Characterization of Recent Isolates of Newcastle Disease virus

Amani A. Saleh [*]; **Rola R. Ali**; **Fawzy, M.** [**] **; Eltarabily, M. M.** [**]

[*]*Department of NDV, Veterinary serum vaccine research institute (VSVRI), Abassia, Egypt* [**] *Virology Department, Faculty of Veterinary Medicine, Suez Canal University, Ismailia, Egypt*

Abstract:

Fifty five tissue samples were collected aseptically from 5 flocks suspected to be naturally infected with NDV in EL- Ismalia, Al-sharkia, El- Gharbia, kafr-Elshiekh & El-Geza and subjected to trials of virus isolation. Nine field samples from 2 flocks (Kafr-Elshiekh and Giza) were positive for virus isolation. Inoculation of SPF chicken eggs induced death of the embryo within 48-72 hours post inoculation. HA test applied on the obtained allantoic fluids showed positive results suggesting that inoculated samples contained NDV. It was noticed that highest infectivity titer of the virus was 9.5 $\log^{10}$ EID_{50}/ml by the 5^{th} passage in SPF chicken eggs. Cell culture passage of the obtained virus isolate from the allantoic fluid through ten consecutive passages in BHK cell line**.** the CPE developed at the 3^{rd} passage within 24-48 hours post cell infection where polykaryocytosis, cell syncitia, rounding of cells. Tissue cultured fluids (TCF) were found to be direct HA possitive. The virus titer was 3 log10 $TCID_{50}$ /ml at the 3^{rd} passage and reach to 7 log10 $TCID_{50}$ /ml by the 6^{th} passage. At the 9^{th} passage, the virus titer was reached to 8.5 log10 $TCID_{50}$ /ml. Identification of the obtained virus isolate was confirmed by VNT using specific anti-NDV in BHK cell. The isolate of NDV with HA-positive allantoic fluids of El-Giza flockes were virulent NDVand the F gene give expected size of 724 bp. The isolate of this study also compared against the reference and vaccinal strains from gene bank and the results revealed that the isolate NDV/chicken/Egypt/Giza/2015 was velogenic type resembling the genotype VIId strains. F gene analysis of the NDV/chicken/Egypt/Giza/2015 revealed that the most nucleotides identity from gene bank with this isolate were NDV/Chicken/GC/IS/2010/1224 and NDV/buzzard/Israel/714/2011 with a nucleotide identity of 92.7%. It was also closely related to Chicken/China/SDWF07/2011; Chicken/China/Shandong/01/2012 and NDV/Turkey/Israel/111/2011 with a nucleotide identity of 92.5%. Otherwise nucleotides identity between the selected NDV field isolate in this study and NDV/Lasota was 72.4%. Phylogenetic analysis of NDV/chicken/Egypt/Giza/2015 with other reference and vaccinal strains of NDV revealed that this isolate was related to

NDV/B7/RLQP/CH/EG/12 and present in the same group with NDV/F388/RLQP/CH/EG/14 and NDV/F460/RLQP/CH/EG/13.
Regarding evaluation of the prepared experimental vaccines, it was found that the NDV infected tissue culture fluid, were completely inactivated with 3% BEI at 30 °C for 18 hrs post treatments showing no pathological changes in the ECE and no CPE in the BHK cell culture. Humoral immune response of chickens to the prepared vaccines showed that vaccinated chicks with NDV-Montanide ISA 70 adjuvanted vaccine exhibited antibody titer of 8.3 log2 three weeks post vaccination recording the higher titer of (10.6 log2) at the 6^{th} week post vaccination with and 3^{rd} week post challenge test. The high antibodies values were persist till the 12^{th} weeks post NDV-Montanide ISA 70 vaccination followed by gradual decrease till the end of the experiment (24^{th} weeks). On the other hand, vaccination of chicks with inactivated NDV isolate without adjuvant failed to induce protective HI antibodies (3 log2) all over the experiment. Challenge test showed that chickens vaccinated with ISA-70 adjuvant vaccine showed no sign of disease and no mortalities while the antigen without adjuvant could not provide any protective efficiency to SPF chickens.

Introduction:

Newcastle disease (ND), is important disease that infect different types of birds and cause sever economic losses.it caused by APMV-1, ssRNA virus, family *Paramyxoviridae*. ***(Alexander and Senne, 2008).*** NDV strains were classified into the highly pathogenic (velogenic), intermediate or moderately pathogenic (mesogenic), and lowly pathogenic (lentogenic) categories. Some lentogenic strains of NDV are avirulent, whereas velogenic forms were further classified as viscerotropic velogenic (VV) and neurotropic velogenic (NV) types based on clinical manifestation and lesions ***(Alexander 1997).*** The primary molecular determinant for NDV pathogenicity is the amino acids of the F protein cleavage site ***(Gotoh et al., 1992).*** This difference in pathogenicity is primarily due to differences in the cleavage site within the F protein. This protein is synthesized as a precursor (F_0) in non-functional state, which then is cleaved by host proteases into two functionally active polypeptides (F_1 and F_2). All the mesogenic and velogenic strains of NDV carry an amino acid sequence of ^{112}R/K-R-Q-R/K-R-F^{117} within the F protein whereas lentogenic viruses have ^{112} G/E-K/R-Q-G/E-R-L^{117} ***(Collins et al.,1993).***

The main objective of this study is:

1- Newly NDV isolates were characterized this isolates can cause

outbreaks or frequently circulate worldwide .

2- Sequencing of F gene of isolated virus & compare it with reference viruses in gene bank.

3- Qualification of prepared inactivated vaccine from the local circulating virulent NDV, efficient in protecting the vaccinated birds.

Material and Methods:

Samples

Fifty five samples (proventriculus, lung, trachea, kidneys, cecal tonsils, spleen, brain, and liver tissues) were collected aseptically from broilers chickens flocks suspected to be naturally infected with NDV in different areas in Egypt. Five flocks represented Egyptian governorates (EL- Ismalia, Al-sharkia, El-Gharbia, kafr-Elshiekh & El-Geza.)

Diseased birds were suffered from watery green diarrhea, feed intake, ruffled feathers, nervous symptoms, incoordination (e.g. backward movement of the bird & falling to one side or the other), with sudden deaths. The morbidity rate was ranged between 10 – 15% while the mortality rate was ranged between 20- 30%.

Isolation and Propagation of NDV:

In SPF eggs:

Isolation of virus was done according to ***(Terregino and Capua, 2009).*** 9 -11 day old SPF chicken eggs were inoculated with 0.1 ml / egg of tissue homogenate supernatant fluid via allantoic sac rout. The inoculated eggs were incubated at 37°C for 5–7 days with daily observing the embryo viability. Then the chicken embryos were examined for gross NDV lesion. Five blind passages were done to all the samples and from the last passage a rapid slide HA test was carried out.

In BHK cell line:

Culture flasks with confluent monolayers were washed with HBSS and one ml of positive rapid slide HA allantoic fluid was layed over the monolayer, then incubated at 37°C for 1 hour with intermittent shaking to ensure viral adsorption. Normal non- infected cell culture controls were included. The infected flasks were washed twice with HBSS then maintenance medium was added and incubated at 37°C and examined daily under inverted microscope for detection of induced CPE. Post CPE detection, the cells freeze tile next passage. Ten serial passages were obtained.

Heamagglutination test*:*

Slide heamagglutination test (slide HA test):

For quick detection of haemagglutinins in allantoic fluid, 10% washed chicken red blood cell suspension in saline was used. One drop of the fluid (harvested allantoic fluid) to be examined was mixed with one drop of 10% chicken RBCs suspension onto a microscopic slide. Results were recorded within two minutes ***Anon, (1971*)**.

Micro-plate hemagglutination (HA) test*:*

The negative samples in direct HA test were discarded and the positive samples carried out for microplate HA test (1% chicken RBCs)which carried out according to ***(OIE, 2008)***

Demonstration of NDV cytopathic effect (CPE):

BHK cell culture were grown in Lighten's tubes containing cover slips. The tubes were infected with NDV. Post appearance of clear CPE, the infected cells and normal cell cultures were stained with Haematoxaline and Eosin (H&E) according to ***Carleton (1967).***

Virus neutralization:

It was carried out according to ***Gelb, et al. (2010)*** using specific antiserum in BHK cell culture as confirmatory test ensure that obtained isolates were NDV.

RNA extraction

the allantoic fluid sent to lab to extract RNA from it by using RNA Mini kits. RNA extraction kits were obtained from **Qiagen, Valencia, California, USA. Cat. No. 52904**. The methodology according to the manufacturer's instruction.

PCR procedures and conditions of amplification of Fgene

The RT-PCR Kit supplied by **QIAGEN, USA Cat. No. 210212**. The primers used were targeting F gene .

The product was detected by 1.7% agarose then the band examined by UV.The expected amplification band is 724 b.p.

Sequencing of F gene:

The analysis was applied with specific software, for sequence analysis we used Seqscape® software for the primary analysis of the row data. A comparative analysis of sequences was performed using the Clustal W multiple sequence alignments program (Madison, Wisconsin, USA).

A phylogenetic tree of the nucleotide was constructed using MEGA 5 software **(Tamura et al., 2011).** Serotype reference sequences were obtained from the NCBI gene bank.

Virus titration:

Titration of the virus (harvested allantoic fluid) in SPF-ECE:

The virus titrated in ECE then the end point and EID50 according to ***Reed and Muench (1938).***

In BHK cell culture:

The $TCID_{50}$ was carried out by ***Ahamed et al. (2004)***

Virus inactivation:

Inactivation of the tissue culture propagated virus suspension with binary ethylenimine (BEI):

The inactivation process was carried out on the tenth BHK cell culture passage according to ***(Razmaraii, et al. 2012).*** harvested infected tissue culture fluid was treated with BEI at a final concentration of 3% v/v then The BEI reactions were stopped after the compilation of inactivation by using 20% sodium thiosulfate solution .

Completion of Inactivation/Virus Infectivity Assay.

Samples from the inactivated virus before addition of adjuvant were tested by at least two passages in 9-11 day old SPF ECE (0.1 ml /egg) via the allantoic cavity. The complete inactivation of the virus was considered if no mortality or HA activities were negative.

Inactivated NDV infected fluid adjuvanted with Montanide ISA 70:

It was prepared at a ratio of 3 / 7 (v/v) aqueous /oil ratio. Methodology according to the protocol of SEPPIC and manufacture instruction.

Quality control of the prepared vaccines:

The Quality control was carried out by sterility &safety tests .

Experimental design for evaluation the potency of prepared vaccines:

Seventy five, one day old SPF chicks were housed in brooder units within isolation facilities till they became 21 days of age.

Evaluation of humoral immune response in vaccinated chicks by Hemagglutination Inhibition (HI) Test:

Serum samples were collected from all chicks (vaccinated and nonvaccinated) weekly till 12th week post vaccination then HI test which was carried out according to *OIE-Manual, (2008).*

Challenge test:

used as a challenge virus in the present study. 3 wks after a single immunization, chickens were challenged by I/M injection with 1 ml of $10^{5.5}$ EID_{50}/ ml of the isolate strain of Newcastle disease virus. To evaluate the protective efficacy of the NDV vaccine, mortality and signs were observed for 14 days post challenge (dpc).

Table (1) *summarizes the data concerning the history of the flocks suspected to be infected with NDV infection.*

Flock No.	Locality	Total No	Age (days)	Breed	Age of vaccination	Used vaccine
1	El-Ismalia	5000	21	Sasso	1d- 7d- 21d	Hichner, lasota, Killed
2	El-sharkia	7500	34	Cobb	1 d 5 d 13d	Killed Hitchiner Lasota
3	Kafr-Elshiekh	5000	27	Ross	1 d 5 d 13d	Killed Hitchiner Lasota
4	EL -GIZA	8000	40	Sasso	7d- 21d	Lasota, killed
5	El- Gharbia	7500	30	Avian 48	1 d 5 d 13d	Killed Hitchiner Lasota

Table 2: *Sequence and location of used primers*

Primer	Orientation	Sequence (5'- 3')	Strains specificity	Genomic location	Genomic target
M2	Forward	TGGAGCCAAACCCGCACCTGCGG	velogenic	4241-4264	980-1003
F2	Reverse	GGAGGATGTTGGCAGCATT	velogenic	4988-5006	503-485

Table 3: *Groups of experimental vaccinated chicks*

Group	Treatment	Chicks	Dose	Route	Age of vaccination	Age of infection
1	Inactivated NDV	25	0.5 ml	S/C	1 day	21 day
2	Inactivated NDV-ISA 70	25	0.5 ml	S/C	1 day	21 day
3	Control (non-vaccinated)	25	-	-	1 day	21 day

Results:

Trials of NDV isolation:

Inoculation in SPF embryonated chicken eggs:

Among the 5 flocks, 9 field samples from 2 flocks resulted death of embryo within 72 hours post inoculation in ECE with hemorrhage, congestion and oedema of the embryo that were characteristics for NDV. Allantoic fluids collected from these embryos were found positive for NDV by the direct HA test suggesting that inoculated samples contained NDV.

Inoculation into BHK cell line:

The positive (HA test) allantoic fluid was inoculated into BHK cell line for the adaptation; ten consecutive passages were given. The CPE developed at the 3rd passage in the BHK-21 cell line within 24-48 hours of inoculation; where polykaryocytosis, cell syncitia, rounding of cell and multinucleated giant cells formation (photo 1 A & B) then dendritic shaped cells were formed. At the end of CPE, the whole monolayer appeared maximum degeneration of cells and plaque formation(after infection by 50-60 hr.). Marked detachment of cells were recorded 72- 96 hours post inoculation (C). TCF (Tissue cultured fluid) were also found positive for NDV by the direct HA test. Control non infected monolayers did not show any changes throughout the observation (D).

Titration of the obtained virus isolate:

Titration in SPF embryonated eggs:

After five blind passages in the SPF embryonated eggs, the NDV isolate was titrated. It was noticed that highest infectivity titer of virus was 9.5 $\log^{10}$ EID_{50}.

Titration in BHK cell culture:

Titration of the NDV isolate in BHK cells revealed gradual increasing in the virus titer through the successive passages (Table 5).

Identification of the NDV isolate:

Identification of the obtained isolate was carried out through the application of virus neutralization test (VNT) by using specific anti-NDV in BHK cell. The inoculated cells did not show any cellular changes.

Molecular characterization of the obtained NDV isolates*:*

RT-PCR:

The isolate with HA-positive allantoic fluids of El-Giza flocks were virulent NDV. By using the primers M2 and F2, the expected size was 724 bp in length as ascertained by agarose gel electrophoresis (Photo 2). The isolate of this study also compared against the reference and vaccinal strains from gene bank (Fig.3). The results revealed that the isolate NDV/chicken/Egypt/Giza/2015 was velogenic type resembling the genotype VIId.

Nucleotide and amino acid sequence analysis of the F gene of NDV:

Nucleotides analysis of F gene of the NDV/chicken/Egypt/Giza/2015 revealed that the most nucleotides identity from gene bank with this isolate were NDV/Chicken/GC/IS/2010/1224 with accession number KF650612 and NDV/buzzard/Israel/714/2011/ IN638354 with a nucleotide identity of 92.7%.

The isolate NDV/chicken/Egypt/Giza/2015 was also closely related to Chicken/China/SDWF07/2011 with accession number JQ015295, Chicken/China/Shandong/01/2012 with accession number KC542912 and NDV/Turkey/Israel/111/2011/IN97 9564 with a nucleotide identity of 92.5%. Nucleotides identity between the selected NDV field isolate in this study and NDV/Lasota was 72.4%. Phylogenetic analysis of NDV/ chicken/ Egypt/ Giza/ 2015 with other reference and vaccine strains of NDV revealed that this isolate was related to NDV/B7/RLQP/CH/EG/12 and present in the same group with NDV/F388/RLQP/CH/EG/14 and NDV/F460/RLQP/CH/EG/13.

Table 4: *Identification of NDV by Hemagglutination test (HA) in inoculated ECE.*

Flock No.	Locality	Slide HA test	Hemagglutination titer (log^2)
1	**El-Ismalia**	**-ve**	**-**
2	**El-sharkia**	**-ve**	**-**
3	**Kafr-Elshiekh**	**+ve**	**4**
4	**EL -GIZA**	**+ve**	**5**
5	**El- Gharbia**	**-ve**	**-**
Control		**-ve**	**-**

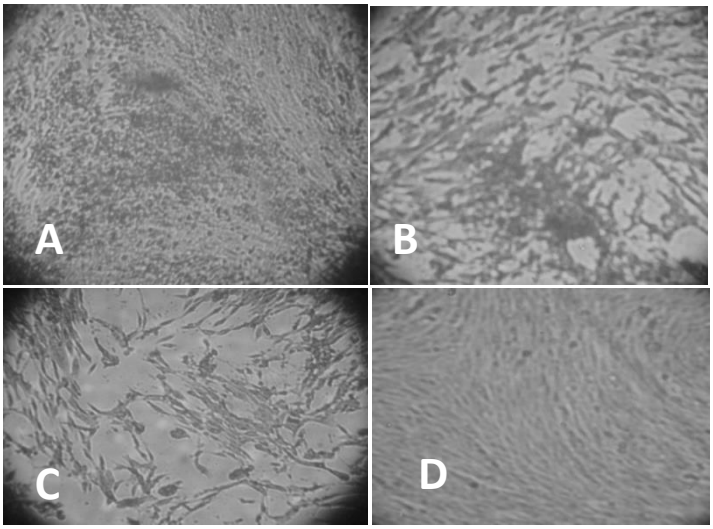

Photo. 1: *BHK cells 24 Hr. post infection (A). Giant cell formation 30 to 40 hours post infection by NDV (B). Plaque formation in BHK monolayer with marked detachment of cells following 60 to 72 hours of infection by NDV (C). Control non infected BHK monolayer (D).*

Table 5: Titer of isolated NDV in BHK cell culture

Passage No.	**1**	**2**	**3**	**4**	**4**	**6**	**7**	**8**	**9**	**10**
Virus titer (log10 $TCID_{50}$)	**-**	**-**	**3**	**5**	**6**	**7**	**7**	**8**	**8.5**	**8.5**

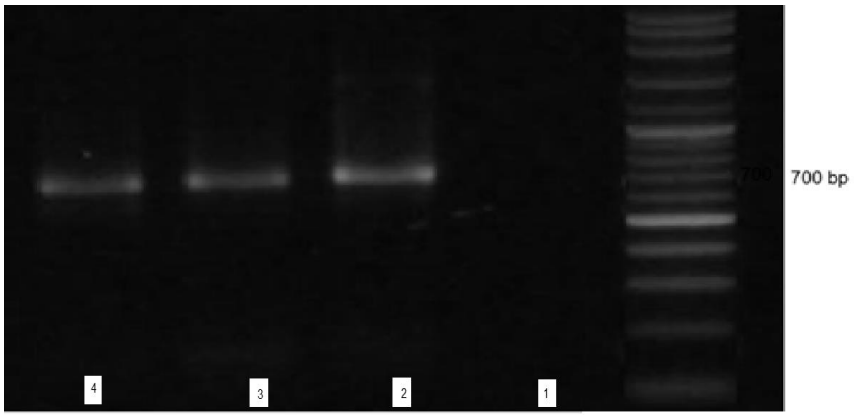

photo (2): Agarose gel electrophoresis pattern of the amplified products

1- Negative control
2- Sample no. 1
3- Sample no. 2
4-Positive control

% Of sequences identity																				
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
1- NDV-Chicken/Egypt/ Giza /2015		92.5%	92.5%	92.1%	92.7%	92.7%	88.8%	89.9%	89.3%	92.5%	87.1%	87.1%	90.1%	82.7%	84.9%	83.6%	81.2%	77.9%	80.3%	79.4%
2- NDV_Chicken/China/SDWF07/2011._JQ015295	34		99.5%	99.5%	99.5%	99.5%	93.6%	90.6%	93.0%	99.7%	91.9%	91.9%	94.3%	85.8%	89.0%	87.7%	84.4%	79.4%	83.8%	81.0%
3- NDV_Chicken/China/Shandong/01/2012._KC542912	34	2		99.1%	99.1%	99.1%	93.2%	90.1%	92.5%	99.3%	91.4%	91.4%	93.8%	85.3%	88.6%	87.5%	84.0%	79.0%	83.4%	80.7%
4- NDV_chicken/China/SDZB11/2013._KJ567597	36	2	4		99.1%	99.1%	93.2%	90.1%	92.5%	99.3%	91.4%	91.4%	93.8%	85.3%	89.0%	87.3%	84.2%	79.4%	83.8%	81.0%
5- NDV_buzzard/Israel/714/2011._JN638354	33	2	4	4		100.0%	93.8%	91.0%	93.2%	99.7%	92.1%	92.1%	94.7%	86.2%	89.3%	87.7%	84.9%	79.6%	84.2%	81.4%
6- NDV/CHICKEN/GC/IS/2010/1224._KF650612	33	2	4	4	0		93.8%	91.0%	93.2%	99.7%	92.1%	92.1%	94.7%	86.2%	89.3%	87.7%	84.9%	79.6%	84.2%	81.4%
7- NDV-F388-RLQP-CH-EG-14._KP316016	51	29	31	31	28	28		87.7%	98.3%	93.8%	95.4%	95.4%	88.8%	81.2%	84.2%	82.5%	79.6%	74.6%	79.0%	76.2%
8- NDV-B7-RLQP-CH-EG-12._KM288609	46	43	45	45	41	41	56		88.2%	90.8%	85.1%	85.1%	86.8%	79.2%	82.0%	80.7%	77.9%	74.0%	78.8%	75.7%
9- NDV-F460-RLQP-CH-EG-13._KP316015	49	32	34	34	31	31	7	54		93.2%	94.7%	94.7%	88.8%	80.7%	83.8%	81.8%	79.2%	74.2%	78.3%	75.9%
10- NDV-turkey/Israel/111/2011._JN979564	34	1	3	3	1	1	28	42	31		92.1%	92.1%	94.5%	86.0%	89.3%	87.9%	84.7%	79.4%	84.0%	81.2%
11- NDV-apmv1/chicken/Jordan/Jo11/2011._JQ176687	59	37	39	39	36	36	20	68	23	36		100.0%	87.5%	79.9%	82.0%	80.5%	77.5%	72.2%	77.0%	73.7%
12- NDV/chicken/VRLCU138/Egypt/2012._JX885868	59	37	39	39	36	36	20	68	23	36	0		87.5%	79.9%	82.0%	80.5%	77.5%	72.2%	77.0%	73.7%
13- NDV-FJ-2/99-20152947._AF458012	45	26	28	28	24	24	51	60	51	25	57	57		91.0%	92.3%	91.0%	88.6%	79.4%	84.7%	82.3%
14- NDV-chicken/SPVC/Karachi/NDV/33/2007._GU182331	79	65	67	67	63	63	86	95	88	64	92	92	41		89.9%	89.0%	87.3%	78.8%	82.9%	80.7%
15- NDV-GD450/2011._JN627508	69	50	52	50	49	49	72	82	74	49	82	82	35	46		91.9%	88.2%	81.4%	85.5%	83.4%
16- NDV-ASTR/74._Y19012	75	56	57	58	56	56	80	88	83	55	89	89	41	50	37		88.2%	82.7%	84.7%	83.4%
17- NDV-chicken-2602-605-Niger-2008._FJ772475	86	71	73	72	69	69	93	101	95	70	103	103	52	58	54	54		79.9%	82.9%	81.0%
18- NDV-MG_MEOLA_08._HQ266604	101	94	96	94	93	93	116	119	118	94	127	127	94	97	85	79	92		81.2%	80.1%
19- NDV-mallard/US(OH)/04-411/2004._FJ705464	90	74	76	74	72	72	96	97	99	73	105	105	70	78	66	70	78	86		88.8%
20- NDV-Lasota._DQ195265	94	87	88	87	85	85	109	111	110	86	120	120	81	88	76	76	87	91	51	
No. of sequences difference count																				

Fig.(1)*Nucleotide identities and divergences of the partial F0 sequence of the NDV strains* .

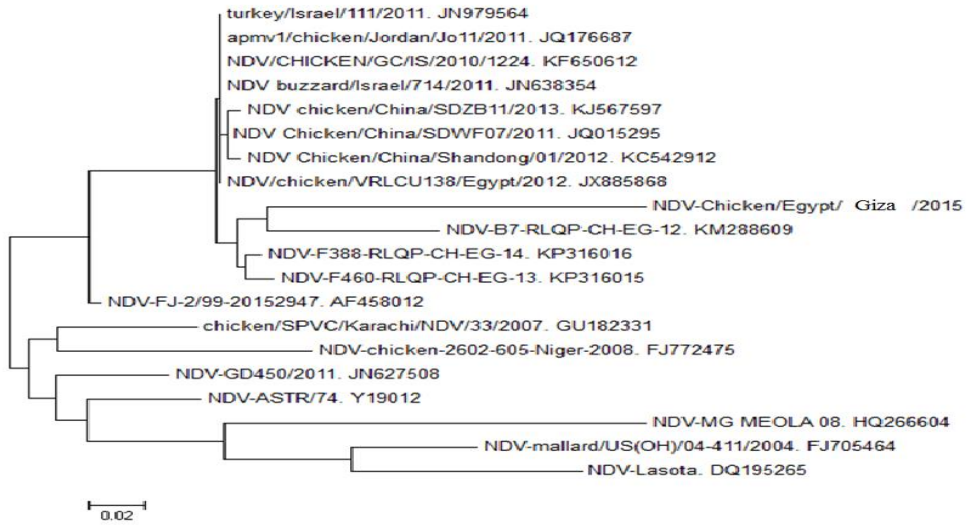

Fig. 2 : *Phylogenetic tree of NDV strains from gene bank*

Discussion:

ND causes a serious economic loses in the poultry industry although the intensive vaccination regimes carried out in Egypt. However, many outbreaks of NDV still reported so frequently. Great efforts had been done globally to combat

NDV including application of quarantine and hygienic measures and intensive vaccination programs ***(Claassen, et al 2004).***

The all isolates from two infected flocks were positive to both slide and micro-plate hemagglutination (HA) test post propagation in ECE following the standard procedure ***(Grimes, 2002)*** suggested that the two flocks were infected with NDV of velogenic type. This result was confirmed with virus neutralization test on BHK cell line.

In our study, nucleotide sequence analysis of F gene of the NDV/chicken/Egypt/Giza/2015 was closely related to Chicken/China/SDWF07/2011, Chicken/China/Shandong/01/2012 and NDV/Turkey/Israel/111/2011 with a nucleotide identity of 92.7-92.5% and this come in agreement with ***Lomniczi et al., (1998) and Radwan et al. (2013)*** who recorded that all of the NDV isolates that have caused outbreaks in eastern Africa and southern Africa originated from the middle east and Asia.the phylogenetic analysis showed that NDV strains isolated in Egypt are closely related with the NDV strains isolated in China after nucleotide sequence analysis ***(Mahmoud et al ., 2009).***

In addition to, our result come in the same manner with ***Ahamed et al. (2004)*** who adapted NDV on Vero cell line with five consecutive passages. in third passage changes in the characteristics of cell monolayer were observed. They observed clear and consistent CPE within 50 to 60 hours of infection during fourth and fifth passages. The CPE of Vero characterized by formation of syncytium& finaly plaque with the titer of 3.9 log10 $TCID_{50}$ /ml.

From a long time the viral inactivation occurred using formaldhyde compound ***(Razmaraii, et al. 2012).*** Similar results were obtained for the formaldehyde inactivating of various viruses by ***Kai & Chi (2008).*** So that formaldehyde was replaced by aziridin compounds which is more safety and antigenicity to other commonly used viral chemical inactivates ***(Rueda et al 2001).***

In this study, NDV was completely inactivated by BEI at a final concentration of 3% when the infected tissue culture fluid was incubated at 30 ºC for 18 hrs. the lower concentrations of BEI is good for make it acting ***as powerful inactivatant (Mondal et al 2005). Razmaraii, et al. (2012)*** .found that the reaction of aziridines will be more effective when incubated at 30ºC comparing to the reaction at lower temperatures. The chemical agents will faster insert into viral particles when the temperature is rising ***(Burrage et al 2000).***

Montanide adjuvants are patent contain its own surfactant which enable an easy manufacturing of vaccines by mixing the aqueous medium into the montanide oil at room temperature manually,

however in mass production to obtain a stable preparation vigorous stirring and the use of high shear mixer is necessary. Many workers had used montanide adjuvants for poultry vaccines preparations, ***(Mohammadi, et al., 1996)*** developed and manufactured inactivated oil emulsion against ND.

In our study, humoral immune response was assessed for chicks vaccinated with Inactivated NDV isolate alone and with Montanide ISA70 oil as adjuvant by HI test. The results revealed that, the higher titer of (10.6 log2) was recorded at the 6^{th} weeks post vaccination in NDV-Montanide ISA 70 group. However the titer showed higher value till 12^{th} weeks post followed by gradual decrease in HI titers till the end of the experiment (24^{th} weeks). On the other hand, vaccination of chicks with inactivated NDV isolate without adjuvant failed to induce protective HI antibodies (3 log2) all over the experiment.

These results were come in agreement with ***Chun et al. (2011)*** who record that the good vaccine not depend only on good antigen but also good adjuvant to stimulate the immunogenicity of antigen & immunoresponce.

Similar observation was found by ***(Aucotuier and Ganne, 2000)*** where they found that ND vaccine based on ISA 70 still induce antibody response with even 1/100 fractional dose.

Conclusion:

The NDV is distributed among broiler flocks in the two governrate (Giza & Kafr El-Sheikh).

The isolated strain showed marked variation and unidentity to circulating strains.

The production of inactivated vaccine (NDV-ISA 70) candidate from the local circulating virulent NDV was efficient in protecting the birds from morbidity and mortality against the challenge virus.

References:

Ahamed, T., Hossain, K. M. Billah, M. M. Islam, K. M. D. Ahasan M. M.and Islam, M. E. (2004): Adaptation of Newcastle disease virus (NDV) on Vero cell line. Int. J. Poult. Sci., 3(2): 153-156.

Alexander,D.J. (**1997):** Newcastle disease and other avian Paramyxoviridae infections. In: Diseases of Poultry 10th Edition. Edited by B.W. Calnek, H.J. Barnes, C.W. Beard, L.R. McDougald & Y.M. Saif. pp 541-570.

Alexander, D.J.,& Senne, D.A. (2008): Newcastle disease, other avian paramyxoviruses, and pneumovirus infections, p 75–98 *In* Saif M, et al., editors. (ed), Diseases of poultry, 12th ed. Blackwell Publishing, Ames, IA.

Amani, A. Saleh, Abd El-Khalek, M. and Michael, A. (2006): The effect of live attenuated anticoccidial vaccine on live infectious bronchitis and NDV

vaccines in chicks. Benha Vet. Med. J., Vol 17, No. 2, 201-210.

Anon (1971): Methods for examining poultry biologics and for identifying and quantifying avian pathogens. In: Newcastle disease. National Academy of Sci., Washington DC; p. 66.

Aucotuier, J. and Ganne, V. (2000): Assessment of oil adjuvants in Newcastle disease vaccine. In: Proceedings of the 20th World Poultry Congress, Montreal, Canada, August 20–25.

Burrage, T. , Kramer, E. ,and Brown, F. (2000): Structural differences between foot-and-mouth disease and papillomyelitis viruses influence their inactivation by aziridines. Vaccine 18:2454-2461.

Carleton (1967): structure and development of Rabies virus in T.C, journal of virology, Feb, 1(1):152-170.

Chun, G., Ming, L., Fei, L., Liu, F., Yun, Z., Wei Q., Hao, C., Chun, H., Sun, E., Hong, T. and Wen, H. (2011): Evaluation of several adjuvants in avian influenza vaccine to chickens and ducks. Virology Journal. 8:321.

Claassen, I., Maas, R., Oei, H., Daas, A., Milne, C. (2004): Validation study to evaluate the reproducibility of a candidate in vitro potency assay of newcastle disease vaccines and to establish the suitability of a candidate biological reference preparation. Pharmeuropa Bio. 2004 (1) :1-15.

Collins, M.S., Bashiruddin, J.B. & Alexander, D.J. (1993): Deduced amino acid sequences at the fusion protein cleavage site of Newcastle disease viruses showing variation in antigenicity and pathogenicity. *Archives of Virology* 128: 363-370.

Gelb, J., Preskenis, L.A., Landman, B. S. , Spackman, E. and Pope, C. R. (2010): Shore-bird origen low pathogenicity H7 avian influenza virus differs in its replication in ducks vs. Land-bassed poultry. Proc. 147th American Assn. Avian pathologists Ann. Mtg. Aug. 2-4.

Gotoh, B., Ohnisi, Y., Inocencio, N.M.E., Nakayama, K., Barr, P.J., Thomas,G., and Nagai ,Y. (1992): Mammalian subtilisin – related protease in cleavage activation of paramyxo virus fusion glycoprotein : Superiority of furin LPACE to PC2 or PC1/PC3.J. Virol.66, (11), 6391-6397.

Grimes, S.E., (2002): A basic laboratory manual for the small-scale production and testing of I-2 Newcastle disease vaccine. In FAO Regional Office for Asia and the Pacific publication, Bangkok, Thailand Senior Animal Production and Health Officer and Secretary of APHCA and FAO Regional Office for Asia and the Pacific (RAP), Thailand, pp: 139.

Kai, Y.H. and Chi, S.C. (2008): Efficacies of inactivated vaccines against betanodavirus in grouper larvae (Epinephelus coioides) by bath immunization. Vaccine 26:1450-1457.

Lomniczi, B., Wehmann, E., Herczeg, J. Ballagi-Pordany, A., Kaleta, E.F., Werner, O. , Meulemans, G., Jorgensen, P.H. , Mante, A.P., Gielkens, A.L.J., Capua,I. & Damoser. (1998): Newcastle disease outbreaks in recent years in Western Europe were caused by an old (VI) and a novel genotype (VII). Archives of Virology 143:49-64.

Mahmoud .H.A. Mohamed, Sachin Kumar , Anandan Paldurai , Mohamed. M. Megahed , Ibrahim.A. Ghanem, Mohamed .A. Lebdah and Siba.K.Samal, (2009): complete genome sequence of avirulent Newcastle disease virus isolated from an outbreak in chickens in Egypt . virus genes (2009) 39:234-237.

Mohammadi, A.A, Hooshamand Rad, P., Ahoural, p. , Momyies Siahkl , R. , Shoostary , A. , Charkhaka S. , Ebrahimi , M. , and Ghodism , N. (1996): Development and manufacture of an inactive oil emulsion. Newcastle disease vaccine in Iran. Arch Inst, Razi. 91-99.

Mondal, S.K. Neelima, M. Seetha, S. Ananda Rao, K. And Srinivasan, V.A. (2005): Validation of the inactivant binary ethylenimine for inactivating rabies virus for veterinary rabies vaccine production. Biologicals 33:185-189.

OIE,(2008): Update of Highly pathogenic Avian influenza in animals (typeH5 and H7) . http://www.oie.int/downld/avian%20INFLUENZA/A AL-Asia.htm.

Radwan, M.M., Darwish, S.F., El-Sabagh, I.M., El-Sanosi, A.A., Shalaby, M.A. (2013): Isolation and molecular characterization of NDV genotype II and VIId in Egypt between 2011 and 2012 , Virus genes 47, 311-316.

Razmaraii , N. ; Sadigh-Eteghad,S. Khalili , I.; Babaei H. ; Toroghi R. ; Razmaraii N.; Froghy L. (2012) : Immunogenicity of commercial, formaldehyde and binary ethylenimine inactivated Newcastle disease virus vaccines in specific pathogen free chickensArchives of Razi Institute, Vol. 67, No. 1, June (2012): 19-25.

Reed, L.J. & Muench, H. (1938): "A simple method of estimating fifty percent endpoints", The American Journal of Hygiene 27, 1938, pp 493–497.

Rueda, P. , Fominaya, J., Langeveld, J., Bruschke, C. ,Vela, C. and Casal , J. (2001): Effect of differentbaculovirus inactivation procedures on the integrity and immunogenicity of porcine parvovirus-like particles. Vaccine 19:726-734.

Terregino, C. and Capua, I. (2009): Conventional diagnosis of Newcastle disease virus infection In: Avian influenza and Newcastle disease, afield and laboratory manual. I. Capua and D.J. Alexander, ed., Springer Milan, Milan, Italy, pp: 123-125.

Tumura , K., Peterson,D., Peterson,N., Stecher,G., Nei, M., Kumar, S.,(2011): MEGA5: Molecular evolutionary genetics analysis using maximum parsimony methods. Mol. Biol. Evol. 28(10), 2731-2739.

الملخص العربي

تم دراسة معزولات حقلية من مرض النيوكاسل مع تصنيفها وتمريرها على خلايا كلى صغير اليربوع السورى مع امكانية تحضير لقاح مثبط من العترة المعزوله. وقد اشتملت الدراسه على خمسة وخمسين عينه من الانسجه المختلفه لخمسة قطعان دواجن من سلالات واعمار مختلفه تظهر عليها اعراض مرض النيوكاسل فى كل من محافظة الاسماعيلية ، الشرقية ،الغربية ، كفر الشيخ والجيزة. وقد اظهرت النتائج العمليه ايجابية تسعة عينات حقليه من قطيعين (كفر الشيخ والجيزة) لاختبار التلزن الدموى بعد تمريرها فى البيض المخصب الخالى من المسببات المرضيه عند عمر ٩- ١١ يوم . وقد اظهرت اجنة البيض وفيات خلال ٤٨- ٧٢ ساعة بعد الحقن مع وجود الاعراض المصاحبة لفيروس النيوكاسل من احتقان وبقع نزفيه واستسقاء على الاجنة الميته. هذا وقد تم تمرير العترة المعزوله على خلايا كلى صغير اليربوع السورى عدة تمريرات (١٠) وقد وجد ارتفاع تدريجى فى القوة العياريه للفيروس من التمريرة الثالثه وحتى التمريرة التاسعه مع ايجابية الوسط الخلوى لاختبار التلزن الدموى. كما تم التعرف على الفيروس بواسطة الاختبار الفيروسى- المتعادل باستخدام مضاد فيروس النيوكاسل على المزارع النسيجيه. وباجراء اختبار البلمرة المتسلسل للمعزوله المتحصل عليها من الجيزة وجد انها تندرج تحت العترات عالية الشراسة لفيروس النيوكاسل. وبتحليل ترتيب النيوكلوتيد بالنسبه للمعزوله ومقارنتها بعترات فيروس النيوكاسل وجد تشابه بنسبة ٩٢,٧٪ مع عترات NDV/Chicken/GC/IS/2010/1224 ، NDV/buzzard/Israel/714/2011 وبنسبة تشابه ٩٢,٥٪ مع عترات Chicken/China/SDWF07/2011; NDV/Turkey/Israel/111/2011 Chicken/China/Shandong/01/2012 . وقد وجد ان نسبة التشابه بين المعزوله وعترة اللاسوتا هى ٧٢,٤٪. هذا وباجراء تحليل النشوء والتطور وجد ان المعزوله تندرج تحت نفس المجموعه الموجود بها NDV/F388/RLQP/CH/EG/14 , NDV/F460/RLQP/CH/EG/13 .

وباجراء عملية تثبيط عترة النيوكاسل المعزوله بمعالجته بمادة البيناري بتركيز ٣٪ فى المزارع النسيجيه , وجد ان الفيروس حدث له تثبيطا كاملا بعد ١٨ ساعه من المعالجة وعند ٣٠ درجة مئوية. هذا وقد تم تحضير لقاح من العترة المعزوله المثبطه بعد تمريرها على المزارع النسيجيه بخلطها مع زيت المونتانيد ISA70 كمحسن لقاح. وقد وجد ان اللقاح امن ولا يسبب وفيات او اعراض مرضية بعد الحقن فى مجموعات الافراخ الخاليه من المسببات المرضيه. هذا وقد وجد ان الاستجابة المناعيه للمجموعه المحقونه من الافراخ باللقاح المثبط مع زيت المونتانيد باجراء اختبار مانع التلزن الدموى كانت ٨,٣ لوغاريتم٢ بعد ثلاثة اسابيع بعد التحصين وقد وصلت لاعلى قيمة (١٠,٦ لوغاريتم٢) بعد ستة اسابيع بعد التحصين وثلاثة اسابيع بعد اختبار التحدى وظلت واضحه حتى ١٢ اسبوع من التحصين تبعها انخفاض تدريجى وحتى نهاية التجربة بعد ٢٤ اسبوع من الحقن. على الجانب الاخر فشلت المجموعه المحصنه بالفيروس المثبط بدون اضافة زيت المونتانيد فى احداث الاستجابة المناعيه اللتى تحمى من الاصابة بالمرض (٣ لوغاريتم ٢) خلال التجربة.

وعند اجراء اختبار التحدى , اظهرت المجموعه المحصنة باللقاح المثبط مع المونتانيد نسبة حماية ١٠٠٪ ضد العدوى التجريبيه لفيروس النيوكاسل مع عدم ظهور اى اعراض مرضيه او نفوق وعلى الجانب الاخر اظهرت المجموعه المحصنه بالفيروس المثبط فقط ومجموعة الضوابط نسبة وفيات ١٠٠٪ مصاحبة ظهور الاعراض المرضية لفيروس النيوكاسل.