

## EVALUATION AND COMPARISON OF HYDROPERICARDIUM SYNDROME VACCINES IN BROILER CHICKS

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### ABSTRACT

Humoral immune responses against Hydropericardium syndrome (HPS) were measured in broilers following single vaccination with a commercial oil-based vaccine, three commercial formalized inactivated and one self prepared formalized inactivated vaccine and correlated with challenge protection. In all vaccinated groups, 100 per cent birds became seropositive eight days post vaccination on indirect haemagglutination test (76-128). Non significant difference was observed in groups given formalized vaccines, that peaked (512) on day 16<sup>th</sup> and showed decreasing trend (153-256) from day 32 to 38. Whereas in case of oil-based vaccine titre was still higher (307). Non vaccinated groups could not survive the challenge and there was 100 per cent mortality in this group. Single shot of formalized inactivated vaccines gave protection, about 80 per cent in challenged groups, whereas booster dose of formalized inactivated vaccine gave about 97 per cent protection in challenged groups. Single shot of oil-based vaccine gave best protection, that was up to 98.5 per cent. It was concluded that oil-based vaccine was best due to single shot and long lasting immune response and all formalized inactivated vaccines gave almost equal results.

### INTRODUCTION

"Angara" disease or hydropericardium syndrome (HPS), first observed in 1987 at "Angara" Goth is now widespread all over Pakistan, mainly seen in broilers and broiler breeders (Ahmed *et al.*, 1989). The characteristic lesions of the disease include accumulation of transudate in the pericardial sac, swollen and friable liver (Cheema *et al.*, 1989; Anjum *et al.*, 1989). The disease is infectious (Afzal and Ahmed, 1990). Recently, it has been reported that the causative agent of the HPS is an adenovirus with eight polypeptides and nuclear mass of 23 KDa, which is DNA (Haq *et al.*, 1997). Formalized (Chishti *et al.*, 1989; Afzal and Ahmed, 1990) and oil emulsion vaccine (Hussain *et al.*, 1996), are available and are being used for immunization against HPS, but still outbreaks of the disease are seen. The present project was designed to test the efficacy of various HPS marketed vaccines in terms of immune response and challenge protection.

### MATERIALS AND METHODS

#### Birds

Three hundred, day-old broiler chicks were purchased from a commercial hatchery. The chicks were divided into six groups each having fifty birds.

#### Vaccines

Five different makes of Hydropericardium syndrome (HPS) vaccines were used for the comparative evaluation of immune response induced by the vaccines in chickens. Out of these different vaccines, 3 formalin inactivated (a,b,c) and 1 oil-based (d) were procured from the local market. One formalin inactivated HPS vaccine (e) was prepared in the Department of Veterinary Microbiology, University of Agriculture, Faisalabad, following the method described by Afzal and Ahmed (1990).

#### Collection of samples

Thirty liver samples from clinically infected birds were collected from outbreaks, and were used for the isolation and confirmation of virus and preparation of vaccine and experimental challenge.

#### Vaccine preparation

Confirmation of HPS was done by indirect hemagglutination test (IHA) as described by Rahman *et al.* (1989). After confirmation, 0.2 ml of pure formalin was added in 100 ml of sample for inactivation.

#### Experimental design

On day 12 of age, vaccines against HPS were injected at a dose rate of 0.5 ml S/C to the chicks according to the following schedule.

Group	Vaccine injected	
A	Commercial formalized vaccine	(a)
B	Commercial formalized vaccine	(b)
C	Commercial formalized vaccine	(c)
D	Commercial oil-based vaccine	(d)
E	Self prepared formalized vaccine	(e)
F	Unvaccinated control	

Serum samples were collected randomly from 5 birds of each group on 8th, 10th, 12th, 14th and 16th days postvaccination and stored at  $-20^{\circ}\text{C}$ , till detection of antibody titres through IHA (Rahman *et al.*, 1989).

#### Challenge

Experimental challenge was given using 20% liver homogenate, 0.25 ml, S/C to birds of all groups on day 28 of age (Afzal and Ahmed, 1990). On the same day, half of the birds of group A, B, C and E were given booster dose of vaccine, respectively. The groups were designated as A.1, B.1, C.1 and E.1. Group D was not boosted. Serum samples were collected from birds of all groups on day 4, 6, 8 and 10 post challenge. Protection percentage after challenge was recorded. Birds of the groups receiving booster vaccination were challenged on the 38th day of age, serum samples were collected on day 42, 44 and 46 of life for antibody titration.

## RESULTS

#### Non-challenged vaccinated groups

There was gradual increase in the level of antibodies starting from day 20<sup>th</sup> to 26<sup>th</sup>. On day 26<sup>th</sup> antibody titre was maximum in all 5 groups i.e. from 256-512. On day 28<sup>th</sup>, titre started declining in groups A, B, C and E, but it remained high as 512 in group D, in group F titre was zero (Table 1).

#### Challenged non-boosted groups

Birds of groups A, B, C and E showed a decrease trend of antibody level from day 32<sup>th</sup> to 38<sup>th</sup>. Birds of group D still had high antibody titre than rest of the groups (Table 1). Mortality percentage was 8, 12, 8, 4, 4 and 100 in groups A, B, C, D, E and F, respectively.

#### Challenged boosted groups

Groups A-1, B-1, C-1 and E-1 were given booster dose of vaccines a, b, c and e, respectively. Antibody titre in all groups was 512. Mortality percentage was 4 in groups A and C, whereas it was zero in groups B and E. Mortality percentage was more in non boosted birds as compared to boosted groups.

#### Re-challenged groups

IHA titres after re-challenge are given in Table 2. There was little mortality which was 2.08 per cent in group D only while it was zero in rest of the groups.

## DISCUSSION

The antibody titres against HPS were measured on day 12 of age in all 6 groups before vaccination. All the groups showed equal titres, that was 16. This indicated that there were maternal antibodies present in the chicks, but these antibodies were not of protective level, because unvaccinated birds died in case of challenge. Immune response to vaccines b and d was much quicker than others, but till day 24, titres of all vaccines ranged from 256-512. This age is the most susceptible to HPS, but the titres at this age obtained as a result of vaccination were protective due to which there was very little mortality as also reported by Chishti *et al.* (1989). Statistical analysis showed that all 5 vaccines and their time intervals were significant. When challenge was given to non vaccinated birds, all of them died. This was due to non protective level of antibodies in unvaccinated group.

Groups A, B, C and E were given booster dose of the respective vaccines and antibody titre in all these groups was 512. This indicated that antibody level peaked to maximum in short time, when booster dose was given. There was no effect of challenge on titre, as very little decrease in titre was observed in 2 birds only. Mortality percentage was more in non boosted birds as compared to boosted challenge groups. This factor showed that booster dose had a protective effect against disease in a better way than that provided by single shot, as also reported by Hussain *et al.* (1996). Titre decreased gradually after challenge but it remained protective up to the most susceptible period of the disease. It was noted that the maternal antibodies against HPS in chicks were not of protective level. The chicks became protective against the disease in the most susceptible age, when they were vaccinated at 12 days of age. The titre peaked to maximum 12<sup>th</sup> days after 1st vaccination with all types of vaccines. It was also noted that all vaccines except oil-based, provided almost equal protection. Oil-based vaccine had better and long lasting protective effects than rest of the vaccines. Vaccinated birds were protected against challenge, whereas non vaccinated groups could not survive the challenge.

Studies conducted showed that all formalized HPS vaccines gave almost equal immune response but all needed a second shot, as their titres became low after some days within the most susceptible age of the disease. Whereas the oil-based vaccine gave protective levels for longer period and there was no need of second shot of this vaccine.

Table 1: Average mean titre values for non-challenged vaccinated and challenged non-boosted groups

Days Post Vaccination	Antibody titres of groups					
	A	B	C	D	E	F
<b>Non challenged and vaccinated</b>						
8	115	128	89	128	76	16
10	230	256	204	204	256	16
12	460	512	409	460	512	8
14	409	512	460	512	512	-
16	307	409	358	512	460	-
<b>Challenged non-boosted</b>						
4	230	256	256	307	256	Died
6	204	256	204	307	256	do
8	204	256	204	307	204	do
10	153	230	153	307	153	do

Table 2: Average mean titre values for re-challenged groups

Days Post Vaccination	Antibody titres of groups				
	A-1	B-1	C-1	D	E-1
4	409	460	460	256	460
6	358	409	358	256	409
8	307	358	358	256	358

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