Effects of various adjuvants on efficacy of a vaccine against Streptococcus bovis and Lactobacillus spp in cattle

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Objective—To determine efficacy of vaccines incorporating QuilA, alum, dextran combined with mineral oil, or Freund adjuvant for immunization of feedlot cattle against Streptococcus bovis and Lactobacillus spp.

Animals—24 steers housed under feedlot conditions.

Procedure—Steers were randomly assigned to 4 experimental groups and a control group. Animals in experimental groups were inoculated on days 0 and 26 with vaccines containing Freund adjuvant (FCA), QuilA, dextran combined with mineral oil (Dex), or alum as adjuvant. Serum anti-S bovis and anti-Lactobacillus IgG concentrations were measured, along with fecal pH, ruminal fluid pH, and number of S bovis and Lactobacillus spp in ruminal fluid.

Results—Throughout the study, serum anti-S bovis and anti-Lactobacillus IgG concentrations for animals in the Dex, QuilA, and alum groups were similar to or significantly higher than concentrations for animals in the FCA group. Serum anti-S bovis and anti-Lactobacillus IgG concentrations were significantly increased on days 26 through 75 in all 4 experimental groups, and there was a linear relationship between anti-S bovis and anti-Lactobacillus IgG concentrations. For animals in the QuilA and Dex groups, mean pH of feces throughout the period of experiment were significantly higher and numbers of S bovis and Lactobacillus spp in ruminal fluid on day 47 were significantly lower than values for control cattle.

Conclusions and Clinical Relevance—Results suggest that immunization of feedlot steers against S bovis and Lactobacillus spp with vaccines incorporating Freund adjuvant, QuilA, dextran, or alum as an adjuvant effectively induced high, long-lasting serum anti-S bovis and anti-Lactobacillus IgG concentrations. Of the adjuvants tested, dextran may be the most effective. (Am J Vet Res 2000;61:839–843)

One of the major digestive disorders faced by the beef cattle feedlot industry is lactic acidosis, which can adversely affect animal production by reducing growth rate and increasing mortality rate. Lactic acidosis results from accumulation of lactic acid produced by Streptococcus bovis and Lactobacillus organisms in the rumen. Several feed additives have been used in conjunction with management practices to control lactic acidosis. At present, however, none of these feed additives are entirely satisfactory for use in feedlot cattle. For example, although antibiotics are effective in preventing lactic acidosis by reducing the numbers of S bovis and Lactobacillus spp, their use is limited because of practical considerations related to emergence of drug-resistant microbial strains and the risk of antibiotic residues in animal products.

Gnanasampanthan has demonstrated the feasibility of using immunization to control rumen protozoa and has suggested that inducing a saliva antibody response is critical for establishing immunologic control of rumen microorganisms. In addition, he found that specific saliva antibodies could survive the proteolytic activity in the rumen fluid. Another study found that immunization could potentially protect sheep from lactic acidosis. Shu et al have shown that immunization against S bovis and Lactobacillus spp resulted in detectable saliva and serum IgG responses and protected cattle from lactic acidosis and suggested that the serum antibody concentration could be used as an indicator of the saliva antibody response.

In these studies, animals were immunized before being introduced to a grain-based diet. Freund complete adjuvant was used in the vaccine used for primary immunization, and Freund incomplete adjuvant was used in the vaccine used for booster immunization. The purpose of the study reported here was to determine efficacy of vaccines incorporating QuilA, alum, dextran combined with mineral oil, or Freund adjuvant in preventing lactic acidosis in feedlot cattle. Vaccine efficacy was assessed by measuring serum IgG response and changes in pH of rumen fluid, pH of feces, body weight, and numbers of S bovis and Lactobacillus spp in rumen fluid.

Materials and Methods

Vaccine preparation—Streptococcus bovis strain Sh-5 and Lactobacillus spp isolate LB-27, isolated from ruminal contents of cattle on grain-based diets, were used for preparing vaccines. The Sh-5 isolate was a pure strain of S bovis, whereas the LB-27 isolate was determined in preliminary studies to be a mixture of Lactobacillus spp. Also, in preliminary studies, Sh-5 and LB-27 were found to cross-react with other S bovis and Lactobacillus strains.

Bacterial isolates were grown and processed for vaccine preparation as described. The final bacterial suspension used for vaccine preparation contained 2 × 10^10 Sh-5 cells and 1 × 10^10 LB-27 cells/2.5 ml. Each vaccine batch was prepared with 17.5 ml of this bacterial suspension and a different adju-
vant. For the vaccine incorporating Freund adjuvant, 17.5 ml of the bacterial suspension was emulsified with an equal volume of Freund complete adjuvant for the primary immunization and with an equal volume of Freund incomplete adjuvant for the booster immunization. For the vaccine incorporating the QuilA adjuvant, 7 mg of QuilA was dissolved in 17.5 ml of sterile phosphate-buffered saline solution, and 17.5 ml of the bacterial suspension was added in drops over a period of 5 minutes while the solution was vigorously mixed. The solution was mixed for another 20 minutes after addition of the bacterial suspension. For the vaccine incorporating dextran as the adjuvant, 14.5 ml of a suspension of Sb-5 cells was centrifuged at 10,000 g for 20 minutes, and 12.25 ml of the supernatant was removed. The pellet was resuspended by adding 3.5 ml of a pension of Sb-5 cells was centrifuged at 10,000 g for 20 minutes, and 12.25 ml of the supernatant was removed. The pellet was resuspended by adding 3.5 ml of a suspension of the LB-27 cells. The solution was mixed thoroughly, and 5.23 ml of 20% DEAE-dextran solution (pH 7.5) was added. This mixture was then emulsified with 24.3 ml of mineral oil. For the vaccine incorporating alum as the adjuvant, 8.75 ml of an alum adjuvant was mixed well with 8.75 ml of sterile phosphate-buffered saline solution, and 17.5 ml of the bacterial solution was added in drops over a period of 5 minutes while the solution was vigorously mixed. The solution was mixed for another 30 minutes after addition of the bacterial suspension.

Animals and immunization procedures—Twenty-four 1-year-old Angus steers were used in the study. Steers had been adapted to a finishing ration (75% barley, 10% Lucerne hay, 8% molos, 5% protein meal, 1% limestone, 0.5% bicarbonate, and 0.3% ammonium sulfate) prior to the study. They were penned together under feedlot conditions and had access to water at all times. Animals were fed in the morning (8 AM) and afternoon (4 PM) and were fed the same ration throughout the study. The trial was conducted from Nov 1995 through May 1996. Animals were randomly assigned to 1 of 5 groups consisting of 4 experimental groups (n = 5/group) and a control group (4). Animals in the 4 experimental groups were immunized on days 0 and 26. Vaccines were administered IM in divided doses in the muscles of the neck. Cattle in the FCA group were immunized with a vaccine incorporating Freund complete adjuvant on day 0 and with a vaccine incorporating Freund incomplete adjuvant on day 26. Cattle in the QuilA group were immunized with the vaccine incorporating QuilA as the adjuvant. Cattle in the Dex group were immunized with the vaccine incorporating DEAE-dextran combined with mineral oil as the adjuvant. Cattle in the alum group were immunized with the vaccine incorporating alum as the adjuvant. Control cattle were not immunized.

Sample collection and analysis—Blood samples for determination of IgG concentrations were collected on days –9, 12, 26, 33, 47, 61, 75, 103, and 138. Blood samples (approx 10 ml) were collected by gently inverting tubules 5 times and centrifuged at 2,000 X g and 4 C for 15 minutes. Serum was collected and stored at –20 C until analyzed. To determine serum IgG concentrations, an aliquot of each serum sample was thawed in a cold room (4 C) overnight. Samples were mixed thoroughly, and IgG concentration was measured by the method described by Shu et al.11

Samples of rumen fluid (approx 20 to 100 ml) were collected via a stomach tube on days –9, 12, 26, 47, and 138, and pH was measured with a portable meter. The pH meter was calibrated with standard pH buffers (pH 4.0 and 7.0).

Fecal samples were collected from the rectum on days 12, 26, 33, 40, 47, 54, 61, 73, 89, 103, and 138. One gram of each sample was immediately mixed thoroughly with 8 ml of distilled water (pH 7.0), and pH was measured.

Numbers of S bovis and Lactobacillus spp in rumen fluid collected on days –9, 47, and 138 from animals in the control, QuilA, and Dex groups were determined as described.13 Numbers of S bovis and Lactobacillus spp in rumen fluid were not determined for animals in the FCA and alum groups because of cost and time limitations.

Animals were weighed on days –2, 5, 12, 19, 26, 33, 40, 47, 54, 61, 75, 89, 103, and 138, and weight gain was determined.

Results

Serum antibody responses—Low serum anti-S bovis and anti-Lactobacillus IgG concentrations were detected in all animals on day –9 (ie, 9 days prior to immunization) and in control animals throughout the study (Fig 1 and 2). Compared with the control group, serum anti-S bovis and anti-Lactobacillus IgG concentrations were both significantly increased on day 12 for steers in the FCA, QuilA, and Dex groups, but only serum anti-Lactobacillus IgG concentration was significantly increased on day 12 for steers in the alum group. Serum anti-S bovis and anti-Lactobacillus IgG concentrations were significantly increased on days 26 through 75 in all 4 experimental groups. On day 103, serum anti-S bovis IgG concentration was not significantly increased in any of the experimental groups, and on day 138, serum anti-S bovis IgG concentration was not significantly increased among animals in the FCA and alum groups. Serum anti-Lactobacillus IgG concentration was significantly increased in all experimental groups on days 103 and 138. Throughout the study,
serum anti-\textit{S} \textit{bovis} and anti-\textit{Lactobacillus} IgG concentrations for animals in the Dex, QuilA, and alum groups were similar to or significantly higher than concentrations for animals in the FCA group.

When changes in antibody concentrations over time were examined, serum anti-\textit{S} \textit{bovis} IgG and anti-\textit{Lactobacillus} IgG concentrations remained constant from day 33 through day 75 and decreased significantly on day 103 for animals in all 4 experimental groups, except anti-\textit{S} \textit{bovis} IgG concentration was significantly decreased on day 61 for animals in the QuilA and Dex groups, and there was no further decrease in anti-\textit{S} \textit{bovis} IgG concentration for animals in the QuilA group after day 61.

Regression analysis indicated that there is a significant linear relationship ($R^2 = 0.56$) between serum anti-\textit{S} \textit{bovis} IgG concentration and serum anti-\textit{Lactobacillus} IgG concentration.

After booster immunization, mean (from day 33 to day 138) anti-\textit{S} \textit{bovis} IgG concentration for animals in the Dex group was significantly higher than mean concentration for animals in the FCA group. Mean anti-\textit{Lactobacillus} IgG concentration for animals in the Dex group was significantly higher than mean concentrations for animals in the FCA and QuilA groups. Other significant differences in mean antibody concentrations among immunization groups were not found.

Other responses—Mean pH of ruminal fluid (on days 9, 12, 26, 47, and 138) for animals in the control, FCA, QuilA, Dex, and alum groups were 6.21, 6.31, 6.31, 6.27, and 6.23, respectively (SE ranged from 0.09 to 0.10). Significant differences in pH of ruminal fluid between groups and over time were not detected ($P > 0.05$). However, mean pH of feces from animals in the QuilA and Dex groups were significantly higher than mean pH of feces from control animals (Fig 3).

On day 47, numbers of \textit{S} \textit{bovis} and \textit{Lactobacillus} spp in ruminal fluid from steers in the QuilA and Dex groups were significantly lower than numbers in ruminal fluid from control cattle (Fig 4 and 5). However, significant differences among groups were no longer found on day 138.
Discussion

Results of the present study indicate that immunization of feedlot steers against *S. bovis* and *Lactobacillus* spp with vaccines incorporating Freund adjuvant, QuilA, dextran with mineral oil, or alum as an adjuvant effectively induced high, long-lasting serum anti-*S. bovis* and anti-*Lactobacillus* IgG responses. The *S. bovis* and *Lactobacillus* cells used to prepare vaccines for this study were not killed by formalin or heat treatment, because in preliminary studies, it was found that a vaccine containing such *S. bovis* cells conferred a significantly higher protection against lactic acid accumulation than a vaccine containing the killed *S. bovis* cells. The vaccine that incorporated DEAE-dextran combined with mineral oil as an adjuvant induced the highest serum IgG responses, which is consistent with findings of Watson, who reported that the most promising mastitis vaccine incorporated dextran sulfate combined with mineral oil as the adjuvant. This is probably attributable to the combined effects of mineral oil and dextran, because both mineral oil and dextran can induce significant antibody responses on their own. Surprisingly, throughout the study, serum anti-*S. bovis* and anti-*Lactobacillus* IgG concentrations for animals in the QuilA and alum groups were also similar to or significantly higher than concentrations for animals in the FCA group. The reasons for this are not fully understood, because Freund adjuvant is considered to be the most potent experimental adjuvant in terms of its ability to stimulate high levels of long-lasting immunity. However, our results do suggest that vaccines against *S. bovis* and *Lactobacillus* spp that incorporate Freund adjuvant, QuilA, dextran, or alum as an adjuvant should provide similar levels of protection against lactic acidosis.

Antibody concentrations attained in steers in the present study, which were housed under feedlot conditions, were similar to those induced in cattle grazing pasture in a previous study. This suggests that level antibody concentrations can be achieved in animals fed a forage-based or a grain-based diet. This may be important clinically, because risk of lactic acidosis is generally highest when cattle are first introduced to a grain-based diet, and if cattle are immunized against *S. bovis* and *Lactobacillus* spp to prevent lactic acidosis, they should, whenever possible, be immunized before they are first introduced to a grain-based diet.

Direct biological benefits of immunization against *S. bovis* and *Lactobacillus* spp were evident in the present study. In cattle adapted to a high grain ration, some grain can pass undigested to the colon, where fermentation can lead to accumulation of lactic acid. The higher pH for feces from cattle in the Dex and QuilA groups in the present study suggests that there were subtle, yet significant, differences in lactic acid accumulation in the colon between groups, possibly because of differences in antibody concentrations in colonic contents. Numbers of *S. bovis* and *Lactobacillus* spp in ruminal fluid from animals in the QuilA and Dex groups were significantly decreased, compared with control animals, 3 weeks after booster immunization, supporting results of a previous study indicating that immunization can reduce numbers of *S. bovis* and *Lactobacillus* spp in ruminal fluid, thereby reducing lactic acid-producing capacity.

Results of the present study indicated that immunization did not completely remove all *S. bovis* and *Lactobacillus* spp from the rumen. Numbers of *S. bovis* and *Lactobacillus* spp in ruminal fluid from cattle in the QuilA and Dex groups were comparable to numbers in ruminal fluid from healthy animals adapted to high concentrate rations. Streptococcus bovis and *Lactobacillus* spp actively degrade starch and may be important components of the normal ruminal flora. These bacteria represent a high proportion (25 to 70%) of the total amylolytic bacteria in ruminal fluid from animals adapted to grain-based diets. In the present study, immunization of animals against *S. bovis* and *Lactobacillus* spp in this study significantly reduced the numbers of these bacteria in ruminal fluid but apparently allowed enough of these bacteria to survive to allow for normal starch fermentation. Importantly, however, it is not known whether functions of *S. bovis* and *Lactobacillus* spp are altered by immunization. For example, it has been shown that saliva anti-protozoa antibodies can immobilize protozoa in ruminal fluid and reduce the rate of predation by ciliates on radiolabeled bacteria. Therefore, it is possible that immunization against *S. bovis* and *Lactobacillus* spp may affect function of these bacteria as well as decrease their numbers.

Serum antibody responses in the present study were similar to responses in a similar study. There was a decrease in serum anti-*S. bovis* IgG concentrations on day 61, and there were further decreases in anti-*S. bovis* and anti-*Lactobacillus* IgG concentrations on day 103. This suggests that a second booster immunization may be required 2 months after the first booster immunization to maintain high levels of antibodies if cattle are fed a grain diet for more than 103 days. We did not find significant differences in numbers of *S. bovis* and *Lactobacillus* spp in ruminal fluid among groups on day 138, possibly because of the decrease in antibody concentrations. It would be interesting to compare numbers of *S. bovis* and *Lactobacillus* spp in ruminal fluid from control and immunized cattle if a second booster immunization were given.

We did not detect significant differences in pH of ruminal fluid among groups or over time in this study. This was not surprising, as pH of ruminal fluid from control animals was never < 6.0. A ruminal fluid pH > 6.0 suggests that lactic acid may not be accumulating in the rumen. Changes in ruminal fluid pH may also have not been detected because of infrequent sample collection.

In the present study, weight gain of cattle in the 4 experimental groups was not significantly different from weight gain of the control cattle. Cattle used in this experiment had already been adapted to a 75%
grain diet before immunization, and management procedures to prevent lactic acidosis by maintaining ruminal fluid pH > 6.0 were used. Therefore, differences in weight gain associated with lactic acidosis were expected to be small, and a larger sample size would have been needed to detect significant differences in weight gain.

References


