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Characterization and isolation and molecular study of newcastle virus (achieved in 2017 in Iraq)

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Abstract

Objective: This research was achieved to update the available data about Newcastle in Iraq, the research was done on chickens and pigeon. Samples were gathered from farms in Baghdad, and were collected from certain organs precisely (lungs, brain and trachea), these organs were suspended in a saline buffer solution (pH 7.2) and then centrifuged and incubated for 10 days and chilled for one day, and then tested to find the molecular configuration which were calculated with DNA STAR software and analyzing with PHYML software. The mean death time for chicken was 35 hours while it was 48 for pigeon, the limited F gene frequency to chickens shows 88% to Newcastle virus chicken china / Jilin / YS03, sequencing analysis program shows identity of 96% of the Russian pigeon, and shows individuality of 89% to china chickens, whereas Iraqi chickens show individuality of 85%. The differences in results could be related to differences in antigenic and genetic among the strains vaccine, and the flow strains fields.

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Introduction

Newcastle virus consider as one of important virus that attack poultry and lead to death and cause a big wastage economically in money and in animal wealth (Jalob *et al.,* 2011).

Genetically, L and F genes which has a certain amino acid sequences considered as class 2 and class one viruses consequently, these viruses considered as a main viruses that cause Newcastle Disease to all poultries like chicken, pigeon and other birds as well (Mayo 2002; Miller *et al.,* 2009).

Pathogenically, this happened due to the formation of a certain protein F gene whose facility increases in the presence of certain amino acids like 112 R/K-R-Q-K/R-R 116 and 112 G/E-K/R-Q-G/E 116 and L (Leucine) at residue 117 Kim *et al.,* 2008), as well as At C terminus of F2 protein and F (phenylalanine) at residue 117 which is the N-terminus of F1 protein (Alexander 1989).

This study was achieved to calculate and updated data regarding Newcastle disease which was done since 1968 and try to figure out the most recent immunize vaccine.

Materials and methods

Isolation of newcastle virus

Gathering samples was done during the year of 2017 from poultries from farms that were surrounded Baghdad, these samples were taken from (Lungs, Brain and trachea) of chickens and pigeons and were subjected to a very standard assay as depicted in 2013 by (Office of International Epizootes 2013) by which the samples were suspended in a saline solution with pH around 7.2 and inoculated for 10 days, the eggs were examined every one day, undesirable eggs (with death embryo) were isolated and removed while the other samples were chilled at 4°C for one night. Hemagglutination assay (HA) and the hemagglutination inhibition assay (Hi) assays were done according to a certain protocol ((Office of International Epizootes 2013)) by which (La Sota Strains antigen were used as positive control. The average time of death (MDT) to isolated samples were carried out according to the protocol of Alexander (Alexander 2000.).

Molecular classification

Extraction of RNA viruses was done according to the extraction kit to the spin virus genes (DNA/RNA) was (Intron. Biotechnology, Korea). Two RT-PCR assess aiming the fusion genes to classify the types of NDVs straining was carried out (Nanthakumar *et al.,* 2000; Gould *et al.,* 2011).

Samples sequencing

Purification to Rt-PCR product was achieved by ABI Prsm 310 (a gene analyzer from Jordan, Jovac).Nucleotides resemblance percent was determined by DNA STAR software.

Results

It was found 5° -GGAGGATGTTGGCAGCATT-3° and NDVD 5° GTCAACATATACACCTCATC- 3° were used to give RT-PCR product of 318 bp (Stauberet *et al.,* 1995l) (Fig 1).

Table 1. MDT and ICPI as well as related virus.

ND Iraqi isolate	ICPI value	MTD value	Virus related on Gene bank	Percentage of identity	Accession no.
Chicken/ Iraq /2/2017	1.9	35 hours	Chicken China jilin/yso3/2017	96%	Ku200238.1
Chicken /Iraqi/3 /2017	1.8	36 hours	Chicken Iran HG 2017	88%	Jx131352.1
Chicken/ Iraq/4/ 2017	1.8	36 hours	Chicken Chinese SD4 / 2017	85%	HM748947.1
Pigeon /Iraqi /1/ 2017	1.4	48 hours	Pigeon Russia / Moscow 407/04	85%	Jf824018.1

The isolation of haemagglutinating was found from far poultry farmhouses outbreak and were identified as 1.9, 1.8, 1.8 and 1.4 correspondingly whereas the average time to death (MDT) as depicted in (Table 1). the sequence of F gene was similar in pigeons and chickens, and range between 86-82% , the range was figured out with DNA STAR software, clear in Figure 2.

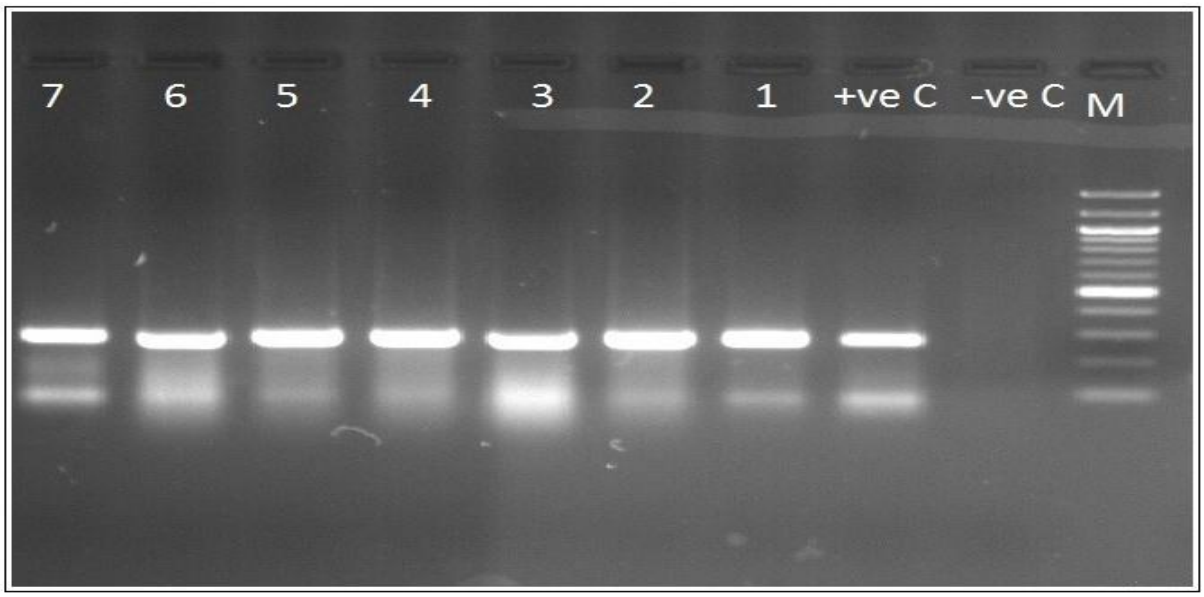

Fig. 1. PCR amplification of F protein gene (318bp) of NDV M ladder of 100 bp. Negative control, positive control well 1 - 6 NDV chicken and well 7 NDV pigeon.

The program of sequence analysis displays individuality of 96% of the Russian pigeon, it shows similarity of 89% to china chickens, whereas Iraqi chicken showed 85% similarity.

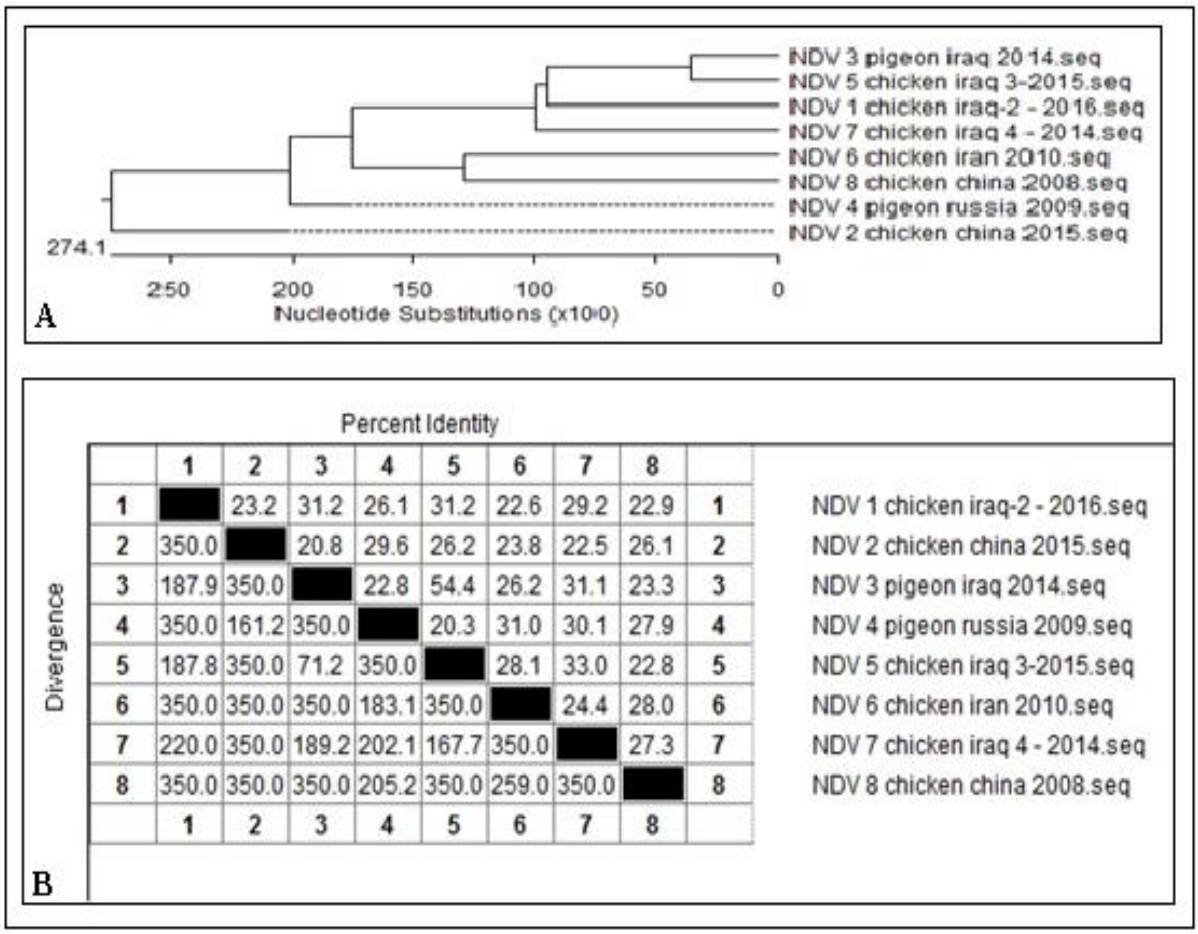

Fig. 2. Nucleotide percentage comparing to the 4 most closely related Iraqi NDV isolates was calculated using DNA STAR software.

Discussion

Table 1 indicates the results was gathered to compare the MDT, ICPI, MDT as well as all the correlated viruses on gene bank.

This result indicates, separate pigeons were displayed individuality among straining in Russian which was (96%) besides China which shows (85%) which indicates the concern of pigeons isolates in transference the infects viruses and microbes to the farm were chickens grow up (poultry), this process cannot be completed because of facilities lacking, on the other hand there is a possibility to substitute ICPI.

If it cannot be demonstrated it is required to achieve the identification and distinguishing residues of amino acids from each other's (precisely among 116 and 113), this can be processed throughout ICPI test (Bogoyavlenskiy *et al.,* 2009).

Vaccine accompanied B1 as binder and La Sota strains be appropriate and genetically belong to the similar type II as well as type I) both are differing than NDVs which can be the reason to cause the viruses attacks (Hassan *et al.,* 2008; Hu *et al.,* 2009). This conclusions can be related and accompanying to antigens and genes deviation among inoculation strains and the mingling field strains.

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