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RESEARCH COMMUNICATION

ANTIBODY RESPONSE IN CATTLE TO OIL EMULSION RABIES AND EPHEMERAL FEVER VACCINES

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ABSTRACT

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A stable oil emulsion rabies vaccine with a low viscosity was composed by a formula previously employed for Newcastle disease vaccine. Cattle developed high and sustained antibody levels, and guinea pigs were found to be solidly immune after a single injection of this vaccine.

Antibody responses in cattle to 2 oil emulsion ephemeral fever vaccines were not satisfactory after a single injection, and severe local reactions were encountered when booster injections were applied.

INTRODUCTION

Effective immunization of cattle against rabies requires that they be given 2 injections of live Flury HEP vaccine (Barnard, unpublished report, 1983). In remote and sparsely populated areas, this requirement poses considerable practical problems involving the mustering of cattle over long distances. An alternative practical method of immunization was therefore required.

The use of reconstituted, freeze-dried vaccine, emulsified in oil (Barnard, unpublished data, 1984), showed that cattle respond very well serologically to an oil emulsion vaccine. The distribution on a large scale of such a product, which would require 3 separate components, however, is impractical. The object of this investigation was therefore to investigate the possiblity of formulating an effective oil emulsion rabies vaccine which could be issued for immunization of cattle against rabies.

Similar problems arise with regard to ephemeral fever vaccine.

MATERIALS AND METHODS

Production of vaccines

Rabies HEP vaccine was produced in 500 mℓ volumes in BHK 9/23 cells in roller flasks (Barnard, unpublished report, 1985). The yield had a virus tire of $10^{6,9}$ mouse LD_{50}/mℓ. The virus was not inactivated in any way but kept at 4 °C until purity and sterility had been confirmed.

Oil emulsion with different formulae were prepared, as is shown in Table 1. In all instances, the water phase 'b' was added gradually to the oil phase 'a' during vigorous mixing in a Waring blender, until a satisfactory emulsion was obtained. The physical features of the emulsions are given in Table 1.

Formula A was used in initial experiments (Barnard, unpublished results, 1983), and formula B was furnished by a commercial company.

Formula C has been used for Newcastle disease vaccine (Brugh, Stone & Lupton, 1983), whereas formulae D and E are currently used for Onderstepoort ephemeral fever and *Chlamydia* vaccines, respectively.

Ephemeral fever vaccine was produced in the same way as formulae D and E using an attenuated virus strain.

Immunization of animals

Rabies. Groups of 10 guinea pigs were immunized with 0,06 mℓ (1/80th cattle dose) of the respective vaccines.

TABLE 1 Composition and characteristics of rabies oil emulsion vaccines

Designation	Formulae			Viscosity centistokes	Stability		
					4 °C for 19 days	37 °C for 19 days	3 freeze-thaw cycles
A	Bayol 72[1] Arlaecel C[2]	54 % 6 %	a	137,5	Not tested	Unstable	Unstable
	Antigen Tween 80[2]	39 % 1 %	b				
B	Marcol 52[1] Span 85[2]	60 % 6 %	a	53,4	Not tested	Unstable	Unstable
	Antigen Tween 85[2]	30 % 4 %	b				
C*	Marcol 52 Arlacel C	71 % 8 %	a	17,8	Stable Remains fluid	Stable	Stable
	Antigen Tween 80	20 % 0,65 %	b				
D	Marcol 42 Montanide[3]	54 % 6 %	a	Variable	Stable Gel formation	Unstable	Stable
	Antigen Tween 80	39 % 1 %	b				
E	Marcol 52 Cirasol[2]	72 % 8 %	a	13,1	Stable Gel formation	Stable	Stable
	Antigen Tween 80	19 % 1 %	b				

[1] Esso (SA), P.O. Box 1453, Johannesburg 2000
[2] I.C.I., P.O. Box 11270, Johannesburg 2000
[3] Chemag (SA), P.O. Box 41368, Craighall 2024
* Brugh, Stone & Lupton (1983) Formula OE-1

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They were bled and challenged 4 weeks later with 4 LD 50 of CVS virus.

Two groups of 8 cattle were immunized with a single subcutaneous injecton of vaccines C and E, since these formulations had the best physical features. One group received 2,0 mℓ and the other 5,0 mℓ of the respective vaccines. They were bled intermittently over a period of 12 months, and the virus-neutralizing titres were determined by conventional techniques.

Ephemeral fever. Groups of 8 cattle were immunized as in the case of the rabies vaccines, using vaccines prepared according to formulae B and E.

RESULTS

Rabies vaccines

Guinea pigs. The antibody titres and protection obtained in guinea pigs with the 5 vaccines are shown in Table 2.

TABLE 2 Antibody response in guinea pigs to and protection provided by various rabies vaccines

Vaccine formula[1]	Reciprocal mean antibody titre	Protection %
A	15	90
B	4	90
C	115	100
D	68	90
E	128	100
Live HEP vaccine only	115	80
Non-immunized controls	0	10

[1] See Table 1

From these results it is evident that guinea pigs can be readily immunized with all the oil emulsion vaccines.

Cattle. The antibody responses of cattle to rabies vaccines C and E are shown in Table 3.

TABLE 3 Mean antibody response in cattle after a single injection of oil emulsion rabies vaccines

Vaccine formula[1]	Dosage (mℓ)	Reciprocal titre					
		Months after injection					
		1	4	6	8	10	12
C	2	512	344	336	384	274	360
C	5	512	368	392	328	292	324
E	2	184	112	35	29	18	21
E	5	480	148	96	82	65	80

[1] See Table 1

The response induced by vaccine C is clearly superior to that of vaccine E. Even a dose of 2,0 mℓ resulted in a high antibody titre which persisted for at least 12 months.

Ephemeral fever

The antibody response to vaccines B and E is shown in Table 4.

TABLE 4 Mean antibody response in cattle after a single injection of oil emulsion ephemeral fever vaccine

Vaccine formula	Dosage (mℓ)	Reciprocal titre						
		Months after 1st injection						3 months after 2nd injection
		1	3	4	7	10	13	
B	2	137	27	54	32	50	46	470
B	5	160	41	97	25	41	40	343
E	2	57	7	21	6	8	10	290
E	5	123	29	51	21	30	22	412

[1] See Table 1

After a single injection, neither of the vaccines induced a sustained antibody response. However, a 2nd injection a year later resulted in a very good antibody response, but this was accompanied by extremely severe reactions at the injection site.

DISCUSSION

The high antibody response induced particularly by a single injection of rabies oil emulsion vaccine C indicates that this product could probably be used beneficially to immunize cattle in remote areas. This prospect is justified by the good immunity the vaccine induced in guinea pigs. However, possible adverse reactions which might follow repeated injections should be investigated. The sustained antibody levels over a period of 12 months suggest that, in practice, a booster injection would probably be superfluous.

The response of cattle to 2 ephemeral fever oil emulsion vaccines was not satisfactory, both because of the low titres obtained after a single injection and the adverse local reactions experienced subsequent to booster injections. In the light of the good results obtained with rabies vaccines, other formulations, e.g. formula C (Brugh *et al.*, 1983), could possibly be fruitfully investigated. There is evidence that immunity to ephemeral fever may be primarily cellular in nature. Reliance should therefore not be placed on antibody responses alone, and all promising results should be confirmed by active challenge of immunized animals.

REFERENCES

BRUGH, M., STONE, H. D. & LUPTON, H. W., 1983. Comparison of inactivated newcastle disease viral vaccines containing different emulsion adjuvants. *American Journal of Veterinary Research*, 44, 72–75.

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