

Original article

THERMOSTABILITY OF RECONSTITUTED NEWCASTLE DISEASE VIRUS STRAINS AT 36⁰C TEMPERATURE

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Haemagglutination (HA) test was employed to determine the stability of HA titers of reconstituted form of Hitchner – B1 (B1), LaSota (L) and Komarov (K) strains of Newcastle Disease Vaccine (NDV) at 36⁰c. The temperature treatment method was through incubation (in water bath) of the reconstituted vaccines at selected temperature and sequential sampling of each vaccine vial for the determination of pre – and post – temperature exposure HA titers. Thus, on the basis of a two-step (2log₂) decline in titer as evidence of loss of stability of HA titers (LST), the LST therefore, occurred at 50th, 24th and 95th hour for B1, L and K strains, respectively, post – temperature exposure. The data, therefore, showed that the NDV – K strain was the most stable at the test temperature. It is believed that the findings will enhance the understanding of the potential of this strain in the developed and application of a thermostable MDV target on village poultry. In rural settings.

Keywords: Haemagglutination, Thermostability, Newcastle Disease Virus, Reconstituted and Strain.

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INTRODUCTION

Newcastle disease (ND) is a ribonucleic acid (RNA) virus infection of birds, which can cause up to 100 percent mortality in susceptible chickens. It is world wide in distribution (Lancaster, 1966; Lancaster and Alexander, 1975). While many avian species may become infected, dramatic losses are seen most often in the domestic fowl and to a lesser extent in turkeys and pheasants (Rosenberger, et al., 1975; Hanson, 1978; Lancarter, 1981; Vickers and Hanson, 1982; Gordon and Jordan, 1982). ND is caused by a paramyxovirus, 100 – 200nm in diameter and is classified as avian paramyxovirus type 1 (Alexander, 1980; Nagai et al., 1976b). The known surface antigens are the haemagglutinin and the enzyme neuramidase (Nagai., et al., 1976a). The haemagglutinin causes dimer formation with avian and other red blood cells and the resulting haemagglutination is the basis of the most commonly used laboratory tests, the identification of viral haemagglutinin and the demonstration of its specificity using the haemagglutination inhibition (HI) test (Allan and Gough, 1974).

The stability of the ND virus is crucial to its value as a vaccine strain (Allan., et al, 1973). Recent developments on ND vaccine studies have been focussed on the thermostability of vaccine strains and its suitability for village operational settings. Available reports on the only widely known thermostable ND vaccine, the Australian V4 strain, indicate the need for further work on aspects like the vaccine strain, immunogenicity and compatibility with feed or vaccine-delivery

medium (Copland, 1992; Spradbrow, 1992). This study was designed to provide information on the thermostability of three reconstituted strains of ND virus at a temperature (36⁰) which equates that of the tropical environment especially Nigeria with the aim of establishing and adopting a vaccination programme suitable for rural poultry.

MATERIALS AND METHODS

Vaccine Strains and Temperature (36⁰) Treatment

Representative vials of three common strains of ND vaccine i.e. Hitchner – B1 and LaSota (both lentogenic strains) and Komaro (mesogenic strain) but with varying degrees of immunogenicity were procured from proprietary outlets of the manufacturer – Nigeria Veterinary Research Institute (NVRI), Vom, Jos, Nigeria.

A 200 dose vial of each strain of the lyophilized vaccine was reconstituted in 8ml of fresh normal saline as diluent, recapped and then gently shaken to homogenize before placement on a rack inside a water bath at 36⁰C. A pre – treatment sampling had been taken so as to determine the initial (stock) haemagglutination (HA) titer for each vaccine strain.

Sampling and Titration

A pre – treatment HA titer for each vaccine strain was determined according to the procedure described by (Allan and Gough, 1974a; Brugh and Beard, 1980) and slightly modified through the use of micro-titration tools as stated below.

Haemagglutination: In the HA test, the microtitration format was employed, using U –

bottom polystyrene microtiter plates, 50µl calibre steel-alloy microdiluters and 50µl plastic micropipettes. Thus, a two-fold serial dilution was carried out from 1:2 dilution, in wells A1 and B1 down to 1:1,024 dilution in wells A12 and B12. Finally using the 50µl plastic micropipette, a drop of 0.5 percent chicken red blood cell (rbc) suspension (as indicator) was added to each well. The control consisted of a pair of wells with same volumes of normal saline and rbc but no antigen. The microtiter plate was shaken for about five seconds on a microshaker, then incubated at room temperature and read after 30 – 45 minutes or as soon as the RBCc in the control wells have settled. The end-point was taken as the dilution in the last pair of wells that showed complete (100 percent end point) haemagglutination. The HA titer was the reciprocal of that value as recommended by (Allan, et al., 1973).

Subsequently, sampling and titration was done at selected interval every hour (minimum of 3 hours daily) post – temperature exposure, until the haemagglutinative activity of the investigated antigen(s) titer had declined by two-step ($2\log_2$) titers or more.

RESULTS

The persistence of haemagglutination (HA) titer was adopted as a measure of antigen stability. The starting titer and the trend in decline in titer when the three strains of ND vaccines were maintained at 36°C are shown in figure 1.

Hitchner – B1 strain with an initial (stock) titer of $5\log_2$, within a few hours of post – temperature exposure (PTE) experienced one-step decline in titer to $4\log_2$. The said titer was stable until the 50th hour when a two-step decline in titer to $3\log_2$

was observed and subsequently, complete loss of titer was recorded on the 71st hour PTE.

Similarly, the L strain with a pre-temperature exposure titer of $6\log_2$ soon lose its titer after the first three hours to $5\log_2$. The antigen was fairly stable at this titer until the 24th hour when a further decline to $3\log_2$ and below was experienced.

The K strain with a stock titer of $5\log_2$ was indeed stable at this titer right from the onset of exposure at 36°C until the 28th hour PTE. A 2nd phase of stability at $4\log_2$ was maintained by K strain, which lasted until the 95th hour when a two-step decline in titer was recorded. Complete loss of HA titers were observed subsequently thereafter.

Using the criterion of decline by at least two-step ($2\log_2$) in titer as a signal for the loss of stability (LOS), the figure shows that the LOS started at the 50th, 24th and 95th hour PTE for B1, L and K strains, respectively. This result thus portrays K strain as the most stable of the three strains of ND vaccine at 36°C PTE.

DISCUSSION

Considering the trend in HA titers of the three ND strain from the initial (i.e. pre-temperature exposure) phase up to the 49th hour, showed that although the L strain had an initial advantaged titer of $6\log_2$, the rapid decline in titer portrays it as a less stable strain than the B1. Meanwhile, the B1 strain was maintained at a reasonably stable titer of $4\log_2$ from the 5th to 49th hour post-temperature exposure before experiencing a decline. The sum of results from this study which suggest the K strain as the most stable is perhaps indicative of reversal of the comparative stability of the K and B1 strains at lower temperatures (Nssien and Adene, 2000).

There is a dearth of current information on investigation on the thermostability of HA titers of ND virus strains in reconstituted form at 36°C. Nevertheless, previous report by Frerichs and Herberts (1974) on International Reference Preparation of lyophilized ND live vaccine stated that titers were reduced by 26% after one month at 37°C. Panina and Nardelli (1965). Likewise, working with F strain, reported that storage at 37°C for one week produced a reduction in titer. In this study, the vaccines were investigated in their reconstituted form at 36°C and the results were presented graphically. Furthermore, K strain portrayed a stability period, which lasted between 49 – 95 hours PTE in reconstituted form at a temperature (36°C) equating that which obtains in a country like Nigeria. This, therefore, has lend allegiance to guaranteed availability of the antigen to rural poultry prior to loss of stability and immunogenicity.

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