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Pilot field trial of a recombinant *Taenia ovis* vaccine in lambs exposed to natural infection

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Abstract

Previous trials of an experimental *Taenia ovis* vaccine using the recombinant antigen GST–45W(B/X) established that it was possible to achieve >90% protection against a single artificial challenge of *T. ovis* eggs. This trial was undertaken to assess vaccine efficacy against artificial challenge and natural infection acquired by lambs grazing contaminated pasture. Two hundred Romney lambs were vaccinated at 6 and 12 weeks of age. One hundred control lambs were not vaccinated but were allowed to run with the vaccinated mob. At 15 weeks of age, 10 controls and 18 vaccinated lambs were artificially challenged with 2000 *T. ovis* eggs. The remaining control and vaccinated lambs were allowed to graze contaminated pasture for 3 weeks and were then moved to clean pasture for 5 months. The artificially challenged lambs plus 24 of the field-infected lambs were slaughtered and the carcasses dissected to obtain cyst counts. The remaining field-infected lambs were slaughtered at a commercial processing plant and the carcasses examined by conventional meat inspection.

The results showed that the vaccine provided a high level of protection against artificial challenge (92%) and natural infection (98%) when assessed by carcass dissection.

The data from commercial meat inspection showed that vaccination provided 89% efficacy against downgrading or condemnation compared to non-vaccinated control lambs. The average difference in carcass values between vaccinated and non-vaccinated groups was $4.36, representing a 35% loss in value due to *T.ovis* infection in non-vaccinated lambs.

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Introduction

Taenia ovis is a cestode parasite existing as an adult tapeworm in dogs and as an encysted larva in sheep and goats. The cystic stage is of economic importance to the New Zealand meat industry because its presence in the musculature ("sheep measles") results in downgrading or condemnation of carcasses.

Taenia ovis has been subject to a control programme in New Zealand since 1970, including dosing of rural dogs with a cestocidal drug, serological monitoring of dogs and meat inspection of sheep carcasses.

The expression by a cloned gene of a *T. ovis* host-protective antigen[1] which gave greater than 90% protection against experimental challenge meant that immunisation of lambs could be considered as an adjunct to other control procedures. A process for the commercial manufacture of the vaccine has been described[2]. Here we describe a pilot scale trial to test vaccine efficacy in lambs exposed to naturally acquired infection from heavily contaminated pasture or given a single artificial challenge. Vaccine efficacy was assessed by conventional meat inspection at a commercial meat processing plant or by dissection.

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Materials and Methods

Vaccine

The vaccine was prepared from the fusion protein GST–45W(B/X) obtained from solubilised inclusion bodies of genetically modified *Escherichia coli* as described elsewhere[2]. The vaccine was formulated to contain 100 μg of antigen and 1 mg of saponin adjuvant (Quest International Ltd) per 1 ml vaccine dose.

Trial protocol

At 6 weeks of age, 300 Romney lambs were randomly sorted into one group of 200 and one group of 100 lambs. All lambs were identified by ear tagging and the 200 lamb group was vaccinated with 1 ml of vaccine by subcutaneous injection into the dorsal neck region. The 100 lamb control group was not vaccinated but was run with the vaccinated group. At 12 weeks of age, the 200 lambs were re-vaccinated as described above. One hundred control lambs and 185 vaccinated lambs were transported from the Flock House research farm, Bulls to the Kaitoke research farm, Upper Hutt 1 week later. One vaccinated lamb died in transit.

At 15 weeks of age, 10 control lambs and 18 vaccinated lambs were given a single oral challenge infection of 2000 mature *T. ovis* eggs. All the other lambs were moved on to pasture previously contaminated with *T. ovis* eggs. This was achieved by housing two infected dogs on the paddock for 1 week before the lambs were allowed to graze. Each dog had been infected 8 weeks earlier with two cysts obtained from an infected sheep and both were excreting eggs. The dogs were moved daily to different sites around the paddock to increase the spread of eggs.

Lambs were allowed to graze contaminated pasture for 3 weeks before being moved to clean pasture. Five control and five vaccinated lambs were slaughtered 9 weeks after initial exposure to field infection and their carcasses examined by dissection as follows. Cyst counts were obtained for each carcass by finely slicing the heart,

diaphragm, masseter muscles and the skeletal muscles from half the carcass. Five control and ten vaccinated lambs that had been artificially challenged were slaughtered 6 weeks after infection and the carcasses examined as described above. Five control and eight vaccinated lambs artificially challenged and seven control and seven vaccinated lambs exposed to field infection were slaughtered 5 months after infection and carcasses examined as described above. During this 5-month period, six lambs lost their ear tags and were excluded from the trial and three lambs did not reach an acceptable weight for commercial processing.

The remaining field-infected lambs were slaughtered at a commercial meat processing plant 5 months after infection. At the plant, the carcasses and offal were examined at normal chain speed (7 seconds per carcass), with each group of lambs processed at different times on the same day, for ease of identification. After inspection, hearts were collected for dissection and counting of cyst numbers.

Antibody response

Blood samples were taken from ten control and ten vaccinated lambs on the days of first vaccination, revaccination and on the day of infection. The sera were stored at -20 °C until assayed by ELISA to assess antibody response following vaccination. Microtitre plates were coated with 10 µg/ml of *T. ovis* antigen 45W(B/X) prepared from the recombinant fusion protein cleaved with thrombin to remove the GST fusion protein[3]. After blocking and washing the plates, sera diluted 1000-fold were reacted with the antigen for 2 h. Unbound antibody was removed by washing and bound antibody was detected by addition of peroxidase-conjugated rabbit anti-sheep IgG (Cappel) and the substrate tetramethylbenzidine. Absorbance was read at 450 nm.

Table I. Cyst numbers in control and vaccinated sheep slaughtered 9 weeks after initial field infection or 6 weeks after artificial challenge

Group	Cyst counts per sheep					Mean	% protection[ab]
Field infection							
Controls	36	46	65	95	96	68	
Vaccinated	0	0	0	0	0	0	100**
Artifical challenge							
Controls	106	141	178	196	254	175	
Vaccinated	0	0	0	0	0		
	0	0	0	0	203	20	88**

a % protection expressed as $\left[\frac{C-T}{C}\right] \times 100$ where C = mean number of cysts in control group and T = mean number of cysts in test group.

b ** = significantly different from the respective controls ($p<0.01$, Mann-Whitney test, two-tailed).

Table II. Cyst numbers in control and vaccinated sheep slaughtered 5 months after field infection or artificial challenge

Group	Cyst counts per sheep								Mean	% protection[ab]
Field infection										
Controls	17	18	35	43	82	268	348		116	
Vaccinated	0	0	0	0	3	8	11		3	97**
Artifical challenge										
Controls	74	134	144	152	182				137	
Vaccinated	0	0	0	0	0	0	0	8	1	99**

a % protection expressed as $\left[\frac{C-T}{C}\right] \times 100$ where C = mean number of cysts in control group and T = mean number of cysts in test group.

b ** = significantly different from the respective controls ($p<0.01$, Mann-Whitney test, two-tailed).

Results

Cyst counts from control and vaccinated lambs slaughtered 6 weeks after artificial challenge or 9 weeks after initial exposure to field infection are shown in Table I. The results show that vaccination was highly effective against either method of infection, with 14 out of 15 vaccinated lambs free from infection. One vaccinated lamb was not protected against artificial challenge.

Cyst counts from control and vaccinated lambs slaughtered 5 months after artificial challenge or field infection are shown in Table II. Eleven of 15 vaccinated lambs were free from infection and vaccination reduced infection by 97% overall.

The distribution of cysts in muscle tissues of control sheep exposed to field infection is shown in Table III. This result shows that all the control carcasses contained cysts in the skeletal muscles, diaphragm and masseter muscles, as well as the heart.

Table IV shows the data provided by MAF meat inspectors at the commercial meat processing plant, together with the cyst count data obtained by fine slicing hearts recovered at the processing plant. Of the 76 control lambs examined, 75 had at least 1 cyst in either the carcass or offal during inspection. In

Table III. Distribution of cysts in control sheep exposed to field infection

	Cyst numbers				
Sheep number	Heart	Masseters	Diaphragm	Muscle[a]	Total
218	435	7	5	27	82
224	5	3	7	20	35
229	6	3	1	8	18
230	18	15	19	44	96
231	14	5	10	14	43
240	8	7	11	20	46
243	5	4	4	4	17
259	120	100	85	43	348
272	40	28	56	144	268
283	13	9	12	61	95
288	10	0	8	18	36
297	19	3	16	27	65
Total	301	234	184	430	1149

a = Skeletal muscles from half the carcass.

Table IV. Numbers of control and vaccinated sheep with *T. ovis* infection detected by MAF meat inspectors at a commercial processing plant, and proportion of infected hearts

	Controls	Vaccinated	% efficacy[a]
Number of carcasses	76	149	
C. ovis detected[b]	75	2	85
Downgraded[b]	15	5	89
Condemned[b]	17	2	
Number of hearts	75	146	
Number of infected hearts	75	19	
Total cysts in hearts	1267	136	87
Mean cysts per heart	16.9	0.9	

a Efficacy = $\left[\frac{C-V}{C}\right] \times 100$ where C = % of controls affected; V = % of vaccinates affected

b Definitions: detected = a *C.ovis (T.ovis)* cyst was seen in either the carcass or offal. Downgraded = 1–6 cysts were cut from the carcass. Condemned = too many cysts to cut out, carcass discarded.

Table V. Summary of processing sheets for control and vaccinated lines

	Controls	Vaccinated
Number of carcasses	76	149
Downgraded due to *T. ovis*	15 (20%)	5 (3%)
Downgraded due to other causes[a]	4 (5%)	8 (5%)
Condemned due to *T. ovis*	17 (22%)	2 (1%)
Condemned, other causes	0	0
Total gross value	$879.24	$2263.38
Net return	$701.55	$2107.18
Average carcass weight	12.1 kg	11.8 kg
Average net value per carcass	$8.21	$12.57

a For example, arthritis, wounds, pleurisy, pyogenic lesion.

contrast, this was the case in only 23 out of 149 vaccinated lamb carcasses. Fifteen control carcasses (20%) were downgraded and 17 were condemned (22%), compared with five carcasses downgraded (3%) and two condemned (1%) from the vaccinated group. Fine slicing hearts showed that all the hearts from control lambs were infected, compared with 13% infected from the vaccinated group.

Vaccination reduced the numbers of lambs found infected with *T. ovis* by 85%, reduced downgrading and condemnation by 89%, and reduced the number of infected hearts by 87%.

A summary of the inspection data provided by the plant is shown in Table V. The average net value of control carcasses was $4.36 less than that of carcasses from vaccinated lambs.

Antibody responses of 10 lambs randomly selected from the control group showed that none of the control lambs had antibody to the recombinant antigen 45W(B/X). The mean absorbance value for control sera taken on the day of challenge was 0.01. Antibody responses of 10 lambs randomly selected from the vaccinated group showed that nine out of ten lambs had an absorbance value 0.7 and one lamb had a value of 0.3 in sera taken on the day of challenge.

Discussion

The results of carcass examinations carried out 6 weeks and 5 months after challenge confirmed that the vaccine was highly effective in providing protection against artificial challenge and equally effective in protecting against infection acquired by grazing pasture heavily contaminated with *T. ovis* eggs. This result validates the artificial challenge method used to assess immunity in previous trials [1][2]. The use of infected dogs to contaminate the pasture with *T. ovis* eggs proved to be very successful. All the control lambs became infected, thus ensuring that the vaccine was subjected to a stringent efficacy test, as the vaccinated lambs were exposed to the same level of infection as the controls.

At the processing plant, meat inspectors recorded that 99% of controls were infected and 42% were either downgraded or condemned because of *T. ovis* infection. The remaining control carcasses (57%) were processed without penalty. Previous work[4] has shown that carcasses can contain substantial numbers of cysts without these cysts necessarily being exposed on the surface of the carcass. Thus, even with stringent meat inspection, some infected carcasses may not be detected. The data obtained in this trial supports these observations, because dissecting the muscles of twelve randomly selected control sheep exposed to field infection revealed that they all contained many cysts. At the plant, only 32 of 76 controls were recorded as having cysts in the carcass. One benefit of vaccination could be to reduce or eliminate these "hidden" cysts in carcasses and provide greater assurance of meat quality.

There was a clear economic benefit of vaccination in this instance, where the average difference in value was over $4 per carcass in favour of the vaccinated group. The main contributing factor to lost revenue in the control group was from *T. ovis* infection, where 22% of carcasses were condemned and 20% were downgraded. This loss, plus the condemnation charge of $4.05 per carcass, amounted to over $400 for the 76 control carcasses processed. This result demonstrates that vaccination can be cost-effective under conditions where lambs are vaccinated prior to exposure to a heavy infection. Consequently, vaccination could be effective in eliminating "cysticercosis storms"[5].

Meat inspection data showed that vaccination did not give complete protection to all lambs. When a random sample of vaccinated lambs was analysed for antibody response, it was found that one out of ten lambs had not responded to the same degree as the others. If the same proportion of the other sheep in the vaccinated group also had a poor antibody response, this could explain why they were not fully protected, because antibody response is correlated with protection[6]. Poor antibody response could result from either errors in injection technique at the time of vaccination or from genetic restriction of the immune response to a single polypeptide antigen [7].

The results presented show that the *T. ovis* vaccine provided substantial protection (greater than 85%) against infection acquired from grazing contaminated pasture, under controlled conditions. It remains to be determined what level of efficacy can be achieved in the field, where there would be less control over vaccination technique and the lambs would be exposed to variable intake of parasite eggs over a longer period.

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