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Evaluation of Some Vaccination Programs in Protection of Experimentally Challenged Broiler Chicken against Newcastle Disease Virus

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Abstract: The current study was carried out to evaluate efficiency of different vaccination programs in protecting broiler chicken against Virulent Viscerotropic Strain of Newcastle disease virus. Different live and inactivated NDV vaccines were used throughout the experiment including; ND-HB1,Elite, LaSota, Inactivated GII and Inactivated GVII. Broiler chicks were divided into 8 groups; 6 groups undergo different vaccination programs against NDV while two groups were kept without vaccination to be the control groups. Challenge was done at day 30th of age via intranasal administration of NDV velogenic GVII (NDV/CK/Egypt/567F/2012). Abs titers were determined at days 1, 7, 14, 21, 28 and 35 days of the experiment. The obtained results of shedding titers of NDV clarified that the lowest shedding titer was recorded in group vaccinated by HB + double shots of Inactivated GVII + Elite + LaSota and HB + double shots of Inactivated GII + Elite + LaSota, then group vaccinated by HB + one shot of Inactivated GVII + Elite + LaSota and HB + one shot of Inactivated GII + Elite + LaSota at days 3, 5, 7 and 9 post challenge day at 30th, respectively compared to those vaccinated by live vaccines HB + LaSota only and HB + Elite + LaSota only. Also, no mortalities (100% protection rate) were recorded in groups vaccinated with both live and inactivated NDV vaccines compared to low mortality rates recorded in groups vaccinated with live vaccines only. Based on the recorded results, it was concluded that application of ND vaccination programs containing both live and double inactivated vaccines (either GII or GVII) was found to be more effective than those depending on one shot of inactivated vaccine (either GII or GVII) plus live vaccines and more effective than program including live vaccines only.

Keywords: ND, Vaccination Programs, Live and Inactivated Vaccines, Shedding

Introduction

In Egypt, poultry sector is the fastest growing industry, with over 90 billion Egyptian pound investments. (1$ = approximately 17 L.E.) (Sabry, 2019). It provides the country with the greatest part of its meat production, in addition to job opportunities for approximately 3 million employees (Sabry, 2019). Several pathogenic agents affect the poultry and associated with severe losses in the poultry production in Egypt specially Newcastle virus, Avian influenza, infectious bronchitis, Infectious bursal disease virus and multidrug resistant bacterial pathogens (Diab *et al*., 2019; Eid *et al*., 2016; Eid *et al*., 2019; Elhady *et al*., 2018; Sedeik *et al*., 2018; Sedeik *et al*., 2019; Sultan *et al*., 2019). So, presence of diseases like ND that was described in Egypt for the first time in 1948 (Daubney and Mansy, 1948) represents a real threat to Egyptian poultry industry. Newcastle disease (ND) is considered one of the most important infectious diseases all over the world. Nearly it affects birds in the six continents causing major economic losses, prompting Office International des Epizooties to classify it as list A disease (OIE, 2015). The causative agent Newcastle disease virus (NDV) is an enveloped, single stranded, non-segmented RNA of negative sense virus. NDV is the only member of the genus *Avulavirus*,

subfamily *Paramyxoviridae* that belonged to family *Paramyxoviridae* in the order *Mononegavirales* and is designated Avian Paramyxovirus-1 (APMV-1) (Alexander and Senne, 2008). According to virulence, NDV isolates can be classified into three main pathotypes; velogenic (virulent), mesogenic (of moderate virulence) and lentogenic (of low virulence) (Orsi *et al*., 2009). Virulent NDV is responsible for the severe clinical symptoms in affected chickens including; diarrhea, nervous and respiratory manifestations, egg problems like low production and production of deformed eggs, hemorrhagic lesions in the trachea, intestine and proventriculus and mortality (Desingu *et al*., 2017; Guo *et al*., 2014; Liu *et al*., 2015). The infected and carrier chickens are the main source of infection to other healthy birds in the flock through shedding the virus in their droppings and respiratory secretions (Nwanta *et al*., 2008). Vaccination is still the most important tool in controlling ND. There are two main types of vaccines used against NDV; live vaccines and inactivated vaccines. The inactivated type is not practical in application especially in large flocks as they need to be injected and have low immunogeniticity but prevent viral shedding (Mansur-Ud-Din *et al*., 2007). On the other hand, live low virulent strains are used as live vaccines to give protection against virulent strains depending on the fact of the antigenic similarity between all NDV strains (Miller *et al*., 2013a). Different studies proved the ability of live NDV vaccines to reduce or prevent clinical disease and mortality, decrease the amount of virus shedding into the environment and increase the amount of virus needed to infect the vaccinated birds (Marangon and Busani, 2007; Miller *et al*., 2009). In addition to inducing better immunity, live vaccines are more convenient in dealing with large flocks as they can be applied via spraying or drinking water (Dimitrov *et al*., 2017; Perozo *et al*., 2012). However, these application methods may result in wide variation in the immune responses of vaccinated birds (Senne *et al*., 2004). Even with suing live ND vaccines and all its core values, NDV can cause infection in vaccinated susceptible birds and shed virus and increase the epidemiological load and increase risk of infected surround susceptible birds (Kapczynski and King, 2005). Moreover, vaccination with live vaccines may induce disease occurrence and reduce growth rates of vaccinated birds (Swayne and Glisson, 2013). In Egypt, where the ND is endemic, vaccination is applied as a routine prophylactic measure using both live attenuated and inactivated vaccines from day zero of chick age; even with this preventive measure ND outbreak are still known to occur hence we suggest improving the vaccination strategy. So, the current study was carried out to evaluate the efficiency of different vaccination programs in protecting experimentally challenged broiler chicken against VV strain of Newcastle disease virus (NDV) and determination of virus shedding of different groups indicating the extent and period of infectiousness by qRT-PCR, comparison of Abs titer using Hemagglutination Inhibition (HI) test and feed conversion ratio.

Materials and Methods

Commercial Broiler Chicks and Experimental Area

A total of 1200 one-day old Arbor Acres broiler chicks with an average weight of 42 g obtained from Cairo Poultry Company (CPC) were allotted randomly into eight groups (150 birds /group). Floor rearing under hygienic conditions was applied to birds in previously cleaned and disinfected isolated experimental pens where biosecurity measures were implemented. Birds were fed a commercial fattening feed produced by Cairo Poultry Company (CPC) by average of 3.5 kg food / bird throughout the experiment time, it can be divided into: 700 g starter / bird of 23% protein, 2000 g grower / bird of 21% protein and 800 g / bird finisher of 19% protein. Water and feed were provided ad-libitum. Maternal immunity was measured for 60 randomly selected 1-day old chicks before being divided into groups. Each group was weighted weekly till the end of the experiment where the final body weights were determined.

ND Virus, Vaccines, Reference Antigens and Antisera Used in the Experiment

Newcastle Disease Challenge Virus

The vNDV (10^6 EID_{50}/0.5ml) challenge virus NDV/CK/Egypt/567F/2012 (acc. no. JX647839) belongs to genotype VIId currently circulating in Egypt. All viruses were propagated and titrated in 10-day-old SPF eggs. The virus challenge dose equal 6-Log-10 EID_{50} given 0.5 ml / bird (OIE, 2015).

Vaccines

Different live and inactivated NDV vaccines were used throughout the experiment as illustrated in **Table (1).** Live vaccines were administrated through eye distillation while inactivated vaccines were administrated through subcutaneous injection in skin fold of the neck.

NDV Reference Antigens and Antisera

ND LaSota vicinal strain was propagated in ECE and diluted to 4 HAU to be used as HA antigen in hemagglutination inhibition (HI) titration of ND antibody, was obtained from ME-VAC Egypt, friendly dedicated. Known positive and negative NDV antisera were provided.

Experiment Design

Broiler Broiler chicks were divided into 8 groups (150 chicks / group). Groups from 1 to 6 undergo different vaccination programs against NDV as illustrated in **Table (2)** while groups 7 and 8 were

unvaccinated control groups. Ab titers were determined at days 1,7,14,21,28 and 35 of the experiment in 15 randomly selected chicks from each group. At day 28th of age (2 days before challenge), 15 birds from each group were randomly selected and moved to controlled isolated rooms where they were examined for presence of Avian Influenza virus (H5 & H9), Newcastle (ND) virus and Infectious Bronchitis (IB) virus using one step RT-PCR at day zero and the two successive days post isolation to ensure their freedom from these pathogens. Challenge was done at day 30 to groups from 1 to 7 via intranasal administration of NDV (10^6EID$_{50}$) velogenic GVII (NDV/CK/Egypt/567F/2012). Finally, shedding percentage of NDV was detected in Oropharyngeal (tracheal) swabs by using qRT-PCR at days 3, 5, 7 and 9 post challenges. Chicken blood were collected from wing vein or by slaughtering and kept in slop position at 37°C for one hour then at 4°C overnight. Sera then separated by centrifugation at 3000 rpm/10 minutes and stored at -20°C till tested by HI as previously described (OIE, 2015).

Molecular Identification of Viral Pathogens

The kits used flowing manufacturer instructions as shown in Tabel 3.

Assessment Parameters

Shedding Titer

Virus shedding was determined in Oropharyngeal (tracheal) swabs by using qRT-PCR as previously described (Miller *et al*., 2007) and the virus titers were calculated using the Spearman-Kärber method (Kärber, 1931):

$$Mean\ of\ shedding\ titer = \frac{SUM\ of\ virus\ shedding}{Total\ Number\ of\ shedders}$$

NB. Virus shedding titer (titer±SD) as EID$_{50}$ using qRT-PCR with absolute standard curve and the engine detection threshold 0.7 EID$_{50}$.

- *Humoral Immunity*

  Expressed by titration of Abs by HI test at age 1,7,14,21,28 of age and 5 days post challenge.

- *Mortality Rate*

$$Mortality\ rate = \frac{Number\ of\ dead\ birds}{total\ number\ of\ birds\ in\ each\ group} \times 100$$

- *Feed Conversion Ratio*

  FCR for each week interval was estimated:

$$FCR = \frac{Feed\ intake\ (g)\ in\ a\ given\ period}{Body\ weight\ gain\ (g)\ in\ the\ same\ period}$$

Statistical Analysis

It was made using Chi^2 test to examine the significant differences of the detection rate of antibodies among different groups studied using SAS software version 9.4 (Institute, 2014).

Table 1: Description of NDV vaccines used in different vaccination programs

Vaccine used	Company	Dose	Strain used/dose
ND-HB1	ME-VAC (Egypt)	1000 d/vail	10^6 EID$_{50}$ of ND strain HB
ND-Elite	ME-VAC (Egypt)	1000 d/vail	10^6 EID$_{50}$ of ND strain ND-60
ND LaSota	ME-VAC (Egypt)	1000 d/vail	10^6 EID$_{50}$ of ND strain LaSota
Inactivated GII	ME-VAC (Egypt)	1000 d/vail 0.3 cc	NDV/Chicken/Egypt/11478AF/2011(ND)10^8
Inactivated GVII (Dalguban)	KBNP (Korea)	1000 d/vail0.5 cc	NDV Genotype VII, KBNP-C4152R2L

Table 2: Description of vaccination programs applied to the experimental groups

Age of vaccination Experimental groups	Day 1	Day 7	Day 10	Day 18	Day30
Group 1	HB	ND Elite		LaSota	Challenge by
Group2	HB			LaSota	NDV (10^6 EID$_{50}$)
Group3	HB	ND Elite + Inactivated GII		LaSota	velogenic GVII
Group4	HB	ND Elite + Inactivated GVII		LaSota	
Group5	HB+ Inactivated GVII	ND Elite	Inactivated GVII	LaSota	
Group6	HB+ Inactivated GII	ND Elite	Inactivated GII	LaSota	Intranasal
Group 7 (control + ve)	No vaccination program				
Group 8 (control - ve)	No vaccination program				No challenge

Table 3: Ready used kits for Q-RT-PCR

Gene fragment	Kit name	Company
NDV- F gene	ABT NDV-F gene QPCR	Applied Biotechnology, Egypt
AIV-H5	ABT AIV-H5 QPCR	Applied Biotechnology, Egypt
AIV-H9	ABT AIV-H9 QPCR	Applied Biotechnology, Egypt
IBV- S1 gene	ABT IBV-S1 QPCR	Applied Biotechnology, Egypt

Results

The The recorded results in showed the Oropharyngeal (tracheal) shedding of challenged NDV from broiler chicken at 3rd day post challenge. Chi^2 analysis of the obtained results showed significant difference between the shedding titers of NDV of different experimental groups where the lowest shedding titer was recorded in experimental group that was vaccinated by HB + Inactivated GVII, Elite, Inactivated GVII and LaSota vaccines at days 1, 7, 10 and 18 of age **(G5)**, respectively followed by the experimental group vaccinated by HB + Inactivated GII, Elite, Inactivated GII and LaSota at the same age **(G6)**, respectively. On the other hand, the highest shedding titer was recorded in experimental group that was not vaccinated and challenged by NDV **(G7)** followed by the experimental group that was vaccinated by HB, and LaSota vaccines at days 1 and 18 of age **(G2)**, respectively as shown in **Table (5)**.

As shown in **Table (5)**, it was observed that experimental groups that was vaccinated by HB + Inactivated GVII, Elite, Inactivated GVII and LaSota vaccines **(G5)** and HB + Inactivated GII, Elite, Inactivated GII and LaSota **(G6)** at days 1, 7, 10 and 18 of age, respectively showed no shedding of the NDV at 5th DPC reflecting efficient vaccination program. On the other hand, the highest shedding titer (4.27±0.15) was recorded in experimental group that was not vaccinated and challenged by NDV **(G7)** followed by the experimental group that was vaccinated by HB and LaSota vaccines (2.92±1.21) at days 1 and 18 of age **(G2)**, respectively. Moreover, it was recorded that birds of the group that was not vaccinated and challenged by NDV were totally dead as a result of ND infection **(G7)**.

The presented data in Table (5) clarified the Oropharyngeal (tracheal) shedding of challenged NDV from broiler chicken at 7th DPC. As 5th DPC, it was observed that experimental groups that was vaccinated by HB + Inactivated GVII, Elite, Inactivated GVII and LaSota vaccines **(G5)** and HB + Inactivated GII, Elite, Inactivated GII and LaSota **(G6)** at days 1, 7, 10 and 18 of age, respectively showed no shedding of the NDV reflecting efficient vaccination program. On the other hand, the highest shedding titer (1.86±0.56) was recorded in experimental group that was vaccinated by HB and LaSota vaccines at days 1 and 18 of age **(G2)**, respectively followed by experimental group that was vaccinated by HB, Elite and LaSota **(G1)** (1.68±0.53).

At 9th DPC, viral shedding occurred only in the group vaccinated by HB and LaSota at days of 1 and 18 of age **(G2)** by shedding birds (30.7 %) and shedding titer of (1.25±0.83) and the group vaccinated by HB, Elite and LaSota at days of 1, 7 and 18 of age **(G1)** by only shedding birds (21.4 %) and shedding titer of (1.05±0.41) **(Table 5)**.

Mortality rate of broiler chicken during the experiment was tabulated in **Tables (4)**. Concerning mortality rate of broiler chicken after challenge by NDV, the highest mortality rate (100%) was observed in non-vaccinated challenged group **(G7)** where no vaccination programs were applied. On contrary, no mortalities (100% protection rate) were recorded in groups vaccinated with both live and inactivated NDV vaccines **(G3, G4, G5**, and **G6)**. In addition, low mortality rates were recorded in groups vaccinated with live vaccines only **(G1, G2)** by (HB, Elite and LaSota).

The presented data in **Fig. (1)** Showed that 15 randomly selected chicks from each experimental group (150 chicks) at 14 days old were tested by HI test for determination of Ab titers against VDV vaccine. Statistical analysis clarified significant difference at ($P < 0.05$) among different groups in X log. The highest Ab titers was observed in group vaccinated with HB + Inactivated GII, Elite, Inactivated GII and LaSota vaccines **(G6)** (Live and double inactivated GII vaccine) (X log 4.06±0.44A) followed by the group vaccinated with HB + Inactivated GVII, Elite, Inactivated GVII and LaSota **(G5)** (Live and double shot of inactivated GVII vaccine) (X log 3.86±0.85B). On contrary, positive and negative control groups (non-vaccinated) showed the lowest Ab titers (X Log 1.26±0.20F and 1.33±0.20F, respectively) **(G7, G8)**.

At 28 days old, statistical analysis clarified significant difference at ($P < 0.05$) among different groups in X log. vaccinated groups with live and double shot of inactivated vaccines continued to show the highest Ab titers among different groups as well as the levels of titers were increased compared to their level at age of 14 days reflecting higher protection level (X log 6.13±0.63A and 6.13±0.62A) **(G5, G6)** compared to groups vaccinated with live vaccine only (X log 4.33±0.34B and 4.26±0.33B) **(G1, G2)**. Non vaccinated groups **(G7, G8)** are not only showed the lowest Ab levels but also the titers were decreased compared to those determined in 14 days old chicks **(Table 1)**.

On the other side, immune response to NDV vaccines at 35 days old (5 days post challenge) was tabulated in **Fig. (1)** Where statistical analysis clarified significant difference at ($P < 0.05$) among different groups in X log. Vaccinated groups with live and double shot of inactivated vaccines continued to show the highest Ab titers among different groups but the levels of titers were decreased compared to their level at age of 28 days (X log 5.4±0.52A and 5.53±0.53A) **(G5, G6)** compared to groups vaccinated with live vaccine only (X log 3.53±0.55C and 3.26±0.32C) **(G1, G2)**. Moreover, it was recorded that chicks of control positive group (non-vaccinated and challenged by NDV) **(G7)** were totally dead. Administration of double shot of inactivated NDV vaccines (Inactivated GVII or Inactivated GII) and immunization with live NDV vaccine HB, ELITE and LaSota in 1,7, 10 and 18 days of life (G5, G6) induced high levels of NDV specific humoral antibodies and completely protect chicken from death post challenge with VV NDV challenge by the oculonasal route at 30th-day of age and reduce significantly the shedding of virus to the environment which subsequently decrease the No. of secondary infected birds.

Effects of different NDV vaccination programs on productive performance of broiler chicken during the experiment was recorded in Table (6), statistical analysis clarified significant difference among estimated FCR of all experimental groups. It was observed that FCR was better in groups vaccinated with live and inactivated vaccines (G3, G4, G5, G6) compared to those vaccinated with live vaccines only (G1, G2) (1.62 vs 1.7) respectively indicated that combination of live and inactivated vaccines in immunization against NDV did not affect feed conversion ratio negatively.

Table 4: Mortality rate of chicken in different experimental groups after challenge (from day 1 post challenge to day 9 post challenge)

Experimental Groups (15 birds/group)	Dead birds		Protection %	
	No.	%	No.	%
Group 1	1	6.6	14	93.40
Group 2	2	13.3	13	86.60
Group 3	0	0.0	15	100.00
Group 4	0	0.0	15	100.00
Group 5	0	0.0	15	100.00
Group 6	0	0.0	15	100.00
Group 7 (control+ve)	15	100.0	0	0.00

Table 5: Oropharyngeal (tracheal) shedding of NDV from broiler chicken of different experimental groups at 3rd, 5th, 7th and 9th day post challenge (DOC)

G DPC	G 1	G2	G 3	G 4	G 5	G 6	G 7	G 8
3DPC	15/15	15/15	10/15	10/15	10/15	10/15	15/15	0%
	100%	100%	66.60%	66.60%	66.60%	66.60%	100%	
	3.29±1.03	3.91±0.10	2.92±0.81	2.75±0.78	1.67±0.76	1.86±0.56	4.27±0.15	
5DPC	15/15	15/15	10/15	10/15	0%	0%	13/13	0%
	100%	100%	66.60%	66.60%			100%	
	2.55±0.78	2.92±1.21	1.71±0.83	1.65±0.83			4.27±0.15	
7DPC	4/14	10/13	5/15	5/15	0%	0%	0%	0%
	28.50%	77%	33.30%	33.30%				
	1.68±0.53	1.86±0.56	1.67±0.31	1.05±0.43				
9DPC	3/14	4/13	0%	0%	0%	0%	0%	0%
	21.40%	30.77%						
	1.05±0.41	1.25±0.83						

*The mean shedding titer =SUM of virus shedding/ total Number of shedders. Virus shedding titer (titer ± SD) as EID_{50} using qRT-PCR with absolute stander curve and the engine detection threshold 0.7 EID_{50}

Table 6: Effects of different NDV vaccination programs on productive performance of broiler chicken in different experimental groups

Experimental Groups	Final Body weight (g) at 39 days old	Total Feed consumption (g)	Feed conversion rate (FCR)
Group 1	2120±21.20C	3610±34.35D	1.7±0.18A
Group 2	2095±25.55D	3600±33.35D	1.71±0.17A
Group 3	2220±22.24B	3650±35.60C	1.64±0.16B
Group 4	2235±23.24B	3660±36.60C	1.63±0.18B
Group 5	2275±27.25B	3700±33.37B	1.62±.16B
Group 6	2265±26.27B	3690±33.38B	1.62±.16B
Group 7 (control + ve)	All chicken dead		
Group 8 (control - ve)	2440±22.34A	3760±33.63A	1.54±0.17B

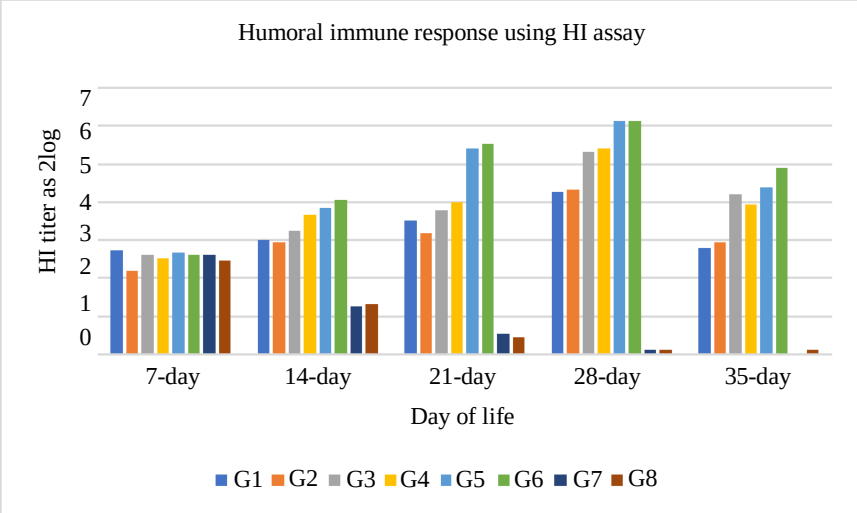

Fig. 1: Humoral Immune response for ND using HI assay along the experiment period Discussion

Discusion

Newcastle Newcastle disease (ND) is one of the most important contagious diseases of poultry and other bird species and considered as a global threat to commercial poultry production (Alexander and Senne, 2008). Velogenic strains of ND virus cause a devastating disease of poultry. Genotype VII (class II genotype VII) was firstly categorized into two sub genotypes: VIIa, which represents viruses that emerged in the 1990s in the Far East and spread to Europe and Asia; and VIIb, which represents viruses that emerged in the Far East and spread to South Africa. Later, the sub genotypes of VII are classified into VIIc, d, and e, which represent isolates from China, Kazakhstan and South Africa and VIIf, g, h and i, which represent African isolates (de Almeida *et al*., 2009; Miller *et al*., 2009). The phylogenetic analysis showed Egyptian NDV isolates are closely related with the genotype II of class II NDV strains. So, sequences of the F genes of 2006 Egypt isolates are closely related to that of the 2005 suggesting that these strains are probably circulating in the vaccinated bird population in Egypt until development of an outbreak (Mohamed *et al*., 2011). Different live and inactivated NDV vaccines were used throughout the experiment including; ND-HB1, ELITE, LaSota, Inactivated GII and Inactivated GVII. Broiler chicks were divided into 8 groups (150 chicks / group); 6 groups undergo different vaccination programs against NDV while 2 groups were kept without vaccination to be the control groups. Challenge was done at day 30 to groups from 1 to 7 via intranasal administration of NDV (10^6EID_{50}) velogenic GVII (NDV/CK/Egypt/567F/2012). Firstly, it was found that broiler chicken of the control negative group that was neither vaccinated nor challenged were all alive throughout the experiment **(G8)**. There are two methods for detection of ND Virus shedding, NDV titration in viral samples in embryonated chicken eggs (ECE) or in tissue culture, which is time consuming and costly. Recently, a Quantitative real-time RT-PCR (RRT-PCR) based assay has been developed and validated for detection of NDV shedding (Perozo *et al*., 2012). The amount of NDV shed into the environment by vaccinated birds has arisen as a potential indicator of vaccine efficacy (Miller *et al*., 2009; Miller *et al*., 2007). Using vaccines formulated with a NDV with the same (homologous) genotype of NDV challenge virus, for both genotype II and genotype V NDV isolates, is possible to decrease not only the number of birds shedding NDV, but also the amount of NDV shed from individual birds by evaluating oropharyngeal swab material, However, in those studies, the amount of virus shed from the birds vaccinated with vaccines heterologous to the genotype of the challenge virus was also decreased but at lower amounts (Miller *et al*., 2007). Like most vaccines, NDV vaccines do not prevent vaccinated birds from becoming infected with NDV and subsequent shedding of the virus (Kapczynski and King, 2005). However, most vaccines will significantly decrease the amount of virus shed in saliva and feces compared to unvaccinated birds (Miller *et al*., 2009). The amount shed will depend on the immunity of the host, the host species infected, the amount and virulence of the challenge virus, the dose and type of ND vaccine and the time between vaccination and challenge, While the amount will vary depending on the NDV isolate and the host species (Miller *et al*., 2013b). The recorded results in **Table (5)** showed the Oropharyngeal (tracheal) shedding of challenged NDV from broiler chicken at 3rd day post challenge. Chi^2 analysis of the obtained results showed significant difference between the shedding titers of NDV of different experimental groups where the

lowest shedding titer was recorded in experimental group that was vaccinated by HB + Inactivated GVII, Elite, Inactivated GVII and LaSota vaccines at days 1, 7, 10 and 18 of age **(G5)**, respectively followed by the experimental group vaccinated by HB + Inactivated GII, Elite, Inactivated GII and LaSota at the same age **(G6)**, respectively. On the other hand, the highest shedding titer was recorded in experimental group that was not vaccinated and challenged by NDV **(G7)** followed by the experimental group that was vaccinated by HB and LaSota vaccines at days 1 and 18 of age **(G2)**, respectively. As shown in **Table (5)**, it was observed that experimental groups that was vaccinated by HB + Inactivated GVII, Elite, Inactivated GVII and LaSota vaccines **(G5)** and HB + Inactivated GII, Elite, Inactivated GII and LaSota **(G6)** at days 1, 7, 10 and 18 of age, respectively showed no shedding of the NDV at 5th DPC reflecting efficient vaccination program. On the other hand, the highest shedding titer (4.27±0.15A) was recorded in experimental group that was not vaccinated and challenged by NDV **(G7)** followed by the experimental group that was vaccinated by HB and LaSota vaccines (2.92±1.21B) at days 1 and 18 of age **(G2)**, respectively. Moreover, it was recorded that birds of the group that was not vaccinated and challenged by NDV were all dead as a result of ND infection **(G7)**. The presented data in **Table (5)** clarified the Oropharyngeal (tracheal) shedding of challenged NDV from broiler chicken at 7th DPC. As 5th DPC, it was observed that experimental groups that was vaccinated by HB + Inactivated GVII, Elite, Inactivated GVII and LaSota vaccines **(G5)** and HB + Inactivated GII, Elite, Inactivated GII and LaSota **(G6)** at days 1, 7, 10 and 18 of age, respectively showed no shedding of the NDV reflecting efficient vaccination program. On the other hand, the highest shedding titer (1.86±0.56A) was recorded in experimental group that was vaccinated by HB and LaSota vaccines at days 1 and 18 of age **(G2)**, respectively followed by experimental group that was vaccinated by HB, Elite and LaSota **(G1)** (1.68±0.53A). At 9th DPC, viral shedding occurred only in the group vaccinated by HB and LaSota at days of 1 and 18 of age **(G2)** by shedding birds (30.7 %) and shedding titer of (1.25±0.83A) and the group vaccinated by HB, Elite and LaSota at days of 1,7 and 18 of age **(G1)** by only shedding birds (21.4 %) and shedding titer of (1.05±0.41A) **(Table 5)**, which agreed with Similar findings were in previous reports (Cho *et al*., 2007; Jeon *et al*., 2008; Kapczynski and King, 2005; Van Boven *et al*., 2008; Yu *et al*., 2001). Collectively, the results indicated that the used vaccination program provides chicken with protection from disease caused by the genotype VII virus, as no mortality or disease symptoms were observed in any of the vaccinated chicken. The results of this study therefore contrast with the concerns in the field and published reports that, current ND vaccines may not produce adequate protection against velogenic challenge (Liu *et al*., 2003). Mortality rate of broiler chicken during the experiment was tabulated in Tables (4). Concerning mortality rate of broiler chicken after challenge by NDV, the highest mortality rate (100%) was observed in non-vaccinated challenged group **(G7)** where no vaccination programs were applied. On contrary, no mortalities (100% protection rate) were recorded in groups vaccinated with both live and inactivated NDV vaccines **(G3, G4, G5,** and **G6)**. In-addition, low mortality rates recorded in groups vaccinated with live vaccines only **(G1, G2)** by (HB, Elite and LaSota). Yi *et al*., (2011) proved that LaSota and ELITE strain of NDV vaccines not effective after VIId NDV strain isolated from chicken in Shanghai, China which has caused several breaks in China and Taiwan (Yi *et al*., 2011). Blood samples were taken from chicken groups before and after challenge, antibodies to NDV were quantified by hemagglutination inhibition test (HI), serum samples were collected and prepared 5-day post challenge from vaccinated challenged and non-challenged chicks. Protection from ND correlates with HI antibody titers at the day of challenge (Kapczynski and King, 2005; Kapczynski *et al*., 2006), All birds in the vaccinated groups developed protective titers from humoral immune response measured by HI assay in comparison to non-vaccinated group as previously described " over 4 $\log_2$" (Saidu *et al*., 2003). The immune response to NDV vaccines was determined using HI test. Approximately, 5 % (60/1200) of 1-day old randomly selected chicks were tested by HI test for determination of maternal immunity and it was found that X Log titer was 5.8. The presented data in **Fig. (1)** Showed that 15 randomly selected chicks from each experimental group (150 chicks) at 14 days old were tested by HI test for determination of Ab titers against VDV vaccine. Statistical analysis clarified significant difference at ($P < 0.05$) among different groups in X log. The highest Ab titers was observed in group vaccinated with HB + Inactivated GII, Elite, Inactivated GII and LaSota vaccines **(G6)** (Live and double inactivated GII vaccine) (X log 4.06±0.44A) followed by the group vaccinated with HB + Inactivated GVII, Elite, Inactivated GVII and LaSota **(G5)** (Live and double shot of inactivated GVII vaccine) (X log 3.86±0.85B). On contrary, positive and negative control groups (non-vaccinated) showed the lowest Ab titers (X Log 1.26±0.20F and 1.33±0.20F, respectively) **(G7, G8)**. At 28 days old, statistical analysis clarified significant difference at ($P < 0.05$) among different groups in X log. vaccinated groups with live and double shot of inactivated vaccines continued to show the highest Ab titers among different groups as well as the levels of titers were increased compared to their level at age of 14 days reflecting higher protection level (X log 6.13±0.63A and 6.13±0.62A) **(G5, G6)** compared to groups vaccinated with live vaccine only (X log 4.33±0.34B and 4.26±0.33B) **(G1, G2)**. Non vaccinated groups **(G7, G8)** are not only showed the lowest Ab levels but also the titers were decreased compared to

those determined in 14 days old chicks **Fig. (1)**. On the other side, immune response to NDV vaccines at 35 days old (5 days post challenge) was tabulated in **Fig. (1)** Where statistical analysis clarified significant difference at ($P < 0.05$) among different groups in X log. Vaccinated groups with live and double shot of inactivated vaccines continued to show the highest Ab titers among different groups but the levels of titers were decreased compared to their level at age of 28 days (X log 5.4±0.52A and 5.53±0.53A) **(G5, G6)** compared to groups vaccinated with live vaccine only (X log 3.53±0.55C and 3.26±0.32C) **(G1, G2)**. Moreover, it was recorded that chicks of control positive group (non-vaccinated and challenged by NDV) **(G7)** were totally dead. Administration of double shot of inactivated NDV vaccines (Inactivated GVII or Inactivated GII) and immunization with live NDV vaccine HB, Elite and LaSota in 1,7, 10 and 18 days of life **(G5, G6)** induced high levels of NDV specific humoral antibodies and completely protect chicken from death post challenge with VV NDV challenge by the oculonasal route at 30^{th}-day of age and reduce significantly the shedding of virus to the environment which subsequently decrease the No. of secondary infected birds. The level of protection achieved in this study demonstrates the efficacy of the test vaccine program based on combination between live and inactivated vaccines (either GVII or GII) and its ability to protect against the clinical disease of ND provide significant higher protection level in comparison to using live vaccine alone. This immunization procedure can be recommended for prevention of GVII NDV strain and this finding agreed with previous reports (Bennejean *et al*., 1978); who proved that simultaneous vaccination of day-old chicks with live and inactivated ND vaccines results in better protection as compared with a single vaccination. Poor vaccine immune response is responsible for viral shedding rather than variation in virus antigenicity and the proper vaccine program can reduce shedding and in turn decrease the environmental virus load and decrease the chance to develop reservoirs. Effects of different NDV vaccination programs on productive performance of broiler chicken during the experiment was recorded in **Table (6)**, statistical analysis clarified significant difference among estimated FCR of all experimental groups. It was observed that FCR was better in groups vaccinated with live and inactivated vaccines **(G3, G4, G5, G6)** compared to those vaccinated with live vaccines only **(G1, G2)** (1.62 vs 1.71) respectively indicated that combination of live and inactivated vaccines in immunization against NDV did not affect feed conversion ratio negatively.

Conclusion

The the combination between live and inactivated ND vaccine provide significantly better protection in broiler chicken in comparison of using live vaccine alone and there is no significant difference between the different genotypes of inactivated ND vaccine as both inactivated genotype-II and Genotype-VII within the vaccination program of ND virus have similar protective levels in broiler chicks.

Author's Contributions

Mousa A. Ayoub, Wael Elfeil, Diaa El Boraey: Conceived and designed the experiments.

Mousa A. Ayoub, Wael Elfeil, Diaa El Boraey: performed the experiments.

Mousa A. Ayoub, Wael Elfeil, Diaa El Boraey Haitham Hammam and Mohamed, A. Nossair: Analyzed the data.

Mousa A. Ayoub, Wael Elfeil, Diaa El Boraey: Contributed reagents/materials/analysis tools.

Wael Elfeil, Haitham Hammam and Mohamed, A. Nossair: Wrote and review the manuscript.

Conflict of Interest

The authors declare that there is no conflict of interests regarding the publication of this paper.

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