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RESEARCH COMMUNICATION

EFFECTIVE IMMUNIZATION OF LAMBS AGAINST ENTEROTOXAEMIA

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ABSTRACT

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In contrast to adult sheep, 2- to 3-month-old lambs do not respond well to a single injection of *Clostridium perfringens* Type D oil adjuvant epsilon toxoid. This unresponsiveness can be overcome, however, by administering 2 injections of oil adjuvant vaccine or one injection of oil adjuvant followed 4 weeks later by an injection of alum-precipitated toxoid. The latter procedure evokes protective antitoxin levels which persist for 8 months, and a booster injection of alum-precipitated toxoid given at this stage results in an immunity which lasts for at least 1 year.

Résumé

L'IMMUNISATION EFFECTIVE DES AGNEAUX CONTRE L'ENTEROTOXAEMIA

Contrairement aux moutons adultes, les agneaux de 2 à 3 mois ne repondent pas bien à une seule injection de Clostridium perfringens *type D avec adjuvant de toxoïde epsilon huileux. Ce manque de reponse peut cependant être surmonté en administrant 2 injections de vaccin à adjuvant huileux ou une seule injection d'huile adjuvante suivie 4 semaines plus tard d'une injection de toxoïde précipité à l'alun. Le dernière protocole évoque des niveaux d'antitoxine protectrice qui persistent pendant huit mois et une injection de rappel de toxoïde précipité à l'alun donnée à ce stade résulte en une immunité qui dure pendant au moins un an.*

INTRODUCTION

Sheep can effectivley be protected against enterotoxaemia by an injection of 2 doses of a potent alum-precipitated toxoid (Jansen, 1967a). This basic immunity is of comparatively short duration, however, and, in order to maintain a constant level of protection, repeated injections of alum-precipitated toxoid are required (Jansen, 1967b). The above-mentioned problem can largely be overcome with the use of oil adjuvant vaccines. Jansen (1962) showed that the incorporation of epsilon toxoid into Freund's complete adjuvant evokes a high level of antitoxin in adult sheep and that a single injection of toxoid in Freund's incomplete adjuvant gives rise to protective antitoxin levels which persist for at least 2 years (Jansen, 1967b). The latter product unfortunately gives rise to unacceptable local reactions and for this reason Thomson & Batty (1967) formulated an oil emulsion using Arlacel A and Tween 80 as emuslifiers which has superior physical properties without marked loss of adjuvantcy. Subsequent studies (Thomson, Batty, Thomson, Kerry, Epps & Foster, 1969) proved that their formulation was most effective even in the form of a multicomponent product. As the emulsifiers used in the above-mentioned studies were not available in large quantities, N. Claassen, K. E. Weiss & P. C. Knoetze (personal communication, 1975) composed an oil emulsion vaccine using Lubrol and Lissapol as emulsifiers. The product proved to be stable and of acceptable viscosity, and, in addition, did not produce unacceptable local reactions unless it was administered repeatedly. Adult sheep immunized by means of a single injection of this product developed protective levels of epsilon antitoxin which persisted for at least 2 years, although the antitoxin level was not as high as those obtained with vaccines in which Arlacel A and Tween 80 were used as emulsifiers (K. E. Weiss & P. C. Knoetze, personal communication, 1976).

Although good results were obtained in adult sheep, reports were received from the field that the vaccine did not give good results in young lambs (A. P. Schutte, personal communication, 1978) and these reports were in fact substantiated by antitoxin assays done on sera from the animals in question (P. C. Knoetze & C. M. Cameron, unpublished results, 1978). In an investigation into this anomaly, experiments were done to determine the reason for the poor response in lambs and to devise means of overcoming it.

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MATERIALS AND METHODS

Vaccine production

Clostridium perfringens Type D epsilon toxoid was produced essentially as described by Jansen (1967a) except that the material was produced in an 800 ℓ fermenter tank instead of in flasks. Furthermore, the medium contained no large meat particles. After inoculation of the tank the contents were continuously stirred and growth was allowed to proceed for 4 h, at which point the pH was adjusted to 8,0 with 10 N NaOH. Incubation at 37 °C was continued for a further 20 h after which the material was trypsinized, formalinized and subsequently clarified by passage through a continuous flow centrifuge to remove larger particles. The potency of the toxoid was assayed by means of an Lf test, using standardized epsilon antitoxin (Jansen, 1967a), and the results were expressed as units/mℓ.

For the preparation of the oil emulsion vaccine the following formulation was used:

Solution A:

Toxoid: 30%
Lissapol NX(1): 2%

Solution B:

Bayol 72(2): 60%
Lubrol MOA(1): 8%

Solutions A and B were separately compounded, solution A was then added to solution B and the mixture emulsified by means of an electric impeller fitted to a 800 ℓ stainless steel tank.

Alum-precipitated toxoid was prepared according to Jansen (1967a).

(1) Imperial Chemical Industries, P.O. Box 3784, Alrode 1451, R.S.A.
(2) Esso Petroleum Co. Ltd, P.O. Box 78011, Sandton 2146, R.S.A.

Antitoxon assays

Antitoxin assays on sheep sera were done by using the L+ test as described by Jansen (1967a). There is no unanimity regarding the minimal level of epsilon antitoxin that is required to ensure complete protection against enterotoxaemia (Oxer, Minty & Liefman, 1971), but for the purposes of this study the level of 0,15 units/mℓ as determined by Jansen (1960) was taken as the norm.

Experiments in sheep

Comparison of antitoxin response in adult sheep and lambs. Two groups of 8 fully susceptible 12-month-old Merino wethers and 2 groups of 8 3-month-old Merino lambs containing no detectable antitoxin in their sera were each injected with 2 batches of vaccine containing 80 Lf and 125 Lf per dose respectively. The volume was 1,0 mℓ throughout and a single subcutaneous injection only was applied.

All the animals were bled 4 weeks later and the serum antitoxin titres determined as outlined above.

Antitoxin response of young lambs to alum-precipitated toxoid and oil adjuvant vaccine. Forty Merino lambs from immunized ewes were divided into 4 groups of 10 each and immunized at 2 months of age as follows:

Group 1: 2 injections of alum-precipitated toxoid (100 Lf/dose) at 4-weeks-interval.

Group 2: 2 injections of oil adjuvant vaccine (125 Lf/dose) at 4-weeks-interval.

Group 3: One injection of alum-precipitated toxoid (100 Lf/dose) followed by an injection of oil adjuvant vaccine (125 Lf/dose) 4 weeks later.

Group 4: One injection of oil adjuvant vaccine (125 Lf/dose) followed by an injection of alum-precipitated toxoid (100 Lf/dose) 4 weeks later.

All the lambs were bled when the 1st injection of vaccine was given as well as at 4-week-intervals after the 2nd injection, and the antitoxin titres of the sera were then determined. A 3rd (booster) injection of alum-precipitated toxoid was given to all the animals 33 weeks (approximately 8 months) after the 2nd injection and the antitoxin titres were determined subsequently at 3, 19, 24, 28 and 50 weeks respectively.

TABLE 1 Comparison of the immune response of adult sheep and young lambs 6 weeks after application of a single injection of oil adjuvant *Cl. perfringens* Type D vaccines

Vaccine Lf/dose	Adult sheep		3-month-old lambs	
	Sheep No.	Epsilon antitoxin units/mℓ	Lamb No.	Epsilon antitoxin units/mℓ
80	944	4,0	7038	4,0
	995	4,0	7039	1,0
	1017	4,0	7042	4,0
	2226	4,0	7063	1,0
	2227	4,0	7080	4,0
	2235	4,0	7052	0,4
	2366	4,0	7051	0,2
	2487	4,0	7068	0,2
125	2568	4,0	7082	1,0
	3129	4,0	7088	4,0
	3171	4,0	7095	0,0
	3172	4,0	7097	0,0
	4339	4,0	7098	0,0
	4919	1,0	7099	0,0
	5751	4,0	7192	4,0
	5756	4,0	7198	0,0

TABLE 2 Immune response of 2-month-old lambs to various immunization schedules

Group	Initial immunization schedule[1]	Geometric mean epsilon antitoxin titres. Units/mℓ											
		No. of weeks after 2nd injection							No. of weeks after 3rd injection[2]				
		0	4	8	16	20	24	28	3	19	24	28	50
1	Two injections of alum-precipitated toxoid	4,82	2,70	3,25	0,42	0,18	0,10	0,10	8,21	8,21	4,68	0,77	0,70
2	Two injections of oil adjuvant vaccine	1,29	6,15	6,82	1,25	1,24	1,16	1,16	7,76	7,94	3,76	1,36	1,07
3	One injection of alum-precipitated toxoid followed by oil adjuvant vaccine	2,34	2,93	4,09	1,73	2,07	1,22	0,38	10,13	6,47	5,45	1,56	1,28
4	One injection of oil adjuvant vaccine folowed by alum-precipitated toxoid	3,89	6,82	7,55	2,93	1,40	1,15	0,79	10,53	14,13	6,97	2,89	1,73

[1] The injections were given at 4-weeks-interval

[2] All the sheep were given a booster (3rd) injection of alum-precipitated toxoid 33 weeks after the 2nd injection

Results

Antitoxin response in adult sheep and lambs

The antitoxin titres obtained in the adult sheep and lambs that were immunized with 2 different batches of oil adjuvant vaccine are shown in Table 1. With the exception of a single animal, all the adult sheep developed antitoxin titres of at least 4 antitoxin units/mℓ. Some lambs did develop adequate antitoxin levels, but the titres in 7 out of 16 lambs were either very low (0,2 units/mℓ) or entirely absent.

Antitoxin response of young lambs to successive injection of oil adjuvant vaccine and alum-precipitated toxoid

The antitoxin levels elicited in 2-month-old lambs given either 2 injections of alum-precipitated toxoid, 2 injections of oil adjuvant vaccine or combinations of the above, are given in Table 2. The group which received 2 injections of alum-precipitated toxoid showed the most rapid antitoxin response but also the fastest decline in titres, which dropped to a sub-protective level by 24 weeks. The other 3 groups developed more persistent titres. The group (No. 4) that was given 1 injection of oil adjuvant vaccine followed by 1 injection of alum-precipitated toxoid developed a high average titre which remained above 0,15 units/mℓ for 28 weeks. After a booster injection of alum-precipitated toxoid the average titre rose to over 10 units/mℓ and the group exhibited a geometric mean titre of 1,73 units/mℓ 50 weeks later.

Discussion

It is a well-established fact that lambs under the age of 2 months do not respond well to immunization against enterotoxaemia (Smith & Marsh, 1953). This lack of response can be partially overcome, however, if 2 injections of alum-precipitated toxin are applied. By this procedure a satisfactory immunity can be obtained for 13–15 weeks (Hepple, Chodnik & Price, 1959; Jansen, 1967b), a result which is in agreement with the findings reported in this paper that an average antitoxin level of more than 0,15 units/mℓ (Jansen, 1960) was present 20 weeks after immunization. The rapid decline in antitoxin levels therefore requires repreated injections of toxoid to maintain an adequate level of immunity for longer than 5 or 6 months.

In the light of the excellent response in adult sheep to a single injection of oil emulsion vaccine (Thomson & Batty, 1967), it was hoped that similar results could also be obtained in lambs. This did, however, not prove to be the case (Table 1). It is noteworthy that the response to the vaccine containing 125 Lf/mℓ was poorer than to the vaccine containing 80 Lf/mℓ, but this discrepancy can possibly be accounted for by variations in the physical quality of the emulsions.

Since the lambs were obtained from non-immunized ewes, had no antitoxin in their sera and were therefore completely susceptible, their poor response cannot be attributed to the possible interference effect of maternal antibodies.

The application of 2 doses of oil adjuvant or oil adjuvant vaccine followed by alum-precipitated toxoid did, however, give rise to a very good immunity. Moreover, a booster injection given approximately 8 months after the first 2 injections gave rise to high and persistent titres for 50 weeks.

In the light of the very gradual decrease in titres from 28 to 50 weeks, it would be reasonable to deduce that antitoxin levels thus obtained would persist for much longer than a year. Since lambs born from immunized ewes are passively protected from 8–13 weeks of age (Jansen, 1967b; Oxer *et al.*, 1971), the use of an oil ajduvant vaccine at 2 months of age followed by an alum-precipitated toxoid a month later and a booster injection 6–8 months thereafter, offers the opportunity of establishing uninterrupted immunity to enterotoxaemia in sheep. A further advantage of the abovementioned schedule is that the untoward local reactions which often follow a 2nd injection of oil emulsion vaccine are avoided.

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