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PRODUCTION AND STANDARDIZATION OF TWO TYPES OF CLOSTRIDIAL VACCINES FOR SHEEP AND CATTLE

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SUMMARY

Two types of Clostridial vaccines, one consisting of Cl.perfringens types, B,C,D and Cl.oedematiens, the other comprising Cl.chauvoei were prepared and tested in rabbits and sheep. The method of preparation, concentration and standardization of the vaccines are described. Both vaccines developed high antibody titre in injected animals according to the British Veterinary Pharmacopoeia and field reports.

Introduction

The clostridial infections among sheep, goats and cattle have been diagnosed since 1936 in Iran. Lamb dysentery, haemorrhagic enteritis of sheep and goats, enterotoxemia of lambs and adult sheep, black disease of sheep and blackleg of cattle have been encountered and the causative agents isolated from infected animals from different parts of Iran(1).

Clostridial infections have caused economic losses each year among domestic animals in this country.

The object of this study was to prepare and test an experimental batch of highly immunogenic combined vaccine against clostridial infections of sheep and cattle in Iran.

Materials and Methods

Two types of vaccines were prepared.

1- A polyvalent vaccine against enterotoxemia and necrotic hepatitis of sheep and goats.

II- A bovine blackleg vaccine.

Preparation of the media

The following culture media were prepared for the production of clostridial vaccines with some modification according to the formula described by Katic et al. (2)

1- Cl. perfringens type B

Peptone (Biokar type II*) 3%
Glucose 1%
Trace elements 0.5%
Vitamins 0.2%
pH-7.6

II- Cl. perfringens type C

Peptone (Biokar type II) 4%
Glucose 1%
Trace elements 0.5%
Vitamins 0.2%
pH-7.6

III- Cl. perfringens type D

Peptone (Biokar type II)..... 3%
Dextrine 1%
Trace elements 0.5%
Vitamins 0.2%
pH-8.2

IV- Cl. oedematiens

Peptone (Biokar type II)..... 4%
Maltose 2%
pH-7.6

V- Cl. chauvoei

Peptone (Biokar type II)..... 4%
Glucose 2%
pH-7.5

The peptones were dissolved in distilled water and distributed into 10 litre bottles (8 litre per bottle). The bottles were autoclaved at 110°C for 40 min. The solutions of trace elements, vitamins, glucose, dextrine and maltose were prepared separately and sterilized by filtration and then added to sterilized bottles (3)

Preparation of vaccines

Strains: The highly toxigenic strains of Cl.perfringens types B,C,D and Cl.oedematiens type B and also pathogenic strains of Cl.chauvoei were used for the preparation of vaccines.

Seed culture. The strains were maintained in freeze dried state and each organism was inoculated into a tube of fresh liver medium as seed culture. It was controlled for purity by subculturing in broth and slope agar medium. Then after an active growth, it was subcultured in 200 ml of the liver medium. The bottles containing 8 litres of liver medium were inoculated with 1% of the starter and were then incubated at 37°C. The optimum times required for maximum production of each toxin were as follow:

(a) Cl.perfringens type B and C: 18 hours

(b) Cl.perfringens type D: After 18 hours of incubation, the samples were removed for the determination of minimum lethal dose of toxin (filtrate) in mice and then the culture was treated with 0.005 grams per cent of trypsin and incubated for another 2 hours (4).

(c) Cl.septicum and Cl.chauvoei: 48 hours.

(d) Cl.oedematiens: 3 days.

The samples were removed from each bottle and determination of minimum lethal dose of toxin (filtrate) in mice was done in case of Cl.perfringens and Cl.oedematiens. Smears were taken to check growth and contamination. The cultures were checked for purity by subculturing in broth and also slope agar media. Toxigenic test was done by injecting 0.5 ml of the culture from each strain into two mice subcutaneously, and the result was observed for 24 hours. But in case of Cl.chauvoei, pathogenicity test was done by injecting 0.5 ml of the culture into two mice subcutaneously, and 2.5 ml into a guinea-pig intramuscularly (300-400grams).

Formolizing. Before formolizing, the pH of the cultures were adjusted to 7.0 by adding 10% NaOH solution and the bottles were shaken well, 40% commercial formaldehyde was first diluted with equal volume of distilled water and it was then added to the containers to a final concentration of 0.5%. The addition was performed gradually and the containers were shaken constantly to bring about a homogeneous mixture. The formolized cultures were left in incubator to be detoxified and transformed to anaculture-toxoid as follow:

(a) Cl.perfringens types B and D, Cl.septicum and Cl.chauvoei for 3 days.

(b) Cl.perfringens type C for 7-14 days.

(c) Cl.oedematiens type B for 4 days.

Test for detoxification. The bottles were taken out from incubator and samples were taken from each bottle; 0.5 ml. of which was injected subcutaneously into two mice and the result was observed for 3 days.

The mice should remain alive during this time, and if any died, the bottle was either returned to incubator for more days or the test was repeated as room temperature detoxification still continued.

All injected mice were alive in this test, under our experimental conditions.

Addition of adjuvant. Addition of adjuvant was done after completing anaculture test. Twenty four hours before adding the adjuvant, all the vaccine bottles, together with the bottle of aluminium hydroxide were put in incubator for 24 hours. Then the pH of each vaccine was adjusted to 6.0 by IN HCL. 15% of aluminium hydroxide was added slowly to each bottle while it was being shaken, until the required amount was totally introduced. The contents were once again mixed thoroughly, after which the containers were returned to the incubator for a period of 4 hours.

After 4 hours, the bottles were shaken again and were kept in refrigerator until the other preparations also went through the same stage.

Concentration of vaccine. The bottles were taken out from refrigerator very carefully and the supernatees were syphoned off and discarded under sterile condition (approximately two litres each). The remaining vaccine (approximately 7 litres) in the bottles were filled upto 10 litres by the same type of vaccine and they were kept at refrigerator +4°C.

For blending of a polyvalent enterotoxemia and black disease vaccine, types of the prepared vaccines were mixed with the proportion of 35% of Cl.perfringens types B and C and 40% of Cl.pertringens type D and 25% of Cl.oedematiens. The dose of vaccine is 3 ml per animal.

II- Quality control of vaccines

I- Safety test. Four susceptible healthy sheep were selected for the safety test of each type of vaccine. Each group of two sheep were injected subcutaneously with 5 ml. and 10 ml. of the combined clostridial and blackleg vaccines.

II- Potency test.

(a) Enterotoxemeia and black disease vaccine — The immunogenic potency of Cl. perfringens types B, C and D and Cl. oedematiens

combined vaccine was determined according to the British Veterinary codex (5). A group of twelve rabbits, three to six months old, were injected subcutaneously with 3 ml. of vaccine. Four weeks later, a second injection of the same dose was given. Fourteen days after the second injection, the rabbits were bled, sera were separated from the collected blood and one ml. of each serum was taken and pooled. The units of antitoxin present in the pooled serum were estimated by titration in Swiss white mice 18-20 grams.

(b) Blackleg vaccine — Twelve guinepigs were injected subcutaneously with 3 ml. of the vaccine. Three weeks later, a second injection of the same dose was given. Ten days after the second injection, the guinea pigs were challenged with 1 ml. of the 24 hour-culture of Cl. chauvoei. For control, two guinea pigs were injected subcutaneously with 0.25 ml. and 0.5 ml. of an active growth of 24 hr culture of Cl.chauvoei.

Results and Discussion

The results of the minimum lethal dose were 500/ml, 2,000/ml, 9000/ml and 1,500/ml for Cl.perfringens type B, Cl.perfringens type C, Cl.perfringens type D and Cl.oedematiens respectively.

The results of potency tests obtained from the above mentioned vaccines showed that the pooled rabbit sera contained an average of 30 International units of beta antitoxin per ml., 5 Int. units of epsilon antitoxin per ml. and 7.5 Int. units of Cl. oedematiens alpha antitoxin per ml. The enterotoxemia and black disease combined vaccines produced high level of antitoxin in vaccinated animals. The titre of antitoxin after second injection in rabbits pooled sera developed high level of immunity of beta of Cl. perfringens and alpha antitoxin of Cl. oedematiens and the same level of Cl. perfringens epsilon antitoxin as suggested by the British Veterinary codex and recommended for the individual vaccine in rabbits (5) (Table 1).

The results of potency test of blackleg vaccine on twelve guinea pigs showed that all vaccinated animals survived the challenged dose, but control guinea pigs died after 24 hours. The potency test proved that the prepared vaccine was quite satisfactory (5).

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Table 1

Titres of rabbits pooled sera to the batch of the combined clostridial vaccine in International Units per ml.

Type of vaccine	Dose of vaccine	Cl. perfringens beta	Cl. perfringens epsilon	Cl. oedematiens aplha
Clostridial combined vaccine	3 ml	30	5	7.5
Recommended by British vet. Pharmacopoea	minimum recomend-ed dose	10	5	3.5

References

1- Ardehali, M., Derakhshan, H and Moosawi, M. (1982). The existance and present situation of clostridial disease of domestic animals in Iran. O.I.E. proceeding of the 4th International symposium on animal disease caused by anaerobes, p. 65.

2- Katic, R.V. and Katrinka, M. (1978). Clostridial infections in cattle reared by modern methods and their prevention by immunization. Veterinarski Glasnik, Vol. 32, p. 6.

3- Ardehali, M. and Derakhshan, M. (1976). Production and standardization of polyvalent clostridium perfringens vaccine in Iran. Journal of Develop. Biol Standard, Vol. 32, p. 31.

5- Katic, R.V., Katrinka, M., Vukicevic, Z., Gadanski, G. and Mijatov, LJ. (1977). The results of investigations of the effect of residual quantity of trypsin on alpha and beta factor toxin and antitoxin of germs from perfringens agni group. Veterinarski glasnik, Vol. 31, p. 8.

5- British Pharmacopeoia (veterinary) 1977. Clostridial vaccines, p. 155-157, A10 appendix XIV B. All printed in England at the University Press, Cambridge.