

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

L. Sharma\*, U. Biswas, C. Guha, A. Chatterjee, P.S. Jana and R. Pandey

Department of Veterinary Epidemiology & Preventive Medicine, West Bengal University of Animal and Fishery Sciences, 37, K. B. Sarani, Kolkata-700037, India.

<http://dx.doi.org/10.22207/JPAM.11.2.40>

(Received: 25 September 2016; accepted: 03 December 2016)

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<sub>50</sub> per bird oronasally at the age of day 5 (primary vaccination) followed by vaccination through drinking water on day 26 (boosting) in farm condition and thereafter every 45 days interval. The vaccine was also inoculated intranasally @ $10^{6.5}$  EID<sub>50</sub> in Two thousand backyard birds (Haringhata Black and non-descriptive) and were boosted after 21 days of 1<sup>st</sup> 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  $1.50 \pm 00$ . 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 3<sup>rd</sup> largest egg (56 billion eggs) and 5<sup>th</sup> 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<sub>4</sub> and I<sub>2</sub> 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

---

\* To whom all correspondence should be addressed.

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 (EID<sub>50</sub>) 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 (EID<sub>50</sub>) 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 45<sup>th</sup> passage. For confirmation of attenuation, the passaged virus was inoculated @ 10<sup>6.5</sup> EID<sub>50</sub> oro-nasally to 10, five days old SPF chicks and observed no abnormality/mortality for 21 days post inoculation. The 44<sup>th</sup> 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 EID<sub>50</sub> was estimated. The master seed virus was given three serial passages in SPF fowl eggs. Allantoic fluid was harvested after 5 days of incubation and HA titre was estimated. Finally, the allantoic fluids of 3 passages were pooled together considered as working seed virus and the HA titre and EID<sub>50</sub> was estimated. The working seed virus was serially inoculated for

three 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 EID<sub>50</sub> 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 EID<sub>50</sub> of locally isolated velogenic pathotype ND challenge virus**

EID<sub>50</sub> 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<sup>6.5</sup> EID<sub>50</sub> per bird oronasally at the age of day 5 (primary vaccination) followed by vaccination through drinking water on day 26 (boosting) in farm condition and thereafter every 45 days interval.

#### **For backyard bird**

Two thousand backyard birds (Haringhata Black and non-descriptive) were inoculated oronasally @ 10<sup>6.5</sup> EID<sub>50</sub> of prepared vaccine to each bird ignoring the age of the bird and were boosted after 21 days of 1<sup>st</sup> 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/1<sup>st</sup> vaccination, before boosting 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 adlib. Vaccinated experimental group of birds (consisted of 6 birds) were challenged with velogenic pathotype of ND virus intra-nasally at the dose rate of  $10^6$  EID<sub>50</sub> 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 boosting (26 days of age)	Thereafter every 45 days interval
1	800x3	1.220±0.03 <sup>a</sup>	-	-
2	800x3		1.561±0.03 <sup>b</sup>	-
3	800x3			1.704±0.03 <sup>c</sup>
4	800x3			1.996±0.05 <sup>d</sup>
5	800x3			1.932±0.05 <sup>de</sup>
6	800x3			1.866±0.06 <sup>e</sup>
7	800x3			1.714±0.04 <sup>c</sup>
8	800x3			2.081±0.06 <sup>d</sup>
9	800x3			2.800±0.06 <sup>f</sup>
10	800x3			1.634±0.02 <sup>c</sup>
11	800x3			2.050±0.06 <sup>d</sup>
12	800x3			1.905±0.06 <sup>de</sup>
13	800x3			1.938±0.03 <sup>de</sup>
14	800x3			1.756±0.02 <sup>c</sup>
15	800x2			2.020±0.03 <sup>d</sup>
16	800x1			2.348±0.04 <sup>g</sup>

(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 boosting	Thereafter every 45 days interval
1	2000	0.312±0.16 <sup>a</sup>		
2	2000		1.157±0.03 <sup>b</sup>	
3	2000			1.350±0.03 <sup>c</sup>
4	2000			1.794±0.02 <sup>d</sup>
5	2000			1.714±0.04 <sup>d</sup>
6	2000			1.320±0.05 <sup>c</sup>
7	2000			1.252±0.03 <sup>c</sup>
8	2000			1.500±0.04 <sup>e</sup>
9	2000			1.844±0.02 <sup>d</sup>
10	2000			1.728±0.06 <sup>d</sup>
11	2000			2.02±0.03 <sup>f</sup>

(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 V<sub>4</sub> 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. 1.634±0.02) but not below protective level at the age of 12<sup>th</sup> 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

Showing 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±0.06 <sup>a</sup>		
2	2000		1.544±0.03 <sup>b</sup>	
3	2000			1.858±0.02 <sup>c</sup>
4	2000			1.840±0.05 <sup>c</sup>
5	2000			1.524±0.03 <sup>d</sup>
7	2000			1.820±0.05 <sup>c</sup>
8	2000			1.890±0.06 <sup>c</sup>

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

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

(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.  $1.820 \pm 0.05$  and  $1.890 \pm 0.06$  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.  $2.11 \pm 0.04$ . After challenge the mean antibody titres of the vaccinated experimental group decreased significantly ( $P < 0.01$ ) i.e.  $1.53 \pm 0.03$  on 7<sup>th</sup> day with no mortality/abnormality. Afterwards, the mean antibody titres increased significantly ( $P < 0.05$ ) on 14<sup>th</sup> day (i.e.  $1.68 \pm 0.04$ ) and at the end of the observation period (i.e.  $1.76 \pm 0.03$ ) 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  $0.42 \pm 0.03$  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.

#### REFERENCES

1. Alexander, D.J., Newcastle disease. In: Rweyemamu, M.M., Palya, V., Tin Win (Eds.), Proceedings of a Workshop on Newcastle Disease Vaccines for Rural Africa, 1991. Pan African Veterinary Vaccine Centre, Debre Zeit, Addis Ababa, Ethiopia, 1991; pp. 7-45.
2. Alexander, D.J. Newcastle disease and other avian Paramyxoviruses infections In: Saif Y. M. (ed.): Disease of poultry, 11th ed. Ames, Iowa: Iowa State Press: 2003; 63-87.
3. Bensink, Z. and Spardbrow, P. B. Newcastle disease virus strain  $I_2$ , a prospective thermostable vaccine for use in developing countries. *Veterinary Microbiology*, 1999; **68**: 131-139.
4. Bouzari, M. and Spardbrow, P. Early Events Following Oral Administration of Newcastle Disease Virus Strain  $V_4$ . *The Journal of Poultry Science*, 2006; **43**: 408-414.
5. FAO. Basic laboratory manual of the small-scale production and testing of  $I_2$  Newcastle disease vaccine 2002.
6. Hassan, E. R., Mahgoob, K.M., Zeinab, E., Amin Girh, M. S. and Mekky, H. M. Comparative studies between the effects of antibiotic (oxytetracycline); probiotic and acidifier on *E. coli* infection and immune response in broiler chicken. *Journal of American Science*, 2012; **8**(4):795-801]. (ISSN: 1545-1003).
7. Ideris A., Ibrahim A.L., Spardbrow P.B. and Hung Seng C. Development of food pellet Newcastle disease vaccine. In: Copland J.W. (ed.): Newcastle disease in poultry: a new food pellet vaccine. Canberra, ACIAR, 1987; pp. 20-23.
8. Iroegbu, C. U., Okeke, M. I. and Ike, A. C. Relationship between antibody titres from  $V_4$ -in-cassava vaccination and protection against velogenic virus challenge in laboratory chicken. *African Journal of Microbiology Research*. 2014; **8**(6): 539-544.
9. OIE Manual (2009). Newcastle disease. Chapter 2.1.15. In: Office of International des Epizooties. J PURE APPL MICROBIO, **11**(2), JUNE 2017.

10. Reed, L.J. and Muench, L.H. A simple method of estimating fifty percentend points. *Amer. J. Hygeine*. 1938; **27**: 493-497.
11. Rahman, M. B. and Khan, M. S. R. Investigation on Baby Chick RanikhetDisease vaccine administration in chicks of vaccinated and non vaccinate origin. *Bangladesh. J. Vet. Med.*, 2004; **4**: 93-96.
12. Spradbrow, P. B. Newcastle disease in village chicken: control with thermostable oral vaccines. *Proceedings of an international workshop held in Kuala Lumpur, Malaysia*. ACIAR, 1992; **39**:189.
13. Spradbrow, P.B. Protection against important diseases including Newcastle disease. In: *Proceedings of XXth World's Congress*, vol. 1, 2±5 September 1996, New Delhi, India, 1996; pp. 31±34.
14. Spradbrow, P.B., Copland, J.W. Production of thermostable Newcastle disease virus in developing countries. *Prev. Vet. Med.*, 1996; **29**: 157±159.
15. Usman, M. Effects of vaccination of chickens against Newcastle disease with thermostable V<sub>4</sub> and Lasota vaccines using different grains and their brans as vehicles. M.Sc. Thesis, Department of Veterinary Surgery and Medicine, Ahmadu Bello Unviersity, Zaria, Nigeria 2002.