Potential Interference of Maternally Derived Antibodies (MDA) of Commercial Broiler Chicks on the Efficacy of Different Vectored –ND Vaccines

Samir A. Nassif, Esraa Fouad, Sahar M. Saber, Mahmoud M. Abotaleb, Ahlam Mourad
Central Laboratory for Evaluation of Veterinary Biologics, Agricultural Research Center (ARC), Abbassia, Cairo Egypt, P.O.B.131

ABSTRACT

Key words:
Newcastle disease virus, Maternally-derived antibodies, Recombinant ND vaccine, immune response and protection.

Using of recombinant vaccines are increased nowadays in broiler chickens due to its easy application at the hatchery. However, there are many factors could hinder its efficacy, one of them is the Maternal Derived Antibodies (MDA) against both the vector and the insert of a vaccine. To investigate this point, three different commercial recombinant vaccines used with only one insert (F gene of NDV) and the three different vectors (MDV, FPV and NDV). Two hundred and forty, one day old commercial broiler chicks were divided into eight groups, each of thirty chicks. The immune response and protection percentages offered by each vaccine were studied when applied as recommended by its manufacturer company and two vaccination strategies were suggested according to the results including prime boost strategy with the use of inactivated ND vaccine besides the recombinant vaccines/or changing the age of vaccination by the recombinant vaccine especially the FP-NDV vaccine to be 10 days instead of one day old (DO).

1. INTRODUCTION
Newcastle disease (ND) is a highly contagious viral disease affecting many domestic and wild avian species that caused by avian Paramyxovirus serotype 1 (APMV1), which has been placed in the genus Avulavirus, family Paramyxoviridae (Naveen, et al. 2013). ND is a major obstacle threatens broiler industry in Egypt. The disease was characterized by gastrointestinal disorder and respiratory signs often associated with nervous disorders and high mortality (up to 100 %) (OIE, 2012). ND can vary from clinically inapparent to highly virulent forms depending on the virus strains and host species (Alexander, 1997). Newcastle disease virus (NDV) has been categorized into five pathotypes based on clinical signs in infected chickens, designated: (viscerotropic velogenic, neurotropic velogenic, mesogenic, lentogenic and asymptomatic) (Tulu, 2020).
Vaccination and biosecurity are widely used as management practices for the protection against ND virus (OIE, 2018). Vaccination is mandatory for commercial and backyard poultry. Vaccination with inactivated oil- vaccine and different low-virulence live vaccine strains, such as Hitchner B1, Clone 30 and LaSota, have been extensively used in commercial farms. With the progress of immunology, molecular biology and microbiology, the technologies for vaccine development evolve rapidly. Recombinant virus vectors represent a powerful and promising platform to produce efficient vaccines. The first and foremost reason for using a vectored vaccine is safety. Some live vaccines used in the poultry industry have some disadvantages such as (interference with maternal immunity, horizontal spreading, reversion to virulence and vaccine reactions, any of which may result in disease or production loss) (OIE, 2018). While the vectored vaccines, the donor gene is inserted into a ‘safe' vaccine vector, thus separating the key protective antigen from the live donor organism. Since the protective gene is removed from the donor organism and is inserted into a vector, the characteristics of the donor organism are
no longer a factor, while the characteristics of the vector become more important. Initially, DNA viruses, such as herpesvirus and adenovirus, were used as vaccine vectors (Willemsen, et al., 1996). After that, numerous RNA viruses have been explored as vehicles for foreign immunogens (Lundstrom, et al 2019). The small size of the NDV genome, along with the low likelihood of genetic recombination, facilitates the use of NDV as a vaccine vector. Several characteristics of NDV suggest that recombinant NDVs expressing a protective antigen of another avian pathogen would be a very good multivalent vaccine for poultry. Different of these vaccine batches are submitted periodically to the Central Laboratory for Evaluation of Veterinary Biologics (CLEVB), Cairo, Egypt for evaluating its efficacy. Potency of these vaccines was tested following the standard evaluation protocols (European Pharmacopoeia, 2017; OIE, 2018). So, the interesting questions arise, why the velogenic ND has been enzootic in commercial poultry despite, application of intensive vaccination programs. Maternally derived antibodies (MDA) were considered as a very important factor that affect vaccine efficacy in broiler chickens (Sedeik et al., 2019). Although MDA can prevent or reduce clinical disease by passive immunization during the first weeks of the chick’s life (Al-Natour, et al., 2004), they can also reduce the production of neutralizing antibodies as seen with NDV. Previous literatures proved that efficacy of ND vaccines was poor in commercial chickens with MDA (Eidson, et al., 1976). (Suardana, 2023) said that the high level of maternal antibodies in the young chickens may interfere with the efficacy of the vaccines and reduce the amount of produced immunity. Subsequently, the designing of good vaccination program is very important to avoid vaccination failure and able to completely prevent outbreaks against the currently circulating viruses. 

The aim of the present study was to evaluate potential interference of maternally derived antibodies (MDA) of commercial broiler chicks on the efficacy of different vectored–ND vaccines (Recombinant Fowl Pox-ND (rFP-ND), Recombinant ND(Clone30) - ND (G7), Recombinant ND (Lasota) – ND (G7), Recombinant HVT-ND vaccines) against a local velogenic ND virus genotype VII.1.1 challenge.

2. MATERIAL AND METHOD
2.1 Ethical approval
All methods in the study were performed according to relevant guidelines and regulations. All experiments were carried out according to ARRIVE 2.0 guidelines and were approved by the Institutional Animal Care and Use Committee (IACUC) in the Faculty of Veterinary Medicine, Cairo University (Code: VetCU01102020217).”

2.2 Vaccines

2.2.1 Commercial recombinant Fowl - Pox + ND vaccine
Suspension vaccine contains a Fowl pox virus which used as vector and containing an insert of the F protein gene of ND VII. It was administrated according to manufacturer instructions.

2.2.2 Commercial recombinant HVT + ND
The vaccine contains one active ingredient: the modified live turkey herpes virus. (rHVT/ND) which is a recombinant serotype 3 Marek’s disease virus (MDV) (also called Meleagris Herpesvirus 1). It was constructed using the FC-126 HVT vaccine strain as a vector, genetically modified to express the immunogenic Fusion (F) gene of Newcastle disease virus (NDV) genotype II. The parent MDV strain (FC-126) is naturally avirulent and has already been used to prepare vector vaccines for chickens. It was administrated according to manufacturer instructions.

2.2.3 Commercial recombinant Lasota (ND) + ND (G7)
Each dose contains at least 106.5 EID50 of rND: LaSota F2+F7 as a vector and rNDV2/G7/2017 which act as an insert. It was administrated according to manufacturer instructions.  

2.2.4 Commercial recombinant Clone (ND) + ND (G7)
Each dose contains at least 106.5 EID50 of rND: Clone VG/GA-F7 as a vector and rNDV2/G7/2017 which act as an insert. It was administrated according to manufacturer instructions.

2.2.5 Inactivated commercial ND vaccine
The commercial inactivated NDV vaccine used for vaccination of chickens was contained (LaSota) . It was administrated according to manufacturer instructions.

2.3 Potency
Potency test was done through titration of the vector of each of the tested vectored ND vaccines according to OIE (2018).

2.2 Commercial chicks and experimental design
Two hundred and forty, Two hundred and forty, one day old commercial broiler chicks (Arbor Acres) were kindly provided from Cairo Company for commercial poultry production in Egypt. During the experiment
period, the chicks were housed in BSL3 chicken isolators. Birds were divided into eight groups as illustrated in Table 2. Ten individual blood samples were collected at one (DO) before grouping then collected from all groups at 10\textsuperscript{th}, 17\textsuperscript{th}, 24\textsuperscript{th}, 31\textsuperscript{st} and 38\textsuperscript{th} DO and sera were examined for detecting of antibodies using ELISA test. Challenge test were applied for vaccinated and control groups at the age of 31\textsuperscript{st} day old. Following challenge, birds were monitored daily for the development of either clinical signs or mortality. Cloacal swabs were collected from challenged vaccinated as well as control groups at 3\textsuperscript{rd}, 5\textsuperscript{th}, 7\textsuperscript{th} and 10\textsuperscript{th} days post challenge (DPC) for detection of the NDV shedding.

2.3 Challenge Virus
Local NDV genotype VII.1.1 was obtained from Strain Bank Department of Central Laboratory for Evaluation of Veterinary Biologics (CLEVB) which has accession no. KM288609 to be used as challenge virus. Viral propagation and titration [calculation of Egg infective dose/50 (EID\textsubscript{50})] were carried out according to OIE (2018). The test was carried out according to (OIE. 2018) through inoculation of NDV genotype VII.1.1 at a dose of 106 EID50/bird for 20 chickens in each vaccinated group and 10 chickens in control group intramuscularly with a dose 0.5ml/bird.

3.5 Enzyme Linked Immunosorbent Assay (ELISA)
The test was done using indirect Newcastle disease virus - fusion protein Antibody test kit (NDV-F-ELISA) for monitoring of the post vaccination immune response of the ND vector vaccines (Biocheck, Cat. No. CK122). ELISA test was performed according to the manufacturer instructions and the results were interpreted as, sample with antibody titer of 993 or more (2.997 log10) was positive while lower than this titer is referred to no antibody detected (negative).

3.9 Measurement of viral shedding
Individual cloacal swabs were taken before challenge at 31 DO from 10 vaccinated chickens of groups (4,5) to check presence or absence of the vaccinal NDV before challenge using real time RT-PCR. Also, individual swabs were taken from 10 chickens from all vaccinated challenged groups and 5 from non-vaccinated challenged control group at 3\textsuperscript{rd}, 5\textsuperscript{th}, 7\textsuperscript{th} and 10\textsuperscript{th} DPC and prepared, then kept at -80C till tested using real time RT-PCR which carried out according (OIE. 2018).

3.10 RT-qPCR:
RNA was extracted from swabs using QIAamp Viral RNA Mini Kit that supplied from (Qiagen, Valencia, Calif., and USA) Cat. No.52906. RNA was amplified using Invitrogen superscript® III platinum® one- step Quantitative RT-PCR kit (Cat. No 11732-088) to investigate the presence or absence of ND virus following the manufacturer instructions. The primers and probe were specific for the M gene of NDV following Wise et al., 2004; the test was conducted in a CFX 96 Deep well TM Real Time system.

Table 2. Experimental design

<table>
<thead>
<tr>
<th>Group No.</th>
<th>Age of vaccination (Day old age)</th>
<th>Vaccine</th>
<th>Route of administration</th>
<th>Blood sampling (days)</th>
<th>Challenge (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>One day</td>
<td>Recombinant Fowl-Pox +ND</td>
<td>S/C</td>
<td>10,17,24, 31 and 38</td>
<td>31</td>
</tr>
<tr>
<td>2</td>
<td>Ten days</td>
<td>Recombinant Fowl-Pox +ND</td>
<td>S/C</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>One day- Ten days</td>
<td>(Priming) Recombinant Fowl-Pox +ND (Boosting) Inactivated ND</td>
<td>S/C + I/M</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Ten days</td>
<td>Recombinant ND (Clone)+GD7</td>
<td>ED</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Ten days</td>
<td>Recombinant ND (Lasota)+GD7</td>
<td>ED</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>One day</td>
<td>Recombinant HVT+ND</td>
<td>S/C</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>Ten days</td>
<td>Inactivated ND</td>
<td>I/M</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>Control</td>
<td>Non-vaccinated</td>
<td></td>
<td>1,10,17,24, 31 and 38</td>
<td></td>
</tr>
</tbody>
</table>

S/C: subcutaneous; I/M: intramuscular; ED: eye drop
Table 1. Oligonucleotide primers are used in RT-PCR for detection of NDV M-gene.

<table>
<thead>
<tr>
<th>Primer</th>
<th>Sequence (5’ - 3’ )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Forward ND-M+4100</td>
<td>AGTGATGTGCTCGGACCTTC</td>
</tr>
<tr>
<td>Reverse ND-M-4220</td>
<td>CCTGAGGAGAGGCATTTTGCTA</td>
</tr>
<tr>
<td>Probe</td>
<td>HEX-TTCTCTAGCAGTGAGAGCTG-BHQ</td>
</tr>
</tbody>
</table>

4. RESULTS

4.1 Antibody titer of vaccinated chickens using ELISA test.

The table 3 illustrated that the maternal antibody titer was high at the 1st day old (4.42 log10). After that, the titer was declined gradually till became negative at 24th DO. On the other hand, groups (1 and 3) which vaccinated at 1st DO show lower antibody titer recording 3.5 and 3.51 log 10 respectively when compared to group 6 and control group. At 17th DO antibody titer of all vaccinated chicken groups were decreased and become negative. After that, antibody titers of all vaccinated groups were increased gradually till reach satisfactory antibody titer in the 24th DO except group 7. It was noticed that at 31st DO, all vaccinated groups become positive with antibody titer ranged from 3.2 to 3.8 log10 then peaked at 38th DO. Meanwhile, there is a significant difference (P < 0.05) among vaccinated groups at 31st and 38th DO and group 3 achieved the highest ELISA antibody titer among all those vaccinated groups.

4.2 Protection % of vaccinated and non-vaccinated chicken groups against local virulent NDV genotype VII

The protection% was illustrated in table (3). It was found that post-challenge survival of chickens experimentally infected with virulent NDV genotype VII was significantly increased (P < 0.05) in vaccinated groups compared to the controls. The protection % were 80, 95, 95, 85, 85 and 90% for G1, G2, G3, G4, G5 and G6, respectively. While in G7 that received inactivated ND vaccine alone it was 60%. The protection% was 0% in the non-vaccinated chicken group.

Table 3. ELISA antibody titers and protection % of all chicken groups.

<table>
<thead>
<tr>
<th>Chicken group</th>
<th>Mean ELISA antibody titer (Log10)</th>
<th>Protection %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1st DO</td>
<td>10th DO</td>
</tr>
<tr>
<td>Group 1</td>
<td>4.42±.05</td>
<td>3.5±.07</td>
</tr>
<tr>
<td>Group 2</td>
<td>3.8±.12</td>
<td>2.67±.12</td>
</tr>
<tr>
<td>Group 3</td>
<td>3.51±.06</td>
<td>2.56±.13</td>
</tr>
<tr>
<td>Group 4</td>
<td>3.8±.12</td>
<td>2.28±.15</td>
</tr>
<tr>
<td>Group 5</td>
<td>3.8±.12</td>
<td>2.3±.15</td>
</tr>
<tr>
<td>Group 6</td>
<td>3.8±.12</td>
<td>2.86±.06</td>
</tr>
<tr>
<td>Group 7</td>
<td>3.8±.12</td>
<td>2.63±.09</td>
</tr>
<tr>
<td>Group 8</td>
<td>3.8±.12</td>
<td>3.19±.18</td>
</tr>
</tbody>
</table>

The arithmetic means and ± standard deviation of ELISA antibody titer. Cut off value of used kit for antibody titer ≥2.997 (log 10).

* = statistically significant difference at P < 0.05
**Figure 1.** Antibody titer of chicken groups using ELISA test

**Figure 2.** Protection % of chicken groups

**Table 4.** Viral shedding titer

<table>
<thead>
<tr>
<th>Chicken group</th>
<th>Titer of Virus Shedding (log10)</th>
<th>Mean titer of viral shedding</th>
<th>Mean titer of reduction of viral shedding</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3rd DPC</td>
<td>5th DPC</td>
<td>7th DPC</td>
</tr>
<tr>
<td>Group 1</td>
<td>3±.13*</td>
<td>3.4±.12 *</td>
<td>1.8±.16</td>
</tr>
<tr>
<td>Group 2</td>
<td>1.5±.14</td>
<td>1.7±.1</td>
<td>-</td>
</tr>
<tr>
<td>Group 3</td>
<td>1.4±.18</td>
<td>1.5±.15</td>
<td>-</td>
</tr>
<tr>
<td>Group 4</td>
<td>2.9±.22</td>
<td>3.1±.18</td>
<td>1.5±.14</td>
</tr>
<tr>
<td>Group 5</td>
<td>2±.17</td>
<td>2.5±.16</td>
<td>1.3±.11</td>
</tr>
<tr>
<td>Group 6</td>
<td>3.5±.08*</td>
<td>3.7±.23 *</td>
<td>2.5±.18</td>
</tr>
<tr>
<td>Group 7</td>
<td>5.1±.19</td>
<td>5.3±.08</td>
<td>-</td>
</tr>
</tbody>
</table>

Data represent arithmetic mean ± standard deviation of NDV M-protein gene copies in 1 ml of swab

*=statistically significant difference at P< 0.05
4.3 Viral shedding titer

It was noticed that the viral shedding titer was reached to the maximum at 5th DPC of all challenged vaccinated groups with a variable titer (table 4). Mean shedding titer in vaccinated challenged groups were significantly reduced (P < 0.05) compared to control challenged group. Also, Groups 1 and 7 shed significantly (P < 0.05) more virus among vaccinated challenged groups at 3rd and 5th DPC. The mean Viral shedding titer from vaccinated groups (1-7) were 2.05, 0.8, 0.725, 1.85, 1.8, 1.125 and 2.97 log10 respectively, while it was 5.2 log10 for non-vaccinated challenged group therefore there is a significant reduction in viral shedding titer in vaccinated challenged groups compared to the control non-vaccinated challenged group with more than 2 log10. Concerning groups 4& 5, there is no vaccinal strain viral shedding at the 31st DO.

5. DISCUSSION

Newcastle disease (ND) is one of the most important diseases of poultry in the world. The infection causes sudden death with high mortality up to 100%. The history of combating viral diseases proves that vaccination is undoubtedly the most protective means to save lives from infection. Several live, inactivated, and recombinant ND vaccines were licensed to control the disease. Despite intensive vaccination, the continued outbreaks of NDV in domestic poultry were recorded in Egypt. So, the need for sustainable surveillance for variant field viruses and updating vaccines strains were become necessary to improve vaccine protection. Moreover, new vaccines and vaccination programs using recombinant ND vector vaccines can also potentiate infection control for multiple diseases at the same time, especially for the vaccines have HVT as a vector, but for the other vectored ND vaccines like FPV-ND, Clone ND and LaSota-ND vaccines, the interference of MDA need more investigations. So, this study aimed to evaluate potential interference of maternally derived antibodies (MDA) of commercial broiler chicks on the efficacy of different vectored –ND vaccines. Concerning control chickens (group 8), it was found that the obtained antibody titer at 1st DO was the maximum. After that, the antibody titer declined gradually till reaching a negative at 24th DO. These findings were in concordance with those previously published by (Bertran et al., 2018) who proved that both AIV and NDV MDA completely declined to non-detectable titers by 3 weeks post-hatch. The post vaccination (PV) immune response (ELISA antibody titer), protection percentages and reduction in virulent NDV shedding in groups 1, 2 and 3 that received commercial recombinant FP-ND at 1st, 10th and prime post at 1st day then inactivated NDV vaccine at 10th day old were 3.42, 3.69 and 3.8 log10 antibody titer respectively and 80%, 95% and 95% respectively. On the other hand, the reduction of virulent NDV shedding PC was 3.15, 4.4 and 4.475 log10 respectively. All the above results indicate the obvious interference of the MDA with the rFP-ND vaccine in group 1 that received the vaccine at first day old. This finding was confirmed by the absence of that interference in SPF chicken group that received the same vaccine at the 1st DO (previous unpublished data). Also in the current study in group 2 that received the same vaccine at 10th DO, there is no interference of the MDA with the efficacy of that vaccine. These results were agreed with that of (Shahin et al. 2019) was recommended to use a live recombinant vaccine to obtain a satisfactory protection level during the first 3 weeks of life. After that, the immunity exhibited by inactivated oil adjuvant vaccine maintains the level of protection. NDV
strains primarily replicate in the respiratory tract and elicit mucosal immunity and subsequently humoral and cellular immunity (Peeters et al., 1999).

So, if consider the application of the recombinant FP-ND vaccine in broiler chickens, vaccination at hatchery is easier and more practical, so the prime post strategy was designed and applied in the current study (group 3) which revealed significantly higher immune response 3.8 log10 antibody titer, 95% protection% and reduction in viral shedding by 4.4 log10 compared to group 1.

While in our study, it was found that the two groups (4 and 5) which inoculated at 10 DO by rND (Clone)-G7 and rND (Lasota) -G7 respectively, compared to the groups vaccinated by other vectored ND vaccines, confer 85% protection%, immune response 3.44 & 3.4 log10 antibody titer and reduction in viral shedding by 3.35 & 3.4 log10 respectively due to partial neutralization of these vectors with MDA which have a partial negative effect of on insert transcription.

It was found that the vaccinated chicks by rHVT-ND vaccine in group 6 can produce satisfactory immune response 3.7 log10 antibody titer, 90% protection% and reduction in viral shedding by 4 log10 despite high level of MDA. This may be attributed to the ability of Marek’s disease virus to escape the MDA (Reddy et al., 2016).

Regarding chickens in group 7 which vaccinated subcutaneously at 10th DO by inactivated ND vaccine had exhibited unsatisfactory immune response, protection and reduction in viral shedding when compared to vectored ND vaccines, these results were agreed with (Pan et al., 2022) who suggested that high or medium titers of MDAs might explain the inactivated vaccine failure in the field.

From the above-mentioned results, it was noticed that there is a gap in the antibody titer between 17th to the 20th DO in all vaccinated groups, so further studies were requested to overcome this problem.

6. CONCLUSION

It was concluded that vaccination with vectored FP-ND vaccine at 1-day old broiler chicks in hatchery affected with MDA and exhibited lower immune response (antibody titers and protection percentage) than that vaccinated at 10 DO. Therefore, when using Recombinant FPV-ND vaccines in broiler chicks at hatchery, it is recommended to be supported by inactivated NDV vaccine at 10th DO (prime-boost strategy) to obtain the maximum protection against NDV-VII.1.1. In addition, vectored HVT-ND vaccine proved to escape the MDA and confer satisfactory immune response and protection percentage when administrated at one day old in hatchery. Also, Vaccination with vectored LaSota-ND & Clone-ND vaccines at 10th DO confer satisfactory immune response and protection percentage.

Conflict of Interest: None

Funding Information: All authors declare that the works conducted in the present study did not receive funds from internal or external funders.

7. REFERENCE


Pan, X., Xin, Su., Pingyun, D., Jinhua, Z., Hongrui, C., Dawei, Y., Qiaoyang, T., Xuesong, L., Nancy, B.,


