PEER REVIEWED

Onset of Serum Antibodies to *Pasteurella* (*Mannheimia*) *haemolytica* Following Vaccination with Five Commercial Vaccines

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Abstract

The purpose of this study was to compare the antibody responses of cattle vaccinated with one of five commercial non-living *P. haemolytica* vaccines during the first two weeks after a single vaccination. Antibodies to surface antigens (whole cells) and to the leukotoxin (LKT) were quantified at Days 7 and 14 after vaccination. In addition, antibodies to specific *P. haemolytica* whole cell protein antigens were determined by immunoblots using sera from vaccinated and control calves. Three hundred pre-weaned, mixed beef breed steers and heifers, greater than three months old, were equally divided among five vaccinated and one placebo groups. Cattle were subcutaneously injected with one of the following commercial P. *haemolytica* vaccines: One Shot®, One Shot Ultra™ 8, Pyramid® 4+Presponse SQ®, Pulmo-guard™ PH-M or Poly-Bac B® 1. Antibody responses to both LKT and whole cells were highest on Day 7 for One Shot and One Shot Ultra. By Day 14, Pulmo-Guard stimulated serum antibodies to both antigen preparations that were equivalent to those for One Shot and One Shot Ultra. Serum antibodies to LKT and whole cells were detected in the Pyramid/Presponse- and Poly-Bac 1 - vaccinated groups; however, those responses were not significantly different from the placebo group. Each vaccine, except for the Pyramid 4/ Presponse - vaccinated group, stimulated antibodies to similar *P. haemolytica* somatic proteins, particularly at 200, 100, 60-70, 45, 35 and 25 kDa. Sera from the Pyramid 4/Presponse-vaccinated group recognized fewer protein bands, mainly those at 200, 70-75, 48 and 35 kDa. In conclusion, various commercial vaccines induced differences in the rapidity and the intensity of serum antibody responses to *P. haemolytica* whole cells and LKT.

Résumé

Le but de cet étude était de comparer la réaction immunitaire du bétail vacciné avec cinq différents types de vaccins commerciaux avec *P. haemolytica* tué durant les deux premières semaines suivant une simple vaccination. Les anticorps aux antigènes de surface (cellules entières) et aux leucotoxines (LCT) ont été quantifiés au jour 7 et au jour 14 suivant la vaccination. De plus, les anticorps aux antigènes de protéines de cellules entières spécifiques à *P. haemolytica* ont été examinés par immunoblot à l'aide de sérum provenant de veaux vaccinés et contrôles. Un total de 300 taures et bouvillons d'origine hybride pré-sevrés et n'ayant pas plus de trois mois d'âge ont été divisés en cinq groupes vaccinés et un groupe contrôle. Les animaux ont été injectés sous-cutanés avec les vaccins commerciaux suivants contre *P. haemolytica* : One Shot, One Shot Ultra 8, Pyramid 4+Presponse SQ, Pulmo-guard PH-M ou Poly-Bac B 1. La réaction immunologique aux LCT et aux cellules entières était plus grande au jour 7 pour le One Shot et le One Shot Ultra. Au jour 14, Pulmo-guard a stimulé la production d'anticorps sériques aux deux types de préparation d'antigène au même niveau que le One Shot et le One Shot Ultra. Des anticorps sériques aux LCT et aux cellules entières ont été détectés dans les groupes vaccinés avec Pyramid/Presponse et Poly-Bac. Toutefois, ces

réactions immunologiques n'étaient pas différentes de celle du groupe contrôle. Chaque type de vaccin, excepté le vaccin Pyramid/Presponse, a stimulé la production d'anticorps aux protéines somatiques similaires de *P. haemolytica* particulièrement dans les bandes protéiques de 200, 100, 60-70, 45, 35 et 25 kDa. Les sérums provenant du groupe Pyramid/Presponse ont reconnu moins de bandes protéiques, principalement à 200, 70-75, 48 et 35 kDa. En conclusion, l'étude de différents types de vaccins commerciaux a mis en évidence des différences dans la rapidité et l'intensité de la production d'anticorps sériques aux cellules entières et aux LCT de *P. haemolytica*.

Introduction

Pasteurella haemolytica (recently renamed *Mannheimia haemolytica*) is an important bacterial pathogen associated with shipping fever (pneumonic pasteurellosis) in cattle.[14] *P. haemolytica* A1 is the major bacterium responsible for severe pneumonia and economic losses in shipping fever.[45] The bacterium contains numerous virulence factors or potential virulence factors, including leukotoxin (LKT), lipopolysaccharide, neuraminidase, capsular polysaccharide, iron-regulated outer membrane proteins and sialoglycoprotease.[4,5,38] External stress factors such as shipping, weaning, inclement weather and viral infections enhance colonization of the respiratory tract with *P. haemolytica*. Replication of *P. haemolytica* in the lung with elaboration of several of these virulence factors leads to production of severe fibrinous pleuropneumonia.[14,38]

In previous studies, it appeared that immunity to *P. haemolytica* required serum antibody to LKT and to surface antigens.[40] The specific important surface antigens have not been determined with surety; however, outer membrane proteins, capsular polysaccharide and lipopolysaccharide have been investigated.[11-13,23,26,27] Antibodies to surface antigens are usually detected either by an enzyme-linked immunosorbent assay (ELISA) against formalin-fixed whole *P. haemolytica* or various purified antigens, or with serum agglutination assays.[9,15,40] Antibodies to LKT can be detected by LKT serum neutralization assays using bovine leukocytes as target cells or by ELISA using highly purified LKT as the antigen.[8,9,40,41]

Currently available *P. haemolytica* vaccines vary in composition. These include bacterins, bacterins with LKT, bacterial extracts, culture supernatants containing LKT, or a live streptomycin-dependent mutant.[20,41] Published studies have demonstrated that vaccination with commercial and experimental vaccines stimulate serum antibody responses to several *P. haemolytica* antigens, and vaccination usually significantly enhances resistance to experimental *P. haemolytica* challenge.[7,20,23,25,36,40,41] Field efficacy of *P. haemolytica* vaccines is often more difficult to demonstrate, and published results of field trials have often demonstrated responses to vaccination that varied from negative, no or positive effects on respiratory disease or production.[1,2,19,22,34,37,43,44]

It has been theorized that cattle would be better protected against shipping fever when vaccinated prior to shipment to allow for maximum antibody responses to develop and, therefore, to provide maximum protection during and after shipment.[9,40] In fact, several studies have demonstrated that cattle that have high serum antibodies to *P. haemolytica* upon entry into a feedlot usually have less morbidity and mortality than do those that have low serum antibodies.[15,42] However, *P. haemolytica* vaccines are often given upon entry into a feedlot. Most experimental and field studies have demonstrated enhanced serum antibody responses and immunity in cattle within a few weeks following *P. haemolytica* vaccination.[5] In a recent study, cattle vaccinated with one of several commercial *P. haemolytica* vaccines developed serum antibodies to surface antigens and to LKT with maximum responses between seven and 14 days after vaccination.[9] Those antibody responses, however, had frequently returned to pre-vaccination values between four and six weeks after vaccination. This led to the conclusion that for maximum protection against *P. haemolytica,* cattle should be vaccinated within two-to-three weeks before shipment to assure optimal antibodies at the time of arrival in a feedlot. In that previous study, several vaccines were given twice, whereas many of the current *P. haemolytica* vaccines are licensed for only a single dose.

From experimental and field data, it appears desirable for cattle to enter feedlots with pre-existing antibodies to *P. haemolytica* surface antigens and LKT, and *P. haemolytica* vaccines should induce rapid antibody responses whether cattle are vaccinated prior to shipment or upon entry into a feedlot. Therefore, this study was undertaken to compare the antibody responses during the first two weeks after a single vaccination for cattle vaccinated with one of five commercial, non-living *P. haemolytica* vaccines. Antibodies to surface antigens and to the LKT were quantified at Days 7 and 14 after vaccination using ELISAs. Antibodies to specific *P. haemolytica* whole cell protein antigens were determined by immunoblots using sera from vaccinated and control calves.

Materials and Methods

Cattle. Three hundred pre-weaned, mixed beef breed steers and heifers, greater than three months old and in good health, were used. Cattle were from a single source and were born, raised and housed on one pas-

ture in one location in Breien, Sioux County, North Dakota. All cattle were provided water *ad libitum* and a protein supplement. Cattle had not received any previous vaccinations for *P. haemolytica*.

Vaccines. Five commercial *P. haemolytica* vaccines were used (Table 1). These were: *P. haemolytica* bacterin-toxoid (One Shot®)[a], clostridia-*P. haemolytica* bacterin-toxoid (One Shot Ultra™ 8)[a], *P. haemolytica* toxoid, IBR-BVD-PI_3-BRSV vaccine (Pyramid® 4+Presponse SQ®)[b], *P. haemolytica-multocida* bacterin-toxoid (Pulmo-guard™ PH-M)[c], and *P. haemolytica-multocida-Salmonella typhimurium* bacterin-toxoid (Poly-Bac B® 1)[d]. The presence of viral or bacterial antigens other than *P. haemolytica* in four of the vaccines should not affect the calves' responses to *P. haemolytica* antigens, because USDA licensing requires demonstration that the addition of other vaccine components does not negatively affect vaccine safety or efficacy.

Serology. Antibodies to *P. haemolytica* whole cells and to LKT were determined by enzyme-linked immunosorbent assays (ELISAs).[8,9] The *P. haemolytica* A1 strain used for antigen preparation was originally isolated from a feedlot calf.[30] Formalinized *P. haemolytica* was prepared from a washed 24-hour culture by suspending cells in 0.4% formalinized saline at a concentration determined spectrophotometrically to be 1.850 OD_{650}. LKT was prepared from culture supernatant from a 3-hour culture of *P. haemolytica* A1 grown in RPMI-1640 medium at 99.6°F (37°C) in a shaking incubator. The LKT was partially purified by precipitation with 40-60% ammonium sulfate as previously described.[3] The precipitate was re-suspended in 3M guanidine containing 59 mM $NaHPO_4$ and 100 mM NaCl. By SDS-PAGE of the LKT preparation, one intensely staining band was identified at 105 kDa and confirmed to be LKT on a western blot using an anti-LKT monoclonal antibody.[9] Leukotoxic activity was 10^4 LKT Units per ml.[3] The 2-keto-3-deoxyoctonate concentration was 7.5 μg per mg of protein.[29] Wells of 96-well microtiter plates were coated with whole cells at an optical density reading equivalent to 10^8 CFU of a 24-hour culture or with LKT at 50 ng per well. Sera were diluted in PBS-Tween 20 containing 1% BSA and tested at dilutions of 1:800 for whole cells and 1:1600 for LKT. The extent of antibody binding was detected using a 1:400 dilution of horseradish peroxidase-conjugated, affinity purified rabbit anti-bovine IgG. Antibody responses are expressed as ng of immunoglobulin binding using a set of immunoglobulin standards.

Table 1. Experimental design.

Vaccine	No. of cattle	Days of antibody testing
Placebo	50	0, 7, & 14
One Shot	50	0, 7, & 14
One Shot Ultra	50	0, 7, & 14
Pyramid 4/Presponse	50	0, 7, & 14
Pulmo-guard PH-M	50	0, 7, & 14
Poly-Bac B 1	50	0, 7, & 14

[a]Pfizer Animal Health, Exton, PA 19341
[b]Fort Dodge Animal Health, Fort Dodge, IA 50501
[c]Boehringer Ingelheim Vetmedica, St. Joseph, MO 64506
[d]Texas Vet Labs, San Angelo, TX 76903
[e]Sigma Co., St. Louis, MO 63178

Electrophoresis and immunoblot analysis. To compare the antigens to which each vaccine stimulated an antibody response, *P. haemolytica* whole cells were equilibrated to 1 mg protein/ml and subjected to discontinuous SDS-PAGE. Proteins were transferred to nitrocellulose membranes.[27] Antigens were identified immunologically, using sera (1:25 dilution) from three calves in each of the vaccine and placebo groups. Immunoglobulins binding to specific antigens were detected with horseradish peroxidase-conjugated, affinity-purified rabbit anti-bovine IgG[e]; hydrogen peroxide; and a color reagent.[26]

Experimental design. Sera were collected from each calf 14 days prior to vaccination, and antibodies to *P. haemolytica* whole cells measured by ELISA. All 300 calves were randomly allotted to six groups of 50 calves each, using a randomized complete block design with blocking based on serum antibodies to *P. haemolytica* whole cells.

On Day 0, 250 cattle were vaccinated subcutaneously on the right side of the neck with one of five commercial *P. haemolytica* vaccines. One group of 50 cattle was vaccinated with sterile diluent and served as a negative control (placebo). All calves were evaluated for post-vaccination systemic reactions and clinically evaluated at each time point in the 14-day experiment. Cattle that were judged to be sick were treated appropriately by a licensed veterinarian.

Blood samples were collected, and sera removed and stored from all cattle on Days 0, 7 and 14. Each serum sample was tested for anti-*P. haemolytica* whole cell and LKT antibodies by ELISA.

Statistics. Antibodies to *P. haemolytica* whole cells and LKT were analyzed using a general linear repeated measures mixed model with fixed effect terms treatment, study day, and treatment by study day interaction.[36] Antibodies were log-transformed prior to analysis. Contrasts among treatment groups on each study day were made after detecting a significant ($p \leq 0.05$) treatment or treatment by study day interaction. The 0.05 level of significance was used to determine statistical significance. A 95% confidence interval was calculated for each

treatment group at each study day. The least squares means and 95% confidence intervals were back transformed for presentation.

Results

Clinical evaluation. No adverse reactions were noted after the administration of either the placebo or the experimental vaccines. A total of 10 calves (3.33% of total) were assessed to be clinically abnormal on days 0, 7 or 14. Between one and two cattle per vaccine group were treated. Clinical signs consisted of depression (n = 2), lameness (n = 6), diarrhea (n = 1) or otitis (n = 1). Cattle were treated with a long-acting antibiotic and returned to pasture. In addition on Day 14, one calf vaccinated with Pyramid 4 / Presponse developed an abscess in the area of the injection. That abscess was lanced, and recovery was uneventful.

Serological responses. Mean serum antibodies to *P. haemolytica* whole cells as determined 14 days prior to vaccination were similar for each vaccine group. Mean immunoglobulin values ranged from 0.309 ± 0.276 (standard deviation) ng for the Pyramid 4/Presponse group to 0.316 ± 0.293 ng for the One Shot Ultra group.

Antibodies to *P. haemolytica* whole cells were determined on Days 0, 7, and 14 for all treatment groups. On Day 0, there were no significant differences ($p > 0.05$) in serum antibodies to *P. haemolytica* whole cells among the vaccine groups (Figure 1). Subsequently, there were no significant changes ($p > 0.05$) in serum antibodies to *P. haemolytica* whole cells for the placebo group on any day of the study. For the One Shot and One Shot Ultra groups, antibody responses on Days 7 significantly ($p < 0.05$) increased over their Day 0 antibody values and were greater than the antibody responses for any of the other groups (Figure 1). Antibody responses for the One Shot and One Shot Ultra groups increased on Day 14 and were significantly greater than were the responses for placebo, Pyramid 4 / Presponse, and Poly-Bac B 1 groups. On Days 7 and 14, the antibody responses to *P. haemolytica* whole cells for the Pyramid 4 / Presponse treatment group were numerically increased, but those responses were not statistically different from the values for the placebo group. For the Pulmo-guard PH-M treatment group, the antibody response on Day 7 was not significantly different from that for the placebo group. The antibody response for the Pulmo-guard PH-M group, however, increased significantly ($p < 0.05$) on Day 14 such that there were no statistical differences in responses among the One Shot, One Shot Ultra, and Pulmo-guard PH-M groups. Although the antibody responses to *P. haemolytica* whole cells increased from Day 0 to Day 14 for Poly-Bac B 1, those responses were not significantly different from the responses of the placebo group.

There were no significant differences in mean antibody responses to LKT among the treatment groups on Day 0 (Figure 2). The mean antibody response for the placebo group did not change significantly ($p > 0.05$) during the study (Figure 2). On Days 7 and 14, the antibody responses to LKT for the One Shot and One Shot Ultra groups increased significantly ($p < 0.05$) from the Day 0 values and were the highest antibody responses among the vaccine groups. The responses of the anti-LKT antibody responses for the One Shot and One Shot

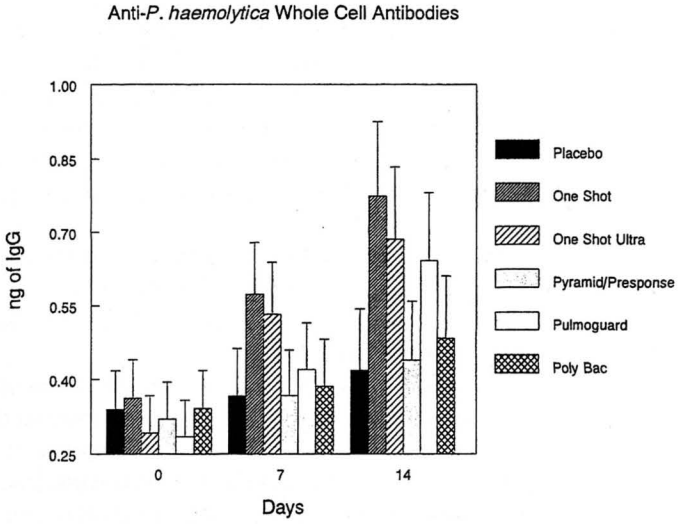

Figure 1. Serum antibody responses of calves to *P. haemolytica* whole cells after vaccination with commercial vaccines. Each point represents the least-squares mean. Error bars represent the 95% confidence interval.

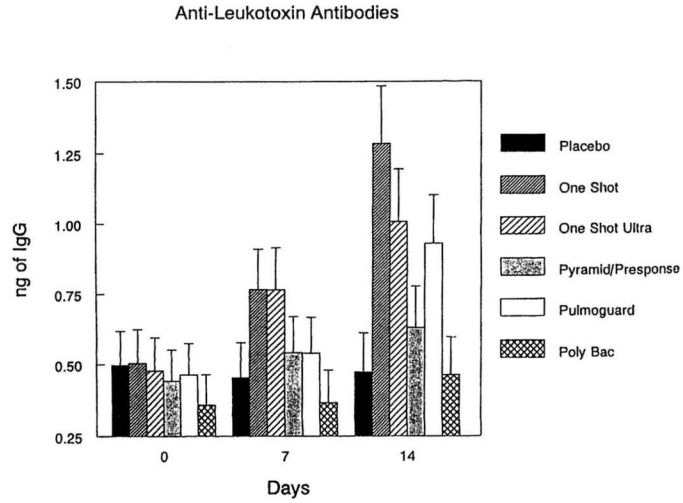

Figure 2. Serum antibody responses of calves to *P. haemolytica* leukotoxin after vaccination with commercial vaccines. Each point represents the least-squares mean. Error bars represent the 95% confidence interval.

Ultra groups were statistically greater ($p < 0.05$) than those for the placebo group on Day 7 and Day 14, and for all other groups on Day 7. On Day 14, the antibody response for One Shot was significantly higher than the response for One Shot Ultra. The mean antibody responses to LKT increased for the cattle vaccinated with Pyramid 4 / Presponse; however, those responses were not significantly different from the responses of the placebo group on any day of the study. Vaccination with Pulmo-guard PH-M stimulated increased antibody responses throughout the study. Those responses were not significantly different ($p > 0.05$) from the placebo group until Day 14, when the Pulmo-guard PH-M group response was significantly different ($p < 0.05$) from the response of the placebo group and similar to that for the One Shot Ultra group. The antibody response to LKT for the Poly-Bac B 1 group post-vaccination was not statistically different ($p > 0.05$) from that for the placebo group on any day of the study.

Immunoblots. Immunoblots against *P. haemolytica* whole cells and culture supernatant were examined using sera from Days 0, 7, and 14 from three calves from each of the vaccine and placebo groups. Sera were selected from three calves in the placebo group that had spontaneously developed antibody responses to *P. haemolytica* of >0.5 ng of immunoglobulin. Therefore, the antigens recognized by vaccine-induced antibody responses could be compared to those recognized by serum from cattle with a naturally induced antibody response.

Only a few sera collected at Day 0 recognized an occasional protein band from *P. haemolytica* whole cells. Those samples were randomly distributed among the groups (data not shown). For sera collected on Day 7, only those from One Shot- and One Shot Ultra-vaccinated cattle recognized numerous protein bands in *P. haemolytica* whole cells. For Day 14 samples, two placebo cattle and cattle from each vaccinated group recognized multiple protein antigens in the *P. haemolytica* whole cell preparation (Figure 3). Each vaccine, except the Pyramid 4/Presponse-vaccinated group, stimulated antibodies to similar major *P. haemolytica* proteins, particularly at 200, 100, 60-70, 45, 35 and 25 kDa. Sera from a few cattle in the placebo group that developed antibodies naturally recognized similar antigens. Sera from the Pyramid 4/Presponse-vaccinated group recognized fewer protein bands, mainly those at 200, 70-75, 48 and 35 kDa.

Discussion

Correlations between antibody responses to certain *P. haemolytica* antigens and enhanced resistance to experimental challenge have been demonstrated.[7,10,11,16,24,26,27] A strong correlation between LKT-neutralization antibodies and resistance to experimental challenge has been demonstrated.[16,24,27] The LKT-neutralization assay is labor-intensive and can give variable results because it uses live bovine leukocytes. ELISA assays to purified LKT have been adapted for use in studies that involve large numbers of cattle or numerous sampling points.[8,41] Studies in our laboratory demonstrated a highly significant ($p < 0.01$) correlation between results of the ELISA and LKT-neutralization assays (Confer and Clinkenbeard, 1997, unpublished data).[24] Therefore, use of the ELISA for detection of antibodies to LKT, although not a functional antibody assay like an LKT-neutralization, produces valid results that can serve as a potential indicator of immunity against *P. haemolytica*.

The surface antigens of importance in stimulating immunity to *P. haemolytica* have not been determined with surety. Antibody responses to several outer membrane protein antigens have correlated with or stimulated resistance to *P. haemolytica*,[6,23,27,35] whereas antibodies to lipopolysaccharide and capsular polysaccharides have either not correlated or inconsistently cor-

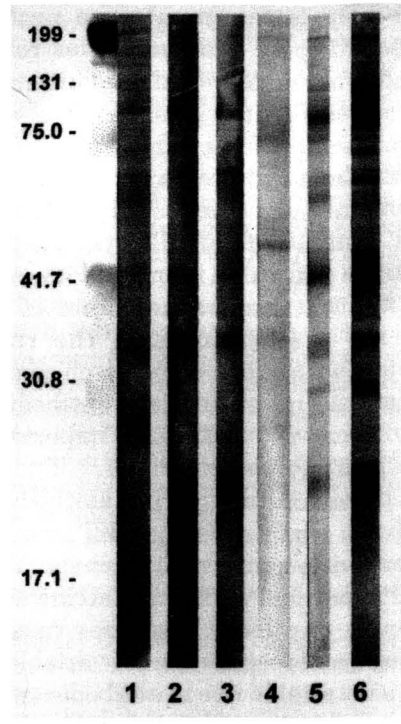

Figure 3. Immunoblots against *P. haemolytica* whole cells using sera from representatives of each vaccine group. The sample from the placebo group was selected because it developed antibodies to *P. haemolytica* spontaneously. Lane 1 = placebo, lane 2 = One Shot, lane 3 = One Shot Ultra, lane 4 = Pyramid 4/Presponse, lane 5 = Pulmo-guard PH-M, lane 6 = Poly-Bac B 1. The numbers to the left of lane 1 represent molecular weights in kiloDaltons.

related with resistance.[11-13,28] Use of formalin-killed *P. haemolytica* whole cells as the antigen of choice for detecting anti-surface antigens has been reported previously using indirect bacterial agglutination, quantitative fluorometric or ELISA assays.[9,17,18,27,31,39,40] In several studies, correlations between anti-whole cell antibodies and resistance to experimental challenge were demonstrated.[10,27,31] Therefore, the use of an ELISA against *P. haemolytica* whole cells is a valid method for measuring antibodies to surface antigens. It is especially useful when examining sera from large numbers of cattle with repeated measures.

As many as 21 surface-exposed, immunogenic *P. haemolytica* outer membrane proteins, ranging in size from 23 to 90 kDa, were recently identified.[33] Previously, Mosier *et al*[27] demonstrated significant correlations between resistance to experimental challenge and antibodies to *P. haemolytica* surface protein antigens of 86, 66, 51, 49, 34, 31 and 16kDa. Mahasreshti *et al*[21] demonstrated that antibodies to a heat-modifiable 32-35 kDa outer membrane protein of *P. haemolytica* correlated with resistance to experimental challenge. Pandher *et al*[32] identified an immunogenic, surface exposed 45-kDa outer membrane protein from *P. haemolytica* (PlpE), and antibodies to that protein stimulated complement–mediated killing of the bacteria. In the present study, immunoblots with sera from cattle vaccinated with the various vaccines induced antibodies in whole cell preparations that were equivalent to several surface exposed antigens previously identified. Only Pyramid 4/Presponse identified a different antigen pattern in immunoblots. Although challenge of the cattle was not done in the present experiment, if one compares the results of the immunoblots with previous research communications, the current vaccines stimulate antibodies to several surface antigens that are likely important for immunity to respiratory pasteurellosis.

In a previous study, serum antibody responses to *P. haemolytica* whole cells and LKT were induced with several non-living commercial vaccines.[9] That study was conducted in parallel with viral vaccine administration for all groups. Antibody responses varied among the vaccines used and responses were variable among cattle such that a detectable rise in antibodies was often short lived. Those data suggested that if cattle were to be vaccinated with a *P. haemolytica* vaccine prior to shipment, vaccines should be given within two-to-three weeks of shipment to maximize antibodies at the time of shipment stress. The data reported herein was designed to compare several commercial *P. haemolytica* vaccines to determine which vaccines might induce the most rapid response so that it could be used prior to shipment or at the time of shipment of cattle. Overall, on Day 7 only One Shot and One Shot Ultra induced the significant antibody responses to both whole cells and LKT. By Day 14, each vaccine had stimulated measurable antibody responses to *P. haemolytica* whole cells; however, the responses induced by One Shot and One Shot Ultra remained the greatest. Poly-Bac 1 failed to stimulate detectable antibodies to LKT.

Of the commercial vaccines studied herein, Presponse has been used the most in previously published field trials. Results of those studies, which used two doses of vaccine, indicated either enhanced resistance to respiratory disease and/or increased economic gain or no effects.[1,2,19,22,38] Those studies were conducted prior to the approval for one-dose administration of Presponse, and there were no evaluations of serum antibody responses to *P. haemolytica* antigens. In the present study, antibody response to LKT for Presponse-vaccinated calves was lower than in two previously published experiments, wherein Presponse-vaccinated cattle had significant antibody responses to LKT on Day 14 after initial vaccination, compared to non-vaccinated controls and to Day 0 values.[9] In another study wherein two doses of Presponse were given 21 days apart, significant antibody responses to surface antigens and to LKT were not detected until between Days 21 and 49.[39] In recent studies by Hodgins and Shewen,[17,18] colostrum-derived Holstein calves were vaccinated twice with Presponse at two and four weeks or at six and eight weeks of age. Serum LKT-neutralizing antibody responses were low in the cattle vaccinated at two and four weeks. Higher responses were seen in calves vaccinated at six and eight weeks. In that previous study,[9] cattle were older (> six months of age) compared to the cattle used in the present study. Therefore, it is possible that the differences seen in the present study and that of the previous one[9] could be accounted for by age differences of cattle between the two studies.

Conclusions

Various commercial vaccines induced differences in the rapidity and the intensity of serum antibody responses to *P. haemolytica* whole cells and LKT. One Shot and One Shot Ultra stimulated the highest responses on Day 7 to both whole cells, LKT and to a number of specific surface antigens identified by immunoblots. Pulmo-guard stimulated similar responses by Day 14. Therefore, one of these vaccines should be given consideration for use in cattle immediately prior to shipping or upon entry to a feedyard when rapid onset of immunity is desired. However, antibody data alone is not necessarily a fair measure of how a vaccine will perform in the field. We firmly believe that there is a need for well-controlled field studies to compare efficacy of *P. haemolytica* vaccines.

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