IN VIVO PATHOGENICITY OF HYDROPERICARDIUM-HEPATITIS SYNDROME (ANGARA DISEASE) AND EFFICACY OF VACCINES

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ABSTRACT

The pathogenicity of recent field isolate (PRI-10) of Hydropericardium-Hepatitis Syndrome (HHS) showed reduced pathogenicity as compared to the isolates studied on the inception of the syndrome in 1987-88. Formalinized vaccines for HHS including Aqua Base Liver Organ (ABLO) vaccine and Oil Base Tissue Culture (OBTC) vaccine are effective for the control of the syndrome. ABLO vaccine gives an early immune response as compared to OBTC vaccine, but OBTC vaccine gives better immune response. The vaccination against HHS has positive effect on the immune response of the chicks against Newcastle disease virus.

INTRODUCTION

Hydropericardium-Hepatitis Syndrome (HHS) was first recognized in broiler flocks in Angara Goth (Goth means small town or village) near Karachi Metropolitan City of Pakistan, in late 1987 (Jaffery, 1988). Because the disease emerged in this specific geographic area, HHS was initially referred to as "Angara Disease". The syndrome was spread in the densely populated broiler growing areas all over the country within six months. The outbreaks of HHS were also recorded in Mexico in 1989 in the high-density poultry producing states (Borrego and Soto, 1995).

The preliminary work on the pathogenicity and vaccine development has been described by Ahmad *et al.* (1989), Anjum *et al.* (1989), Cheema *et al.*(1989), and Khawaja *et al.* (1989). The formalinized vaccines including aqua base liver organ (ABLO) and oil base tissue culture (OBTC) 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 were reported to provide 100% protection in vaccinated flocks (Ahmad *et al.*, 1990; Gay *et al.*, 1995).

The objective of the present study was to evaluate the pathogenicity of the recent field isolate named PRI-10 and to determine the comparative efficacy of ABLO and OBTC vaccines.

MATERIALS AND METHODS

Experimental chicks

1230 day-old quality broiler chicks were purchased and reared in isolation units of Poultry Research Institute, Rawalpindi. The chicks were randomly divided into three major groups named A, B and C having 50, 970 and 210 chicks in each, respectively. A was further divided into five groups named A1, A2, A3, A4 and A5 having 10 chicks in each. Group C was having 210 chicks, which were further divided into three groups named C1, C2 and C3 having 70 chicks in each.

Virus

The liver tissue showing gross pathological lesions (swollen and necrosed) were collected from freshly dead broiler chickens typically affected with HHS. A 40% suspension of liver tissue was prepared in phosphate buffer saline (PBS). The suspension was sonicated at 40 MHz for 3 minutes and centrifuged at 3000rpm for 15 minutes. The supernatant was used as virus source in the study.

HHS Vaccines

Formalinized HHS vaccines, aqua base liver organ (ABLO) and oil base tissue culture (OBTC) obtained from the market (Vety vac_Hydro of Vetycare Pharmaceuticals (Pvt) Ltd, Islamabad; H.C.I. of Avimex Laboratorio Avi_mex S.A. DE C.V., Mexico) were used as ABLO and OBTC vaccines, respectively.

Pathogenicity

Biological Titre (LD_{50})

The biological titre i.e., lethal dose for 50% (LD_{50}) of the virus suspension per 1 mL was determined in 28 days old broiler chickens of groups A1, A2, A3, A4 and A5 by inoculating subcutaneously and dilutions 10^{-1} ,10^{-2}, 10^{-3} & 10^{-4} respectively. LD_{50} was calculated by the method described by Reed and Muench (1938).

Mortality pattern

The chicks of Group B were inoculated with the viral suspension @ 1 mL subcutaneously at the age of day 28. The chicks were observed upto 8 days (192 hours) post inoculation to determine the infectivity and mortality pattern.

Comparative efficacy of vaccines

The chicks of groups C1 and C2 were vaccinated with ABLO and OBTC vaccines with recommended dose of 0.25 mL and 0.5 mL per chick, respectively. The chicks of group C3 were kept without vaccination as negative control. The chicks of each group were further sub-grouped in to three groups named C1a, C1b and C1c, and similarly C2a, C2b, C2c, C3a, C3b and C3c comprising 30, 30, 10, 30, 30, 10, 30, 30 and 10 chicks, respectively at the age of day 25.

Challenge protection

The chicks of subgroups C1a, C2a and C3a were subjected to challenge protection with the viral suspension @ 1 mL subcutaneously per chick 5 days postvaccination. The chicks of groups C1b, C2b and C3b were subjected to challenge protection 10 days postvaccination with the same dose and route as described for first challenge.

Serology

The blood was drawn from the chicks of groups C1c, C2c and C3c, 20 days post vaccination. The sera were separated.

The serum antibody titres against the HHS and ND viruses were determined by the Indirect Heamagglutination Test and Heamagglutination Inhibition Test respectively.

The Geometric Mean Titres (GMT) were calculated by the method described by Brugh (1977).

RESULTS AND DISCUSSION

Pathogenicity

Biological Titre (LD_{50})

The biological titre i.e., LD_{50} of the viral suspension was determined as $10^{-1.1}$ per mL inoculated subcutaneously in 28 days old broiler chicks (Table 1).

This finding is significantly different from the previously reported viral titres of 10^4 to 10^5 per ml of 20% liver filtrate studied at the inception of the disease problem (Anonymous, 1989). The natural outbreaks of the disease have also reduced to a significantly low level. The reduction in the pathogenicity of the HHS causing virus may be due to intensive vaccination to control the disease.

Mortality

A high rate of mortality was observed in chicks of group B. A total of 77.1 per cent chicks died due to HHS between 12 to 180 hours postinoculation with the peak between 36 to 48 hours (Table 2). The symptoms and lesions observed were representative of typical field cases. The negative control did not show any mortality due to HHS throughout the trail. The high rate of mortality with less pathogenic isolate (PRI-10) may be due to the additional effect of oral and aerosol transmission of the virus within the experimental chicks which may contribute 20 - 40% (Ahmad *et al.*, 1992).

Table 1: Determination of Biological Titre LD_{50}.

Group	Dilution	Nos. of chicks Inoculated	Nos. of chicks survived	Nos. of chicks died	Proportion death ratio	% age
A1	Undiluted	10	5	5	17/22	77.3
A2	10^{-1}	10	6	4	12/23	52.2
A3	10^{-2}	10	7	3	8/26	30.8
A4	10^{-3}	10	7	3	5/30	16.7
A5	10^{-4}	10	8	2	2/34	5.9

Proportionate Distance (PD) = 52.2 - 50.0 / 52.2 - 30.8 = 0.1 so, $LD_{50} = 10^{-1.1}$

Table 2: Mortality pattern in experimentally infected 970 chicks

Hours post inoculation	Nos. of chick died	Cummulative daily mortality
0	0	0
12	5	5
24	50	55
36	175	230
48	250	480
60	90	570
72	55	625
84	40	665
96	35	700
108	14	714
120	12	726
132	8	734
144	4	738
156	4	742
168	4	746
180	2	748
192	0	0
TOTAL	748 (77.1 %)	

Efficacy of vaccines

ABLO vaccine gave 90 and 100% protection in subgroups C1a & C1b on days 5th and 10th post vaccination (PV) respectively. The chicks of group C2a vaccinated with OBTC vaccine showed no protection at day 5th PV while comparing with the subgroup C3a (non vaccinated chicks) but gave solid protection 10 days PV in the chicks of group C2b (Table 3). The serum antibodies level against HHS virus was better in OBTC vaccinated chicks as compared to ABLO vaccinated chicks at 20 days PV (Table 4).

In the light of present study, it is recommended that OBTC and ABLO vaccines should be used between the age of 10 to 15 days and 15 to 20 days, respectively to achieve a maximum protection level in 3rd week of age. Moreover, vaccination against HHS has a good effect on the development of the serum antibody titres against NDV (Table 4). The HHS virus is an immunosuppressive (Naeem *et al.*, 1995). The vaccination against HHS gives protection against the subclinical infection also. The presence of antibodies against HHS in the HHS un vaccinated subgroup C3c is suggestive of the subclinical infection in the control group. Good performance of the inactivated HHS vaccines without priming with live vaccine is also suggestive of the exposure of the chicks to HHS virus at early days of their age. The low antibody titres against NDV in the same subgroup of chicks are also supportive of our conclusions that subclinical infection of HHS is immunosuppressive. The chicks should be vaccinated against HHS even in the absence of epidemic of the disease to control the subclinical infection of the HHS virus.

Table 3: Comparative efficacy of ABLO and OBTC vaccines

Group	Subgroup	Days post vaccination	Nos. of chicks died	Nos. of chicks survived	Per cent protection
C1	a	5	3	27	90
	b	10	0	30	100
C2	a	5	18	12	39
	b	10	0	30	100
C3	a	5	18	12	39
	b	10	14	16	53

Table 4: Immune response against HHS and ND vaccines

Group	Vaccinated with	GMT of antibodies in serum against	
		HHS virus	ND virus
C1	ABLO + NDV	19	11
C2	OBTC + NDV	21	20
C3	NDV	11	8

ABLO = Aqua Base Liver Organ HHS vaccine; OBTC = Oil Base Tissue Culture HHS vaccine; NDV = Newcastle Disease Virus vaccine

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