

## THE EFFICACY OF EXPERIMENTAL ANGARA DISEASE VACCINES

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### ABSTRACT

Formalinized aqua based liver organ vaccines for Angara disease (AD)/hydropericardium-hepatitis syndrome (HHS) are effective for the control of the disease. The experimental formalinized 10, 7.5 and 5% liver organ vaccines showed significant differences in efficacy from 2.5 and 1% liver organ vaccines. However, 10, 7.5 and 5% liver organ vaccines differed non significantly from one another. The vaccines of 10 and 7.5% liver organ concentrations showed the best results in terms of reducing mortality among the given groups, while 5% liver organ vaccine proved the best in both efficacy and economy. The vaccination of AD showed positive effect on the immune response of the chicks against Newcastle disease virus.

**Key words:** Angara disease, hydropericardium-hepatitis syndrome, efficacy, vaccines.

### INTRODUCTION

Angara disease (AD)/hydropericardium-hepatitis syndrome (HHS) was first recognized in broiler flocks in Angara Goth near Karachi, Pakistan, during late 1987 (Jaffery, 1988). Because the disease emerged in that specific geographic area, HHS was locally referred to as "Angara disease". Since its first outbreak, AD widely prevails in the country. The outbreaks of the disease were also recorded in Mexico in 1989 in the highly dense poultry producing states.

The preliminary work on the aetiology and pathogenicity of the causative agent and controlling the problem was described by Ahmad *et al.* (1989), Anjum *et al.* (1989) and Cheema *et al.* (1989). The vaccines, including formalinized aqua based liver tissue vaccines and oil based tissue culture vaccines, were developed for the control of the syndrome (Chishti *et al.*, 1989; Afzal and Ahmad, 1990; Ahmad *et al.*, 1990; 1991). Both types of vaccines on challenge were reported to show 100% protection in vaccinated flocks (Ahmad *et al.*, 1990; Ahmad, 1999). Since sporadic outbreaks despite AD vaccination are reported in the field, the present study was undertaken to re-evaluate the efficacy of Angara disease vaccine produced at Poultry Research Institute, Rawalpindi and to improve its formulation for efficacy and economy.

### MATERIALS AND METHODS

#### Experimental chicks

For the present study, day-old chicks were procured from a local hatchery and reared at Poultry

Research Institute, Rawalpindi till the age of 45 days. The chicks were given feed and water *ad libitum*. Afterwards the chicks were randomly divided and separated.

#### i) Experimental chicks for biological titre (LD<sub>50</sub>) of AD virus

For this purpose, a total of 50 chicks were included in the trial. The chicks were divided into five groups named I, II, III, IV and V, with 10 chicks in each group.

#### ii) Experimental chicks for vaccination

In this trial a total of 180 chicks were included. The chicks were divided into six groups named 1, 2, 3, 4, 5 and 6, with 30 chicks in each group.

#### Infectious liver organ filtrate as virus source

The liver organ showing gross lesions including inflammation, typical discoloration and necrosis were collected from the freshly dead broiler chickens exhibiting typical symptoms of AD. A 20% suspension of liver tissue was prepared in phosphate buffer saline. The suspension was sonicated at 40 MHz for 3 minutes and centrifuged at 3000 rpm for 15 minutes. The supernatant was used as virus source in the study.

#### Biological titre (LD<sub>50</sub>) of AD virus filtrate

The biological titre i.e. lethal dose for 50% (LD<sub>50</sub>) of the virus suspension per 1 ml was determined in 25 days old broiler chickens of groups I, II, III, IV and V by inoculating subcutaneously, undiluted 20% filtrate and its dilutions of 10<sup>-1</sup>, 10<sup>-2</sup>, 10<sup>-3</sup> and 10<sup>-4</sup>, respectively.

LD<sub>50</sub> was calculated by the method described by Ahmad (1999).

#### AD vaccines

Five different concentrations of AD vaccine as 1.0, 2.5, 5.0, 7.5 and 10.0% aqua based infectious liver organ tissue filtrate, inactivated with 0.1% formalin, were prepared and named as vaccines I, II, III, IV and V, respectively, for further reference in the study.

#### Comparative efficacy of vaccines

The chicks of groups 1, 2, 3, 4 and 5 were vaccinated with vaccines I, II, III, IV and V respectively, at the recommended dose of 0.30 ml per chick, at the age of 18 days. The chicks of group 6 were kept without vaccination and were used as negative control. At the age of 25 days, the chicks of each group were further sub-grouped into three groups named 1a, 1b, 1c, 2a, 2b, 2c, 3a, 3b, 3c, 4a, 4b, 4c, 5a, 5b, 5c, 6a, 6b and 6c, having 10 chicks each.

#### Challenge protection

The chicks in all subgroups were subjected to challenge protection with the 20% viral suspension at the rate of 1 ml subcutaneously per chick 7 days post vaccination at the age of 25 days.

#### Newcastle disease vaccination and serology

All the experimental chicks were vaccinated twice with Newcastle disease (ND) at the age of 7 and 21 days at the recommended dose rate and route of vaccination to evaluate the immune response of the AD affected birds. Blood samples were collected from the chicks of groups 5 and 6, 20 days after second ND vaccination, sera were separated and antibodies titres against ND virus were determined by the haemagglutination inhibition test. Geometric mean titres (GMT) were calculated by the method described by Burgh (1977).

#### Statistical analysis

The data on the challenge protection test were subjected to the statistical analysis for the interpretation of results by using one way analysis of variance (Steel and Torrie, 1984).

## RESULTS AND DISCUSSION

#### Biological titre (LD<sub>50</sub>)

The biological titre (LD<sub>50</sub>) of the 20% viral filtrate was determined as 10<sup>1.1</sup> per ml inoculated subcutaneously in 25 days old broiler chicks (Table 1). This finding is different from the previously reported viral titres of 10<sup>4</sup> to 10<sup>5</sup> per ml of 20% liver filtrate studied at the inception of the disease problem (Anonymous, 1989). The reduced number of outbreaks of the disease due to intensive and widely adopted vaccination might have reduced the pathogenicity of the causative agent (Ahmad, 1999).

#### Efficacy of vaccines

Formalized aqua based infected liver organ filtrate vaccines for Angara Disease (AD) were effective for the control of the disease. Statistical analysis of the data showed that the 10, 7.5 and 5% infected liver organ vaccines had significantly better efficacy than 2.5 and 1% infected liver organ vaccines. Their results were, however, non significantly different from one another. Vaccine I (10%) and Vaccine II (7.5%) showed the best results in terms of protection among the given groups (Table 2). However, in the light of the present study and keeping in view the cost element in the production of infectious liver, vaccine III (5%) can be recommended as the best in efficacy and economy. Moreover, vaccination against AD had a good effect on the development of the serum antibodies titres against NDV (Table 3). The low antibodies titres against NDV in the group 6 chicks is supportive of the finding of Naeem *et al.* (1995) that infection of AD is immunosuppressive. The vaccination against AD gives protection against the subclinical infection also. Good performance of the inactivated AD vaccines without priming with live vaccine is also suggestive of the exposure of the chicks to AD virus at early days of their age.

**Table 1: Determination of biological titre LD<sub>50</sub>**

Group	Dilution	No. of chicks inoculated	No. of chicks died	No. of chicks survived	Proportion death ratio	% age
1	undiluted	10	5	5	17/22	77.3
2	10 <sup>-1</sup>	10	4	6	12/23	52.2
3	10 <sup>-2</sup>	10	3	7	8/26	30.8
4	10 <sup>-3</sup>	10	3	7	5/30	16.7
5	10 <sup>-4</sup>	10	2	8	2/34	5.9

Proportionate distance (PD) = 52.2 - 50.0 / 52.2 - 30.8 = 0.1 so, LD<sub>50</sub> = 10<sup>1.1</sup>

**Table 2: Comparative efficacy of AD vaccines of different concentrations in challenge protection test**

Subgroup <sup>†</sup>	Mortality in AD vaccine treatment groups					
	VAC-I	VAC-II	VAC-III	VAC-IV	VAC-V	NC
A	1	2	0	0	0	4
B	1	1	0	0	0	5
C	2	2	1	0	0	5
Total	4	5	1**	0**	0**	14

AD = Angara disease. VAC = Vaccine. NC = Negative control. <sup>†</sup> 10 birds in each sub group. \*\* Statistically significant from VAC I & II (P < 0.05).

**Table 3: Immune response against Newcastle disease vaccines**

Group	Vaccinated against	GMT of antibodies against ND virus*
5	AD + NDV	11
6	NDV	8

\* = 10 HA units used in HI test.

AD = Angara diseases. NDV = Newcastle disease virus. GMT = Geometric mean titre.

Based on the findings of the present study it can be concluded that the chicks should be vaccinated against AD even in the absence of wide spread clinical prevalence of the disease to control the subclinical AD virus infection and the related complications. Furthermore, since different standard vaccines are in use in the field, the efficacy of the vaccine should be evaluated from time to time.

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