

Immunisation Against Lactic Acidosis in Sheep and Cattle

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Declaration

I certify that the substance of this thesis has not already been submitted for any degree and is not currently being submitted for any other degree or qualification.

I certify that any help received in preparing this thesis, and all sources used, have been acknowledged in this thesis.

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Signature

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Summary

Introduction

Lactic acidosis is due to the over production of lactic acid in the rumen by the bacteria, *S. bovis* or a combination of *S. bovis* and *Lactobacillus* when sheep and cattle consume large amounts of grain. It was hypothesised that the risk of lactic acidosis could be reduced by immunisation against the lactic acid producing bacteria. The present studies were conducted to test this hypothesis and investigate several key factors for developing an immunisation strategy against lactic acidosis.

Review of the literature

The review covers aspects of ruminant digestion, lactic acidosis, antibody-mediated immunity, and some of the important factors influencing immune responses.

General materials and methods

This chapter includes general bacterial media and methods for preparing antigenic cells, and measuring antibody, rumen pH, rumen lactate, rumen *S. bovis* and *Lactobacillus*, severity of diarrhoea, and statistical analysis.

Isolation and selection of *S. bovis* and *Lactobacillus* for vaccine preparation

This chapter describes the isolation and selection of the vaccine antigen bacteria. Five single strains of *S. bovis* and five isolates of *Lactobacillus* were obtained from the rumen contents of sheep and cattle. A strain of *S. bovis* (Sb-5) and an isolate of *Lactobacillus* (LB-27) had higher lactate-producing capacity than the other strains or isolates and were selected for vaccine preparation in the following experiments.

Immunisation with either a live or a killed vaccine against lactic acidosis in sheep

The first experiment was conducted in sheep to determine the efficacy of live and killed vaccines. Fifteen wethers were allocated to 3 treatment groups. Two groups were immunised with either formalin killed or live Sb-5 vaccine, and the other was control. The vaccines (Freund's complete adjuvant for primary immunisation and Freund's incomplete adjuvant for boosters) were injected

intramuscularly. After the primary immunisation, three boosters were administered at 2-4 week intervals. Anti-*S. bovis* antibody concentration in saliva was measured prior to animals being challenged with wheat grain.

The antibody level induced by the live Sb-5 vaccine (after three booster immunisations) was higher ($P<0.05$) than the killed Sb-5 vaccine. A significant increase ($P<0.05$) in the antibody concentration was observed after each booster. Compared with the control, significantly higher rumen pH and lower L-lactate concentrations were found in the immunised groups. The rumen pH in the group immunised with the live Sb-5 was higher than that in those sheep given the killed Sb-5 ($P<0.05$). The results support the hypothesis that the risk of lactic acidosis can be reduced by immunisation against *S. bovis* and that live Sb-5 vaccine is more effective than the killed one.

Immunisation with a *S. bovis* vaccine primed either intramuscularly or intraperitoneally against lactic acidosis in sheep

A second experiment in sheep was designed to investigate the relative effectiveness of immunisation primed intramuscularly or intraperitoneally. Forty five wethers were allocated to 3 treatment groups. Two groups were immunised with Sb-5 vaccines, and the other was control. The vaccines were prepared using live Sb-5 with Freund's complete adjuvant for primary immunisation and with Freund's incomplete adjuvant for booster injections. The primary immunisation was injected either intramuscularly (IM) or intraperitoneally (IP), and the boosters were administered intramuscularly at 2-4 week intervals. Killed Sb-5 cells were also administered orally at the same time of the 2nd and 3rd booster injections. Anti-*S. bovis* antibody concentration in saliva was measured prior to and following animals being challenged by feeding wheat grain.

The average antibody level in the IM group was higher ($P<0.05$) than in the IP group. A significant increase ($P<0.01$) in the antibody concentration was observed in the immunised groups after the 1st booster immunisation. No significant differences in antibody concentrations ($P>0.05$) were observed in the IM group between subsequent boosters (before grain feeding). Compared with the control, there were significantly ($P<0.05$) lower diarrhoea scores and less increase in blood packed cell volumes (%) in the immunised animals. The liveweight loss in the IP group was higher ($P<0.05$) than that of the IM and control groups. The results confirmed that the risk of lactic acidosis can be reduced by immunisation against *S.*

bovis and indicated that the immunisation primed intramuscularly was more effective than that primed intraperitoneally.

Comparison of adjuvants in sheep grazing pasture

Having established that effective vaccination against lactic acidosis was possible using Freund's complete adjuvant and multiple boosters, this experiment was undertaken to investigate the efficacy of a range of commercially acceptable adjuvants using one booster following a primary immunisation. Thirty five lambs were allocated to 7 treatment groups. Six groups were immunised using live Sb-5 vaccines, and the other was control. One booster was given 4 weeks after primary immunisation. Five adjuvants (Freund's incomplete adjuvant, QuilA, Dextran sulphate, Imject Alum, and Gerbu adjuvant) were compared with the Freund's complete/incomplete adjuvant (Freund's complete adjuvant for primary injection and Freund's incomplete adjuvant for booster). Anti-*S. bovis* antibody concentration in saliva and serum was measured weekly. The experiment was carried out under grazing conditions and animals were not challenged with grain.

The commercially acceptable adjuvants were effective in inducing high level and lasting anti-*S. bovis* antibody responses except that the use of Gerbu adjuvant stimulated a relatively low level and short lasting response. On some occasions the antibody levels induced by either QuilA or Freund's incomplete adjuvant were comparable ($P>0.05$) with the level stimulated by Freund's complete adjuvant. There was a positive correlation ($r=0.874$) between saliva and serum antibodies. No difference ($P>0.05$) was observed in liveweight gain between treatment groups. The results suggest that immunisation (a single booster following the primary injection) with a live vaccine containing one of the commercially acceptable adjuvants (including FIA, QuilA, Alum, and Dex) is safe and likely to be successful against clinical lactic acidosis. Results from this study also indicated that the serum antibody response is a good indicator of efficacy of immunisation.

Immunisation with a combination of *S. bovis* and *Lactobacillus* vaccine against lactic acidosis in cattle

Having shown effective immunisation against *S. bovis* in sheep, an experiment was conducted to determine the effectiveness of this technique in cattle using a combination of *S. bovis* (Sb-5) and *Lactobacillus* (LB-27). Ten steers were allocated to 2 treatment groups. One group was immunised with a vaccine containing live Sb-

5 and LB-27 cells, and the other was control. The vaccine (Freund's complete adjuvant for primary immunisation and Freund's incomplete adjuvant for boosters) was injected intramuscularly. After primary immunisation, boosters were administered at 2-4 week intervals. Antibody isotype IgG concentration in saliva and serum was measured over the period of experiment.

Both anti-*S. bovis* and anti-*Lactobacillus* IgG in saliva increased significantly ($P < 0.01$) after the 1st booster, which was lower ($P < 0.05$) than the IgG levels after the 2nd and 3rd boosters. However, it was not significantly different ($P > 0.05$) from the IgG concentration prior to the grain challenge (after the 4th booster). There was a positive correlation between the anti-*S. bovis* and anti-*Lactobacillus* IgG in serum and saliva. Compared with the control, higher feed intake, lower rumen concentrations of lactate and numbers of *S. bovis* and *Lactobacillus* were observed in the immunised group ($P < 0.05$). These results support the hypothesis that the risk of lactic acidosis can be reduced by immunisation against *S. bovis* and *Lactobacillus*, and provided further evidence that one booster following a primary immunisation is likely to be successful against clinical lactic acidosis.

Further comparison of adjuvants in cattle under feedlot conditions

This experiment was conducted to further test commercially acceptable adjuvants in cattle. Twenty four steers were allocated to 5 treatment groups under feedlot conditions. Four groups were immunised with vaccines containing live Sb-5 and LB-27, and the other was control. One booster was given following 4 weeks after primary immunisation. Three commercially acceptable adjuvants (QuilA, Alum, and Dextran combined with mineral oil) were compared with the Freund's complete/incomplete adjuvant. Serum antibody IgG concentration was measured over the period of the experiment.

Compared with Freund's complete/incomplete adjuvant, higher ($P < 0.05$) or similar ($P > 0.05$) IgG responses were observed when using the 3 commercially acceptable adjuvants. There was a positive correlation between the anti-*S. bovis* IgG and anti-*Lactobacillus* IgG. Compared with the control, a significantly ($P < 0.05$) higher faecal pH was found in the animals immunised using either DEAE-Dextran combined with mineral oil adjuvant or QuilA adjuvant. The numbers of *S. bovis* and *Lactobacillus* in the rumen in these two groups were lower than in the control. These results suggest that using any of the 3 commercially acceptable adjuvants can

induce high level and lasting IgG responses, with the DEAE-dextran combined with mineral oil being the most promising. The biological parameters point to vaccination reducing acid in the gut and reducing the risk of lactic acidosis.

Immunological cross-reactivity between the vaccine and other isolates of *S. bovis* and *Lactobacillus*

The above studies were based on the use of Sb-5 or a combination of Sb-5 and LB-27. In order to examine the potential for a vaccine to protect sheep and cattle from a number of strains of *S. bovis* and *Lactobacillus spp.* which may cause lactic acidosis, this study was conducted to determine the degree of immunological cross-reactivity between the Sb-5 and 8 other strains of *S. bovis*; and between the LB-27 and 4 other isolates of *Lactobacillus*. The cross-reactivity index (CRIs) ranged from 7.3 to 56.1% between the strains of *S. bovis* (the encapsulated strains with CRIs ranging from 7.3 to 12.4%). The CRIs ranged from 11.5 to 72.2% between the isolates of *Lactobacillus*. The results provide evidence that there is considerable antigenic variation between the vaccine and other isolates of *S. bovis* and *Lactobacillus*. However, the results also indicate that all the strains tested cross-react with the vaccine reference strain to some extent. Because *S. bovis* in the rumen is encapsulated, the low CRIs (indicating the high degree of immunological cross-reactivity) of the encapsulated strains suggest that the vaccine containing Sb-5 may be effective against a wide range of strains of *S. bovis* in sheep and cattle. The results also suggest that further work is needed to optimise the vaccine strain(s).

General discussion

Results from the studies in sheep and cattle support the hypothesis that the risk of lactic acidosis can be reduced by immunisation against *S. bovis* or *S. bovis* and *Lactobacillus*. Live vaccine (using DEAE-dextran combined with mineral oil as an adjuvant) may provide a suitable protection using one booster following a primary immunisation administered intramuscularly. This novel approach to reducing the risk of lactic acidosis associated with grain feeding offers a promising alternative to current practices of using feed additives, such as antibiotics active against the lactic acid-producing bacteria.

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