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Pathogenicity of Intraocularly Administrated Newcastle Disease Virus in Pigeons

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Abstract: Newcastle disease (ND) virus was isolated and identified from the field outbreaks of ND in chickens showing typical signs and lesions. These isolates were characterized on the basis of MDT, ICPI and IVPI. ELD_{50} of velogenic isolate was $10^{-4.82}$/0.1ml. ND virus was inoculated intraocularly in pigeons for the production of disease. Velogenic strain of ND produced starvation, greenish white droppings, difficult respiration, paralysis and leg weakness. Postmortem lesions of dead birds showed hemorrhages in trachea, proventriculus, intestine and spleen. Spleanomegaly was also observed.

Key words: Pathogenicity, NDV, pigeons

Introduction

Poultry farming is one of the most profitable industries of Pakistan but the development of this enterprise is restricted by different diseases but the respiratory problems are a major hazard to the development of this industry. Among these respiratory problems Newcastle disease is causing heavy economic losses in Pakistan poultry (Anjum, 1990).

Newcastle disease is a highly contagious Paramyxovirus-1 infection of poultry and has devastating effects on economical poultry production (Alexander, 1980). Newcastle disease virus strains are known as lentogenic, mesogenic and velogenic on the basis of virulence. These strains respectively produce mild, moderate and highly acute types of infection in the birds (Calneck, 1991).

Lentogenic and mesogenic strains of NDV are responsible for the disease production even in vaccinated flocks (Akram *et al.*, 2000).ND has reported in chickens, pigeons, turkeys, partridges, pheasants, doves, sparrows, geese, starlings and other free flying birds (Vindevogel *et al.*, 1972). It has been observed that in Pakistan various wild birds like sparrows, crows and pigeons even may have access to the poultry feed and may be incriminated for many disease outbreaks in chickens.

A little work has been done on the pathogenicity of NDV in pigeons, although along with chickens, ND is a serious problem in pigeons in Pakistan (Arshad, 1984). Keeping in view the situation a project was planned to isolate NDV strain from chicken and to determine its pathogenicity in pigeons.

Materials and Methods

From ten different poultry farms of Faisalabad, trachea, lungs and spleen were collected from chickens infected with Newcastle disease and stored at −20°C until further use.

Suspected tissues were diluted in normal saline at the rate of 10 per cent and then homogenized. In the homogenate Gentamycin (1 mg ml^{-1}) was added according to Senne (1989). This homogenate was centrifuged at 1000 x g for 20 minutes at 4°C. The supernatant thus collected was stored in aliquots at −20°C till further use.

Isolation and identification of newcastle disease virus: Ten field isolates were inoculated into ten 9-days old embryonated chicken eggs via allantoic cavity route (Hitchner, 1975). The allantoic fluid of the eggs were harvested and tested for haemagglutination activity by spot agglutination test and confirmed by haemagglutination inhibition test with known standard antiserum (MAFF, 1984).

Characterization of pathogenicity of field isolates: The pathogenicity of ten field isolates of NDV were characterized on the basis of mean death time, Intracereberal pathogenicity index and Intravenous pathogenicity index (Alexander, 1989).

Determination of ELD 50: Embryo lethal $dose_{50}$ (ELD_{50}) of field isolates were determined following the method of Reed and Muench (1938) as described by Villegas and Graham (1989).

Experimental production of disease in pigeons: Twenty one pigeons of almost same age were purchased from local market of Faisalabad and divided into three equal groups A, B and C. The group C served as control.

The birds were kept under observation for two weeks in the Department of Veterinary. Pathology, University of Agriculture, Faisalabad, for any disease problem. At the end of two weeks about 5 ml of the blood of these birds was drawn for haematology and antibody titre against Newcastle disease virus. The hematological parameters included erythrocyte count (Natt and Herrick, 1952), hemoglobin concentration determination, packed cell volume (Benjamin, 1978) and leukocyte count (Natt and Herrick, 1952).

The pigeons of the group B and C were vaccinated against NDV (LaSota strain) subcutaneously. Antibody titre and haematology of these groups were determined at the end of two weeks of vaccination. Velogenic isolate was inoculated with ELD_{50}/0.1ml of NDV in the pigeons of group A and B intraocularly (Erickson *et al.*, 1980) 14 days post vaccination. Antibody titre and haematology was determined weekly after inoculation of infection. Morbidity and mortality were observed daily. The dead birds were examined for the gross lesions and the morbid tissues were preserved for histopathology (Bancroft and Stevens, 1990).

Results and Discussion

Clinical signs and lesions: In non vaccinated pigeons, challenged with NDV anorexia, greenish white droppings leg weakness and ruffled feathers were observed. The birds were paralyzed and unable to fly. The mortality rate in this group was 57.14 per cent. Postmortem lesions of the dead birds showed proventricular and tracheal hemorrhages. Lungs of these birds were also hemorrhagic. Intestinal contents were greenish white and intestine was hemorrhagic in all birds. Spleanomagaly was also observed. Banerjee *et al.* (1994) and Duque and Estupinan (1976) also observed the same signs and lesions in pigeons and parrots infected with NDV. However, Barton *et al.* (1992) reported neurological disease with encephalitis in pigeons infected with NDV.

Haematology: There was non-significant difference in erythrocyte count of pigeons before and after vaccination. Erythrocyte count (Table 1) was significantly decreased in unvaccinated pigeons as compared to the vaccinated group during first and second week post infection.

Hemoglobin concentration and packed cell volume (Table 2,3) were not effected in all groups pre and post vaccination and after infection. Total leukocyte count (Table 4) was not affected in all groups before and after vaccination. TLC was significantly increased in unvaccinated group infected with NDV as compared to the vaccinated groups during two weeks post challenge. Fredrickson and Cute (1958) also observed leukocytosis in chicks infected with NDV, while Ergene *et al.* (1988) observed leucopenia in non vaccinated chicken challenged with NDV.

Antibody titre: GMT (Table 5) was almost similar in all groups before vaccination. GMT increased in vaccinated pigeons while it

Table 1: Erythrocyte count of experimental pigeons challenged with NDV through intraocular route

	Control vaccinated	Vaccinated plus infected	Unvaccinated plus infected
Before vaccination	2.544±2.847	1.360±0.081	1.339±0.123
After vaccination	1.824±0.042	1.881±0.040	1.339±1.123
After infection (1st week)	1.608±0.063	1.230±0.111a***	0.820±0.095b***
After infection (2nd week)	1.755±0.046	1.308±0.046a***	1.100±0.032b***

Each figure represents mean (standard deviation) of seven pigeons. Data subjected to analysis of variance revealed significant difference among the groups. ***Significant difference (P<0.001) compared with the control group.

Table 2: Hemoglobin concentration of experimental pigeons challenged with NDV through intraocular route

	Control vaccinated	Vaccinated plus infected	Unvaccinated plus infected
Before vaccination	12.42±0.838	12.057±0.526	12.771±0.801
After vaccination	13.800±0.57	13.406±0.383	12.771±0.801
After infection (Ist week)	15.600±0.55	16.871±0.170	16.143±0.010
After infection (2nd week)	12.571±0.48	12.614±0.530	13.550±1.226

Each figure represents mean (standard deviation) of seven pigeons. Data subjected to analysis of variance revealed non-significant difference among the groups.

Table 3: Packed cell volume of experimental pigeons challenged with NDV through intraocular route

	Control vaccinated	Vaccinated plus infected	Unvaccinated plus infected
Before vaccination	20.826±2.43	20.571±1.902	23.429±2.760
After vaccination	42.143±1.86	42.571±1.864	23.429±2.760
After infection (Ist week)	37.714±4.57	32.286±1.380	33.286±3.638
After infection (2nd week)	29.000±1.83	31.000±1.823	30.500±3.638

Each figure represents mean (standard deviation) of seven pigeons. Data subjected to analysis of variance revealed non-significant difference among the groups.

Table 4: Total leucocytes count of experimental pigeons challenged with NDV through intraocular route

	Control vaccinated	Vaccinated plus infected	Unvaccinated plus infected
Before vaccination	40.143±2.34	39.143±3.024	38.143±2.34
After vaccination	36.143±2.48	35.286±1.797	38.143±2.34
After infection (Ist week)	33.286±1.11	33.571±1.134a	46.000±6.191b***
After infection (2nd week)	29.143±1.84	31.429±1.272a	50.250±2.062b***

Each figure represents mean (standard deviation) of seven pigeons. Data subjected to analysis of variance revealed significant difference among the groups. ***Significant difference (P<0.001) compared with the control group.

Table 5: Geometric mean titre of experimental pigeons challenged with NDV through intraocular route

	Control vaccinated	Vaccinated plus infected	Unvaccinated plus infected
Before vaccination	4	3.5	5.3
After vaccination	388	256	5.3
After infection (Ist week)	675.6	194	45.3
After infection (2nd week)	512	294.1	*64.1

Each figure represents GMT of seven pigeons * represents GMT of three pigeons.

remained same in non vaccinated group. GMT during 1st and 2nd week post infection increased in vaccinated plus infected pigeons as compared to the non vaccinated group challenged with NDV. This increase in GMT probably helped in protecting the birds against NDV. Duchatel *et al.* (1992) reported that aqueous suspension of LaSota vaccine could provide protection against ND. Chen *et al.* (1993) observed that formalin inactivated oil emulsion vaccine can produce immunity in birds against ND. While Heil (1984) observe that inactivated oil based vaccine can give protection to the pigeons for 6-7 months.

From this study, it can be concluded that pigeons are responsible for the transmission of Newcastle disease in chickens and LaSota vaccine can give protection to pigeons against Newcastle disease.

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