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

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ABSTRACT


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 venerealis. 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, Cremaske & 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 venerealis 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 venerealis, 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% CO₂. The nomenclature used is that employed by Clark et al. (1976).

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
- CaCl₂ (2% sol.) 1,0 mℓ
- MgSO₄.7H₂O (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
- MgSO₄.7H₂O 0,04 g
- CaCl₂.2H₂O 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 inoculate 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.

<table>
<thead>
<tr>
<th>TABLE 1 C. fetus strains used</th>
</tr>
</thead>
<tbody>
<tr>
<td>Strain No.</td>
</tr>
<tr>
<td>-------------</td>
</tr>
<tr>
<td>68/4</td>
</tr>
<tr>
<td>7572</td>
</tr>
<tr>
<td>661</td>
</tr>
<tr>
<td>7961</td>
</tr>
<tr>
<td>8735</td>
</tr>
</tbody>
</table>

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For the preparation of oil emulsion vaccines, 5% 'Tween 80' was added to the bacterial suspension (water phase), whereas the oil phase contained 10% 'Cirrasol' and 90% Bayrol 72* or Marcol 52* 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 received 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 both'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% CO₂. 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 between agitation or aeration was commenced. By using these techniques and the appropriate media, yields of 0.2% packed cells can consistently be obtained.

**Serological relationship between strains**

The results in Table 2 show that Strain 661 (*C. fetus ss intestinals*) is poorly antigenic and serologically unrelated to the other strains. The same applies to Strain 68/4 (*C. fetus ss intestinals*), although it exhibited some cross reaction to Strain 796/1. Strain 796/1 (*C. fetus ss intestinals*) 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 intestinals*) antisera reacted only poorly with the other strains, but Strain 7572 antigen cross-reacted with Strain 873/5 antisem.

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

<table>
<thead>
<tr>
<th>Antigen strain</th>
<th>Antiserum</th>
<th>68/4</th>
<th>7572</th>
<th>661</th>
<th>796/1</th>
<th>873/5</th>
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<tbody>
<tr>
<td>68/4</td>
<td>0.16</td>
<td>86</td>
<td>6</td>
<td>5</td>
<td>288</td>
<td></td>
</tr>
<tr>
<td>7572</td>
<td>604</td>
<td>520</td>
<td>190</td>
<td>312</td>
<td>2944</td>
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<tr>
<td>661</td>
<td>7</td>
<td>17</td>
<td>136</td>
<td>20</td>
<td>n.t.</td>
<td></td>
</tr>
<tr>
<td>796/1</td>
<td>20</td>
<td>18</td>
<td>28</td>
<td>9729</td>
<td>1508</td>
<td></td>
</tr>
<tr>
<td>873/5</td>
<td>178</td>
<td>75</td>
<td>38</td>
<td>2432</td>
<td>2678</td>
<td></td>
</tr>
</tbody>
</table>

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**

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

* '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**

<table>
<thead>
<tr>
<th>Vaccine composition</th>
<th>Experimental Vaccine 1</th>
<th>Experimental Vaccine 2</th>
<th>Onderstepoort production Batch 82</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antigen strain</td>
<td>'Bayol' 72 oil</td>
<td>'Marcol' 52 oil</td>
<td>'Marcol 52' oil</td>
</tr>
<tr>
<td>Mean agglutination titres</td>
<td>7120</td>
<td>7680</td>
<td>2880</td>
</tr>
<tr>
<td>7572</td>
<td>7680</td>
<td>4038</td>
<td>6640</td>
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<tr>
<td>873/5</td>
<td>3680</td>
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<td>8320</td>
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<tr>
<td>7572</td>
<td>8320</td>
<td>8046</td>
<td>8960</td>
</tr>
</tbody>
</table>

* NBC, Cleveland, Ohio, USA
* IC, P.O. Box 3784, Atrode 1451, RSA
* Esso, P.O. Box 78011, Sandton 2146, RSA
* 'Vibrin' Norden Laboratories, Lincoln, Nebraska, USA
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 vaccine

<table>
<thead>
<tr>
<th>Antigen strain</th>
<th>Onderstepoort vaccine: Batch 82</th>
<th>Commercial vaccine</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 injection</td>
<td>2 injections</td>
</tr>
<tr>
<td>68/4</td>
<td>693</td>
<td>6400</td>
</tr>
<tr>
<td>7572</td>
<td>83</td>
<td>457</td>
</tr>
<tr>
<td>796/1</td>
<td>520</td>
<td>496</td>
</tr>
<tr>
<td>873/5</td>
<td>6790</td>
<td>10240</td>
</tr>
</tbody>
</table>

| Mean agglutination titres |

Discussion

C. fetus ss venerialis is the most common cause of Campylobacter infection 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).

Acknowledgements

I wish to thank Mr B. H. J. Smit and Mr J. H. Schoeman for producing the bulk vaccine and Dr Leslie Te Brugge for confirming the identity of the C. fetus strains used. Special tribute is due to the late Mr W. J. P. Fuls, who prepared the experimental vaccines and conducted the serological tests.

The Director, Transvaal Region, Department of Agriculture, is thanked for making the experimental cattle available.

References


