

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 ₅₂)4%
2 - Maltose	1%
3 - Na ₂ HPO ₄	0.5%
4 - Liver powder	0.04%
5 - L-cysteine005%

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

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.

No.of sheep	dose of vaccine	Titre of sheep pooled sera of <i>C.oedematiens</i> alpha antitoxin in International Unit per ml.		
		Preinoculation	2 weeks after 1st inoculation	2 weeks after second inoculation
5	3 ml	0.2	0.7	2
5	5 ml	0.2	1	3

Table 2

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

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.

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