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COMPARISON OF OIL ADJUVANT AND ALUMINIUM PHOSPHATE-ADSORBED TOXOID FOR THE PASSIVE IMMUNIZATION OF LAMBS AGAINST TETANUS

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ABSTRACT

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Immunization of ewes with oil emulsion toxoid followed by an aluminium phosphate-adsorbed toxoid both containing 10 Lf per dose, resulted in a very high antitoxin level in their lambs. Two injections of aluminium phosphate-adsorbed toxoid also imparted a passive immunity to lambs which is considered to be adequate to protect them against tetanus for 4 weeks after birth.

INTRODUCTION

In South Africa, tetanus commonly occurs in young lambs as a sequel to docking by the elastrator method. Since the disease occures within the first week of life, reliance on passive immunity imparted to the lamb by the ewe is the only practical method of prophylaxis.

The immunization of horses against tetanus is common practice and the relevant literature has recently been reviewed by Jansen & Knoetze (1979). There is, however, little information on the immunization of sheep. Chodnick, Jull & Addison (1960) effectively protected lambs by immunizing their dams with an aluminium hydroxide-adsorbed toxoid while Wallace (1963), using a combined vaccine, was likewise successful. Roberts, Güven & Worrall (1971) studied the immune response to ewes to tetanus toxoid, but they did not report either on the effect of adjuvants or on the passive transfer of immunity.

The object of this investigation was therefore to determine whether lambs could be effectively protected against tetanus by immunization of their dams, either with an oil emulsion toxoid or an aluminium phosphate-adsorbed toxoid (APAT).

MATERIALS AND METHODS

Experimental animals

Sixty adult Dorper ewes, which had not previously been immunized against tetanus, were used in the experiment. They were kept in a pen with a concrete floor, fed a balanced ration and given water *ad libitum*. They were bled prior to the commencement of the experiment to ascertain whether or not their sera contained tetanus antitoxin.

Preparation of vaccines

Aluminium phosphate-adsorbed tetanus toxoid was produced according to the procedure described by Sterne & Wentzel (1950) for the production of *Clostridium botulinum* formol-toxoid as adapted by Jansen & Knoetze (1979). The Lf value of the toxoid was determined by the Ramon Flocculation test as employed by Jansen & Knoetze (1979) and Jansen (1961). The final vaccine contained 10 Lf per 1 mℓ dose.

An oil emulsion vaccine was prepared also by the method described by Jansen & Knoetze (1979). It contained 10 Lf per 2 mℓ dose.

Immunization of sheep

All 60 ewes were synchronized by 2 intramuscular injections of 250 micrograms Estrumate* at an interval of 9 days and served by 6 rams 19 days after the 2nd injection. They were subsequently divided into 4 groups of 15 animals each and immunized as follows:

Group I was given 2 injections of 1,0 mℓ of aluminium phosphate-adsorbed toxoid 8 weeks and 4 weeks before the expected lambing date.

Group II was immunized by 3 injections of aluminium phosphate-adsorbed toxoid. The first injection was given when the ewes were served, the 2nd 4 weeks later, and the 3rd 2 weeks before lambing.

Group III was given 2,0 mℓ of oil emulsion vaccine when the ewes were served and a booster injection of 1,0 mℓ aluminium phosphate-adsorbed toxoid 2 weeks before lambing.

Group IV was given a single 2,0 mℓ injection of oil emulsion toxoid 7 weeks before lambing.

All the ewes were bled 1 week before lambing to assess their immune status at that time. The lambs from the first 10 ewes that lambed in each group were bled approximately 24 h after birth and every 2 weeks thereafter until the termination of the experiment at 18 weeks.

The sera were stored at −20 °C until they were tested.

Estimation of antitoxin titres

Antitoxin titres were determined by means of neutralization tests in mice as described by Jansen & Knoetze (1979) and Jansen (1961).

Antitoxin titres were expressed in IU/mℓ and the geometric mean was calculated for each group.

RESULTS

Antitoxin response in ewes

The geometric mean titres as well as the individual titres of the ewes in each of the 4 experimental groups are shown in Table 1.

The antitoxin response of the group that received the oil adjuvant toxoid, followed by a booster injection of aluminium phosphate-adsorbed toxoid, was far superior to that of the other groups.

Group IV, which received a single injection of oil emulsion vaccine, did not respond any better than the group that received APAT only. The antitoxin titres of the lambs from this group were therefore not determined.

Duration of passive immunity in lambs

The geometric mean antitoxin values of the lambs of ewes in Groups I, II and III over a period of 18 weeks are given in Fig. 1.

As was anticipated, the values in the lambs from the ewes in Group III were far higher than those of the other 2 groups. The latter, nevertheless, had antitoxin titres that would afford protection against tetanus.

DISCUSSION

From the results given above, it is clear that an oil adjuvant tetanus toxoid followed by a booster of aluminium phosphate-adsorbed toxoid results in a spectacular antitoxin response in ewes which is effectively transferred to their lambs. However, in practice, such a regimen would require that 2 separate products be available, since a single injection of oil emulsion toxoid was not as effective.

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TABLE 1 Antitoxin response of ewes given different toxoids containing 10 Lf/dose

Group I	Ewe No.	Antitoxin titre IU/mℓ	Group II	Ewe No.	Antitoxin titre IU/mℓ
Two injections of APAT	1	10,0	Three injections of APAT	1	500,0
	2	20,0		2	10,0
	3	2,5		3	Died
	4	16,6		4	5,0
	5	16,6		5	25,0
	6	12,5		6	12,5
	7	1,0		7	100,0
	8	12,5		8	0,5
	9	2,5		9	1,0
	10	2,5		10	5,0
Geometric mean		6,50	Geometric mean		10,77
Group III	**Ewe No.**	**Antitoxin titre IU/mℓ**	**Group IV**	**Ewe No.**	**Antitoxin titre IU/mℓ**
Oil emulsion toxoid followed by APAT	1	250,0	One injection of oil emulsion toxoid	1	12,5
	2	100,0		2	12,5
	3	500,0		3	10,0
	4	100,0		4	10,0
	5	500,0		5	2,5
	6	1000,0		6	1,0
	7	1000,0		7	16,6
	8	250,0		8	10,0
	9	200,0		9	10,0
	10	125,0		10	2,5
Geometric mean		287,82	Geometric mean		6,62

APAT = aluminium phosphate-adsorbed toxoid
IU = International units

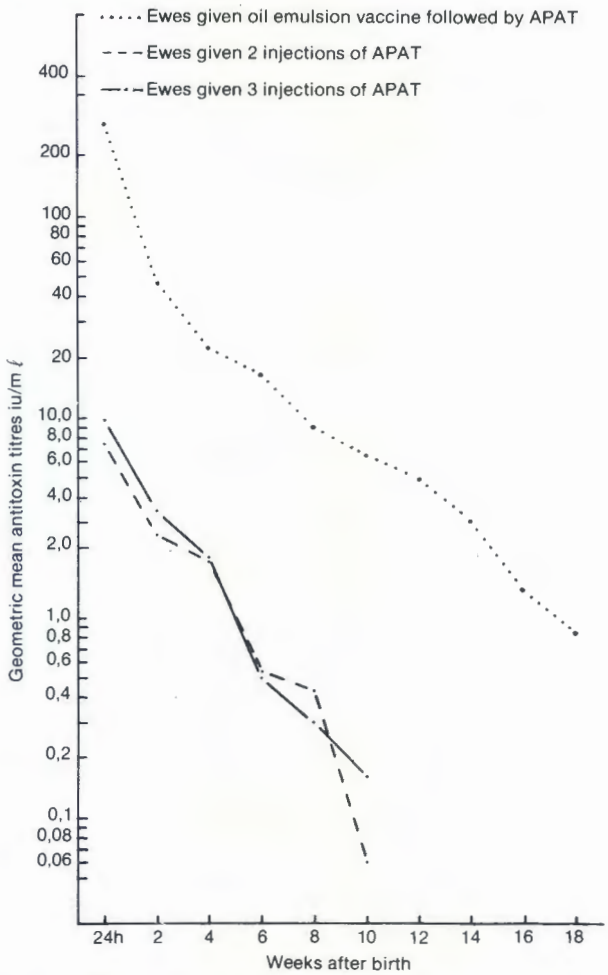

FIG. 1 Antitoxin levels in lambs born from ewes that were given different toxoids

..... Ewes given oil emulsion vaccine followed by APAT
---------- Ewes given 2 injections of APAT
———— Ewes given 3 injections of APAT

Ewes that received 3 injections of aluminium phlosphate-adsorbed toxoid developed a higher mean antitoxin titre than ewes that received only 2 injections. This difference, however, can be explained by the very high titre of a single animal. The difference was not reflected in their lambs, since both groups had essentially the same antitoxin levels over a period of 8 weeks.

Lambs are normally only seriously at risk for approximately the first month after birth. Up to that time the group from the ewes given injections of APTD had a mean antitoxin tire of 1,5 IU/mℓ, which is more than adequate for solid protection. Even at 8 weeks of age they had mean antitoxin levels of 0,2 IU/mℓ, which is considered to be adequate for protection (Jansen & Knoetze, personal communication, 1983).

In the light of the above it can be stated that lambs can be effectively passively immunized by the administration of 2 injections of aluminium phosphate-adsorbed tetanus toxoid containing 10 Lf per dose to their dams. Our results therefore agree with the findings of Chodnick *et al.* (1960) and of Wallace (1963). The 1st injection should be given approximately 8 weeks before lambing and the 2nd injection approximately 3–4 weeks before lambing.

In the light of the findings of Roberts *et al.* (1971) it can be anticipated that ewes given 2 injections of APAT will require only a single booster injection 7–14 days prior to each subsequent lambing.

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