YERSINIOSIS VACCINE RESEARCH

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Introduction

Yersiniosis, caused by Yersinia pseudotuberculosis (Y.pstb) continues to be a major cause of death in young red deer in their first winter. It also causes diarrhoea and death in dairy calves, lambs and goat kids, sporadic abortion in ewes, and deaths in laboratory animal colonies, especially in rabbits and guinea pigs, and in zoo collections, especially in primates and birds. The epidemiology of yersiniosis in deer has been presented previously (Mackintosh and Henderson, 1984). Research at Invermay has been directed primarily at young red deer with the objective of protecting them from clinical disease, although probably not subclinical infection.

Previous Studies

The initial Yersinia vaccine studies at Invermay had the following objectives: firstly to produce a simple killed Y.pstb bacterin based on methods used for making plague (Y.pestis) vaccines for humans, secondly to investigate the serological and cell-mediated immune (CMI) responses of deer to this bacterin combined with various adjuvants and thirdly to challenge these vaccines with yersiniosis experimentally or in field trials. The results have been discussed previously in full (Mackintosh et al, 1986; Mackintosh, 1988) but in summary we found that oil adjuvanted vaccines produced measurable antibody and CMI responses but aluminium hydroxide adjuvanted vaccines only gave small transient rises in macroscopic agglutination test (MAT) titres. Experimental challenge of 8 vaccinated deer with a subcutaneous injection of a serotype I strain of Y.pstb resulted in the death of 2 calves with the lowest MAT and CMI responses to vaccination. A second trial which used a serotype III Y.pstb subcutaneous challenge in a larger number of animals failed to produce clinical yersiniosis although most animals developed temporary pain and swelling at the site of injection. A field trial involving 2216 red deer calves on 30 farms using an aluminium hydroxide adjuvanted killed vaccine failed to give a significant result because of the unusually low incidence (0.8%) of yersiniosis. Only one farm experienced an outbreak with more than 2 deaths and on this property Y.pstb was isolated from 3 control animals and not from any vaccinated calves.

These trials left a number of questions unanswered and highlighted a number of problems, namely:

a) The MAT appeared to lack sensitivity because titres were low and short-lived. It detects only agglutinating antibody which may not relate well with protection. In particular it does not specifically detect antibodies to the virulence (V) antigens which appear to be of particular importance for the organism to resist being killed by macrophages. The ability of an animal to resist infection is associated with the development of immunity to V antigens (Une and Brubaker, 1984), therefore a test for the measurement of antibody to V antigens in deer was required.

b) The lack of a laboratory animal model for yersiniosis in deer hampered the research. The development of such a model would simplify quality control of vaccine efficacy and virulence testing of Yersinia strains.

c) The aluminium hydroxide adjuvant, although well tolerated by deer, did not provoke a significantly greater response than saline when combined with the bacterin. Oil adjuvant appeared to promote significantly higher MAT response to the somatic antigens.

d) There was some suggestion that heat killing of the organisms gave a better MAT response than formalin killing but the relative effects on virulence antigens were unknown.

e) Clinical yersiniosis was difficult to induce experimentally. It seemed necessary to challenge a stressed animal with a large infective dose of virulent organisms. If experimental infections could be consistently produced this would provide an efficient model for testing vaccine efficacy.

f) Field trials needed to be large, ie >10,000 animals to increase the chance of getting sufficient outbreaks to test the vaccine. These trials were also likely to be biased against a significant result by the fact that the best farmers were more likely to be involved in trials and were less likely to have outbreaks. Laboratory confirmation of yersiniosis would also be expensive.

Recent studies

The studies over the last 2 years set out to resolve these problems but retained the primary objective of producing a multi-strain Yersinia vaccine for use in deer. The work has been jointly planned and conducted by the Yersinia Management Group comprising Drs C.G. Mackintosh, Invermay, B. Buddle, Wallaceville and F. Griffin, Otago University.

Plasmid analysis: The V antigens are coded for by plasmid associated DNA rather than nuclear DNA. Y.pstb organisms which lose the virulence associated plasmid become avirulent. Therefore it is essential that strains of Y.pstb used in vaccine manufacture are checked to ensure they retain their plasmids, because repeated subculturing and growth at 37°C can quickly cause loss of the plasmid. This work has been carried out at Wallaceville.

Somatic serotyping and vaccine production: The 3 main somatic serotypes of Y.pstb are I, II and III. The majority of clinical cases of yersiniosis in the South Island are caused by Serotypes I and II, while III is more common in the North Island. The relative importance of the somatic antigens with regards to protection is not known and therefore all 3 serotypes are included in the vaccine. The vaccine is produced by growing these 3 strains individually under conditions which favour expression of the virulence antigens and then equal antigenic loads are combined. Currently vaccine batches are produced at Wallaceville using semi-commercial methods, although only small batches are made.

ELISA test development: Because of the shortcomings of the MAT, the development of an ELISA test for detecting antibodies against Y.pstb antigens in deer became a high priority. A student, Sarah Hook, working in Dr Griffin's laboratory at Otago University, developed an ELISA test for somatic and virulence antigens based on methods developed by Hibma and Griffin (1987) and modified specifically for deer (Hibma and Griffin, 1988). These tests are very specific, sensitive and repeatable and allow the monitoring of circulating antibody levels to both somatic and V antigens in infected and vaccinated animals. Currently the ELISA tests are being modified to detect class specific antibody responses in order to investigate the roles of IgM, IgG and IgA in protection against yersiniosis.

Mouse model: Dr Buddle has developed a laboratory model for testing the virulence of Y.pstb strains and the efficacy of vaccines in mice. Testing the virulence involves the intraperitoneal injection of mice with live Y.pstb, assessing their clinical state over the next few days and then killing the mice and estimating the number of viable organisms in the spleen. In our work so far, there appears to be good correlation between the virulence of Y.pstb strains in mice and deer. Likewise the efficacy of vaccines in mice corresponds with that in deer, although a 0.2 ml dose of the adjuvant DEAE dextran is lethal in mice whereas a 2 ml dose it is well tolerated in deer and sheep.

A mouse passive protection test is currently being investigated. This involves the injection of serum from deer immunised with the Yersinia vaccine into mice which are then challenged with virulent Y.pstb. If the serum gives significant protection to the mice it will demonstrate that this test can provide a more precise and convenient method for testing vaccine efficacy than challenging deer directly. However, studies are being undertaken to verify that the results of tests involving parenteral challenge in mice correlate with tests involving gut borne infection in deer.

Stress-challenge test in deer: Because of the cost and difficulties of running a field trial to test a Yersinia vaccine it was decided to try to induce experimentally an outbreak of yersiniosis in a large group of vaccinated and unvaccinated deer at Invermay.

A mob of 139 deer were randomly allocated to 2 equal groups one of which received a single subcutaneous dose of an oil adjuvanted 3 strain killed Y.pstb vaccine and the other remained unvaccinated. Eight weeks later in early June all the deer were yarded, fasted for 24 hours, transported in a truck for 2 hours, deposited in another set of yards and challenged with an oral dose of 8×10^{10} live virulent Y.pstb serotype I organisms. They were then run at pasture on maintenance feed rations and yarded daily for close examination. All clinically affected animals were treated in an effort to prevent any deaths.

The vaccine gave significant protection ($P<0.05$) against the development of clinical yersiniosis, with 54% of non-vaccinates being affected compared with 31% of vaccinates. Of 9 in-contact deer which were not orally challenged, none developed clinical yersiniosis from natural exposure to affected deer. This suggests that the experimental challenge was far greater than would be naturally experienced and was therefore a severe test of the protection given by a single dose of vaccine.

Treatment: There were two important lessons learnt during the stress-challenge trial. Firstly, it is very easy to miss signs of scouring in deer with yersiniosis because often there is very little evidence of scour material on the tail, perineum or hocks, especially if it is a very watery scour. Secondly, intensive fluid therapy is absolutely essential in severe yersiniosis, and the simplest method of treatment is to give 2-3 litres of oral electrolytes and gut protectants orally via a stomach tube.

Genetic susceptibility/resistance: An unexpected finding of the stress-challenge trial was that there appears to be some inherited susceptibility or resistance to yersiniosis. Some of the weaners used in this trial were the offspring of 3 known sires. Their calves had rates of severe yersiniosis of 30% (6/20), 23% (3/13) and 0% (0/12) respectively. The latter result is significantly different ($P<0.01$) from the other two and the results are not confounded by gender, liveweight or vaccination status. In future it may be possible to select for resistance to yersiniosis, especially if gene markers that relate to resistance can be identified.

1990 Trials

This year's work is in two parts. The first is a similar trial to last year's stress-challenge trial except that there will be a control group and 2 vaccinated groups testing different adjuvants. Two doses of vaccine will be given 3 weeks apart and the challenge dose will be reduced. The second part will be a serological comparison of 3 different adjuvants including one supplied by a commercial company.

Laboratory tests on blood samples to measure antibody and cell mediated immune responses, and parallel mouse vaccination studies will be compared with the clinical responses of the deer to experimental challenge with Y.pstb in order to test the efficacy of the vaccine and provide parameters to obviate the need for live deer challenges for vaccine testing in the future.

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