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MASS PRODUCTION AND STANDARDIZATION OF CLOSTRIDIUM OEDEMATIENS VACCINE AGAINST BLACK DISEASE (INFECTIOUS NECROTIC HEPATITIS) OF SHEEP (*)

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ABSTRACT

The object of this study was to prepare a potent vaccine against black disease of sheep. Attempts were made to prepare and formulate the ingredients in order to obtain high yield of toxin. A batch of mass production of Clostridium oedematiens (Cl.novyi) vaccine was prepared in volume of 600 litres with ingredients consisted of 4% peptone, 0.25% NaCl, 0.5% liver extract, 0.1 % L-cysteine, 1% maltose, 0.02% sodium dithionite and 0.25% ferrous sulphate solution.

The prepared vaccine was diluted at a rate of 20, 40, 60 and 80 per cent of antigens. Potassium alum was added as an adjuvant. The potency test of the prepared vaccine was determined in a group of forty rabbits according to the British Pharmacopoeia (Veterinary). Maximum titre was obtained in 80 per cent with the level of 33 units per ml of alpha antitoxin in rabbits pooled serum. 20, 16 and 8 units per ml of alpha antitoxin were also obtained in 60, 40 and 20 per cent of diluted antigen in rabbits pooled serum respectively. Sheep have been vaccinated in black disease areas by this vaccine, the reports obtained from the field indicated that black disease in sheep could be effectively controlled by this vaccine in Iran.

INTRODUCTION

Black disease is an acute and fatal disease of sheep and goats in Iran. It occurs in some areas where the animals are affected with liver fluke infestation. Cl. oedematiens types A, B and D have been isolated and studied from liver lesions among the black disease cases of sheep.(1)

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The object of this study was to prepare and test an experimental batch of potent Cl. oedematiens potash alum adjuvant vaccine to immunise animals against black disease of sheep and goats in Iran.

There are less references in the literature concerning the production of Cl. oedematiens vaccine. An excellent medium suggested by Nishida and Nakagawara(2) contains peptone, maltose, di-sodium hydrogen phosphate and meat particles but meat particles cause some difficulties in mass production of Cl.oedematiens vaccine.

Attempts were made to prepare and formulate the ingredients in order to obtain high titre of toxin.

MATERIALS AND METHODS

Preparation of vaccine

After several experimental tests for composition of the ingredients, the following medium was prepared for production of large scale of Cl.oedematiens vaccine:

1- Peptone (Oxoid L52) 4%
2- NaCl 0.25%
3- Liver extract 0.5%
4- L-cysteine 0.1%
5- Maltose 1%
6- Ferrous sulphate ..... 0.25%(*)
7-Sodium dithionite 0.25%

The ingredients were dissolved in 200 litres of distilled water at 37°C, the pH of the medium was adjusted to 8. The temperature of the medium was raised up to boiling for ten minutes. For rapid precipitation of non specific materials, the cold water was circulated in double tank for rapid cooling and after one hour the concentrated supernatant was distributed in volume of 5 liter in each of 40 twenty litre bottles and the volume was raised up to 16 litres by adding distilled water. The bottles were autoclaved at 110°C for 40 minutes. For preparation of 640 litres of vaccine, forty of twenty litres bottles were used, each one with 16 litres of medium.

(*) used of 4% ferrous sulphate diluted in sulfuric acid

Seed culture

Cl.oedematiens strain (C.N.804) was inoculated in a tube of fresh liver medium as seed culture. After an active growth, it was subcultured in the flasks of 300 ml liver medium. The 16 litres bottles were inoculated with 0.5% of the seed cultures. The period of incubation for production of maximum toxin was 72 hours. The samples were removed from some bottles for the determination of minimum lethal dose of toxin in mice (18-20 grs). The cultures were checked for non specific organisms by subculturing in broth and slope agar medium.

Toxoiding

The volume of 96 ml of 40% of commercial formaldehyde solution was added to each bottle. The formolised cultures were left for 7-10 days more in incubator to be detoxified and transformed to bacterin. The anaculture after being removed from incubator, sample were taken from each container and mixed together for safety and potency tests of vaccine.

Blending of vaccine

The collected samples were prepared for testing as follow:

1- Twenty per cent of anaculture plus twenty per cent of 2.5% of sterile potash alum as adjuvant and sixty per cent of distilled water were well mixed to give a final concentration of 0.5 per cent alum at pH 6.5.
Sterile alum and twenty per cent caustic soda were added at the same time.

2- Forty per cent of anaculture plus twenty per cent of alum and forty per cent distilled water as above.

3- Sixty per cent of anaculture plus twenty per cent of alum and twenty per cent distilled water as above.

4- Eighty per cent of anaculture and 20 per cent of potash alum without distilled water as above.

Potency test

The following materials were prepared for potency test of Cl.oedematiens vaccine.

Preparation of Cl.oedematiens concentrated toxin

A highly toxigenic strain of Cl.oedematiens was grown in a medium consisting of 3% peptone (Oxoid L52), 0.5% Na2 HPO4, 1% maltose and 5% chopped meat at pH-8. (2) After three days incubation, the culture was centrifuged and the supernatant was transferred in a celuphan sac and was concentrated by polyethylene glycolecompound 20-M (Carbowax). The titre of concentrated toxin obtained was 20,000 minimum lethal dose per ml using 18-20 grams mice.

Cl.oedematiens standard antitoxin

The standard antitoxin was supplied by Statene Seruminstitute.(*)

Potency of the vaccine was determined according to the British Pharmacopoeia Veterinary. (3)

Forty healthy rabbits (three to six month old) were selected for potency test of the Cl.oedematiens experimental vaccine.

1- First group of ten rabbits were vaccinated with 3 ml dose of No. 1 prepared vaccine.
2- Second group of ten rabbits were vaccinated with 3 ml dose of No. 2 prepared vaccine.
3- Third group of ten rabbits were vaccinated with 3 ml dose of No. 3 prepared vaccine.
4- Fourth group of ten rabbits were vaccinated with 3 ml dose of No. 4 vaccine.

After four weeks the same quantity of the vaccines were injected to each group of ten rabbits. After fourteen days of the second injection, the rabbits were bled. Sera were separated from the collected blood of each group of the injected rabbits, then one ml of each serum was taken and pooled. The units of antitoxic value of the pooled sera was determined by standard method for titration of Cl.oedematiens alpha antitoxin, British Pharmacopoeia.(3)

The prepared vaccines were injected subcutaneously with 3 ml dose in 800 sheep during an outbreak of black disease around Razi Institute.

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RESULTS AND DISCUSSION

The titre of the toxin produced by cultures of Cl.oedematiens was 1500-2000 minimum lethal dose per ml for mice.

The results of the test on forty rabbits vaccinated with different composition of antigens are summarised in Table No. 1.

The titre of immunised rabbits showed that the level of antitoxic values were very high. According to the British Pharmacopoeia each mililitre of immunised rabbits pooled serum should contain 3.5 units per ml of Cl.oedematiens alpha antitoxin. The prepared vaccine which contained only twenty per cent of antigen (No. 1), the alpha antitoxic value was 8 International units per ml in rabbits pooled serum. Maximum units was obtained in 80 per cent antigen with the level of alpha antitoxin 33 units per ml in the rabbits pooled serum.

The rate of sixty and forty per cent of antigens gave high titres as 20 and 10 international units of alpha antitoxin in the rabbits pooled serum in immunised animals. The results of this experiments proved that the minimum composition of the prepared vaccine produced high titre of antitoxin in the vaccinated animals.

The reports obtained from the field where the vaccines had been injected indicated the black disease could be effectively controlled by this vaccine.

Table No. 1

The responses of rabbits to an experimental batch of Cl.oedematiens vaccine

Cl.cedematiens vaccine consisted of:	**Responses in International units per ml to Cl.odematiens alpha antitoxin**
80 per cent antigen	33 units/ml
60 per cent antigen	20 units/ml
40 per cent antigen	16 units/ml
20 per cent antigen	8 units/ml
Recommended by British Vet. Codex	3.5 " "

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(3) British Pharmacopoeia (Veterinary) (1979). Clostridium oedematiens type B vaccine, 156, A10 Appendix XIV B A10 University Press, Cambridge.