

## ISOLATION AND CHARACTERIZATION OF NEWCASTLE DISEASE VIRUS FROM OSTRICHES (*STRUTHIO CAMELUS*) IN EGYPT

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### ABSTRACT

Newcastle disease (ND) is a highly contagious disease of poultry that causes high mortality in poultry of all ages as well as in young ostriches. Therefore, this study was designed in order to explore ND in ostriches in Egypt. Samples from died ostriches were collected from different farms in five localities (AL-Ismailia, AL-Behera, AL-Sharkia, Helwan and Cairo) and were examined by inoculation in SPF embryonated chicken eggs. Newcastle disease virus (NDV) was isolated from kidney samples collected from AL-Ismailia farm (13/23). Negative NDV isolation was recorded from other farms. Interestingly, Intracerebral pathogenicity index (ICPI) of NDV isolate was 0.8 that indicated virulence of this virus while the isolate had the lentogenic motif 111G-R-N-Q-G-R-L117 at the cleavage site of fusion protein. To the best of our knowledge, this study seems to be the first report of NDV isolation from ostriches in Egypt.

**KEY WORDS:** F protein Cleavage site, ICPI, kidney, Newcastle disease virus (NDV), Ostrich.

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### 1. INTRODUCTION

Newcastle disease virus is one of the most important infectious agent influence poultry production [27] that belongs to the genus Rubulavirus of the family Paramyxoviridae [2, 3, 18, 22]. Newcastle disease virus (NDV) occurs worldwide and has a considerable economic impact on the world poultry industry, ranging from losses due to disease and the expense of vaccination to the significant cost of diagnostic laboratory investigations [17]. The first report of Newcastle disease (ND) in ostriches was in zoo birds in Germany in the 1950 [21]. Many reports about ND outbreaks showed significant losses in ostrich's farms in many localities worldwide for examples in Zimbabwe [5], South Africa [4] and Italy [6].

Although, ND is endemic in Egypt there is no any report of ND disease in ostriches

and this study seems to be the first report of NDV isolation from ostriches in Egypt.

### 2. MATERIALS AND METHODS

#### 2.1. Sample collection

Tissue samples were collected separately from different organs including trachea, lungs, intestine, spleen, kidney, brain, liver and heart from a total of 92 freshly dead birds (32 from AL-Ismailia, 6 from AL-Behera, 8 from AL-Sharkia, 14 from Helwan and 32 from Cairo). All samples were submitted to Central Laboratory for Veterinary Quality Control on Poultry Production (CLQP), Animal Health Research Institute for viral isolation and identification.

#### 2.2. Virus isolation

Samples were homogenized individually to give approximately 10% (w/v)

suspension in PBS pH 7.2 containing 2000 units/ml penicillin, 2 mg /ml Streptomycin, 50 µg/ml gentamycin and 1000 IU/ml mycostatin. The homogenized samples were centrifuged at 2500 rpm/10 min. and then filtered through a 0.2 µm filter membrane, the supernates was inoculated at 0.2ml via the allantoic sac of 9-11 day-old SPF eggs. Allantoic Fluids from inoculated eggs were harvested four days Post inoculation and subsequently tested for hemagglutination (HA) using 0.1% chicken erythrocyte OIE, [20].

### 2.3. Identification of haemagglutinating agents

Haemagglutinating agents were identified by haemagglutination inhibition (HI) test; using reference antisera (NDV.VLDIA053, HAR-NDL–HI serum. NDV strain Lasota. Lot no. 07142-200907) supplied from local agency of (GD Lab., Holland) which is the regional reference OIE lab; the procedure was carried according to OIE, [20].

### 2.4. Pathogenicity test

The isolate was tested for virulence by intracerebral pathogenicity index test in day-old 10 SPF chicks OIE [20]

### 2.5. RNA isolation and partial F gene amplification

Total RNA was extracted from harvested allantoic fluids using BIOZOL® Total RNA Extraction Reagent (Bioflux, Japan). Extracted RNA was subjected to RT-PCR using primers targeting the fusion protein gene of NDV. The RNA was amplified using internal primers specific to the F gene of NDV [19]. PCR product was electrophoresed on 1.2% agarose gel. The NDV positive PCR products was purified using a PCR purification kit (Qiagen, Valencia,CA) and then sequenced in both directions at the Macrogen Inc., Korea.

## 3. RESULTS

### 3.1. Clinical signs

During April 2010 a commercial ostrich farm located at AL-Ismailia province showed signs of disease include depression, inappetance, drowsiness, greenish diarrhea, respiratory distress, high mortalities especially in young birds (6 days-1 month) in addition to drop of egg production in adults. No vaccination programs were adopted for ND before developing the fore mentioned clinical signs.

### 3.2. Gross findings.

Post mortem examination revealed facial hemorrhages, edema of neck and enlargement of abdomen. These lesions were seen externally. Internally the lesions were recorded in respiratory tract as redness in tracheal wall and lungs and thickness in air sacs. The gastrointestinal lesions revealed characteristic lesions of hemorrhagic spots in proventriculus gland (fig. 1), haemorrhages in gizzard (fig. 2) and ulceration in junction between gizzard and proventriculus. Interestingly, the renal lesions were the most striking lesions and were seen in the form of sever enlargement in kidney (fig. 3) scattered areas of paleness mixed with areas of redness in kidney tissue (fig. 4) and the two ureters filled with urates.

### 3.3. Virus isolation and identification

Surviving embryos from chilled eggs exhibiting marked congestion and urates deposition in chorioallantoic membranes and were identified by hemagglutination (HA) activity in allantoic fluid harvested as log 29. Positive HA allantoic fluids were identified as avian paramyxovirus-1 (APMV-1). In AL-Ismailia farm positive NDV isolates were recovered only from kidneys with percentage of 40.6% (13/32) with mean HI titers (log 2) of 9 while negative isolation were recorded from other organs (Table 1). NDV isolation was negative from samples collected from other farms.

### 3.4. Pathogenicity of Newcastle diseases virus isolate recovered from ostrich

Based on intracerebral pathogenicity index (ICPI) isolated strain was inoculated through intracerebral route in day-old SPF 10 chicks. Mortality started from third day post-inoculation. The ICPI was 0.8 (Table 2) indicating the virulence of the isolate OIE [20]. All dead chicks showed severe deposited urates in both ureters (fig. 5). Based on Sequence of the F protein cleavage site Deduced amino acid sequence of the cleavage site of fusion protein (1-445) revealed that the isolate has avirulent sequence motifs of 111GRNQGRL117 at the cleavage site.

Table 1 Prevalence of identified NDV isolates recovered from different examined organs collected from freshly dead ostriches from AL-Ismailia farm.

Examined organs	No of samples	No. of positive	%* of positive
Trachea	32	0	0%
Lung	32	0	0%
Heart	32	0	0%
Liver	32	0	0%
Intestine	32	0	0%
Kidney	32	13	40.6%
Spleen	32	0	0%
Brain	32	0	0%

\*%of positive=percentage of positive NDV isolates /no of samples /organ.



Fig. 1 Six days old naturally infected ostrich with NDV showed hemorrhagic spots in proventriculus gland.



Fig. 2 Six days old naturally infected ostrich with NDV showed hemorrhage in gizzard.



Fig. 3 Six days old naturally infected ostrich with NDV showed sever enlargement of kidney.

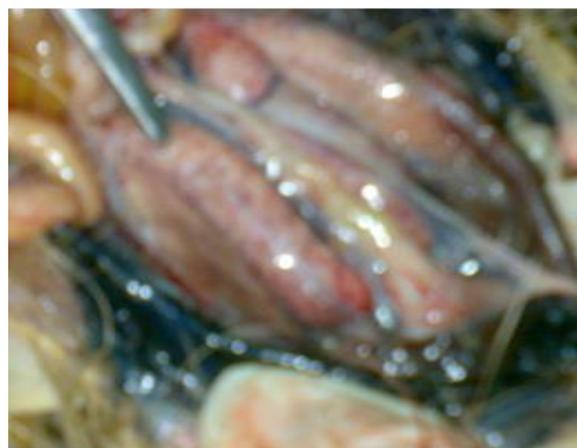


Fig. 4 Thirteen days old naturally infected ostrich with NDV showed scattered areas of paleness mixed with areas of redness in renal tissue.



Fig. 5 Four days old SPF chick showed redness of kidney with sever urate deposition in two ureters after intracerebral inoculation with 50 $\mu$ l of 104.3 ELD50/0.1 $\mu$ l of isolated NDV.

#### 4. DISCUSSION

Several outbreaks of ND were reported in different countries raised ostriches causing losses which have worse impact on their international trade and its products [14, 26]. Current study reported and characterized Newcastle disease virus which was associated with high morbidity and mortalities in an ostrich farm in Egypt. Our findings revealed that most of young affected bird suffered from green diarrhea and/or respiratory distress accompanied with poor appetite, loss of body weights and depression. Similar signs were described in ostriches infected with NDV [10, 23]. Although the source of infection was unknown but we suspected to be the wild birds which attracted to ostrich feed and water, as ostrich reared in open camps. This observation agrees with Deeming [8]. At necropsy, the hemorrhagic lesions in respiratory and intestinal tract are suggestive for NDV infection in ostriches

[10, 23]. Interestingly, the renal lesions were sever and predominant. The severity of renal lesion suggested the extensive virus replication in the kidney and selectivity of virus to kidney tissue. The obtained PM findings disagreed with former authors [4, 15] stated that ostriches which have died from Newcastle virus disease do not reveal any typical pathological and histopathological lesions. Virus isolation was attempted on SPF embryonated chicken eggs which represent an extremely sensitive and convenient vehicle for the propagation of NDV [1, 20]. Many authors isolated NDV from different organs from ostriches as intestine [11, 17, 24]; lung and brain [10]; spleen and liver [13] or from brain only [12]. In our study NDV was isolated from kidney only with percentage of 40.6% (13/32). The high prevalence rate from kidney suggested the high tropism of the virus to kidney tissue. Similar findings were obtained by Jørgensen *et al.* [17] who isolated NDV from one pool of kidneys tissues collected from four ostriches died within the first 23 days in quarantine in Denmark.

The current definition of ND of the World Organization for Animal Health (OIE) relies on ICPI value determined in day old chicken or on the amino acid sequence at the fusion protein cleavage site, these are the two parameters of OIE to determine the virulence and used to differentiate between virulent isolates (notifiable disease) and avirulent isolates (not notifiable).

Table 2 Intracerebral Pathogenicity Index (ICPI) of field isolate of NDV isolated from ostrich in ten birds one day old SPF chicks.

Clinical view	Days post inoculation									total	Score	sum
	1	2	3	4	5	6	7	8				
Normal	9	8	6	0	0	0	0	0	0	23	0	0
Sick	1	2	3	6	2	0	0	0	0	14	1	14
Dead	0	0	1	3	4	2	0	0	0	10	2	20
Total recording										47	3	34

Calculated as weight mean/ number of observation (47)

$$\text{ICPI} = \frac{\text{Mean weight}}{\text{Number of observations}} = \frac{3 + 34}{47} = 0.8$$

An isolate with ICPI values equal to or greater than 0.7 or having a multiple basic amino acids motif at the F protein cleavage site is classified as virulent and its presence should be reportable OIE [20]. Interestingly, in present study, although the isolate had the lentogenic motif 111G-R-N-Q-G-R-L117 at the cleavage site of fusion protein the ICPI in one day SPF chicks gives index of 0.8. Our findings were supported by [25] who revealed that, three cases of Newcastle disease virus (NDV) found in nature had the lentogenic motif 112G-R-Q-G-R-L117 in their fusion protein cleavage sites however, (ICPI) showed that these NDV isolates were virulent. The author noted that these viruses had significant genetic variations in the hemagglutinin-neuraminidase gene. However, viruses with low levels of virulence had the sequence 112G/E-K/R-Q-G/E-R116 at the C terminus of the F2 protein and leucine at residue 117 at the N-terminus of the F1 protein [7 and 16]. Our results showed that the recovered isolate from ostrich revealed the presence of arginine (R) at residue 112 at the C terminus of the f2 protein instead of 112G/E may explain the increase in the virulence as mentioned by De Leeuw et al. [9] who confirmed that the presence of one or both arginines (R) at positions 112 and 115 and/or the phenylalanine at residue 117 are necessary for efficient cleavage of the F0 protein and result in an increase in virulence. Also to the best of our knowledge with the already available data on the amino acid sequences of the F0 cleavage site, the presence of asparagine (N), polar amino acid, at residue 113 at the C terminus of the F2 protein is the first record in the cleavage site motifs of our NDV isolate. The two later amino acids substitutions may be attributed to increase the virulence of the isolate. Regarding with this variant strain isolated from ostriches permit us to suppose that the virus might be circulating in local poultry farms and overcome vaccination barrier causing new outbreaks.

## 5. CONCLUSION

Newcastle disease virus was isolated from ostriches from kidney samples from AL-Ismailia farm. The isolate had the lentogenic motif 111G-R-N-Q-G-R-L117 at the cleavage site of fusion protein.

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