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Short Communication

Development of Inactivated Whole Cell Vaccine against Ovine Foot Rot

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ABSTRACT

The serogroups B and I of *Dichelobacter nodosus* were selected as whole cell vaccine strains based on isolation and serogrouping studies in Chittoor district of Andhra Pradesh. The available serogroups B and I of *D.nodosus* were revived in TASH agar media with 2% hoof powder. The purity of antigens were checked and adjusted to a concentration of 1×10^8 mg/ml and 2×10^9 mg/ml. Later inactivated with Formalin and were adjuvanted separately using Montanide and Alum. Experimental trails were conducted in Sheep and Goat with two different adjuvants, namely Montanide and Alum and with the different antigenic concentrations i.e 1×10^9 organisms/ml and 2×10^9 organisms/ml. The immune response was high in vaccines prepared using antigenic concentration of 2×10^9 organisms/ml compared with antigenic concentration of $1x10^9$ organisms/ml. Similarly among two adjuvants Montanide and Alum, Montanide adjuvanted vaccines were found to be better in eliciting immune response.

Key words: Selection of vaccine strains, TASH agar medium, Concentration of antigen, Inactivation and adjuvantation, Standardization, Immune response

INTRODUCTION

Foot rot is an infectious and contagious disease of Sheep and Goat caused by an anaerobic bacterium *Dichelobacter nodosus.* The disease results in lameness of affected sheep and goat leading to production losses. The disease is being reported in Andhra Pradesh regularly during the rainy season. Treatment of affected Sheep appears to be costly and not economical. Serogroup specific vaccination found to have excellent therapeutic efficacy. Hence the study was taken up to develop an inactivated whole cell vaccine against ovine foot rot using local isolates belonging to serogroups B and I.

MATERIALS AND METHODS

Selection of vaccine strains

The serogroups B & I of *Dichelobacter nodosus* were selected as vaccine strains based on isolation and serogrouping studies in Andhra Pradesh.

Revival of vaccine strains

The selected vaccine strains of B & I sero-groups of *D.nodosus* were revived in TASH agar medium with 2% hoof powder.

Concentration of antigen

The purity of the antigens of vaccine strains B & I sero groups of *D.nodosus* was checked and the concentrations of antigens were adjusted to $1X10^9$ organisms / ml. and $2X10^9$ organisms / ml. respectively.

Inactivation and adjuvantation

The antigens of vaccine strains were inactivated using 0.5% formalin and adjuvanted separately using Montanide and Alum.

Standardization of vaccine strains

The prepared vaccines subjected for sterility, safety according to the *British Pharmacopoeia*, *1985*.

Evaluation of vaccine

Trails were conducted in lab animal, Rabbit and also in Sheep and Goat to study the efficacy of the vaccine using the micro agglutination and ELISA.

RESULTS

An attempt was made to develop an inactivated whole cell vaccine with local serogroups B & I of *D.nodosus*. The *D.nodosus* sero groups B & I were grown in TASH agar media plates and whole cell antigens of *D. nodosus*

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were extracted in TASH agar media plates in PBS and concentration was adjusted at $1X10^9$ organisms / ml. and $2X10^9$ organisms / ml. respectively. Both the vaccine strains B and I were inactivated by using 0.5% formalin and keeping overnight. Adjuvantation of vaccine strains was done with two different adjuvants namely Alum and Montanide with both the concentration of antigens i.e. with $1X10^9$ and $2X10^9$ organisms / ml. respectively. (Table 1). Adjuvanted vaccines namely vaccines A & B were tested for sterility and safety tests. Blood agar, Nutrient agar, Sabourauds agar and Thioglycolate medium were inoculated with Vaccine A, B and Vaccine I, II, III, IV did not shown any growth of micro organisms and fungi during incubation of 14 days at 30^0C and 37^0C respectively. Regarding safety tests all the six rabbits inoculated with the vaccine preparations did not shown any local and systemic reaction up to 20 days, the period of observation. The vaccine was found to be safe in rabbits. The thermal response of sheep and goat to the formalin inactivated whole cell vaccines of *D. nodosus* were recorded and results are shown in (Table 2). No significant change in daily rectal temperatures could be observed during period of observation. The efficacy of vaccine was studied. in Rabbits and the titers were ranged from 80- 320 on 30^{th} day and from 320-640 on 60thday using microplate agglutination test.

Later two experimental trials were conducted under laboratory conditions in sheep and goat. In trial-1 no clinical signs were observed in all vaccinated sheep and goat including thermal response. A slight skin thickness of 2-5cm diameter was observed during the safety test in sheep. In trial – 1, Immune responses were studied in sheep and goats using microtiter plate agglutination test. The Mean microtiter plate agglutination titers of monovalent vaccines and bivalent vaccine in trial-1 were shown in Table 3 and Fig. 1. Similarly in trial-2 both Montanide and alum were used as adjuvants with antigenic concentration of $2x10^9$ org/ml using serogroups B, I of *D.nodosus.* (Table 4).

DISCUSSION

The efficacy of the vaccine could be improved by proper adjuvantation and frequency of vaccination. In the present study an attempt was made to develop serogroup specific vaccine using the local serogroups B and I isolated in Chittor and Nellore districts of Andhra Pradesh. During the study local serogroups B & I of *D. nodosus* were grown in TASH agar media plates for the preparation of antigen. TASH agar media was also used by Skerman (1975); Depiazzi and Richards (1985); Pitman (1994); Teshale Sore (2005) and Hussain *et al.* (2009) for the growth of *D. nodosus* organisms for preparation of vaccine. During the study the formalin was used at a concentration of 0.5% for inactivation of D. nodosus organisms in the preparation of vaccine. The organisms were completely inactivated by exposing them to formalin overnight. Earlier Skermann and Cairney (1972) and Dhungyel *et al*., (2008) reported that proper inactivation of organisms without any adverese effects was achieved by using formalin as an inactivating agent in the preparation of Foot rot vaccine. The prepared vaccines were tested for sterility and safety as per British pharmacopoeia (1985). No bacterial growth could be seen in plates as well as in broth indicating the sterility. The safety test conducted in rabbits did not show any local and systemic reactions indicating that vaccine preparations are safe. Similarly vaccine was proved to be safe in sheep as no change in the rectal temperature was recorded up to 6 days period of observation. Rectal temperatures recorded daily for about six consecutive days were shown in Table 2. Martin-Palomino *et al.,* (2004) also recorded the similar observations.

During the study of evaluation of the vaccine in Sheep and Goats slight local skin thickening was observed at the site of inoculation of vaccine adjuvanted with Montanide. This may be due to irritant effect of oil and *D. nodosus* antigen. Similar observations were reported by Hindmarsh *et al*. (1989), Lambell (1986) and Every and Skerman (1983) in their findings.

Table 1: Types of vaccine preparations and their compositions

S. No	Type of vaccine	Trial No.	Serogroup	Antigen concentration	Adjuvant
1	Vaccine-A		B	1x 10^8 org/ml	Montanide
2	Vaccine -B	Trial - 1	I	1x 10^8 org/ml	Montanide
3	Vaccine - I		B	2x 10^9 org/ml	Montanide
4	Vaccine - II		B	2x 10^9 org/ml	Alum
5	Vaccine - III	Trial -2	I	2x 10^9 org/ml	Montanide
6	Vaccine - IV		I	2x 10^9 org/ml	Alum

Table 2: Thermal response of sheep and goat to inactivated whole cell foot rot vaccines – Trial-2

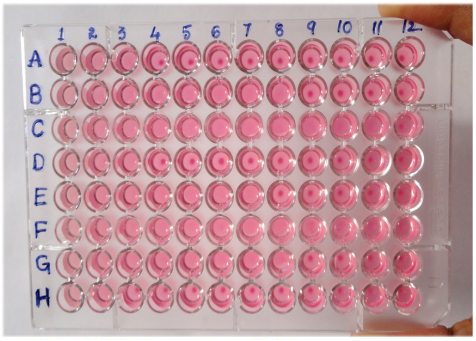
S. No.	Type of vaccine	Animal No.	Rectal temperatures in ^{0}C						
			Days after vaccination						
			0	1	2	3	4	5	6
1.		282	102.6	102.3	102.9	102.6	102.7	102.6	102.5
2.	Vaccine-I	37	102.1	104.0	104.5	104.6	103.8	103.5	103.5
3.		283	102.5	102.6	102.8	103.0	102.8	102.5	102.9
4.	Vaccine –II	38	102.4	103.0	103	103.0	103.2	103.4	103.7
5.		284	104.0	104.0	104.8	104.0	104.0	104.5	104.0
6.	Vaccine -III	39	102.4	102.8	104	103.1	103.5	103.6	103.4
7.		285	102.8	102.4	103.3	104.2	104.2	104.0	104.2
8.	Vaccine -IV	40	102.4	102.9	103.2	103.2	104.2	103.0	103.8
9.		230	102.8	102.4	103.3	102.6	101.2	103.0	102.8
10.	Controls	18	102.4	102.4	102.8	103.2	103.0	103.5	103.0

Table 3: Microtiter plate agglutination titers of Montanide adjuvanted Foot rot vaccines with antigenic concentration of $1x10^8$ org/ml - Trail-1

S. No	Animal group	Type of vaccine	Antigen (Serogroup)	Adjuvant used	No. of animals (Sheep and Goats)	Mean Agglutination titers		
						0 day	30day	60day
1.	A_1	Vaccine –A	B	Montanide	4	20	640	320
2.	B_1	Vaccine -B	I	Montanide	4	10	320	160
3.	C_1	Vaccine –A+B (Bivalent)	B+I	Montanide	4	20 10	640 160	160 80
4.	D_1	Control	----	-----	4	10	10	10

Table 4: Microtiter plate agglutination titers of sera of sheep and goats inoculated with Foot rot vaccines with antigenic concentration of $2x10^9$ org/ml - Trial-2

S. No.	Animal group	Type of vaccine	Antigen (serogroup)	Adjuvant Used	No. of animals (sheep and goats)	Mean Agglutination titers		
						0 day	30day	60day
1.	A_2	Vaccine –I	B	Montanide	2	8	1024	2048
2.	B_2	Vaccine -II	B	Alum	2	16	256	512
3.	C_2	Vaccine – III	I	Montanide	2	16	1024	2048
4.	D_2	Vaccine - IV	I	Alum	2	8	256	256
5.	E_2	Control	---	----	2	8	8	8



Serum dilutions : 1:2

Antigen concentration : $1x10^8$ org/ml

ROWS – Antibody titers

A, D : 8

B : 256

C, F : 2048

E : 128

F : 2048

G : 512

H : 4

Fig.1: Microtitre plate agglutination test for serum samples of vaccinated animals

In the present study two different adjuvants namely Montanide and Alum were used. In trial – 1 only Montanide ISA-206 alone and both Montanide and Alum in trail -2. Gurung *et al.* (2006) used the same adjuvant in the preparation of vaccine in his studies. In trail-1, eight sheep and goats each were tested, with monovalent (B-serogroup) vaccine, monovalent (I-serogroup) vaccine and bivalent (B+I) serogroup vaccine. In this trail all vaccine preparations were prepared using Montanide as adjuvant. Each animal was given 1ml of vaccine subcutaneously high below the ear. Booster was given after 30days of primary dose. Similar schedule was followed by Gurung *et al.*, (2006), Egerton *et al.*, (2002), Dhungyel *et al.*, (2008) in their experimental trails. In trail-2, Vaccine responses using microtiter plate agglutination titers were found to be higher compared to the trail-1. It could be attributed to higher antigenic concentration used in preparation of vaccine $2x10^9$ org/ml. Thorley and Egerton (1981) prepared the vaccine using antigen concentration of $5x10^9$ org/ml, but in the present study with montanide adjuvant, the antigenic concentration of $2x10^9$ org/ml was used to avoid the problems associated with endotoxins since *D.nodosus* is a gram negative organism. It elicited a protective titre in sheep with a mean agglutination titre of 3413.3.

During study it was observed that high antibody titers were reported with Montanide adjuvanted vaccines of both serogroups when compared to alum adjuvanted vaccines with microtiter plate agglutination test. On comparing the two adjuvants it was found that Montanide was more ideal for preparation of ovine Foot rot vaccine when compared to alum.

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