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Comparative Immune Response of Formalin Inactivated and Binnary Ethyleneimine Inactivated Angara Disease Vaccines

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Abstract: Immune response of formalin inactivated and binnary ethyleneimine inactivated Angara disease (hydropericardium) vaccines were compared using indirect hemagglutination (IHA) test. A four fold increase in IHA antibody titre was recorded with binnary ethyleneimine (BEI) inactivated vaccine. Agar gel diffusion test gave a strong precipitation line with serum of chicks vaccinated with BEI inactivated vaccine whereas a weak precipitation line was observed with serum from chicks vaccinated with formalin inactivated vaccine. The results of challenge test were promising and no adverse effects were seen in vaccinated chicks. BEI as inactivating agent produced antigenically superior vaccine.

Key words: Immune response, formaline, Binnary Ethyleneimine Angara Disease Vaccines

Introduction

Angara disease commonly known as hydropericardium was reported for the first time in Pakistan in the region of Angara Goth in 1986 and further reported in Iraq, Mexico and Chille. In 1994, it was reported in India in the region of Jammu and Punjab and in 1996 it has attacked the southern part of India, precisely in Tamilnadu. In 1996, the disease was also reported in several regions of the Indonesia. Different attempts have been made in Pakistan to control Angara disease by using formalinized liver homogenate (autogenous vaccine; Chishti *et al*., 1989; Afzal and Ahmad, 1990), oil emulsion (Hussain *et al*., 1996), cell culture and egg passaged (Naeem *et al*., 1995) vaccines. But only the liver homogenate vaccine reported to provide good protection and is available on commercial scale. The discomfort of farmers remain after application of this vaccine and at many occasions, it caused the disease. Present paper reports the evaluation of immune responses following single vaccination with formalinized liver homogenate and Binary ethyleneimine (BEI) inactivated liver homogenate vaccines of Angara disease.

Materials and Methods

Hydropericardium vaccines: The vaccines were prepared from liver collected from birds showing symptoms of hydropericardium syndrome (HPS) after experimental infection according to the procedure described by Cheema *et al.* (1989).

Vaccine I: Inactivated with formalin following the methods described by Ahmad *et al.* (1990).

Vaccine II: Inactivated with Binary ethyleneimine (BEI) according to the method described by Brown and Crick (1959). BEI (0.1 M solution) was prepared by dissolving Bromoethylamine hydrobromide 25.5 gram per liter of 0.175% sodium hydroxide at 37°C for 30 minutes. A 0.1 M BEI solution was added to infected liver homogenate/suspension at a concentration 1-3%. This gave a final concentration of 1-3 mM BEI at 37°C under slow stirring for 20 minutes. The residual BEI was hydrolyzed with a sterile (autoclaved) 1 M sodium-thiosulphate which was added at equi-molar concentration to BEI solution. The sterility and safety of the vaccines were checked as described by Afzal and Ahmad (1990).

Experimental chicks: Three hundred, day old broiler chicks were purchased from local market. The chicks were reared under standard managemental conditions and routinely vaccinated against Newcastle disease on day 7 and 21. On day 10, chicks were divided into three equal groups. Group I and Group II were injected Vaccine I and Vaccine II, respectively and group III was served as unvaccinated control. A 0.25 mL of Vaccine I and II was given subcutaneously.

Serum samples were collected from 10 chicks of each group at random at day 0, 5, 10, 15, 25 and 30 post-vaccination and stored at -20°C till antibody titration using indirect haemaggulatination test (Rahman *et al*., 1989) and Agar gel diffusion (Brown and Crick, 1959).

Challenge test: Challenge test was conducted using 20% infected liver homogenate in normal saline with antibiotics following the method of Afzal and Ahmad (1990). Five birds from each group were injected 0.5 mL of the inoculum intramuscularly. Chicks from each group were kept separately. Postmortem was conducted of all the dead chicks and all the surviving chicks were slaughtered on day 7th post challenge and observed for Angara disease lesions.

Results and Discussion

Inactivation of Angara disease vaccine by treatment with formalin (aqueous formaldehyde solution) has been in use since, 1988. Despite of vaccination, disease occurred in most of the vaccinated flocks. Similar reports also indicate about the inactivation of other viral vaccines with formalin. Inactivation of virus is a complex process and not a linear reaction. Formalin react with many chemical group of proteins which leads to the phenomenon of "membrane effects". The membrane effect alters the surface protein of the virus and modifies/reduces the antigenicity of an antigen. This can cause or even lead to disease in vaccinated flocks. Many veterinary viral vaccines have been produced with BEI as inactivant (Brown and Crick, 1959). Infact BEI is an alkylating substance which react very little with proteins and therefore, do not alter the antigenic components of the virus. The results of the present study (Table 1) depict the above explanation.

The two vaccines were prepared and immune response of both the vaccines (formalin inactivated and binnary ethyleneimine inactivated) were compared using indirect hemagglutination (IHA) test. IHA antibody titre with vaccine II were detected on day 10 post vaccination (pv) and peaked on day 25th pv. Whereas the IHA antibody response with vaccine I was also

Table 1: Results of indirect hemagglutination, agar gel diffusion and challenge test

	IHA antibody titre*		Agar gel diffusion		Challenge	
Days	Vaccine I	Vaccine II	Vaccine I	Vaccine II	Vaccine I	Vaccine II
0	0	0	0	0	0	0
5	8	8	0	0	0	0
10	16	32	0	+	10	40
15	32	125	+	+ + +	60	90
20	64	256	+	+ + +	80	90
25	16	512	+	+ + +	10	100
30	8	256	+	+ + +	0	100

*Minimum antibody title

detected on day 10 pv and maximum on day 20th pv but antibody titre dropped abruptly on 25th day pv. Similar results have also been reported previously by Afzal and Ahmad (1990), Ahmad *et al.* (1990), Hassan *et al.* (1994) and Hussain *et al.* (1996). Chicks in control group remained negative for HPS antibody through out the experimental period.

Results of the agar gel diffusion test with serum from chicks immunized with vaccine I and vaccine II were observed on day 15 pv. Strong precipitation line were observed with serum of chicks vaccinated with BEI inactivated vaccine whereas a weak precipitation line was recorded with serum from chicks vaccinated with formalin inactivated vaccine.

Results of the challenge experiments revealed 100 percent mortality of the control chicks after challenge on different days. Died birds showed typical lesions of HPS as reported previously by (Afzal and Ahmad, 1990; Hassan *et al.*, 1994). Maximum protection which persisted was achieved with vaccine II.

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