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ROLE OF ALUM AS ADJUVANT IN CLOSTRIDIAL VACCINES

M. Ardehali and H. Darakhshan

INTRODUCTION

The incidence of Clostridial infections among sheep has been diagnosed in Iran since 1938 (Kaveh 1957) and several strains of Clostridium-perfringens types B,C and D, Clostridium oedematiens, Clostridium chauvoei and Clostridium septicum have been isolated and identified from specimens and carcasses of infected animals from different parts of the country (Ardehali, 1967., Ardehali and Dowran 1969., Ardehali and Darakhshan, 1975). During recent years, there has been an increasing of demand for vaccination of sheep against anaerobic diseases in Iran. Since 1939, a large batch of formolised whole culture vaccine has been prepared and delivered to Veterinary Organisation for immunising of sheep against Clostridial diseases. Three kinds of vaccine are produced: A polyvalent vaccine against lamb dysentery and pulpy kidney, a monovalent vaccine against black disease and a monovalent vaccine against blackleg.

The object of this study was to prepare and examine several batches of Clostridial combined vaccines containing of Cl. perfringens types B,C, and D,Cl. oedematiens by adding potash alum as an adjuvant and to determine the minimum immunogenic dose in sheep.

MATERIALS AND METHODS

Preparation of vaccine

Clostridium perfringens types B,C and D vaccines were prepared according to the method described previously (Ardehali and Darakhshan 1975). The Cl. oedemations vaccine was prepared by using the following medium:

1 – Peptone 4%

2 – Na2HPO4 ..... 0.5%

3 – Maltose 1%

4 – Cysteine hydrochloride 0.05%

Final pH =8

The constituents were boiled for five minutes and filtered through filter paper. The medium was distributed in 20 pyrex-bottles (15 litres each) and autoclaved at 100°C for 40 minutes.

Seed culture: One freeze dried ampoule of Cl. oedematiens strain (Col. No.C.N.804 Evans, England) was transfered into a tube of fresh liver medium and incubated at 37°C. for 24 hours. The seed material was inoculated into 500 ml. capacity flask containing 300 ml. of fresh liver medium which had been boiled before use and then incubated at 37°C for 24 hours. Each flask was inoculated into 15 twenty litres capacity bottles and the maximum toxicity of the cultures were obtained within 60–70 hours for Cl. oedematiens. At the end of inoulation period, samples were taken from each bottle for the control of non-specific organisms. The samples were inoculated into nutrient broth and slope agar with a volume of 0.2 ml. of the cultures.

The samples were also taken randomly from several bottle for determination of toxicity in mice (M.L.D.).

Toxoiding: Formaldehyde (40–37%) solution was added to each container. The toxoiding took place during 7–14 days. On removing from incubator the containers were well stirred and 5 ml. were taken from each container for Safety and potency tests of the vaccine. The containers were kept at 4°C to 10°C for two months to insure the complete detoxification.

Blending: The collected samples from each bottle of Cl. perfringens types B,C,D and Cl. oedematiens were divided in two parts:

1 – Anaculture plus one percent potash alum. The final product consisted of :

52 per cent of Cl. perfringens type D

12 per cent of Cl. perfringens type C

8 per cent of Cl. perfringens type B

20 per cent of an 5 per cent solution of potash alum.

The combined formolised cultures were well mixed by means of magnetic stirrer with five per cent of sterile potash alum, as adjuvant, to give a final concentration of one per cent at pH–6 to pH 6.5 (Jansen 1960). Sterile potash alum and 20 per cent caustic soda were added at the same time.

2 – Anaculture. The final products consisted of:

60 per cent of Cl. perfringens type D

15 per cent of Cl. perfringens type B

15 per cent of Cl. perfringens type C

The combined formolised cultures were well mixed at pH.7.0 by using 20 per cent caustic soda.

To find out which rate of each vaccine is suitable for combination of final product, several batches of alum precipitated were prepared and tested in rabbit according to the method described in British Veterinary Codex (1970) for potency of Clostridial vaccine.

Experiment with sheep. A group of forty healthy sheep aged 1 to 3 years were selected randomly to assess vaccine efficacy. The animals were divided in four groups, each of ten sheep were inoculated with the following materials:

1 – First group were vaccinated with 3 ml. dose of combined vaccine containing one per cent potash alum.

2 – Second group were inoculated with 5 ml. dose of the combined vaccine containing one per cent potash alum.

3 – Third group were inoculated with 3 ml. dose of anaculture vaccine.

4 – Fourth group were inoculated with 5 ml. dose of anaculture vaccine.

Serum sampling and antitoxin assays.

The animals were kept in Kordan experimental farm during the experiment. Serum were collected before and two weeks after each vaccination up to three months. The serum samples of each sheep were stored at 20°C until required for testing.

One ml. of the serum of each group of sheep were pooled together. The antitoxin values of the pooled serum of each group of sheep were determined according to the British Veterinary Codex (1970) for Clostridial Vaccines, which requires:

1 – Clostridium perfringens type C dried toxin and International Standard of Cl. perfringens type B antitoxin used(*) for titration of beta antitoxin in pooled sheep serum.

2 – Clostridium perfringens type D dried toxin and International Standard of Cl. perfringens type D antitoxin (*) used for titration of epsilon antitoxin in pooled sheep serum.

(*) Clostridium perfringens type B antitoxin and Clostridium perfringens type D antitoxin obtained from the International Laboratory for Biological Standard, Central Veterinary Laboratory Weybridge, England.

Clostridium oedematiens concentrated toxin and International of Cl. oedematiens alpha antitoxin (*) used for titration of alpha antitoxin in pooled sheep serum. All titrations were determined by using Swiss white mice by the method described in British Veterinary Codex (1970).

RESULTS AND DISCUSSION

The results of the tests on 40 sheep vaccinated with varying doses of two kinds of the Clostridial vaccine summarises in Table 2. As the titres of the injected animals shows, the responses to a 3 ml. and a 5 ml. doses after first and second injections were quite satisfactory. The vaccine produced appreciable level of immunity in vaccinated animals even with a dose of a 3 ml. Hepple et al (1959) and Jansen (1960) showed that a figure of 0.3 to 0.2 International Unit of Cl. perfringens epsilon antitoxin per ml. in the serum of vaccinated sheep would be enough to protect animals against experimental challenge with Cl. perfringens type D. Macheak et al (1972) proved that Cl. oedematiens titre of 1.6 International unit per ml. of sheep serum protect most animals against Cl. oedematiens challenge. The appreciable level of immunity obtained after two weeks of vaccination of sheep with 3 ml. and 5 ml. doses of the combined vaccine, but the second injection increased the level of immunity. The response to the 5 ml. dose of anaculture plus one per cent alum was excellent. The level of antibody titre fell down 3 months after second inoculation. The results of this experiment proved that the combined vaccine of anaculture and anaculture plus one per cent alum developed antibody titre in injected animals but the vaccine containing alum in 5 ml. dose gave greater immunity in vaccinated animals. Sterne et al. (1962) reported that immunisation of sheep with alum precipitated Clostridial vaccine produced better immunity in injected animals.

The results of routin batches with 2 ml. doses of Clostridial polyvalent vaccine precipitated with alum for potency test and to find out the rate of combination of vaccine in rabbit are shown in Table 2. The titre of antitoxin after second injection were about the same level suggested by the British Veterinary Codex and recommended for the individual vaccine in rabbits (British Vet. Codex 1970).

SUMMARY

Two types of the Clostridial vaccines were prepared and tested in sheep for determination of the level of immunity. All injected animals developed high

(*) Statens Seruminstitut, Copenhagen S-Denmark.

antibody titre, but the greater response was obtained in anaculture plus one per cent potash alum in 5 ml. dose of the vaccine.

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Table 2.

The responses of rabbits to 10 batches of the combined Enterotoxemia polyvalent vaccine.

No.of Rabbit	Titres of rabbits sera in Int. unit per ml. two weeks after second inoculation.			
	Dose of vaccine	Cl. perfringens		Cl. oedematiens
		beta	epsilon	alpha
10	2 lm. (Batch No. 112)	15	3.5	4.5
10	2 ml. (Batch No. 113)	8	3	4
10	2 ml. (Batch No. 114)	20	6	4.5
10	2 ml. (Batch No. 115)	6	2.5	4.5
10	2 ml. (Batch No. 116)	45	2.5	5
10	2 ml. (Batch No. 117)	18/8	3.5	7/2
10	2 ml. (Batch No. 118)	6	5	4.5
10	2 ml. (Batch No. 121)	15	6	3.5
10	2 ml. (Batch No. 122)	20	5	7
10	2 ml. (Batch No. 123	16	6	ç

The responses of sheep to a 3 ml. and a 5 ml. dose of the combined vaccines, anaculture and anaculture plus one per cent potash alum

No. of sheep	Type and dose of vaccine	Titre of sheep in international units per ml.													
		Preinoculation		2 weeks after 1st inoculation		2 weeks after 2nd inoculation		4 weeks after 2nd inoculation		6 weeks after 2nd inoculation		8 weeks after 2nd inoculation		10 weeks after 2nd inoculation	
		Cl. perfr.	Cl. oed.	Cl. perfr.	Cl. oed.	Cl. perfr.	Cl. oed.	Cl. perfr.	Cl. oed.	Cl. perfr.	Cl. oed.	Cl. perfr.	Cl. oed.	Cl. perfr.	Cl. oed.
		β ε	α	β ε	α	β ε	α	β ε	α	β ε	α	β ε	α	β ε	α
10	3 ml. Anaculture	0.02 0.08	0.02	0.2 3	1	9 12	1.6	6 5	0.7	5 1.6	0.3	0.9 1.2	0.2	0.8 1	0.1
10	3 ml. Anaculture plus alum	0.07 0.08	—	0.6 3	0.09	7 9	0.8	5 3.6	0.7	2 0.8	0.6	1 0.5	0.45	0.8 0.4	0.15
10	5 ml. Anaculture	0.08 0.02	—	1.5 5	—	9 9	0.17	6 7.2	0.11	3 2	0.2	1 1.5	0.2	0.6 1	0.06
10	5 ml. Anaculutre plus alum	0.06 0.06	0.3	9 5	0.5	24 30	12	12 7.2	5	9 4	4.5	8 3	3	6 2	0.75

— = not tested

β = beta

ε = epsilon

α = alpha