Onderstepoort J. vet. Res., 53, 51–53 (1986)

IMMUNIZATION OF GUINEA-PIGS AND CATTLE WITH A REDUCED DOSE *CLOSTRIDIUM CHAUVOEI* VACCINE PRODUCED IN A SEMI-SYNTHETIC MEDIUM

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ABSTRACT

CAMERON, C. M., BOTHA, W. J. S. & SCHOEMAN, J. H., 1986. Immunization of guinea-pigs and cattle with a reduced dose *Clostridium chauvoei* vaccine produced in a semi-synthetic medium. *Onderstepoort Journal of Veterinary Research*, 53, 51–53 (1986)

A semi-synthetic culture medium and method are described for the production of a reduced dose *Clostridium chauvoei* vaccine. The vaccine gave excellent results in guinea-pigs, and 2 injections of 2,0 mℓ protected cattle against challenge with 2 M.L.D. of a virulent culture for at least 12 months.

The suitability of *C. chauvoei* Strain OP64 as a vaccine strain was confirmed.

INTRODUCTION

Clostridium chauvoei vaccine comprising a so-called "cultural aggressin" was issued by the Veterinary Research Institute from 1922 to 1934 (Viljoen & Scheuber, 1927). This product was superceded by an anaculture produced on ox-liver infusion broth (Mason & Scheuber, 1936) which when precipitated with alum, was found to be superior to the cultural aggressin (Scheuber, 1944). Sterne, Thorold & Scheuber (1951) described a method for producing *C. chauvoei* vaccine in cellophane tubing but this procedure was never adopted for large scale production.

The method of Mason & Scheuber (1936), utilizing flasks for production purposes, was employed until 1972, when it was adapted to fermentation tanks (B. H. J. Smit, 1985, personal communication). Although the vaccine thus produced has given excellent results, the preparation of the culture medium is laborius, it tends to vary in quality and is difficult to process. The final product also contains undesirable fine meat particles. A more refined culture medium for production purposes was considered necessary. Simultaneously, it was thought expeditious to attempt to reduce the dosage from the current 5,0 mℓ to 2,0 mℓ.

C. chauvoei Strain OP64 (Mason & Scheuber, 1936) has been used for production purposes with success for the last 50 years. Largely unsubstantiated reports are occasionally received, however, suggesting that in rare instances the vaccine does not afford adequate immunity.There is no evidence in the literature to suggest that different immunotypes of *C. chauvoei* exist, but certain strains, e.g., CH3, are more immunogenic than others (Chandler & Gulasekharam, 1970). Nevertheless, it has been found that although there is a marked degree of cross protection among strains, minor differences do occur (Kerry, 1967). It has, in fact, been necessary to augment vaccines with additional strains for the above-mentioned reason (Reed & Reynolds, 1977).

In the light of these considerations the suitability of Strain OP64 as a vaccine strain was re-investigated.

MATERIALS AND METHODS

Bacterial strains

C. chauvoei Strain OP64 was used for all vaccine production. It was originally isolated from a case of blackquarter in a heifer in the Waterberg district (Transvaal) in 1929 (Mason & Scheuber, 1936) and has since been maintained in Von Hibler's medium and periodically passaged in guinea-pigs.

The following strains were isolated from local cases of black-quarter and used for the cross protection tests: Ball (Bandelier-kop, Transvaal; 81 (Potgietersrus, Transvaal); Estcourt (Natal); 213 (Botswana). In addition, strains CH1, CH2 and CH4 were included for comparison. The latter 3 strains were supplied by the courtesy of Dr H. M. Candler, Commonwealth Serum Laboratories, Parkville, Australia.

Vaccine production

(i) *Culture media*

Liver: meat broth was prepared as described by Mason & Scheuber (1936).

The semi-synthetic medium (CAM-medium), based on the formulae of Jones & Clifton (1953) and Buddle (1953), was composed as follows:

Tryptone (Biolab)*	8 g/ℓ
Peptone (Biolab)	4 g/ℓ
Meat extract (Biolab)	2 g/ℓ
Yeast extract (Biolab)	2 g/ℓ
Casein Hydrolysate (Biolab)	6 g/ℓ
L-cysteine hydrochloride (Biolab)	1 g/ℓ
$Na_2HPO_4.12H_2O$ (Merck)**	0,4 g/ℓ
50 % sterile glucose solution (added later)	20 mℓ/ℓ
pH 7,2	

In one experiment a culture medium used by a private pharmaceutical company for *C. chauvoei* vaccine production was also incorporated.

(ii) *Method*

For small scale production, media were prepared in 8 ℓ volumes in 9 ℓ aspirator bottles, and for large scale production a 500 ℓ fermentation tank was employed. In both instances, the medium was sterilized by steam and the required quantity of sterile 50 % glucose solution was added to give a final concentration of 1,0 %. The pH was adjusted to 7,2. After inoculation, growth was allowed to proceed at 37 °C. The growth cycle was normally completed after 16–18 h.

The whole cultures were inactivated by the addition of 0,7 % formalin and holding the cultures for 6 days at 37 °C. After inactivation, concentration was achieved by the addition of potassium alum to a final concentration of 1,0 %; $Al(OH)_3$ gel to 6 % (120 mℓ of a 50 % gel/ℓ); polyethylene glycol to 4 % (Cameron & Weiss, 1974) or by centrifugation. In all instances, the required volume of supernatant fluid was siphoned off to give a 2,5 fold concentration.

Immunization of experimental animals

(i) *Guinea-pigs:* The potency of all the vaccines was first assayed in guinea-pigs. Fivefold dilutions (1/5–1/125) of the vaccines were made in 0,15 M NaCl. Groups of 5 guinea-pigs were immunized respectively

Received 24 September 1985—Editor

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with each dilution. They were given 2 subcutaneous injections of 2,0 mℓ for the standard vaccine and 0,8 mℓ for the concentrated vaccines 4 weeks apart. All the groups as well as non-immunized controls were challenged with 50 M.L.D. of standardized spore suspension 4 weeks after the 2nd injection.

(ii) *Cattle:* Groups of 15 3–6-month-old Bonsmara calves were used. They were given 2 injections of the various vaccines 4 weeks apart. As is shown in Table 4, a number of animals from each group were challenged 6, 9 and 12 months after the 2nd injection of vaccine with either 2 or 10 M.L.D.

Challenge of experimental animals

Stabilized spore suspensions were prepared by growing the respective strains in Von Hibler's brain medium for 5 days at 37 °C. The cultures were then kept at room temperature for 3 weeks, and minced. For the experiments in guinea-pigs the suspensions were kept at room temperature, whereas for the cattle it was distributed in vials and stored in liquid nitrogen.

The minimal lethal dose (M.L.D.) of the suspensions was determined in guinea-pigs and cattle. Appropriate dilutions of the respective spore suspensions were mixed with a 5 % calcium chloride solution in a ratio of 2:1 and 2 mℓ volumes were injected intramuscularly. Deaths were recorded after 5 days.

All experimental guinea-pigs were challenged with 50 M.L.D. and the cattle with either 10 or 2 M.L.D., as is shown in Table 4.

RESULTS

Spectrum of immunity afforded by C. chauvoei *Strain OP64*

The results of an experiment in which guinea-pigs that were immunized by vaccine prepared from *C. chauvoei* Strain OP64 produced in liver:meat broth and challenged with a series of heterologous strains are shown in Table 1. With the exception of the Australian isolate CH1, Strain OP64 afforded good protection against all the other strains, including the 4 recent local isolates.

TABLE 1 Protection in guinea-pigs afforded by *C. chauvoei* Strain OP64 (vaccine) to heterologous challenge strains

Challenge Strain	Dilution of vaccine used	Protection afforded %
Australia CH1	Undiluted	60
	1/5	20
	1/25	0
Australia CH2	Undiluted	80
	1/5	60
	1/25	80
Australia CH4	Undiluted	60
	1/5	60
	1/25	40
Ball	Undiluted	100
	1/5	100
	1/25	80
Potgietersrus 81	Undiluted	100
	1/5	80
	1/25	20
Estcourt	Undiluted	100
	1/5	80
	1/25	80
Botswana 213	Undiluted	100
	1/5	100
	1/25	40
OP64	Undiluted	80
	1/5	80
	1/25	20
Minimum standard	Undiluted	80
	1/5	60
	1/25	20

TABLE 2 Comparison of different culture media for the production of *C. chauvoei* vaccines

Culture medium	Dilution of vaccine used	Unconcentrated 2,0 mℓ dose	Concentrated with alum 0,8 mℓ dose
"CAM"	Undiluted	100	100
	1/5	100	100
	1/25	80	80
	1/125	60	100
Commercial	Undiluted	80	100
	1/5	80	80
	1/25	80	20
	1/125	0	0
Liver:meat broth	Undiluted	100	100
	1/5	100	100
	1/25	100	100
	1/125	60	40
Minimum requirement	Undiluted	80	80
	1/5	60	60
	1/25	20	20

TABLE 3 Comparison in guinea-pigs of vaccines concentrated by different methods

Method of concentration	Dosage for guinea-pigs mℓ	Dilution of vaccine used	% protection afforded	
			C. chauvoei alone	*C. chauvoei* + *C. botulinum* toxoid
None	2,0	Undiluted	100	80
		1/5	100	80
		1/25	100	nt
		1/125	60	nt
Alum—not concentrated	2,0	Undiluted	100	nt
		1/5	100	nt
		1/25	60	nt
		1/125	0	nt
Alum	0,8	Undiluted	60	100
		1/5	100	100
		1/25	100	nt
		1/125	0	nt
$Al(OH)_3$	0,8	Undiluted	100	100
		1/5	100	100
		1/25	60	nt
		1/125	60	nt
PEG	0,8	Undiluted	100	nt
		1/5	100	nt
		1/25	80	nt
		1/125	0	nt
Centrifugation	0,8	Undiluted	60	nt
		1/5	60	nt
		1/25	60	nt
		1/125	60	nt

nt = not tested

Influence of culture media on the immunogenicity of C. chauvoei *vaccine*

In the experiment, the results of which are shown in Table 2, the vaccine produced in liver:meat broth gave exceptionally good results. It is nevertheless also evident that the vaccine produced on CAM medium also afforded a degree of protection in guinea-pigs which is noticeably above the minimum standard requirement. The same pattern was observed for both the unconcentrated vaccine (2,0 mℓ dose) and alum concentrated vaccine (0,8 mℓ dose).

Effect of concentration method on the immunogenicity of C. chauvoei *vaccines*

All the chemical sedimentation procedures gave similar, good results, but the vaccine that was concentrated by centrifugation was unsatisfactory. Because of economic considerations, further experiments in cattle were done with alum concentrated vaccines.

TABLE 4 Duration of protection in cattle afforded by *C. chauvoei* vaccines produced by different methods

Interval between immunization and challenge	Challenge	Vaccine composition	Survivors after challenge				
			Group I	Group II	Group III	Group IV	Group V
		Culture medium: production method: concentration: dosage (mℓ):	Liver:meat fermenter None 5	CAM fermenter None 5	CAM flasks × 2,5 2	CAM fermenter × 2,5 2	Non-immunized controls
Months	M.L.D.						
6	10		2/2	2/2	2/2	2/2	0/1
	2		nt	nt	nt	nt	nt
9	10		1/2	2/2	2/2	1/2	0/1
	2		0/1	1/1	0/1	1/1	0/1
12	10		nt	nt	nt	nt	nt
	2		2/4	3/4	4/4	4/4	0/2

nt = not tested

The results given in Table 3 also show that the addition of *C. botulinum* toxoid does not adversely effect the potency of *C. chauvoei* vaccine.

Duration of immunity in cattle

In a preliminary experiment it was established that a 2,0 mℓ dose of alum concentrated vaccine, prepared in liver:meat broth, gave an immunity in cattle equivalent to that obtained by a 5,0 mℓ dose.

In a subsequent experiment, the duration of immunity afforded by 2 injections of a 2,0 mℓ dose of vaccine that was produced in CAM medium and concentrated with potassium alum was evaluated. The results given in Table 4 show that all the cattle could resist a challange of 10 M.L.D. 6 months after immunization. After 9 months, the immunity in the various groups was not as solid. At 12 months the cattle in the groups that were immunized with concentrated vaccine prepared in CAM medium (Groups III and IV) were still able to resist a challenge with 2 M.L.D.

Discussion

The findings ensuing from the current investigation clearly demonstrate that *C. chauvoei* Strain OP64 is a suitable vaccine strain since it afforded adequate protection against a number of strains isolated from different geographic areas in southern Africa.

It was also established that a satisfactory vaccine can be produced in fermentation tanks in a suitable semi-synthetic culture medium. When concentrated by means of alum precipitation, 2 injections of such a product afforded a good immunity in cattle for at least 12 months. Although the challenge dosage was only 2 M.L.D., it is probably still appreciably higher than would be encountered under natural circumstances. By inference, it can be stated that apparent vaccine breaks can be ascribed to failure to observe the recommendations for administration. It is nevertheless necessary to note that immunity to *C. chauvoei* decreases with time. This observation supports the recommendation that immunization should be repeated annually until the animals are 3 years of age.

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