Assessment of Immunity of Arian Broiler Breeders to Newcastle Disease Virus after Vaccination by Hi: A Retrospective Study

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Abstract

Newcastle disease (ND) is one of the most important poultry diseases and distributed worldwide causing devastating losses in poultry industry. Vaccination against ND is widely practiced. It is essential that the effectiveness of vaccination programs be evaluated. In this study, Arian broiler breeder flocks vaccinated with the same vaccination program during rearing period. At 18 weeks old, inactivated oil emulsion multivalent vaccines containing NDV antigen administered to these flocks. Blood samples of birds of these flocks were taken four times during production phase. Sera were subjected to hemagglutination inhibition (HI) assay to determine level of antibodies against NDV. Statistical analysis was carried out using SPSS where applicable. Results of this study reveal that use of three live NDV vaccine and two inactivated NDV vaccines provide sufficient immunity during production period of breeder flocks. Use of inactivated vaccines after live primary vaccination increase amplitude and duration of immune response against NDV.

\textbf{Keywords:} Newcastle Disease, Hemagglutination Inhibition (HI), Broiler Breeder, Vaccine.
Introduction

Newcastle disease (ND) is one of the most important infectious diseases of poultry. Newcastle disease virus (NDV) is synonymous with avian paramyxovirus type 1 and infects over 200 bird species (Alexander, 2000). Strains of NDV have been classified into five pathotypes known as viscerotropic velogenic, neurotropic velogenic, mesogenic, lentogenic, and asymptomatic enteric, based on the clinical signs seen in infected chickens (Alexander & Senne, 2008). Eleven serotypes of avian paramyxoviruses have been recognized. Among them, avian paramyxovirus type 1 causes ND in poultry (Miller & Koch, 2013). NDV infections range from asymptomatic to rapidly fatal (Alexander and Senne, 2008). ND causes massive financial loss to global poultry industry. Different strategies have been applied to reduce losses caused by ND. Orajaka et al., reported vaccination as the only safe option in control strategies of infection (Orajaka et al., 1999). In countries that ND is endemic, like Iran, vaccines offer good option for protection of poultry flocks against ND. In Iran, live and killed vaccines are used for protection of poultry flocks against ND. It must be noted that vaccination without good management practices and biosecurity protocols will not eliminate risk of infection.

Laboratory tests should be used to confirm clinical suspicion (Cattoli et al., 2011). Molecular assays, viral isolation and presence of specific antibodies are indicative of exposure to NDV. Hemagglutination inhibition (HI) test is the serologic assay most often used for evaluation of immune response in affected birds (Alexander and Senne, 2008). Value of serology in diagnosis of disease depends on vaccination history of birds and on prevailing disease conditions (OIE Terrestrial Manual 2012).

There are several reports of seroprevalence of NDV in poultry flocks (Ghaniei & Mohammadzadeh, 2012; Hossain, 2010; Ali, 2015). Efficacy of different vaccination programs against NDV in poultry flocks evaluated by researchers (Roy et al., 2003; Hassanzadeh & Bozorgmeri Fard, 2004). It is known that vaccination of poultry provides a suitable tool to lessen clinical signs of NDV infection (Senne et al., 2004). It has also been known for a long time that vaccination itself (with live vaccines based on non-virulent virus strains) may cause disease and reduced growth in vaccinated birds (Alexander, 2003). As a consequence, there has been a trend to use ever less virulent strains as the seed viruses for vaccine production. Although this strategy has reduced the disease rates after vaccination, it also may have contributed to the fact that current vaccines and vaccination campaigns are not maximally effective in preventing infection and transmission (Alexander, Senne et al., 2004). Hence, it is not clear whether the ultimate goal of prevention of major outbreaks after primary virus introductions can be achieved with current vaccines and vaccination programmes. So, more research needed to ascertain efficacy of vaccination programs in poultry flocks.

This study was conducted to determine immune response of Arian broiler breeders to ND vaccines. The results of such surveys can be useful in designing vaccination programs, with regard to NDV infections in broiler breeders of Iran.

Methodology

This study was carried out in Arian broiler breeder flocks of Mazandaran province (north of Iran). Vaccination program against ND in the birds of these flocks were identical. In this case, the first live vaccine at 8-14 days, second live vaccine at 18-24 days, and third live vaccine at 28-32 days administered to birds. These flocks also vaccinated at 7 and 18 weeks old with oil emulsion inactivated vaccines.

Immune response to NDV vaccination in breeder flocks was determined by HI. Blood samples were taken from wing veins of 16-24 birds of each flock on 18-25 weeks old, 25-35 weeks old, 35-45 weeks old, and 45-60 weeks old. Sera was separated by centrifugation of blood samples, transported to laboratory in a refrigerator box and subsequently stored at -20°C until titer determination for antibodies against NDV by a HI assay using the method of Alexander (1988). Briefly, two fold serial dilutions of sera were made and 8 HAU NDV with equal volume (25 µL) of diluted sera was used in each well of 96 well microplates. After 45 min incubation at room temperature, 25 µL 1% chicken red blood cell was added and after 30 min incubation at room temperature, the last well which had a complete inhibition, was considered as the antibody titer. Mean of HI antibody titers, CV%, and standard deviation of titers also calculated. Statistical analysis was carried out using SPSS version 16.0 (SPSS, Chicago, IL, USA) where applicable.
**Results**

Mean antibody titers and CV% (coefficient of variation) in group 1 (18-25 weeks old) represented in Figure 1. Minimum titer, maximum titer, median titer, and total CV% of the group were 6.12, 8.59, 7.46, and 11.15, respectively. Distribution of the antibody titers of group 1 showed 75% of them were in the range of Mean ± Standard Deviation (SD) = 7.46±0.67. 8.3% of data were in higher range, and 16.6% of data were in lower range. Assessment of data revealed that they are not normally distributed. Mean antibody titer of 5 may be considered protective. So, mean antibody titer of this group is good. CV% of titers also revealed that they were uniform.

![Figure 1. NDV HI antibody titer and CV% related to birds of group 1 (18-25 weeks old).](image)

<table>
<thead>
<tr>
<th>Mean</th>
<th>Std Dev</th>
<th>Variance</th>
<th>Range</th>
<th>Minimum m</th>
<th>Maximum m</th>
<th>Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>7.46</td>
<td>0.67</td>
<td>0.45</td>
<td>2.47</td>
<td>6.12</td>
<td>8.59</td>
<td>12</td>
</tr>
</tbody>
</table>

Mean antibody titers and CV% of titers of group 2 (25-35 weeks old) represented in figure 2. NDV antibody titers were in the range of 5.53-8.25. Mean of titers and CV% were 6.88 and 10.71%, respectively. Distribution of the antibody titers of group 2 showed only 20% of them were in the range of Mean ± SD = 6.88 ± 0.77. 25% of data were in higher range, and 55% of data were in lower range. Assessment of data revealed that they are not normally distributed. Due to mean antibody titer of 6.88 and minimum titer of 5.53, it may be concluded that antibody titers were protective.

![Figure 2. NDV HI antibody titer and CV% related to birds of group 2 (25-35 weeks old).](image)

<table>
<thead>
<tr>
<th>Mean</th>
<th>Std Dev</th>
<th>Variance</th>
<th>Range</th>
<th>Minimum m</th>
<th>Maximum m</th>
<th>Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>6.88</td>
<td>0.77</td>
<td>0.59</td>
<td>2.72</td>
<td>5.53</td>
<td>8.25</td>
<td>20</td>
</tr>
</tbody>
</table>
Data related to birds of group 3 (35-45 weeks old) presented in figure 3. CV% of titers increased and mean HI antibody titers of samples showed a descending trend. Mean HI titer of NDV, minimum, maximum and CV% were 6.33, 4.33, 8.0, and 14.37%, respectively.

![Figure 3. NDV HI antibody titer and CV% related to birds of group 3 (35-45 weeks old).](image)

Data related to birds of group 4 (45-60 weeks old) presented in figure 4. Mean HI titer of NDV, minimum, and maximum titer were 6.57, 4.06, and 8.59, respectively.

![Figure 4. NDV HI antibody titer and CV% related to birds of group 4 (45-60 weeks old).](image)

Data related to whole period of production of these flocks represented in figure 5. Mean HI titer of NDV, minimum, and maximum titer were 6.57, 4.06, and 8.59, respectively.
Discussion and Conclusion

The virulent strains of avian paramyxovirus type 1 (APMV-1) cause a serious disease in chickens and other birds known as ND. Flock mortalities due to NDV approach 100% in fully susceptible birds (Alexander et al., 2012). In response to the threat presented by ND, several countries, like Iran, have put in place vaccination campaigns to prevent epizootics. However, outbreaks have been reported in vaccinated populations despite the fact that vaccination is widely applied (Alexander, 2003). In order to achieve protective immune status in vaccinated birds, efficacy of vaccination programs should be evaluated by serologic and virology assays.

In this study, we evaluate efficiency of a vaccination program against NDV in Arian broiler breeder flocks. Results of present study revealed that multivalent inactivated virus water-in-oil vaccines are valuable adjunct to poultry vaccination programs when used in combination with live virus primary vaccination.

The results of NDV surveys showed that NDV are prevalent in backyard and commercial chicken flocks of Iran. So, preventive measures must be applied in every poultry farm. NDV vaccines are commonly used in poultry farms of Iran. But, incidence of ND in vaccinated flocks may be due to inadequate vaccination practices (Dortmans et al., 2012) or biosecurity faults. Rezaeianzadeh and coworkers conducted a survey on NDV prevalence in village chickens of Fars province using molecular and serological tests. Results showed that chickens in 13 villages (61.9%) were seropositive, but all of RT-PCR results were negative (Rezaeianzadeh et al., 2011). Hadipour (2009) examined 350 blood samples of backyard chickens for NDV antibodies. He stated that 37.56% of samples were positive, and means HI titer was 5.21. Ghaniei and Mohammadzadeh evaluate seroprevalence of NDV antibodies in serum of broilers of North-west of Iran. 309 out of the 383 collected broiler sera were positive for NDV antibodies in HI test.

Mean antibody titre for NDV was 5.31 (Ghaniei & Mohammadzadeh, 2012). Some researchers focus on efficacy of vaccination programs in poultry flocks. Ghahramani et al., (2014) evaluated efficacy of two vaccination program in broiler breeders by HI. Another study conducted on twenty-week old broiler breeder chickens that had received previous live virus vaccination with NDV and IBDV were injected intramuscularly with the monovalent or bivalent vaccine. The antibody titers to either the monovalent vaccine or bivalent vaccine increased rapidly and then remained at high levels for the duration of the 40-week trial. There were no practical differences in amplitude or duration of the antibody response to either antigen used alone compared to that of the bivalent combination (Thayer et al., 1983).

It is clear that infection, shedding, and transmission of virulent NDV in vaccinated birds may occur without overt disease signs (Boven et al., 2008; Kapczynski and King 2005). Due to this reason, if preventive vaccination
programmers are to be implemented, they should go together with a monitoring program ensuring that sufficient flock immunity levels are achieved.

Immune response following vaccination must be monitored by serological assays to make sure that the vaccination was proper and evoked adequate immunity.

Conflict of interest
The authors declare no conflict of interest

References

