



Original Research Article

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## Studies on Thermostability of Newcastle Disease Viruses (Local Isolates) for Preparation of Vaccine

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

\*Corresponding author

### ABSTRACT

#### Keywords

Haemagglutination, EID<sub>50</sub>, Thermostability and Newcastle Disease Virus

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The study was carried out to assess the thermo stability of Newcastle Disease viruses (local isolates) which were isolated, characterised and provided by the Department of Veterinary Epidemiology and Preventive Medicine, West Bengal university of animal and Fishery Sciences. The thermo stability procedure was carried out at 25<sup>0</sup>C for 36 days, 37<sup>0</sup>C for 29 days, 40<sup>0</sup>C for 30 days and 43<sup>0</sup>C for 30 days respectively for each isolates. The results showed that all the five isolates tolerated 25<sup>0</sup>C and 37<sup>0</sup>C temperature for 36 days and 30 days respectively. The L4 isolate tolerated the 40<sup>0</sup>C temperature for 18 days but the L5 isolate tolerated the 43<sup>0</sup>C temperature for 12 days (showed HA titre 2<sup>4</sup> and EID<sub>50</sub> 10<sup>7.5</sup>) was detected in 30 days heat exposure. The L5 isolate showed the longest viability after exposure to the highest temperature and can be used as seed virus for preparation of vaccine at room temperature after attenuation.

### Introduction

Newcastle Disease (ND) is a major constraint to village poultry production throughout developing countries, frequently causing mortality rates of 75% to 100% in unvaccinated flocks (Spradbrow, 1992).

Vaccination has been reported as the only safeguard against endemic ND (Usman, 2002). Inactivated vaccines give very good immunity without vaccinal reactions and have been widely used, but are relatively expensive and require considerable attention to training when used by non-veterinary personnel. Live vaccines are easy to apply and relatively

inexpensive, and give moderately good immunity. Vaccinal reactions to them vary according to the vaccine strain. Among the live vaccines, the heat resistant vaccines require less stringent transport requirements in the field, and they have also been widely used in villages.

The selection of a ND vaccine for use in rural chicken depends on the local conditions in each country. Selection criteria include – ease of use, cost, thermo stability, immunogenicity, availability and transportability.

In circumstances where the cold chain is weak or absent, the only reliable option will be the

use of thermo stable ND vaccines; i.e. the live vaccines NDV<sub>4</sub>-HR (Ideris *et al.*, 1987) and I<sub>2</sub> (Bensink and Spradbrow, 1999), or inactivated vaccines such as ITANEW and Newcavac. The ACIAR sponsored project for vaccination against ND by using Australian V4 and I2 strains (ND asymptomatic pathotypes) in thirty countries in SEA, Africa and Austria (Spradbrow *et al.*, 1995).

The main objective of the present study is to test the thermo stability in different temperatures of the isolated ND viruses.

## **Materials and Methods**

### **Source of the virus for the vaccine production**

Viruses used in this study for thermo stability 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 (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).

### **Thermo stability testing of the virus isolates**

In the first cycle, vials containing virus were placed in B.O.D incubator at 25<sup>0</sup>C for 36 days. The HA titre and EID<sub>50</sub> was performed at every 6 days interval. At the end of the first cycle, the virus was passaged in embryonated SPF eggs. In the second cycle, the virus was kept at 37<sup>0</sup>C for 29 days and HA titre and EID<sub>50</sub> was checked at every 6 days interval. After the end of the second cycle the virus again inoculated in embryonated SPF eggs.

In the third cycle, the virus was exposed to 40<sup>0</sup>C for 30 days and the HA titre and EID<sub>50</sub> was performed at every 6 days interval. At the end of the first cycle the virus was passaged in embryonated SPF eggs. In the fourth cycle, the virus was exposed to 43<sup>0</sup>C for 30 days. The HA titre and EID<sub>50</sub> was performed at every 6 days interval. At the end of the cycle the virus was passaged in embryonated SPF eggs and HA activity was checked and virus was stored at -70<sup>0</sup>c for further use.

## **Results and Discussion**

All the 5 isolates i.e. L1, L2, L3, L4 and L5 of lentogenic pathotypes of NDV tolerated 25<sup>0</sup>C and 37<sup>0</sup>C temperature for 36 days and 30 days respectively (Table 1 and 2). The L4 isolate tolerated the 40<sup>0</sup>C temperature for 18 days but the L<sub>5</sub> isolate showed the HA titre up to the

end of 30 days cycle. On the other hand, the EID<sub>50</sub> of all the five isolates detected up to the end of the cycle. The L<sub>5</sub> isolate tolerated the 43°C temperature for 12 days (showed HA titre) and EID<sub>50</sub> was detected in 30 days heat exposure which means that the L<sub>5</sub> isolate showed the longest viability after exposure to the highest temperature and partially fulfilled the objectives and recommendations of the Commission of the European Communities (1993) and OIE (2004) (ICPI of <0.4 if no fewer than 10<sup>7</sup>EID<sub>50</sub> or <0.5 if no fewer than 10<sup>8</sup>EID<sub>50</sub>) for using as seed virus for preparation of vaccine at room temperature. Here, the lentogenic strain L<sub>5</sub> (local isolate) showed the longest viability (showed HA titre up to 12 days and EID<sub>50</sub> up to 30 days) exposed to highest temperature (43°C) having

the MDT 98 hours 40 minutes, ICPI 0.06 and IVPI 0.00 along with EID<sub>50</sub> above 10<sup>7</sup> (Biswas, 2006). Therefore, the L<sub>5</sub> isolate has been considered as seed virus after attenuation for preparation of vaccine.

Similar observations were expressed by Chakraborty (2013) and Bhagat (2014) at different temperatures.

Nassien and Adene (2002) reported that Hitchner-B<sub>1</sub> strain, K strain completely lost the titre at 72 hours and 95 hours respectively following thermal exposure at 36°C temperature. Therefore, from the result it was concluded that all the isolates withstood 37°C for long time (30 days) without losing their HA titre (Table 2).

**Table.1** Showing HA titre and EID<sub>50</sub> of the viruses exposed at 25°C for 36 days in the first thermal cycle

Sl. no.	Period of thermal exposure (in days)	HA titre					EID <sub>50</sub>				
		L <sub>1</sub>	L <sub>2</sub>	L <sub>3</sub>	L <sub>4</sub>	L <sub>5</sub>	L <sub>1</sub>	L <sub>2</sub>	L <sub>3</sub>	L <sub>4</sub>	L <sub>5</sub>
1.	0 (before thermal exposure)	2 <sup>9</sup>	2 <sup>10</sup>	2 <sup>10</sup>	2 <sup>10</sup>	2 <sup>9</sup>	10 <sup>8.67</sup>	10 <sup>9.54</sup>	10 <sup>9.55</sup>	10 <sup>10.32</sup>	10 <sup>9</sup>
2.	6	2 <sup>9</sup>	2 <sup>8</sup>	2 <sup>10</sup>	2 <sup>10</sup>	2 <sup>8</sup>	10 <sup>9.25</sup>	10 <sup>8.83</sup>	10 <sup>10.10</sup>	10 <sup>9.55</sup>	10 <sup>9.25</sup>
3.	12	2 <sup>9</sup>	2 <sup>8</sup>	2 <sup>10</sup>	2 <sup>9</sup>	2 <sup>8</sup>	10 <sup>9.16</sup>	10 <sup>9.61</sup>	10 <sup>10.33</sup>	10 <sup>9.24</sup>	10 <sup>9.29</sup>
4.	18	2 <sup>9</sup>	2 <sup>8</sup>	2 <sup>9</sup>	2 <sup>8</sup>	2 <sup>8</sup>	10 <sup>9.24</sup>	10 <sup>9.42</sup>	10 <sup>9.42</sup>	10 <sup>8.42</sup>	10 <sup>8.75</sup>
5.	24	2 <sup>8</sup>	2 <sup>8</sup>	2 <sup>7</sup>	2 <sup>7</sup>	2 <sup>8</sup>	10 <sup>9.79</sup>	10 <sup>8.66</sup>	10 <sup>10.16</sup>	10 <sup>9.5</sup>	10 <sup>8.75</sup>
6.	30	2 <sup>8</sup>	2 <sup>7</sup>	2 <sup>7</sup>	2 <sup>7</sup>	2 <sup>8</sup>	10 <sup>8.5</sup>	10 <sup>9.33</sup>	10 <sup>10.33</sup>	10 <sup>8.77</sup>	10 <sup>8.50</sup>
7.	36	2 <sup>7</sup>	2 <sup>7</sup>	2 <sup>6</sup>	2 <sup>5</sup>	2 <sup>8</sup>	10 <sup>8.45</sup>	10 <sup>7.65</sup>	10 <sup>9.25</sup>	10 <sup>7.42</sup>	10 <sup>8.50</sup>

**Table.2** The HA titre and EID<sub>50</sub> of the viruses exposed at 37°C for 30 days in the second thermal cycle

Sl. no.	Period of thermal exposure (in days)	HA titre					EID <sub>50</sub>				
		L <sub>1</sub>	L <sub>2</sub>	L <sub>3</sub>	L <sub>4</sub>	L <sub>5</sub>	L <sub>1</sub>	L <sub>2</sub>	L <sub>3</sub>	L <sub>4</sub>	L <sub>5</sub>
1.	0 (before thermal exposure)	2 <sup>10</sup>	2 <sup>9</sup>	2 <sup>10</sup>	2 <sup>10</sup>	2 <sup>9</sup>	10 <sup>9.54</sup>	10 <sup>8.83</sup>	10 <sup>10.5</sup>	10 <sup>10.76</sup>	10 <sup>10.16</sup>
2.	6	2 <sup>8</sup>	2 <sup>9</sup>	2 <sup>9</sup>	2 <sup>9</sup>	2 <sup>8</sup>	10 <sup>8.66</sup>	10 <sup>9.16</sup>	10 <sup>10.25</sup>	10 <sup>8.78</sup>	10 <sup>9</sup>
3.	12	2 <sup>7</sup>	2 <sup>8</sup>	2 <sup>7</sup>	2 <sup>8</sup>	2 <sup>8</sup>	10 <sup>8.61</sup>	10 <sup>9.7</sup>	10 <sup>10</sup>	10 <sup>9.56</sup>	10 <sup>8.64</sup>
4.	18	2 <sup>6</sup>	2 <sup>8</sup>	2 <sup>6</sup>	2 <sup>6</sup>	2 <sup>7</sup>	10 <sup>9.43</sup>	10 <sup>9.5</sup>	10 <sup>9.62</sup>	10 <sup>10</sup>	10 <sup>8.67</sup>
5.	24	2 <sup>6</sup>	2 <sup>7</sup>	2 <sup>6</sup>	2 <sup>6</sup>	2 <sup>7</sup>	10 <sup>9.16</sup>	10 <sup>9.33</sup>	10 <sup>10</sup>	10 <sup>9.24</sup>	10 <sup>8.53</sup>
6.	30	2 <sup>5</sup>	2 <sup>6</sup>	2 <sup>5</sup>	2 <sup>5</sup>	2 <sup>7</sup>	10 <sup>9.24</sup>	10 <sup>8.87</sup>	10 <sup>9.39</sup>	10 <sup>9.25</sup>	10 <sup>8.50</sup>

**Table.3** HA titre and EID<sub>50</sub> of the viruses exposed at 40<sup>0</sup>C for 30 days in the third thermal cycle

Sl. no.	Period of thermal exposure (in days)	HA titre					EID <sub>50</sub>				
		L <sub>1</sub>	L <sub>2</sub>	L <sub>3</sub>	L <sub>4</sub>	L <sub>5</sub>	L <sub>1</sub>	L <sub>2</sub>	L <sub>3</sub>	L <sub>4</sub>	L <sub>5</sub>
1.	0 (before thermal exposure)	2 <sup>9</sup>	2 <sup>9</sup>	2 <sup>9</sup>	2 <sup>9</sup>	2 <sup>9</sup>	10 <sup>9.54</sup>	10 <sup>8.83</sup>	10 <sup>10.5</sup>	10 <sup>10.76</sup>	10 <sup>10.20</sup>
2.	6	2 <sup>8</sup>	2 <sup>6</sup>	2 <sup>7</sup>	2 <sup>5</sup>	2 <sup>7</sup>	10 <sup>8.66</sup>	10 <sup>9.16</sup>	10 <sup>10.25</sup>	10 <sup>8.78</sup>	10 <sup>9.58</sup>
3.	12	2 <sup>6</sup>	2 <sup>4</sup>	2 <sup>5</sup>	2 <sup>3</sup>	2 <sup>7</sup>	10 <sup>8.61</sup>	10 <sup>9.7</sup>	10 <sup>10</sup>	10 <sup>9.56</sup>	10 <sup>10</sup>
4.	18	2 <sup>3</sup>	2 <sup>1</sup>	2 <sup>1</sup>	Nil	2 <sup>7</sup>	10 <sup>9.43</sup>	10 <sup>9.5</sup>	10 <sup>9.62</sup>	10 <sup>10</sup>	10 <sup>10</sup>
5.	24	Nil	Nil	Nil	Nil	2 <sup>7</sup>	10 <sup>9.16</sup>	10 <sup>9.33</sup>	10 <sup>10</sup>	10 <sup>9.24</sup>	10 <sup>10</sup>
6.	30	Nil	Nil	Nil	Nil	2 <sup>7</sup>	10 <sup>9.24</sup>	10 <sup>8.87</sup>	10 <sup>9.39</sup>	10 <sup>9.25</sup>	10 <sup>9.62</sup>

**Table.4** the HA titre and EID<sub>50</sub> of the virus in the fourth thermal cycle exposed at 43<sup>0</sup>C for 30 days

Sl. no.	Period of thermal exposure (in days)	HA titre	EID <sub>50</sub>
1.	0 (before thermal exposure)	2 <sup>10</sup>	10 <sup>9.25</sup>
2.	6	2 <sup>6</sup>	10 <sup>9.24</sup>
3.	12	2 <sup>4</sup>	10 <sup>8.25</sup>
4.	18	-	10 <sup>7.43</sup>
5.	24	-	10 <sup>7.69</sup>
6.	30	-	10 <sup>7.5</sup>

The selected 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> oronasally 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 (Table 3 and 4).

The selected lentogenic strain ND virus (local isolate) could withstand the thermal exposure of 40<sup>0</sup>C for 30 days and 43<sup>0</sup>C for 12 days without losing its viability and infectivity in

laboratory condition and used as vaccine after attenuation.

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