Onderstepoort J. vet. Res. 45, 143–147 (1978)

METHOD OF PRODUCTION OF AND ANTIBODY RESPONSE TO AN OIL ADJUVANT *BACTEROIDES NODOSUS* BACTERIN

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ABSTRACT

CAMERON, C. M. & FULS, W. J. P., 1978. Method of production of and antibody response to an oil adjuvant *Bacteroides nodosus* bacterin. *Onderstepoort Journal of Veterinary Research* 45, 143–147 (1978)

A procedure is described for the production of a highly concentrated *Bacteroides nodosus* bacterin.

An oil adjuvant bacterin which elicited the best antibody response in rabbits produced a response in sheep which lasted for at least 6 months.

Résumé

PRÉPARATION D'UN VACCIN À BACTEROIDES NODOSUS *EN ÉMULSION HUILEUSE ET PRODUCTION D'ANTICORPS CONSÉCUTIVE À SON EMPLOI*

On décrit une technique de préparation d'un vaccin à Bacteroides nodosus *à forte concentration.*

Un vaccin en émulsion huileuse qui était la plus efficace pour la production d'anticorps chez le lapin a provoqué chez le mouton une réponse d'une durée minimale de 6 mois.

INTRODUCTION

Egerton & Roberts (1971) reported that sheep could be successfully immunized against experimental footrot with a bacterin emulsified with Freund's incomplete adjuvant. Further investigation showed that vaccination with an oil wool-wax adjuvant vaccine was effective under field conditions and that the application of this vaccine also had some therapeutic value (Egerton, 1970; Egerton & Burrell, 1970). These results were confirmed in subsequent, more elaborate investigations (Egerton, Morgan & Burrell, 1972; Egerton & Morgan, 1972). Skerman (1971) also reported good results when a similar vaccine was used.

Oil emulsion vaccines unfortunately give undesirable local reactions. To avoid these reactions, Roberts, Foster, Kerry & Calder (1972) composed an alum-treated bacterin which afforded good protection. Satisfactory (Kerry & Craig, 1976) and encouraging (Eikelenboom, 1976) results were reported when this vaccine was used under field conditions.

Since the production technique of this vaccine described by Egerton & Burrell (1970) is cumbersome and unsuitable for mass production, we proposed to develop a more practical production procedure and with the vaccine thus prepared to assay the antibody response following the application of alum-precipitated and various oil emulsion bacterins.

MATERIALS AND METHODS

Bacterial strain

The strain of *B. nodosus* (Strain 198 type A) which was used in these studies was kindly supplied by Dr M. D. Murray*[1].

Media and containers

Plates and tubes: Hoof broth was prepared as described by Egerton, Parsonson & Graham (1968) and hoof agar as described by Egerton & Parsonson (1969).

The hoof broth, containing trypsin and thioglycollate, was distributed in 10 mℓ quantities in tubes as well as 100 mℓ quantities in 300 mℓ Erlenmeyer flasks. The tubes and flasks were fitted with gauze-covered cotton wool stoppers and in addition the Erlenmeyer flasks were fitted with a siphon tube and an inoculation tube (Fig. 1).

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Received 20 March 1978—Editor

FIG. 1 Erlenmeyer flask with approximately 100 mℓ of hoof broth

Seed flasks: The medium used in the seed flasks was prepared according to the formulation and technique described by Egerton (1974), except that soy pepton (Oxoid)*[2] was used instead of soy extract and bovine pancreas instead of sheep's pancreas. The basal medium was distributed in 1 ℓ volumes in 2 ℓ pressure flasks (Fig. 2).

The pancreas, glucose and yeast extract (PGY) was prepared separately, sterilized by filtration through Zeits EKS 2 filter pads and distributed in flat-bottomed boiling flasks (Fig. 3).

Each pressure flask was fitted with a rubber stopper and 4 tubes as shown in Fig. 2. One of the 3 tubes that were passed through the stopper was longer than the other 2 and was used to seed the production flasks while the latter were used for introducing the PGY and inoculum, respectively. The 4th tube was attached to the nozzle of the flask and used for introducing the gas mixture.

*[2] Oxoid Ltd, London, England

FIG. 2 Seed flask containing 1 ℓ basal Egerton medium (2 ℓ pressure flask)

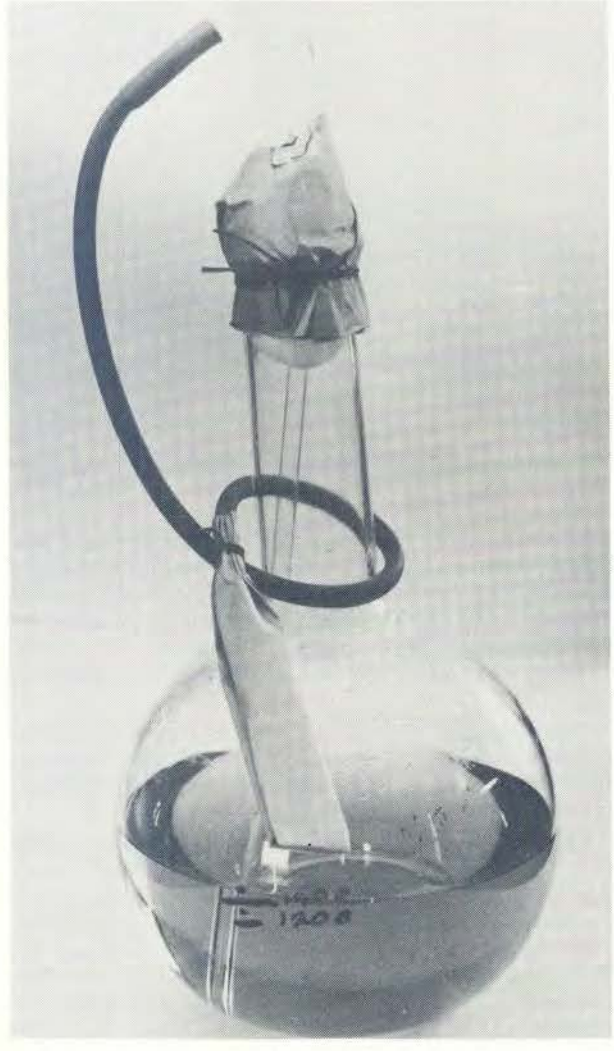

FIG. 3 Round-bottomed flask containing filtered PGY

The basal medium was sterilized by autoclaving (30 min at 120 °C) and, while it was still hot, the tubes were tightly clamped so that a vacuum was created in the flasks as the medium cooled. After cooling the required volume of PGY was siphoned into the flask by suction, care being taken to utilize as little of the vacuum capacity as possible.

Production bottles: The production flasks comprised 10 ℓ bottles containing 8 ℓ Egerton medium and were prepared in the same way as the seed flasks. They, however, were fitted with only 3 tubes, 2 for introducing the PGY and inoculum respectively and the 3rd for introducing the gas mixture.

The principle of preparing the bottles and flasks with a vacuum was based on a description for the preparation of *Fusobacterium necrophorum* bacterin (Garcia & McKay, 1973). The collapsed state of the rubber tubes seen in Fig. 4 clearly indicates the existence of a vacuum.

FIG. 4 Production flask (10 ℓ) containing 8 ℓ of complete Egerton medium. Note collapsed state of rubber tubes indicating a vacuum

Culture technique

Bacterin production was initiated by growing *B. nodosus* on hoof agar plates in McIntosh & Fildes anaerobic jars filled with a gas mixture composed of 90% H_2 and 10% N_2. The jars were incubated for 48 h at 37 °C.

The growth from the plates was used to inoculate the tubes containing 10 mℓ of hoof broth which were incubated under anaerobic conditions for 48 h. The growth from these tubes was used to inoculate the Erlenmeyer flasks which were also incubated in jars containing 90% H_2 and 10% N_2.

The contents of the Erlenmeyer flasks were then used to inoculate the seed flasks (Fig. 2). This was accomplished by utilizing the vacuum in the seed flasks to siphon the culture in the Erlenmeyer flasks through one of the short tubes. Care was again taken to use as little of the vacuum as possible and to avoid an accidental introduction of air.

Once the seeding was completed, the flasks were filled with the 90% H_2 10% N_2 mixture from a gas cylinder. The gas was slowly introduced into the flasks through the tube attached to the nozzle until the collapsed rubber tubes had regained their normal diameter, but as far as possible no internal pressure was allowed to build up. These seed flasks were incubated at 37 °C for 48 h.

The production bottles were inoculated with the contents of the seed flasks in the same way, the siphon tube of the seed flask being attached to one of the short tubes of the production bottles. Gas was then introduced into the bottles by the tube fitted with the cotton wool filter (Fig. 4).

The production bottles were then incubated for 48 h at 37 °C, although 24 h incubation would probably suffice.

Preparation of bacterins

A haemocytometer was used to determine the number of bacteria in the production bottles and the whole culture was inactivated by the addition of 0,3% formalin and by maintaining the bottles at room temperature overnight.

The culture was concentrated by sedimenting the bacteria with 4% polyethylene glycol (p.e.g.) (Cameron & Weiss, 1974). After being allowed to stand at room temperature for 72 h, 8 ℓ of the supernatant fluid was siphoned off, thus giving approximately an 8-fold concentration.

This concentrated suspension of bacteria was used for the preparation of the following bacterins:

A. *Untreated bacterin*

B. *Alum-precipitated bacterin*

Ten mℓ of an 11% solution of potassium alum was added to 100 mℓ of the bacterial suspension.

C. *Bluetongue (BT) oil adjuvant bacterin*

The following solutions were prepared:

Solution 1.	Bayol 72*1	45 mℓ	50 mℓ
	Cirrasol*2	5 mℓ	
Solution 2.	Tween 80*3	0,5 mℓ	50 mℓ
	Distilled water	49,5 mℓ	
Solution 3.	Bacterial suspension	50 mℓ	

Solutions 2 and 3 were mixed, gradually added to solution 1 and emulsified by means of a syringe.

D. *Haemophilus oil adjuvant bacterin*

This bacterin was prepared as recommended by Dr S. B. Buys (personal communication, 1976).

The following components were mixed and emulsified by means of a syringe:

Bayol 72	52 mℓ
Cirrasol	13 mℓ
Tween 80	1 mℓ
Bacterial suspension	34 mℓ

*1 Esso, P.O. Box 78011, Sandton 2146, R.S.A.
*2 ICI, P.O. Box 3784, Alrode 1451, R.S.A.
*3 NBC, Cleveland, Ohio, U.S.A.

E. *B.W. Oil adjuvant bacterin*

The formulation used for this bacterin was based on that of Thomson, Batty, Thomson, Kerry, Epps & Foster (1969), but the components were altered and it was finally composed as follows:

Solution 1.	Bayol 72	72 mℓ	80 mℓ
	Cirrasol	8 mℓ	
Solution 2.	Bacterial suspension	19 mℓ	20 mℓ
	Tween 80	1,0 mℓ	

Solution 2 was slowly added to Solution 1 and emulsified by vigorous shaking.

F. *Freund Incomplete*

Bayol 72 (17 mℓ) and Arlacel A*4 (3,0 mℓ) were mixed and emulsified with 20 mℓ of bacterial suspension by means of a syringe.

G. *Commercial bacterin*

The product*5 used was an alum-precipitated bacterin.

Animal experiments

Rabbits: Groups of 3 adult albino rabbits were used for testing the antibody response to bacterins A to G. Each animal was given 2 subcutaneous injections of 1,0 mℓ at intervals of 4 weeks. They were bled 4 weeks after the 1st and 2nd injections as well as 6 weeks after the 2nd injection. The sera were stored at –20 °C until they were tested.

Sheep: Two groups of 6 adult Dorper wethers were used. The sheep in Group A were given a single subcutaneous injection of 2,0 mℓ of BW oil adjuvant bacterin while Group B were given 2 injections with a 10-week interval in between. Both groups were bled 1, 2, 3, 6 and 12 months after the 1st injection.

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

Serological test

The serum antibody response was assayed as described by Egerton (1974), washed *B. nodosus* strain 198 cells being used as antigen in order to detect "O" agglutinins.

Results

Bacterin production

The production flasks yielded approximately 1,2–1,6 × 10^8 organisms/mℓ which, when concentrated by p.e.g., gave a concentration of approximately 10^9 organisms/mℓ in the concentrated bacterial suspension.

Effect of adjuvants

The agglutinin titres established in rabbits after the administration of various adjuvant bacterins are given in Table 1.

The BW formulation gave the best results while both alum-precipitated bacterins elicited only a poor antibody response.

Duration of antibody response in sheep

As shown in Table 2, the antibody response was slow but lasted for at least 6 months irrespective of whether 1 or 2 injections were given. By 12 months the titres in both groups had dropped appreciably.

*4 Hilltop Laboratories Inc., Cincinnati, Ohio, U.S.A.
*5 Clovax, Foot-rot vaccine, Burroughs Wellcome & Co., Berkhamsted, Herts, England

TABLE 1 Average reciprocal serum agglutination titres of rabbits given different bacterins

Bacterin	Average reciprocal agglutination titre		
	Four weeks after 1st injection	Four weeks after 2nd injection	Six weeks after 2nd injection
A. No adjuvant	0	20,0	25,4
B. Alum	0	20,0	63,5
C. B. T. adjuvant	50,4	1 016,0	508,1
D. *Haemophilus* adjuvant	160,0	1 016,0	2 032,0
E. B. W. adjuvant	452,7	806,9	3 225,0
F. Freund incomplete	320,0	403,3	10 240,0
G. Commercial foot-rot vaccine	10,0	40,0	25,2
H. Controls	0	0	0

TABLE 2 Antibody response in sheep given *B. nodosus* BW oil adjuvant bacterin

Group	Average reciprocal agglutination titres					
	Before injection	Months after 1st injection				
		1	2	3	6	12
A. One injection only	14,8	35,6	57,9	376,3	330,0	33,6
B. Two injections at 10-week-intervals	14,8	28,3	50,4	172,8	452,6	22,5

Discussion

The technique described in this paper is suitable for medium scale production of vaccine. It may not be the ideal procedure, however, and the improved medium formulated by Skerman (1975) may give better results. The specific growth phase at which the bacteria are harvested may also be important in the light of the fact that pili have been shown to be closely associated with the antigenicity of *B. nodosus* (Walker, Short, Thomson & Roberts, 1973).

Since the "O" agglutinin levels of antibody in the sera of vaccinated sheep are correlated with immunity (Egerton & Merritt, 1973; Egerton, 1974) while cellular immune mechanisms are probably not involved (Moriarty, Cooper & Ingram, 1976), the assay of these antibodies should be a fair measure of the immune status of an animal. In sheep our oil emulsion bacterin (BW) did not elicit antibody levels as high as those reported by Egerton & Merritt (1973) and Egerton (1974). The titres obtained in rabbits with a commercial bacterin were also much lower than those recorded by Roberts *et al.* (1972) using the same product. It thus appears that the observed discrepancy is due to differences in technique and the proof of the efficacy of our product will have to be established by means of exposure experiments. Serological assays should also preferably employ tests which detect antibodies to pili since these antigenic structures are responsible for the production of agglutinins associated with immunity (Walker *et al.*, 1973).

The existence of at least 3 different serotypes of *B. nodosus* is a further complicating factor, as cross-immunity between them is negligible and any bacterin should therefore contain all the serotypes present in a particular area or country.

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