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IMMUNISATION OF SHEEP AND GOATS WITH A COMBINED CLOSTRIDIAL AND ANTHRAX VACCINE. *

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ABSTRACT. **Clostridial and anthrax combined vaccine was prepared with two types of adjuvants,aluminium hydroxide and saponine. Safety and potency of the combined vaccine were tested on fifty two sheep and goats and fourty rabbits. Each type of the combined vaccine developed high titres of beta, epsilon antitoxins of Cl.perfringens types B,C,D and alpha of Cl.oedematiens type B in vaccinated animals. All vaccinated animals were resistant to the challenge dose of virulent B.anthracis The greater response was obtained from a 5 ml dose of the combined vaccine.**

Key words: SHEEP/GOATS/IMMUNISATION/VACCINES/COMBINED VACCINES/INOCULATION

INTRODUCTION

There are some reports in the literature regarding

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a combined vaccine against aerobic and anaerobic infections in domestic animals. Kadmove et al (1975) reported production of a combined vaccine against anthrax , sheep pox and clostridial infections in sheep with satisfactory results obtained in the challenged animals. Provost et al (1962) reported a combined vaccine against anaerobic and aerobic diseases in farm animals.

Clostridial and anthrax diseases are prevalent among sheep and goats in Iran. During the recent years , there has been increasing demand of vaccination of sheep and goats against clostridial and anthrax diseases in Iran .

The object of this study was to prepare and standardize an effective combined vaccine for immunisation of sheep and goats.

MATERIALS AND METHODS

The methods of preparation of clostridial vaccines in Iran were previously described by Ardehali et al(1976 1982) and anthrax attenuated live spore vaccine reported by Sotoodehnia et al (1984).

Preparation of the combined vaccine. Two types of the combined vaccines were prepared,each one in three litres. For comparison of safety and potency of the prepared combined vaccine , two types of adjuvants,aluminium hydroxide (gel) and saponine were used in each of the combined vaccine. The ingredients of each type of the formalised culture vaccine were as follow:

A- Combined vaccine containing aluminium hydroxide adjuvant:

1- Cl.perfringens type B vaccine 8%

2- Cl.perfringens type C vaccine 10%

3- Cl.perfringens type D vaccine 45%

4- Cl.oedematiens type B vaccine 27%

5- Aluminium hydroxide (gel) 10%

B- Combined vaccine containing saponine adjuvant:

1- Cl.perfringens type B vaccine 8%

2- Cl.perfringens type C vaccine 10%

3- Cl.perfringens type D vaccine 45%

4- Cl.oedematiens type B vaccine 27%

5- Saponine 0.1%

Neutralization of excess formalin in the combined clostridial vaccine : The combined clostridial vaccine consisting of excess formalin, kills live spores of B.anthracis when mixed with anthrax vaccine. The excess formalin of two types of clostridial combined vaccines were neutralized with 3% of sterile suspension of 50% sodium-metabisulphite (Jansen 1975). The vaccines were kept at 4°C for five days, then in each type of the combined vaccine, anthrax live spores vaccine was added to a final concentration of $3x10^6$ /ml. The quantity of anthrax spores was determined by colony count method after six days No remarkable decrease was observed in quantity of the spores.

Quantity control of the combined vaccine:

A- Potency tests of clostridial vaccines . The potency tests of clostridial vaccines were determined according to the methods described in British pharmacopoeia (Veterinary) (1977). Fourty rabbits (3-6 months of age) were selected and divided into four groups of ten rabbits.

First and second groups were vaccinated with a 3 ml and a 5 ml doses of the combined vaccine containing ten per cent aluminium hydroxide as adjuvant. Third and fourth groups were vaccinated with 3 ml and a 5 ml doses of the combined vaccine containing 0.1 per cent saponine as adjuvant . Each group of the rabbits was given two injections with an interval of four weeks. Fourteen days after the second injection the animals were bled. Sera separated from the collected blood and then, one ml of each serum was taken and pooled. The level of antibody in pooled sera of rabbits, sheep and goats were assayed by titration in mice (18-20 grams), according to the methods described in the British pharmacopoeia (Veterinary)(1977).

Safety and potency tests of the combined vaccine in sheep and goats. A group of 52 sheep and goats aged 1-3 years were selected for this experiment. The animals were not vaccinated before with either enterotoxemia or anthrax vaccines. The animals were divided into four groups, each group of sheep and goats were inoculated with the following materials:

The first and second groups were vaccinated subcutaneously with a 3ml and a 5 ml doses of the combined vaccine containing ten percent aluminium hydroxide. The same doses of the vaccine were injected two weeks later (Sterne et al 1962). Third and fourth groups were vaccinated subcutaneously with a 3 ml and a 5ml doses of the combined vaccine containing 0.1 percent saponine.

Serum sampling and antitoxin assays of clostridial antigens*. Each animal was bled before vaccination two

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* Clostridium perfringens types B and D antitoxins ⟶

weeks after the first injection and ten weeks after the second inoculation.

The serum samples were stored at 4°C. one ml of the collected serum of each sheep and goat was pooled together for the determination of antitoxins level.

The following materials were prepared and used for antitoxin assays of sheep and rabbits pooled sera.

1- Clostridium perfringens type C dried toxin and international standard of Cl.perfringens type B antitoxin* used for titration of beta antitoxin in pooled rabbits and sheep sera.

2- Clostridium perfringens type D dried toxin and international of Cl.oedematiens alpha antitoxin* used for titration of pooled rabbits and sheep sera.

3- Cl.oedematiens concentrated toxin and international of Cl.oedematiens alpha antitoxin* used for titration of pooled rabbits and sheep sera. The above titrations were determined by using white mice (18-20) according to the methods described by British pharmacopoeia (1977).

Potency test of anthrax vaccine: All four groups of the vaccinated animals inoculated with a 3ml and a 5ml doses of the combined vaccine were challenged subcutaneously with 200 MLD of virulent strain C_2 of B. - anthrax (British Veterinary Codex 1965). In each group, one control goat was kept separately and inoculated subcutaneously with 1 MLD of the same strain which

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→ obtained from International Laboratoy, Weybridge.

* Statens seruminstitut, Copehhagen S. Denmark .

contained approximately 20000-30000 viable spores of B. anthracis.

RESULTS AND DISCUSSIONS

The results of the responses of rabbits, sheep and goats, vaccinated with a 3ml and a 5ml doses of two types of the combined vaccines are summarised in the Tables Nos.1 and 2 . The Table No.1 shows the responses of the vaccinated rabbits to a 3ml and a 5ml doses of the combined vaccine. The combined vaccine produced appreciable level of immunity in the rabbits pooled sera.

The titres of Cl.perfringens beta and Cl.oedematiens alpha antitoxins were higher than the level suggested by the British Pharmacopoeia (1977), as recommended for the individual vaccine.(Table 1). Cl. perfringens epsilon antitoxin was close to the level recommended by British Pharmacopoeia for single vaccine. Table 2 summarises the results of the tests on 52 sheep and goats which were vaccinated with a 3ml and a 5ml doses of two types of aluminium hydroxide and saponine adjuvants of the combined vaccine. As the titres of Cl.perfringens beta,epsilon and Cl.oedematiens alpha antitoxin shown in the Table No.2 the responses to a 3ml and a 5ml doses after first and second injections were quite satisfactory. Each of the combined vaccine developed appreciable levels of immunity in vaccinated animals (Sterne et al 1962).

Before vaccination there was no remarkable level of immunity in the pooled sheep and goats sera. After the first injection, the vaccinated animals developed Cl.perfringens beta and epsilon antitoxins and the significant level of

immunity reached peak after the second injection up to 15 Int/units per ml for beta and 6 units per ml for epsilon antitoxins. Hepple et al (1959) and Jansen (1962) showed that a figure of 0.3 to 0.2 International unit per ml of Cl.perfringens beta and epsilon antitoxins in the serum of vaccinated sheep would be enough to protect the animals against experimental challenges. As the results show, the vaccinated animals could develop beta and epsilon antitoxins even with a single injection which could protect the sheep against enterotoxemia. Cl.oedematiens developed appreciable level of antitoxin in vaccinated animals up to 1.5 units/ ml. (Macheak et al 1962). Ten weeks after the second injection, the beta and epsilon antitoxins did not fall bellow the acceptable level.A 5 ml dose of both combined vaccines, developed more titre in the injected animals than a 3 ml dose vaccine.

The results of potency test of the anthrax vaccine indicated that the combined vaccine prepared either with saponine or aluminium hydroxide was highly potent and the vaccinated animals survived the challenge test. A 5 ml and a 3ml doses of the combined vaccine confered a solid immunity in vaccinated animals. All control animals died of anthrax during 3-5 days, and in post challenge, bacillus anthracis was isolated from the bone marrow. The results of the challenge tests of anthrax vaccine are summarised in the Table No.3.

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