

<http://www.pjbs.org>

PJBS

ISSN 1028-8880

**Pakistan
Journal of Biological Sciences**

ANSI*net*

Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Pathogenicity of Field Isolates of Newcastle Disease Virus

Rizwana Akram, Farzana Rizvi, A.D. Anjum and Abeera Mubarak

Department of Veterinary Pathology, University of Agriculture, Faisalabad, Pakistan

Abstract: Six Newcastle disease virus (NDV) isolates were recovered from field outbreaks of the disease in and around Faisalabad. Viral isolates were confirmed by haemagglutination activity and haemagglutination inhibition test. On the basis of mean death time (MDT), intracerebral pathogenicity index (ICPI) and intravenous pathogenicity index (IVPI), two isolates were categorized as lento genic and four as misogynic. Non was velogenic. Haemorrhages were observed on body of dead embryos and urates were deposited in allantoic cavity of lento genic and misogynic group. In chicks of lento genic group lesions were observed in proventriculus, intestine and lungs, while haemorrhages were observed on proventriculus, intestine, lungs, brain and liver and spongiomagny of chicks of misogynic group.

Key words: Pathogenicity, Newcastle disease

Introduction

The poultry industry in Pakistan is confronted by the prevalence of various disease problems and a number of newly emerging diseases. Newcastle disease is one of the major respiratory problem (Alexander, 1980; Anjum, 1990) having devastating effects on economy of poultry production. It is responsible for high morbidity and mortality in susceptible birds of all age groups (Calnek, 1991) and its outbreaks are reported even in vaccinated flocks (Siddique *et al.*, 1986). Keeping in view the present situation of ND, a project was designed to isolate the ND virus from various field outbreaks and to characterize their pathogenicity.

Materials and Methods

Morbid organs (lungs, trachea and spleen) were collected from suspected field outbreaks of Newcastle disease. Tissues were homogenized in normal saline (1:10) and gentamicin was added at the rate of 1 mg/ml (Senne, 1989). The homogenate was centrifuged at 1000 × g for 20 minutes at 4°C and supernant was stored in aliquates at -20°C until further processed. Suspected homogenate was inoculated into 9-11 days-old embryonated eggs using allantoic cavity route (Hitchner, 1980). Amino-allantoic fluid of dead embryos was harvested and tested for presence of virus by haemagglutination test (Allan *et al.*, 1978) and confirmed by hemagglutination inhibition test (MAFF/ADAS, 1984).

Isolates were further processed for pathogenicity test following the criteria described by Alexander (1989). The viral isolates were characterized on the basis of mean death time (MDT) in 10-days old chicken embryos, intracerebral pathogenicity index (ICPI) in day-old chick and intravenous pathogenicity index (IVPI) in six weeks old birds.

Results

Haemagglutination and haemagglutination inhibition test: Haemagglutination (HA) titre of the six isolates was 1:128, 1:64, 1:16, 1:32, 1:256 and 1:256. These isolates were inhibited by specific hyper immune sera against Newcastle disease virus.

Mean death time of chick embryos: Mean death time (MDT) of various isolates is shown in Table 1. MDT of two isolates was 136 and 143 hours with minimum lethal dose (MLD) dilution 10^{-3} and these isolates were categorized as lento genic. Haemagglutination titre of these two isolates was 1:128 and

1:64. MDT of one isolate was 63 hours with MLD dilution 10^{-3} and it was categorized as misogynic. Three isolates were misogynic having MDT of 63, 68 and 64 hours, respectively with MLD dilution 10^{-4} . Haemagglutination activity in amino allantoic fluid from dead embryos of the four misogynic isolates was 1:16, 1:64, 1:128 and 1:512. All viral isolates were inhibited by known hyper immune sera against NDV.

Intra cerebral Pathogenicity index (ICPI): Intra cerebral pathogenicity index (ICPI) of field isolates is shown in Table 2. With the two lento genic isolates morbidity was observed from 3rd day post-inoculation. Mortality started from 4th and 6th day post-inoculation. ICPI value was 0.46 and 0.4. With the misogynic isolates morbidity and mortality were observed from 1st and 2nd day post-inoculation. ICPI value of these isolates was 1.52, 1.49, 1.34 and 1.72.

Intravenous Pathogenicity index (IVPI): Intravenous pathogenicity index (IVPI) of field isolates is shown in Table 3. In chicks inoculated with lento genic isolates morbidity and mortality started from 4th to 7th day post-inoculation. The IVPI values were 0.50 and 0.25. In chicks inoculated with misogynic isolates morbidity started 2nd or 3rd day post-inoculation and mortality was observed from 2nd or 3rd day post-inoculation. The IVPI values of these isolates were 0.76, 0.89, 0.82 and 0.99.

Pathological changes of patbotypes of ND: Haemorrhages were observed on occipital, cervical regions, trunk and limbs of ten dead embryos of lento genic group. Urates were deposited in the allantoic fluid of eight embryonating eggs. In ICPI test haemorrhagic proventriculus and enteritis was observed in four chicks and lungs were pneumonic in three chicks. In chicks of IVPI group proventricular haemorrhages were recorded in three chicks and enteritis was present in two chick only. Haemorrhages were observed on occipital, cervical region, trunk and limbs of nearly all the dead embryos of misogynic group. In chicks of ICPI test proventricular haemorrhages were observed in seven chicks, enteritis was present in four chicks, lungs were pneumonic in five chicks. Liver and brain were congested in three and six chicks, respectively and in two chicks spleen was enlarged. In six chicks of IVPI test proventricular haemorrhages and enteritis was observed. Spongiomagny was present in five chicks and lungs were pneumonic in three chicks of misogynic group.

Akram *et al.*: Pathogenicity of field isolates of Newcastle disease virus

Table 1: Mean death time (MDT) of chicken embryos inoculated with field isolates of Newcastle disease virus (NDV)

Isolate	Hours post-inoculation													MDT
	24	36	48	60	72	84	96	108	120	132	144	156	168	
Al	-	-	2	5	5	-	-	-	-	-	-	-	-	63
B1	-	-	-	-	-	-	-	-	2	4	6	-	-	136
C1	1	-	-	-	-	-	-	-	-	4	4	5	-	143
D2	-	-	7	5	-	-	-	-	-	-	-	-	-	63
E2	1	-	3	3	5	-	-	-	-	-	-	-	-	68
F2	-	-	2	7	3	-	-	-	-	-	-	-	-	64

Each figure represents number of embryos died out of 12 embryos inoculated with 0.1 ml doses of field isolate of field NOV¹ MLD 10⁻³ 2MLD 10⁻⁴

Table 2: Intra-cerebral pathogenicity index (ICPI) of field isolates of Newcastle disease virus (NDV) in day old chicks

Isolate	Days post-inoculation								Mortality	Survival	ICPI
	1	2	3	4	5	6	7	8			
A	-	1	5	4	-	-	-	-	10	0	1.52
B	-	-	-	1	6	1	1	-	9	1	0.46
C	-	-	-	-	1	2	2	-	5	5	0.40
D	4	-	-	2	4	-	-	-	10	0	1.49
E	-	1	2	4	1	1	1	-	10	0	1.34
F	-	7	3	-	-	-	-	-	10	0	1.70
Cont	-	-	-	-	-	-	-	-	0	10	0.00

Table 3: Intravenous pathogenicity index (IVPI) of field isolates of New castle disease virus (NOV) in 6-weeks-old broilers

Isolate	Days post-inoculation										Mortality	Survival	ICPI
	1	2	3	4	5	6	7	8	9	10			
A	-	-	1	-	1	-	-	1	-	-	4	6	0.76
B	-	-	-	-	1	-	1	-	-	-	3	7	0.50
C	-	-	-	-	-	1	-	1	-	-	2	8	0.25
D	-	-	2	1	-	1	-	-	-	-	4	6	0.89
E	-	1	2	-	-	-	-	-	1	-	4	6	0.82
F	-	1	2	1	-	-	1	1	-	-	5	5	0.99
Cant	-	-	1	-	-	-	-	-	-	-	0	10	0.00

Ten, 6-weeks-old, broilers inoculated intravenously with 0.1 ml of the field isolate of NDV. Figure indicate number of birds died/survived

Discussion

Newcastle disease (ND) is one of the major and continuing respiratory problems in poultry in Pakistan (Bhatti, 1989; Anjum, 1990). Outbreaks of ND are reported even in vaccinated in flocks (Siddique *et al.*, 1986). This study confirms severe outbreaks of ND causing high mortality in vaccinated flocks.

A widely accepted protocol for classifying field isolates is based on MDT, ICPI and IVPI. According to this criteria lento genic strains possess MDT >90 hours, ICPI value <0.5 and IVPI <0.5 (Samuel *et al.*, 1979; Azam *et al.*, 1984; Shirai *et al.*, 1986; Khalafalla, 1994; King, 1996) misogynic strains possess MDT 60-90 h, ICPI value 1.0 to 1.5 and IVPI value 1 to 1.5 (Afzal, 1990; Reddy *et al.*, 1993; King, 1996; Parimal *et al.*, 1997; Shirai *et al.*, 1986) and velogenic strains possess MDT <60 h, ICPI 1.5 to 2.0 and IVPI 1.5 to 2.0 (Namita *et al.*, 1995; Parimal *et al.*, 1997). In the present study the two isolates had 136 and 143 hours MDT, 0.4 and 1.49 (CPI and 0.25 and 0.52 ICP1, thus they were categorized as lento genic. Two strains had 63 and 68 hours MDT, 1.49 and 1.34 ICPI, 0.89 and 0.82 IVPI and thus were categorized as misogynic. Two of the six field isolates had 63 and 64 hours MDT, 1.52 and 1.72 ICPI and 0.76 and 0.99 IVPI. The ICPI values in these two isolates suggest velogenic potential (Table 2). However based on overall analysis, these isolates could not qualify for velogenic character and therefore, were considered misogynic. Other workers has also reported high ICPI values in misogynic strains (Azam *et al.*, 1984; Afzal, 1990).

Haemorrhages were observed on body of dead embryos and urates were deposited in allantoic cavity of lento genic and misogynic group. Such lesions were also observed by

Reddy *et al.* (1993), Afzal (1990) and Namita *et al.* (1995). But they did not find urates deposition in allantoic cavity. In chicks of ICPI and IVPI test of lento genic group proventricular haemorrhages, enteritis and pneumonic lungs were observed. On all the chicks of ICPI and IVPI test proventricular haemorrhages, enteritis and pneumonic lungs, congestion of brain and liver and splenomagly was observed in lento genic group. It indicates that misogynic strain is more pathogenic and affects all the visceral organs of the body as compared to lento genic isolates (Calnek, 1991).

References

Afzal, S., 1990. Isolation and characterization of NDV strains in poultry. M.Sc. Thesis, University of Veterinary and Animal Sciences, Lahore, Pakistan.
 Alexander, J.D., 1980. Avian paramyxoviruses: A review. Vet. Bull., 50: 737-751.
 Alexander, J.D., 1989. A Laboratory Manual for the Isolation and Identification of Avian Pathogens. 3rd Edn., AAAP, Pennsylvania.
 Allan, W.H., J.E. Lancaster and B. Toth, 1978. Newcastle Disease Vaccines: Their Production and Use. FAO, Rome, ISBN: 9789251004845, Pages: 163.
 Anjum, A.D., 1990. Weather and disease: 1. Prevalence of poultry diseases in and around Faisalabad and their relationship to weather. Pak. Vet. J., 10: 42-45.
 Azam, M., M. Ashfaq, M. Irfan and M.D. Ahmad, 1984. Isolation and typing of Newcastle disease virus in and around Faisalabad. Pak. Vet. J., 4: 207-209.

Akram *et al.*: Pathogenicity of field isolates of Newcastle disease virus

- Bhatti, B.M., 1989. Incidence of Poultry diseases and their importance in poultry production in Pakistan. *Pak. Vet. J.*, 9: 194-197.
- Calnek, B.W., 1991. *Diseases of Poultry*. 9th Edn., Iowa State University Press, Iowa, USA.
- Hitchner, S.B., 1980. Virus Propagation in Embryonating Eggs. In: *Isolation and Identification of Avian Pathogens*, Hitchner, S.B. (Ed.). AAAP, College Sta, Texas.
- Khalafalla, A.I., 1994. Isolation and characterization of lentogenic Newcastle disease viruses from apparently healthy chickens in the Sudan. *Bull. Anim. Health Prod. Africa*, 42: 179-182.
- King, D.J., 1996. Avian paramyxovirus type 1 from pigeons: isolate characterization and pathogenicity after chicken or embryo passage of selected isolates. *Avian Dis.*, 40: 707-714.
- MAFF/ADAS., 1984. *Manual of Veterinary Investigation Laboratory Techniques*. 3rd Edn., ADAS Ministry of Agriculture, London.
- Namita, P., V.D.P. Rao and S.K. Gorg, 1995. Characterization of field isolates of Newcastle disease virus. *Indian J. Virol.*, 14: 39-41.
- Parimal, R., A.L. Venugopalan and P. Roy, 1997. Characterization of Newcastle disease viruses obtained from outbreaks in Tamil Nadu. *Int. J. Anim. Sci.*, 12: 115-117.
- Reddy, Y.K., A. Kotaeswaran and N. Dorairajan, 1993. Characterization of Ranikhat disease virus isolates from Namakkal. *Indian Vet. J.*, 70: 1097-1100.
- Samuel, R., V.D. Padmanaban and Nachimuthu, 1979. General characterization of LDF-66 strain of Newcastle disease virus in comparison to F and K strains. *Indian Vet. J.*, 56: 346-355.
- Senne, A.D., 1989. *A Laboratory Manual for the Isolation and Identification of Avian Pathogens*. 3rd Edn., American Association of Avian Pathologists, USA.
- Shirai, J., K. Tsukamoto and H. Hihara, 1986. Newcastle disease viruses isolated from racing pigeons in Japan. *Jap. J. Vet. Sci.*, 48: 1091-1095.
- Siddique, M., M.A. Sabri and M. Zargham, 1986. Outbreaks of ND in vaccinated chicken flocks in and around Faisalabad. *Pak. Vet. J.*, 6: 41-45.