Evaluation of Antibody Production against Newcastle Disease Virus after Immunization with Different Vaccines in Fayoumi Chicks

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Introduction

Newcastle disease (ND) is a highly contagious viral ailment belonging to Paramyxovirus type 1 of the genus Avula virus, sub-family Paramyxovirinae of the family Paramyxoviridae. Strains of Newcastle disease virus (NDV) are distinguished into five pathotypes on the basis of the clinical signs in infected chickens and other birds. These are viscerotropic velogenic virus (vvNDV), neurotropic velogenic virus (nvNDV), mesogenic virus, lentogenic virus and asymptomatic enteric viruses.

The disease is kept under control by vaccination and other preventive measures. Vaccination of birds as a means of prevention of ND in Bangladesh is being conducted by the Department of Livestock Services (DLS), Government of People’s Republic of Bangladesh and non-government organizations (NGO). Currently practiced vaccination programme against ND includes administration of two types of live vaccines of either lentogenic (F, B1, LaSota strain) or Mesogenic (M-strain) and inactivated (killed) vaccines. Such vaccines and vaccination programme are sometimes found ineffective to protect chickens against ND. Among the various causes of vaccination failure, the age of birds at the time of vaccination is an important one. The priming time depends on the level of maternally-derived antibody (MDA). In spite of the fact that vaccination is thoroughly followed, incidence of ND with varied degrees has been recorded by different investigators such as 18.65% by Kamal, 4.80% by Bhattacharjee et al., 17.20% by Islam et al., 10.24% by Talha et al., 9.80% by Saleque et al. and 19.50% by Das et al.

For the control of ND in Bangladesh, vaccines are produced with F strain (lentogenic) and M strain (Mukteswar, mesogenic) by Department of Livestock Services (DLS). Beside these, vaccines containing B1, LaSota, VG/GA strains etc. are imported and marketed in Bangladesh. These are administered under different schedule of primary and followed by either live or inactivated vaccine as a secondary one. The schedule of vaccination however, differs with type of flock such as broiler, layer, parent, and grandparent stock and those reared under backyard system. As...
stated earlier, Fayoumi day-old chicks (DOC) are being kept by rural farmers and are getting priority day-by-day. These farmers required to be encouraged with continuous support of disease preventive measures. As such prophylaxis against ND comes under consideration where the schedule of vaccination demands evaluation on the performance or efficacy of vaccines.

The present research program was undertaken to record the persistence of maternally-derived antibody (MDA) against NDV in Fayoumi chicks, and also to compare the antibody production level to BCRDV (F strains), Izovac B1 Hitchner® (B1 strain), Cevac New L® (LaSota strain) and Avinew (VG/GA strain) in Fayoumi chicks.

**Materials and Methods**

**Experimental chicks**

A total 110 apparently healthy day-old-chicks of Fayoumi breed were collected from the Government Poultry Farm, Mirpur, Dhaka and carried to the experimental houses of the Department of Microbiology and Hygiene, Bangladesh Agricultural University (BAU), Mymensingh. The birds were supplied with feed (Quality Feed Co Ltd) and water adlibitum maintaining strict biosecurity.

**Newcastle disease vaccine (NDV)**

Lyophilized ND vaccines baby chick ranikhet disease vaccine (BCRDV, F-strain, lentogenic), Izovac B1 Hitchner®, (B1 strain, lentogenic), Cevac New L® (LaSota strain, lentogenic) and Avinew® (VG/GA strain, lentogenic) were collected from local market and were used during the experiment. The vaccines were stored and diluted during use according to the instruction of manufacturers.

**Experimental design**

One hundred and ten-day-old Fayoumi chicks were grouped into five groups; viz., A, B, C, D and E, where group A, B, C and D contained 20 birds each and group E contained 30 birds. Prior to grouping and vaccination blood samples were collected to measure the maternally-derived antibody (MDA) on day 3 and day 7 of age of chicks, which were then divided into five groups (n = 5) as required. The birds of group A, B, C and D were vaccinated with BCRDV (F-strain, lentogenic), Izovac Hitchner B1® (B1 strain, lentogenic), Cevac New L® (LaSota strain, lentogenic) and Avinew®, (VG/GA strain, lentogenic) vaccines respectively through intraocular (i/o) route while birds of group E was kept as unvaccinated control. The vaccines were administrated at 7 days of age according to manufacturer’s direction. Blood samples were collected for sera from day 3 until day 22 and the titres are presented in Table 1. The mean ± SD of haemagglutination inhibition (HI) titre were 89.60 ± 33.05, 108.80 ± 30.91, 198.40 ± 76.62, 96.00 ± 33.73, 64.00 ± 36.95 and 41.60 ± 20.24 in case of group A; 67.20 ± 35.22, 102.40 ± 33.05, 268.80 ± 183.02, 86.40 ± 45.38, 48.00 ± 16.87 and 32.00 ± 18.48 in case of group B; 54.40 ± 15.46, 99.20 ± 38.31, 172.80 ± 74.21, 83.20 ± 30.91, 59.20 ± 30.22 and 40.00 ± 21.66 in case of group C, and 48.00 ± 16.87, 102.40 ± 33.05, 153.60 ± 75.12, 300.80 ± 190.93, 192.00 ± 134.92 and 108.80 ± 66.10 in case of group D at 7, 13, 15, 17, 19 and 21 days post-vaccination (DPV) respectively. On the other hand the unvaccinated control group E maintained a mean ± SD of HI titre of 96.00 ± 33.73, 41.60 ± 15.46, 32.00 ± 18.48, 13.60 ± 3.86, 10.40 ± 5.06, 7.20 ± 3.67, 6.00 ± 2.11 and 2.60 ± 0.97 at the age of day 3, 7, 10, 14, 16, 18, 20 and 22 respectively.

**Results and Discussion**

In the present study persistence of maternally (passive)-derived antibody (MDA) was measured on a number of occasions starting from day 3 until day 22 and the titres are presented in Table 1. The mean ± SD of haemagglutination inhibition (HI) were 96.00 ± 33.73, 41.60 ± 15.46, 32.00 ± 18.48, 13.60 ± 3.86, 10.40 ± 5.06, 7.20 ± 3.67, 6.00 ± 2.11 and 2.60 ± 0.97 at the age of day 3, 7, 10, 14, 16, 18, 20 and 22 respectively.

It was observed that such antibody remains minimal in chicks until the age of day 20 and none at the age of day 22 and afterwards. The finding of this investigation in respect of MDA is closely related with Begum et al. who stated that MDA remains protective until 18 days of age. On the other hand, Chowdhury et al.3, Islam et al.12 and Shil13 stated the period of its existence was 12, 15 and 17 days of age respectively. The level of HI antibody titres exists during first two weeks of life. However, Balla16 and Mahmud17 reported that MDA persisted until day 27 of age of chicks but Saeed et al.18 found that MDA declined to zero after the age of 25 days of chicks. According to Islam et al.12, MDA can be considered as an effective means of protection of the chickens till two weeks of age.

**Slide haemagglutination (HA) test**

Slide haemagglutination (HA) test was performed to determine the presence of NDV in the allantoic fluid of the infected embryos. The clumping of chicken RBC in allantoic fluid appearing within a minute was considered as positive for the presence of virus.

**Micro-plate haemagglutination (HA) test**

The test was carried out by two-fold serial dilutions of the viral suspension in a U-shaped plate to determine 4 HA units (4 HA/25 μl).

**Micro-plate haemagglutination inhibition (HI) test**

The haemagglutination inhibition (HI) test was performed to determine the HI titre of the sera samples collected from the chicks. The HI titre of sera samples of control group of chicks was determined to measure the maternal antibody and its persistence. The test was conducted by using constant 4 HA unit antigen and decreasing serum method (β procedure).

**Statistical analysis**

The data were analysed by using Statistical Package for Social Sciences (SPSS) program. Differences among the vaccinated groups were determined by F-test.
One of the principal objectives of this study was to determine the comparative performance of BCRDV (F strain), Izovac B1 Hitchner® (B1 strain), Cevac New L® (LaSota strain) and Avinew® (VG/GA) in chicks. As such a relative statement of HI antibody titres of sera samples obtained from birds of group A (vaccinated with BCRDV), group B (vaccinated Izovac B1 Hitchner®), group C (Cevac New L®) and group D (Avinew®) together with that of non-vaccinated control group E (control) is depicted in Table 2. It may be noted that the mean HI titres of the four vaccinated groups of birds are more or less of similar order when measured at 7, 13, 15, 17, 19 and 21 DPV. It was observed that the F-value of HI titres among four vaccines was significantly different (p <0.01). Peak level of HI titres was found on 15 DPV in group A, B and C, which is similar to observation of Rahman et al.19 where the author stated that HI antibody titres reached the peak levels between 2 to 3 weeks of vaccination.

Among the four vaccines, Avinew® (VG/GA strain) engendered highest HI titres, which is in agreement with the previous work done by Beard et al.20 who stated VG/GA strain produced better immunity than B1. It is also similar to the findings of Mahmud17 who noted that mean HI titres was higher in birds vaccinated with Avinew® (VG/GA strain) than BCRDV (F-strain).

In this study, BCRDV (F strain), Izovac B1 Hitchner® (B1 strain) and Cevac New L® (LaSota strain) produced more or less similar degree of HI titres. In this regard it may be mentioned that Ibrahim et al.21 who also compared F strain of ND vaccine with B1 and LaSota and found no significant difference among these three strains.

The peak HI titre was higher in the group of chicks vaccinated with Izovac B1 Hitchner® than BCRDV and Cevac New L® (Table 2). However, BCRDV and Cevac New L®, birds vaccinated with BCRDV (F strain) showed higher HI titre than Cevac New L® (LoSota strain) though previous result of Shil13 indicated that Cevac New L® provide more immunity than B1.22 The lentogenic F and Lasota strains provided superior antibody production after vaccination compared to B1.23 In addition to the antigenic characteristics of the vaccine virus, the titre and the dose of virus play an important role in the level of immunity produced24-27.

### Table 1. Results of persistence of maternally-derived antibody (MDA) titre to Newcastle disease vaccine in Fayoumi chicks

<table>
<thead>
<tr>
<th>Bird serial No.</th>
<th>Haemagglutination inhibition titre (HI unit) at different periods</th>
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<tbody>
<tr>
<td></td>
<td>Day 3</td>
</tr>
<tr>
<td>1</td>
<td>128</td>
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<tr>
<td>2</td>
<td>128</td>
</tr>
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<td>3</td>
<td>128</td>
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<td>9</td>
<td>64</td>
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<td>10</td>
<td>64</td>
</tr>
</tbody>
</table>

Mean ± SD 96.0 ± 33.73 41.6 ± 15.46 32.0 ± 18.48 13.6 ± 3.86 10.4 ± 5.06 7.20 ± 3.67 6.00 ± 2.11 2.60 ± 0.9

SD = Standard deviation; HI = Haemagglutination inhibition.

### Table 2. Comparative haemagglutination inhibition (HI) titre in sera of Fayoumi chicks following vaccination with different vaccines

<table>
<thead>
<tr>
<th>Bird group</th>
<th>Vaccine</th>
<th>HI titre (Mean HI unit ± SD) at different periods</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>BCRDV</td>
<td>89.60 ± 33.05 108.80 ± 30.91 198.40 ± 76.62 96.0 ± 33.73 64.00 ± 36.95 41.60 ± 20.24</td>
</tr>
<tr>
<td>B</td>
<td>Izovac B1 Hitchner®</td>
<td>67.20 ± 35.22 102.40 ± 33.05 268.80 ± 183.02 86.40 ± 45.38 48.00 ± 16.87 32.00 ± 18.48</td>
</tr>
<tr>
<td>C</td>
<td>Cevac New L®</td>
<td>54.40 ± 15.46 99.20 ± 38.31 172.80 ± 74.21 83.20 ± 30.91 59.20 ± 30.22 40.00 ± 21.66</td>
</tr>
<tr>
<td>D</td>
<td>Avinew®</td>
<td>48.00 ± 16.87 102.40 ± 33.05 153.60 ± 75.12 300.80 ± 190.93 192.00 ± 134.92 108.80 ± 66.10</td>
</tr>
<tr>
<td>E</td>
<td>Control (non-vaccinated)</td>
<td>13.60 ± 3.86 6.00 ± 2.11 2.60 ± 0.97 1.40 ± 0.97 0.40 ± 0.84 0.00 ± 0.00</td>
</tr>
</tbody>
</table>

F-Value 13.54** 20.62** 9.47** 15.27** 12.24** 14.09**

DPV = Days post-vaccination; SD= Standard deviation, ** = Significant (p <0.01)
As regards the prime objectives of the present study, it may be stated that production of HI antibody was higher in birds of group D vaccinated with Avinew® compared to those of group A vaccinated with BCRDV, group B vaccinated with Izovac B1 Hitchner® and group C vaccinated with Cevac New L® (LaSota strain). However, it was earlier stated that among the lentogenic strains, the LaSota strain is more virulent in the host than B1 or F and causes more post-vaccination respiratory symptoms28-29, whereas B1 was a little more virulent and effective than F30. In the statistical analysis, it was observed that the F-value of HI titres among four vaccines were significantly different (p <0.01).

In view of above, further investigation, as requires to be performed include the influence of maternal immunity in chicks on the efficacy of F, B1, LaSota and VG/GA strains vaccine, effect of different environmental and climatic condition on the performance of ND vaccines in chickens, and assessment of genetic reversion of F, B1, LaSota and VG/GA strain after vaccination.

Newcastle disease (ND) is recognized as one of the serious threat to the developing poultry industry in Bangladesh. Vaccination is extensively used to protect birds against ND. Therefore, the types of vaccine, the efficacy of the vaccines and vaccination schedule are important factors for the success of vaccination. Moreover, antigenicity of vaccine virus, virulence of field virus, route of administration of vaccine and age of birds etc. are also considered as a matter of great consequence. To protect the chickens against ND, mostly the lentogenic BCRDV and mesogenic live (RDV) vaccines are mostly used in Bangladesh. Beside these, B1, LaSota, VG/GA, cloned and inactivated vaccines are also imported. Vaccination is an effective control measure relies on two aspects. Firstly vaccination of the parent stock to ensure that the progeny chicks are hatched with a high level of maternally-derived antibody (MDA) and secondly, vaccination of the chickens with a suitable vaccine at the correct age. The resultant high level of MDA found in the progeny chickens is important in protecting the chicken during 10 to 14 days of age from NDV infection. However, as MDA level declines gradually and chickens become susceptible to NDV therefore, immunity must be boosted by secondary vaccination.

References

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17. Mahmud MS. 2006. Comparative efficacy of Avinew® (VG/GA strain) and BCRDV (F strain) vaccines in broiler chicks. MS Thesis. Department of Microbiology and Hygiene, Bangladesh Agricultural University (BAU), Mymensingh.
Immunization against Newcastle Disease in Chicks


