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Evaluation for Efficacy of Commercially Available Vaccines Against Challenge with Newcastle Disease Virus Genotype VII in Broilers

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THE STUDY evaluated the protective efficacy of commercially available live and inactivated Newcastle Disease Virus (NDV) vaccines. Moreover, study also highlighted the implementation of genotypically-matched NDV vaccines to the currently circulated velogenic genotype VII strain in commercial broilers vaccination regimes in Egypt. Serum antibodies level was assessed by Haemagglutination Inhibition method .Efficacy of Newcastle Disease killed and live vaccine was also determined using challenge test. Group A immunized with live attenuated and inactivated NDV vaccines genotype VII homologous to challenge virus provided non-significant protection against mortality .It proved 20 % as compared to 15 % mortalities in group B immunized with live attenuated and inactivated NDV vaccines genotype II heterologous to challenge virus. Meanwhile, group Aredult proved a significant reduction in viral shedding by cloacal swabs in compared with group B ,7 days post-challenge. Final Results of the present work concluded that live and inactivated NDV genotype VII vaccines antigenically matched to currently epidemic NDV genotype VII providing better control on virus shedding if compared with different genotype II vaccines. However, both vaccines provided good protection against likely virulent challenge in commercial broilers.

Keywords: Vaccines, Newcastle, Disease Virus, Broilers, Genotype VII.

Introduction

Newcastle Disease virus (NDV) is a worldwide distributed virus, which is the sole member of Avian Paramyxovirus type 1 (APMV-1) of genus Avulavirus, subfamily Paramyxovirinae and family Paramyxoviridae and it affects almost all species of wildas well as domestic birds (1) ND is still amajor threat to the poultry industry in developing countries and currently NDV is widely present in all areas of Egypt and out breaks have been increased since NDV genotype VII was firstly identified in Egypt 2012 by (2).

ND vaccination in field include useing of attenuated live and killed vaccines for induceing protection against contagious diseases. Additionally, mutation changes in the circulating field virus after a period of time, makingthis phylogenetically different from classical vaccine strain that reduced the efficacy of ND live vaccine. Then, to improve the efficacy of commercial vaccine, new vaccines candidates must be investigated in the laboratories (3). Even with wide spreaduse of different types of commercially availableattenuated live and inactivated NDV vaccines more antigenically matched to currently circulated virus for better control of NDV epidemics which found to provide better protectionand reduce virus shedding from infected birds against challenge with virulent genotype VII NDV (4).

Therefore, present study was conducted to evaluate the efficacy of NDVcommercially available live and killed vaccine, using circulating

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isolate of NDV genotype VII, collected from recent outbreak in Egypt.

Materials and Methods

Ethics statement

The animal experiment was conducted in strict accordance with and adherence to the relevant policies regarding animal handling as mandated under international, national, and/or institutional guide lines for the care of animals and was approved by the Research Ethical Committee at the National Research Centre, Cairo, Egypt.

Chickens

Oe -day old commercial broiler chicks (Cobb 500®) were provided by certified local hatchery, ,chicks were divided intoequal three groups of 20 birds of each in separate units with strict biosecurity level. Conventional animal welfare regulations and food standards were taken into account.

Viruses and vaccines

NDV used in the challenge was characterized by sequencing as VNDV genotype VIId designated as "NDV/Chicken/EG-MN/NRC/2015" under accession no, (MF418020.1) on Gene bank. The virus was propagated in 9-day-old specific pathogen freeembryonated chicken eggsvia allantoic cavity inoculation and the virus challenge dose equal 6-Log-$_{10}$ embryo infective dose **(**EID $_{50}$**)** given 0.5 ml / bird via intramuscular route (I/M) (5).

Live attenuated NDVvaccines: freeze-dried vaccines containing live attenuated Newcastle Disease virus genotype VII (KBNP-C4152R2L strain, Himmvac®Dalguban N Plus2000 doses) and genotype II (LaSota strain, Jovac ND LaSota® 1000 doses) supplied by local agencies. The vaccinal doses equal 6-Log-$_{10}$ EID$_{50}$/ bird in 20 µlwere given via occulonasal route as recommended by manufacturers.

Inactivated NDV genotype VIId and genotype II vaccines (KBNP-C4152R2L strain,Himmvac® Oil Vaccine 1000 doses and LaSota strain, Jovac ND LaSota® 1000 doses, respectively) supplied by local agency. The vaccinal dose equal 8.2-Log-$_{10}$ EID$_{50}$ given 0.5 ml / bird via subcutaneous route (S/C) as recommended by manufacturer.

Serology

Blood sampleswas taken from all birdsandSerum was extraced.at designated days andtested by hemagglutination inhibition (HI) assay. The HI assaywas performed using inactivated NDV antigen (LaSota strain) with four haemagglutinatingunitsvirus/antigen in 0.050 ml according to standard procedures (5).

Virus shedding

Shedding of the virus was determined by collecting oropharyngeal swabs on day 3 and cloacal swabs on day 7 post-challenge. The presence of virus was determined by inoculating clarified swab samplesin 9-days-old embryonatedspecific pathogen free (SPF) chicken eggs and conducting HA assay three days later. Pools of swabs (n = 3 per group) from the same group was clarified via centrifugation at 1000 × g for 15 minutes. Virus titerswere calculated by titration using the standard methods (5) and are reported as mean embryo infectious dose (EID$_{50}$/0.1 ml) on a Log-$_{10}$ scale.

Statistical analysis

Data were analyzed by one way ANOVA with Tukey's post hoc test analyzed by SPSS 21 software to determine the significance of differences between individual treatments and corresponding controls. A probability (p) value ≤ 0.05 was considered statically significant.

Challenge experiment

Sixty day-old commercial broiler chicks were divided into three groups (A, B & C) of twenty birds. Chickens in group A vaccinated with live attenuated NDV vaccine (genotype VIId strain) at 5 and 19 days-old via occulonasal route and inactivated NDV vaccine (genotype VIId strain) at 7 days-old via subcutaneous route. Meanwhile, group B received live attenuated NDV vaccine genotype II (LaSota strain) also at 5 and 19 days-old via occulonasal route and inactivated NDV vaccine genotype II (LaSota strain) at 7 days-old via subcutaneous route. In addition, control group C of twenty non-vaccinated broilers was included. The three groups were challenged with VNDV genotype VIId at 28 days of age (Table.1).

Results

Protective efficacy of vaccination study in commercial broilers:

All chickens in non-vaccinated infected group C were died all during 4th and 5th days (n=20/20) post-challenge (pch) developing severe depression, marked respiratory manifestations and neurological signs of paralysis and torticollis. The autopsy lesions confirmed NDV infection depicted by hemorrhages in proventriculus, small

intestine and caecal tonsils with spleenomegally. While,no-significant different in mortality rate of chickens in vaccinated challenged group A and B were from each other ($P < 0.05$) with (20%, n= 4/20) and (15%, n=3/20), respectively 7 days pch (Table 2) . In addition, groups A and B revealed much less clinical signs as compared to unvaccinated controls showing reduced activity, mild depression, mild respiratory manifestations 7 days pch.

Serology

Mean Log $_2$ serum antibody titers against NDV antigen (LaSota) collected at 14, 21, 28 and 35 days-old in all groups are presented in (Table 3). Mean HI titers of unvaccinated control group C were reduced from Log $_2$ on the day 14 prior to challenge day (28 days-old) that were not able to protect chickens from NDV infection. On the other hand, non-significant difference in antibody titers were observed between vaccinated infected groups A and B at the same designated days that exhibited positive HI titers for NDV antigen (LaSota) which increased throughout the vaccination course with significantly higher titers from control group C. HI titers for Groups A and B were comparable to each other ranging from 3.14, 4.31, 5 and 6.12 Log $_2$ titers Vs 3.93, 4.86, 5.21 and 6.64 Log $_2$ titers in groups A and B, respectively pre- and post-challenge.

Virus shedding

To evaluate the capacity of live and inactivated NDV vaccines of different genotypes (VII and II) to inhibit viral shedding, or opharyngeal swabs were taken on day 3 pch and cloacal swabs on day 7 pch for virus titration by mean embryo infective dose 50. Oral swabs collected at day 3 pch exhibited positive with clearly detectable titers of 4.2, 4.7 and 8.5Log-$_{10}$ (EID_{50}/0.1 ml) in

TABLE1. Vaccination schedule for evaluation of different genotypes of NDV vaccines against challenge with velogenic NDV genotype VIId in commercial broilers.

Group	Birds no.	Vaccination regime		Challenge at age / day [e]
		Type	Age / days	
A	20	Live. GVII[a] Inact.NDVGVII[b]	5 & 19 7	28
B	20	Live. GII [c] Inact.NDV GII [d]	5 & 19 7	28
C	20	None	None	28

[a] Live recombinant NDV vaccine genotype VIId. The vaccinal dose equal 6-log-$_{10}$ EID_{50} / bird given via occulonasal route.
[b] Inactivated recombinant oil emulsion NDV vaccine genotype VIId. The vaccinal dose equal 8.2-Log-$_{10}$ EID_{50} given 0.5 ml / bird via subcutaneous route (S/C).
[c] Live NDV vaccine genotype II LaSota strain. The vaccinal dose equal 6-log-$_{10}$ EID_{50} / bird given via occulonasal route.
[d] Inactivated oil emulsion NDV vaccine genotype IILaSota strain. The vaccinal dose equal 8.2-Log-$_{10}$ EID_{50} given 0.5 ml / bird via subcutaneous route (S/C).
[e]Challenge with velogenic Newcastle disease virus (genotype VIId). The virus challenge dose equal 6-Log-$_{10}$ EID_{50} given 0.5 ml / bird via intramuscular route (I/M).

TABLE 2. Mortalitiespost-challenge with Velogenic NDV (Genotype VII):

Group	Birds no.	Challenge at age / day	Mortalities 7 days Post-challenge	
			no	%
A*	20	28	4	20
B *	20	28	3	15
C	20	28	20	100

*Denotes significant difference from control group C at ($P < 0.05$).
no number

groups A, B and C, respectively pch revealed significantly reduction of shedding in vaccinated groups A and B as compared to non-vaccinated controls C. Meanwhile, no significant difference was detected in oral shedding between group A and B 3 days pch. While, it was clearly detected that group A significantly reduced in cloacal shedding in compared with group B in titers 2.0 VS 3.6 Log-$_{10}$(EID_{50}/0.1 ml), respectively 7 days pch, as shown in (Table 4).

Discussion

The virulent NDV genotype VII has become the dominant genotype since 2012 in Egypt with many successful reported outbreaks up till now (2). The antigenic differences between the vaccine strain (exclusively genotype II) and circulating strain (genotype VII) may contribute to the outbreaks of disease. Therefore, the new vaccines based on currently circulating virus strain are urgently needed to control spread of the disease (4).

Although all NDV isolates are considered belongs to one serotype, vaccination with any NDV strain couldn't provide equal protection against all isolates. Therefore, in thoroughly controlled vaccination experiments, vaccination with the available attenuated vaccinestrain is not sufficient to protect birds against challenge with virulent field isolate, especially in respect to viral shedding, which contributes to viral spread (6). None the less, vaccinesa composed of strains which are more homologous to the challenge virus are more efficient at decreasing the number of infected birds and amount of virus shedding (7,8). Subsequently, in the present work levels of protection induced by the live and inactivated genotype VII genotype II NDV vaccines were compared following challenge with the recently

TABLE 3. Titer of antibodies against VNDV(Genotype VII) pre -challenge.

Group	Birds no.	HI titre means Log-2 at age / days (N = 8)			
		14	21	28	35
A *	20	3.24	4.31	5.00	6.12
B *	20	3.93	4.86	5.21	6.64
C	20	1.73▶	1.61▶	1.91▶	NT

* Denotes significant difference from control group C ($P < 0.05$).
▸HI titre ≤ 2 Log-$_2$considered negative (OIE, 2012).
NNumber of tested samples.
NT Nottested(All Birds of this group died at 4 and 5 days post challenge).
no number.

TABLE 4. Viral shedding post-challenge with VNDV (Genotype VII):

Group	Birds no.	Challenge at age / day	Virus Shedding at days post challenge *	
			3 days (oropharyngeal)	7 days (Cloacal)
A	20	28	4.2 [a]	2.0[b]
B	20	28	4.7[a]	3.6
C	20	28	8.5	NT

*Viral titers (log-10) expressed as mean embryo infectious doses per 0.1 ml from pool of oral or cloacal swabs (n=3 per group) taken at day 3 and 7, respectively post-challenge.
[a]Denotes significant difference from control group C 3dpc ($P < 0.05$).
[b]Denotes significant difference from group B 7dpc ($P < 0.05$).
NT None tested (All Birds of this group died at 4 and 5 days post challenge).

acquired genotype VIINDV isolatein commercial broiler chickens.

Almost all NDV genotype VII isolates are velogenic strains and resulting in higher mortality rates in poultry reached to 100 % (9). Accordingly, trials of vaccination against genotype VII NDV challenge have been carried out by (10 .1) concluded that, adequate application of live attenuated or inactivated NDV vaccines provided a sufficient protection in chickens challenged with velogenic NDV. Vaccination of chickens with live or inactivated LaSota strain has been used to control virulent Newcastle for several years, providing adequate protection against mortality (11, 12. 6). In the present study, the birds vaccinated with live and inactivated genotype II or genotype VII vaccines were reasonably protected from death and clinical signs, and no significant differences were recorded between Group A and B20% and 15% mortalities, respectively in compared with non- vaccinated infected group C that revealed serious clinical disease, post-mortem gross lesions with higher mortality rate (100%) 7 days pch. Similar results by (4) who suggested that both oil emulsion inactivated genotype VIId vaccine (aSG10 strain) and inactivated oil emulsion LaSota vaccine provided similar protection from deaths against challenge with NDV genotype VIId (SG10 strain). On the other hand (13 &14) indicated that recombinant genotype VII vaccines offered better protection from deaths against challenge with genotype VII NDV than classical genotype II vaccine.

Expecting that protection rate may peak approximately 3 weeks after primo-vaccination and then gradually decline (15), vaccinated groups received single dose of inactivated NDV vaccine at 7 days-old and the first NDV live vaccine 5 days-old to avoid its probable neutralization by maternal derived antibodies which are decreased by half every 3-4 days (16), and the second dose two weeks later on with the aim to test whether the protection rate will be maintained until the end of production period. Consequently, the challenge test was performed at 28 days-old, which is the case of birds vaccinated two times two weeks interval and challenged after 21 days from the first dose. Titers of anti-NDV antibodies were measured from second week post-initial vaccinal dose and every week prior challenge moreover one week after. Both live attenuated and inactivated genotype VII and II NDV vaccines induced a significant immune response in compared to non-vaccinated controls. While, no significant difference was detected between the vaccinated groups. However, genotype II group B showed little higher HI titers than genotype VII group A may be due to LaSota used as an antigen as mentioned by (7) when detected higher HI titers when the antigen used in the assay was homologous to the vaccine antigen. More commonly, it needs to be stressed that level of protective anti-NDV antibodies is not always the optimum estimate of protection of birds against NDV challenge. More commonly, HI titers of 6 Log-2 or higher are what typically thought of being protective (17). Our results emphasized this and further revealed that even mortalities and virus shedding following challenge with velogenic NDV were not completely inhibited when HI titers of both NDV vaccines genotype VII and II were 5.0 and 5.21, respectively at challenge day.

Like most vaccines, NDV vaccines do not prevent vaccinated birds from becoming infected with a vNDV and subsequently shedding the virus (18).However, most vaccines will significantly decrease the amount of virus shed in saliva and feces compared to non-vaccinated birds (19). The amount shed will depend on the immunity of the host, the host species infected, the amount and virulence of the challengevirus, the dose and type of ND vaccine and the time between vaccination and challenge. Virus 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 shed from vaccinated birds infected with NDV could potentially minimize the impact of an outbreak and help to prevent spread of disease (12. & 6). Our results suggested that genotype VII NDV vaccines have conferred a significant protection against virus shedding in compared with the genotype II vaccines when challenged with velogenic genotype VII NDV, in which it was clearly detected that group A significantly reduced in cloacal shedding in compared with group B in titers 2.0 VS 3.6 Log-$_{10}$ (EID_{50}/0.1 ml), respectively 7 days pch, as shown in (Table 4). Meanwhile, no significant difference was detected in oral shedding between group A and B 3days pch.Similar studies by (21. 7 , 20, 4) whom demonstrated that the NDV vaccine homologous with the challenge virus reduced viral shedding significantly more than the heterologous vaccines.

In conclusion, the live attenuated and inactivated genotype VII NDV vaccines which

formulated to be phylogenetically closer to potential outbreak viruses provided significant protection virus shedding than classical genotype II NDV vaccines. However, both genotypes conferred adequate protection from clinical disease and mortality against challenge with Velogenic Newcastle disease virus (Genotype VII) in commercial chicken broilers.

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Conflict of interest

Noconflict of interest

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تقييم كفاءة بعض اللقاحات المثبطة ضد فيروس النيوكاسل من النمط الوراثى السابع فى دجاج التسمين

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بالدراسات الفعليه الوقائيه لمرض النيوكاسل الفيروسى الحى (NDV المتوفره تجاريا عن طريق دراسة اللقاحات المطابقة وراثيا الحقلى المتداول لانظمة تحصين التسمين فى مصر كما تم تقييم الاجسام المضادة فى الدم وتحديد اللقاح الحى باستخدام اختبار التحدى حيث تم تقسيم الى مجموعات حيث حصنت المجموعة الاولى لفيروس النيوكاسل الحى المثبت كالاهما من النمط الجينى السابع والذى يتوافق مع العترة الفيروسية المستوطنة وأظهرت نتائجها نسبة نفوق ٢٠٪ والتى بدورها لم تختلف معنويا عن المجموعه المحصنه الثانيه والتى تلقت لقاح فيروس النيوكاسل الحى والمثبت من النمط الجينى بنسبة نفوق ١٥٪ خلال اسبوع بعد العدوة الصناعية تستخلص من نتائج الدراسة ان لقاحات فيروس النيوكاسل الحية والمثبطة والتى تتوافق جينيا مع العترة الفيروسية المستوطنة لها قدرة معنوية عالية على تخفيض الذرف والفيروسى مقارنة باللقاحات التقليدية من النمط الجينى الثانى والتى تختلف جينيا من الفيروسى المستوطنة ومع ذلك كل اللقاحات النمط الجينى الثانى والسابع توفر حماية جديد لطيور التسمين ضد النفوق.