

Protective Efficacy of Some Newcastle Disease Virus (NDV) Vaccination Programs Against Velogenic NDV Genotype VII in Broiler Chickens.

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ABSTRACT

The protective efficacy of live and inactivated NDV- genotype II vaccines against challenge with velogenic Newcastle disease virus (VNDV) genotype VII (Chicken/USC/Egypt/2014), was evaluated in vaccinated groups (G1, G2, G3, G4 and G5) with inactivated vaccine on day 7 and live vaccine (LaSota) on days 7 and 15, in addition, with live V4 strain either on day 1 (G1) or on day 25 (G2) or in both days 1 and 25 (G3) and challenged on day 32 of age. The protection percentages were 96%, 96%, 96% and 88% in G1, G2, G3 & G4, respectively, vs 4% in non-vaccinated challenged chickens (G5). Moreover, the tracheal and cloacal viral shedding were 30% & 100% ; 0% & 0% ; 0% & 30%; 0% & 30% in vaccinated challenged G1, G2, G3 & G4, respectively, vs 100% & 60% in non-vaccinated challenged G5 at 3 DPC, whereas, the tracheal and cloacal viral shedding were 0% & 30% ; 0% & 0% ; 0% & 0% ; 30% & 60% in vaccinated challenged G1, G2, G3 & G4, respectively, vs 100% & 100% non-vaccinated challenged G5 at 7 dpc. However, the clinical signs, gross lesions and serological response were monitored till 14 dpc. Our results revealed that the inclusion of V4 vaccine strain in vaccination program is providing superior protection against NDV genotype VII and good safely replace live (LaSota) in early and late vaccination practices.

Key words: Vaccination, Newcastle disease, Genotype VII, V4 strain.

INTRODUCTION

Newcastle disease (ND) is highly contagious disease in chickens and turkeys and one of the most important diseases of poultry in the world, the infection causes sudden death with high mortality (Ahmed *et al.*, 2017). The causative agent of Newcastle disease virus (NDV) is an enveloped, single stranded, non-segmented and negative sense RNA virus, which belongs to genus Avulavirus, subfamily Paramyxovirinae and family paramyxoviridae in the order Mononegavirales and is designated as avian paramyxovirus 1 (APMV-1) (Mayo, 2002). In addition, NDV genome is of approximately 15 kb that contains six genes major encoding major structural proteins such as: nucleocapsid protein (NP), phosphoprotein (P), matrix protein (M), fusion protein (F), hemagglutinin-neuraminidase (HN), and the RNA dependent

RNA polymerase (L) (Kattenbelt *et al.*, 2006). Genotypically, APMV-1 is classified into two classes designated as class I and class II. The majority of APMV-1 strains, including both virulent and non-pathogenic strains, belong to class II within which are classified the viruses of economic importance to chickens, while class I isolates mainly circulate in wild waterfowl (Aldous *et al.*, 2003). APMV-1 strains have traditionally been classified as lentogenic (low virulence), mesogenic (intermediate virulence), or velogenic (highly virulent) based on the presentation of clinical signs in susceptible specific pathogen free chickens (Alexander and senne, 2008). Virulent APMV-1 strains are the causative agents of Newcastle disease (ND), one of the most economically important diseases for poultry production worldwide (Alexander and senne,

2008). Virulent viruses belong to genotypes V, VI and VII are highly mobile, spread globally and are responsible for the majority of recent outbreaks in poultry and wild birds worldwide (Dimitrov *et al.*, 2016). Recently VNDV genotype VII infection has been reported to cause outbreaks in commercial poultry farms and showed more severe symptoms and high mortalities (Ahmed *et al.*, 2017). In many countries, prophylactic vaccination is applied to control ND (Sonoda *et al.*, 2000). Nowadays, the strains of NDV used to produce ND vaccines, such as LaSota and B1, are phylogenetically in the same genotype of viruses isolated in the 1940s but are phylogenetically divergent or distant from strains causing the recent outbreaks of ND (Miller *et al.*, 2007 & Hu *et al.*, 2009). In some regions in the world vaccines containing lentogenic virus strains such as Ulster 2C, F and V4 are also used (Senne *et al.*, 2004). Furthermore, application of an intensive vaccination policy and annual use of ND vaccine has increased, but ND outbreaks have still occurred periodically across the world, even in well-vaccinated farms have raised questions about the antigenic variation of NDV and the efficacy of conventional vaccines (Cho *et al.*, 2008).

MATERIALS AND METHODS

Chickens:

One hundred and twenty-five, 1-day-old broiler chicks were obtained from a commercial hatchery, which possessed maternal derived antibody (MDA) against NDV using HI test. The chicks were floor reared under natural day light in strictly isolated experimental rooms previously cleaned, disinfected, and provided with commercial broiler starter ration. Water and feed both provided ad-libitum.

Newcastle disease virus and vaccines used in experiment:

- Live freeze –dried NDV vaccine genotype II V4 strain (Vaxsafe), vaccinal dose equal 6-log_{10} EID₅₀/ bird, obtained from (Bioproperities, Australia).

- Live bivalent virus vaccine against Newcastle disease virus (NDV) and Infectious bronchitis virus (IBV) diseases, vaccinal dose contains 6-log_{10} EID₅₀ of the NDV strain LaSota and 3-log_{10} EID₅₀ IBV (serotype Massachusetts), obtained from (Boehringer Ingelheim, Germany).

- Live LaSota strain genotype II, vaccinal dose contains 6-log_{10} EID₅₀ / bird, obtained from (Boehringer Ingelheim, Germany).

- Inactivated oil emulsion NDV (Volvac® B.E.S.T.) (AI+ND) genotype II LaSota strain vaccine, vaccinal dose equal EID₅₀ = 8.2-Log_{10} / bird, obtained from (Boehringer Ingelheim, Germany).

All vaccines were obtained under clean and complete aseptic conditions from local agency (IFT) international and were used in different vaccination regimes in this study as shown in Table 1.

Newcastle disease challenge virus:

Velogenic ND virus used in the challenge, was kindly obtained from Prof. Dr. Hesham Sultan, professor of birds and rabbit diseases, Faculty of Veterinary Medicine, University of Sadat City, and was characterized by sequencing as VNDV genotype VII_d designated as (Chicken/USC/Egypt/2014) under accession no.; (KM659400) on GenBank.

Sampling

Blood samples:

Chicken blood was collected from wing vein and kept in slop position at 37°C for one hour then at 4° C over night. Sera were separated by centrifugation at 3000 rpm /10 minutes and stored at -20 C till tested. Sera were inactivated at 56°C for 30 minutes before testing.

Tracheal and cloacal swabs:

Swabs were collected 3- and 7-days post challenge in sterile phosphate buffer saline, swabs were routinely processed individually. Infected materials were pooled and centrifuged at 500 rpm or 10 minutes.

NDV reference antigens and antisera:

- ND LaSota vaccinal strain was propagated in ECE and diluted to 4 HAU to be used as HA

antigen in haemagglutination inhibition (HI) titration of ND antibody.

- Known positive and negative NDV antisera, were obtained from MSD international, B.V. Boxmeer, Holland, supplied by local agency, and used in HI test according to (OIE, 2012).
Haemagglutination Inhibition (HI) test:

HI test was carried out according to the OIE manual, 2012 using 1% suspension of chicken red blood cells. The HI antibody titer was determined as the reciprocal of the highest serum dilution that had complete inhibition of haemagglutination.

Assessment of viral shedding by RT-PCR:

RNA from tracheal and cloacal swabs was extracted using Intron viral gene-spin viral RNA extraction kits according to the manufacturer's instructions. Two primers were used to amplify 254bp specific fragment in the F gene of VNDV according to (Kant *et al.*, 1997). The primers sequences were; ND A F 5'-TTG ATG GCA GGC CTC TTG C-3', and ND C R 5'-AGC GTY TCT GTC TCC T 3'b.

Thermo Scientific Verso® 1-Step RT-PCR Ready-mix Kit was used. First-strand cDNA synthesis was accomplished by incubating the mixture for 15 minutes at 50°C then at 94°C for 4 minutes for inactivation of Verso RT enzyme and initial denaturation. 40 amplification cycles of 94°C for 1 minute (denaturation), 50°C for 1 minute (annealing) and 72°C for 1 minute (extension) were conducted followed by a final extension cycle of 5 minutes at 72°C. After the end of PCR run the PCR product was analyzed with Agarose gel Electrophoresis using 2% Agarose gel stained with Ethidium bromide.

Statistical analysis:

Whenever necessary, analysis of variance –test (ANOVA) two way ANOVA was used for study the significant differences. The statistical analysis was carried-out using SPSSPC+ version (16)-computer program.

RESULTS

Clinical signs, post-mortem gross lesions, and mortalities:

Birds were monitored for two weeks (Wk) post challenge for presence of clinical signs and post-mortem gross lesions (PM). The birds in vaccinated challenged groups showed signs of dull appearance, decreased in water and feed intake at 3 days post challenge (PCh), also Mild greenish diarrhea was observed.

Clinical signs in non-vaccinated challenged (G5) started 2 days pch with marked decrease in feed and water intake, marked depression with sleepy appearance at 3 days pch. Severe respiratory sounds, conjunctivitis, nasal discharge 4 days pch, and paresis ended by nervous signs (tremors in head, neck and torticollis in head) observed in the remaining live chicken (Fig. 1).

Postmortem lesions varied from mild to severe lesions. Non-vaccinated challenged (G5) showing characteristic pm lesions of severe tracheitis, severe congestion of chest muscle, liver, pancreas and swollen kidneys, mottled spleen, Hemorrhagic spots and/or peticheal heamorrhage on the proventriculus (Fig. 2). Whereas, in vaccinated challenged (G1, G2, G3, G4 and G5) showed pm lesions of mild tracheitis and congestion of chest muscle (Fig. 3). Mortalities were recorded in all groups for 14 days post challenge, varied from 0% to 96% and started at 3 days pch. Mortalities in G1, G2, G3, G4 and G5 were 1/25 (4%), 1/25 (4%), 1/25 (4%), 3/25 (12%) and 24/25 (96%), respectively, as shown in Table 1.

Serology:

Our results observed in (Table 2 & Fig. 4), revealed that, there is a significant differences ($P < 0.05$) of the serological response among different types of vaccinated groups and also among different day after vaccination.

The serological response of all groups was monitored before challenge (24 and 31 days of age) and after challenge (39 and 46 days of age) using HI test. Blood samples were collected from each group, HI geometric means at 24 days of age in (G1, G2, G3, G4 and G5) were (6.4, 5.7, 5.7, 5.2 and 0.2) and at 31 days of age were (6.7, 6.9, 7.1, 6.2 and 0), respectively.

After challenge with velogenic Newcastle disease virus genotype VII "chicken/USC/Egypt/2014", blood samples were collected at 39 & 46 days of age whereas, HI geometric means after 7 dpc in (G1, G2, G3 & G4) were (7.6, 8.3, 7.4 and 8.3) and after 14 dpc, were (9.5, 9, 7.9 and 9), respectively, (Table 2 & Fig. 4).

Detection of viral shedding by RT-PCR:

Results were summarized in Table 3 & Fig. 5, 6 and 7, we observed significant differences ($P < 0.1$) among different groups in virus shedding in trachea and cloacal route at the 3rd and the 7th day post-challenge.

The viral shedding in tracheal and cloacal swabs were; 30% & 100%, 0% & 0%, 0% & 30%, 0% & 30% in vaccinated challenged G1, G2, G3 and G4, respectively, while it was 100% and 60% in non-vaccinated challenged G5 at 3 dpc. On the other hand, at 7 days post challenge the viral shedding in tracheal and cloacal swabs were; 0% & 30%, 0% & 0%, 0% & 0%, 30% & 60% in vaccinated challenged G1, G2, G3 & G4, respectively, while it was 100% & 100% non-vaccinated challenged G5.

DISCUSSION

Newcastle disease (ND) is one of the most important diseases of poultry and our area and is a global threat to commercial poultry production, especially velogenic strains of ND virus which cause a devastating disease of poultry. ND viruses of genotype VII had been associated with the most recent worldwide outbreaks in Asia, Europe, Africa, the Middle East and South America, that can be further divided into five (VIIa-e) sub-genotypes according to amino acid sequences of F gene (Liu *et al.*, 2003; Rui *et al.*, 2010; Tan *et al.*, 2010; Zhang *et al.*, 2011 and Perozo *et al.*, 2012).

The isolation and molecular identification of NDV genotype VII d in Egypt was reported for the first time between 2011 and 2012 through active surveillance from outbreaks affecting poultry farms (Radwan *et al.*, 2013). Available NDV vaccines induce protection against morbidity and mortality from a challenge with velogenic NDV strains, but several studies have

shown that they do not prevent infection and virus shedding, which may result in silent spread to other vaccinated birds. This might be due to insufficient immunity as a result of antigenic divergence between the vaccine strains and the circulating strains. Vaccines should provide optimal protection even to viruses that have drifted away antigenically from conventional ND vaccines based on genotype I and II strains (Kapczynski and King, 2005; Miller *et al.*, 2007; Hu *et al.*, 2009 and Miller *et al.*, 2009).

Since all APMV-1 isolates are of one serotype because antibodies to 1 strain can neutralize all NDV isolates. However, antigenic variation does occur and it has been suggested that the specificity of antibodies induced by a vaccine to the challenge or outbreak virus is important in reducing the clinical signs and the amount of virus shed from vaccinated bird (Miller *et al.*, 2007). It is essential to use a live vaccine in order to ensure a satisfactory level of protection during the first 3 weeks of life. After that time the immunity induced by inactivated oil adjuvant vaccine maintains the level of protection (Bennejean *et al.*, 1978). Now, there are many commercial live and inactivated oil adjuvant vaccines prepared from genotype I and/or genotype II NDV which are very effective as vaccines but repeated outbreaks of virulent NDV among vaccinated chickens indicate the need to revise the NDV vaccination strategy. Furthermore, several underlying factors may have contributed to vaccination failure such as the presence of immunosuppressive diseases as well as poor cross-immunity between the vaccines and field challenge virus strains (Yi *et al.*, 2011).

Recently VNDV genotype VII infection has been reported to cause outbreaks in commercial poultry farms and showed more severe symptoms and high mortalities (Ahmed, 2017).

Since, the goal of this experiment was to further explore the effect of different types of live vaccines in different ages against velogenic NDV challenge virus (Genotype VII).

In the present study, we assessed the degree of protection of different types of live vaccines and inactivated oil emulsion vaccine (genotype II) against challenge with velogenic Newcastle

disease virus (Genotype VII d) "chicken/USC/Egypt/2014" at 32- days of age in broiler chickens via intramuscular route. The degree of protection was assessed by; clinical signs, post-mortem gross lesions, mortalities, seroconversion and viral shedding.

Due to the greater potential for LaSota vaccines to cause respiratory disease, they are normally used for boosting NDV vaccines in chickens (Eidson and Kleven, 1980). In the present study, all groups vaccinated with inactivated oil adjuvant vaccine (B.E.S.T) on day 7 and live NDV vaccine (LaSota) on day 7 and 15, in addition, with live V4 strain either on day 1 (G1) or on day 25 (G2) or in both days 1 and 25 (G3) and challenged with velogenic NDV (chicken/USC/Egypt/2014) (Genotype VII) at 32 day-old and monitored for two weeks (Wks) post challenge for presence of clinical signs and gross lesions (PM). The birds in vaccinated challenged groups showed signs of dull appearance, decreased in water and feed intake at 3 days pch. Mild greenish diarrhea observed in all vaccinated challenged groups. Clinical signs in non-vaccinated challenged (G5) started 2 days pch with marked decrease in feed and water intake, marked depression with sleepy appearance at 3 days pch Severe respiratory sounds, conjunctivitis, nasal discharge 4 days pch, and paresis ended by nervous signs in remaining live chicken. These findings come in agreement with (Susta *et al.*, 2011 and Moussa, 2015) who observed the same clinical signs in SPF chickens after challenge with Genotype VII.

Clinical signs and post-mortem gross lesions are an important factors in assessment of protection against VNDV genotype VII, Since in poultry commonly pm lesions seen as hemorrhages in the spleen, trachea, proventriculus Payer's patches, cecal tonsils, bursa, and thymus. In addition, the spleen may be swollen, mottled and necrotic (Wakamatsu *et al.*, 2006; Susta *et al.*, 2011 and Miller and Koch, 2013). Moreover, splenomegaly, multifocal splenic necrosis and hemorrhagic spots on the proventricular glands were clearly detected by (Susta *et al.*, 2014). Similar results to these studies were detected in our study. All vaccinated challenged groups showed pm

lesions of mild tracheitis and congestion of chest muscles While, in non-vaccinated challenged groups showing characteristic gross lesions of velogenic NDV like severe tracheitis, severe congestion of chest muscle, swollen kidneys, heamorrhagic spots and/or peticheal haemorrhage on the proventricular glands

Almost all velogenic NDV isolates result in higher mortality rates in poultry may reach up to 100 % (Yang *et al.*, 1999 and Zhang *et al.*, 2010). In our study, the vaccinated groups showed decreasing mortalities compared to control group (96%). This agreed with (Dortmans *et al.*, 2014 and Susta *et al.*, 2014) who reported that adequate application of live attenuated and inactivated NDV vaccines provide a sufficient protection reached 100% in chickens challenged with VNDV. In the present study, the protection percentages were 96%, 96%, 96% and 88% in vaccinated chickens G1, G2, G3 & G4, respectively, vs 4% in non-vaccinated challenged chickens G5 (Table 1), and these results explained that the protective efficacy obtained from V4 strain vaccination (G1, G2, G3) was better than that obtained from vaccination with LaSota (G4). This higher protection by V4 strain may be due to mucosal immunity according to (Jayawardane and Spradbrow, 1995) who reported that oral vaccination of chickens with V4 virus induces a mucosal immune response

Haemagglutination inhibition antibody titre is one of the most direct factors to estimate the protection induced by ND vaccines, as it corresponds with protection level (Kapczynski and King 2005).

The serological response of all groups was monitored before challenge (24 & 31 days of age) and after challenge (39 and 46 days of age) using (HI) test. Blood samples were collected from each group, HI geometric means at 24 days of age in (G1, G2, G3, G4 and G5) were (6.4, 5.7, 5.7, 5.2 and 0.2) and at 31 days of age were (6.7, 6.9, 7.1, 6.2 and 0), respectively. Higher HI titer tested on day 24 was observed in G1 (V4 strain on day 1) due to mucosal immunity according to (Jayawardane and Spradbrow, 1995) who reported that oral vaccination of chickens with V4 virus induces a mucosal immune response.

After challenge with velogenic Newcastle disease virus genotype VII "chicken/USC/Egypt/2014", blood samples were collected at 39 & 46 days of age whereas, HI geometric means after 7 dpc in (G1, G2, G3 & G4) were (7.6, 8.3, 7.4 and 8.3) and after 14 dpc, were 9.5, 9, 7.9 and 9, respectively,

In previous study, Live LaSota strain was given through eye dropping route on day 7 and another dose on day 15 to produce secondary immune response (Timms and Alexander, 1977). The immune response of live ND vaccine by eye dropping route is reported to be superior to the drinking water route (Degefa *et al.*, 2004).

Another study was carried out by (Rauw *et al.*, 2009) who reported that the virulence of the virus appears to play a role in CMI stimulation and demonstrated an earlier and shorter CMI induced by a less virulent NDV vaccine strain, compared to a stronger and longer CMI mediated by a more virulent vaccines strain. Thus, the more virulent strain persisted longer in the bird and therefore was able to increase magnitude and duration of CMI.

Viral shedding is a highly relevant indicator of NDV vaccine efficacy, to effectively control NDV infections in the poultry industry, as well as reduction of viral shedding from vaccinated birds infected with NDV could potentially minimize the impact of an outbreak and help to prevent spread of disease (Kapczynski *et al.*, 2013).

Viral shedding was detected at 3 and 7 dpc by RT-PCR using specific primers for F gene of velogenic Newcastle disease virus (VNDV) in 10 tracheal and 10 cloacal swabs from each group.

The tracheal and cloacal viral shedding were 30% & 100% ; 0% & 0% ; 0% & 30%; 0% & 30% in vaccinated challenged G1, G2, G3 and G4, respectively, vs 100% & 60% in non-vaccinated challenged G5 at 3 DPC, whereas, the tracheal and cloacal viral shedding were 0% & 30% ; 0% & 0% ; 0% & 0% ; 30% & 60% in vaccinated challenged G1, G2, G3 & G4, respectively, vs 100% & 100% in non-vaccinated challenged G5 at 7 dpc

The tracheal shedding of the groups vaccinated with V4 strain on day 7 pch reduced to 0%, this finding disagreed with (Miller *et al.*, 2007),

who reported that ND vaccines do not protect vaccinated chicken from infection and viral shedding after challenge.

This result gives us a solid conclusion that using of V4 strain plus LaSota vaccines in vaccination programs against NDV is better than using LaSota alone. Also, it is preferred to use V4 strain in young age as it's less pathogenic than LaSota.



Fig (1): 34-days old chickens showing marked depression with sleepy appearance in non-vaccinated challenged chickens 2 days post challenge.

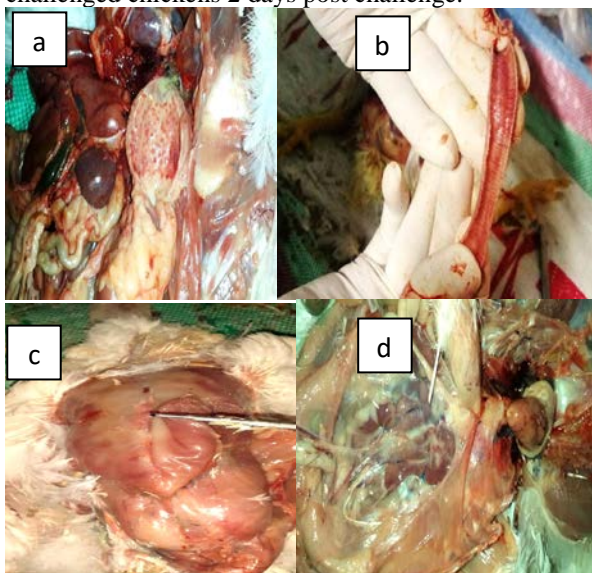


Fig (2): Gross lesions 3 days pch in G5 (non- vaccinated and challenged), a: showing hemorrhagic spots on proventricular glands and swollen mottled spleen. b: Severe tracheitis. c: severe congestion of chest muscle. d: showing swollen kidneys.



Fig (3): a: showing mild congestion in vaccinated and challenged G2. b: showing mild tracheitis in vaccinated and challenged G3.

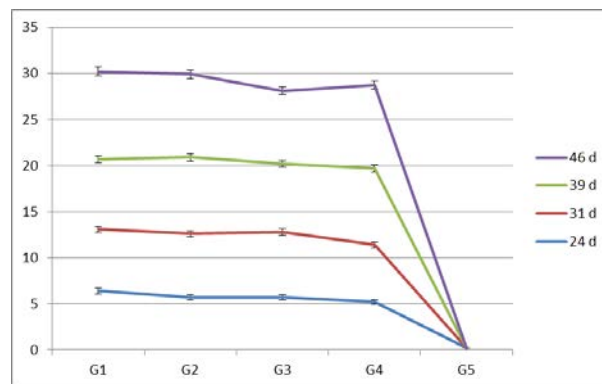


Fig (4): serological response of broiler chickens after vaccination with live lentogenic genotype II NDV vaccines with inactivated oil emulsion NDV vaccine genotype II and challenged with velogenic Newcastle disease virus (Genotype VII) "chicken/USC/Egypt/2014" at 32 days of age.

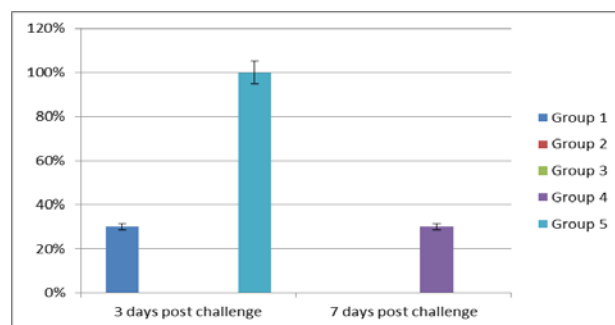


Fig (5): Tracheal viral shedding titre (\pm SD) in broiler chickens after vaccination with live lentogenic genotype II NDV vaccines and inactivated oil emulsion NDV vaccine genotype II and challenged with velogenic Newcastle disease virus (Genotype VII) "chicken/USC/Egypt/2014" at 32 days of age.

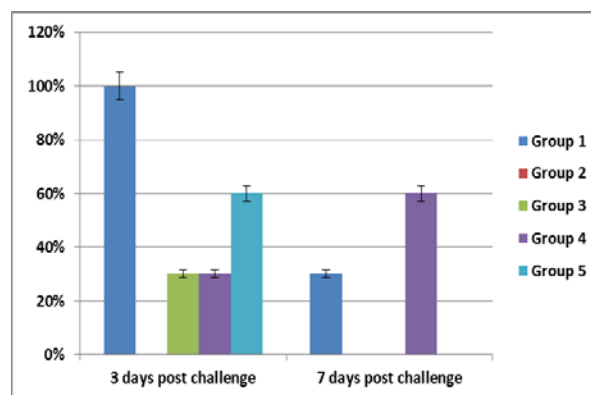


Fig (6): Cloacal viral shedding titre (\pm SD) in broiler chickens after vaccination with live lentogenic genotype II NDV vaccines and inactivated oil emulsion NDV vaccine genotype II and challenged with velogenic Newcastle disease virus (Genotype VII) "Chicken/USC/Egypt/2014" at 32 days of age.

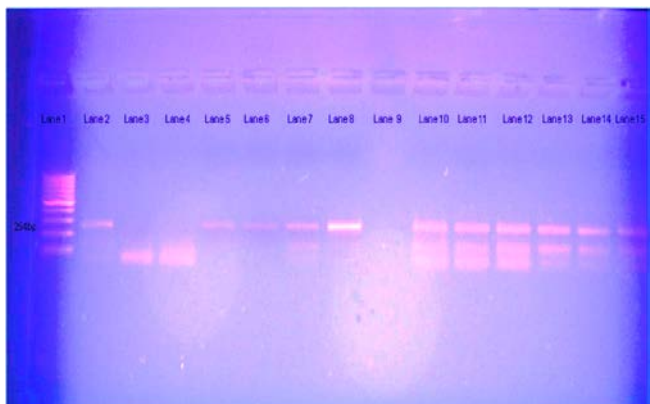


Fig (7): Agarose Gel Electrophoresis showing 254 bp RT-PCR Products of VNDV Fusion protein gene; lane 1: 100bp marker, lane 2: control positive, lane 5, 6, 7, 8, 10, 11,12, 13, 14 &15 are positive swabs for VNDV, lane 3, 4,&9 are VNDV negative swabs.

Table (1): Results of mortalities in broiler chickens after vaccination with live lentogenic with inactivated oil emulsion NDV vaccines genotype II and challenged with velogenic Newcastle disease virus genotype VII at 32-days of age:

Group no.	No. of birds	Vaccination regime			Challenge at 32 age/day ⁵ Mortalities at age/days pch ⁶														Mortality	
		Type	Age/day	Route of administration	1	2	3	4	5	6	7	8	9	10	11	12	13	14	No.	%
1	25	Vaxsafe ¹ LaSota IB ² B.E.S.T ³ LaSota ⁴	1 7 7 15	S/C oculonasal S/C Oculonasal	-	-	-	-	-	-	-	-	-	-	-	-	1	-	1	4 ^a
2	25	LaSota IB ² B.E.S.T ³ LaSota ⁴ Vaxsafe ¹	7 7 15 25	oculonasal S/C oculonasal oculonasal	-	-	-	-	-	-	-	-	-	-	-	1	-	-	1	4 ^a
3	25	Vaxsafe ¹ LaSota IB ² B.E.S.T ³ LaSota ⁴ Vaxsafe ¹	1 7 7 15 25	S/C oculonasal S/C oculonasal oculonasal	-	-	-	-	-	-	-	-	-	-	-	-	-	1	1	4 ^a
4	25	LaSota IB ² B.E.S.T ³ LaSota ⁴	7 7 15	oculonasal S/C oculonasal	-	-	-	1	-	1	-	-	1	-	-	-	-	-	3	12 ^b
5	25	None vaccinated			-	-	7	14	1	2	-	-	-	-	-	-	-	-	24	96 ^c

- Live NDV vaccine genotype II V4 strain (Vaxsafe), with vaccinal dose equal 6-log₁₀ EID₅₀/ bird given via subcutaneous route (0.3 ml/bird) or oculonasal route.
- Live bivalent vaccine against Newcastle disease virus (NDV) and infectious bronchitis virus (IBV), with vaccinal dose equal 6-log₁₀ EID₅₀ of the NDV strain LaSota and 3-log₁₀ EID₅₀ IBV (serotype Massachusetts), given via oculonasal route.

- Inactivated oil emulsion NDV genotype II LaSota strain vaccine. The vaccinal dose equal $EID_{50} = 8.2 - \text{Log}_{-10}$ vaccine given 0.5ml/bird via subcutaneous route(s/c).
- Live NDV vaccine LaSota strain, with vaccinal dose equal $6 - \text{log}_{-10} EID_{50}$ / bird given via oculonasal route.
- Challenge with velogenic Newcastle disease virus (genotype VII) .The challenge virus dose was $5.5 - \text{Log}_{-10} EID_{50}$ given 0.5 ml / bird via intramuscular route (I/M).
- All dead birds' swabs were VNDV positive by RT- PCR post challenge.
- no.: Number
- Small letters mean significant at ($P < 0.05$)

Table (2): Results of serological response after vaccination with live lentogenic genotype II NDV vaccines with inactivated oil emulsion NDV vaccine genotypeII and challenged with velogenic Newcastle disease virus genotype VII "chicken/USC/Egypt/2014" at 32- days of age. (N = 10).

Group no.	HI ⁶ . Titer means Log_{-2} at age/day			
	24	31	39	46
1	Ac	Bc	Cb	Aa
	6.4±0.40	6.7±0.70	7.6±0.60	9.5±0.50
2	Bd	Bc	Bb	Ba
	5.7±0.82	6.9±0.91	8.3±0.41	9±0.44
3	Bc	Ab	Cb	Ca
	5.7±0.57	7.1±0.15	7.4±0.44	7.9±0.19
4	Cd	Cc	Bb	Ba
	5.2±0.51	6.2±0.66	8.3±0.75	9±0.45
5	Ea	Db	Db	Db
	0.2±0.20	0	0	0

HI: heamagglutination inhibition

NT: not tested.

N: number of tested samples 10.

-Capital litters means: Means within the same column of different litters are significantly different at ($P < 0.05$).

-Small litters means: Means within the same row of different litters are significantly different at ($P < 0.05$).

Table (3): Results of viral shedding in broiler chickens after vaccination with live lentogenic genotype II NDV vaccines with inactivated oil emulsion NDV vaccine genotype II and challenged with velogenic Newcastle disease virus (Genotype VII) "Chicken/USC/Egypt/2014" at 32- days of age:

Group no.	Viral shedding at 3 and 7 days post challenge by RT-PCR (Positive no. / Examined no.)							
	3 days pch				7 days pch			
	TR		CL		TR		CL	
	no.	%	no.	%	no.	%	no.	%
1	3/10	30 ^a	10/10	100 ^a	0/10	0 ^a	3/10	30 ^a
2	0/10	0 ^b	0/10	0 ^b	0/10	0 ^a	0/10	0 ^b
3	0/10	0 ^b	3/10	30 ^c	0/10	0 ^a	0/10	0 ^b
4	0/10	0 ^b	3/10	30 ^c	3/10	30 ^b	6/10	60 ^c
5	10/10	100 ^c	6/10	60 ^d	1/1	100 ^c	1/1	100 ^d

- no: Number.
- TR: Tracheal swabs.
- Cl: Cloacal swabs.
- RT-PCR: conventional polymerase chain reaction with VNDV specific primers.
- NT: not tested.

Small litters mean significant at ($P < 0.01$)

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