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ANSInet
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan
Mob: +92 300 3008585, Fax: +92 41 8815544
E-mail: editorijps@gmail.com

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Thermostability Profile of Newcastle Disease Viruses Isolated from Wild Birds in Central Nigeria and the Selection of a Thermostable Clone from the Sub-Population

O.J. Ibu[1], J.O.A. Okoye[2], S.S. Baba[3], S.V.O. Soyinka[2], K.F. Chah[2],
J. Antiabong[1], D. Eze[2], E.A. Salihu[1] and S.B. Oladele[4]
[1]National Veterinary Research Institute, Vom, Nigeria
[2]Faculty of Veterinary Medicine, University of Nigeria, Nsukka, Nigeria
[3]Faculty of Veterinary Medicine, University of Maiduguri
[4]Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria, Nigeria

Abstract: The study was carried out to assess the Haemagglutinin thermostability of Newcastle disease virus isolates obtained from wild birds in three climatically distinct states in central Nigeria. Identification of heat stable ND virus isolates from the locality will provide environmentally friendly thermostable vaccine candidates for rural poultry. The 12 field virus isolates and the 5 vaccine virus strains showed variable degrees of heat stability. Three field isolates each was inactivated in 5 min, three in 10 min and one in 15 min. One isolate was inactivated in 20 min while two and three strains got inactivated in 25 and 30 min respectively. The most thermostable of the field isolates was inactivated in 40 min. A more thermostable clone was subsequently derived from the latter strain as a local vaccine candidate. For the vaccine strains, NDV (I/O) and NDV (K) were inactivated in 20 min while NDV (L) was inactivated in 25 min. The velogenic strain (Herts) was inactivated in 40 min. The two established thermostable strains, NDV_4 and $NDVI_2$ were inactivated in 90 min each. The thermostable profile of the field virus strains did not vary with the climatic background of the isolates.

Key words: Nigeria, newcastle disease viruses, heat stability, clone

INTRODUCTION

Newcastle diseases is a highly contagious disease of poultry and other avian species caused by the Avian Paramyxovirus 1, the Newcastle disease virus (OIE, 2004). The disease is endemic in subbaharam Africa including Nigeria. Both commercial and free-range village chickens suffer periodic outbreaks in the country (Okeke and Lamorde, 1988).

Whereas vaccines are available for the control of the disease in commercial flocks, effort is still needed to identify heat-stable strains of the virus from the local environment for vaccine preparation to control the disease in the scavenging rural flocks. The search for such thermostable strains in different ecologically diverse localities for the purpose of developing vaccines for rural tropical poultry is a continues one. It has also been observed that progenies derived from passing ND virus strains through thermal cycles show greater heat stability than the parent virus (Kinde *et al*., 2000).

In this study, the haemagglutinin thermostability of ND viruses which were previously isolated from wild birds in three climatically distinct localities of central Nigeria was determined. The most thermostable of the isolates was then subjected to further cycles of heat treatment to derive a more heat stable clone from the sub-population. Such a locally derived clone could serve as a thermostable vaccine candidate for village poultry.

MATERIALS AND METHODS

A total of 12 ND virus field isolates (one mesogenic and eleven lentogenic strains) were previously obtained from Plateau, Benue and Kaduna States in Central Nigeria (Ibu *et al*., 2009). These three States represented different climatic conditions. Plateau state (located on the Jos plateau), has a cold mountain climate; Benue state (located in the Benue river valley), has a warm humid climate; while Kaduna state (located on the vast plain land of central Nigeria), a dry warm climate. Of the 12 ND virus isolates used in this study, 8 were from the Plateau (Pl 016, Pl 029, Pl 032, Pl 038, Jz 2, Jz 4, Jz 6, Jz 13), three from Benue (Bn 2, Bn 7, Bn 11) and one (KD 4) from Kaduna State. All 12 isolates were exposed to a predetermined temperature of 56°C in a water bath for varying time intervals to determine their thermostability. Also, 5 NDV vaccine strains, Lasota, Kamorov, B_1 (intraocular), V4 and I_2 as well as a standard velogenic strain (Herts) were included in the test for comparison.

The virus strains were first propagated in 9-11 days-old embryonated chicken eggs via the allantonic route. Following 96 h of incubation at 37°C, the infective

Corresponding Author: O.J. Ibu, National Veterinary Research Institute, Vom, Nigeria

allantoic fluid was harvested and the Haemagglutination (HA) titre determined according to standard procedures (OIE, 2004).

The allantoic fluid harvest containing a minimum Haemagglutination titre of 7 logs base 2 (1:128) was aliquoted in 1 ml volumes into sterile vials and corked. Two pairs of vials were removed at 5 min intervals and chilled immediately on ice. A HA test was carried out on each aliquot and the titre determined. Where slight differences exist in the HA titres between the aliquots, the lower limit was taken. The longest duration (in minutes) it takes for a virus strain to show HA activity was taken as the thermostability time.

The field ND virus strain that showed the highest thermostability at 56°C was consequently taken and subjected to four further cycles of heat treatment. Briefly, the infective allantoic fluid (containing the virus with HA titre > 1:256) was dispensed into 1 ml vials. The vials were placed in a water bath pre-heated to 56°C. A set of vials was removed after every 5 minutes and placed on ice. A standard HA test was conducted on each aliquote. The aliquote with the highest heating time showing HA titre was noted and taken for subsequent heating cycle. Following each heating cycle, the product was injected into 9-11 days old embryonated chicken eggs and incubated for 96 h at 37°C. The incubated eggs were chilled and spot-tested for viral haemagglutinins. The infective allantoic fluid was then harvested, pooled and dispensed into vaccine vials of 1 ml aliquots. The vials were placed in the water bath for another cycle of heating. The cycle of heating, egg inoculation, harvesting, dispensing, pooling, chilling and testing were repeated three more times.

A known thermostable ND virus strain I_2 was used as control.

RESULTS

All the field isolates from the three states showed similar patterns of heat stability. With the exception of isolate Bn 11 from Benue State, all other isolates were inactivated in 30 min and below (Table 1 and 2).

Generally, three of the Strains (Pl 032, Pl 038 and Jz 4) were inactivated in 5 min and two others (Pl016, Bn 2) in 10 min.

One isolate (Jz 6) was inactivated in 20 min while two isolates (Pl 029 and Bn 8) were inactivated in 25 min. Three isolates (Bn 7, Jz 13 and KD 4) were inactivated in 30 min. The most thermostable strain among the field isolates (Bn11) was inactivated in 40 min.

For the vaccine strains, two (NDV1/O and NDVK) were the most heat sensitive being inactivated in 20 min. The NDVL was inactivated in 25 min while the only velogenic strain (Herts) was inactivated in 40 min. The two thermostable vaccine strains ND V4 and NDVI2 were inactivated in 115 min and 90 min respectively.

In comparison, the haemagglutinin thermostability of 46.15% of the 12 field isolates was higher than that of NDV I/O reference strain. The same percentage, (46.15%) had haemagglutinin thermostability lower than that of NDVL. Only 7.69% of the isolates had their haemagglutinin thermostability equal to that of NDV I/O vaccine strain. Similarly, 30.77% of the field isolates had higher haemagglutinin thermostability titres than the NDVL strain. The haemaggluntinin thermostability titre of 53.85% of the field isolates was lower than that of the NDVL strain while 15.38% of the field isolates had their haemagglutinin thermostability equal to that of the lentogenic vaccine (NDVL) strain.

The thermostability HA titre levels were found to increase after each passage of isolate Bn I1 through a cycle of heating, testing and propagation (Table 1). The first cycle produced an isolate within a time interval of 40 min. After the second heating cycle, the time interval increased to 55 min. The third cycle of heating and propagation resulted in time interval of 75 min. A maximum time interval of 90 min was achieved after the 4th cycle.

DISCUSSION

From the results, it is obvious that there are some variations in the levels of haemagglutinin thermostability among the field virus strains. Other workers elsewhere had observed similar variations and had used thermostability of HA activity as a characterization test of ND virus isolates. This property has proved to be a useful tool in epizootiological studies and a rapid method for distinguishing between some avirulent and virulent viruses (Hanson and Spalatin, 1978).

Although the selection of initial samples from the three States of Central Nigeria was based on climatic differences, the thermostability titre pattern did not reflect same. For example, the thermostability of isolates from Plateau State with a cold mountain climate ranged between 5 and 30 min while the heat stability of isolates from Benue, a hot humid climate ranged between 10 and 40 min. The sole isolate from Kaduna State, a relatively hot dry climate had a thermostability of 30 min. From the foregoing, it appears that the thermostability of the haemagglutinins does not depend on the source and environmental condition where the isolates were derived.

It is also observed in this study that 46% of the field isolates had greater thermostable haemagglutinin than the lentogenic vaccine NDV I/O strain while the haemagglutinins of 31% of the isolates were more thermostable than the NDVL vaccine strain. These findings are similar to those of King (2001) who found 38% of field ND virus isolates to be more thermostable than the two reference strains, NDV I/O and NDVL.

The haemagglutinins of the only mesogenic strain (isolate Pl038) in the present study was found to be more thermolabile (5 min) than the reference mesogenic strain, the NDV Kamorov (20 min) and most of the lentogenic strains as well as the reference velogenic strain (Herts).

Table 1: The pattern of thermostability of haemagglutinins of Newcastle disease field virus isolates and vaccine strains

State of origin of virus isolates	Time (min) 5	10	15	20	25	30	35	40	70	80	90	100	110	115
Plateau	++	+	+		+	+								
Benue		+				+		+						
Kaduna						+								
NDV I/O				+										
NDVL					+									
NDVK				+										
NDV4														+
NDV(I2)											+			
NDVHerts								+						

Key: + = Virus isolates and vaccine strains

Table 2: HA titres of the 12 NDV isolates after exposure to temperature of 56°C over time

Virus strain	Time (min) 0	5	10	15	20	25	30	35	40	45	50	55	60	65	70	75	80	85	90	95	min
PI 016	2^9	26	2^2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	10
PI 029	2^9	2^5	2^8	-	2^2	2^2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	25
PI 032	2^{10}	2^5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	5
PI 038	2^9	2^2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	5
Bn 2	2^{10}	2^9	2^4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	10
Bn 7	2^9	2^9	2^8	2^6	2^6	2^4	2^2	-	-	-	-	-	-	-	-	-	-	-	-	-	30
Bn 11	2^{12}	2^{12}	$>2^{12}$	$>2^{12}$	$>2^{11}$	$>2^{12}$	2^{10}	2^8	2^1				-	-	-	-	-	-	-	-	40
Jz 2	2^9	2^6	2^5	2^5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	15
Jz 4	2^6	2^6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	5
Jz 6	2^7	2^{10}	2^{10}	2^9	2^5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	20
Jz 13	2^9	2^6	2^4	2^4	2^2	2^2	2^3	-	-	-	-	-	-	-	-	-	-	-	-	-	30
KD 4	2^8	2^5	2^3	2^3	2^4	2^3	2^2	-	-	-	-	-	-	-	-	-	-	-	-	-	30
NDV(L)	2^{10}	2^8	2^7	2^7	2^7	2^1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	25
NDV(1/0)	2^9	2^8	2^8	2^5	2^5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	20
NDV4	2^9	2^9	2^{10}	2^7	2^6	2^6	2^6	2^6	2	2^4	2^5	2^5	2^5	2^4	2^4	2^4	2^3	2^3	2^4	2^3	115
NDVI2	2^{11}	2^{10}	2^{10}	2^9	2^{10}	2^9	2^8	2^5	2^6	2^4	2^4	2^3	2^3	2^2	2^1	2^1	2^4	2^3	2^2	-	90
NDV(K)	2^{10}	2^8	2^8	2^9	2^8	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	20
Herts	2^{12}	2^8	2^7	2^6	2^6	2^5	2^5	2^4	2^4	-	-	-	-	-	-	-	-	-	-	-	40

Table 3: Thermal cycles passed by NDV strain Bn11 over time

Isolate Bn11 Passage cycle	Time (min) 0	5	10	15	20	25	30	35	40	45	50	55
1st	2^{12}	2^{12}	2^{12}	2^{12}	2^{11}	2^{11}	2^{10}	2^8	2^1	-	-	-
2nd	2^9	2^{10}	2^8	2^8	2^7	2^7	2^5	2^5	2^5	2^2	2^2	2^1
3rd	2^{11}	2^{11}	2^{10}	2^{10}	2^9	2^8	2^8	2^8	2^8	2^6	2^6	2^6
4th	2^7	2^7	2^7	2^6	2^6	2^6	2^6	2^6	2^6	2^6	2^6	2^5
Isolate NDVI2	2^{11}	2^{10}	2^{10}	2^{10}	2^{10}	2^9	2^8	2^5	2^5	2^4	2^4	2^3

Isolate Bn11 Passage cycle	Time (min) 60	65	70	75	80	85	90	100	Min		115	120
1st	-	-	-	-	-	-	-	-	40	-	110	40
2nd	-	-	-	-	-	-	-	-	55	-	-	55
3rd	2^5	2^5	2^4	2^4	-	-	-	-	75	-	-	75
4th	2^4	2^4	2^2	2^2	2^2	2^2	2^2	-	90	-	-	90
Isolate NDVI2	2^3	2^3	2^2	2^2	2^2	2^2	2^2	-	90			

These findings seem to agree with the observations made by other workers that haemagglutinin thermostability does not depend on viral virulence or pathogenecity (Lomniczi, 1975; Hanson and Spalatin, 1978).

The selection and subsequent passage of strain Bn 11 through thermal cycles was based on its thermostable property. The over two-fold increase in thermostability time after four passage cycles shows that this strain could be a source of a heat stable virus. It also demonstrates that NDV with heat stable haemagglutinins are derivable through selection from heterogenous NDV subpopulations. Similar observations were made previously where four cycles of

heat treatment at 56°C resulted in the selection of variants more thermostable than the parent vaccine strains (King, 2001).

Currently, $NDVI_2$ and NDV_4 are produced at the National Veterinary Research Institute, Vom for field use in Nigeria. Since these vaccines are live attenuated, their use in the field amount to the introduction of foreign virus strains into the subpopulation. The identification of thermostable local strain with suitable characteristics as obtained in this study will provide a more adaptable and ecologically friendly alternative.

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