Mass Production of Clostridium oedematiens Vaccine Against Black Disease of Sheep

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Summary: Attempts were made to produce and formulate the ingredients of a culture medium suitable for obtaining a highly immunogenic C. oedematiens type B vaccine for immunisation of sheep and goats against black disease. Large-scale production of C.oedematiens toxin was achieved in a culture medium consisting of 4% peptone, 1% maltose, 0.5% di-sodium hydrogen phosphate, 0.05% L-cysteine and 0.04% liver powder. The immunogenicity of prepared vaccine was determined in sheep and rabbits according to the British Veterinary pharmacopoeia and field reports.

Keywords: Sheep diseases / Clostridium / Vaccines / Braxy

Introduction

Black disease is an acute and fatal disease of sheep and goats in Iran (1). C.oedematiens type B has been isolated and typed from liver lesions obtained from different parts of Iran (2).

For induction of black disease in sheep, invasion of sheep liver by immature fluke is an essential factor and must be controlled by efficient flukicides. The object of this study was to prepare a potent large scale vaccine against black disease of sheep and goats. Nishida et al (3) suggested an excellent medium containing peptone, maltose, di-sodium hydrogen phosphate and meat particles for production of C.oedematiens toxin but meat particles cause some
difficulties in mass production. Ardehali et al (4) prepared experimentally a highly potent vaccine against black disease but this type of vaccine is very costly in mass production for veterinary use.

**Materials and Methods**

Medium: Several media with different ingredients were tested for production of C. oedematiens vaccine. The following medium was selected and used for a large-scale production.

1 - Peptone (Oxoid L.) .................. 4%
2 - Maltose ............................ 1%
3 - NaHPO₄ ................................ 0.5%
4 - Liver powder ...................... 0.04%
5 - L-cysteine .......................... 0.05%

Preparation of vaccine: The method of production of C. oedematiens was the same as described by Ardehali et al (4). The aluminium hydroxide gel has been selected as adjuvant for production of Clostridial vaccines (5). The final products consisted of 60% C. oedematiens vaccine, 10% aluminium hydroxide gel and 30% distilled water.

**Quality control of the vaccine:**

*Potency test:*

1 - Sheep: Ten healthy sheep were selected and given two doses of a 3 ml and a 5 ml of the vaccine with an interval of two weeks. The animals were bled before inoculation, two weeks after the first injection and two weeks after the second injection.

The collected sera were tested for determination of the level of antibody against C. oedematiens alpha toxin according to the British Veterinary Pharmacopoeia (6).

2 - Rabbits: Twelve healthy rabbits were vaccinated with two doses of 3 ml

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1 - Final concentration consisted of 1.5 mg Al(OH)₃ per ml in vaccine.
each (vaccinal dose) with an interval of four weeks. After fourteen days the rabbits were bled and the pooled sera were tested for determination of the level of C. oedematiens alpha antitoxin according to the British Veterinary pharmacopoeia (6).

Results and Discussion

The level of C. oedematiens alpha antitoxin of pooled sera in unvaccinated sheep was less than 0.2 International Units per ml. Two weeks after 1st inoculation of the vaccine, the level of antitoxin increased to 0.7 and 1 International Units per ml in the pooled sera of vaccinated sheep respectively. Two weeks after second inoculation, the level of antitoxin in sheep pooled sera reached 2 and 3 International Units per ml. Two doses of five ml of vaccine produced higher level of antibody than three ml dose (Table 1). Macheak et al (7) proved that 1.6 International Units of C. oedematiens alpha antitoxin of sheep serum protected most animals against C. oedematiens challenge.

The result of the level of C. oedematiens alpha antitoxin in the pooled rabbits sera was 10 International units per ml, which was three times higher than recommended by British Veterinary Pharmacopoeia for C. oedematiens alpha antitoxin (Table 2).

Table 1: The responses of sheep to a 3 ml and a 5 ml dose of C. oedematiens aluminium hydroxide gel vaccine.

<table>
<thead>
<tr>
<th>No. of sheep</th>
<th>dose of vaccine</th>
<th>Titre of sheep pooled sera of C. oedematiens alpha antitoxin in International Unit per ml.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Preinoculation</td>
</tr>
<tr>
<td>5</td>
<td>3 ml</td>
<td>0.2</td>
</tr>
<tr>
<td>5</td>
<td>5 ml</td>
<td>0.2</td>
</tr>
</tbody>
</table>
Table 2

<table>
<thead>
<tr>
<th>No.of rabbits</th>
<th>Dose of vaccine</th>
<th>Titre of rabbits pooled sera of C.oedematiens alpha antitoxin in International unit per ml.</th>
</tr>
</thead>
<tbody>
<tr>
<td>12 Veterinary Codex Standard</td>
<td>3 ml</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3.5</td>
</tr>
</tbody>
</table>

The results of the prepared C.oedematiens vaccine which was tested in sheep and rabbits showed that a 3 ml dose of the vaccine which had been used in the field for immunization of sheep and goats, produced significant titre of C.oedematiens antitoxin.

Acknowledgements

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References


6 - British Pharmacopei (Veterinary) 1985. Clostridium oedematiens type B vaccine, 166, appendix XIV B A113.