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*Immunisation of Cattle with Clostridium chauvoei Vaccine**

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Summary. Blackleg is one of the important diseases of cattle in Iran. Sporadic outbreaks have been observed in different parts of the country which cause economical losses in cattle flocks. The main object of this study was to prepare and formulate a vaccine for immunisation of cattle against blackleg. A medium consisted of peptone, sodium chloride, glucose and L-cysteine, was selected and used for cultivation of Clostridium chauvoei. The method of large scale production, safety and potency tests of aluminium hydroxide gel vaccine are described in this paper. The vaccine is highly immunogenic as determined in laboratory animals and sheep.

Keywords: *Cattle / Clostridium Chauvoei / Vaccines*

Introduction

Blackleg is generally known to affect cattle in enzootic form in Iran, but in 1988, a severe and extensive outbreak of blackleg occured in a vast area among cattle flocks in fifteen villages, which killed 400 cattle *(1)*.

More than thirty strains of C.chauvoei, the causal agent of blackleg have been isolated from infected animals, received from different parts of Iran. The characterization of the isolated strains were studied by Ardehali et al *(2)*.

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The fluorescent labelled antibody technique is used as a routine for detection of C.chauvoei from pathological materials.

The main object of this study was to prepare and formulate a vaccine for immunisation of cattle against blackleg. There are a few references in the literature concerning mass production of C.chauvoei vaccine *(3,4)*. Various media and methods were attempted for production of a potent C.chauvoei vaccine. The following medium was selected and used for large scale production of C.chauvoei vaccine *(3)*.

Materials and Methods

Selected medium for production of 672 liters of C.chauvoei vaccine:

1 - Peptone (Oxoid L_{52}) . . . . . 25 Kg
2 - NaCl . . . . . 3.3 Kg
3 - Glucose . . . . . 3.3 Kg
4 - L-Cysteine . . . . . 330 Grams
pH.7.5

For preparation of a batch of 672 litres of blackleg vaccine, the above ingredients were dissolved first in 200 litres of distilled water, then five litres of the concentrated medium was distributed into each of the forty two bottles and each twenty litres bottle was filled up to sixteen litres with distilled water. The bottles were autoclaved at 115°C for forty minutes. For the production of a batch of 672 liters of vaccine, forty two bottles were used each containing 16 liters of medium.

Seed culture: The highly pathogenic strain (C.N.701) of C.chauvoei was inoculated in a tube of fresh liver medium as seed culture.

A 24 hr culture of the seed was used as the second seed culture in 250 ml flasks of the liver broth and again a 24 hr. culture was transfered in 500 ml flasks of the liver broth and used as third seed culture. The sixteen liter bottles after, being in optimal temperature, were sown with flask of 500 ml, after which the culture starts to grow, and generally the growth is completed within two days at 37°C in incubator. Samples were taken from each bottle to check the purity of cultures with nutrient broth and nutrient slope agar. The pathogenicity of cultures were checked by intramuscular injection into hind legs of the guinea pigs.

Toxoiding: A volume of 96 ml (0.6 per cent of 40% commercial formaldehyde) solution were added to each bottle. The formolised cultures were left for ten days in incubator to be detoxified and transformed to anacultures. Sample was removed from each bottle and mixed together with aluminium hydroxide gel as adjuvant *(5)*, to be injected to the susceptible animals for safety and potency tests.

Blending of the vaccine: The prepared vaccine was blended as follows:

1 - Anaculture 70%
2 - Aluminium hydroxide gel. 10%
3 - distilled water 20%

Final concentration of aluminium hydroxide gel consisted of 1.5 mg $Al(OH)_3$ per ml in the prepared vaccine.

Quality control of the vaccine:

The safety and potency tests of the vaccine were determined according to the British Pharmacoepia (Veterinary)1985, *(6)* .

Six healthy sheep were selected for potency test of the vaccine. Five healthy sheep were injected with 3 ml dose (Vaccinal dose) of the vaccine and one sheep remained as control. All vaccinated and unvaccinated sheep were challenged after three weeks with 4 MLD in the vaccinated and 1 MLD in unvaccinated (control)animals with 24 hr virulent culture of C.chauvoei. (Table 1).

Ten healthy guinea pigs (300-400 grs) were injected subcutaneously with a quantity of 2 ml of the vaccine as primary dose, followed by three weeks later a second injection of the same quantity of the vaccine as the secondry dose. After two weeks the vaccinated and unvaccinated guinea pigs were injected intramuscularly with 4 and 1 MLD of 24 hr virulent culture of C.chauvoei respectively *(6)* , (Table 11).

Results and Discussion

High yield of C.chauvoei cultures were obtained after 48 hr and the cultures were highly pathogenic. 0.25 ml of the mixed sample which was taken from each bottle killed guinea pig with typical lesions of C.chauvoei infection

after 36 hr. The results of the safety and potency tests on susceptible healthy sheep and guinea pigs were quite satisfactory. None of the injected sheep showed any general or local reactions. All vaccinated sheep and guinea pigs resisted to the challenge dose of C.chauvoei virulent culture while the unvaccinated (control) sheep and guinea pig died of typical lesions of C.chauvoei infections

Table No.1 : Challenge of C.chauvoei vaccine in sheep

No of sheep	Vaccinal dose	Reaction	Challenge	
			Material	Results
5	3 ml	0	1 ml (2MLD) of 24 hr C.chauvoei Virulent culture	Resisted
1 (control)	0	0	0.5 ml (1 MLD) 24 hr C.chauvoei Virulent culture	Died of C.chauvoei infection

Table No.2 : Challenge of C.chauvoei vaccine in guinea pigs

No of guinea pigs	Minimum vaccinal dose	Reaction	Challenge	
			Material	Results
10	2 ml.	0	1 ml 24 hr C.chauvoei Virulent culture	1 ml (4 MLD)
2	0	0	0.25 ml (1 mld) 24 hr C.chauvoei	Died of C.chauvoei
Control)	0	0	Virulent culture	infection

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