8 colony forming units per ml. The calves in the PM vaccine trials received 35

ml of PM culture that contained 3 x 10^9 colony forming units per ml.

Microbiologic procedures

Nasal specimens were collected prior to vaccination and at weekly intervals for six weeks and cultured for *Pasteurella* sp., *Mycroplasma* sp. and viral isolation. The nasal specimens were cultured on bovine blood agar to detect *Pasteurella* sp., and Frey's mycoplasma agar to detect *Mycoplasma* sp. The specimens were subjected to three blind passages in bovine kidney tissue culture for viral isolation.

Serologic procedures

Blood samples were collected prior to vaccination and weekly intervals for six weeks for serum antibody analysis. A KSCN extract of PH or PM was used as an antigen for the ELISA to assay the serum antibody to the somatic antigens of PH or PM.[7,8]

Cytotoxin assay

The cytotoxin neutralization assay was measured using the neutral red dye uptake[4] of sheep alveolar macrophages. The alveolar macrophages were subjected to the PH cytotoxin or the PH cytotoxin neutralizing antibody and the cytotoxin. The survival of the alveolar macrophages was quantitated by measuring the neutral red dye uptake.

Field observations

Calves were moved through commercial sale channels to evaluate the vaccines under field conditions. The animals were divided by alternate selection and vaccinated with the PH vaccine or not vaccinated.

Results

Clinical observations

Pasteurella haemolytica vaccine

Six calves exhibited clinical signs of respiratory disease during the 14 day observation period following infection. All five nonvaccinated calves and one calf from group A vaccinated with 4.5 x 10^4 PH exhibited clinical signs of respiratory disease (Table 1). These cases of disease developed during the 3-7 day post-infection period. One animal in the nonvaccinated group developed clinical signs of respiratory disease at day one post-infection and their signs persisted until death on the ninth day following infection.

TABLE 1. The incidence of clinical respiratory disease among Pasteurella haemolytica vaccinated and nonvaccinated calves challenged with Pasteurella haemolytica.

Group	Vaccine level	no.	No. Cases	% Case	No. Deaths	No. case days
Control	0	5	5	100	1	19
A-vac	4.5 x 10^4	6	1	16	0	1
B-Vac	4.5 x 10^5	6	0	0	0	0
C-Vac	4.5 x 10^6	6	0	0	0	0

*Case-days — cases of disease X days exhibiting clinical signs.

The average daily gain of the calves in the PH vaccinated groups was greater than the calves of the nonvaccinated group during each weekly period (Table 2). The accumulative average daily gain for the four-week period for the nonvaccinated group was 0.86 KG while the accumulative average daily gain of the PH vaccinated group were 1.45 kg, 1.58 kg, and 1.81 kg for those vaccinated with 4.5 x 10^4, 4.5 x 10^5, and 4.5 x 10^6 PH respectively. The greatest difference in weight gain was evident during the first week post-infection. The PH vaccinated calves gained an average of 2.67 kg per day while the nonvaccinated calves gained an average of 0.95 kg per day.

TABLE 2. The average daily weight gains of vaccinated and nonvaccinated calves during the four-week period after challenge with P. haemolytica.

Group	Week 1	2	3	4	1-4
Control	0.95*	0.77	1.17	0.54	0.86
A-Vac	2.30	1.08	1.81	0.58	1.45
B-Vac	2.90	1.40	1.31	0.68	1.58
C-Vac	2.18	1.58	1.22	1.54	1.81

*Kilograms

Pasteurella multocida vaccine

Fifteen calves were vaccinated with 1.6 — 2.2 10^6 PM and 20 calves were vaccinated with 1 x 10^7 PM. Fourteen of the nineteen nonvaccinated calves developed clinical signs of respiratory disease and one vaccinated calf exhibited clinical signs of respiratory disease. The nonvaccinated calves were observed to be sick a total of 52 case days. The one PM vaccinated calf was ill for three days.

TABLE 3. The incidence of clinical respiratory disease among Pasteurella multocida vaccinated and nonvaccinated calves challenged with Pasteurella multocida.

Group	Vaccine Titer	No.	No. Cases	% Case	No. Deaths	No. case days*
Non-Vac	0	10	6	60	0	19
D-Vac	1 x 10^7	20	0	0	0	0
Non-Vac	0	9	8	89	0	33
E-Vac	2.2 x 10^6	5	0	0	0	0
F-Vac	1.7 x 10^6	5	1	20	0	3
G-Vac	1.6 x 10^6	5	0	0	0	0

*Case-days — cases of respiratory disease x days exhibiting signs.

The calves in group D-Vac and their nonvaccinated controls were weighed at the time of weaning and at the

conclusion of the trial. The ten nonvaccinated calves decreased an average of 0.02 kg while the PM D-Vac calves gained an average of 6.16 kg during the experimental period. Six of the ten nonvaccinated calves lost weight during the trial.

Serologic response

Serum antibody response to *Pasteurella haemolytica* vaccination. All of the groups of calves had a detectable level of antibody to PH in their sera at the time of the initiation of the trial. The antibody titers did not change in a significant manner during the two week period following vaccination with PH. After the calves received the intratracheal challenge of PH at two weeks post vaccination the serum samples exhibited an increase in antibody to PH. The immune response was greater and persisted longer among the groups of vaccinated calves than the nonvaccinated calves. Fourteen of eighteen calves showed a seroconversion by the fourth week while only one of the five calves in the nonvaccinated group exhibited a similar seroconversion.

Serum antibody response to *Pasteurella multocida* vaccination.

All of the calves had detectable levels of PM antibody at the time of the initiation of the trial. The calves vaccinated with 1 x 10^7 PM, demonstrated a 1.7 times increase in the GMT of the PM antibody during the two week period following vaccination. The PM vaccinated calves exhibited a 5.2 X response to the PM antigen at two weeks post-challenge while the control calves only demonstrated a 3 X increase in PM antibody. There was not a significant difference in the PM antibody titers between the nonvaccinated calves and the calves that were vaccinated with 1.6 — 2.2 X 10^6 PM throughout the period of this trial.

Pasteurella haemolytica cytotoxin neutralizing antibody.

Eight calves that were vaccinated with PH vaccine were used to study the induction of cytotoxin neutralizing antibody and anti-PH antibody in the serum (Table 4). There was an increase in the cytotoxin neutralizing antibody by seven days after vaccination with the PH vaccine. The titer of the PH cytotoxin neutralizing antibody increased until 14 days post-vacination. Thereafter the level of the PH cytotoxin neutralizing antibody remained at an increased level until 28 days post-vaccination. The serum antibody as detected by the ELISA tended to parallel the anti-cytotoxin antibody.

TABLE 4. Pasteurella haemolytica cytotoxin neutralizing antibody and anti-PH antibody in the sera of calves vaccinated with PRECON-PH.

Day:	0	7	14	21	28
Anti-PH cytotoxin	40*	65	81	78	83
PH antibody	27	140	136	200	152

*Average--8 calves--vaccinated at 0 day.

Immune response of calves to simultaneous vaccination with *Pasteurella haemolytica* and *Pasteurella multocida.*

Forty-seven calves were used to study the immune response to two live vaccines containing *Pasteurella haemolytica* and *Pasteurella multocida.* The vaccinated calves responded to both the PH and PM vaccines as measured by the ELISA and the PH cytotoxin neutralization test (Table 5). The titers of the PM antibody increased 3.5 X the original titer during the seventeen days post-vaccination. The titers of the PH antibody increased 5.2 X the original titer in the seventeen days post-vaccination. The sera of the vaccinated calves showed a marked increase in cytotoxin neutralizing antibody as compared to the nonvaccinated calves.

TABLE 5. The immune response of beef calves to simultaneous vaccination with live Pasteurella haemolytica and Pasteurella multocida at two separate vaccination sites.

Vaccine	Antigen	No.	Days post-vaccination 0	17	29	43
PM-PM Vac	PM	24	88*	304	352	426
Control	PM	23	80	122	183	242
PM-PH Vac	PH	24	68	355	323	400
Control	PH	23	80	112	215	401
			PH cytotoxin neutralizing antibody			
PH-Vac		24	49	70	75	77
Control		23	52	56	64	66

*ELISA titer

Discussion

Vaccination with viable cultures of PH or PM protected the calves against respiratory disease when they were stressed and challenged with PH or PM. Four percent of the vaccinated animals exhibited clinical signs of respiratory diseases while 79 percent of the nonvaccinated animals demonstrated clinical respiratory disease. The antibody response to the PH antigen or the PM antigen as measured by the ELISA increased following vaccination. The injection of the viable culture into the skin apparently creates a micro foci of infection by viable organisms that may persist up to seven days. The PH or PM growing in the skin may produce immunogenic substances that diffuse into the systemic circulation and stimulate the immune system of the calf. The stimulation of the immune system may persist for several days until the protective antibody is formed by the host and this antibody diffuses into the foci of infection and neutralizes the infection. The organism growing in the skin of the host are most likely to be in the correct immunogenic form to produce a protective immune response.[2,3,10]

Summary

Two vaccines have been developed that contain viable *Pasteurella haemolytica* (PH) (PRECON-PH) or *Pasteurella multocida* (PM) (BOVICON-PM). The

experimental calves were vaccinated intradermally with viable organisms 10-14 days before stressing and challenge. Eighteen calves were vaccinated to test the safety and efficacy of the PH vaccine. Calves were vaccinated with either (A) 4.5 x 10^4: (B) 4.5 x 10^5; or (C) 4.5 x 10^6PH. Five nonvaccinated calves and one calf from group A developed clinical signs of respiratory disease after stressing and intratracheal challenge with 15 cc of a PH culture that contained 3.1 x 10^8 colony-forming units/ml. The vaccinated animals demonstrated an increased antibody production following challenge and an increased rate of weight gain when compared to the nonvaccinated calves. PH vaccinated calves have been shown to produce a cytotoxin neutralizing antibody seven days after vaccination. Thirty-five calves were vaccinated with either 1 x 10^7 or 2 x 10^6 PM. Fourteen nonvaccinated calves and one vaccinaged calf developed clinical signs of respiratory disease after stressing and challenge with 35 ml of 3 10^9 PM. The PH and PM vaccines have been shown to be effective in the control of laboratory induced infections and natural outbreaks of respiratory disease.

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