A Tissue Culture-Derived *Pasteurella Haemolytica* Vaccine

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Introduction

Available methods for the prevention of bovine respiratory disease (BRD) do not meet the current needs of the beef cattle industry. The reasons for this include unsatisfactory products, product use requirements that do not fit with established management practices, lack of cost effectiveness and the complex etiology of BRD. In those instances where vaccines have been initially administered at the sale yard or feed yard, no benefit or an adverse effect has been observed.[5,13] When vaccines were administered in advance of weaning and shipping, significant benefits have been observed in some trials,[9,10] but not in others.

With the recognition of the important role of *Pasteurella haemolytica* as an etiologic agent in BRD, much effort has been directed toward prophylaxis. A live *P. haemolytica* vaccine for intradermal injection is available commercially.[7,14] Relatively difficult administration and the requirement for special pre-weaning processing have limited the use of this vaccine in beef cattle. More recently, oil-adjuvanted *P. haemolytica* bacterins have been reported to afford protection against intrapulmonic injection of homologous bacteria.[8,4,2] Loan and Purdy[8] reported that an oil-adjuvanted tissue culture-derived *P. haemolytica* bacterin afforded protection against field exposure to BRD. Further studies of protection afforded by a tissue culture-derived *P. haemolytica* bacterin against experimental challenge with virulent bacteria and against field exposure to BRD are reported herein.

Materials and Methods

Vaccine Preparation and Use

One serial lot of tissue culture-derived *P. haemolytica* bacterin designated PASTEURELLA BACTERIN-TC was used throughout the study. This bacterin was prepared by methods previously reported[8] with minor modifications. Briefly identified field isolates of *P. haemolytica* biotype A, serotype 1 were stored in the fifth serial passage at –70°C. These cultures were re-isolated on blood agar, inoculated into bovine tissue cultures and incubated at 37°C to the approximate peak of the logarithmic growth phase. The cultures were harvested by the addition of a final concentration of 0.38% formalin.

Calf Vaccination and Challenge

Trials 1 and 2 were laboratory studies of vaccine efficacy. Calves weighing 400 to 500 pounds were vaccinated 2 times with 5.0 ml PASTEURELLA BACTERIN-TC at 28-day intervals. This was followed by challenge of immunity by transthoracic inoculation of 10^{10} (Trial 1) and 10^{8} (Trial 2) colony-forming units (cfu) of *P. haemolytica* Al, 7 days following the second vaccination. The calves that died of septicemia were examined postmortem. Vaccinated and nonvaccinated calves that survived were killed four days after challenge inoculation and the lung lesions were scored and compared according to the method of Panciera and Corstvet,[11] In Trial 3, the calves were vaccinated at 1-4 months old, left on the dam three additional months and then inoculated transthoracically with 10^{9} cfu virulent bacteria and examined as above.

For testing the efficacy of PASTEURELLA BACTERIN-TC in the field, calves from three farms in eastern Tennessee were vaccinated 28 days prior to weaning. The calves were revaccinated at weaning. They were then shipped 1,770 km to an experimental feedlot in Bushland, Texas. Upon arrival at the feedlot, all calves were processed by generally accepted procedures and observed for signs of BRD for 28-days.[8]

Statistical Calculations

The incidence of clinical illness and death and the lung lesion scores were analyzed by Chi square and analysis of variance as a completely randomized design. Differences in serologic responses to vaccination were analyzed by analysis of variance using the Duncan multiple range test.

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Results

Trial 1

The indirect hemagglutination (IHA) antibody response to *P. haemolytica* was low following the first vaccination. Two days following the second vaccination, the IHA titer of vaccinated calves averaged 350 (Table 1). Anticytotoxin titers of serum samples taken 7 days after the second vaccination averaged 109 in vaccinated calves versus 8 in nonvaccinated calves (Table 1). Upon transthoracic challenge of immunity, all 10 control calves died of septicemic *P. haemolytica* infection within 24 hours. Four of the 10 vaccinated calves died at a slower rate over a period of approximately 2 days (Table 2). Six vaccinated calves survived ($p<.01$). No marked differences were observed in the lungs of calves that died acutely of septicemia.

TABLE 1. Serologic response of calves following vaccination with PASTEURELLA BACTERIN-TC.

Treatment Group	Indirect Hemagglutination Titer*	Anti-Cytotoxin Titer**
Vaccinated	350	109
Not Vaccinated	5	8

* Two days after second vaccination

** Seven days after second vaccination

Trial 3

Seven of 8 control calves in this experiment died of septicemia following transthoracic challenge of immunity (Table 2). Of the calves initially vaccinated at 1 to 4 months of age, revaccinated 3 months later and subsequently inoculated transthoracically, all survived ($p<.001$). Lung lesions of vaccinated versus nonvaccinated calves were not compared due to lack of surviving control calves for comparison.

Field Protection Study

Results of this study are presented in Table 3. Vaccination reduced the incidence of BRD by 70% ($p<.05$) and the number of days sick by 80% ($p<.001$).

Trial 2

The results of transthoracic challenge of immunity are presented in Table 2. Nonvaccinated calves had lung lesions which averaged 831 cm^3 and included 1 calf with an apparent rapidly developing lesion consisting of multiple foci. By contrast, the vaccinated calves had an average lung lesion volume of 58 cm^3 ($p<.001$).

TABLE 2. Protection afforded by PASTEURELLA BACTERIN-TC vaccination against transthoracic inoculation of Pasteurella haemolytica.

Trial Number	Treatment	Number of Calves	Number Immune	Percent Reduction of BRD
1	Vaccinated	10	6	60
	Not Vaccinated	10	0	
2	Vaccinated	8	8	100
	Not Vaccinated	8	0*	
3	Vaccinated	8	8	88
	Not Vaccinated	8	1	

* Based on lung lesion volumes.

TABLE 3. Protection afforded by PASTEURELLA BACTERIN-TC vaccination against field exposure to BRD.

Treatment	Number of Calves	Number Sick	Treatment Days
Vaccinated	50	3	9
Not Vaccinated	50	10	45

Postmortem Findings

Calves that died acutely following transthoracic injection of *P. haemolytica* had heavy, moist, congested lungs with froth in the larger air passages. Histologically the lesions consisted predominantly of much congestion, fibrin and cellular debris. Many bacteria were present. In these calves, *P. haemolytica* was readily recovered from bronchial lymph nodes, heart blood and splenic tissue. *P. haemolytica* was recovered regularly from the lung lesions of surviving calves, but was seldom recovered from bronchial lymph nodes, heart blood or splenic tissues. Histologically the lesion sites of lungs of surviving calves were characterized by increased thickness of the alveolar septa, an increase in plasma cells and macrophages and a marked increase in polymorphonuclear cells. There were also necrotic foci, fibrin and debris.

Discussion

An ideal preventive for BRD under North American stocker/feeder calf management systems would be a vaccine administered as a single injection upon arrival of calves at the stocker facility or feedlot. Both the literature cited[5,13] and extensive experience in the cattle feeding

industry has indicated that this procedure is not effective. Vaccination at branding time and at the initial point of assembly, however, could be effective as shown in these studies and could be accomplished within the existing calf production and marketing systems. Thus, this latter is a viable option for the prevention of BRD.

Previous reports that killed bacterins do not prevent BRD suggest that protective antigens are not present in the vaccines used or are not present in optimally immunogenic form. This could be due to the failure of *P. haemolytica* to produce protective antigens when grown on bacteriological media, instability of the antigens once they are produced, or lack of effective adjuvants to properly present the antigens to the immune system. The timing of vaccination and revaccination also may be important since IgM and possibly IgG_1 are likely candidates for important protective roles in BRD.[8] These antibodies, especially IgM, have short biological half-lives.

Living *P. haemolytica* used as a vaccine protects against BRD, indicating that protective antigens are produced during clinical and subclinical infection. However, the promising value of commercially available living *P. haemolytica* vaccines has not been fully realized in field studies.[12] Several potential disadvantages are inherent in the use of living vaccines. These include difficult administration, the potential for abscess formation, the potential for causing disease in some herds and the possibility that the timing of administration of the vaccine does not fit with commonly used livestock management practices. Unless a vaccination practice can be adapted to current livestock management procedures, it is unlikely that its use will be cost effective to the calf producer.

Many pathogenic bacteria undergo changes toward non-pathogenicity when grown on artificial, lifeless media. These changes are well documented for *Brucella abortus*[15] and *Pasteurella multocida*[1] and involve changes in the outer portions of the bacterial cell accompanied by changes in virulence and antigenic structure. There are indications that such changes also occur in *P. haemolytica* grown on lifeless media. Culture of *P. haemolytica* on lifeless media often results in loss of hemolytic capacity. This returns following passage in mice or embryonating eggs.[3]

In the studies reported here, a tissue culture-derived, oil-adjuvanted bacterin inoculated 2 times protected calves against experimental transthoracic challenge with *P. haemolytica* and markedly reduced sickness in an experimental feedlot trial. Effective prophylaxis requires that the first vaccination be done at the same time as blackleg vaccination, anytime between 2 months old and 1 month prior to weaning. The second vaccination should be done at weaning. Thus, the vaccination schedule for PASTEURELLA BACTERIN-TC is adaptable to present-day calf management practices of many parts of the world.

Summary

A tissue culture-derived *P. haemolytica* bacterin in oil adjuvant provided up to 100% protection in calves against experimental challenge inoculation of homologous bacteria. In a field study, vaccination reduced morbidity by 70% and reduced treatment days by 80%.

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