YERSINIA VACCINATION TRIALS IN RED DEER (*CERVUS ELAPHUS*)

C.G. Mackintosh, T.G. Henderson, and J.F.T. Griffin

INTRODUCTION

Yersiniosis continues to be one of the most common causes of death in young red deer in their first winter. The epidemiology of the disease in deer in New Zealand has been discussed previously (Mackintosh & Henderson, 1984). Young deer entering their first winter are usually exposed to *Yersinia pseudotuberculosis (Y. pstb)* for the first time. The majority appear to experience a subclinical infection and develop natural immunity to subsequent infection. However, if the calves are stressed they may succumb to an acute fulminating enteritis. Investigations at Invermay have been aimed at producing a vaccine which will prevent deer calves from developing clinical yersiniosis.

This paper will discuss the pathogenesis and immunology of *Y. pstb* infection in animals, review the literature on vaccines against *Yersinia* spp. and similar organisms and present a summary of the results of vaccination trials at Invermay.

INFECTION AND PATHOGENESIS

Infection normally occurs by the faecal/oral route. In subclinical *Y. pstb* infections there appears to be little evidence of damage to the intestine. Faeces usually appear normal and there is no apparent ill-health despite the deer excreting *Y. pstb* organisms for a period of time. Apparently normal calves at Invermay have been shown to excrete *Y. pstb* intermittently for up to 3 months. In clinical yersiniosis there can be profound damage to the gastro-intestinal tract (Beatson & Hutton, 1982). Usually the ileum, caecum and colon are most severely affected and occasionally the changes extend to the upper small intestine and abomasum. Histopathological changes in the intestine can include "acute to subacute enteritis, with patchy ulcerations, oedema and inflammation of the lamina propria and submucosa". This may be accompanied by mesenteric lymphadenitis with focal necrosis. This profound damage to the gut lining allows the *Y. pstb* organisms to invade the body via the lymphatic drainage to the mesenteric lymph nodes and thence to the visceral organs. *Y. pstb* is often isolated from the liver in fatal cases of yersiniosis in deer. Death is probably due to dehydration and endotoxic shock.

SEROLOGICAL RESPONSE TO INFECTION

Blood and faecal samples from 38 red deer calves on Invermay were monitored at 3 to 4 weekly intervals from March to October, 1983. A total of 20 (53%) calves had positive faecal cultures on at least one occasion and they yielded 23 Serogroup I, 2 Serogroup II and 2 Serogroup III isolates from 17, 2 and 2 animals respectively (I and III were isolated from one calf on 2 different occasions 8 weeks apart). Of the calves, 36 had titres to Serogroup I ranging from 1:40 to 1:640. Over half had titres of 1:80 while the Geometric Mean Titre (GMT) was 1:113. Titres which peaked at <1:80 were usually undetectable 3-4 weeks later. Peak titres of 1:160, 1:320 and 1:640 had usually declined to undetectable levels by 8, 12 and 16 weeks respectively. Antibody titres in most of the animals peaked initially in May/June and 14 calves had a second peak between July and October. This peak was usually within one dilution of the original peak titre. This suggests that these animals experienced another

challenge with the same organism. It is interesting to note that there did not appear to be an anamnestic response to subsequent challenges.

During this period 20 calves also developed Serogroup II titres (1:40 to 1:160) and 6 had titres to Serogroup III (1:40). Interestingly neither of the 2 deer from which Serogroup III isolates were cultured from faeces developed measurable Serogroup III titres. This may indicate that these last strains were of low pathogenicity.

Thus, in general it appears that these low transient serological responses are a feature of natural exposure to *Y. pstb* and are thought to be largely immunoglobulin M (IgM). Unfortunately it was not possible to monitor cell mediated immunity (CMI) in these calves.

For comparison, it has been shown that *Y. pstb* titres in humans usually disappear in 3 to 4 months. However, persistent titres at high levels are thought to indicate persistent infection in the lymphatic system and may be accompanied by recurrent symptoms of mesenteric lymphadenitis (Mair, 1968).

A REVIEW OF VACCINATION AGAINST Y. PSTB AND SIMILAR BACTERIA

A number of experiments have been conducted overseas in which "killed" virulent strains of *Y. pstb* have failed to produce immunity in guinea pigs (Thal, 1954) whereas live avirulent vaccines gave good protection (Thal, 1962). However, these trials may not be relevant to vaccination in deer because the challenge involved intraperitoneal (IP) injections of a large challenge dose, and the disease in guinea pigs is usually of a more chronic form, with extensive abscess formation indicating prolonged intracellular survival and high CMI involvement.

Thal (1973) has also shown that the vaccination of guinea pigs with some live avirulent *Y. pstb* strains gives good protection against *Y. pestis*, the organism which causes bubonic plague and is closely related to *Y. pstb*. However, vaccination with avirulent plague vaccines apparently does not protect against *Y. pstb*. The reason for this is not clear.

Some Serogroup III strains of *Y. pstb* produce an exotoxin which enhances their virulence. A toxoid has been shown to protect guinea pigs against this exotoxin (Thal, 1973).

Simple *Y. pstb* bacterins have been used in zoo situations in Europe to protect birds (Zwart *et al.* 1981) and other animals (G.H.K. Lawson, *pers. comm*). The latter author gained the impression that the vaccines gave some protection although they were usually used in uncontrolled field situations where efficacy could not be proved. One controlled trial with red deer in Scotland proved nothing as clinical disease did not appear in non-immunised calves. The vaccines were usually from homologous strains isolated in previous outbreaks on the property. The vaccines incorporated incomplete Freunds adjuvant. Vaccinated animals included beavers, monkeys, baboons, squirrel monkeys and deer. (G.H.K. Lawson, *pers. comm.*)

Plague vaccines (formalin killed *Y. pestis* non-adjuvanted bacterins) have been made and used for U.S. military personnel and civilians for over 40 years with only minor modifications to the medium and strains of *Y. pestis* used (Bartelloni et al., 1973). Three doses of vaccine are recommended: 1:0 ml primary vaccination IM with boosters of 0.2 ml IM at 90 and 270 days (Anon, 1982). Serological and clinical trials in human volunteers have demonstrated measurable serological responses in 90% of people after the first booster and 93% after the second booster. Titres declined 4 fold in the 6 months following the first booster but reached a higher level and had a slower rate of decline after the second booster. Mouse protection indices using serum from vaccinated people indicate that there is a high degree of correlation between protection against fatal infection and the presence of passive-haemagglutination antibodies >1:128. Based on this relationship 83% of human volunteers were protected after 2 vaccinations.

Y. pestis and *Y. pstb* are regarded by some people as 2 subspecies of the same species on the basis of being indistinguishable by DNA relatedness (Bercovier *et al.*, 1980). Because of this similarity *Y. pstb* bacterins produced in the same way may also promote a similar protective immune response. Consequently, the experimental *Y. pstb* vaccines used in the Invermay trials were made by a similar method to that described by Bartelloni *et al.*, (1973).

Similar simple killed bacterins have been used for some years to protect livestock against various diseases including salmonellosis, which has a similar pathogenesis to that of yersiniosis. Killed *Salmonella* bacterins are of benefit, but the immunity may be overwhelmed by a large challenge inoculum. Live avirulent vaccines administered parenterally or orally give better protection against severe oral challenge (Smith *et al.*, 1984).

Parenterally administered vaccines raise the circulating immunoglobulin (Ig) level and it is thought that some protection may be mediated by the passage of Ig across the mucosa into the intestinal lumen. This amount is increased markedly during diarrhoeal diseases and this may be one of the reasons that mortality is inversely proportional to serum Ig concentrations in many enzootics of diarrhoea in calves (Moon & McDonald, 1983).

IMMUNISATION OF DEER IN NEW ZEALAND

Invermay Trial 1984 - feasability study

The first objective was to determine if a single-strain *Y. pstb* bacterin would produce detectable agglutinating antibody and CMI responses in deer. The second objective was to ascertain the response of vaccinated calves to a live *Y. pstb* challenge. In this challenge work the subcutaneous (SC) route was chosen, since experimentally the oral route had failed to produce an infection in trials the previous winter.

Experimental

Thirteen 10-month-old red deer calves being run at pasture received a single 2 ml SC injection of either a heat killed (HK) or a formalin killed (FK)

bacterin containing approximately 5 x 10^8 *Y. pstb* Serogroup I organisms plus either an oil or aluminium hydroxide (AlOH) adjuvant or saline. Serological and lymphocyte transformation tests (LTT) were conducted weekly on blood samples and the calves were skin-tested with a crude preparation of FK or HK homologous strain *Y. pstb* (0.1 ml intradermal) and read 2 days later.

Results and Discussion

In the winter, 3 to 4 months prior to the immunisation, the calves had shown transient titres and some CMI activity indicating exposure to *Y. pstb*. At the start of the trial (September) all calves were seronegative and had low background CMI activity except for one calf which had low to moderate activity. The saline and AlOH adjuvanted bacterins produced similar (1:80-1:320) transient antibody responses which peaked at around 1 week and were detectable for only 3 weeks after inoculation. Oil adjuvanted bacterin gave higher titres (1:160-1:640) which were more prolonged: after 8 weeks the titres ranged from <1:40 to 1:320. Although 2 animals failed to develop a measurable titre, both showed high CMI responses as measured by both LTT and skin test. FK antigen with either adjuvant produced a consistently high CMI response whereas HK antigen was inconsistent, with over half the deer producing a low response. The skin test correlated well with the LTT. Eight of the deer were grouped into 4 pairs, on the basis of their serological and LTT results as high antibody/high CMI, high/low, low/high and low/low responders. They were all challenged with a 2 ml SC dose of homologous *Y. pstb* serogroup I (5 x 10^8 organisms/ml). Two animals died, 2 and 8 days post challenge, from systemic yersinia infections. The first was a low antibody/high CMI responder and the second a low/low responder.

Because all these animals appear to have had prior exposure to *Y. pstb* I in the winter the vaccination probably acted as a booster. However, it was useful to demonstrate the feasability of using serology and the LTT to monitor the response of deer to immunisation. It also appears that the higher CMI response to FK antigens is probably due to the higher level of intact lipoprotein because heat is more likely to denature protein.

The subcutaneous challenge with live *Y. pstb* was unnatural compared to natural oral field challenge, but it was the only practical method available. The results of the SC challenge suggest that humoral immunity (as measured by serological titres) may be more important than CMI for protection against systemic *Y. pstb* infection. This may not necessarily be the case in enteric infections.

Invermay Trial 1985

This experiment was designed firstly to investigate the serological responses of deer to 2 doses of a single strain *Y. pstb* bacterin mixed with a variety of adjuvants, and secondly to observe their response to a subsequent challenge with live *Y. pstb* organisms.

Experimental

Twenty newly-weaned 3-month-old calves were used in this trial. A type III organism was chosen because this Serogroup is rarely isolated from Invermay animals and it was thought unlikely that the deer would receive a field challenge that would interfere with or be superimposed upon the serological response to the vaccine. In early March a *Y. pstb* Serogroup III field strain mixed with either oil, muramyl dipeptide (MDP), AlOH or saline was given to 4 groups of 4 calves while a fifth group were unvaccinated controls. The vaccinations were repeated 6 weeks later in mid-June and 7 weeks later all calves received a 2 ml SC challenge dose of 10^9 *Y. pstb* III homologous strain organisms.

Results and Discussion

Prior to inoculation 8 calves had titres ranging from 1:20 - 1:80. Two weeks after vaccination all 4 calves which received oil adjuvanted bacterin had titres 1:80 to 1:160, while the MDP, AlOH and Saline (plus antigen) groups had similar titres to the control group (0 to 1:80). After the booster the oil group again had the most consistent titres (4 x 1:80) while all the deer in the other groups had titres of 0 to 1:80. One week after challenge all titres rose to 1:320 to 1:640 and all had fallen to 0 to 1:80 4 weeks post challenge. Just prior to the challenge 3 calves yielded Serogroup I isolates from faecal samples and one of these also yielded a Serogroup II isolate. In the 4 weeks after the challenge 6 calves yielded 5 x I, 3 x II and 3 x III Serogroup isolates. These latter III isolates were each from a calf from the MDP, AlOH and Saline groups.

There were no deaths resulting from the challenge, although most of the calves appeared depressed and had sore necks at the site of the challenge injection for a few days.

Surprisingly, it appears that the calves were naturally exposed to either *Y. pstb* III or to other bacteria that cross-react with this Serogroup (e.g. *Y. enterocolitica*, other *Yersinia spp.* or *Salmonella spp.*) because the control calves developed titres similar to the vaccinated calves, except for those receiving the oil adjuvanted bacterin which had higher titres.

The fact that none of the control calves died as a result of the challenge may have been due to the fact that they had been naturally exposed to a similar organism and developed natural immunity or due to low virulence in the challenge strain. However, the subcultures were grown at 26°C in an attempt to avoid the loss of virulence associated with the prolonged culture of *Y. pstb* at 37°C. An IP dose of 10^8 organisms of the challenge strain killed 2 guinea pigs within 3 days indicating some degree of virulence.

These 2 trials above demonstrate the difficulties associated with conducting trials with vaccines against organisms which are ubiquitous in the environment, and which appear to produce disease in deer calves which are concurrently being stressed. Reproducing these challenge conditions is extremely difficult. Add to this the fact that bacterins of this kind against enteric infections rarely produce immunity in 100% of animals. Consequently it was decided that a field trial of such a vaccine with natural challenge would be the most practical way to test its efficacy.

Field Trial 1985

A total of 2216 calves on 30 farms were included in a trial conducted by 8 veterinarins. Half of the deer received Yersiniavax (Yvax), an experimental multi-strain, formalin-killed, AlOH adjuvanted, *Y. pstb* vaccine. The other half received a sham vaccine (Dvax) which did not contain *Y. pstb* organisms. Two doses were given 4 to 8 weeks apart between March and July. Between April 1 and September 31 there were 18 recorded deaths: 10 Yvax and 8 Dvax calves. However, *Y. pstb* was isolated from 4 Dvax and 2 Yvax calves (both of which had only received one dose of Yvax prior to their death). Other causes of death were pneumonia or lungworm (4 Yvax, 1 Dvax) malignant catarrhal fever (1 Yvax), broken-neck (1 Yvax, 1 Dvax), and undiagnosed (2 Yvax, 2 Dvax).

On one farm 1/30 Dvax calves died of yersiniosis while no Yvax calves were affected. Only one farm experienced a yersiniosis outbreak with more than 2 deaths. *Y. pstb* was isolated from 3 Dvax calves and not from Yvax calves.

There were no adverse reactions to the vaccination reported.

Invermay calves, which were also vaccinated, all had titres <1:40 4 weeks after both 1° and 2° vaccinations, suggesting that measurable titres are not maintained for long.

The results are not significant because of the relatively small number of animals in the trial, and the low incidence (0.8%) of yersiniosis. This latter factor was presumably due to good management and a mild winter.

Unfortunately, many of the booster vaccinations were not completed until July. By this time the majority of calves would have been exposed to *Y. pstb* and it is past the peak of clinical cases which is usually observed in June (Mackintosh & Henderson, 1984). This could explain the 2 deaths which occurred in Yvax calves from which *Y. pstb* was isolated, although one of the calves had some histopathological features of MCF as well.

It can be argued that these conditions may be an unfair test for a vaccine. To conduct a trial it is necessary to vaccinate only a proportion of a herd and leave the rest as untested controls. If the vaccine gives some protection it would be expected that there would be greater losses in the control deer. However, the fact that a proportion of the herd are unvaccinated and may shed large numbers of organisms may provide an unfair challenge to the vaccinated proportion. If, as would be the case in a normal herd, all the calves were vaccinated the phenomenon of "herd immunity" may completely prevent an outbreak of infection from sweeping through the herd. However, the vaccinated calves would still be exposed to the "normal" level of contamination in the environment which, if at a relatively low level, may tend to boost the acquired immunity without causing clinical disease.

Other problems with field trials are:

(a) Usually only good reliable farmers are invited to take part in a trial and these farmers tend to have the lowest losses due to yersiniosis.

(b) Because the animals are so valuable farmers tend to want to intervene if any deaths occur and treat all unvaccinated calves.

(c) Involvement in a vaccine trial tends to increase a farmer's awareness of the possiblity of yersiniosis and he may unconsciously improve conditions and minimise stress, thus preventing an outbeak which would test the vaccine.

The fact that measurable agglutination titres did not last more than 4 weeks does not necessarily mean that protection is lost. It has been shown in growth inhibition tests (Thal, 1966) that protective humoral immunity may be present in the serum of vaccinated animals even when agglutinating antibody is no longer detectable. In addition, memory cells are also probably formed after vaccination and subsequent challenge may result in the immediate production of protective antibodies. There is also probably stimulation of the CMI system, which may give some protection against natural challenge via the small intestine and lymphatic system.

GENERAL DISCUSSION

It is obvious from the above that it is no simple matter to conduct meaningful trials of *Y. pstb* vaccines. The measurement of serological and CMI responses are difficult due to their brief nature and to the complications of environmental exposure causing interference with the vaccine exposure. There is also no simple laboratory measurement that exactly equates with protection. The use of laboratory animals for vaccine testing is fraught with dangers because the clinical expression of the disease is so different between them and deer. The challenge of vaccinated deer under experimental conditions is also difficult and unless the oral route is used the results may not truly indicate protection against enteric infection. The virulence of challenge organisms must be maintained by minimising the number of preliminary subcultures, which should be grown at 26°C to prevent the loss of the virulence plasmid, and then the challenge inoculum should be grown at 37°C so that there is full expression of virulence antigens (Brubaker, 1967). However, the most important factors determining the pathogenicity of a challenge appear to be animal related factors such as stress, food intake, body condition, environmental conditions, concurrent disease, genetic susceptibility etc., most of which are complex and cannot be manipulated predictably.

Therefore, it still appears that the most meaningful test of *Y. pstb* vaccine is an extensive field trial where natural challenge will occur and a proportion of vaccinated and unvaccinated animals will be subjected to stressors liable to precipitate yersiniosis.

FUTURE WORK

Field Trial - 1987

It is planned to conduct a larger field trial involving up to 10,000 calves next autumn/winter.

The new vaccine will have a higher antigenic load (10^9 orgs/ml) than that used in 1985. Further work will depend on the 1987 trial but should the AlOH adjuvant not produce sufficient immunity then it will be necessary to use an alternative adjuvant (probably oil-based).

A long term objective is the production of an avirulent live vaccine which would be expected to produce the best immunity in deer.

REFERENCES

Anon, 1982. Plague Vaccine. Recommendation of the Immunization Practices Advisory Committee (ACIP) *Morbidity Mortality Weekly Reports 31 (22)*: 301-304.

Bartelloni, P.J.; Marshall, J.D.; Cavanagh, D.C. 1973. Clinical & serological responses to plague Vaccine U.S.P. *USP Mills Med. 138*: 720-2.

Beatson, N.S.; Hutton, J.B. 1981. An outbreak of yersiniosis in farmed red deer. *Deer Advisory Panel N.Z.V.A. Deer Seminar Queenstown*: 136-139.

Bercovier, H.; Mollaret, H.H., Alonso, J.M., Brault, J., Fanning, G.R., Steiger-walt, A.G.; Brenner, D.J. 1980. Intra - and interspecies relatedness of *Y. pestis* by DNA hybridisation and its relationship to *Y. pstb. Current Micro. 4*: 225-9.

Brubaker, R.R. 1967. Growth of *Pasteurella pseudotuberculosis* in simulated intracellular and extracellular environments *J. Inf. Dis. 117*: 403-417.

Mackintosh, C.G., Henderson, T.G. 1984. The epidemiology of yersiniosis in deer. *Proc. Deer Branch N.Z.V.A. Course No. 1*: 34-42.

Mair, N.S. 1968. *Pasturella pseudotuberculosis* infection in Great Britain 1959-66. *In* Int. Symp. on Pseudotuberculosis. Paris 1967. Symp. Series Immunobiol. Std. *Vol. 9* (Karger, Basel./New York, 1968) pp 121-128.

Moon, H.W.; McDonald, J.S. 1983. Antibody response of cows to *E. coli* pilus antigen K99 after oral vaccination with live or dead bacteria. *Am. J. Vet. Res. 44*: 493-496.

Smith, B.P.; Reina-Guerra, M.; Stocker, B.A.D.; Hoiseth, S.K.; Johnson, E.H. 1984. Vaccination of calves against *S. dublin* with aromatic-dependent *S. typhimurium. Am. J. Vet. Res. 45*: 1858-1861.

Thal, E. 1954. Unters uchungen uber *Pasteurella pstb* - unter Beruchsichtigung ihres immunologischec Verhalten. *Nordisk Vet. 6*: 829-32.

Thal, E. 1962. Oral immunisation of guinea pigs with avirulent *P. pstb. Nature 194* (4827): 490-91.

Thal, E. 1966. Immunobiologische Studien an *Y. pstb. Schweizer archiv fur tiesheilkunde, 108*: 372-88.

Thal, E. 1973. Observations on immunity in *Y. pstb. Contrib. to Micro. & Immuno. Vol 2.* (Karger Basel, 1973): 190-5.

Zwart, P.; Wiesner, H.; Goltenboth, R. 1981. Experience from use of inactivated pseudotuberculosis vaccine in birds. *In* Erkrankungen der Zootiere. Verhandlungsbericht des XXIII. Int. Symp. Erkrankungen der Zoo., 24-28 Juni, 1981. Halle/Saale. *Ed* R. Ippen and H.D. Schroder. Berlin, GDR. Akademie Verlag. pp. 73-76.