Comparative Efficacy and Duration of Immunity of Commercial *Pasteurella haemolytica* Vaccines[1,2]

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Bovine respiratory disease complex (BRDC) continues to cause substantial losses in spite of improved understanding of the disease and the development of new vaccines (Loan *et al.*, 1992). Many viruses have been implicated in BRDC, several of which are more important than others (Rosenquist, 1984; Storz *et al.*, 1996). Stress is an important, albeit ill-defined, component of the complex (Babiuk and Acres, 1984). *Pasteurella haemolytica* (PHA1) is recognized as the most frequent bacterial pathogen in BRDC and the major cause of morbidity and mortality (Frank, 1979). Despite the very considerable defenses of the bovine lung (Loan, 1985) and recent advances for augmenting these defenses, BRDC is still the most important feedlot disease problem in North America. Also, because of the importance of PHA1 in the pathogenesis of BRDC, this pathogen continues to be a promising focus for immunoprophylaxis as part of effective health management programs for stocker-feeder calves.

There is convincing evidence of the efficacy of some of the newer PHA1 vaccines (Confer *et al.*, 1988; Loan and Purdy, 1996), however, only limited direct comparisons of efficacy have been made and duration of immunity studies are virtually nonexistent. In a limited evaluation of PHA1 vaccines, Cardella *et al.* (1987) demonstrated that live PHA1 vaccine administered intradermally and an oil-adjuvanted bacterin protected against experimental challenge of immunity. Aluminum hydroxide-adjuvanted vaccine did not provide similar protection.

Comparisons of some of the new generation commercially-available PHA1 vaccines were included in a statistical reanalysis (Srinand *et al.*, 1995) and a review of field efficacy (Perino and Hunsaker, 1996). These reevaluations of mostly individual reports of vaccine efficacy did not evaluate experimental and licensed vaccines separately, did not differentiate vaccine types within broad vaccine classifications and did not evaluate duration of immunity. More recently, Srinand *et al.* (1996) evaluated protection against experimental challenge of immunity induced by One Shot®, Presponse™ HM and Once PMH™. As determined in this latter study the vaccines "did not confer optimal protection," however, One Shot® stimulated the best protective immunity compared to the other two.

This report extends and broadens the comparative studies of PHA1 vaccine efficacy through duration of immunity studies. The data indicate superior efficacy of specific PHA1 bacterin-toxoids.

Materials and Methods

Calves: Two hundred-thirty seven nursing mixed-breed calves approximately 4 months old on native East Texas pasture were available for the study. The calves were identified by numbered tags in both ears and prescreened for PHA1 antibody by the cell associated agglutination test (CAAT) and cytotoxin neutralization (CN) test. One hundred-thirty nine calves with antibody titers of 16 or lower in both tests were assigned to the study. The calves were randomly assigned to groups 1 through 6 as shown in Table 1. For protection tests calves were selected by ascending ear tag number from those assigned to each group. There were no signs of respiratory disease in the herd. The calves were vaccinated and allowed to remain with their dams throughout the study.

Vaccination of calves: Calves were vaccinated according to label directions for each product used (Table 1). Groups 1 through 5 were vaccinated on day 0 (July 16, 1996). To synchronize the study, calves in group 4,

[1] From the Texas Agricultural Experiment Station and Texas A&M University, College Station, Texas (Loan) and American Animal Health, Inc., Grand Prairie, Texas (Tung, Payne).

[2] Products used in this study: One Shot® - Pfizer Animal Health, Lincoln, Nebraska (Pfizer); Once PMH™- Bayer Corporation, Shawnee Mission, Kansas (Bayer); Presponse™ HM - Fort Dodge Animal Health, Fort Dodge, Iowa (American Home); LeukoTox™ M and LeukoTox™ 1 - American Animal Health, Grand Prairie, Texas (AAH). Pulmo-guard™ PH-M and Pulmo-guard™ PH-1 are trademarks of Boehringer Ingelheim (BI), St. Joseph, Missouri for LeukoTox™ M and LeukoTox™ 1 respectively and are marketed by Boehringer Ingelheim.

Table 1. Distribution of eligible calves in six experimental groups.

Group	No. of Calves	Vaccine	Vaccine Serial No.	No. of Calves Challenged on Postvaccination Day Indicated*	
				83	97
1	24	One Shot® (Pfizer)	104034050 - vaccine 105446050 - dilutent	5	5
2	23	Once PMH™ (Bayer)	112025	5	5
3	23	Presponse™ HM (American Home)	376207A	5	5
4	23	LeukoTox™ M (AAH)** Pulmo-guard™ PH-M (BI)	42006	5	5
5	23	LeukoTox™ 1 (AAH) Pulmo-guard™ PH-1 (BI)	45007	5	5
6	23	None (Control)	None	5	5

* Protection tests were by transthoracic challenge of immunity
** Two vaccinations, first two weeks prior to other vaccinations

vaccinated with the two-dose product LeukoTox™ M, were given the first injection two weeks earlier (July 2, 1996). Calves in group 6 were nonvaccinated controls.

Serum samples: Serum samples were collected from all calves for prescreening antibody titer determinations on day 0 minus 42 (June 4, 1996). In addition, samples were obtained from groups 4 and 6 coincident with the first vaccination of group 4, (July 2, 1996) and from all vaccinated, revaccinated (group 4) and control calves on the day of synchronized vaccination (July 16, 1996). Samples were also collected on the day of challenge of immunity on postvaccination days 83 and 97. Serum samples from all remaining calves were collected on postvaccination day 132 in anticipation of challenge studies at 139 days postvaccination.

Serological tests: Antibodies measured by the cell associated agglutination test (CAAT) were determined by a modification of the method of Reggiardo (1979). Antibody dilution endpoints measured by the cytotoxin neutralization (CN) assay were determined microscopically (Fann, 1994) in a modification of the method of Greer and Shewen (1986).

Protection tests: At 83 days postvaccination (October 7, 1996), serum samples were collected for antibody determinations from 5 calves with the lowest sequential eartag numbers from each of the 6 groups. Each calf was injected transthoracically at that time with 5.0 ml of PHA1 challenge inoculum in each lung (10 ml per calf) according to the method of Panciera and Corstvet (1984). Again at 97 days postvaccination (October 21, 1996) the procedure was repeated with the next 5 sequentially numbered calves in each group. The scheduled challenge of immunity of all remaining calves on postvaccination day 139 was cancelled due to seroconversion to PHA1 in 3 of 8 remaining control calves.

Challenge inocula: The challenge inoculations were prepared from 20- to 24 hour cultures of PHA1 grown on blood agar. The bacteria were collected by washing the culture plates and diluting the resulting suspension to an optical density$_{630}$ of 1.08. The first challenge conducted on postvaccination day 83 was with PHA1 cultures washed and standardized in tryptose soy broth. The second challenge conducted on postvaccination day 97 was with PHA1 cultures washed and standardized with phosphate buffered saline. The challenge inocula were kept in an ice bath throughout each protection test procedure. Triplicate plate counts on blood agar were conducted pre- and post-challenge to quantitate colony forming units (cfu). The total challenge dose at 83 days postvaccination was 2.30×10^{10} at the beginning of the procedure; 2.20×10^{10} at the end (average 2.25×10^{10}). The total challenge dose at 97 days postvaccination was 2.50×10^{10} at the beginning of the procedure; 2.35×10^{10} at the end (average 2.43×10^{10} per calf).

Post Mortem Examination: All calves inoculated transthoracically were observed at least 2 times daily and clinical signs were recorded. The lungs of calves that died prior to day 4 post-challenge were removed and refrigerated. Surviving calves were euthanized 4 days post-challenge. Lungs of the surviving and dead calves were examined, swab samples of lesions were collected, and the volumes of the foci of infection were measured on day 4 post-challenge (Panciera and Corstvet, 1984). For statistical analyses, measurements of the foci of infection were used for comparisons.

Statistics: In protection studies, differences in mortality in vaccinates vs. nonvaccinates following challenge of immunity were analyzed for significance by chi square. For determining significance of lung lesions as indicators of protection, the combined lung lesion volumes of individual calves were ranked. These rankings were used to calculate probabilities for the groups associated with the U value in the Mann-Whitney test. Lung lesions of calves that died prior to termination of the study were assigned the highest rankings, since they were considered least protected.

Results and Discussion

This comparative study of duration of immunity involved vaccination of stocker-feeder calves with 5 commercially available PHA1 vaccines from 4 companies according to label directions (Table 1). This was followed by challenge of immunity approximately three months later, at the customary age for marketing and shipping calves to stocker operations or feedlots. Typi-

cally, clinical signs following challenge inoculation did not correlate well with lesion scores or subsequent deaths. Where clinical signs were observed in calves that subsequently died, depression was followed by labored breathing, recumbency and death within a few hours.

The results (Table 2) indicate significant protection of calves from mortality following vaccination with the bacterin-toxoids LeukoTox™ M and LeukoTox™ 1 at postvaccination day 83 ($p < 0.01$). Reduction in mortality following challenge of immunity on postvaccination day 97 appeared to be similar, but the survival of one control calf reduced the significance of this to $p< 0.10$. Nevertheless, combined, the above results indicate a 70 percentage point reduction in mortality attributable to the use of either product ($p < 0.005$). Significant protection ($p < 0.01$) was observed from Presponse™ HM on postvaccination day 83, but no protection was observed on postvaccination day 97. Combined, these results suggest a 40 percentage point reduction in mortality. One Shot® did not provide significant protection on either post-inoculation day 83 or 97. Combined, the results suggest that One Shot® may have reduced mortality by 30 percentage points. Once PMH™ gave no protection.

As determined by lung lesion scores, LeukoTox™ M, LeukoTox™ 1 and Presponse™ HM gave significant protection ($p=0.022$) at 83 days postvaccination compared to nonvaccinates. Results after challenge of immunity 97 days postvaccination also suggested protection resulting from vaccination with LeukoTox™ M ($p=0.075$), LeukoTox™ 1 ($p=0.075$) and One Shot® ($p=0.111$) although these results are not significant by the conventional standard ($p\#0.05$).

Table 2. Deaths and lung lesions following transthoracic challenge of immunity in vaccinated and nonvaccinated calves.

Vaccine	Protection of Calves 83 days postvaccination*		Protection of Calves 97 days postvaccination*	
	Dead Total	Geometric means of lung lesion volumes (p value**)	Dead Total	Geometric means of lung lesion volumes (p value**)
One Shot®	4/5	1848 (> 0.6)	2/5	474 (0.111)
Once PMH™	4/5	3584 (> 0.6)	5/5	540 (> 0.6)
Presponse™ HM	1/5†	460 (0.022)††	4/5	911 (0.5)
LeukoTox™ M (Pulmo-guard™ PH-M)	1/5†	455 (0.022)††	1/5	332 (0.075)
LeukoTox™ 1 (Pulmo-guard™ PH-1)	1/5†	819 (0.022)††	1/5	458 (0.075)
Control	5/5	5202	4/5	1436

* Transthoracic challenge of immunity; ** p value based on Mann-Whitney U tests of ranked lesion scores when compared to controls; † Significantly different ($p< 0.01$) from controls (chi square); †† Significantly different from controls.

The CAAT antibody titers at the levels observed were not associated with protection. Ten of the 50 vaccinated calves seroconverted (4-fold or greater increase in antibody titers) as measured by the CAAT. Of those that seroconverted, 5 died and 5 survived following challenge of immunity. Similarly, as regards CN antibody titers, 15 of the 50 vaccinated calves seroconverted. Following challenge of immunity, 5 of these died and 10 survived suggesting little or no difference in protection between those with or without CN antibody seroconversion. These observations, however, may not negate the value of these measures of immunity since protection and duration of immunity may depend upon a combination of these and other immune mechanisms.

None of the cohabiting control calves seroconverted during the 83 and 97 day postvaccination periods. In anticipation of the third scheduled challenge study on postvaccination day 139, and because of the high cost involved, serum samples were collected on day 132. At this sampling three of eight remaining control calves had seroconverted with 4-fold or greater increases in CN antibody. None of the control calves seroconverted in the CAAT. However, because of the possibility of PHA1 infection in the herd, although there were no clinical signs of such and there were no seroconversions indicated in the CAAT antibody titers, further protection tests were canceled.

The CAAT and CN antibody increases in vaccinated calves during the 83 and 96 day postvaccination period probably was the result of vaccination since cohabiting control calves did not seroconvert. However, the presence of these antibodies did not appear to be correlated with resistance in protection tests. As regards the apparent seroconversions after 96 days postvaccination, it is possible that as the calves grew older, *P. haemolytica* leukotoxin production by serotypes other than PHA1 contributed to increases in CN antibodies. *Pasteurella haemolytica* A2 and A6 are common inhabitants of the nasal and pharyngeal mucosa (Frank and Smith, 1983; Frank *et al.*, 1996) and are known to produce serologically cross-reacting cytotoxins. Also, since there is extensive homology between PHA1 leukotoxins and the alpha haemolysin of *Escherichia coli* (Strathdee and Lo, 1987), this latter could be a source of cross-reactive antibody-inducing antigen. In retrospect, an additional protection study at postvaccination day 139 or beyond might have yielded interesting results.

There is now general agreement that protective immunity to PHA1 is directed against multiple antigens of the bacteria (Confer *et al.*, 1988; McVey *et al.*, 1989). Bacterin-toxoids potentially are highly effective for delivering multiple protective antigens. In this study, the

Table 3. Serological responses of calves to vaccination.

Vaccine	Postvaccination / Prevaccination Ratio of CAAT titers* (Calves Seroconverting/Total†)		Postvaccination / Prevaccination Ratio of CN titers** (Calves Seroconverting/Total †)	
	83 days ††	97 days ††	83 days ††	97 days ††
One Shot®	3.48 (2/5)	1.52 (1/5)	2.00 (3/5)	1.15 (1/5)
Once PMH™	0.66 (0/5)	1.24 (1/5)	2.30 (2/5)	0.87 (0/5)
Presponse™ HM	0.87 (0/5)	1.15 (1/5)	2.64 (2/5)	1.52 (1/5)
LeukoTox™ M (Pulmo-guard™ PH-M)	2.64 (1/5)	1.33 (1/5)	1.99 (2/5)	2.00 (2/5)
LeukoTox™ 1 (Pulmo-guard™ PH-1)	2.00 (3/5)	0.66 (1/5)	5.29 (4/5)	1.52 (1/5)
Control	0.66 (0/5)	0.76 (0/5)	1.75 (0/5)	0.66 (0/5)

* Ratio of geometric mean titers determined by cell associated agglutination test (CAAT)
** Ratio of geometric mean titers determined by cytotoxin neutralization (CN)
† Calves developing 4-fold increase or greater in antibody titer compared to prevaccination titer
†† Days postvaccination

antigenic masses of two of the products, LeukoTox™ M and LeukoTox™ 1, were known and were similar. The equivalent performance of LeukoTox™ 1 (one-dose product) compared to LeukoTox™ M (two-dose product) from an equivalent amount of antigen in each dose may be due, in part, to the use of a proprietary potentiator. Overall, the study supports the concept that successful vaccination depends on the use of high-quality antigens, in sufficient quantity, with appropriate adjuvants, given at appropriate times. The ability of bacterin-toxoids to do this efficiently has been recognized in several certified calf programs that have stipulated bacterin-toxoids (Cornett 1994).

Summary

Nursing crossbred calves on range were assigned to 6 groups. Five of these groups were vaccinated with different commercially available *Pasteurella haemolytica* A1 (PHA1) vaccines. Calves in group 6 were not vaccinated and served as controls. At 83 and 97 days postvaccination the protection of 5 calves from each group was challenged by transthoracic inoculation of live, highly pathogenic PHA1. Based on survival of challenged calves and lung lesion size there were significant differences in protection compared to controls in one or more comparisons of LeukoTox™ M, LeukoTox™ 1 and Presponse™ HM. Overall, vaccination with two of the bacterin-toxoids, LeukoTox™ M and LeukoTox™ 1 (American Animal Health), resulted in 70 percentage point reductions in mortality. Reduction in mortality from Presponse™ HM (American Home) was 40 percentage points; from One Shot® (Pfizer), 30 percentage points. Once PMH™ (Bayer) did not protect. Lung lesions were significantly smaller in calves vaccinated with LeukoTox™ M, LeukoTox™ 1 and Presponse™ HM compared to controls. In general, lung lesion size paralleled mortality in protection tests. Antibody titers measured by cell-associated agglutination tests and cytotoxin neutralization assays were not strongly indicative of protection suggesting that other immune mechanisms also are involved in duration of immunity.

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