Paratuberculosis vaccination and Tb testing trial in red deer

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Abstract

Paratuberculosis or Johne's disease (JD) has emerged as a serious disease of farmed deer in New Zealand and immunisation with an effective vaccine is likely to be the most practical means of controlling it However, for any proposed vaccine it is essential to determine (a) if the vaccine is safe, (b) the degree to which it will cause cross-reactivity with the bovine tuberculin Tb test and (c) to determine whether or not approved ancillary tests can clear false-positives.

This paper summarises the results of a trial, using red weaner deer, that compared (A) a single dose of "Neoparasec" vaccine, a live attenuated vaccine plus an oil adjuvant, which is currently licensed for sheep and cattle and (B) two doses, 6 weeks apart, of a Novel JD vaccine, which does not contain an oil adjuvant

This study showed that both vaccines gave significant increases in johnin/avian tuberculin (PPD) sensitivity in lymphocyte transformation tests and skin tests However, the oil adjuvanted "Neoparasec" vaccine caused moderate injection site reactions and produced high levels of bovine sensitisation as well, with large skin test reactions at both sites in the CCT and high, persistent levels of antibody to avian, johnin and bovine PPD By contrast, the Novel vaccine produced smaller transient injection site lesions, less cross-reactivity and low levels of antibody to bovine, avian and johnin PPD that were similar to those of the unvaccinated Control animals. At 36 weeks after vaccination, over half the deer receiving "Neoparasec" had CCT bovine skin reactions of >4 mm, although in all cases the increase in skin thickness at the avian site was larger than the bovine site By contrast, at 36 weeks after vaccination with the Novel vaccine none of the deer had skin thickness increases >4mm at the bovine site and all the avian sites were greater than the bovine sites.

The oil based vaccine is also likely to cause greater interference with the skin test used in the National Tuberculosis Control Programme and the CCT and BTB may be unable to clear reactors because of the high degree of cross-reactivity The Novel vaccine caused less non-specific sensitisation and the CCT and BTB should be able to identify vaccinated animals as "non-specific reactors" if there is adequate time (ie at least 36 weeks) between vaccination and testing

At slaughter, the majority of vaccinated animals had small nodules or scars trimmed off the necks, but no gross lesions were found in any of the prescapular lymph nodes.

The cell-mediated immune response and the absence of antibody in deer vaccinated with the Novel vaccine is indicative of a protective Type 1 response, whereas the high antibody and cell-mediated response after the oil-based vaccine indicates a mixed Type 1/Type 2 response Our results suggest that two doses of the Novel vaccine should give a protective response without some of the adverse side effects of "Neoparasec"

Introduction

Paratuberculosis or Johne's disease (JD) has emerged as a serious disease of farmed deer in New Zealand and overseas (Mackintosh et al , 1998, 1999, 2000). Agriculture New Zealand undertook a review of JD in New Zealand livestock (Brett, 1998) commissioned by the Chief Veterinary Officer of MAF, and it acknowledges that, of all the farmed animals, the least is known about JD in deer

The first confirmed case of JD in deer was reported in the mid-80s Since then JD has been confirmed on at least 200 deer farms and is suspected to have occurred on many more The JD review estimated that JD costs the NZ deer industry $200-300K per annum based on estimated 1998 figures However, this is likely to be a gross underestimate of the future annual costs, because the disease is spreading and there has been an increasing number of serious outbreaks in deer aged 8- to 15-months-old These outbreaks have generally involved approximately 10% of yearlings in a group and have caused

serious economic losses on affected properties This type of serious outbreak in yearlings demonstrates a significant difference between JD in deer and JD in cattle and sheep, where losses are usually confined to 2 to 4 year old animals and annual mortalities rarely rise above 3% under NZ conditions. Sporadic losses of adult deer have also occurred on farms throughout NZ and it is acknowledged that there is under-reporting of infection. The effects of finding JD on stud farms can also be financially crippling Thus the annual costs of JD are projected to increase significantly Of even greater concern than the direct cost of JD to individual farmers is the potential for JD to be used against New Zealand venison exporters as a non-tariff-trade barrier in the same way that bovine tuberculosis, a closely related disease, might be

The control of JD in cattle and/or sheep is traditionally by test-and-slaughter, hand rearing of newborn offspring in isolation, depopulation or vaccination. Test-and-slaughter relies on sensitive and specific tests for JD Unfortunately JD tests in general are only sensitive late in the disease and are poor at detecting subclinically infected animals This is especially the case in deer. A previous study showed that none of the serological or cell-mediated-based tests were sensitive or specific enough to be of any value in controlling the disease (Mackintosh et al, 1998, 1999) The handrearing of all newborn deer is possible in specialised situations but is not commercially viable or practicable, especially for stags, which become dangerous as adults if handreared Depopulation will control the disease but is usually uneconomic

Prevention is obviously the best option, but once a deer herd is infected with JD then, as with JD in sheep, vaccination is likely to be the most practical means of control. In Scotland, vaccination of deer with a live attenuated M paratuberculosis oil adjuvanted vaccine ("Weybridge" vaccine) has been used successfully to prevent clinical disease and it is believed that, with continued vaccination over an extended period of time, the infection levels within the herd will decline and may approach zero (Goddard et al , 1994) Currently the only JD vaccine available in NZ is "Neoparasec" (Merial NZ Ltd), which is licensed for use in sheep and cattle One dose of this live attenuated M paratuberculosis oil adjuvanted vaccine has been shown to give significant protection against clinical disease in sheep However, there are problems in sheep associated with lesions at the injection site in the neck and the draining lymph node in the shoulder, which devalue the carcass and create a significant impediment to the export of carcasses from vaccinated animals Some of these problems are alleviated by delaying vaccination from tailing (the preferred time for maximal immune protection) to weaning, when mostly replacement stock are vaccinated

A Novel vaccine, containing a live attenuated strain of M. paratuberculosis, but not containing an oil adjuvant, is currently undergoing field trials in sheep and the results to date look promising. The same Novel vaccine is used in the present study in deer. One of the objectives of this study was to compare the local tissue reactions at the injection site and draining lymph node for "Neoparasec" and the Novel vaccine

An additional problem faced by the cattle industry is that the "Neoparasec" vaccine can cause sensitisation of the cattle to the skin test for Tb, because of the antigenic similarities between M. bovis, M avium and M. paratuberculosis (which is considered to be a subtype of M avium). Currently AgriQuality/Animal Health Board approval is necessary before "Neoparasec" can be used in cattle. A second objective of the current trial was to investigate the potential of JD vaccines to interfere with the test single intradermal skin test for Tb and to test the ability of the comparative skin test (CCT) and the Blood Test for Tb (BTB) to clearly identify vaccinated animals as "avian (or non-specific) reactors"

The third objective was to use these tests to monitor the immune response of animals to vaccination to indicate the likelihood of protection to natural challenge with JD To the best of our knowledge these animals did not receive any artificial or natural challenge with M paratuberculosis.

Ideally the current "Neoparasec" vaccine, which is a single dose, should be administered to animals as young as possible to assist them develop an immunity prior to receiving significant *M. paratuberculosis* challenge, which is assumed to be primarily once the animal is eating substantial amounts of pasture. In lambs and calves it is recommended to vaccinate at 2-4 weeks of age, although the vaccination of lambs at weaning (~12 weeks of age) does give significant protection against

disease. Waiting till the animals are 12 weeks old allows the replacement animals to be selected for vaccination and largely avoids the vaccination of lambs destined for slaughter The vaccination of deer fawns less than 6-12 weeks of age is not practicable on most farms and therefore this trial was conducted shortly after the red deer calves were weaned at 12 weeks of age

Materials and Methods

Animals: A group of 45 newly weaned red deer were randomly allocated to three groups of 15 animals.

- Group A: "Neoparasec" - vaccinated with a single dose of "Neoparasec" vaccine on Day 0.
- Group B: Novel - vaccinated with two doses, 6 weeks apart, of the Novel vaccine.
- Group C: Control - unvaccinated controls

All vaccinations were given by subcutaneous injection in the right side of the neck.

The animals underwent the following schedule of events.

Day -13	Blood sampled and weighed
Day 0	Weighed, allocated to groups and Groups A and B were vaccinated
Week 3	Weighed and injection sites measured
Week 6	Blood sampled, weighed, injection sites measured and Group B boosted.
Week 9	Injection sites measured
Week 12	Blood sampled, weighed and skin tested (MCT see below)
Week 12	MCT Read
Week 15	Blood sampled and weighed
Week 24	Blood sampled, weighed and skin tested (First CCT see below)
Week 24	Blood sampled.
Week 27	Blood sampled and weighed
Week 36	Blood sampled, weighed and skin tested (Second CCT).
Week 39	B/S and weigh
Week 42	Slaughter through DSP, necropsy, histopathology and culture all deer.

The Blood Test for Tuberculosis (BTB) was done on blood samples taken on Day -13, and Weeks 6, 12, 15, 24, 27, 36 and 39 Johnin PPD was used in addition to the normal avian and bovine PPD as antigens in the lymphocyte transformation (LT) and ELISA assays

The mid-cervical skin test (MCT) was performed at Week 12 using a 0.1 ml intradermal injection of 1mg/ml Wallaceville Bovine PPD at a closely clipped site on the left side of the neck.

The comparative cervical skin test (CCT) was performed at Weeks 24 and 36 using intradermal injections of 0.5 mg/ml Wallaceville Avian PPD and 1mg/ml Wallaceville Bovine PPD at an upper and lower closely clipped site on the left side of the neck, away from areas used in previous tests.

Lesions trimmed from the carcasses at slaughter were described grossly and then fixed in formalin and examined histopathologically The prescapular lymph nodes from both sides of the carcass were removed, cleaned of excess fat, weighed and then incised an closely examined

Results

Injection site lesions

The "Neoparasec" vaccine produced hard lumps 8 – 23 mm diameter (mean 16 8 mm) three weeks after injection and these remained as hard lumps averaging 14.6 mm at nine weeks after injection. The Novel vaccine produced hard nodules 9 – 15 mm (mean 11 5 mm) at three weeks and these declined to 5 – 10 mm (mean 7 6 mm) by six weeks after primary vaccination Booster Novel vaccination produced similar reactions with a mean of 10 4 mm three weeks after injection.

The majority of "Neoparasec" vaccinated animals and a few of the Novel vaccinated animals still had a small lump or nodule at the injection site, ten months after vaccination.

Skin test reactivity

At the MCT (see Table 1), all the animals receiving the "Neoparasec" vaccine had obvious reactions, with skin thickness differences of 1 3 – 7 0 mm (mean 3 9 mm) The animals receiving the Novel vaccine animals had skin thickness differences of 0 2 – 4 3 mm (mean 2.9 mm), and five of these 14 animals had increases of <2 mm The Controls had skin thickness differences of -0 7 – 2 4 (mean 1 0)

At the first CCT, there were very strong avian reactions in most vaccinated animals

Group A. The "Neoparasec" vaccine animals had mean avian reactions of 12 5 mm and bovine reactions of 7 3 mm (see Table 2) Fourteen of the 15 animals had bovine increases 2 mm or greater. Twelve of these 14 animals had avian>bovine reactions and two had equal avian and bovine increases (ie these two animals were CCT positive, according to the standard interpretation)

Group B The Novel vaccine animals had mean avian reactions of 5 8 mm and bovine reactions of 3 9 mm Eleven of the 14 had bovine PPD site increases 2 mm or greater. Nine of these 11 animals had avian>bovine reactions and two had equal avian and bovine increases (ie these two animals were technically CCT positive)

Group C· The Controls had mean avian reactions of 0 8 mm and bovine reactions of 0 7 mm Two animals had bovine reactions 2 mm or greater and one of these had similar avian and bovine reactions of 3.7 and 3 6 mm respectively (ie this animal was CCT positive according to the standard interpretation).

At the second CCT, the level of reactivity in vaccinated animals had declined

Group A. "Neoparasec" vaccine animals had mean avian reactions of 8.6 mm and bovine reactions of 4 6 mm. Fourteen of the 15 animals had bovine increases 2 mm or greater and all had avian>bovine reactions

Group B Novel vaccine animals had mean avian reactions of 3.3 mm and bovine reactions of 1 8 mm. Four of the 15 had bovine increases 2 mm or greater and all had avian>bovine reactions.

Group C. The Controls had mean avian reactions of 1 0 mm and bovine reactions of 0.6 mm and all had avian>bovine reactions

Table 1. Skin test results· Group mean double skin thickness differences in mm when read at 72 hours after intradermal injection with bovine (1 mg/ml) or avian (0 5 mg/ml) PPD

	MCT week 12	CCT week 24		CCT week 36	
	Bovine diff.	Avian diff.	Bovine diff.	Avian diff.	Bovine diff.
"Neoparasec"	3.9	12 5	7 3	8 6	4.6
Novel	2 9	5 8	3 9	3 3	1 8
Control	1 0	0 8	0 7	1 0	0 6

Lymphocyte transformation (LT)

The entire group of animals, which originated from the Invermay deer herd, had low levels of bovine and moderate levels of avian reactivity at the start of the trial and these levels were maintained through much of the trial in the controls (see Table 2) The vaccinated animals had high avian/johnin reactivity that was much greater than the bovine levels on most occasions, but on Week 24 there were transient "positive" bovine LT results in 9/15 "Neoparasec" and 7/14 Novel animals due to unexpectedly low Johnin responses by comparison with results at the previous and next sampling A number of control animals had low "positive" bovine LT results at Weeks 12, 15, 24 and 36, but there was no bovine antibody and the MCT and CCT tests remained negative, suggesting non-specific background sensitisation. Prior to the trial starting, two animals from the same source had low bovine reactivity on the LT, but they had no antibody and were negative on a CCT. They were slaughtered as

a precaution and they were NVL and four pools of lymph nodes were culture negative These results are consistent with some environmental organisms causing non-specific bovine reactivity

Group A "Neoparasec" vaccine animals had the highest and most persistent levels of johnin and bovine PPD sensitivity in the LT assay (see Figs 1 and 2 and Table 2). Bovine LT levels appeared to be declining at Week 36.

Group B Novel vaccinated animals moderate johnin sensitisation and low bovine sensitisation in the LT (see Figs 1 and 2 and Table 2) Bovine LT levels appeared to be declining at Week 36

Group C controls maintained low mean levels of johnin and bovine sensitisation (see Figs 1 and 2 and Table 2)

The avian results were very similar to the johnin results and are not presented here

Table 2. Lymphocyte transformation results for the three groups using the standard interpretation for the BTB, but using johnin PPD for this analysis

Group	LT result*	Week 0	Week 6	Week 12	Week 15	Week 24	Week 36
"Neoparasec"	No Johne's positive	9	11	10	15	1	9
N = 15	No Bovine positive	0	0	0	0	9	0
	No equivocal or no data	0	0	4	0	5	4
	No. negative	16	4	1	0	0	2
Novel	No Johne's positive	5	13	12	13	1	9
N = 14	No Bovine positive	0	0	0	0	7	0
	No equivocal or no data	2	0	0	1	2	2
	No negative	7	1	2	0	4	4
Control	No Johne's positive	5	7	8	5	2	0
N = 14	No Bovine positive	0	0	2	4	1	4
	No equivocal or no data	0	0	1	1	4	0
	No negative	9	7	3	4	7	10

*Discriminant equation y = bovine cpms/johnin cpms – 1 0
Johne's positive y > -0 1
Bovine positive y > 0 1
Equivocal 0 1 > y > -0 1
Negative Bovine or Johnin < 2 5 x the negative control (50 – 5,000 cpms)

Fig.1: Mean Johnin LT assay counts for "Neoparasec" (Np), Novel and Control Groups

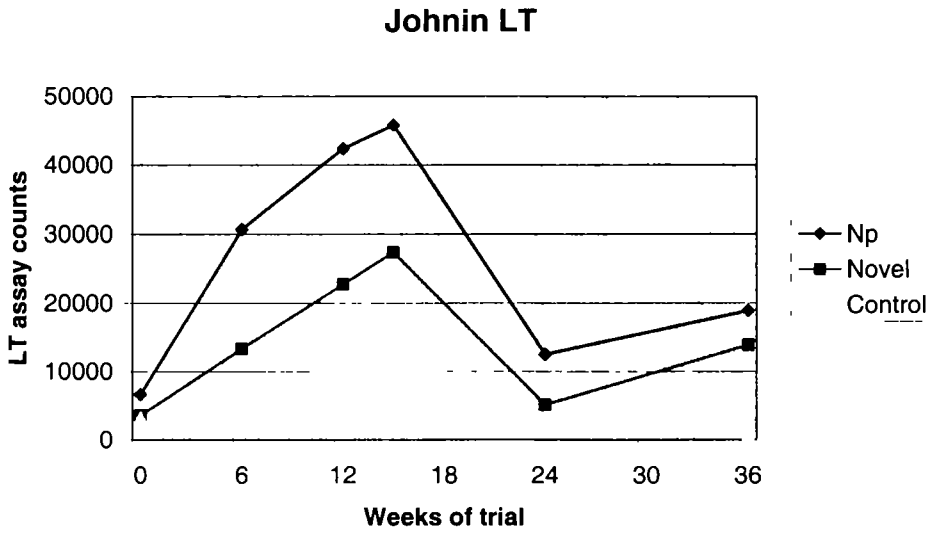
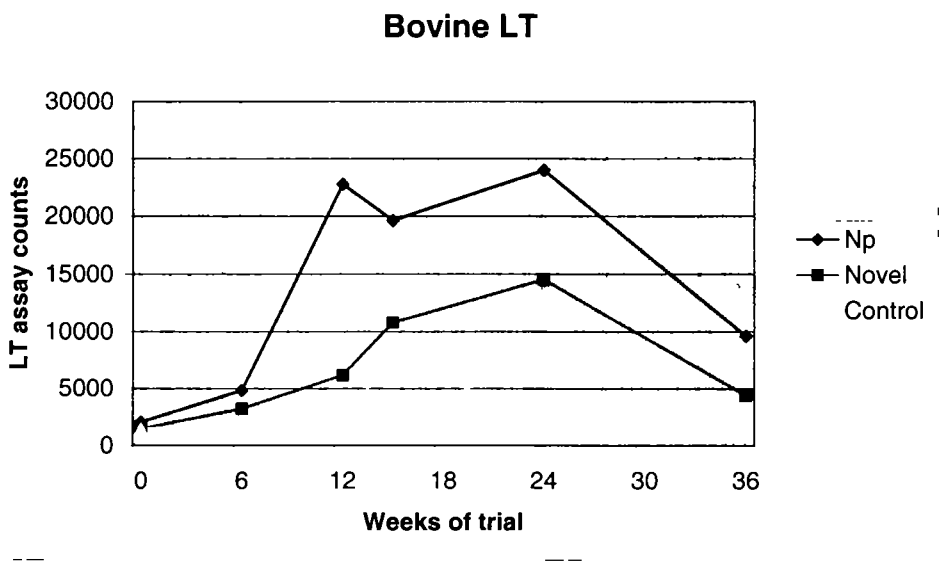


Fig.2: Mean Bovine LT assay counts for "Neoparasec" (Np), Novel and Control Groups.



ELISA

After the start of the trial all three groups had a rise in antibody reactivity against avian, bovine and johnin PPD antigens with the "Neoparasec" group the highest at all time points There were no apparent differences between the mean ELISA values of the Novel and Control groups throughout trial By contrast the mean ELISA value of the "Neoparasec" group initially peaked at 12 weeks, declined slightly and then continued to climb again to reach a maximum at Week 36 (see Figs 3 and 4) After vaccination the "Neoparasec" group had increasing numbers of animals that were bovine ELISA positive or equivocal, peaking at 10 positives and 5 equivocals at Week 27 and then declining to two of each at Week 36 (see Table 3) Neither the Novel nor the Control group had any bovine positives throughout the trial, although the former had one equivocal at Weeks 27 and 36 and the latter had an equivocal at Week 6

Fig. 3. Mean Bovine PPD ELISA optical densities for "Neoparasec" (Np), Novel and Control Groups

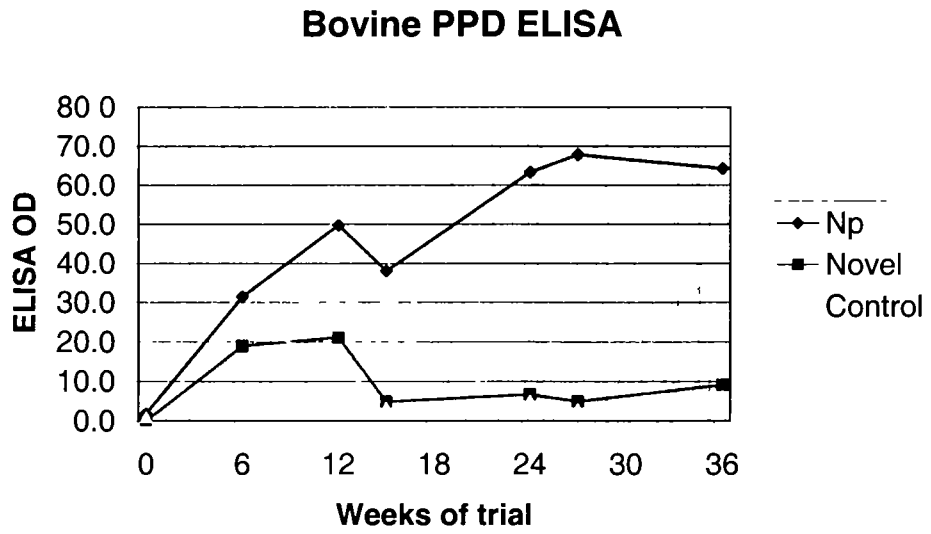


Fig. 4: Mean Johnin PPD ELISA optical densities for "Neoparasec" (Np), Novel and Control Groups.

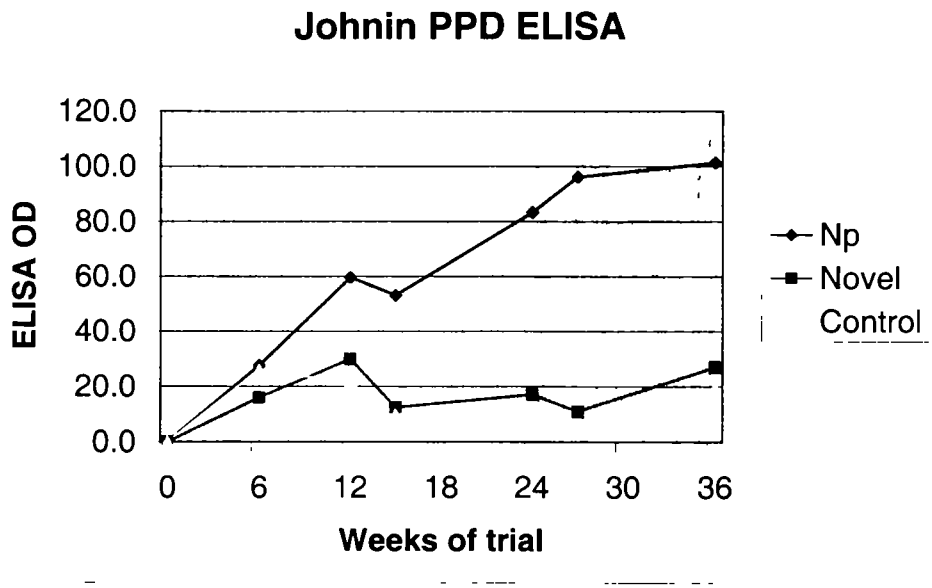


Table 3. Number of animals in each group that had a bovine ELISA test result interpreted as positive or equivocal.

Group	ELISA result*	Week 0	Week 6	Week 12	Week 15	Week 24	Week 27	Week 36
"Neoparasec" N = 15	No positive	0	1	5	8	11	10	2
	No equivocal	0	2	2	4	3	5	2
	No negative	15	12	8	3	1	0	11
Novel N = 14	No positive	0	0	0	0	0	0	0
	No equivocal	0	0	0	0	0	1	1
	No negative	14	14	14	14	14	13	13
Control N = 14	No positive	0	0	0	0	0	0	0
	No equivocal	0	1	0	0	0	0	0
	No negative	14	13	14	14	14	14	14

*Standard interpretation for the BTB ELISA Positive where ELISA (Bovine) Optical Density (OD) – ELISA (Avian) OD was =/> 20
Equivocal where ELISA (Bovine) OD – ELISA (Avian) OD was =/> 10 but < 20

Discussion

"Neoparasec" vaccination resulted in moderate injection site lesions that were more persistent than the Novel vaccination site lesions, which were smaller and regressed more quickly "Neoparasec" vaccination resulted in higher LT, MCT, CCT and ELISA reactivity than two doses of the Novel vaccination All the vaccinated animals reacted to the MCT at 12 weeks. At the CCT at 24 weeks there were two "Neoparasec", two Novel and one of the control animals that had positive bovine reactions (ie >2 mm and A=B responses) However, by 36 weeks the skin test reactivity had declined and 14/15 "Neoparasec", 4/14 Novel and 0/14 control animals had bovine reactions 2 mm or greater, but in all cases the avian sites were greater than the bovine sites The non-specific reactivity declined with time but the risk of false positive reactions appears to be higher with the "Neoparasec" vaccine due to higher and more persistent bovine sensitisation

"Neoparasec" vaccination also resulted in high persistent bovine Tb antibodies, compared with negligible levels in the Novel vaccinated animals after 15 weeks This has implications for testing with the BTB animals vaccinated with "Neoparasec", because bovine antibody is the hallmark of disease in *M. bovis* Tb in deer

On the basis of these results it appears that the Novel vaccine, given as two doses 6 weeks apart to weaners, gives measurable cell-mediated responses that persist for over 36 weeks, but produces negligible bovine antibody when compared with the controls It appears that the risk of equivocal CCT results diminishes with time and false positives are unlikely after 36 weeks

It is probably unwise to use the "Neoparasec" vaccine in farmed red deer because of the prolonged antibody levels and the increased risk of equivocal CCT results, as well as the larger and more persistent injection site reactions

In terms of putative protection against Johne's disease, it appears that the Novel vaccine stimulates a Type 1 (Th1) response, ie good T-cell response (LT) and no B-cell response (antibody) Classically this is the type of response that is protective against Mycobacteria By contrast, "Neoparasec", which has an oil adjuvant, stimulates a mixed Type 1/Type 2 response, resulting in not only cell-mediated responses, but also high levels of antibody that are not considered protective against Mycobacteria Our results suggest that two doses of the Novel vaccine should give a protective response without some of the adverse side effects of "Neoparasec"

Acknowledgements

The authors would like to acknowledge the assistance of Tony Pearse, the Invermay deer farm staff and Ian Spencer who helped with the farm work, of the Disease Research Laboratory staff, Otago University for the BTB assays, of AgResearch Wallaceville staff for Johne's culture and editorial advice, Gary Clark for the histopathology and Peter Johnstone for biometrics The trials were funded by the Foundation for Research Science and Technology The trial was approved by the Invermay Animal Ethics Committee

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