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# Subclinical Johne's disease in sheep

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## ABSTRACT

This paper presents preliminary results from a 5-year study aimed at generating objective data on the effect of subclinical Johne's disease on productivity in sheep. On two properties where Johne's disease was considered to be a problem, replacement ewe lambs from a single generation were allocated to one of three groups. One group was vaccinated with the killed *Mycobacterium avium* subsp. *paratuberculosis* vaccine Neoparasec, another with the live vaccine Gudair, and the other group remained as unvaccinated controls. The vaccinated and control ewes were monitored over the ensuing four years for the following parameters: lesion size, live weight, fleece weight, scanning percentage, lamb production and weight of lambs weaned per ewe. Serology was also performed on a sample of animals from each group. On one property, the size vaccination-site lesions associated with Gudair declined at a more rapid rate than those associated with Neoparasec. However, results to date have failed to detect a significant difference in productivity between vaccinated and unvaccinated ewes in either flock, suggesting that subclinical Johne's disease may not be an important cause of production loss in sheep. The trial is now in its final year.

**Keywords:** Johne's disease; ovine; subclinical.

## INTRODUCTION

Ovine Johne's disease in New Zealand was first diagnosed in the early 1950s in the South Island, and is now widespread in flocks throughout the country. Although official figures (MAF database) indicate that the infection is present in 6.4% of New Zealand's sheep flocks (West, 1997), the true prevalence may be as high as 70% (Brett, 1998). The significance of Johne's disease within an infected flock is uncertain and may vary considerably with management different practices, geographic region, breed of sheep and between seasons. The incidence of clinical disease ranged from 0 to 1.6% of ewes per flock in one survey (Davidson, 1970).

Johne's disease is considered to be of economic importance to the dairy industry in the US (Johnson-Ifearulundu *et al.,* 1999). A study on six dairy herds in New Zealand (de Lisle and Milestone, 1989) estimated annual losses of up to $6 651.38, but with considerable variation between herds and between years. There is no reliable information on the cost of the disease to New Zealand's sheep industry, or even to individual farmers with known infected properties. The number of clinical cases can be estimated with some accuracy but there is no information on the effect of subclinical Johne's disease on the productivity of sheep. As a result, it is impossible to provide objective advice to sheep farmers on the likely benefits of vaccination or other control options. Furthermore, granting agencies and industry representatives have no yardstick by which to rank Johne's disease in their funding priorities.

In this paper we present preliminary data from a study aimed at generating objective data on the subclinical effects of Johne's disease on two properties where the disease was considered by the farmer and his veterinarian to be a problem. Because of the lack of a suitable test for subclinically infected animals, vaccination was used as a means of suppressing or minimising the effects of the disease. This is based on the assumption that vaccination is effective in disease prevention.

## MATERIALS AND METHODS

Two commercial properties on which Johne's disease had been confirmed, and was considered to be a problem, were chosen for the study. One was a hill country property at Kimbolton (near Feilding) running crossbred sheep. The other was a high country property in Marlborough running purebred Merinos. The project commenced on 13 January 1998 with the vaccination and weighing of a total of 1657 replacement ewe lambs, 994 on the Kimbolton property and 663 in Marlborough. On both properties, the lambs were randomly allocated to one of three groups. One group was vaccinated with a live *M. a. paratuberculosis* vaccine (Neoparasec), one with a dead *M. a. paratuberculosis* vaccine (Gudair) and the other group remained as unvaccinated controls. The trial animals on both properties were run together at all times and treated the same.

All lambs were ear tagged at the time of weighing to allow for future recording of data from individual animals. Blood samples were collected from 20 lambs from each group (60 in total) on the Kimbolton property to serve as baseline values for measuring serological responses following vaccination. Surviving animals from this group of 60 were re-sampled on several visits during the next 4 years.

The following measurements were made, and production data collected, throughout the period of the trial:

- size of vaccine-site lesions (regular intervals)
- growth rate of vaccinated and control lambs
- body weight of trial ewes (annually, pre-tup)
- fleeceweights
- scanning percentage
- weight of lambs weaned per ewe (Kimbolton flock only)

In addition to the above measurements, faecal cultures

for *M. a. paratuberculosis* were performed on one occasion on a sample of animals from each group. An effort was made to obtain samples of ileum and ileocaecal lymph node for microscopic examination from trial animals that died or were culled during the trial.

The vaccine-site lesions were scored using the following system:

0 = no lesion
1 = small discrete lesion approx. 1cm diameter
2 = discrete lesion approx. 2cm diameter
3 = lesion approx. 3cm diameter
4 = larger lesion approx. 4cm diameter
5 = very large lesion 5cm or greater in diameter. Some of these lesions were suppurating.

Data were analysed using the SAS statistical package (SAS, 1996). Live weight, fleece weight and ELISA serological data were analysed using GLM procedures, CATMOD procedures were used to analyse pregnancy information and lesion scores were analysed using FREQ procedures.

## RESULTS

### Live weight

Mean live weights for vaccinated and control sheep in the two flocks from the start of the trial are presented in Figures 1 and 2. The mean weight of the Gudair-vaccinated group in the Kimbolton flock was slightly lower than that of the other two groups at all weighing dates, but was only significant (P<0.05) at the most recent weighing in March 2001. No differences have been observed between groups in the Marlborough flock.

**FIGURE 1.** Mean live weights of vaccinated and control sheep in the Kimbolton flock from January 1998 to March 2001.

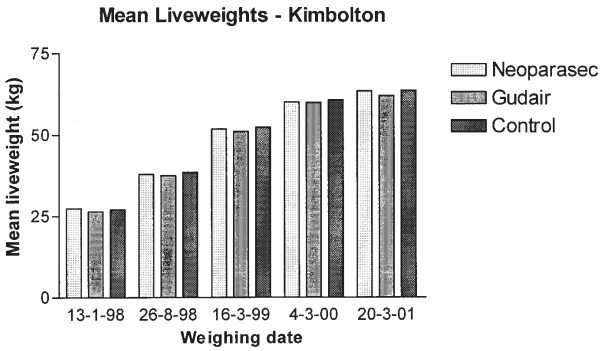
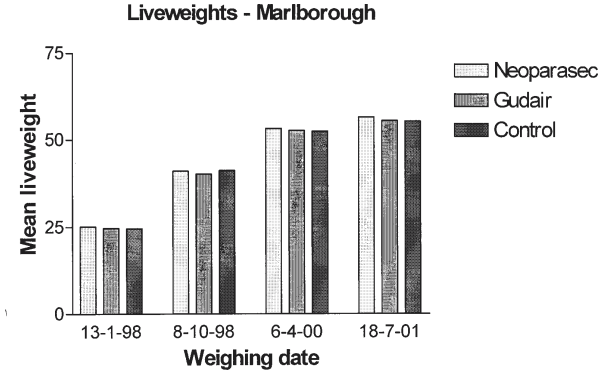


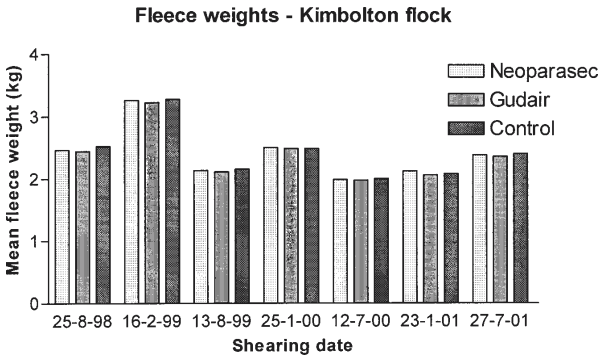

**FIGURE 2.** Mean live weights of vaccinated and control sheep in the Marlborough flock from January 1998 to July 2001.




### Fleece weight

Mean fleece weights for vaccinated and control sheep in the Kimbolton flock from August 1998 to July 2001 are presented in Figure 3. Since the first shearing date in August 1998, when the two vaccinated groups had slightly, but significantly lower mean fleece weights than the control group (P<0.05), no significant differences have been detected.

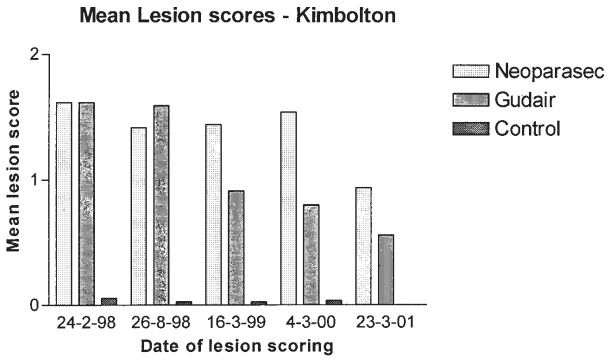
**FIGURE 3**. Mean fleece weights for vaccinated and control sheep in the Kimbolton flock from August 1998 to July 2001.




### Lesion score

Mean scores for vaccination site lesions for sheep on both properties are shown in Figures 4 and 5. In the Kimbolton flock, the mean lesion size for sheep vaccinated with Gudair was significantly smaller than the mean for sheep vaccinated with Neoparasec in March 1999. This difference persisted during the next two years as the lesions declined in size. No significant difference between the two vaccinated groups was observed in sheep on the Marlborough property.

**FIGURE 4.** Comparison of vaccine site lesions in Neoparasec and Gudair-vaccinated sheep in the Kimbolton flock from February 1998 to March 2001.

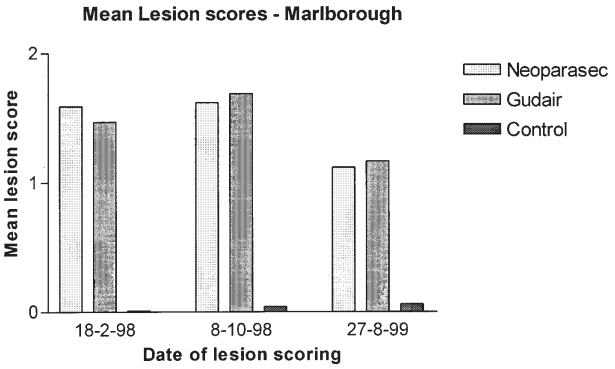



### Reproductive performance

There were no differences in scanning percentages between vaccinated and control groups on either property in 1999, 2000, or 2001.

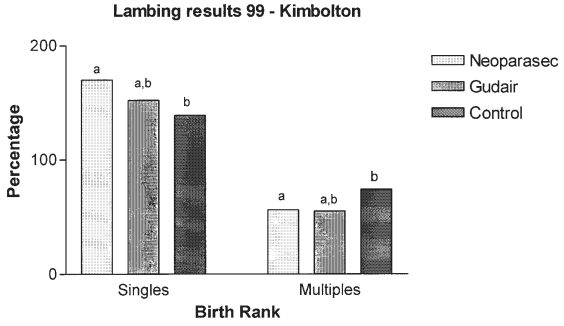
Lambing data for the Kimbolton flock in 1999 is presented in Figure 6. Ewes in the Neoparasec-vaccinated group produced significantly fewer twins than ewes in the control group (P<0.05). The percentage of twins in the Gudair-vaccinated group was also less than that of controls in the 1999 season, but the difference was not

**FIGURE 5.** Comparison of vaccine site lesions in Neoparasec and Gudair-vaccinated sheep in the Marlborough flock from February 1998 to August 1999.




significant. Nor was there a significant difference between the two vaccinated groups.

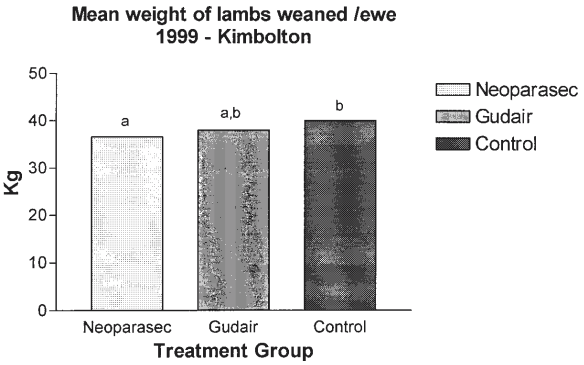
**FIGURE 6.** Lambing results for 1999 season in the Kimbolton flock




The mean weight of lambs weaned per ewe in the 1999 season (Figure 7) was significantly lower in the Neoparasec-vaccinated group than in controls, most likely due to the lower percentage of multiple births.

There were no differences between groups in the percentage of single and multiple lambs, or in the weight of lambs weaned per ewe, in 2000.

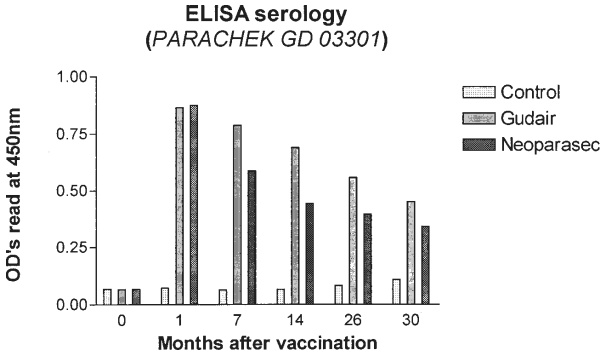
**FIGURE 7.** Mean weight of lambs weaned per ewe in the Kimbolton flock during the 1999 lambing season.




## Serology and gamma interferon assays

ELISA results from a sample of ewes monitored over a 30 month period are presented in Figure 8. The mean ELISA titres in both vaccinated groups peaked one month after vaccination and were significantly higher than in controls. The titres declined more rapidly in ewes vaccinated with Neoparasec and were significantly lower than those in the Gudair-vaccinated group at 14 months. By 30 months, the mean titre of the Neoparasec group was not significantly different from that of the control group.

**FIGURE 8.** Mean ELISA titres in a sample of ewes (initially 20 per group) monitored for 30 months after vaccination.




At 30 months after vaccination, γ-interferon assays in response to Johnin (supplied by CSL) were performed on the same sheep that were tested by ELISA. The results are presented in Figure 9. Ewes vaccinated with Neoparasec showed a greater γ-interferon response than those vaccinated with Gudair. Significant responses were also recorded in some of the control animals, presumably as a result of natural exposure.

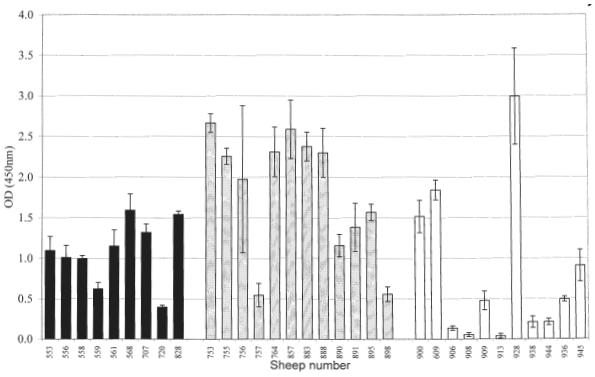
## Post mortem results

A summary of postmortem findings in ewes that either died or were culled for illthrift on the Kimbolton property is presented in Table 1. The table includes 4 ewes that were not part of the trial, but were examined in order to gain additional information on the prevalence of Johne's disease in the flock. Of the 16 ewes examined to date, 7 had advanced lesions of Johne's disease. One of these ewes was in the group that had been vaccinated with Neoparasec, but the others were either from the control group or not included in the trial.from the control group or not included in the trial.

**TABLE 1:** Summary of postmortem findings in ewes that have either died on the Kimbolton property or have been culled after developing illthrift.

| Ewe Number | Age at death | Treatment group | Johne's Disease (+/-) | Comments |
|---|---|---|---|---|
| 986 | 3yrs | Control | -ve | |
| 731 | 3yrs | Control | -ve | |
| 291 | 3.5yrs | Control | +ve | Severe - lepromatous form |
| 854 | 3.5yrs | Neoparasec | -ve | ? Parasitism |
| 242 | 3.5yrs | Neoparasec | -ve | ? Parasitism |
| 362 | 3.5yrs | Neoparasec | -ve | Bearing |
| 921 | 3.5yrs | Control | -ve | ? Parasitism |
| 582 | 3.5yrs | Control | +ve | Mod severe - tuberculoid form |
| 226 | 3.5yrs | Neoparasec | +ve | Severe - lepromatous form |
| Blue 5640 | Aged | Not in trial | +ve | Severe – lepromatous form |
| No tag A | | Not in trial | -ve | ? Parasitism |
| No tag B | | Not in trial | -ve | Footrot/pleurisy/lymphad-enitis |
| CF-Bird | | Not in trial | +ve | Severe - lepromatous form |
| 656 | 4 yrs | Control | +ve | Severe - lepromatous form |
| 120 | 4 yrs | Control | +ve | Severe - lepromatous form |
| 864 | 4 yrs | Gudair | -ve | Cause of illthrift uncertain |

**FIGURE 9.** Gamma-interferon response to Johnin 30 months after vaccination in control sheep and sheep vaccinated with either Neoparasec or Gudair.




A further 22 ewes were culled in August 2001 after being scanned empty. None of these animals were illthrifty and none showed gross or microscopic evidence of either clinical or subclinical Johne's disease.

## DISCUSSION

This trial is now entering its 5th and final year and, to date, there is no evidence to suggest that subclinical Johne's disease is limiting productivity on either property. No consistent differences between control and vaccinated sheep have been detected for growth rate, fleece weight, ewe body weight prior to tupping, scanning percentage or lambs weaned per ewe. In the 1999 season, the mean weight of lambs weaned per ewe on the Kimbolton property, and the percentage of multiples born, were significantly lower in the Neoparasec-vaccinated group than in controls, but this difference was not repeated in other seasons and was most likely an abberation. No differences in scanning percentage have been detected on either property in any of the three seasons.

The trial was also seen as an opportunity to compare the live Neoparasec vaccine for Johne's disease and the killed Gudair vaccine, which was not registered for use in New Zealand when the trial commenced. Both vaccines stimulated a strong humeral immune response, as measured by the "Parachek" ELISA, but the titres of the Neoparasec group declined more rapidly than those of the Gudair-vaccinated animals. By 14 months after vaccination, the mean titre of the Neoparasec group was significantly lower than that of the Gudair group (Figure 8). This does not however imply that Gudair induces more effective immunity to *M. a. paratuberculosis* as cell-mediated immunity is considered to be more important in the defence against this organism. In order to assess the cell-mediated response to the two vaccines, γ-interferon assays were performed 30 months after vaccination on the same animals that were tested by the "Parachek" ELISA. In the γ-interferon assay, the response to Neoparasec was significantly better than that to Gudair (Figure 9), suggesting a better cell-mediated immunity following use of the live vaccine. This does not necessarily mean that Neoparasec induces stronger immunity as the response generated by Gudair may still be sufficient to protect against establishment of infection.

A major disadvantage of the live Neoparasec vaccine is the relatively high prevalence of injection site lesions. One reason for including Gudair in the current trial was to determine whether this killed vaccine induced smaller or fewer lesions than Neoparasec. As shown in Figure 4, the mean lesion score of the Gudair group in the Kimbolton flock declined more rapidly than that of the Neoparasec group. By 15 months after vaccination the mean score of the Gudair group was significantly smaller and the difference was still significant after 39 months. Interestingly, no differences have been detected between mean lesion scores of vaccinated sheep in the Marlborough flock.

The results presented here are not complete as the trial is still proceeding and not all available data has been analysed, but there are indications that the subclinical effects of Johne's disease in sheep may not be as significant as in cattle. It is important to recognise however that only two properties have been included and the level of challenge with *M. a. paratuberculosis* is likely to vary considerably between different properties, as are management and other environmental factors that may influence clinical or subclinical disease.

## ACKNOWLEDGEMENTS

We are indebted to the participating farmers for their cooperation over the last 4 years and to WoolPro for funding the project.

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