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PRELIMINARY FINDINGS ON THE THERMOSTABILITY OF THREE IMMUNOGENIC STRAINS OF NEWCASTLE DISEASE VIRUS UNDER REGIMEN OF HIGH TEMPERATURE

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Target Audience: Scientists, poultry, farmers, veterinary, extension staff, vaccine manufacturers.

ABSTRACT

Haemagglutination (HA) test was employed to determined the stability of HA titers of reconstituted form of Hitchner-BI (B1), Lasota (L) and Komarov (K) strains of Newcastle Disease Virus (NDV) in vaccines at 45°C and 56°C respectively. The temperature treatment method was through incubation (in water bath) of the reconstituted vaccines at selected temperatures and sequential sampling of each vaccines for the determination of HA titers. A two-step ($21og_2$) decline in titer was adopted as evidence of loss of stability . Thus, on the basis of this criterion, loss of stability was after 5 hours for both B1 and L strains and after 23 hours for K strain at 45°C. The corresponding period at 56°C for both B1 and L strains were less than 10 minutes each and longer than the 10 minutes for K strain. The result therefore showed that the NDV-K strain was the most stable at high temperatures. Further studies are required for the characterization of the three strains after reconstitution at low and intermediate temperatures. It is believed that the findings will enhance the understanding of the potentials of these strains in the development and application of thermostable Newcastle Disease (ND) vaccine for rural poultry.

Key words: Newscastle disease; virus, strains, immunogenic, thermostability

DESCRIPTION OF PROBLEM

Newcastle Disease (ND) is an infectious highly contagious viral disease of domestic poultry especially chickens, turkeys and numerous species of wild and captive birds caused by Newcastle Disease Virus (NDV). Many of the lentogenic Newcastle Disease Viruses (NDVs) isolated from waterfowl are heat stable, distinguishing them from the usual lentogenic viruses in poultry (1,2). The virion or mature virus unit, which varies in size from 120-300nm but is usually about 180nm, consists of an envelope and an internal component. The other sensitive and osmotically deformable envelope has a pattern of projections or spikes (80A long) and contains the antigenic components that

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stimulate the hosts to produce haemagglutinatoin-inhibition (HI) and virus-neutralizing (VN) antibodies (3). It was observed that NDV possess several biological properties, the most important ones being haemagglutination and hemolysis of erythrocytes and other cell types (4). The haemagglutinin and enzyme neuramidase are responsible for the hemolytic activity. The stability of NDV can be measured on the basis of alterations in the ability of the virus to infect, to agglutinate cells and to induce an immunogenic response. These abilities can be destroyed at varying rates by exposure to such physical, and chemical treatments as heat, light, ultraviolet, X-rays, oxidation processes, etc, (5). This study was designed to find out the thermostability of three reconstituted immunogenic strains of NDV at 45^{0}C and 56^{0}C temperatures.

MATERIALS AND METHODS

Vaccine strains and temperature (45^{0}C and 56^{0}C) treatments.

Representative vials of three common strains of lyophilized ND vaccine i.e Hitchner-B^1 and Lasota (both lentogenic-strains) and Komarov (mesogenic strain) but with varying degrees of immunogenicity were procured from proprietary outlets of the manufacturer - Nigerian Veterinary Research Institute (NVRI), Vom, Jos. These lyophilised vaccines were stored in the refrigerator for subsequent use.

A 200 dose vial of each strain of the lyophilised vaccine was reconstituted in 8ml of sterile normal saline recapped and gently shaken to homogenise before placement in a waterbath at 56^{0}C. In each case, a pre-treatment sampling had been taken so as to determine the initial (stock) haemagglutination (HA) titer for each strain.

Sampling and Titration.

A pre-treatment HA titer for each strain was determined according to the procedure described (6,7) and slightly modified through the use of micro-titration tools as stated below.

In the haemagglutination (HA) test, the micro-titration format was employed, using U-bottomed polystyrene microtiter plates, 50µl calibre steel-alloy microdiluters and 50µl plastic micro-pipettes. 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 the 0.5% chicken, Red Blood Cell (RBC) supension (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 - 40 minutes or as soon as the RBCs in the control wells had 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 the value as recommended (8).

Subsequently, sampling and titration were done about every hour (at least 5 samplings daily) at 45^{0}C and every 10 minutes at 56^{0}C until the haemagglutinative activity of the investigated antigen(s) had declined by two-step ($2\log_2$) titers or more.

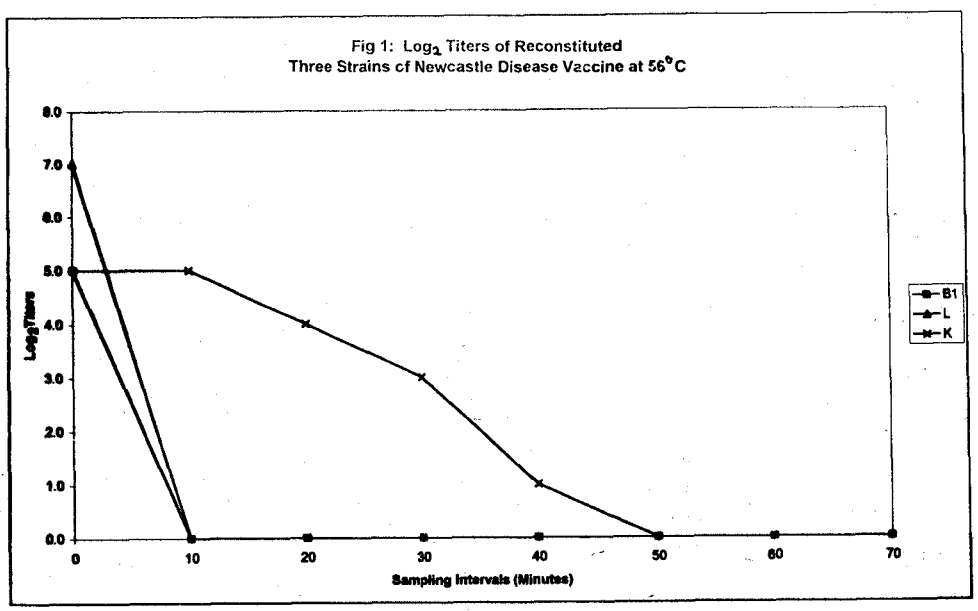
RESULTS AND DISCUSSION

The persistence of HA titers was adopted as a measure of the antigen stability of the three strains of reconstituted ND vaccine under each regime of temperature treatment. At 45°C (Table 1), the Hitchner-B1 strains with an initial pre-heat treatment (HA) titer of $5\log_2$, declined to $4\log_2$ at the 2nd hour. It remained constant till the 5th hour, when it declined to $3\log_2$ at the 6th hour (day-1) lasting till the 50th hour, (days 2 and 3). It further declined to $2\log_2$ at the 51st hour and remained stable at this titer till the 54th hour (day-3). The Lasota strain commencing with an initial (HA) titer of $6\log_2$, declined by the 2nd hour to $5\log_2$ (day-1). This titer remained constant till the 5th hour and it finally terminated at zero on the 6th hour (day-1). The Komarov strain starting with an initial (HA) titer of $5\log_2$ remained stable till the 2nd hour. It further declined by one-step to $4\log_2$ at the 3rd hour, remained stable at this titer till the 23rd hour (day-2) and then to $3\log_2$ at the 24th through to the 30th hour. It finally terminated at zero at the 49th hour and beyond.

Table 1: Log_2 titers of three strains of reconstituted Newcastle Disease Vaccine at 45°C

Daily Sampling	Sampling Interval (Hours)	Log_2 Titers of Reconsituted Newcastle Disease Vaccine Strains		
		B1	L	K
	Pre-heat Treatment	5.0	6.0	5.0
	1hr	5.0	6.0	5.0
Day-1	2hr	4.0	5.0	5.0
	3hr	4.0	5.0	4.0
	4hr	4.0	5.0	4.0
	5hr	4.0	5.0	4.0
	6hr	3.0	0	4.0
	23hr	3.0	0	4.0
	24hr	3.0	0	3.0
Day -2	25hr	3.0	0	3.0
	26hr	3.0	0	3.0
	27hr	3.0	0	3.0
	28hr	3.0	0	3.0
	29hr	3.0	0	3.0
	30hr	3.0	0	2.0
	49hr	3.0	0	0
Day-3	50hr	3.0	0	0
	51hr	2.0	0	0
	52hr	2.0	0	0
	53hr	2.0	0	0
	54hr	2.0	0	0

Also, presented in Figure 1, is the summary of the HA titers of three strains of reconstituted ND vaccines (Hitchner-B1) B1, (Lasota) L and (Komarov) K strains during the 70 minutes post-exposure treatment at 56^{0}C. The B1 strain with an initial (HA) titer of 5log$_2$ terminated at less than 10 minutes post-heat treatment temperature. The Lasota strain commencing with an inital high (HA) titers of 7log$_2$, terminated at less than 10 minutes post-heat treatment temperature. The mesogenic Komarov strain starting with an initial HA titer of 5log$_2$ at the 10th minute, and then to 3log$_2$ at the 20th minute. The subsequent titers were 1log$_2$ at the 30th minute and finally zero at the 40th minute and beyond.



Considering that changes of two-step (2log$_2$) and above are required as a criterion for loss of stability in HA titer during this investigation. The stability of NDV can be measured on the basis of alterations in the ability of the virus to infect, to agglutinate cells, and to induce an immunogenic response (5). The rate at which the reactivity of the virus is destroyed varies with the strain of the virus. It is also dependent on the time of exposure to treatment, the quantity of virus iniatially exposed, the nature of the suspending medium, and interactions among treatment variables (5). Therefore, the initial (stock) titers for the three strains of reconstituted ND vaccines were 5log$_2$, and 6log$_2$ and 5log$_2$ for Hitchner-B1 (B1), LaSota (L) and Komarov (K) strains, respectively for the experiment at 45^{0}C temperature. The loss of stability in HA titers by the said criterion was therefore at the 6th, 6th and 24th hour for B1, L and K strains of ND vaccines or effectively after the 5th, 5th and 23rd hour, respectively. Thus, K strain was therefore the most stable of the three strains at this temperature (45^{0}C). The B1 strain had a better stability over the L

strain, which was the least stable though it had an advantaged high initial titer, thus suggesting a lack of correlation between initial titers and stablility.

Similarly, going by the same criterion, loss of stability in HA titer of the three strains at 56°C post-heat treatment occurred in less than 10 minutes for both B1 and L strains while it occurred at the 20th minute or effectively after 10th minute for K strain. This showed that the K strain was the only relatively stable strain at this high temperature (56°C), while the titers of the other two strains (i.e B1 and L) declined very rapidly and were undetectable.

There is a dearth of current information on the stability of HA titers of ND virus strains especially after reconstitution in diluent at temperatures of 45°C and 56°C. Published data on the stability of various strains of ND virus which do not relate to the stability of reconstituted virus or vaccine, showed that at the temperature of 45°C, the stability time for GB, Roakin and B1 strains with allantoic fluid in sealed glass vials persisted for 12 hours but not more than 96 hours (9). Also previous reports on the stability of NDV at 56°C concluded that the stability varied according to the strains under investigation. A period of 5 minutes to 6 hours has been reported for the stability of the HA titer of NDVs (10,11). Data published elsewhere showed that the strains stabilized for 15 minutes at 60°C with allantoic fluid in sealed glass vials (9).

CONCLUSION AND APPLICATIONS

The data obtained from this investigation on exposure of the reconstituted three strains of ND vaccines at two test temperatures have therefore revealed the potential of the NDV mesogenic Komarov strain as prototype themostable vaccine. Thermostability is a baseline determinant for the selection of ND virus clone targeted for a thermostable vaccine. Furthermore, the results from this experiment have provided substantial justification for further investigation (at low and intermediate temperatures) on the adaptability of this vaccine strain in a new kind of ND vaccination programme specifically targeted on village poultry and the (physical) husbandry and environmental limitations in rural settings.

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