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ANTIBODY RESPONSE OF GUINEA-PIGS AND CATTLE TO A *CAMPYLÖBACTER FETUS* OIL EMULSION VACCINE

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ABSTRACT

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A method is described for the mass production of *Campylobacter fetus* oil emulsion vaccine, using 2 strains of *C. fetus* ss *intestinalis* and a strain of *C. fetus* ss *intermedius* as a substitute for *C. fetus* ss *venerialis*. Heifers, given 2 injections of the vaccine, developed serum antibody titres comparable with the response induced by a commercial product.

INTRODUCTION

It is now generally accepted that heifers can be effectively immunized against *Campylobacter fetus* infections (Firehammer & Berg, 1966; Newhall, 1966; Mitchell, 1968; Kendrick, Williams, Crenshaw & Vestal, 1971; Clark, Dufty & Monsbourgh, 1972 a; Clark, Dufty & Monsbourgh, 1972 b; Clark & Fitzpatrick, 1972; Clark, Dufty, Monsbourgh & Parsonson, 1976; Clark, Dufty & Monsbourgh, 1975). Mass production of vaccines, however, remains a problem, since strains of *C. fetus* ss *venerialis* grow poorly, the media are either complex or expensive (Simon, 1975; Clark, Dufty & Monsbourgh, 1976; Berg & Firehammer, 1978) and specific atmospheric conditions are required (Robertstad & Morrison, 1957; Dennis & Jones, 1959; Clark *et al.*, 1972 a). It was therefore proposed to employ a production technique based on the procedures described by Clark *et al.* (1972 b) and Berg & Firehammer (1978), respectively, to overcome the above-mentioned obstacles.

To obviate the problems of poor growth and the exacting nutritional and physical requirements of *C. fetus* ss *venerialis*, it was further proposed to investigate the possibility of substituting this strain with a less fastidious but serologically related organism (Clark *et al.*, 1975).

MATERIALS AND METHODS

Bacterial strains

The strains of *C. fetus* used in this study are listed in Table 1. They were stored, freeze-dried and activated for use on blood tryptose agar plates under 10% CO_2. The nomenclature used is that employed by Clark *et al.* (1976).

TABLE 1 *C. fetus* strains used

Strain No.	Identity	Source
68/4	*C. fetus* ss *intestinalis*	Heifer vaginal mucus, 1971
7572	*C. fetus* ss *intestinalis*	Dr P. de Keyser*
661	*C. fetus* ss *intestinalis*	Dr P. de Keyser*
796/1	*C. fetus* ss *venerialis*	Bull, sheath washing, 1979
873/5	*C. fetus* ss *venerialis* biotype *intermedius*	Cow, cervical biopsy, 1980

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Vaccine production

Culture media. The following culture media were used:

(i) Botha's medium for production of seed material (A. D. Botha, personal communication, 1981).

Peptone (Biolab)*	20	g
Yeast extract (Biolab)*	5	g
Aspartic acid	2,5	g
Glumatic acid	2,8	g
$CaCl_2$ (2% sol.)	1,0	mℓ
$MgSO_4.7H_2O$ (1% sol.)	1,0	mℓ
NaCl	3,0	g
Distilled water	1 000	mℓ

The above ingredients were dissolved, the pH was adjusted to 7,0, and the solution distributed into Erlenmeyer or boiling flasks, fitted with inoculation tubes and siphons, as required.

(ii) Onderstepoort *C. fetus* vaccine production medium based on the medium of Dennis & Jones (1959).

Peptone (Biolab)*	20	g
Yeast extract (Biolab)*	5	g
NaCl	7	g
$MgSO_4.7H_2O$	0,04	g
$CaCl_2.2H_2O$	0,04	g
Distilled water	1 000	mℓ

The ingredients were dissolved by heat, the pH was adjusted to 7,0, and the solution distributed into production flasks or a fermentation tank, as required.

Method of vaccine production. The *C. fetus* strains were maintained on nutrient broth containing 0,5% agar. After incubation for 72 h at 37 °C, the growth from 3 tubes (± 60 mℓ) was used to inoculate 800 mℓ of Botha's medium in 1 ℓ Erlenmeyer flasks. These were kept static for 24 h at 37 °C and then rotated slowly on a rotary shaker for a further 24–48 h, depending on the density of growth obtained. The 800 mℓ cultures were used to inoculated 15 ℓ volumes of Botha's medium and the growth cycle was repeated. For mass production, the 15 ℓ cultures were used to inoculate 300 ℓ of Onderstepoort vaccine production medium contained in a 800 ℓ fermentation tank. After inoculation, the culture was kept static for ± 18 h at 37 °C and then agitated for a further 26 h by means of a paddle rotating machine at approximately 200 rpm. During the last 18 h of growth the culture was aerated (Clark *et al.*, 1972 b).

The cultures were inactivated by the addition of 0,5% formalin and kept for 4 days at 37 °C. The bacterial cells were then sedimented and concentrated approximately fiftyfold (Clark *et al.*, 1972 b) by the addition of 4% polyethylene glycol, and then kept for 3 days at room temperature to allow the cells to settle (Cameron & Weiss, 1974). The density of each strain was adjusted to 10% packed cell volume and, for combined vaccines, equal volumes of each strain were mixed. The purity of the cultures was monitored throughout by examination of Gram-stained smears and blood tryptose agar cultures.

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For the preparation of oil emulsion vaccines, 5% 'Tween 80'*[1] was added to the bacterial suspension (water phase), whereas the oil phase contained 10% 'Cirrasol'*[2] and 90% Bayol 72* or Marcol 52*[3] oil. The emulsion was prepared by slowly adding 1 part of the water phase to 4 parts of the oil phase, the latter being constantly shaken. The final trivalent vaccine therefore contained approximately 0,8% packed cells per strain. The monovalent vaccines contained 1,0% packed cells. The stability of the emulsion was tested by placing a drop of it on the surface of cold water in a beaker and the sterility of the final product was verified by cultures in thioglycolate and soya broth media.

To examine the effect of the type of oil used on the immune response, three vaccines, all containing *C. fetus* strains 68/4, 7572 and 873/5 but made up either with 'Bayol' 72 or 'Marcol' 52, were prepared. The antigenicity of the vaccines was evaluated in guinea-pigs, as outlined below.

Immunization of guinea-pigs

Groups of 10 guinea-pigs were used to test the antibody response to each experimental vaccine. Each guinea-pig was given a single subcutaneous injection of 1,0 mℓ and bled by cardiac puncture 4 weeks later. The sera were stored at −20 °C until they were tested.

Immunization of heifers

Four groups of 10 Bonsmara heifers were used to test the antibody response to a production batch of Onderstepoort vaccine and a commercial vaccine* after 1 or 2 injections. Group A received a single subcutaneous injection of Onderstepoort Batch 82 vaccine and Group B recieved 2 injections at a 4-week interval. Groups C and D were similarly given the commercial vaccine. The animals were prebled and bled again 6 weeks after the 2nd injection. The sera were stored at −20 °C until they were tested. IgM antibodies were inactivated before testing by heating to 65 °C for 15 min (Border & Firehammer, 1980).

Serological tests

Formalinized antigens for agglutination tests were produced in Botha's medium, as described for vaccine production. *C. fetus* ss *venerialis* (Strain 796/1) antigen was produced on blood tryptose agar plates, incubated under 10% CO_2. The antigen suspensions were made in saline and standardized to contain 4% packed cells.

The test itself was based on the method of Berg & Firehammer (1978), as described by Bryner (J. H. Bryner, personal communication, 1979). For testing cattle sera, the antigen was heated to 65 °C for 15 min to depolymerize the flagella (Border & Firehammer, 1980).

RESULTS

Vaccine production

During preliminary experiments it was found that 2 factors materially influenced the yield or organisms. The first was the need to use fairly large inocula (5%) in the production flasks or fermenter, and the second was to allow at least a 24 h stabilization period before agitation or aeration was commenced. By using these techniques and the appropriate media, yields of 0,2% packed cells can consistently be obtained.

*[1] NBC, Cleveland, Ohio, USA

*[2] ICI, P.O. Box 3784, Alrode 1451, RSA

*[3] Esso, P.O. Box 78011, Sandton 2146, RSA

* 'Vibrin' Norden Laboratories, Lincoln, Nebraska, USA

Serological relationship between strains

The results in Table 2 show that Strain 661 (*C. fetus* ss *intestinalis*) is poorly antigenic and serologically unrelated to the other strains. The same applies to Strain 68/4 (*C. fetus* ss *intestinalis*), although it exhibited some cross reaction to Strain 873/5. Strain 796/1 (*C. fetus* ss *venerialis*) was also distinct, but showed appreciable cross reaction with Strain 873/5 (*C. fetus* ss *intermedius*). The latter was consequently used as a substitute for Strain 796/1 in the subsequent experiment. Strain 7572 (*C. fetus* ss *intestinalis*) antiserum reacted only poorly with the other strains, but Strain 7572 antigen cross-reacted with Strain 873/5 antiserum.

TABLE 2 Serological relationship of *C. fetus* strains used in this study

Antigen strain	Antisera				
	Mean agglutinin titres				
	68/4	7572	661	796/1	873/5
68/4	6016	86	6	5	288
7572	484	520	190	312	2944
661	7	17	136	20	n.t.
796/1	20	18	28	9729	1508
873/5	178	75	38	2432	2678

n.t. = Not tested

Antibody response of guinea-pigs to trivalent vaccine

As is shown in Table 3, guinea-pigs responded well to both vaccines, but in this experiment the substitution of Strain 873/5 for Strain 796/1 did not have the desired effect. In the subsequent experiment, however, high levels of antibodies were obtained to strain 796/1, despite its being omitted from the experimental trivalent and production Batch 82 vaccines.

When 3 trivalent vaccines were prepared with different oils, it was shown that the type of mineral oil used had no significant effect on the results, but, since 'Marcol 52' oil produced a thinner emulsion, it was the one preferred (Table 4).

TABLE 3 Antibody response in guinea-pigs to 3 polyvalent vaccines

Antigen strain	Vaccine composition		
	A: Strains 68/4; 7572 and 796/1	B: Strains 68/4; 7572 and 873/5	C: Commercial vaccine*
	Mean agglutination titres		
68/4	3324	6560	1226
7572	5738	6560	1632
796/1	7680	328	330
873/5	1504	3872	139

* 'Vibrin', Norden Laboratories, Lincoln, Nebraska, USA

TABLE 4 Effect of the type of oil on the antibody response of guinea-pigs to polyvalent *C. fetus* vaccine

Antigen strain	Vaccine		
	Experimental vaccine 1 'Bayol' 72 oil	Experimental vaccine 2 'Marcol' 52 oil	Onderstepoort production batch 82 'Marcol 52' oil
	Mean agglutination titres		
68/4	7120	7680	2880
7572	7680	4938	6640
796/1	3680	6940	8320
873/5	8320	8046	8960

Antibody response of heifers to trivalent vaccine

As is shown in Table 5, 2 injections of the Onderstepoort vaccine generally resulted in a better antibody response than a single injection. In the case of the commercial product, a 2nd injection did not have the same booster effect. The titres remained lower than those of the Onderstepoort vaccine except those of Strain 796/1, which were higher after both a single and 2 injections.

TABLE 5 Antibody response of heifers to *C. fetus* polyvalent vaccines

Antigen strain	Onderstepoort vaccine: Batch 82		Commercial vaccine	
	1 injection	2 injections	1 injection	2 injections
	Mean agglutination titres			
68/4	693	6400	33	54
7572	83	457	264	167
796/1	520	496	1066	800
873/5	6790	10240	2478	8400

Discussion

C. fetus ss *venerialis* is the most common cause of *Campylobacter* infertility in cattle and any vaccine designed to combat this infection should elicit a good antibody response to it. Typical *C. fetus* ss *fetus* organisms, however, grow poorly on artificial media and are therefore difficult to include in a mass-produced vaccine. To solve this problem, a *C. fetus* ss *venerialis biotype intermedius* strain that is serologically related to *C. fetus* ss *venerialis* and grows well was included in a trivalent vaccine. Serological tests in guinea-pigs show that this approach was successful, but the serological results in cattle were less satisfactory. The vaccine described here has nevertheless given satisfactory results in the field (Beutler-Schröder, 1980) and these are in agreement with the findings of Clark *et al.* (1975). Further studies, designed to find a more antigenic strain or to develop an economic and practical method for producing large quantities of *C. fetus* ss *venerialis* cells, however, are necessary. The practicality of using culture supernatants which contain the immunizing K antigens (Myers, 1971; Border & Firehammer, 1980) should also be investigated.

Our results indicate that, for the initial immunization of heifers, 2 injections of vaccine should be used. This is in accordance with the findings of Clark *et al.* (1972 b).

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