Seroconversion of a Thermostable Live Attenuated Lentogenic Strain Newcastle Disease Vaccine (Local Isolate) in Chicken

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Vaccine was prepared by using the isolate, exposed to 43°C for 12 days. The vaccination was performed in two thousand four hundred breeder flocks (Banoraja) in 3 different flocks @10^6.5 EID_{50} per bird oronasally at the age of day 5 (primary vaccination) followed by vaccination through drinking water on day 26 (boostering) in farm condition and thereafter every 45 days interval. The vaccine was also inoculated intranasally @10^6 EID_{50} in Two thousand backyard birds (Haringhata Black and non-descriptive) and were boostered after 21 days of 1st vaccination with the same dose and subsequent vaccination was performed at every 45 days interval. Serum samples were collected for HI titre at regular intervals and it was observed that the mean antibody both in backyard birds and breeder flocks was above 1.50 ± 00. Therefore, it was concluded that the thermostable live attenuated lentogenic strain Newcastle Disease vaccine after inoculation into backyard birds and breeder flocks, provides satisfactory level of immunity.

Keywords: Vaccine, Chicken, Newcastle, Serum sample.

India has emerged on the world poultry map as the 3rd largest egg (56 billion eggs) and 5th largest poultry meat (2.6 million tons) producer. Total chicken population has registered an annual growth of 7.3% in the last decade. While farm chicken grew at the rate of 12.4%, desi chicken showed much lower growth rate of about 2%. Other poultry species showed reduction of 2.3 % per annum between 2003 and 2007. Newcastle disease (ND) is the main killer of these chickens in many developing countries (Alexander, 1991). Ten serogroups of avian paramyxoviruses have been recognized: [APMV-1 to APMV-10] and APMV-1 remains the most important pathogen for poultry while others are known to cause disease in poultry and other types of birds (Alexander, 2003). To counter this deadly disease, proper control methods are needed. Vaccination has been reported as the only safeguard against endemic ND (Usman, 2002). It has been very difficult to vaccinate village chickens against ND using conventional vaccines. However, vaccines with the specific properties of thermostability have become a solution to these problems (Spradbrow, 1996). The Australian Centre for International Agricultural Research (ACIAR) sponsored project for vaccination against ND by using Australian V_4 and I_2 strains (ND asymptomatic pathotypes) in thirty countries in South East Asia, Africa and Australia. A thermostable vaccine enables distributors and users to reduce the problems associated with inadequate cold chains in the field.

MATERIALS AND METHODS

Source of the virus for the vaccine production

Viruses used in this study for thermostability testing were isolated, characterised
and provided by the Department of Veterinary Epidemiology and Preventive Medicine, Faculty of Veterinary and Animal Sciences, West Bengal University of Animal and Fishery Sciences, Kolkata-37.

**Embryonated Specific Pathogen Free (SPF) fowl eggs**

Embryonated SPF fowl eggs were procured from Venky’s (India) Ltd., SPF Eggs Division, Pune for propagation of virus as recommended by OIE, 2009.

**Spot/slide agglutination test**

One drop of allantoic fluid, collected during harvesting of virus, was dropped on a grease free glass slide. Freshly prepared 0.5% chick RBC suspension was added to the allantoic fluid in 1:1 ratio (50% v/v). Both the suspensions were mixed thoroughly by rotating the slide gently. The slide was examined by diffused light to see any haemagglutination.

**Standard Plate Haemagglutination Test (HA) as per OIE (2009)**

**Determination of Embryo Infective Dose fifty (EID50) of the virus as per Reed and Muench (1938) and FAO (2002)**

The HA titre was estimated as per OIE (2009) and the Embryo Infective Dose fifty (EID50) was determined as per the FAO(2002) and the result was calculated as per Reed and Muench (1938).

**Source of the vaccine**

The thermostable virus was serially passaged in 9-11 days old embryonated SPF fowl eggs and observed no embryopathy on 45th passage. For confirmation of attenuation, the passaged virus was inoculated @ $10^{6.5}$ EID50 oro-nasally to 10, five days old SPF chicks and observed no abnormality/ mortality for 21 days post inoculation. The 44th passaged virus was inoculated into five 9-11 days old embryonated SPF fowl eggs and allantoic fluids were harvested after 5 days of post inoculation. After estimation of HA titre separately the allantoic fluid was pooled together considered as master seed virus and the HA titre and EID50 was estimated. The master seed virus was given three serial passages in SPF fowl eggs. After 5 days of incubation, allantoic fluids were harvested from every passage of each inoculated egg and HA titre was determined as per the OIE (2009). Finally, the allantoic fluids of the three passages were pooled together considered as vaccine and HA titre was determined as per the OIE (2009). The vaccine dose was calculated by determining the EID50 as per the method of FAO (2002) and Reed and Muench (1938). For confirmation of vaccine virus as APMV-1, HI test was performed using known positive antisera of APMV-1.

**Determination of EID50 of locally isolated velogenic pathotype ND challenge virus**

EID50 of the locally isolated velogenic pathotype of NDV was determined in 9-11 day old embryonated SPF fowl eggs as per the standard method Reed and Muench, 1938.

**Experimental design**

Study of the seroconversion of the thermostable live attenuated lentogenic strain (local isolate) ND vaccine in layer and backyard birds.

**For layer bird**

Two thousand four hundred commercial layer chicks (Banoraja) in 3 different flocks were vaccinated with live attenuated thermostable lentogenic strain (local isolate) ND vaccine @ $10^{6.5}$ EID50 per bird oro-nasally at the age of day 5 (primary vaccination) followed by vaccination through drinking water on day 26 (boostering) in farm condition and thereafter every 45 days interval.

**For backyard bird**

Two thousand backyard birds (Haringhata Black and non-descriptive) were inoculated oro-nasally @ $10^{6.5}$ EID50 of prepared vaccine to each bird ignoring the age of the bird and were boostered after 21 days of 1st vaccination with the same dose and subsequent vaccination was performed at every 45 days interval.

**Serology**

Serum samples from 10% of the vaccinated birds were assayed by haemagglutination inhibition (HI) antibody test as per OIE (2009) before primary vaccination/1st vaccination, before boostering and then every 45 days interval prior to vaccination.

**Challenge study**

Twelve vaccinated birds were subdivided into two groups i.e. vaccinated experimental group
and vaccinated control group and housed separately with balanced feed and water ad lib. Vaccinated experimental group of birds (consisted of 6 birds) were challenged with velogenic pathotype of ND virus intra-nasally at the dose rate of $10^6 \text{EID}_{50}$ per bird and another group (consisted of 6 birds) was kept as vaccinated control. Both the groups of birds were reared for next 3 weeks and observed for any mortality and or abnormality. Blood samples were collected from all the birds of both the group at the

Showing mean antibody HI titres of 3 different ages of birds vaccinated with live attenuated thermostable lentogenic strain ND vaccine (local isolate):

| S. No. | Flock size | Before primary vaccination (5 days of age) | Before boostering (26 days of age) | Thereafter every 45 days interval |
|--------|------------|------------------------------------------|----------------------------------|---------------------------------|
| 1      | 800x3      | 1.220±0.03a                              | -                                | -                               |
| 2      | 800x3      | 1.561±0.03b                              | -                                | -                               |
| 3      | 800x3      | 1.704±0.03c                              | -                                | -                               |
| 4      | 800x3      | 1.996±0.05d                              | -                                | -                               |
| 5      | 800x3      | 1.932±0.05e                              | 1.866±0.06c                      | -                               |
| 6      | 800x3      | 1.714±0.04f                              | 1.701±0.06e                      | -                               |
| 7      | 800x3      | 2.081±0.06g                              | 2.800±0.06f                      | -                               |
| 8      | 800x3      | 1.634±0.02c                              | 1.905±0.06e                      | -                               |
| 9      | 800x3      | 2.050±0.06d                              | 1.938±0.03e                      | -                               |
| 10     | 800x3      | 1.320±0.05e                              | 1.756±0.02c                      | -                               |
| 11     | 800x3      | 1.252±0.03c                              | 2.020±0.03d                      | -                               |
| 12     | 800x3      | 1.500±0.04e                              | 2.348±0.04f                      | -                               |
| 13     | 800x3      | 1.844±0.02d                              |                                  |                                  |
| 14     | 800x3      | 1.728±0.06d                              |                                  |                                  |
| 15     | 800x2      | 2.020±0.03d                              |                                  |                                  |
| 16     | 800x1      | 2.348±0.04f                              |                                  |                                  |

(Note: Means bearing any one common superscript (i.e. a, b, c, d, e, f and g) in the columns did not differ significantly with each other)

Showing mean HI antibody titre of Haringhata Black backyard birds vaccinated with live attenuated thermostable lentogenic strain ND vaccine (local isolate):

| S. No. | No. of birds | Before first vaccination | Before boostering | Thereafter every 45 days interval |
|--------|--------------|-------------------------|-------------------|---------------------------------|
| 1      | 2000         | 0.312±0.16a             | 1.157±0.03b       |                                 |
| 2      | 2000         | 1.350±0.03c             | 1.794±0.02d       |                                 |
| 3      | 2000         | 1.714±0.04d             | 1.320±0.05e       |                                 |
| 4      | 2000         | 1.252±0.03c             | 1.500±0.04e       |                                 |
| 5      | 2000         | 1.844±0.02d             | 1.728±0.06d       |                                 |
| 6      | 2000         | 2.020±0.03f             |                                  |                                 |

(Note: Means bearing any one common superscript (i.e. a, b, c, d, e and f) in the columns did not differ significantly with each other)
time of prior to challenge and then weekly for 3 weeks. HI antibody titre was determined from each sample as per the method OIE (2009).

Statistical analysis
The results were analyzed statistically by one way ANOVA to study the seroconversion and protectivity of live lentogenic strain (local isolate) ND vaccine through drinking water in commercial broiler birds.

RESULTS AND DISCUSSION

Antibody response in layer birds
Similar finding was also observed by Iroegbu et al. (2014) who reported that unvaccinated laboratory-raised, five week old cockerels were fed V₄ Newcastle disease vaccine in cassava once, twice or thrice; and tested for hemagglutination inhibition (HI) antibody response and observed that the immune status of the chicken flocks improved with number of vaccinations.

During the two years studies the mean antibody titre of one of the three flocks decreased (i.e. 1.63±0.02) but not below protective level at the age of 12th month (11 months, 26 days). Investigation in the farm it was revealed that E. coli and M. gallisepticum organism have been isolated and identified from that flock as no outbreak of NDV or no clinical signs of ND was seen and even no APMV-1 virus was isolated from the lesion of the dead birds during that period.

Antibody response in Haringhata Black backyard birds
After regular revaccination, the mean antibody titre increased significantly (P<0.05) to protective level (i.e.1.5±0.04) and afterwards again increased significantly (P<0.01) above protective level throughout the trial period. By thorough

### Table 1: HI antibody titre of non descriptive backyard birds vaccinated with live attenuated thermostable lentogenic strain ND vaccine (local isolate)

| S. No. | No. of birds | Before first vaccination | Before boosting | Thereafter every 45 days interval |
|--------|--------------|--------------------------|-----------------|---------------------------------|
| 1      | 2000         | 0.600±0.06ᵃ               |                 |                                 |
| 2      | 2000         | 1.544±0.03ᵇ               | 1.858±0.02ᶜ     |                                 |
| 3      | 2000         |                          | 1.840±0.05ᶜ     |                                 |
| 4      | 2000         |                          | 1.524±0.03ᵈ     |                                 |
| 5      | 2000         |                          | 1.820±0.05ᶜ     |                                 |
| 7      | 2000         |                          | 1.890±0.06ᶜ     |                                 |
| 8      | 2000         |                          |                 |                                 |

(Note: Means bearing any one common superscript (i.e. a, b, c and d) in the columns did not differ significantly with each other)

### Table 2: HI titres and abnormality (sick) and mortality of vaccinated experimental and vaccinated control groups before and after challenge

| Birds                  | HI titre before challenge | Post challenge 7th day | Post challenge 14th day | Post challenge 21st day | Post challenge abnormality/mortality 7th day | Post challenge abnormality/mortality 14th day | Post challenge abnormality/mortality 21st day |
|------------------------|---------------------------|-------------------------|-------------------------|-------------------------|---------------------------------------------|---------------------------------------------|---------------------------------------------|
| Vaccinated experimental group | 2.11±0.04ᵃ                 | 1.53±0.03ᵇ              | 1.68±0.04ᶜ              | 1.76±0.03ᵈ              | -                                          | -                                          | -                                          |
| Vaccinated control group  | 2.11±0.04ᵃ                 | 1.63±0.04ᵇ              | 1.22±0.02ᶜ              | 0.42±0.03ᵈ              | -                                          | -                                          | -                                          |

(Note: Means bearing any one common superscript (i.e. a, b, c and d) in the rows did not differ significantly with each other)
investigation in the village, coccidia infection was isolated and identified along with tapeworm infestation among those backyard birds at that time. Therefore, the slight decline in antibody titre might be due to secondary infection of coccidiosis and tapeworm infestation. Similar finding was also observed by Hassan et al. (2012).

**Antibody response in non-descriptive backyard birds**

After revaccination, the mean titre increased significantly ($P<0.01$) i.e. $1.820\pm0.05$ and $1.890\pm0.06$ and persisted above protective level. Thus, it can be concluded that the thermostable vaccine produced significant protective titre when inoculated to the backyard birds.

Bouzari and Spardbrow (2006) also reported that the Heat-resistant strains of Newcastle disease virus, such as strain $V_4$ are being used as vaccine to protect flocks of rural chicken in developing countries.

**Challenge study**

From the table, it was observed that before challenge the mean HI titres of both the groups were same and very good i.e. $2.11\pm0.04$. After challenge the mean antibody titres of the vaccinated experimental group decreased significantly ($P<0.01$) i.e. $1.53\pm0.03$ on 7th day with no mortality/abnormality. Afterwards, the mean antibody titres increased significantly ($P<0.05$) on 14th day (i.e. $1.68\pm0.04$) and at the end of the observation period (i.e. $1.76\pm0.03$) with no mortality/abnormality.

On the other hand, the mean antibody titre of the vaccinated control group decreased gradually with the advancement of time and reached to $0.42\pm0.03$ at the end of the experiment with no mortality/abnormality. Similar finding was also described by Rahman and Khan (2004).

**CONCLUSION**

- The live attenuated lentogenic strain thermostable ND vaccine (local isolate) was absolutely safe and showed desirable potency in layer chicks.
- The thermostable live attenuated lentogenic ND vaccine (local isolate) was highly potent and generated sufficient immune response when administered through drinking water in farm condition. But the immune response was not optimum when the flocks were concomitantly infected with other diseases.
- The vaccine is also highly efficacious and produces optimal immune response in backyard birds when administered intranasally in village level.
- The vaccinated backyard birds became able to withstand the challenge infection of velogenic pathotype of NDV (local isolate).
- The vaccine without any additive (stabilizer) when kept at room temperature, could retain its viability for at least three months.
- The vaccine can be used in field level.

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