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## STUDIES ON THERMOSTABILITY OF NEWCASTLE DISEASE VIRUS (LOCAL ISOLATE) FOR PREPARATION OF VACCINE

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### ABSTRACT

*The study was carried out to assess the thermostability of Newcastle disease virus (local isolate) which was isolated, characterised and provided by the Department of Veterinary Epidemiology & Preventive Medicine, West Bengal university of animal and Fishery Sciences. The thermostability procedure consist of three cycles which were carried out at 25<sup>0</sup>C for 36 days, 37<sup>0</sup>C for 29 days and 56<sup>0</sup>C for time period ranging between 5 minutes to 8 hours respectively. The results showed that the infectivity titre of the virus was remained good at the end of the first thermal cycle i.e. the HA titre i.e. 2<sup>8</sup> and 10<sup>8.5</sup>EID<sub>50</sub>/ml respectively. At the end of the second thermal cycle the infectivity titre of the virus was also in good level i.e. 2<sup>7</sup> and 10<sup>8.50</sup>EID<sub>50</sub>/ml respectively. At last the infectivity titre of the virus decreased to 2<sup>5</sup> HA titre and 10<sup>7.36</sup>EID<sub>50</sub>/ml respectively in 10 minutes of thermal exposure at 56<sup>0</sup>C and the HA titre and EID<sub>50</sub> value became undetectable from 15 minutes and onward till the end of the third thermal cycle.*

**Key words :** *Newcastle disease, Local isolate, Thermostability.*

### INTRODUCTION:

ND or Ranikhet disease or avian pneumoencephalitis or pseudo fowl pest constitutes the most serious epizootic poultry disease throughout the world, particularly in developing countries (Chu and Rizk 1972; Aini 1990; Demey 1990; Spradbrow 1994, 1996; Bell 1996; Guèye 1998 2002b; Branckaert and Guèye 2000).

It is a highly contagious disease of the respiratory and nervous systems, mostly affecting chicken, but sometimes also affecting other poultry species, such as guinea fowls, ducks, turkeys, etc. It often devastates unvaccinated family poultry flocks in periodic outbreaks. The disease is caused by avian paramyxovirus type-I, with the most virulent strains isolated in Africa (Bell 1996; Spradbrow 1996; Verwoerd 1996). Vaccination is a most effective means of controlling ND and has been used throughout the world since 1940 (Beard and Hanson, 1984). Now-a-days, various pharmaceutical private agencies and State Biological are producing ND vaccines in India, consisting of live attenuated LaSota strain, B1 strain, F-strain and ND VH strain. These are lentogenic strains of ND vaccine. These vaccines are used mainly in the commercial poultry sectors (intensive poultry farms) and have limited applications in rural area (extensive production system) due to some problems like i) Heat lability of vaccine strain of viruses, ii) Large dose presentation, iii) Affordability, iv) Cold chain for effective administration of the vaccine and v) Ignorance of the farmers. Moreover, in areas where ND is endemic, disease control through vaccination is greatly cost effective intervention and has been given a high priority by farmers. Yet such measures appear not to be effective in many cases as frequent report of ND outbreak even in vaccinated flocks. Therefore, to prevent such type of economic loss by sudden outbreak of ND, it is very important to develop a simple but absolutely effective vaccine and easy to administration against the disease. 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 thermostable vaccines require less stringent transport requirements in the field.

Thermostability is a relative term. It has been unfortunate that thermostable vaccines have sometimes been considered as another basic commodity, like a sack of rice that needs no special treatment.

The 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 SEA, Africa and Austria (Spradbrow *et al.* 1995). In this context, thermostable strains should be better for local field and use for vaccine preparation that may be more effective against Newcastle disease.

The main objective of the present study is to test the thermostability in different temperatures of the isolated ND virus.

## **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 (EID<sub>50</sub>) of the virus as per Reed and Muench (1938) and FAO (2002).**

### **Thermostability testing of the virus isolate as per Aini Ideris (1992)**

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 test was performed at every 2 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 activity was checked at every 2 days interval. After end of the second cycle the virus again inoculated in embryonated SPF eggs.

In the third cycle, the virus was exposed to 56<sup>0</sup>C for different time period i.e. 5 minutes, 10 minutes, 15 minutes, 30 minutes, 1 hour, 2 hours, 4 hours and 8 hours and HA activity was performed.

At the end of the third cycle the virus was passaged in the embryonated SPF egg and HA activity was checked and virus was stored at -70<sup>0</sup>c for further use.

## **RESULT AND DISCUSSION:**

At the end of the first thermal cycle, the HA titre i.e. 2<sup>9</sup> and EID<sub>50</sub> i.e. 10<sup>9</sup>EID<sub>50</sub>/ml of the heat unexposed virus decreased to 2<sup>8</sup> and 10<sup>8.5</sup>EID<sub>50</sub>/ml respectively. Literature in this aspect was scanty in relation to the 36 days thermal exposure of virus at 25<sup>0</sup>C in the first cycle.

At the end of the first thermal cycle the concentration of virus increased by 3 serial passages in embryonated SPF fowl eggs and the HA titre and EID<sub>50</sub>/ml was detected before using it in the second thermal cycle.

At the end of the second thermal cycle, the HA titre i.e.  $2^9$  and EID<sub>50</sub> i.e.  $10^{10.16}$ EID<sub>50</sub>/ml of the heat unexposed virus decreased to  $2^7$  and  $10^{8.50}$ EID<sub>50</sub>/ml respectively. Literature in this aspect was scanty in relation to the 29 days thermal exposure of virus at 37<sup>0</sup>C in the second cycle.

At the end of the second thermal cycle the concentration of the virus increased by 3 serial passages in embryonated SPF fowl eggs and the HA titre and EID<sub>50</sub>/ml were detected before using it in the third thermal cycle.

In the third cycle, the HA titer i.e.  $2^8$  and EID<sub>50</sub> i.e.  $10^{8.78}$ EID<sub>50</sub>/ml of the heat unexposed virus decreased to  $2^5$  and  $10^{7.36}$ EID<sub>50</sub>/ml respectively in 10 minutes of thermal exposure and the HA titer and EID<sub>50</sub> value became undetectable from 15 minutes and onward till the end of the third thermal cycle.

Islam and Abdellatif (2012) reported that titer of Dongola strain (heat stable) decreased 2 logarithmic order within 15 minutes of incubation at 56<sup>0</sup>C and EID<sub>50</sub>/ml decreased from  $10^{10.0}$ EID<sub>50</sub>/ml of unheated to  $10^{8.0}$ /ml of heated virus.

## CONCLUSION:

From the results of the present study, it can be concluded that the lentogenic strain ND virus (local isolate) could withstand the thermal exposure of 25<sup>0</sup>C for 36 days, 37<sup>0</sup>C for 29 days and 56<sup>0</sup>C for 10 minutes without losing its viability. Therefore, the virus can be used as vaccine strain which will be able to withstand the environmental temperature up to 37<sup>0</sup>C for a month without losing its viability and infectivity.

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