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Full Length Research Paper

Pathogenicity of recent field isolate of Avian Adenovirus Serotype-IV of hydropericardium syndrome (Angara disease)

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The pathogenicity of recent field isolate of Avian Adenovirus Serotype IV (AAS-IV) was determined during the present study. During the present investigations, Hydropericardium Hepatitis Syndrome (HHS) showed reduced pathogenicity as compared to the isolates studied on the inception of the syndrome in 1987-1988. Pathogenicity of the virus was determined through LD_{50} and challenge protection test. The LD_{50} titre of the 10% viral suspension prepared from liver filtrate was found to be $10^{-2.5}$ per ml in 26 days old broiler chicks. None of the chick showed mortality however, Hydropericardium syndrome of Angara disease was observed on postmortem of the challenged chicks and observations grossly observed in the form of lesions on liver, hydronephrosis and water around heart (yellow straw coloured). These findings proved that the injected virus suspension was biologically active. 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. The vaccination against HHS has positive effect on the immune response of the chicks against HHS. Due to extensive vaccination in the field there is a reduction in the virulence of the virus.

Key words: Pathogenicity, adenovirus, Angara disease, LD_{50}.

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 Ahmed et al. (1989), Anjum et al. (1989), Cheema et al. (1989) and Khawaja (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 Ahmed, 1990; Ahmed et al., 1990, 1991). Both types of vaccines were reported to provide 100% protection in vaccinated flocks (Ahmed et al., 1990)

The objective of the present study was to evaluate the pathogenicity of the recent field isolate of Avian

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Adenovirus.

MATERIALS AND METHODS

Rearing of chicks

For the present study 175 day old quality broiler chicks were purchased from the market and were reared at Poultry Research institute (PRI), Rawalpindi and National Veterinary Laboratories (NVL) Islamabad in poultry rearing units. The chicks were further divided into group 1 and 2 containing 25 and 150 chicks respectively. Twenty-five chicks of group 1 were reared at PRI, Rawalpindi and chicks of group 2 were reared at NVL, Islamabad. Feed and water were provided *ad-libitum*.

Twenty-five chicks of group 1 were divided into five subgroups named group a, b, c, d and e having five chicks in each group. These chicks were used to determine the biological titre i.e., LD^{50} of the virus suspension to use further in this study.

150 chicks of group 3 were divided into five sub groups named as k, l, m, n and o with 30 birds in each sub group. These sub groups were used for vaccine pthogenicity testing.

Pathogenicity test

One hundred and fifty day old broiler chicks were housed at Animal House, National Veterinary Laboratory (NVL), Islamabad under standard husbandry conditions. At age of day 10 and 20, blood samples were taken from 08 chicks. At the day 20 the chicks were divided into five sub- groups each having 30 chicks, named k, l, m, n, and o. On the same day, the chicks of group k, l, m, and n were vaccinated. The chicks of subgroup o were kept as non-vaccinated control. Route and dose of vaccination was adopted according to manufacturer's instructions.

Pathogenicity was tested by challenge protection and serology. 10 chicks of subgroup k, l, m, n and o were subjected to challenge protection by 2 ml of 10% infectious virus suspension. These chicks were observed for 05 days post challenge. The serum samples from subgroup k, l, m, n and o were also obtained at day 7, 14 and 21-post vaccination for serology.

Procurement of Angara disease vaccines

Four commercially available Angara disease vaccines were selected and purchased from the market for quality testing.

Procurement of Angara disease virus

10% infectious Angara disease liver suspension was obtained from the Angara disease vaccine production laboratory, disease Section, Poultry Research Institute, Murree Road, Rawalpindi. This virus suspension was used to determined the biological titre i.e., Lethal Dose for 50% and challenge protection test.

Determination of biological titre (LD50) of Angara disease (AD) virus

The biological titre i.e., Lethal Dose for 50% (LD50) of the virus suspension (10% infectious liver suspension) per 1 ml was determined in 26 days old broiler chicks of sub groups a, b, c, d and e. Ten fold dilution of virus suspension was prepared in Phosphate Buffer Saline (PBS) starting from 10-1-10-5 dilution. One ml of each dilution suspension i.e., 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4} and 10^{-5} was injected subcutaneously to five chicks of each sub group of a, b, c, d and e respectively. Mortality was observed up to seven days post injection. Died and survived birds in each group were counted. LD50 was calculated by the method described by Reed and Muench (1938).

Histopathology

Samples collection

All survived chicks of sub subgroup k, l, m, n and o were subjected to post mortem examination after slaughtering at the age of day 42. Livers were collected from all the chicks for histopathological examination. For processing of tissues following methodology was used.

The tissues were further processed for histopathology. For this, the tissue block of about 4-5 mm size representing the pathological lesion, periphery, line of demarcation and normal area were incised and obtained for routine histopathology.

RESULTS AND DISCUSSION

Pathogenicity determination of lethal dose 50 (LD_{50}) of Angara disease virus suspension

Chicks of sub groups a, b, c, d and e were used to determine the LD_{50} of virus suspension. 5, 3, 2, 1 and 1 out of 5 chicks from each subgroup a, b, c, d and e were died respectively. 0, 2, 3, 4 and 4 chicks of sub groups a, b, c, d and e were survived. Biological titre (LD_{50}) of the viral suspension was calculated by the method described by Reed and Muench (1938) (Table 1). The LD_{50} titre of the 10% viral suspension prepared from liver filtrate was found to be $10^{-2.5}$ per ml in 26 days old broiler chicks. This finding is significantly different from the previously reported vial titres of 10^4 and 10^5 per ml of the 10% liver filtrate studied at the inception of the disease problem (Anonymous, 1989). Lesions recorded in the dead chicks were representative of typical field cases of Angara Disease (Hydropericardium Syndrome)

$$\text{Proportionate Distance} = \frac{\text{Percentage infected at dilution next above 50\%-50}}{\text{Percentage infected at dilution next above}}$$

50%-below50%

$$PD = \frac{60-50}{60-40}$$

$$PD = \frac{10}{20}$$

PD = 0.5.

The proportionate distance obtained was corrected by the dilution factor, which was the logarithm of the dilution step employed. Then 50% end point dilution was

Table 1. Determination of Lethal Dose (LD_{50}) of Angara disease (AD) virus.

Sub-groups	Dilution	No. of chicks inoculated	No. of chicks died	No. of chicks survived	Cumulative proportion death rate	% Mortality
A	10^{-1}	5	5	0	12/12	100
B	10^{-2}	5	3	2	7/9	60
C	10^{-3}	5	2	3	4/7	40
D	10^{-4}	5	1	4	2/6	20
E	10^{-5}	5	1	4	1/5	20

Table 2. Gross pathological lesions recorded after challenge dose of viral suspension inoculated in chicks.

Sub-groups of chicks	Vaccines administered	Postmortem lesions
k	AD Vac-I	5 birds with lesions on liver, hydronephrosis and water around heart (yellow colour).
l	AD Vac-II	3 birds with lesions on liver, hydronephrosis and water around heart (yellow colour).
m	AD Vac-III	4 birds with lesions on liver, hydronephrosis and water around heart (yellow colour).
n	AD Vac-IV	1 bird with lesions on liver, hydronephrosis and water around heart (yellow colour).
o	Non-vaccinated	7 birds with lesions on liver, hydronephrosis and water around heart (yellow colour).

calculated as,

Negative logarithm of LD_{50} titre = (Negative logarithm of the next dilution above 50% mortality + PD) × Dilution factor

= (-2+0.5) ×1 = - 2.5

Log of LD_{50} titre =$10^{-2.5}$

Pathogenicity testing was performed by challenge protection test and antibody titration after vaccination of chicks of sub-groups k, l, m and n. Chicks of sub-group o were kept as non-vaccinated control.

The vaccinated and non vaccinated chicks were subjected to challenge protection test with the 10% viral suspension at 2 ml subcutaneously per chick 17 days post vaccination at the age of 37 days. None of the chick in any group showed any mortality. In the previously reported experiments the challenge protection test was performed in the chicks aged different 25-30 days (Ahmed, 1989; Mashkoor et al., 1994) but in this study the chicks were aged to 37 days at the time of challenge protection. Moreover the biological titre i.e., LD_{50} of the viral suspension was determined in chicks at the age of 25 days in this study. This may be one of the reason of failure of mortality in the control group o as well as in vaccinated groups k, l, m and n. However Hydropericardium syndrome of Angara disease was observed on postmortem of the challenged chicks and observations shown in (Table 2). These findings proved that the injected virus suspension was biologically active. Some chicks from sub-group k, l, m, n and o showed lesions of Hydropericardium, which are detailed in Table 2.

Histopathological findings of the vaccinated groups

During the present study the histopathological examination of the liver tissues of the sub-group k, l, m, n and o showed the basophilic intranuclear inclusion bodies, cloudy swelling, fatty degeneration, dilated sinusoidal spaces, necrosis, cytoplasmic blebing and liquefactive necrosis (Table 2). These findings are in line with the previously reported findings of Swati et al. (2000), Fadly and Winterfield (1973) and Cubillos et al. (1986) who experimentally introduced the infectious agent of hydropericardium in 28 day old broiler chicks and confirmed the presence of Fowl Adeno Virus (FAV) by observing basophilic intra-nuclear inclusion bodies in the hepatocytes of all the dead birds. It is clear from the present discussion, that local isolate of adenovirus from Angara disease can produces significant pathological changes in the hepatocytes of liver and other tissues like fatty degeneration, cloudy swelling, basophilic intra-nuclear inclusion bodies and cytoplasmic blebbing. It is further observed with conclusions made by workers that FAV-4 produces straw coloured fluid in the heart and predominantly inclusion bodies in the hepatocytes.

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