EVALUATION OF AN EXPERIMENTAL VACCINE FOR CONTROL OF BOVINE PNEUMONIA INDUCED BY PASTEURELLA HAEMOLYTICA

R. Newsham, T. Kaufman and K. Dayalu. SmithKline Beecham Animal Health, Lincoln, Nebraska, U.S.A.

Introduction

Among the members of the HAP group (Haemophilus, Actinobacillus, and Pasteurella) are bacteria of considerable importance in veterinary medicine. These, over the years, have caused considerable economic losses in cattle and swine herds. The pathogenic peculiarities in these bacteria require investigators to look beyond killed whole cell vaccines for the induction of the immune response. Compounding all of this is the fact that in many instances a strong immune response is not necessarily synonymous with protection.

P. haemolytica Type A-1, a conditional pathogen and normal inhabitant of the nasal cavity of clinically normal cattle, is the most frequent isolate from acute pneumonic pasteurellosis in cattle. Lesions formed by P. haemolytica are characterized by necrosis, vascular damage, fibrin exudation, numerous viable bacteria and variable leukocyte infiltrations. Virulence factors produced by P. haemolytica include; a) leukotoxin (LKT), a potent exotoxin which affects ruminant leukocyte populations; b) capsule, which inhibits complement mediated serum killing; c) lipopolysaccharide, which alters neutrophil function and stimulates leukocyte adherence; and d) fimbriae, which enhances adherence of the bacteria to the upper respiratory mucosa. Increasing knowledge of the growth conditions conducive to expression of virulence factors should help in the development of efficacious vaccines.

A vaccine has been developed which contains many of the virulence factors described above. This vaccine has been tested in a single dose regimen in many vaccination/artificial challenge experiments and has proven to be efficacious and safe. Experiments detailed in this manuscript are representative of results obtained for similar tests during the development of this vaccine.

Materials and Methods

Vaccine

An experimental vaccine that contains leukotoxoid, capsular antigen (Ag) and other cell associated as well as soluble antigens, was prepared using state-of-the-art fermentation technology. Upon assembly, the product was lyophilized. Rehydration of the vaccine was accomplished with an adjuvant-containing diluent.

Immunogenicity Study

Forty calves, weighing 400-550 lbs., received a single dose of vaccine by either intramuscular (IM) (n=20) or subcutaneous (SC) (n=20) route of administration. Ten additional calves were injected SC with a placebo containing sterile culture medium without P. haemolytica antigens. Animals were challenged by intratracheal administration two weeks post-vaccination with 1 x 10^9 colony forming units (cfu) of a heterologous strain of P. haemolytica Type A-1 contained in approximately 500 milliliters (ml) of saline. This system is similar to a challenge system for P. multocida in swine (1). Calves were monitored for febrile response to challenge. Animals which did not succumb to challenge were necropsied six days post-challenge. Lungs were removed and evaluated

for lesions characteristic of P. haemolytica infection. Evaluation included assigning percent involvement based on visual observation and the weights of lesion-bearing areas, and by microbial isolations. Serological analyses were performed using assays for agglutination of whole cells, LKT neutralization, and for antibodies (Ab) to capsular Ag.

Antigen (Ag) Extinction Study

To determine the level of vaccine which was not protective, forty calves were vaccinated by the SC route with one dose of vaccine at full dose (n=10), one-fourth dose (n=10), one-sixteenth dose (n=10), and one-sixty-fourth dose (n=10). Ten additional calves received a SC injection of placebo. Antigens were quantitated with the use of monoclonal antibodies to LKT (obtained from the University of Nebraska) (2) and to capsular Ag (obtained from the University of Minnesota) (3). Remaining steps were the same as described for the Immunogenicity Study.

Duration of Immunity Study

Ten calves were vaccinated by the SC route with one dose of vaccine. Ten more animals received a single dose of placebo. Animals were held four months on a ranch in western Nebraska.

Animals were transported to SmithKline Beecham animal facilities and challenged at four months, seven days post-vaccination. The procedure for challenge was the same as previously described, except the challenge inoculum was reduced to 1 x 10^7 cfu in 500 ml of saline. Calves were necropsied six days post-challenge, lungs evaluated, and serological analyses performed.

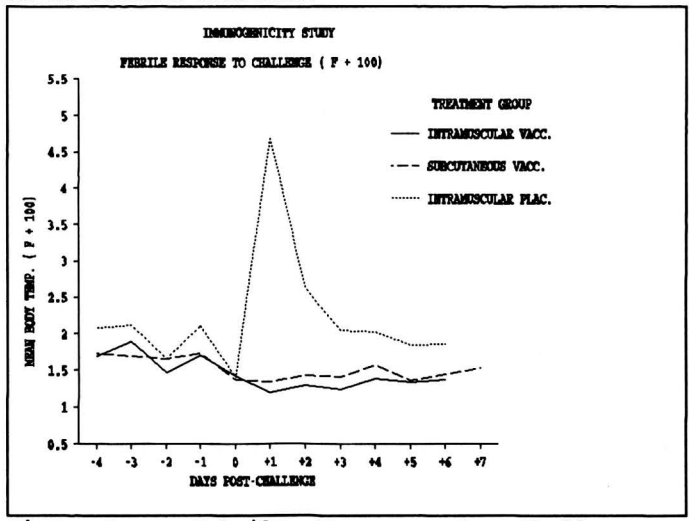

Figure 1. Febrile Response to Challenge - Immunogenicity study (p=0.05)

Results

Immunogenicity Study

Placebo animals exhibited a sharp increase in mean body temperature one day post-challenge, while mean body temperatures of vaccinated animals did not vary appreciably post-challenge (Figure 1). One SC vaccinate and one placebo control succumbed to challenge. The difference in percent lung consolidation between vaccinates and controls was significant, but not between IM and SC vaccinates (Table 1). The resulting reduction in percent lung consolidation was 94% for IM vaccinates, and 81% for SC vaccinates. P. haemolytica was isolated from

1/20 IM vaccinates, 3/20 SC vaccinates, and 6/10 controls. Table 2 displays the Geometric Mean Titers (GMTs) resulting from serological evaluation. Significant differences were observed between pre-vaccination and post-vaccination serum samples of the vaccinates, but not the controls, for all three serological assays.

Table 1. Percent Lung Consolidation Post-Challenge - Immunogenicity study

Treatment Group	*Percent Lung Consolidation (visual)	*Percent Lung Consolidation (weight)
IM Vaccinates	0.79[A]	1.46[A]
SC Vaccinates	4.09[A]	4.77[A]
IM Placebo Controls	19.35[B]	25.32[B]

* Statistically significant difference between A and B (p=0.05)

Table 2. Serological Responses to Vaccination and Challenge - Immunogenicity study

Treatment Group	Whole cell Agglutination GMT	Leukotoxin Neutralization GMT	Ab to Capsular Ag GMT
IM Vaccinates			
Pre-Vacc.	9.8	4.4	63.7
Post-Vacc.	132.5	10.9	414.1
Post-Chall.	34.3	8.9	414.1
SC Vaccinates			
Pre-Vacc.	9.8	4.8	36.6
Post-Vacc.	152.2	13.0	254.9
Post-Chall.	148.1	13.3	619.7
IM Placebo Controls			
Pre-Vacc.	9.2	6.1	107.2
Post-Vacc.	16.0	10.6	100.0
Post-Chall.	21.8	11.8	216.0

Antigen (Ag) Extinction Study

Placebo animals showed the greatest febrile response to challenge, followed in order by one-sixty fourth dose, full dose and one-sixteenth dose, and one-fourth dose (Figure 2). No animals succumbed to challenge in the two highest dosage groups, while in the two lowest dosage groups and controls, one, three, and four animals died, respectively. Percent lung consolidation, shown in Table 3, demonstrates percent reduction in lung damage of the vaccinates compared to the controls ranging from 87% (1/4 dose) to 20% (1/64 dose). P. haemolytica was isolated in 1/10 full-dose calves, 4/10 one-fourth dose calves, 6/10 one-sixteenth dose calves, 7/10 one-sixty-fourth dose calves, and 9/10 controls. GMTs determined by serological analysis indicated that agglutination and capsular Ag antibody titers responded in a dose-dependent manner, while LKT neutralization titers were highest in the one-fourth dose group.

Duration of Immunity Study

Although no animals succumbed to challenge, the placebo controls exhibited a significant febrile response to challenge (Figure 3), while the vaccinates showed no such response. Table 4 shows the percent lung consolidation, demonstrating a significant difference between vaccinates and controls. At four months post-vaccination, an 86% reduction in lung damage was realized in the vaccinates compared to the controls. P.

haemolytica was isolated from 4/10 vaccinates and 8/10 controls. Table 5 shows the serological responses at the time of vaccination, at 4 months post-vaccination, and post-challenge. LKT neutralization GMTs and antibody titers to capsular antigen of the vaccinates at 4 months post-vaccination were higher than pre-vaccination levels. GM agglutination titers of the vaccinates were identical at pre- and post-vaccination samplings. Controls showed no increase in GMTs at four months after receiving placebo. Initial attempts to monitor cell-mediated immunity (CMI) responses employing both non-specific mitogens and specific vaccinal antigens yielded promising results.

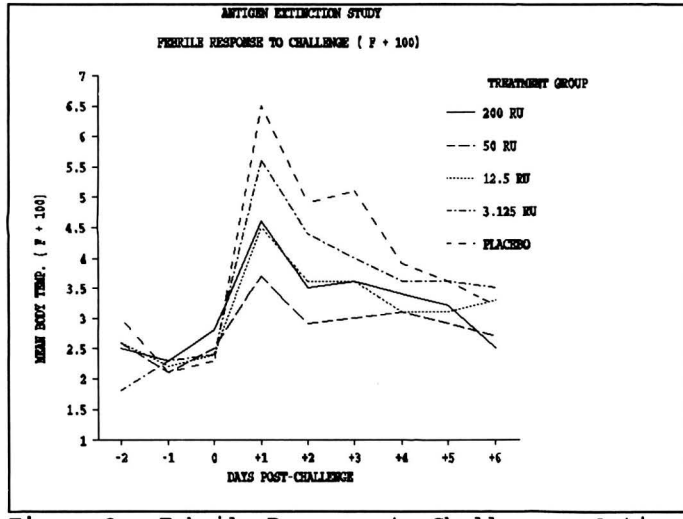

Figure 2. Febrile Response to Challenge - Antigen Extinction study

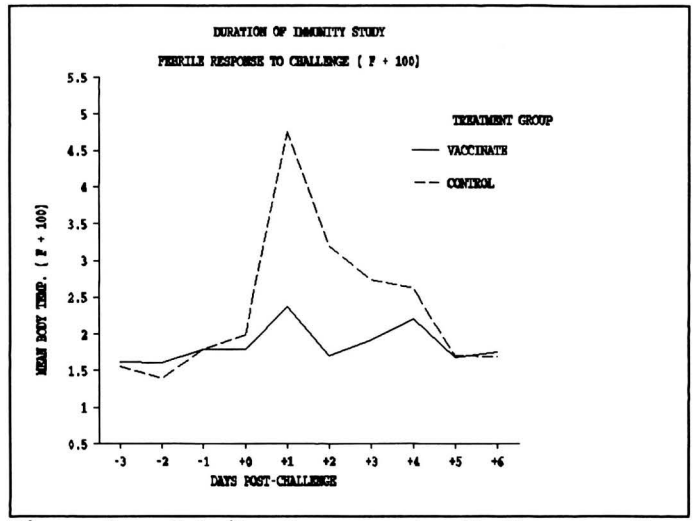

Figure 3. Febrile Response to Challenge - Four Month Duration of Immunity Study

Conclusions

In the studies mentioned in this paper, vaccination and challenge experiments involving a severe experimental challenge system proved the efficacy of the vaccine at two weeks and four months post-vaccination. While placebo controls exhibited a significant febrile response to challenge, vaccinated groups showed no such response. Mortality was reduced by vaccination with a full dose and with one-fourth dose. Percent reduction in lung consolidation was consistently within the 80-95% range. Perhaps most importantly, it

Table 3. Percent Lung Consolidation Post-Challenge - Ag Extinction study

Treatment Group	*Percent Lung Consolidation (visual)	*Percent Lung Consolidation (weight)
Full dose	11.1^{A}	15.6^{A}
1/4 dose	4.3^{A}	7.3^{A}
1/16 dose	19.1^{A}	25.4^{A}
1/64 dose	36.3	44.1
Placebo Controls	43.0^{B}	54.9^{B}

* Statistically significant difference between A and B (p=0.05).

Table 4. Percent Lung Consolidation Post-Challenge - Duration of Immunity study

Treatment Group	*Percent Lung Consolidation (visual)	*Percent Lung Consolidation (weight)
Vaccinate	1.68^{A}	2.65^{A}
Control	13.35^{B}	19.35^{B}

* Statistically significant difference between A and B (p=0.05)

Table 5. Serological Responses to Vaccination and Challenge - Duration of Immunity study

Treatment Group	Whole cell Agglutination GMT	Leukotoxin Neutralization GMT	Ab to Capsular Ag GMT
Vaccinate			
Pre-Vacc.	18.4	3.1	85.7
Post-Vacc.	18.4	6.7	373.2
Post-Chall.	45.3	6.5	800.0
Control			
Pre-Vacc.	13.0	4.8	81.2
Post-Vacc.	17.2	3.7	87.1
Post-Chall.	73.5	5.2	246.2

appears that one vaccination confers at least a four month duration of immunity. Re-isolation of P. haemolytica from challenged animals was significantly reduced in vaccinated animals compared to controls. Serological responses to vaccination were significant for all three parameters tested, but individual titers did not necessarily correlate with protection. LKT neutralization GMTs and antibody titers to capsular antigen were elevated in vaccinates at four months post-vaccination, a phenomenon not observed in control animals. CMI data indicates that it is a major player in the protective mechanism.

Summary - Resumen - Résumé

Pasteurella haemolytica Type A-1, a conditional pathogen and normal inhabitant of the nasal cavity of clinically normal cattle, is the most frequent isolate from acute pneumonic pasteurellosis in cattle. A vaccine has been developed which contains many of the virulence factors described for P. haemolytica. This vaccine has been tested in many vaccination/severe artificial challenge experiments and has proven to be efficacious and safe at two weeks and four months following a single dose administration. Percent reduction in lung consolidation was consistently within the 80-95% range. It appears that one vaccination confers at least a four month duration of immunity. Re-isolation of P. haemolytica from challenged animals was significantly reduced in vaccinated animals compared to controls. Serological responses to vaccination were significant for all three parameters tested, but

individual titers did not necessarily correlate with protection. Cell-mediated immunity data indicates that it is a major participant in the protective mechanism.

Pasteurella haemolytica tipo A-1, un patógeno condicional y habitante normal de la cavidad nasal del ganado bovino clínicamente normal, es el microorganismo aislado con mayor frecuencia en animales con pasteurelosis neumónica aguda. Se ha desarrollado una vacuna que contiene muchos de los factores de virulencia descritos para P. haemolytica. Esta vacuna se ha ensayado en muchos experimentos de vacunación y de agresión artificial severa, habiendo demostrado ser eficaz e inocua a las dos semanas y cuatro meses después de la administración de una sola dosis. El porcentaje de reducción de la consolidació pulmonar estuvo constantemente dentro de los valores del 80 al 95%. Aparentemente, una vacunación confiere inmunidad de por lo menos cuatro meses de duración. El índice de aislamientos posteriores de P. haemolytica a partir de animales agredidos se redujo significativamente en el ganado vacunado en comparación con los animales testigos. Las respuestas serológicas a la vacunación fueron significativas para los tres parámetros ensayados, pero los títulos individuales no se relacionaron, necesarialmente, con la protección. Los datos de inmunidad mediada por células indican que ésta es un factor important en el mecanismo protector.

Pasteurella haemolytica (Type A-1), agent conditionnellement pathogène et commensal normal des fosses nasales du bétail cliniquement normal, est l'organisme isolé le plus fréquemment au cours de la pasteurellose broncho-pulmonaire aiguë des bovidés. Un vaccin contenant de nombreux facteurs de virulence décrits pour P. haemolytica a été mis au point. Ce vaccin a été étudié au cours de nombreuses expériences de vaccination suivie de provocation artificielle sévère, et a fait preuve de son efficacité ainsi que de son innocuité à la deuxième semaine et au quatrième mois après l'administration d'une dose unique. Le pourcentage de réduction des condensations pulmonaires s'est échelonné entre 80 et 95%. Il semble qu'une seule vaccination conférerait une immunité d'une durée minimum de 4 mois. L'isolement ultérieur de P. haemolytica à partir des animaux volontairement exposés à l'agent pathogène a été réduit de facon significative par rapport aux témoins. Les résponses sérologiques à la vaccination ont été significatives en ce qui concerne les trois paramètres étudiés, sans que l'on ait pu observer nécessairement une corrélation entre les titres individuels et la protection. Les données portant sur l'immunité à médiation cellulaire indiquent que celle-ci joue un rôle important dans le mécanisme de la protection.

References

1. Hall, W., Bane, D., Kilroy, C., Essex-Sorlie, D. 1988. A model for the induction of pneumonia caused by Pasteurella multocida type A. In: Proceedings, 10th Congress International Pig Veterinary Society, p. 59.
2. Gentry, M., Srikumaran, S. 1991. Neutralizing monoclonal antibodies to Pasteurella haemolytica leukotoxin affinity-purify the toxin from crude culture supernatants. Microbial Path. 10:411-417.
3. Penaredondo, M., Maheswaran, S., Whiteley, L., Ames, T., Leininger, J. 1988. Production and partial characterization of monoclonal antibodies to Pasteurella haemolytica A1 capsular polysaccharide and lipopolysaccharide. Am. J. Vet. Res. 49(11):1962-1968.