

IMMUNIZATION OF SHEEP AGAINST THE LARVAL STAGE OF *TAENIA MULTICEPS*

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ABSTRACT

VERSTER, ANNA & TUSTIN, R.C., 1987. Immunization of sheep against the larval stage of *Taenia multiceps*. *Onderstepoort Journal of Veterinary Research* 54, 103-105 (1987)

A trial with 200 ewes and 127 lambs showed that vaccination of lambs with 2 doses of Oncosphere Secretory Antigen (OSA) 4 weeks apart, at either 4-8 and 8-12 weeks or at 12-16 and 16-20 weeks of age, was equally effective in protecting them against infestation with the larval stage of *Taenia multiceps*. In this trial, the lambs of ewes, vaccinated with OSA when they were 90 and again 120 days pregnant, were as susceptible to infestation as the lambs of untreated control ewes. Another trial to provoke passive immunity in lambs gave inconclusive results. In a 3rd trial 2 doses of regular OSA and 2 doses of freeze-dried OSA protected all the lambs in each group, while a single dose of regular OSA protected 9 out of 10 lambs against cerebral lesions.

INTRODUCTION

Rickard & Bell (1971) reported that Oncosphere Secretory Antigens (OSA), derived from activated oncospheres of *Taenia ovis*, protected sheep against infestation with the metacestodes of this worm, and Rickard & Adolph (1976) used a similar vaccine to protect calves against infestation with the larval stage of *Taenia saginata*. Rickard, Boddington & McQuade (1977) found that vaccination of pregnant ewes with OSA prepared from oncospheres of *T. ovis* conferred immunity to their lambs, thus confirming the findings of Rickard & Arundel (1974) that ewes naturally infested with *T. ovis* conferred immunity to their lambs via the colostrum. Edwards & Herbert (1982) found that antigen prepared from oncospheres of *Taenia multiceps* protected lambs against infestation with the larval stage of this cestode. The antigen they used was stored at -70 °C and concentrated by freeze-drying.

These findings were confirmed by Verster & Tustin (1982), who found live cerebral coenuri in 5 out of 30 vaccinated sheep while 8 out of 11 untreated controls were infested in the same way.

This paper reports on 3 trials with OSA derived from *T. multiceps* which were conducted to determine whether passive immunity was conferred on the lamb after vaccination of the ewe, the age at which lambs should be vaccinated and, finally, whether the vaccine can be freeze-dried for storage.

MATERIALS AND METHODS

Origin of the eggs

Coenuri of *T. multiceps* from naturally infested sheep originating from Middelburg, Cape Province, were used to infest dogs. Each dog was fed 50-80 scolices and necropsied 6-12 weeks later for recovery of the cestodes. At necropsy, the detached gravid proglottids in the rectum, as well as 10-12 terminal proglottids of each worm, were collected for preparation of the antigen or for infesting sheep. The gravid proglottids were rinsed in physiological saline and placed in a small open Petri dish which, in turn, was placed in a large covered Petri dish lined with moist cotton wool. The proglottids were stored in a refrigerator at 4 °C until they were required. They were subsequently macerated in a small (40 ml) blender to liberate the ova, which were then washed and sterilized as described by Heath (1973).

Preparation of the antigen

Antigen was prepared as described by Rickard & Bell (1971), except that NCTC 135¹ and not Medium 858 was used as the culture medium. In place of foetal serum, serum from sheep known to be free of *T. multiceps* was added to the culture medium. The antigen was stored at -20 °C until it was required.

The antigen used in Trial 3 was freeze-dried 48 h before it was used after reconstitution with sterile water.

Vaccination of the animals

This is described separately under each trial.

Necropsy

The animals were killed by exsanguination.

The brain was removed from the skull and the surface examined for superficial coenuri. The brain was then sectioned at 5 mm intervals for locating coenuri in its substance. The masseters, tongue, diaphragm, heart and lungs were examined for lesions caused by dead metacestodes.

Trial 1 Vaccination of pregnant ewes and lambs of different ages

Materials and Methods

Experimental animals

Ewes

A group of 200 Merino and Merino-cross ewes were flushed by feeding them *inter alia* with lush green oats for 4 weeks before teaser rams were run with them to synchronize their oestrus cycles. When the ewes commenced showing evidence of oestrus, the teaser rams were replaced by 24 intact rams. Eight of these rams were introduced into the flock on a rotational basis for 24 h and then rested for 48 h before rejoining the flock. These rams were fitted with markers and every morning and afternoon the ewes that had mated were removed to a separate camp.

The ewes were divided into 2 groups (A and C) of 100 animals each with the aid of tables of random numbers. The ewes in Group A were injected subcutaneously with OSA when the majority of them were 90 days pregnant and again after 30 days. The ewes in Group C were not treated and acted as control. Although the ewes started lambing during the last half of January, 87 % of the lambs in Group A and 89 % in Group C were born between 10 February and 10 March 1981.

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Received 12 January 1987—Editor

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TABLE 1 Trial 1: Treatment of the ewes and their lambs

Ewes	Group	A (Vaccinated)			C (Controls)		
	Treatment OSA* OSA*	Vaccinated on 80-12-23 Vaccinated on 81-01-21			— —		
Lambs	Group	A1	A2	A3	C1	C2	C3
	No. of animals**	26	28	25	26	25	25
	Treatment OSA* OSA*	81-04-09 81-05-08	81-06-10 81-07-10	— —	81-04-09 81-05-08	81-06-10 81-07-10	— —
	Challenge	81-08-28 4 600 <i>Taenia multiceps</i> ova per os					
	Necropsy	81-11-16-81-12-01					

* Oncosphere Secretary Antigen administered subcutaneously

** Number of animals in each group on 1981-04-09

— No treatment

Lambs

When the majority of the surviving 155 lambs were 4-8 weeks old, the offspring of the ewes in Group A were divided into 3 groups (A1, A2, A3) with the aid of tables of random numbers, while those of Group C ewes (1, 2 and 3) were handled in the same way. The numbers of animals in each group and their subsequent treatment are summarized in Table 1.

On 28 August 1981, when a total of 127 lambs survived, the animals in all 6 groups were dosed orally with 4 600 ova of *T. multiceps*. All the lambs were killed by exsanguination, necropsied during a 16-day period (1981-11-16 and 1981-12-01), which was from 81-96 days post-infestation, and examined for coenuri.

Results

The number of sheep in the various groups with coenuri of *T. multiceps* in the brain and/or lesions caused by metacestodes and their location are listed in Table 2.

TABLE 2 Trial 1: Numbers of sheep with lesions of *T. multiceps*

Group	No. of animals	Sheep with lesions			
		Brain	Myocard	Muscles	Lung
Vaccinates					
A1	21	1	4	1	4
A2	22	1	—	—	1
C1	23	2	—	1	1
C2	19	0	1	—	1
Total	85	4	5	2	7
Controls					
A3	24	13	12	3	5
C3	18	9	13	4	2
Total	42	22	25	7	7

Cerebral lesions were present in 4 out of 85 (4.7%) of the vaccinated lambs, while 22 out of 42 (52.3%) unvaccinated control lambs showed such lesions. The cerebral coenurus in the infested lamb in Group A1 was sterile (i.e. did not contain any scolices), while in Group A3 9 lambs had viable and 4 dead cerebral coenuri. All the coenuri in the brains of the other sheep were viable.

Trial 2 Vaccination of lambs with a single or double dose of OSA or with 2 doses of freeze-dried antigen

Materials and Methods

Experimental animals

Thirty Dorper* and 9 Merino lambs 4-6 months of age, bred at the Veterinary Research Institute, were di-

TABLE 3 Trial 2: Experimental design: Vaccination of lambs with a single and double dose of antigen and with freeze dried antigen compared with undosed controls

Treatment	Group 1	Group 2	Group 3	Group 4
No. of animals	9	10	10	10
Day 0	OSA	OSA	FD ¹	—
Day + 14	—	OSA	FD ¹	—
Day + 42	5 000 <i>T. multiceps</i> ova per os			
Day + 132	Necropsied			

¹FD: Freeze dried antigen

— No treatment

vided into 4 groups and given the treatment summarized in Table 3. The animals in Group 1 were treated with a single dose of OSA, while those in Group 2 received 2 doses of OSA at 14-day intervals. The animals in Group 3 were treated with 2 doses of freeze-dried OSA at 14-day intervals, while those in Group 4 were not treated. All the animals were challenged with 5 000 ova of *T. multiceps* 42 days after the 1st dose of OSA was administered and were necropsied 90 days later.

Results

The number of coenuri recovered from these animals are summarized in Table 4.

TABLE 4 Trial 2: Efficacy of single and double doses of antigen (OSA) and of freeze dried antigen (FD)

Group	Vaccination	Number of animals		
		In group	With cerebral coenuri	
			Alive	Dead
1	OSA x 1	9	1	3
2	OSA x 2	10	0	0
3	FD x 2	10	0	0
4	None	10	8	0

All the animals in Groups 2 and 3 that had received 2 doses of antigen, either normal or freeze dried, were immune to infestation, while 8 out of the 10 unvaccinated animals in Group 4 had viable cerebral coenuri.

* This is a well-established breed of sheep originally founded by crossing Dorset Horns with Blackhead Persians

One of the 9 animals that had received a single dose of normal antigen had a viable cerebral coenurus and 3 further animals had sterile (dead) parasites in their brains.

Trial 3 Further attempt to stimulate transcolostral immunity

Materials and Methods

Experimental animals

Thirty ewes (Merino, Merino-Cross and Dorper) were flushed to synchronize their oestrus cycles. The rams placed with them were fitted with markers so that the date of mating could be recorded. Fifteen of the ewes were vaccinated with freeze-dried OSA on the 90th and again on the 120th day after mating. The remaining ewes were not vaccinated. The dates of birth were recorded, and when the lambs were 21 days old each was dosed with 100 of *T. multiceps* ova. All the lambs were necropsied 7 months later and examined for cerebral coenuri.

Results

A single live coenurus was present in the brain of a lamb of one of the vaccinated ewes. No coenuri were recovered from the other 29 lambs.

DISCUSSION

The results obtained in Trials 1 and 2 confirm reports by Edwards & Herbert (1982) and Verster & Tustin (1982) that OSA may be used as a vaccine to successfully induce a protective immunity against the larval stage of *T. multiceps* in the majority of sheep to which it is administered. Edwards & Herbert (1982) found cerebral coenuri in 1 out of 5 sheep that had been treated with a single dose of OSA. In Trial 2 (Group 1), where 9 sheep were given a single dose of OSA, 1 sheep had a viable cerebral lesion while 3 had dead cerebral lesions. In Trial 1 (Groups A1, A2, C1 and C2) and in Trial 2 (Groups 2 and 3), 105 sheep were each given 2 doses of vaccine, and cerebral lesions were found in 4 (3,8 %) at necropsy. In Trial 2, 10 animals (Group 3) were treated with 2 doses of lyophilized OSA and another 10 (Group 2) with 2 doses of regular OSA. Since at necropsy, none of these animals had cerebral lesions, it appears that 2 doses of the present vaccine gives better protection than a single dose.

Vaccination of lambs at 1-2 months of age (Trial 1: Group A1, C1) and at 3-4 months (Trial 1: Group A2, C2) gave equally good results, and under field conditions it is recommended that animals should be vaccinated as soon after birth as possible.

In Trial 1, one group of ewes (Group A) was vaccinated 30 and 60 days before parturition, and one group of their lambs (Group A3) was not vaccinated before they were challenged with *T. multiceps* ova. Cerebral coenuri were present in 13 out of 24 (54 %) of these lambs and in 9 out of 18 (50 %) of the unvaccinated lambs (Group C3) of the untreated ewes.

In this trial, the ewes and the lambs were treated on a flock basis, the latter being approximately 6 months old when they were challenged. It may be argued that any passive immunity that may have been present would be lost after such a long period. In Trial 3, the ewes as well as the lambs were treated individually to determine whether there was any passive transfer of immunity. Fifteen ewes were inoculated with freeze-dried OSA 90 and again 120 days after mating, and each lamb of these, as well those from the untreated ewes, was challenged when it was 21 days old. At necropsy, only one lamb (the progeny of a treated ewe) had cerebral coenuriasis. As none of the lambs of the untreated ewes were infested, the results are inconclusive.

These unsatisfactory results are probably due to the low number of eggs used for challenge, but the lambs were very young and a higher dose could break down any passive resistance acquired by the lambs of the vaccinated ewes or cause acute coenuriasis in the lambs of the untreated sheep.

Field trials were undertaken on 5 farms in the endemic areas, but owing to floods and/or lack of co-operation from the farmers no reliable results were obtained. On one farm where there had been a serious outbreak, no further cases occurred in the vaccinated animals despite the fact that there were infested dogs on the farm on at least 2 occasions while the trial was being conducted.

ACKNOWLEDGEMENTS

We wish to thank Adri Erasmus, Francois de Villiers, Zennie Haarhoff, Yvonne Kloryga, Duncan Meyer, Phillip Minnaar and Corrie Watermeyer for their technical assistance. Dr L. Kritzinger advised us on the synchronization of the sheep and Dr P. D. Botha carried out pregnancy tests on the ewes.

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