YERSINIOSIS VACCINE UPDATE

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INTRODUCTION

Yersiniosis is a commonly fatal bacterial enteritis of young deer farmed in New Zealand and has also been reported to occur in deer in Australia, Canada and the United Kingdom (Mackintosh & Henderson 1984, Jerrett *et al* 1990, English 1990; Hariharan & Bryenton 1990; Fletcher 1982) The causative organism is *Yersinia pseudotuberculosis* (*Y pstb*) and common farm stressors such as underfeeding, fasting, transport and bad weather predispose young deer in their first winter to clinical disease The majority of unstressed deer experience subclinical infection and become relatively resistant to yersiniosis in subsequent years. Three serotypes (I, II and III) have been reported in New Zealand In an effort to prevent outbreaks of yersiniosis, a programme of investigations into the immune response of deer to *Y pstb* infection and to killed bacterins has been undertaken and reported previously (Mackintosh *et al* 1990) This paper reports the latest developments

In 1989 a stress/challenge protocol was developed which orally exposed young red deer to live *Y. pstb* organisms following a 24 hr period of typical farm stressors including yarding, weighing, fasting for 24 hr, truck transport for 3 hr and a move to a new location Half the 139 calves had received a single dose of an oil adjuvanted killed multistrain *Y. pstb* vaccine 8 weeks previously and the stress/challenge showed that the vaccination gave significant protection against the development of clinical yersiniosis (54% unvaccinated vs 31% vaccinated) However, at the time of challenge the serological titres were low and there was no association between titre and protection The high incidence of yersiniosis even in the vaccinated deer was probably due to the very high oral dose of *Y. pstb* (8×10^{10}) 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 in the field and was therefore a severe test of the protection given by a single dose of vaccine

In order to investigate further the possible relationship between serological titre and protection against yersiniosis another trial was conducted in 1990. Two different adjuvants were used with 2 doses of vaccine and the challenge dose was reduced. In addition, a non-stressed group was also challenged in order to study the effect of the stress component in predisposing deer to yersiniosis.

1990 STRESS/CHALLENGE TRIAL

Protocol

128 newly weaned red deer calves, stratified by sire, were randomly allocated to 4 groups of 32 calves in mid-March Groups 1 and 2 were vaccinated by subcutaneous injection in the neck on March 20 and April 10

Group 1 received 2 doses, 3 weeks apart, of STM oil adjuvanted vaccine[1] and were stressed
Group 2 received 2 doses, 3 weeks apart of DEAE dextran adjuvanted vaccine[1] and were stressed.
Group 3 unvaccinated stress controls.
Group 4 unvaccinated non-stress controls

Three weeks after the time of the second vaccination (day 41, April 30) Groups 1, 2 and 3 were yarded, blood sampled, weighed, fasted for 24 hr and transported for 3 hrs by truck to a new location, ie "stressed" On day 42 (May 1) all four groups received an oral dose of 3×10^{10} live virulent *Y. pstb* serotype I (except for 2 unchallenged controls in each group)

From May 2 all the animals were yarded daily and examined closely for clinical signs of yersiniosis, especially diarrhoea around tail, hocks, perineum and anus Any clinically affected animals were isolated and temperatures, faecal samples and blood samples were taken and dehydration assessed A subjective score of the clinical

[1]Formalin-killed multistrain *Y.pstb* bacterin with total 4×10^{10} organisms plus concentrated culture supernatant

severity of the yersiniosis was made, based on the severity of the diarrhoea, the degree of dehydration and the length of time it took the animal to recover The 8 unchallenged controls were also monitored for temperature and haematological values

All clinically affected animals were kept in covered yards and treated daily with 2-4 ℓ oral electrolytes[2] and diarrhoea powder[3] by stomach tube and parenteral oxytetracycline[4] until fully recovered, then returned to their mob

RESULTS

Clinical effects

Significantly more of the unvaccinated stressed Group 3 calves (60%) developed yersiniosis than either of the vaccinated Group 1 (26%) or Group 2 (33%) calves (Table 1). Group 3 had nearly twice as many cases in 3 of the 4 categories of clinical severity The unstressed Group 4 controls, by contrast, only had mild and moderate cases of yersiniosis and an incidence of only 30%

TABLE 1: Incidence and degree of severity of clinical yersiniosis in red deer calves experimentally challenged with live *Yersinia pseudotuberculosis* organisms.

Groups (n=30/group)	Clinical Yersiniosis			
	Mild	Moderate	Severe	Total
1 Oil vaccine and stressed	3	0	5	8 (26%)
2 DEAE dextran vaccine and stressed	3	2	5	10 (33%)
3 Unvaccinated stressed controls	7	2	9	18 (60%)
4. Unvaccinated non-stressed controls	5	4	0	9 (30%)

All the affected calves showed varying degrees of diarrhoea, dullness, inappetance, elevated temperature and dehydration.

None of the 8 non-challenged control deer developed clinical yersiniosis although haematological changes and seroconversion suggested that 6 of them developed subclinical yersinia infection from natural challenge.

Serology

Serology using somatic (OI, OII and OIII) and virulence (crude "V" and purified "V") antigens in an ELISA test (Voller *et al* 1980, Hibma & Griffin 1987, 1988) showed that the DEAE dextran adjuvanted vaccine produced similar titres to the STM adjuvanted vaccine 3 weeks after 1° vaccination but DEAE gave consistently higher titres 3 weeks after 2° vaccination However, there was no correlation between serological response and the effect of experimental challenge

Cell mediated immunity

There was only low transient CMI response (measured by lymphocyte transformation assays) to vaccination and no correlation with protection

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[2]Trolyte, Ethical Agents Limited, Auckland

[3]Parnell Diarrhoea Powder, Phoenix Pharm Distributors Ltd, Auckland

[4]Oxytetrin LA., Pitman-Moore, Upper Hutt.

Haematology

There was a characteristic picture from blood samples collected when clinical signs of yersiniosis first appeared At this time there were low of eosinophil, basophil, lymphocyte, neutrophil, total white cell and platelet counts, but elevated levels of fibrinogen, haptoglobin and haemoglobin. Levels of fibrinogen, haptoglobin and haemoglobin in these acute cases could be used to predict the severity of the inflammatory response

Both haptoglobin and fibrinogen were clearly raised 48 hours after challenge, before clinical signs appeared The mean platelet volume was reduced in the early phase of the disease, and raised in the convalescent stage, when the platelet count was greater than pre-challenge.

Haematological changes suggested that 6 of the 8 non-challenged controls developed sub-clinical *Y pstb* infection from natural exposure to experimentally infected deer

Sire effects

The weaners were all of known breeding and the 9 sires each had 7-18 offspring distributed across the 4 treatment groups Although none of the differences in serological response or clinical effect were significant at the 5% level, the differences in morbidity rate between the "best" sire (#618 14%) and the "worst" sire (#259 53%) closely approached significance

Vaccination site reactions

Weekly examination of the vaccination sites showed that the STM vaccine produced a 40-60 mm diameter raised lump at the site of injection 1 week after vaccination and 30% developed into a "cold" abscess which burst 1 to 2 weeks later By 8 weeks all reaction sites had regressed to a mild thickening 0 to 20 mm which eventually disappeared DEAE dextran vaccines produced a thickening 30-50 mm across and usually developed a thick leathery scab which lifted off 3-5 weeks later leaving a small area of hair loss and a negligible thickening 0-10 mm diam. 6-7 weeks after vaccination

DISCUSSION

Once again the combination of 24 hours simulated farm stress plus heavy oral challenge with virulent *Y pstb* I resulted in development of clinical yersiniosis in 40% of animals overall (36/90 stress/challenged) The trial showed that vaccination with either STM or DEAE adjuvanted *Y pstb* killed bacterins give significant protection against this challenge (30% vaccinated vs 60% non-vaccinated developed clinical yersiniosis) By contrast only 30% of the unstressed control animals were affected and they had only mild or moderate yersiniosis This emphasises the importance of typical farm stress factors such as fasting, transport and introduction to new surroundings, in predisposing deer to yersiniosis

The oral challenge was intended to be considerably lighter than the 8×10^{10} live organisms used in the 1989 challenge Because the challenge must be used as a fresh subculture the number of live organisms could only be estimated (1×10^{10}) but in fact was 3×10^{10} per dose based on a plate culture which requires 24 hours incubation. The resulting number and severity was very similar to that seen last year The protection given by 2 doses of the vaccines in this trial was also comparable to that afforded by 1 dose of an STM adjuvanted vaccine last year (31% yersiniosis in vaccinates versus 54% in non-vaccinates)

As in the 1989 trial, none of the 8 in-contact non-challenged controls developed clinical yersiniosis, suggesting that the experimental challenge was far greater than would be naturally experienced under field conditions and was therefore a severe test of the protection given by these vaccines

All the cases of yersiniosis were confirmed by *Y pstb* culture and serology, and all had similar haematological pictures in the acute phase

The serological results are intriguing After boosting, the DEAE dextran vaccine promoted higher titres than the STM vaccine but there was no correlation between titre on the day of challenge and protection CMI as measured by lymphocyte transformation does not appear to be related to protection either Further studies are being made to investigate other factors that might relate to protection

Haematological tests on the first day of clinical yersiniosis may be useful for estimating the severity of the condition based on elevated fibrinogen, haptoglobin and haemoglobin levels Haptoglobin is a particularly sensitive measure of acute inflammation but is not quantitative, whereas the level of fibrinogen is associated with the degree of severity of inflammation (Cross *et al*, in prep) Haematological tests of in-contact non-challenged controls suggested that 6 to 8 developed subclinical yersiniosis and the same 6 animals all seroconverted 1 to 2 weeks after the rest were challenged

There is some evidence that there are sire effects relating to serological response to a vaccination and to susceptibility to clinical yersiniosis, although these 2 latter factors do not appear to be associated Similar sire effects were seen in 1989 trial.

The DEAE dextran adjuvanted vaccine gave higher titres, is easily mixed with the antigen and produces an acceptable short-term reaction at the site of injection For these reasons, it has been chosen for use in future commercial vaccines Provisional registration has been obtained for Yersiniavax which is currently being tested on 19 deer farms in a field trial involving 5,000 red deer calves this winter, and if successful, we hope to have the vaccine commercially available next year

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