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 Aadult 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 wilds as 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 inducing protection against contagious diseases. Additionally, mutation changes in the circulating field virus after a period of time, making this 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 spreadue of different types of commercially available attenuated live and inactivated NDV vaccines more antigenically matched to currently circulated virus for better control of NDV epidemics which found to provide better protection and reduce virus shedding from infected birds against challenge with virulent genotype VII NDV (4).

Therefore, present study was conducted to evaluate the efficacy of NDV commercially available live and killed vaccine, using circulating...
isolates 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 200®) were provided by certified local hatchery, chicks were divided into equal three groups of 20 birds in each 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 VIIId 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 free embryonated chicken eggs via allantoic cavity inoculation and the virus challenge dose equal 6-Log10 embryo infective dose (EID50) given 0.5 ml / bird via intramuscular route (I/M) (5).

Live attenuated NDV vaccines: freeze-dried Oil Vaccine 1000 doses and genotype II vaccines (KBNP-C4152R2L strain, Jovac ND LaSota® 1000 doses) supplied by local agencies. The vaccinal doses equal 6-Log10 EID50/ bird in 20 µl were given via ocularonasal route as recommended by manufacturers.

Inactivated NDV genotype VIIId and genotype II vaccines (KBNP-C4152R2L strain, Himmmvac® Oil Vaccine 1000 doses and LaSota strain, Jovac ND LaSota® 1000 doses, respectively) supplied by local agency. The vaccinal dose equal 8.2-Log10 EID50 given 0.5 ml / bird via subcutaneous route (S/C) as recommended by manufacturer.

**Serology**

Blood samples were taken from all birds and Serum was extracted at designated


**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 Log₂ 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 Log₂ 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 Log₂ titers Vs 3.93, 4.86, 5.21 and 6.64 Log₂ 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, oropharyngeal 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 8.5Log₁₀ (EID₅₀/0.1 ml) in

Table 1. Vaccination schedule for evaluation of different genotypes of NDV vaccines against challenge with velogenic NDV genotype VIIId in commercial broilers.

<table>
<thead>
<tr>
<th>Group</th>
<th>Birds no.</th>
<th>Vaccination regime</th>
<th>Challenge at age / day</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Type</td>
<td>Age / days</td>
</tr>
<tr>
<td>A</td>
<td>20</td>
<td>Live. GVII*</td>
<td>5 &amp; 19</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Inact.NDVGVII*</td>
<td>19</td>
</tr>
<tr>
<td>B</td>
<td>20</td>
<td>Live. GII</td>
<td>5 &amp; 19</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Inact.NDV GII</td>
<td>19</td>
</tr>
<tr>
<td>C</td>
<td>20</td>
<td>None</td>
<td>None</td>
</tr>
</tbody>
</table>

*Live recombinant NDV vaccine genotype VIIId. The vaccinal dose equal 6-log₁₀ EID₅₀/ bird given via occulonasal route.

*Inactivated recombinant oil emulsion NDV vaccine genotype VIIId. The vaccinal dose equal 8.2-log₁₀ EID₅₀ given 0.5 ml / bird via subcutaneous route (S/C).

Live NDV vaccine genotype II LaSota strain. The vaccinal dose equal 6-log₁₀ EID₅₀/ bird given via occulonasal route.

Inactivated oil emulsion NDV vaccine genotype IIILaSota strain. The vaccinal dose equal 8.2-Log₁₀ EID₅₀ given 0.5 ml / bird via subcutaneous route (S/C).

Challenge with velogenic Newcastle disease virus (genotype VIIId). The virus challenge dose equal 6-Log₁₀ EID₅₀ given 0.5 ml / bird via intramuscular route (I/M).

Table 2. Mortalities post-challenge with Velogenic NDV (Genotype VII):

<table>
<thead>
<tr>
<th>Group</th>
<th>Birds no.</th>
<th>Challenge at age / day</th>
<th>Mortalities 7 days Post-challenge</th>
</tr>
</thead>
<tbody>
<tr>
<td>A*</td>
<td>20</td>
<td>28</td>
<td>4 20</td>
</tr>
<tr>
<td>B *</td>
<td>20</td>
<td>28</td>
<td>3 15</td>
</tr>
<tr>
<td>C</td>
<td>20</td>
<td>28</td>
<td>20 100</td>
</tr>
</tbody>
</table>

*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\textsubscript{10} (EID\textsubscript{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

<p>| TABLE 3. Titer of antibodies against VNDV (Genotype VII) pre-challenge. |</p>
<table>
<thead>
<tr>
<th>Group</th>
<th>Birds no.</th>
<th>HI titre means Log-2 at age / days (N = 8)</th>
<th>14</th>
<th>21</th>
<th>28</th>
<th>35</th>
</tr>
</thead>
<tbody>
<tr>
<td>A *</td>
<td>20</td>
<td>3.24</td>
<td>4.31</td>
<td>5.00</td>
<td>6.12</td>
<td></td>
</tr>
<tr>
<td>B *</td>
<td>20</td>
<td>3.93</td>
<td>4.86</td>
<td>5.21</td>
<td>6.64</td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>20</td>
<td>1.73</td>
<td>1.61</td>
<td>1.91</td>
<td>NT</td>
<td></td>
</tr>
</tbody>
</table>

* Denotes significant difference from control group C (P < 0.05).
* HI titre ≤ 2 Log\textsubscript{2} considered negative (OIE, 2012).
N Number of tested samples.
NT None tested (All Birds of this group died at 4 and 5 days post challenge).

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

<table>
<thead>
<tr>
<th>Group</th>
<th>Birds no.</th>
<th>Challenge at age / day</th>
<th>Virus Shedding at days post challenge *</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>3 days (oropharyngeal)</td>
</tr>
<tr>
<td>A</td>
<td>20</td>
<td>28</td>
<td>4.2 *</td>
</tr>
<tr>
<td>B</td>
<td>20</td>
<td>28</td>
<td>4.7p</td>
</tr>
<tr>
<td>C</td>
<td>20</td>
<td>28</td>
<td>8.5</td>
</tr>
</tbody>
</table>

*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.
Denotes significant difference from control group C 3dpc (P < 0.05).
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 strains ranging from 100% (9). Accordingly, trials of vaccination against genotype VII NDV challenge have been carried out by (10 .1) concluded that, adequate protection observed in vaccinated or non-vaccinated control groups, suggesting (by maternal immunity) that the NDV genotype VII strain used in the trials showed lower HI titers than genotype VII group A used 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 challenge virus, 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 shedding from vaccinated infected birds which 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.

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

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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
No conflict of interest

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References


