Efficacy of V₄HR Newcastle Disease (V₄HR-ND) Vaccine in Broiler Birds in Bangladesh

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Abstract: Thirty day-old chicks of Cobb-100 breed with the history of vaccination of parent stock against Newcastle disease (ND) were equally divided into two groups such as group A and B. At 7 days of age of birds, group A was vaccinated with experimentally prepared V₄HR-ND vaccine via eye drop @ 10⁶EID₅₀/bird to determine the efficacy of this vaccine while group B was kept as unvaccinated control. The birds were used for the study during the period from October 2003 to December 2003. Each bird of group A was boosted with same vaccine @ same dose at 24 days of age. Both the groups (A and B) were challenged after two weeks of post-booster vaccination with 0.25ml inoculum containing 10³⁵EID₅₀ of virulent field isolates of ND virus intramuscularly. The results of challenge experiment revealed that six birds (40%) of group A succumbed within 3 to 4 days of post-challenge, whereas 15 (100%) unvaccinated control birds of group B showed clinical illness and ultimately died within 3 to 5 days. Thus, the experimental V₄HR-ND vaccine conferred 60% protection of vaccinated birds against challenge infection. The mean values of Haemagglutination inhibition (HI) antibody titres of birds in group A were found to have significantly (P<0.01) increased at two weeks of post-booster vaccination. These results indicated that experimental V₄HR-ND vaccine induced sufficient humoral immune response which gives satisfactory level of protection against ND.

Key words: Broiler, birds, efficacy, Newcastle disease, vaccine, V₄HR

Introduction
Poultry farming in Bangladesh has emerged as a strong agro-based industry during the last two decades and present population of chickens is approximately 140 millions (Rahman, 2003). But the advancement of poultry industry is being hampered by various fatal infectious and non-infectious diseases. One of the major infectious diseases that cause serious economic loss to the poultry is Newcastle disease (ND). ND is caused by genus- Avulovirus, subfamily- Paramyxovirinae, family-Paramyxoviridae (Al-Garib et al., 2003). ND is endemic in Bangladesh with prevalence of velogenic viscerotropic strains (Chowdhury et al., 1981). An avirulent Australian strain of ND virus (V₄HR) seemed suitable as a vaccine strain (Westbury, 1981) which produced an adequate serological response following mass administration to commercial meat chickens (Bell et al., 1991). The V₄ HR virus spread readily among chickens, causes no disease and protect against challenge with virulent strains of NDV (Westbury, 1981; Kafi, 2003). In Bangladesh, there are varieties of mode and means of transport and variation in maintenance of cooling system at all stages of District, Thana, Union, Village and farm level. This is one of the important reasons of vaccination failure causing economic loss to the farmers. To get remedy from these problems heat resistant thermostable V₄HR vaccine is of growing interest. But the efficacy of vaccine needs to be studied in Bangladesh before releasing extensively for field use. In consideration of these, research program was undertaken to determine the efficacy of the experimentally prepared V₄HR Newcastle disease vaccine in broiler birds.

Materials and Methods
Experimental chickens: A total of 30 healthy day old broilers of Cobb-100 breed with previous history of vaccination of parent stock against ND were collected from a commercial hatchery. These chicks were divided into two equal groups (A and B). The chicks were maintained separately with strict biosecurity and adequate commercial feed (Aftab Feed Ltd., Dhaka) and water supply throughout the study period. Vitamin-mineral Premix (RHODIVIT®; Rampart-Power®, Bangladesh) was also supplied with the drinking water.

Chicken eggs: Eggs of Fayoumi breeds were obtained from Bangladesh Agricultural University (BAU) poultry farm. Ten day-old embryonated chicken eggs were used for propagation of the virus.

V₄HR-ND vaccine: Live freeze-dried V₄HR-ND vaccine was prepared by chicken embryo propagation following standard method of vaccine production of FAO (Allan et al., 1978). One bird dose being at least 10⁶⁵⁰ 50% embryo infective doses (EID₅₀). The vaccine was produced in chicken eggs from a purified seedlot derived from the original V₄ isolate received from SLDP-
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Table 1: Results of immunization of chickens with experimentally prepared V4HR-ND vaccine and their state of protection

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Experimental V4HR Newcastle disease vaccine (n = 15)</th>
<th>Control (n=15)</th>
</tr>
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<tbody>
<tr>
<td>Age of primary vaccination</td>
<td>7 days</td>
<td>-</td>
</tr>
<tr>
<td>Dose</td>
<td>$10^{6.0}$ EID₅₀</td>
<td>-</td>
</tr>
<tr>
<td>Route</td>
<td>ED</td>
<td>-</td>
</tr>
<tr>
<td>Age of booster vaccination</td>
<td>24 days</td>
<td>-</td>
</tr>
<tr>
<td>Age of challenged</td>
<td>38 days</td>
<td>38 days</td>
</tr>
<tr>
<td>No. of birds challenge infection</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Challenged with * ID</td>
<td>0.25 ml IM</td>
<td>0.25 ml IM</td>
</tr>
<tr>
<td>Birds showed clinical signs or dead</td>
<td>6/15**</td>
<td>15/15</td>
</tr>
<tr>
<td>Birds survived after challenged</td>
<td>9/15**</td>
<td>0/15</td>
</tr>
</tbody>
</table>

EID₅₀ = Embryo Infective Dose, ED = Eye-drop, IM = Intra-muscular, n = number of birds
* ID (Infective Dose) = $10^{6.0}$ EID₅₀ of virulent field isolate of Newcastle disease virus
** Numerator = Showing clinical signs and symptoms, Denominator = No. of birds challenged

Project, Department of Livestock Services (DLS).

Vaccination: Two doses of vaccine were administered in chicks via eye drops at an interval of 17 days. The chicks of group A (n = 15) was primarily vaccinated at 7 days of age and booster vaccination at 24 days of age with same vaccine and same route. Birds of group B (n = 15) served as unvaccinated and control.

Challenge of experimental chickens: Velogenic local field isolates of ND virus obtained from Department of Microbiology and Hygiene, BAU, was used as challenge virus and 0.25ml of $10^{6.0}$ EID₅₀ virus constituted one bird lethal dose (1 BLD) which was determined following the method of Reed and Muench (1938). All the challenge birds were observed daily for 6 days and the signs, symptoms and morbidity were recorded.

Blood collection: Blood samples were collected by jugular vein four birds randomly selected from each group at intervals shown in Table 1. Blood samples were collected from the birds at pre-vaccination 7, 10, 15 and 17 days after primary vaccination and 7, 10 and 14 days after booster vaccination. Sera were separated from the blood collected without adding any anticoagulant and stored at -20°C until used.

Serology: The haemagglutination inhibition (HI) titre of immune and pre-inoculation sera of the experimental birds were measured by using standard β-procedure of micro plate HI test (Anonymous, 1971). All titres were recorded as log₂ of the reciprocal of the endpoint dilution.

Statistical analysis: The significance of difference of the Log₂ mean titres (LMTs) was assessed using Student's t-test. Standard deviation was calculated for LMTs.

Results and Discussion

Vaccination as a mean of protecting birds against ND is routinely practiced in Bangladesh where the farmers mostly use the lento genic BCRDV (Baby Chick Ranikhet Disease Vaccine) and merogenic live RDV (Ranikhet Disease Vaccine). Despite extensive use of vaccines, outbreaks of ND are still recorded due to failure to follow an effective cold chain system, required for the maintenance of efficacy of vaccines. To get remedy from this problem experimental heat resistant thermostable (V₄HR-ND) vaccine was produced. The immunogenic potential of the V₄HR strain of ND virus as vaccine against velogenic viscerotrophic ND virus (vvNDV) has been reported by several workers (Idris et al., 1990; Bell et al., 1991; Tantawasdi et al., 1992; Aiders et al., 1994; Bell et al., 1995; Biswas et al., 1996) but there is paucity of published data on serological responses and protection due to V₄HR-ND vaccine in Bangladesh. The results of immunization of chickens with experimentally prepared V₄HR-ND vaccine and its protection level are shown in Table 1. The chicks of both vaccinated and nonvaccinated groups were challenged on 14 days after booster vaccination with virulent field isolate of NDV and the results are presented in Table 1. It appeared from the results that after 14 days of booster vaccination with experimental V₄HR-ND vaccine, six out of fifteen birds showed signs of illness and the rest did not exhibit any signs of illness and survived after challenge infection. All the control birds challenged on the same day succumbed to infection. Thus it appeared that vaccination with experimentally prepared NDV₄HR conferred 60% protection. Using V₄HR-ND vaccine similar results were recorded by Aini et al. (1987).

The HI antibody titre in chickens immunized with V₄HR-ND vaccine was found to have (Table 2 and Fig. 1) significantly (P<0.01) increased at post vaccination (5.07±0.50). Thirty-one days after first vaccination and 2 weeks after booster vaccination, a peak titre of about Log₅ was observed in the vaccinated group which is similar to the peak of Log₅ as were observed by Aini et al. (1987) 2 weeks after a single vaccination with V₄-UPM.
Table 2: HI antibody titre (log2 base) after vaccination with experimental V4HR-ND vaccine

<table>
<thead>
<tr>
<th>Age (days)</th>
<th>Log2 HI Antibody Titre (Mean ± SD)</th>
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<tbody>
<tr>
<td></td>
<td>Group A (vaccinated)</td>
</tr>
<tr>
<td>7</td>
<td>-</td>
</tr>
<tr>
<td>14</td>
<td>3.82±0.57 (n=4)</td>
</tr>
<tr>
<td>17</td>
<td>3.82±1.00 (n=4)</td>
</tr>
<tr>
<td>22</td>
<td>3.82±0.57 (n=4)</td>
</tr>
<tr>
<td>24</td>
<td>3.57±0.50 (n=4)</td>
</tr>
<tr>
<td>31</td>
<td>4.32±0.81 (n=4)</td>
</tr>
<tr>
<td>34</td>
<td>4.57±0.95 (n=4)</td>
</tr>
<tr>
<td>38</td>
<td>5.07±0.50* (n=4)</td>
</tr>
</tbody>
</table>

*= Not done. HI = Haemagglutination Inhibition.

heat-resistant clone, and is in the range of 2-4 weeks for a peak titre reported by Spadbow and Samuel (1987). It is difficult to make valid comparisons between the present results and those obtained by other researchers using V4 or other ND vaccines. All the birds did not respond to vaccination in the same manner and individual variation in the production of HI antibody response following both primary and secondary vaccination were observed by Saifuddin et al. (1986) and similar results were observed in the present study after primary vaccination at 7, 10 and 15 days post vaccination when the antibody titres were almost of similar value (3.82). The variation might be due to the presence of variable passive immunity in chicks or to varying degree of susceptibility of immune mechanism to antigen as were also suggested by Toth and Markovits (1964). Haplin (1976) suggested that this might be due to genetical incapability of some birds to produce any reaction to ND virus. It has been reported previously that maternal antibody reduces the immune response to V4 vaccination (Westbury et al., 1984). The birds of the control group developed a slight level of antibody titre that did not protect the birds during the challenge experiment. The cause of the presence of slight antibody titre (Table 2) in the control birds before challenge is thought to be due to very mild exposure of a few birds to ND virus possibly transmitted by feeds and attendants. Allan et al. (1978) and Westbury et al. (1984) reported that the relationship between HI antibody and protection against challenge with virulent virus is such that a Log2 titre of 4 or greater is required for a high probability of survival. This report is in agreement with the present study.

On autopsy, the birds that died after challenge had gross pathological lesions such as tracheal mucus and haemorrhages of the proventriculus and intestine suggesting that Newcastle disease was responsible.

Acknowledgement
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References


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Kafi, M.A., 2003. Vaccination studies on use of Ramikhet inactivated vaccine combined with V1,HR or BCRDV. M. S. Thesis. Department of Microbiology and Hygiene. Bangladesh Agricultural University, Mymensingh-2202, Bangladesh.


