

## EFFECT OF PIGEON ORIGIN NEWCASTLE DISEASE VIRUS ON VARIOUS LIVER ENZYMES AND ASSOCIATED PATHOLOGICAL CHANGES IN EXPERIMENTALLY INFECTED PIGEONS

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### ABSTRACT

Newcastle disease virus (NDV) was isolated from a field outbreak in pigeons. The virus was characterized by haemagglutination test (HA) and confirmed by haemagglutination inhibition test (HAI). The pathotyping was done by mean death time (MDT), intracerebral pathogenicity index (ICPI) and intravenous pathogenicity index (IVPI). The  $ELD_{50}$  of the velogenic strain was  $10^{4.66}/0.1$  ml. Thirty-nine pigeons were randomly divided into three equal groups. Pigeons of one group were vaccinated with ND vaccine (LaSota strain) intraocularly after 14 days of procurement, while the other two groups served as vaccinated and non-vaccinated controls. Birds of these two groups were challenged with velogenic strain of field isolate of NDV 7 days post-vaccination. Birds were kept under observation for 15 days post-challenge. Haemorrhages and congestion were observed in trachea, lungs, liver, proventriculus and intestine of pigeons infected with NDV. Concentrations of AST, ALT and ALP did not differ among pigeons of the three groups.

**Key words:** Pigeons, Newcastle disease, velogenic strain, transaminases, pathology.

### INTRODUCTION

Pigeons are one of the few domesticated birds, which are kept by the humans for variety of purposes such as food, hobby (racing) and treatment of various diseases. A variety of diseases affect pigeons but viral diseases predominate (Liu *et al.*, 2003). Among viral diseases, Newcastle disease (ND) is most important disease (Ballouh *et al.*, 1985). Nervous form of ND in pigeons is colloquially known as 'Jholah' in the Punjab province of Pakistan (Arshad, 1984).

In pigeons, ND is highly contagious disease, caused by pigeon paramyxovirus serotype-1 (PPMV-1), which is a variant of Avian Paramyxovirus serotype-1 (APMV-1), causing ND in poultry (Alexander and Parsons, 1985; Kaleta *et al.*, 1985). Pigeons are also susceptible to Avian Paramyxovirus type-1 (Mubarak *et al.*, 2001).

On the basis of its virulence, Newcastle disease virus (NDV) has been classified into three strains viz. velogenic, mesogenic and lentogenic. These strains produce highly acute, moderate and mild type of infection in poultry, respectively. Only velogenic strain causes disease in pigeons (Saif, 2003). Along with chicken, ND is a serious problem in pigeons in Pakistan (Arshad, 1984). However, no concrete attempt has been carried out to know liver enzymes and associated pathology in naturally or experimentally NDV infected pigeons. Therefore, the present project was planned to

study the effect of NDV on health of pigeons and on liver enzymes.

### MATERIALS AND METHODS

#### Virus isolation and pathotyping

For the isolation of field NDV, trachea, lungs and spleen were collected from 20 pigeons suspected for Newcastle disease (Azam *et al.*, 1984). Suspected tissue were diluted 10 times in normal saline with 10% glycerol and homogenized in a tissue homogenizer at 1000Xg for 20 minutes. Gentamicin sulfate was added at the dose of 1.0 mg/ml and supernatant was inoculated into 20, 9-days old embryonated chicken eggs via allantoic cavity route, as described by Senne (1989). The eggs were incubated at 37°C for 3 days and candled after every 24 hours. The embryos found dead after 24 hours were chilled overnight at 4°C to collect allantoic fluid. The fluid was tested for haemagglutination (HA) activity by spot agglutination test and confirmed by HAI test (MAFF, 1984). The confirmed isolate of NDV was processed for pathotyping (Alexander, 1989) on the basis of mean death time (MDT) in 9-days old embryonated chick eggs, intracerebral pathogenicity index (ICPI) in day-old chicks and intravenous pathogenicity index (IVPI) in six-week-old chicks. The  $ELD_{50}$  of the velogenic isolate was calculated by the method described by Villegas and Purchase (1989).

### Experimental design

Thirty nine adult pigeons, divided randomly into three equal groups ( $G_1$ ,  $G_2$  and  $G_3$ , having 13 birds in each group), were kept under standard managerial conditions for 14 days. Group  $G_1$  was designated as vaccinated control, group  $G_2$  served as non-vaccinated challenged and third group  $G_3$  served as non-vaccinated control. Pigeons of group  $G_1$  were vaccinated with LaSota strain of NDV 14 days after procurement. Pigeons of group  $G_1$  and  $G_2$  were experimentally challenged with velogenic strain of NDV through intraocular route at 21 days after procurement (Erickson *et al.*, 1993), while group  $G_3$  served as control.

Pigeons of all groups were kept under observation for two weeks after challenge. Dead birds were examined for postmortem lesions and organs showing lesions were preserved in 10% buffered formalin for histopathological examination (Bancroft and Stevens, 1990). Blood was collected without EDTA from wing vein of seven birds of each group at first day, 14 and 21 days post-infection. Serum was separated from blood for the estimation of aspartate (AST) and alanine transaminases (ALT) and alkaline phosphatase (ALP), using commercially available diagnostic kits and following the method provided by manufacturer. Data were subjected to statistical analysis using computer statistical package "MSTAT-C. Analysis of variance and Duncan's Multiple range test were applied to see the differences among the groups.

## RESULTS

### Haemagglutination and haemagglutination inhibition tests

Tissue homogenate from pigeons suspected for ND was harvested in embryonated chicken eggs. The

chorio-allantoic fluid was tested for NDV through HA test and confirmed by HAI test. From suspected pigeons four isolates were positive for haemagglutinating activity. They were named as A, B, C and D. Haemagglutination (HA) titers of the isolates A, B, C and D were 1:256, 1:128, 1:512 and 1:64, respectively. These isolates were inhibited by specific hyperimmune sera against NDV.

### Mean death time (MDT) of viral isolate

Field isolates A, B, C and D were inoculated in 9-days old embryonated chicken eggs through chorio-allantoic route to determine MDT. MDT of isolate D was 90 hours with dilutions  $10^{-2}$ ,  $10^{-3}$  and  $10^{-4}$  and was categorized as lentogenic. MDT of A and B isolates ranged from 78 to 90 hours with dilutions,  $10^{-2}$ ,  $10^{-3}$  and  $10^{-4}$ . These isolates were characterized as mesogenic. MDT of isolate C ranged from 54 to 66 hours with dilutions  $10^{-2}$ ,  $10^{-3}$  and  $10^{-4}$ . This isolate was characterized as velogenic (Table 1).

### Intracerebral pathogenicity index (ICPI)

Four field isolates A, B, C and D were inoculated through intracerebral route in day-old chicks. From isolate D, morbidity and mortality started on 5<sup>th</sup> day post-inoculation. ICPI value of this isolate was 0.3 and it was characterized as lentogenic. From isolates A and B, morbidity and mortality started on day 4<sup>th</sup> post-inoculation. The ICPI values of these isolates were 1.2 and 1.0. These isolates were characterized as mesogenic. With isolate C, morbidity and mortality started from third day post-inoculation. The ICPI value was 1.8 and it was characterized as velogenic (Table 2).

### Intravenous pathogenicity index (IVPI)

**Table1: Mean death time of various isolates of NDV isolated from natural cases of pigeons suffering from Newcastle disease.**

Isolate No.	Dilutions	No. of embryos died at different hours post inoculation							MDT
		24	36	48	60	72	84	96	
A	$10^{-2}$	-	-	-	-	4	1	-	78
	$10^{-3}$	-	-	-	-	3	-	2	84
	$10^{-4}$	-	-	-	-	2	-	3	84
B	$10^{-2}$	-	-	-	-	3	2	-	78
	$10^{-3}$	-	-	-	-	2	3	-	78
	$10^{-4}$	-	-	-	-	-	2	3	90
C	$10^{-2}$	-	-	3	2	-	-	-	54
	$10^{-3}$	-	-	2	3	-	-	-	54
	$10^{-4}$	-	-	-	2	3	-	-	66
D	$10^{-2}$	-	-	-	-	-	4	1	90
	$10^{-3}$	-	-	-	-	-	2	3	90
	$10^{-4}$	-	-	-	-	-	1	4	90

**Table 2: Intracerebral pathogenicity index (ICPI) and intravenous pathogenicity index (IVPI) of field isolates of pigeon NDV**

Isolate No.	ICPI	IVPI	Pathotype
A	1.2	0.3	Mesogenic
B	1.0	0.2	Mesogenic
C	1.8	2.5	Velogenic
D	0.3	0.0	Lenotgenic

Four field isolates A, B, C and D of NDV were inoculated intravenously in six-weeks-old chicks. From isolate D morbidity was started on 6<sup>th</sup> day post inoculation. No mortality was observed upto 10 days post-inoculation. IVPI value for this isolate was 0.0 and was characterized as lentogenic. With isolates A and B, morbidity and mortality started on 4<sup>th</sup> and 5<sup>th</sup> day post inoculation, respectively. IVPI values of these isolates were 0.3 and 0.2 and they were mesogenic. From isolate D, morbidity and mortality started third day post-inoculation. IVPI was 2.5 and it was characterized as velogenic (Table 2).

#### Embryo lethal dose<sub>50</sub> (ELD<sub>50</sub>)

To determine the ELD<sub>50</sub>, 10 fold serial dilutions of velogenic strain of NDV were used i.e. 10<sup>-1</sup>, 10<sup>-2</sup>, 10<sup>-3</sup>, 10<sup>-4</sup>, 10<sup>-5</sup> and 10<sup>-6</sup>. Each of these dilutions was inoculated to 10 embryonated chicken eggs. Mortality was recorded upto seven days post-inoculation. The calculated ELD<sub>50</sub> of field velogenic isolate was 10<sup>-4.66</sup>/0.1 ml of virus (Table 3).

#### Serum enzymes and pathology

Serum concentrations of ALT, AST and ALP showed non significant differences among the three groups G<sub>1</sub>, G<sub>2</sub> and G<sub>3</sub> (Table 4). There was a slight increase in the concentrations of ALT, AST and ALP of pigeons of group G<sub>2</sub>.

Eyelids and periocular tissue of pigeons of infected group were oedematous. Necropsy examination revealed mild to moderate congestion of viscera and

enteritis of pigeons of group G<sub>2</sub>. Splenomegaly and hepatomegaly were observed in pigeons of infected group. Liver showed lightened discoloration along with small areas of necrosis. Heart showed pinpoint haemorrhages and slight enlargement. There were congestion and slight swelling in the brain of pigeons of infected group.

Microscopically, haemorrhages and necrosis of epithelial cells of proventriculus of infected pigeons was observed. Alveoli of lungs were filled with exudate and inflammatory cells. Tracheal mucosa was haemorrhagic and congested. There was sloughing of intestinal epithelium and haemorrhages were present in the mucosa and submucosa.

#### DISCUSSION

Newcastle disease is one of the major and continuing respiratory problem in poultry in Pakistan (Anjum, 1990). Pigeons are affected by a variety of diseases, but viral diseases predominate (Liu *et al.*, 2003). Among viral diseases of pigeons, Newcastle disease is an important disease (Ballouh *et al.*, 1985).

A widely accepted protocol for classifying field isolates is based on MDT, ICPI and IVPI. According to these criteria, lentogenic strains possess MDT >90 hours, ICPI value <0.5 and IVPI <0.5 (Shirai *et al.*, 1986; Khalafalla, 1994; King, 1996). Mesogenic strains possess MDT <60-90 h, ICPI value 1.0 to 1.5 and IVPI value 1 to 1.5 (Reddy *et al.*, 1993; King, 1996; Parimal *et al.*, 1997) and velogenic strains possess MDT <60 h,

**Table 3: Embryo lethal dose<sub>50</sub> (ELD<sub>50</sub>) of velogenic strain of NDV of pigeon origin**

Dilutions	Embryos		Accumulated numbers		Proportion dead/total	%age dead
	No. of dead	No. of alive	No. of dead	No. of alive		
10 <sup>-1</sup>	5	0	20	0	20/20	100
10 <sup>-2</sup>	5	0	15	0	15/15	100
10 <sup>-3</sup>	4	1	10	1	10/11	90
10 <sup>-4</sup>	3	2	6	3	6/9	66
10 <sup>-5</sup>	2	3	3	6	3/9	33
10 <sup>-6</sup>	1	4	1	10	1/11	9

Mortality at dilution above 50% - 50

Proportionate distance (PD) = -----

Mortality at dilution above 50% - mortality at dilution below 50%  
87 - 50

Proportionate distance (PD) = ----- = 0.66; ELD<sub>50</sub> = Negative log of dilution above 50% + PD = log

10<sup>-4</sup> + PD = 10<sup>-4.66</sup>/0.1 ml.

**Table 4: Concentrations of liver enzymes (IU/L) in serum of pigeons infected with Newcastle disease virus**

Enzyme	Group	1st day*	7 <sup>th</sup> day*	14 <sup>th</sup> day*
AST	G <sub>1</sub>	159.00 ± 15.87	173.67 ± 10.33	168.17 ± 12.23
	G <sub>2</sub>	157.57 ± 9.10	193.29 ± 15.02	184.71 ± 13.47
	G <sub>3</sub>	145.57 ± 9.31	151.57 ± 8.13	155.43 ± 6.19
ALT	G <sub>1</sub>	52.57 ± 4.70	53.83 ± 4.40	61.67 ± 6.61
	G <sub>2</sub>	58.00 ± 5.83	64.86 ± 7.71	62.00 ± 6.56
	G <sub>3</sub>	51.71 ± 4.42	55.71 ± 6.15	57.57 ± 7.19
ALP	G <sub>1</sub>	371.86 ± 53.96	399.83 ± 52.83	352.33 ± 46.60
	G <sub>2</sub>	379.43 ± 50.96	448.29 ± 74.50	444.86 ± 71.03
	G <sub>3</sub>	374.43 ± 42.73	367.86 ± 56.02	378.14 ± 46.60

Each figure represents mean (± SEM) of 7 chicks. Statistical analysis revealed non significant difference among the groups. \* = Days post-infection.

ICPI 1.5 to 2.0 and IVPI 1.5 to 2.0 (Namita *et al.*, 1995; Parimal *et al.*, 1997). According to this criteria in the present study, from four isolates, one isolate was lentogenic having 90 hours MDT, ICPI value of 0.30 and IVPI value of 0.0. Two isolates were mesogenic having 72-84 hours MDT, ICPI value of 1.00 to 1.20 and IVPI value of 0.20 to 0.30. One isolate was velogenic having MDT 54-60, ICPI 1.8 and IVPI 2.5.

There were non-significant differences in the concentrations of various serum enzymes (AST, ALT and ALP) in the pigeons infected with NDV and control. The slight increase in the concentrations of liver enzymes of infected group may be due to hepatocellular damage caused by the Paramyxovirus, as has also been reported by Tangredi (1985). Moreover, concentrations of enzymes in pigeons vaccinated, nonvaccinated and challenged with NDV remained almost the same as that of control group. This is suggestive of the fact that vaccination prevented the disease and hence no hepatocellular damage occurred.

Gross lesions observed in pigeons are in line with the findings of Banerjee *et al.* (1984), which included edema of eyelids and periocular tissue, pulmonary edema and congestion, marked splenomegaly, hepatic necrosis and scattered haemorrhages in visceral organs.

Haemorrhages and edema were observed in lungs and tracheal mucosa of infected pigeons. Deque and Estupinan (1976) also observed same lesions, while Menedez (1982) observed hyperplasia of lymphoid tissue and laryngotracheitis.

From this study it can be concluded that velogenic strain of NDV can produce haemorrhages in respiratory tract and brain and necrosis of hepatocytes of the unvaccinated pigeons. Vaccination can help in protection of pigeons against NDV.

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