Immunological responses to vaccines in deer: effect of multiple vaccines

P R Wilson, C G Mackintosh, J F T Griffin

Abstract

Multiple strain vaccines are frequently used in deer Multiple organism vaccines are currently not, but their use may increase in future with heightened awareness of leptospirosis and the potential for clostridial and perhaps, other diseases Theoretically, multiple vaccination may reduce immunoreactivity to some or all valents, although evidence in humans is variable, with some suggesting potentiation. This paper reviews a number of issues related to the manufacture and use of multiple vaccines and describes a study into leptospiral and yersinia antibody production after multiple and single vaccinations

Yersinia and clostridial 5-in-1 and a bivalent leptospiral vaccine were applied singly or in combination, to weaners Blood samples were collected day 0, day 28, when a booster was given, and day 49 Somatic and virulence yersinia antigen antibody responses and leptospiral Micro Agglutination Test (MAT) titres were measured There was a statistically significant reduction in antibody response to the pure virulence antigen for yersinia after both the sensitiser and booster vaccines, and after the sensitiser vaccine to the pure virulence antigen. Leptospiral titres were unaffected by multiple vaccination

Since there is no data that correlates immunological responsiveness to protection against clinical disease, these data should alert veterinarians to the possibility of reduced immunoresponsiveness when multiple vaccines are used A cautious approach, until more data is available could be to recommend a short period between vaccinations However, without efficacy data, farmers should not be discouraged from using multiple vaccinations

Introduction

A vaccine against *Yersinia pseudotuberculosis*("Yersiniavax", AgVax NZ Ltd) and two bivalent and one trivalent leptospirosis vaccine ("Leptoshield Vaccine", CSL NZ Ltd, "Leptavoid 2" and "Leptavoid 3", Schering Plough Animal Health Ltd), respectively, are available in New Zealand. Deer are susceptible to a number of other disease for which vaccines are licenced for species other than deer in New Zealand (Mackintosh, 2001) Those vaccines may be advised by veterinarians for use in deer under the *Discretionary Use Criteria of the Agricultural Compounds and Veterinary Medicines* regulations Such use is permitted under the New Zealand Veterinary Association's standard procedures for discretionary use approved by the Agricultural Compounds Unit

For many of reasons, only a minority of deer farmers vaccinate their deer (Wilson, 2001). "Yersiniavax" is the most commonly used vaccine in deer Some farmers use a multivalent clostridial vaccine and some currently use a leptospiral vaccine. Some use more than one vaccine concurrently

Few vaccination programmes are fully effective because of a range of environmental, animal and human factors (Wilson *et al*, 1999) The effectiveness of a recommended yersiniosis vaccination programme has been described in Mackintosh *et al*, (1992). However, there are no data to demonstrate the effectiveness of clostridial or leptospiral vaccination programmes. Indeed, published data suggests that serological responses to both vaccines are lower in deer than in other species (Wilson, 1984, Wilson and Schollum, 1984)

A risk-based evaluation of the appropriateness of vaccination programmes on deer farms may result in a greater number of farmers vaccinating deer to protect their animals and their investment, and potentially themselves (Wilson, 2001) Furthermore, the Occupational Safety and Health section of the Labour Department has recently focused more attention on leptospirosis, and has included deer in their recent publication The deer industry is particularly aware of these concerns, and research and development in this area is likely to occur shortly Furthermore, if an increasing number of farmers adopt the full risk assessment process for decision making about vaccination discussed by Wilson

(2001), it is likely an increasing number of deer farmers will vaccinate deer. It is also increasingly likely that multiple vaccine use will become more common

Presently, many vaccines are multivalent and one multi-genera vaccine ("Ultravax 7-in-One", CSL) has become available for cattle, thus reducing the number of vaccine shots required Sone deer farmers may be hesitant to multiple vaccinate their deer because of repeatedinjections Thus, there may be increasing interest in developing multiple vaccines for use in deer

With these considerations in mind, as part of a broader study of factors affecting the efficacy of vaccination programmes (Mackintosh *et al*, 2001), a study was undertaken into the immunoresponsiveness of deer to single and multiple vaccinations involving "Yersiniavax", a biovalent leptospiral vaccine, and a 5-in-1 clostridial vaccine

General considerations for multiple vaccinations

Precedent

Multiple vaccination has been used for a long time in humans (Goldenthal *et al*, 1995). Vaccines with up to 23 valents of the one organism have been available, and combined vaccines such as measles, mumps, rubella, diphtheria, tetanus toxoid, pertussis and hepatitis B have been shown to be effective (Parkman, 1995) For farm animals in New Zealand some vaccines are monovalent, others contain various serotypes of an organism, eg "Yersiniavax", others contain antigens to threespecies of one genera, eg· clostridial 5-in-1 vaccine, multiple leptospiral vaccines. There is one vaccine available for multiple genera (clostridial 5-in-1 plus Leptospiral 2)

Immunological efficacy

The efficacy of multiple vaccines is an important question (Parkman, 1995). For all vaccines available the monovalent components are usually assessed prior to combination. The assessment is based on clinical effectiveness and immunological responses To evaluate multiple vaccines, comparative immunology is accepted as appropriate (Parkman, 1995) However, it is often difficult to evaluate the correlation between immunological response and clinical effectiveness.

It is necessary to examine antibody responsiveness and clinical effectiveness in each target species, because extrapolation between species is not appropriate For example, antibody responses in deer to clostridial and leptospiral vaccines appear to be lower than those in other species (Wilson, 1984, Wilson and Schollum, 1984)

It has been shown in humans that there may be interference between live vaccines reducing effectiveness Interference between inactivated vaccines is rare To counter that, here is some evidence of enhancement of immunogenicity by combining vaccines for humans (Parkman, 1995)

While there have not been a large number of examples of reduced immuogenicity of combination vaccines, when that phenomena arises there are several potential causes (Insel, 1995). These include physical or chemical interactions, interactions between live viruses and immunological interference There may be physical interactions affecting stability, consistency and immunogenicity Buffers for one vaccine may not provide compatibility with those of others. Adjuvants also differ in their effectiveness Each individual vaccine component needs to be stably absorbed into an adjuvant prior to mixing, and there may be inherent incompatibility between adjuvants Some preservatives interfere with some antigens These effects have been reviewed in detail by Insel (1995) That author noted that while interference between valents of a vaccine is an important issue, there are also prospects of enhancement of reactivity That author also confirms that, when tested, the thoretical possibility of enhanced reactivity or suppression of immune response with vaccine combinations has rarely been observed.

Manufacture

The production of multiple vaccines requires a product which has stability, compatibility between components, appropriate preservatives, appropriate adjuvants, a long shelf life, and which is economic

to produce Clinical and immunological issues include antigenic interference, safety, effectiveness, the need or otherwise for boosters, dose volume and site reactions (Parkman, 1995).

There are a number of technological issues which determine the combination of vaccines (Saldarini, 1995) These are both pharmaceutical and clinical in origin The amount and type of adjuvant differs between valents in a vaccine and may affect the stability. Further issues related to manufacturing multivalent vaccines are compatability of the components, stability and combination, and regulatory concerns in methods for testing (Elliot, 1995)

For practical purposes, multiple vaccines to be used in farm animals would need to be combined at manufacture, ie "one shot does all" Dual chamber administration, as is available for some vaccines in humans, would not be appropriate for herd animals Mixing of vaccines by the end user would be particularly risky and should be discouraged until evidence exists to show effectiveness and safety

Vaccination programme efficacy

For deer, several vaccines are available Some are licensed One significant limiting factor for vaccine recommendations for deer is that only one, "Yersiniavax", has been evaluated clinically While some leptospiral vaccines are licensed for use in deer, they have not been evaluated for their clinical efficacy Therefore the clinical effectiveness of a vaccination programme *per se* is unknown. Some farmers use more than one vaccine and would like a combination, provided they were effective, to obviate the need for multiple injections There is also a belief that they may be more cost effective

Evaluation of multiple vaccines in deer

Materials and Methods

Deer

This trial used nine 0 25 red x 0 75 wapiti and 71 red mixed sex weaner deer on a commercial deer farm Progeny were from five identified sires

This herd had never experienced a case of yersiniosis, leptospirosis or clostridial disease since its establishment in 1992.

Animal management

Prior to weaning, deer were grazed *ad lib* on turnips with free access to pasture Late December they were yarded and ear tagged, and subsequently paired with their dams They were drenched mid-January with oral "Ivomec" Grazing was behind an electric fence and these animals had frequent close contact with humans and were thus very quiet to handle.

Weaning was undertaken on February 22, 2000, and a second "Ivomec" oral anthelmintic was given After weaning, they alternately grazed a special purpose pasture of chicory and red clover and conventional perennial ryegrass/white clover swards Deer were managed as a single group throughout

Experimental procedures

Deer were randomly allocated to one of four treatment groups·

1 Control - no vaccination treatment,
2. Triplevaccine. "Yersiniavax", 5-in-1 clostridial vaccine and a bivalent leptospiral vaccine,
3 Leptospiral vaccine alone,
4 Yersinia vaccine alone

Deer were yarded on February 29 A jugular venipuncture blood sample was taken for serum and deer were vaccinated according to their group allocation A booster vaccine was given and a second blood sample collected on March 28 (day 28) A further blood sample was collected April 18 (day 49)

Vaccines used were

- "Yersiniavax", batch 0 001 2, expiry 01 November 00,

- "Ultravac 5-in-1", CSL Ltd, batch 07110 3206, expiry 08/02
- "Leptoshield", CSL Ltd, batch 0503 12702, expiry 05/01

Vaccination procedures

An area was clipped as appropriate on the anterior half of the neck towards the dorsum The skin was swabbed with alcohol The vaccine was carefully administered from flexipacks using vacciguns set at 2 ml A new needle was used for each vaccination on each animal

Serology

Samples collected Day 1 were analysed for yersinia and leptospiral antibodies. Day 28 and Day 49 samples were analysed for leptospiral antibodies (Groups 1, 2 and 3) and yersinia antibodies (Groups 1, 2 and 4)

Somatic (O) I, II and III and pure and crude virulence antibodies were measured by ELISA at the Deer Research Laboratory, Otago University

Leptospiral titres for *Leptospira pomona* and *L hardjo* were undertaken by the Leptospirosis lab at Massey University using the MAT test

Results

Mean antibody responses to somatic and virulence antigens of yersinia pseudotuberculosis and leptospiral serovars *pomona* and *hardjo* are presented in Tables 1-3

Table 1. Mean optical density increase from day 0, for yersinia somatic antigens OI, OII and OIII, 28 days after sensitiser and at day 49, 21 days after booster vaccine

	OI		OII		OIII	
Day	***28***	***49***	***28***	***49***	***28***	***49***
Control	6	7	4	6	-5	-2
Tripple-vacc	8	14	8	13	-4	3
Yersinia	11	16	15	14	0	4

Differences in optical density relating to all three somatic antigens are negligible and not statistically significant

Table 2. Mean optical density increase from day 0, for yersinia crude and pure virulence antigens 28 days after sensitiser and at day 49, 21 days after booster vaccine

	Crude V		Pure V	
Day	***28***	***49***	***28***	***49***
Control	10^{a}	-10^{a}	0^{a}	2^{a}
Tripple-vacc	17^{b}	75^{b}	29^{b}	86^{b}
Yersinia	53^{bc}	97^{bc}	46^{bc}	99^{b}

*abc = different letters denote statistical significance

Mean crude virulence antibody level in control deer fell marginally while those in vaccinated animals increased significantly above controls Furthermore, the crude antibody level in the yersinia-only vaccine group was significantly higher after both the sensitiser and booster vaccinations than the 3-vaccine group Pure virulence antibody levels in both 3-vaccine and yersinia vaccine groups were significantly higher than controls The yersinia antibody was significantly higher than the 3-vaccine antibody group after the sensitiser vaccine, but not after the booster vaccine

Table 3. Mean MAT titre increase from day 0, 28 days after a sensitiser vaccine and 49 days later, 21 days after a booster vaccine

	Pomona		*Hardjo*	
Day	***28***	***49***	***28***	***49***
Control	-4	-12	-12	-20
3-vacc	34	107	-5	29
Yersinia	21	185	-6	29

There were no significant differences in antibody concentration between the 3-vaccine and the lepto alone vaccine groups for either serovars *pomona* or *hardjo*

Discussion

These results show a statistically significantly reduction of antibody response to crude virulence antigen 21 days after both sensitiser and booster vaccines, and a reduction in pure virulence antibody concentration after the sensitiser vaccine

The clinical significance of this observation is unknown since there are no studies relating antibody concentration to immunoprotection Thus, it is currently not appropriate to draw a conclusion that the 3-vaccine group may be at higher risk of contracting yersiniosis

It is notable that there is no difference in leptospiral vaccine titres between the 3-vaccine and lepto alone vaccine groups The reason for this is not clear, although in considering the results presented it is apparent that the immune system is reacting differently to different antigens For example, there has been no immuno-responsiveness to yersinia somatic antigens, yet there have been significantly different responses to virulence antigens This is likely due to the biochemical nature of antigens: somatic antigens are lippopolysaccharide, while virulence antigens are protein The latter are more antigenic However, the lack of response to somatic antigens in this study is in contrast to those reported in other parts of the broader study of vaccine responses presented elsewhere in these proceedings (Mackintosh et al)

There was considerable variation in optical density readings for yersinia antibody. Some higher concentrations at the first treatment period suggested maternal antibody To test the prospect that maternal antibody may have interfered with immunoresponsiveness to the vaccine, those animals with an optical density value > 40 were removed from statistical analysis. This had very little difference on the final result. It is notable that most of those animals with high somatic antibody OD values at the beginning of the trial showed a decrease in OD during the trial However, the virulence antibody concentrations increased in those animals, but not as much as in the vaccinated animals This is consistent with the pattern seen in the maternal antibody trial by Mackintosh *et al*, (these Proceedings).

Of note was the relatively low antibody response to the leptospiral vaccine for both serovars *pomona* and *hardjo* Falling titres in the unvaccinated control animals probably resulted from maternal antibody, since the dams were vaccinated prior to calving

One variable from this dataset which will be analysed further is the potential influence of sire The progeny used for this trial were identified to five sires by single sire mating groups Data from field trials of yersinia vaccine effectiveness (Mackintosh *et al*, 1992) showed a sire effect in susceptibility to disease Further studies (Wilson *et al*, 1999) demonstrated differences in serological responses of progeny related to sire. Results of that analysis will be published elsewhere

Data from studies of human multiple vaccines indicate variability in response, with some observations suggesting enhancement and some suggesting interference of immune responsiveness This study suggests that while there may have been some interference in antibody responsiveness to virulence antigens of yersinia pseudotuberculosis, a considerable increase in antibody was evident However, substantially more work is required, given the variation reported in other species, before one can have confidence in recommending that multiple vaccines not be used concurrently.

For deer, there are a number of situations where it may be appropriate to use more than one vaccine type Already some farmers use yersinia vaccine, a multiple clostridial and leptospiral vaccines. While the efficacy of the yersinia vaccination programme has been defined, the efficacy of leptospiral and 5-in-1 vaccines have not been researched in deer However, in the absence of that research it would seem the best alternative would be to extrapolate from other species Therefore some farmers currently use yersinia, clostridial and leptospiral vaccines concurrently at weaning, with a booster 3-4 weeks later. If a breakdown of vaccination were to occur in those herds, the possible reduction in immunological response due to multiple vaccination would be a factor to consider in establishing cause. This would provide valuable data There currently appears to be no clinical evidence that multiple vaccination in involved in apparent vaccine failures (Brenton-Rule, these proceedings).

In addition to the need for further research into use of multiple vaccines is the potential to combine vaccines into a one-shot-dose-all vaccine for deer A significant amount of research into formulation, relative antigenic potency, effectiveness of various vaccination strategies, before such a vaccine will be marketed for deer in New Zealand

Acknowledgements

The authors thank Geoff Purchas for field assistance, Chris and Christine Pullar for use of their deer, Ngaire Chinn and Simon Liggett for Elisa assays, Dr Julie Collins-Emmerson for Leptospiral serology, and Dr Peter Johnstone for statistical analyses. Funding was provided by AgVax Developments NZ Ltd , CSL NZ Ltd , the GIB, NZDFA, Massey and Otago Universities, and AgResearch.

References

Elliott RAY (1995) Manufacturing issues for multivalent vaccines In Combined vaccines in simultaneous administration Current issues and perspective *Annals of the New York Academy of Science* 754, XXIII-XXVI

Goldenthal KL, Burns DL, McVittie LD, Lewis BP, Williams JC (1995) Overview - combination vaccines and simultaneous administration past, present and future In Combined vaccines in simultaneous administration Current issues and perspective *Annals of the New York Academy of Science* 754, XI-XV

Insel RA (1995) Potential alterations in immunogenicity by combining or simultaneously administering vaccine components In. Combined vaccines in simultaneous administration Current issues and perspective *Annals of the New York Academy of Science* 754, XXXV-XLVII

Mackintosh CG (2001) Diseases of deer for which vaccines exist Proc of a Deer Course for Veterinarians, Deer Branch NZVA No 18. Ed Wilson PR, (These proceedings)

Mackintosh, CG, Griffin, JFT, Wilson PR (2001) The effects of maternal antibody and stress on responses to Yersiniavax in red deer calves These proceedings

Mackintosh CG, Buddle BM, Griffin JFT (1992) "Yersiniavax" - efficacy under field conditions in weaner red deer Proc of a Deer Course for Veterinarians, Deer Branch NZVA No 9. Ed. Wilson PR, 107-10.

Parkman PD (1995) Combined and simultaneous administrated vaccines a brief history In Combined vaccines in simultaneous administration. Current issues and perspective. *Annals of the New York Academy of Science* 754, I-IX

Saldarini RJ (1995) Strategies for developing combination vaccines In Combined vaccines in simultaneous administration Current issues and perspective *Annals of the New York Academy of Science* 754, XVII-XXII

Wilson PR (1984) Vaccination of deer leptospirosis and clostridial diseases. Proc of a Deer Course for Veterinarians, Deer Branch NZVA No 1 Ed Wilson PR, 110-16

Wilson PR, Schollum LM (1984) Serological responses in farmed deer to a vaccine against *Leptospira interrogans* serovars *hardjo* and *pomona* NZ vet J 32, 117-18

Wilson PR, Mackintosh CG, Griffin JFT (1999) Yersiniosis resistance, susceptibility and vaccination Proc. of a Deer Course for Veterinarians, Deer Branch NZVA No 16 Ed Wilson PR, 133-44 Wilson PR (2001) Vaccination of deer - a pragmatic or philosophical question? Proc of a Deer Course for Veterinarians, Deer Branch NZVA No 18 Ed Wilson PR, (These proceedings)