OPTIMISATION OF ANTIBODY PRODUCTION AND IMMUNISATION SCHEDULES IN FARMED DEER

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INTRODUCTION

Factors which influence the quality of an immune response following immunisation of animals include; the nature of the antigen (immunogenicity), dose administered, route of exposure, regime of primary and booster administration and the addition of adjuvants (Beh and Lascelles, 1981; Spiegelberg, 1974; Waksman,1979). Whereas the classical parameters of immune reactivity have been defined using the natural response of animals exposed to attenuated microorganisms, a more critical understanding of immune reactivity can be gained by immunising animals with well characterised purified antigens, under highly controlled experimental conditions. By understanding the basic immunological mechanisms involved in the response to a given immunisation regime, it is possible to produce conditions appropriate for the production of a defined type of immune reaction.

Full exploitation of vaccine use in preventive veterinary medicine demands a more complete understanding of immune mechanism for each species being treated. Uniquely different patterns of reactivity are seen between given species. Extrapolation from experimental findings obtained in laboratory rodents which differ significantly from larger domestic animals such as ruminants may prove invalid, so it is vital to tailor vaccination systems appropriate for individual species.

Within groups of animals such as ruminants, there appears to be more consistent patterns of responsiveness. It may therefore be possible to use the observations made from cattle or sheep studies to identify the prospects of immune reactivity in a novel domesticated species such as deer. For both cattle and sheep there is a consistent pattern of humoral immune reactivity which involves the production of specific immunoglobulin classes; IgM, IgG1, IgG2 and IgA. In sheep, the route of immunisation provides a heavy bias for the production of specific classes of immunoglobulin (Beh and Lascelles, 1981), with IgA being produced predominantly following immunisation via the intraperitoneal or mucosal route. By contrast the dose of antigen and type of adjuvant used

has a marked influence on the relative quantities of IgM, IgG1 and IgG2 (Reynolds, Suttie and Griffin, 1988). Because different classes of immunoglobulin have markedly different biological activities such as neutralisation, optimisation and colostral transfer, it is important to consider these factors before attempting to design any vaccination system for use in deer.

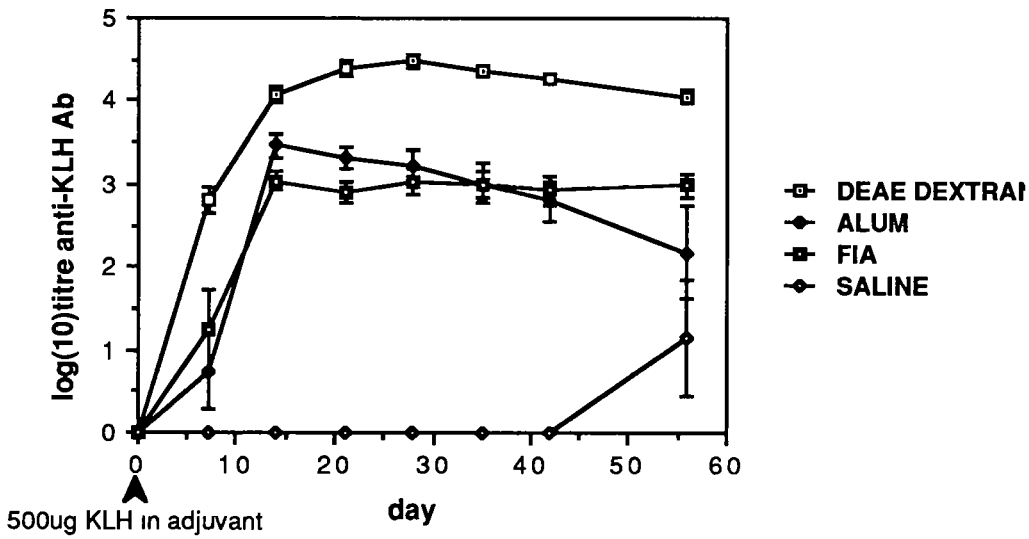
To date there has been reasonably widespread usage of conventional sheep clostridial toxoid vaccines in deer, with reported efficacy in disease control (Van Reenan and Innes, 1985). These findings have been empirical and have not attempted to assess the quality of immune reactivity following vaccination. To date only one experimental study (Wilson, 1984) has attempted to measure antibody production in deer following vaccination with leptospiral (*L. pomona-hardjo*) and clostridial toxoid vaccines. Whereas the animals showed a low response to primary vaccination with leptospiral vaccine all animals showed seroconversion following boosting. Although the responses were similar to that found in cattle they waned more rapidly in deer. By contrast, deer vaccinated with clostridial toxoid vaccines gave poor serological responses with peak titres only 10-20% the predicted levels found routinely in sheep.

Vaccination of deer with *Y. pseudotuberculosis* heat killed 'bacterin' has also been studied (Mackintosh et al., 1986). This study looked at the comparative serological responses in animals vaccinated with antigen mixed in different adjuvants; oil + emulsifier (STM), muramyl dipeptide (MDP), aluminium hydroxide (Alum) or saline. The STM adjuvant gave the highest serological titres and an associated activation of lymphocytes as measured by *in vitro* transformation and skin test reactivity. The findings from this study were confused somewhat by high preimmunisation titres (up to 1/80) to *Y. pseudotuberculosis* found using a serum agglutination test. This experiment highlighted the difficulty in assessing vaccination responses to antigens which occur naturally within the environment unless there is a critical immunological marker present which is uniquely specific for the vaccine strain. Studies being carried out currently (Hook unpublished) which have screened the serum samples from the above study using a highly sensitive ELISA assay and purified serotype specific lipopolysaccharide (LPS) antigens confirms the earlier observation of seroconversion in animals vaccinated with 'bacterin' plus STM. Poor serological responses were evident in animals vaccinated with antigen plus MDP, Alum or saline. Even in the STM group serological titres had dropped off to background within 42 days following vaccination. The advantage of the second assay system was that pre-vaccination titres could be excluded using specific LPS antigens in the ELISA.

EXPERIMENTAL STUDIES

In the context of these findings we recently set up some preliminary immunisation protocols to measure antibody production in deer immunised with a novel protein antigen combined with different adjuvants. Keyhole limpet haemocyanin (KLH) was chosen as an immunogenic protein against which deer would have no prior reactivity. The adjuvants chosen were, Freunds Incomplete Oil Adjuvant (FIA), aluminium hydroxide (Alum) and diethylaminoethyl dextran (DEAE-dextran). Five hinds were included in each treatment group and the animals were bled at weekly intervals after immunisation. Total anti-KLH antibody was measured by ELISA, and the results for the first eight weeks post immunisation are given in Figure 1. A significantly higher response to KLH was shown with the DEAE-dextran group in comparison to FIA or Alum. The response to KLH was not only significantly greater (10-50x) at its peak response but it also found a plateau of reactivity which persisted for 56 days.

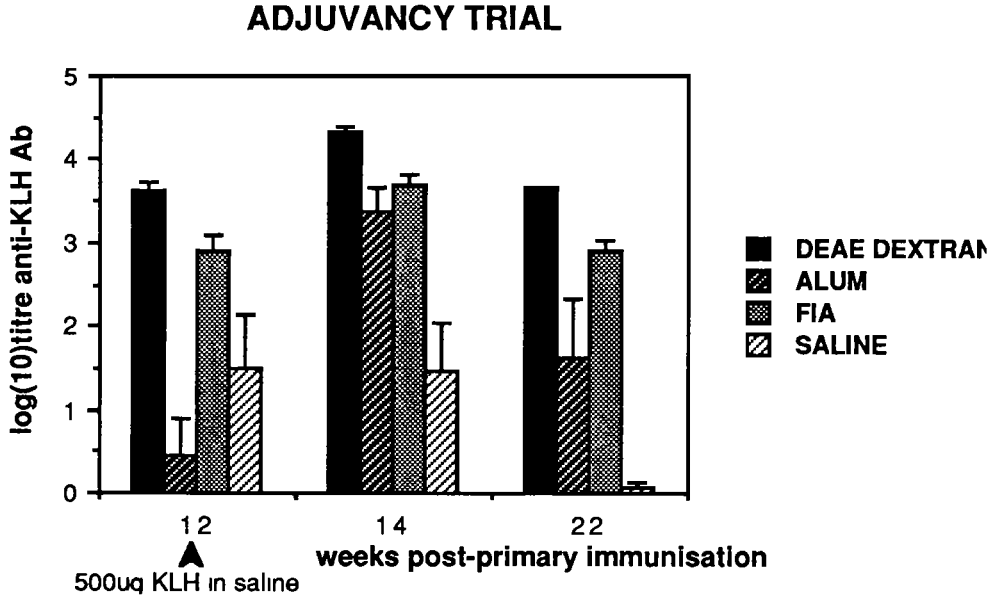
Figure 1.
Antibody Titres to KLH in Hinds for 8 weeks post-immunisation.



Because exposure of animals to primary immunisation results in the production of antibody and the activation of memory B and T cells, the study was extended to examine the effect of challenge by boosting the hinds with KLH in saline. Even though the animals immunised with

KLH in DEAE-dextran and FIA still had significant antibody titres at 12 weeks post primary immunisation they still demonstrated a boost in antibody titres (Figure 2) following secondary immunisation at week 12. Evidence of cellular memory cell activity was especially obvious in the Alum treated group whose titres increased by 1000 x in the two weeks following challenge.

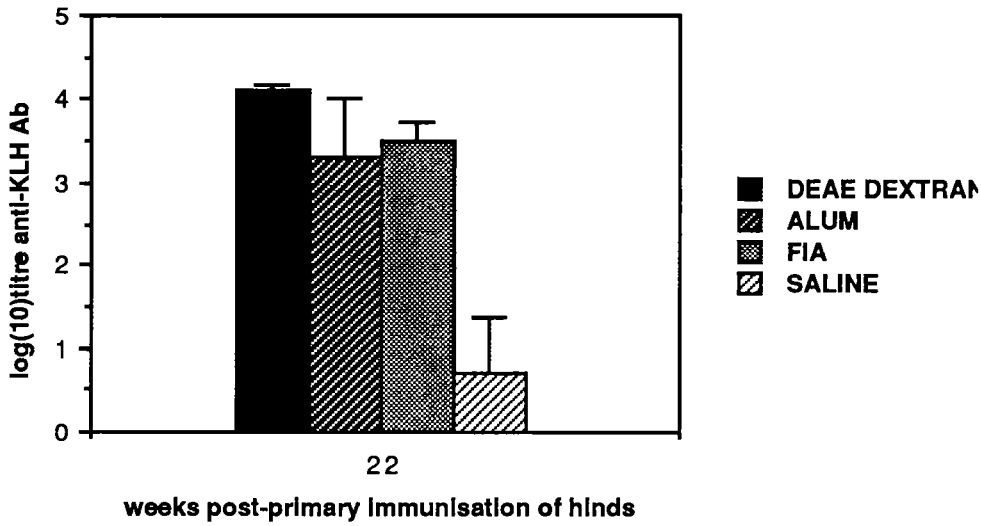
Figure 2.
Comparative Antibody Titres in Hinds at Different times post-secondary Immunisation.



Whereas KLH in saline failed to generate an effective primary antibody response it was quite effective in stimulating good secondary production in all groups of hinds primed with KLH and adjuvant. It can also be seen from Figure 2 that good responses to 2° immunisation were seen in all animals at 2 weeks after boosting. Even 10 weeks later there were high levels of anti-KLH antibody in animals immunised with KLH in DEAE-dextran and FIA. There was a significant reduction in antibody in animals immunised with KLH in Alum between weeks 14 and 22. In an attempt to measure the functional activity of immunoglobulin produced following experimental immunisation, the transfer of immunity from the hinds to their fawns was measured approximately 5 weeks post calving. The titres found in the fawns (Figure 3) show a very efficient transfer of immunity from the hinds to their offspring. The antibody titres in the fawns are significantly higher than the equivalent (22 week) titres in the hinds. Whereas there is a significantly higher titre of antibody in fawns from

hinds immunised with KLH in DEAE-dextran, there are also high antibody titres in the progeny of hinds immunised with KLH in Alum or FIA. Immunisation of hinds with KLH in saline resulted in low levels of passively transformed antibody.

Figure 3.
Passive Transfer of anti-KLH Antibody from Hinds to Fawns.



CONCLUSIONS

The preliminary findings from these studies of antibody production infer that adult deer can produce high titres of antibody which persist for long periods (>12 weeks). DEAE-dextran acts as a very efficient adjuvant for antibody production in deer. There is very efficient transfer of antibody from hinds to their offspring, indicating that this immunisation regime has adequate potential to ensure passive transfer of immunity via colostrum (Fleenor and Stott, 1983). This also infers that antibody of the IgG1 class is likely to dominate the immunoglobulin profile of animals at 2 to 5 weeks following 2° immunisation, as it has been shown for other ruminants (Butler, 1983) that IgG1 is the dominant immunoglobulin transferred via colostrum. The classical switch in immunoglobulin production from IgM to IgG1 indicates that an appropriate maturation of immunoglobulin production has occurred in the primary response and that an efficient memory response occurs when the animals are boosted with the same antigen. The efficient passive transfer of antibody from animals immunised with KLH in Alum suggests clostridial toxoids which

are precipitated by Alum should serve as appropriate vaccines to stimulate protective immunity in deer. The titres found in these deer are similar to those found with similar immunisation regimes in ewes (Reynolds and Griffin, 1988), so it is likely that deer can mount responses to vaccines which are similar in nature to those produced in cattle or sheep.

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