A COMPARISON OF THE V_4 STRAIN WITH THE CONVENTIONAL F_1 AND M STRAIN OF NEWCASTLE DISEASE VACCINE IN RURAL BANGLADESH

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Summary

Bangladeshi indigenous chickens of mixed ages vaccinated twice at a three week interval with either conventional vaccines- F_1 (ocular) and M (mukteswar, Intramuscular), or heat resistant V_4 vaccine administered by either the ocular or oral routes, all showed satisfactory hemagglutination inhibition antibody (HI) responses and protection against Newcastle Disease (NCD) challenge persisting for four months. The antibody response to F_1 and M was higher than for V_4, which was similar whether administered by the ocular or oral routes. All vaccinated treatments have a significant level of protection compare to the control group ($p < 0.01$). No significant difference ($p > 0.05$) in the protection against controlled challenge with virulent NCD virus was found between vaccinated groups.

(**Key Words** : Newcastle Disease Conventional Vaccines (F_1, M Strains), Heat Resistant V_4 Vaccine, Chickens)

Introduction

In Bangladesh the small scale rearing of chickens practiced in villages supplies the bulk of the poultry meat and eggs. Subsistence level households, in the Noakhali district town, belongs to an average of 13 chickens (Beidler, 1990). Scavenging chickens provide supplementary income, employment and a nutritious food at little cost, however the system is not very efficient. Newcastle disease (NCD) is reported to be the most prevalent poultry disease in the Noakhali district and caused mortality of 35% during the year ending June 1989 (Beidler, 1990). Bentz (1993) also found that NCD is clearly considered to be the most important disease of chickens by resource poor households in the Noakhali district. NCD is a major constraint to village level poultry production and is currently second priority after "Gumboro" in the national disease control programme of the Bangladesh government.

The control of NCD relies on vaccination. The current vaccination programme against NCD in Bangladesh includes ocular administration of live lentogenic F_1 strain vaccine to chicks below 2 months of age followed by intramuscular (IM) injection of live mesogenic Mukteswar or 'M' strain to growers and adults.

V_4 vaccine was chosen for this work because considerable evidence showed that the strain is highly immunogenic (Webster et al., 1970; Turner et al., 1976; Spradbrow et al., 1978; Ibrahim et al., 1980; Ibrahim et al., 1981; Ideris et al., 1990) when given in sufficient quantity, of very low virulence and is suitable for administration by mass application procedures due to its transmissibility properties (French et al., 1967; Hall et al., 1967; Westbury, 1981). NDV_4HR has the added advantage that it has been selected for heat resistance. These characteristics of the NDV_4HR vaccine along with the added simplicity of having only one type of vaccine for all ages of chickens makes it potentially more attractive than those currently used in Bangladesh. The objective of this study was to evaluate for the first time the efficacy of the NDV_4HR vaccine compared to the F_1 and M strains when administered in a manner simulating a large scale rural poultry vaccination programme in Bangladesh.

Materials and Methods

Experimental design

The trial was conducted with 5300 indigenous chickens in 446 subsistence level rural household in the Noakhali and Lokhipur districts of Bangladesh. Field activities were implemented by 12 female field workers

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Received June 29, 1995

Accepted November 6, 1995

within the Mennonite Central Committee (MCC) farming systems research and extension programme. Each of the 12 working areas was divided into four parts of approximately equal areas. Four treatments were randomly assigned to the four parts thus making each complete working area one replication. The trial was conducted in areas not previously involved in MCC poultry vaccination programmes. The four treatments therefore became:

I No intervention with NCD vaccine
II F_1 (Eye drop) and Mukteswar (Intramuscular)
III NDV_4HR (Oral)
IV NDV_4HR (Eye drop)

Approximately 10 percent of the household within each treatment of each replication provided their chickens for blood sampling throughout the trial period. These households were initially selected at random, however in practice a bias was added to select the households that were willing to cooperate. All laboratory support was provided by Bangladesh Livestock Research Institute (BLRI), Savar, Dhaka.

Vaccine

A commercial live freeze dried NDV_4HR vaccine in 100 dose vials produced by Arthur Websters Pty Ltd., Sydeny, New South Wales, was purchased and supplied to MCC and BLRI by Checchi and Company Consulting inc. The V_4 strain of Newcastle disease virus (NDV) has been described previously (Simmons, 1967). The F_1 and M strains (Lot numbers 2009 and 2092) were collected from the Sudharam Thana Livestock Office on March 13 and April 5, 1993.

Vaccination

In each of the 3 vaccinated treatments 2 vaccination rounds were administered at a 3 week interval. For the conventional vaccination the F_1 strain was given by the ocular method to all chickens up to 2 months of age, and the M strain was administered by the intramuscular route to all birds aged 2 months or more. Birds were aged according to their owners estimation. The V_4 vaccine was administered by the oral and ocular routes as described in Websters information dossier (Heath et al., 1991). The feed used was parboiled rice that was boiled again and sundried before mixing with the vaccine. The calculated dose for all of the vaccinated treatments was 10^6 EID_{50}. The efficacy of the vaccines were evaluated by monitoring serological response, flock morbidity and mortality, and the protection against controlled on-station challenge with virulent NCD virus.

Serology

A baseline blood sample survey was taken approximately one week before the first vaccination. Following the second vaccination a further three blood sampling rounds were conducted at monthly intervals. During each blood sampling round a minimum of five blood samples were collected from each treatment of each replication. A total of 1291 blood samples were collected. The serum of 974 or 68 percent of the samples collected were titrated for NDV antibody using the hemagglutination inhibition (HI) test as previously described (Allan and Gough, 1973). All titres were recorded as $\log_2$ of the reciprocal of the endpoint dilution.

Challenge

Three birds from each treatment in each replication were purchased from households that had previously provided birds for blood sampling. Chickens were purchased on the basis of having a good vaccination take during both vaccination rounds and having apparent good health. The birds were tagged and sent to BLRI for controlled on-station challenge. The challenge was conducted 17 weeks after the second vaccination round. The challenge virus was a pathogenic Bangladeshi field isolate typical of the endemic strains throughout Bangladesh. The birds were challenged intramuscularly with 0.25 ml of 10^3 EID_{50} virus per bird. Challenged birds were observed for 15 days. Postmortem examinations were conducted on all birds that died during the challenge period.

Results and Discussion

Results of the serological tests conducted after the 1st and 2nd vaccination at the age of 1, 3, 7 and 11 weeks have been presented in table 1. From the present study it was revealed that all the vaccinated groups (Conventional & NDV_4HR) responded to vaccination having geometric mean titres of HI (Hemagglutination inhibition) antibody > 2. The HI antibody titre in chickens vaccinated by conventional vaccines (F_1 & M strain) were found to be higher than all other groups. The percentage of birds having HI antibody titre greater than or equal to 3 also higher in chicken vaccinated by conventional vaccines. However, significant differences of protection were not found between vaccinated groups ($p > 0.05$). Antibody titres in chickens vaccinated by oral and ocular route with NDV_4HR vaccine were found almost similar to each other and were greater than protection titre (> 2).

Results of controlled on-station challenge test and protection rates are presented in table 2. It is evident from the challenge test that all the chickens of non-vaccinated

TABLE 1. HEMAGGLUTINATION INHIBITION (HI) ANTIBODY TITRES (LOG_2) OF CHICKENS AT DIFFERENT INTERVALS OF VACCINATION WITH CONVENTIONAL AND NDV_4HR VACCINES

Treatment	Control		F_1 / M		NDV_4HR Oral		NDV_4HR Ocular	
	$\overline{X}$	% ≥ 3	$\overline{X}$	% ≥ 3	$\overline{X}$	% ≥ 3	$\overline{X}$	% ≥ 3
1 week before vaccination	2.4	0.5	2.8	47.2	1.5	43.5	2.9	21.6
2 to 3 weeks after vaccination	0.5	3.1	5.6	86.0	3.3	62.0	3.6	70.7
7 weeks after vaccination	0.8	14.3	4.1	76.0	3.3	66.7	3.3	69.4
11 weeks after vaccination	0.9	17.0	4.5	85.1	3.3	63.5	3.1	62.9

$\overline{X}$ = Geometric mean; ≥ 3 = Percentage (%) of birds with titre equals or greater than 3.

TABLE 2. RESULTS OF CONTROLLED ON-STATION CHALLENGE TEST AND PROTECTION RATES OF VACCINATED AND NON-VACCINATED (CONTROL) CHICKENS

Treatment	No. of birds challenged	No. of birds survived	% Protection	
			Mean	95% CL
Control	15	0	0	19.56
F_1 / M Strain	35	29	82.9	63.34-102.45
NDV_4HR Oral	35	26	74.3	54.74- 93.86
NDV_4HR Ocular	31	25	80.6	61.04-100.16

group (control) died within 8 days of challenge while the vaccinated chickens survived from 74.3 to 80%. Further it is evident that the percentage of protection is higher in chickens vaccinated by conventional (F_1 / M) vaccines than the NDV_4HR vaccine. Using student 't' test it was found that all vaccinated treatments have a significant level of protection compared to the control group ($p < 0.01$). No significant difference was found between vaccinated groups.

When birds become sick in rural Bangladesh it is common practice for households to kill the birds for home consumption or even sell them in the market, for this reason, along with the practical complexity of collecting and transporting sufficient numbers of samples for postmortem analysis. the experiment was not conducted on the basis of monitoring confirmed cases of NCD. but on overall flock mortality and morbidity. As the trial was conducted during the season of highest NCD incidence in an area where NCD has been identified as the most serious disease for poultry. it was thought reasonable to assume a high correlation would exist between flock mortality and morbidity and the incidence of NCD.

In order to estimate the level of protection offered by the vaccines it was necessary to estimate when each replication might have been subjected to a NCD outbreak. This was done by observing the mortality and morbidity

TABLE 3. ESTIMATED EFFECTIVE FIELD PROTECTION DURING THE NCD EPIDEMIC PERIOD

Groups	Suspected NCD infected replications[1]		No NCD infection replications[2]		Rise due to suspected NCD	Reduction due to vaccination	Estimated effective field protection
	Mean	SD	Mean	SD			
Control	27.5	19.1	1.8	2.8	25.7		
F_1 / M	8.1	9.5	2.2	4.3	5.9	19.8	77.0
V_4HR Oral	11.2	16.9	2.7	7.3	8.5	17.2	66.9
V_4HR eye	11.1	9.1	2.7	5.1	8.4	17.3	67.3

[1] average mortality + morbidity in the non-vaccinated group for the two week period was greater than 10 percent.
[2] average mortality + morbidity in the non-vaccinated group for the two week period was less than or equal to 10 percent.

rates in the non-vaccinated control group. Replications where the control group had a mortality plus were designated as being exposed to a suspected NCD outbreak. The estimated effective field protection levels are shown in table 3. NCD epidemic was only evident during three out of a total of eight period recorded, each period consisting of 15 days. During these three periods between five and seven out of twelve replications appeared to have some infection. Given the large difference between the vaccinated and non-vaccinated groups it seems likely that the mortality and morbidity observed during these periods was at least to a large extent due to NCD. The estimated field protection was highest for the conventional F_1 and M treatment, followed by V_4 (ocular) and V_4 (oral) which were almost the same as each other (table 3). The controlled on-station challenge indicated that all treatments gave similar levels of protection. The estimated effective field protection levels were all within 7.4 percent of the level of protection observed in the controlled on-station challenge.

During the initial blood sampling prior to the first vaccination round NCD was reported by the field workers to be epidemic in many of the replications. The high mean titres of up to 2.9 $\log_2$ indicate that blood samples were collected from infected birds. Maternal antibodies are unlikely to have had a significant affect as blood was collected mainly from growers and adult birds. Several workers have found an HI titre of 3 $\log_2$ to be the lowest reliable indicator of protection against NCD (Allan and Gough, 1974; Ibrahim et al., 1981; Sagild and Haresnape, 1987), although birds with lower titres may also be protected (Ibrahim et al., 1981). As presented in table 1, the percentage of samples with mean $\log_2$ titres of three or more was greatest for the F_1 (ocular) and M (intramuscular) treatment, followed by the V_4 (ocular), then V_4 (oral). This is possibly in part due to IgM responses which are not induced by oral vaccination. Oral vaccination may induce the protection of secretory immunoglobulins by stimulating the intestinal lymphoid tissue, and these could assist in resistance to challenge even when levels of circulatory antibodies are low (Spradbrow and Samuel, 1991). All vaccinated treatments show satisfactory HI responses.

The results do not show any clear difference in the efficacy of the different vaccinated treatments. However, the thermostability and ease of administration of the V_4 vaccine offers distinct advantages relative to the F_1 and M strains. In Bangladesh, chickens are generally free to scavenge around the household area during the daytime and then housed in small baskets at night in the farmers house. Chickens are more accessible for vaccination than in South East Asian countries such as Malaysia where precious work has been conducted with the V_4 vaccine (Ibrahim and Babjee, 1984). It may therefore be found that ocular route of application is equally appropriate to oral administration for a rural vaccination programme in Bangladesh.

For a practical rural vaccination programme the major cost for the vaccinator is transport to and from the Thana Livestock Office for collecting vaccines. The use of V_4 vaccine could enable trained vaccinators to collect vaccine from the Thana Livestock Office and vaccinate at the village level for at least two weeks without relying on refrigerator compared to the two days for the conventional vaccines. The vaccine would not rely on the ownership of insulated vaccine carriers or the availability of ice, and the need for only one type of vaccine for all ages of chickens instead of the two conventional vaccines would accelerate and simplify the vaccination process. These factors could help to create sustainable rural poultry vaccination programmes prerequisite for the development of rural poultry production in Bangladesh.

Acknowledgements

The authors wish to thank the Director, BLRI, Savar, Dhaka and the Agriculture Programme Administrator, MCC, Dhaka, for their cooperation and encouragement throughout the course of work. The authors also wish to thank the female field workers for their important role in implementing the trial with enthusiasm and precision and Mr. Manik Kuri for his invaluable assistance in coordinating the field activities. The authors are grateful to Dr. Chip Stem of Checchi and Consulting inc. for providing the Websters NDV_4HR vaccine to Mennonite Central Committee (MCC) and BLRI. The project was supported by funds from the MCC in Bangladesh with laboratory and technical support from BLRI, Savar, Dhaka, Bangladesh.

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