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Efficacy of V_4HR Newcastle Disease (V_4HR-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 V_4HR-ND vaccine via eye drop @ $10^{6.0}EID_{50}$/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 boostered 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 $10^{5.0}EID_{50}$ 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 V_4HR-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 V_4HR-ND vaccine induced sufficient humoral immune response which gives satisfactory level of protection against ND.

Key words: Broiler, birds, efficacy, Newcastle disease, vaccine, V_4HR

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 (V_4HR) 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 V_4 ND 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 V_4HR 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 V_4HR 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.

V_4HR-ND vaccine: Live freeze-dried V_4HR-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 $10^{6.0}$ 50% embryo infective doses (EID_{50}). The vaccine was produced in chicken eggs from a purified seedlot derived from the original V_4 isolate received from SLDP-

Table 1: Results of immunization of chickens with experimentally prepared V4HR-ND vaccine and their state of protection

Parameters	Experimental V4HR Newcastle disease vaccine (n = 15)	Control (n=15)
Age of primary vaccination	7 days	-
Dose	$10^{6.0}$ EID_{50}	-
Route	ED	-
Age of booster vaccination	24 days	-
Age of challenged	38 days	38 days
No. of birds challenge infection	10	10
Challenged with * ID	0.25 ml IM	0.25 ml IM
Birds showed clinical signs or dead	6/15**	15/15
Birds survived after challenged	9/15**	0/15

EID_{50} = Embryo Infective Dose, ED = Eye-drop, IM = Intra-muscular, n = number of birds
* ID (Infective Dose) = 10^5 EID_{50} 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 $10^{5.0}$ EID_{50} 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 log_2 of the reciprocal of the endpoint dilution.

Statistical analysis: The significance of difference of the Log_2 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 (V_4HR-ND) vaccine was produced. The immunogenic potential of the V_4HR strain of ND virus as vaccine against velogenic viscerotropic ND virus (vvNDV) has been reported by several workers (Ideris *et al.*, 1990; Bell *et al.*, 1991; Tantaswasdi *et al.*, 1992; Alders *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 V_4HR-ND vaccine in Bangladesh.

The results of immunization of chickens with experimentally prepared V_4HR-ND vaccine and its protection level are shown in Table 1. The chickens 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 V_4HR-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 NDV_4HR conferred 60% protection. Using V_4HR-ND vaccine similar results were recorded by Aini *et al.* (1987).

The HI antibody titre in chickens immunized with V_4HR-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 $Log_2$5 was observed in the vaccinated group which is similar to the peak of $Log_2$5 as were observed by Aini *et al.* (1987) 2 weeks after a single vaccination with V_4-UPM

Table 2: HI antibody titre (log2 base) after vaccination with experimental V4HR-ND vaccine

Age (days)	Log2 HI Antibody Titre (Mean ± SD)		
	Group A (vaccinated)	Group B (unvaccinated control)	
7	-	5.32±0.81	(n=4)
14	3.82±0.57 (n=4)	5.07±0.50	(n=4)
17	3.82±1.00 (n=4)	4.07±0.95	(n=4)
22	3.82±0.57 (n=4)	3.32±0.00a	(n=4)
24	3.57±0.50 (n=4)	2.82±0.57	(n=4)
31	4.32±0.81 (n=4)	2.57±0.50	(n=4)
34	4.57±0.95 (n=4)	2.32±0.00a	(n=4)
38	5.07±0.50*(n=4)	2.32±0.00a	(n=4)

- = Not done. HI = Haemagglutination Inhibition.
* = Significantly higher than control. n = number of birds tested. N.B. The birds were vaccinated at 7 (primary) and 24 (boostering) days of age in group A.

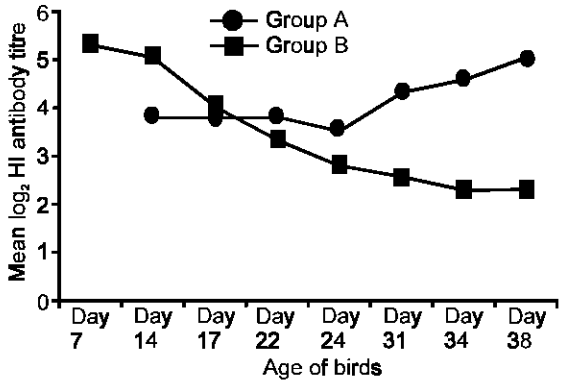

Fig. 1: HI antibody titres of chickens of group A and B

heat-resistant clone, and is in the range of 2-4 weeks for a peak titre reported by Spadbrow and Samuel (1987).

It is difficult to make valid comparisons between the present results and those obtained by other researchers using V_4 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 (1978) 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 V_4 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 Log_2 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.

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References

Aini, I., A.L. Ibrahim, P.B. Spadbrow and C.H. Seng, 1987. Development of food pellet Newcastle disease vaccine. In: Copland J. W. (Ed.), Newcastle Disease in Poultry. A New Food Pellet Vaccine. ACIAR, Canberra, Monograph No. 5, pp: 20-23.

Alders, R.G., S. Inoue and J.C. Katongo, 1994. Prevalence and evaluation of Hitcher B_1 and V_4 vaccines for the control of Newcastle disease in village chickens in Zambia. Prev. Vet. Med., 21: 125-132.

Al-Garib, S.O., A.L.J. Gielkens, E. Gruys and G. Koch, 2003. Review of Newcastle disease virus with particular references to immunity and vaccination. World's Poult. Sci. J., 59: 185-200.

Allan, W.H., J.E. Lancaster and B. Toth, 1978. Newcastle disease vaccines- their production and use. FAO Animal Production and Health Series No. 10. FAO, Rome, Italy.

Anonymous, 1971. Methods for examining Poultry biologics and for identifying and quantifying avian pathogens. National Academy of Sciences, Washington, D. C., pp: 83-87.

Bell, I.G., P.J. Nichells, C. Norman, K. Cooper and G. M. Cross, 1991. The Serological responses of chicken to mass vaccination with a live V_4 Newcastle disease virus vaccine in the field and in the laboratory 1. Meat chickens. Aust. Vet. J., 68: 85-89.

Bell, I. G., T.M. Fotzo, A. Amara and G. Agbede, 1995. A field trial of the heat resistant V_4 vaccine against Newcastle disease by eye-drop inoculation in village poultry in Cameroon. Prev. Vet. Med., 25: 19-25.

Biswas, H.R., M.M. Haoque, M. Oxley and M.A.M. Prodhan, 1996. A comparative study on the protection of indigenous chickens against Newcastle disease induced by Australian NDV_4HR and locally produced conventional vaccines in Bang. Prev. Vet. Med., 26: 157-164.

Chowdhury, T.I. M.F., A.J. Sarkar, M.M. Amin and W.I.M.A. Hossain, 1981. Studies of Newcastle Disease in Bangladesh. Bang. Vet. J., 15: 1-9.

Haplin, B., 1978. Patterns of Animal Disease. The English Language Book Society and Bailliere Tindal. London, pp: 151-157.

Ideris, A., A.L. Ibrahim and P.B. Spadbrow, 1990. Vaccination of chickens against Newcastle disease with a food pellet vaccine. Avian Pathol., 19: 371-374.

Kafi, M.A., 2003. Vaccination studies on use of Ranikhet inactivated vaccine combined with V_4HR or BCRDV. M. S. Thesis. Department of Microbiology and Hygiene. Bangladesh Agricultural University, Mymensingh-2202, Bangladesh.

Rahman, M., 2003. Growth of poultry industry in Bangladesh, poverty alleviation and employment opportunity. Proceeding of the 3rd International poultry show and seminar, Bangladesh China Friendship Conference Centre, Dhaka, Bangladesh, February 28- March 2, 2003, pp: 1-7.

Reed, L.J. and H. Muench, 1938. A simple method for estimating fifty percent endpoints. Am. J. Hyg., 27: 493-497.

Saifuddin, M., A.J.S. Sarker, M.M. Amin and M.A. Rahman, 1986. Studies on the efficacy of Newcastle disease vaccines and their vaccination schedule. Bang. Vet. J., 20: 67-75.

Spadbrow, P.B. and J.L. Samuel, 1987. Oral Newcastle disease vaccine in experimental chickens in Australia. In: Copland J. W. (Ed.), Newcastle Disease in poultry. A New Food Pellet Vaccine. ACIAR, Canberra, Monograph No. 5, pp: 44-49.

Tantaswasdi, U., J. Danvivatanapom, P. Siriwan, A. Chaisingh and P.B. Spadbrow, 1992. Evaluation of an oral Newcastle disease vaccine in Thailand. Prev. Vet. Med., 12: 89-94.

Toth, B. and P. Markovits, 1964. Studies on the immunogenicity of Newcastle disease vaccine strains in growing chickens. Acta. Vet. Hung., 14: 339-345.

Westbury, H.A., 1981. Newcastle disease in Australia. Aust. Vet. J., 57: 292-298.

Westbury, H.A., G. Pearsons and W.H. Allan, 1984. Comparison of the immunogenicity of Newcastle disease virus strains V_4, hitchner B_1 and Ls Sota in chickens. 2. Tests in chickens with maternal antibody to the virus. Aust. Vet. J., 61: 10-13.