

STRAINS OF NEWCASTLE DISEASE VIRUS INVOLVED IN OUTBREAKS OF THE DISEASE IN NSUKKA, NIGERIA.

ABSTRACT:

Aim: Strains of *Newcastle disease virus* (NDV) involved in outbreaks of the disease in Nsukka, Nigeria was studied.

Materials and methods: Sixty three poultry disease outbreaks, characterized by clinical signs and lesions suggestive of Newcastle disease(ND) were confirmed by running haemagglutination test with extracts from tissue homogenates of the sick or dead chickens. The NDV samples, were characterized by combining results of their agglutination of mammalian erythrocytes test and their erythrocyte elution time.

Results: Fifty three of the 63(84%) NDV samples were of the Lentogenic strain while 10(16%) were of the Mesogenic strain. None was of the Velogenic strain.

Conclusion : In Nigeria, attenuated Lentogenic and Mesogenic NDV strains are used as live vaccines but Velogenic strains used as vaccines are inactivated. We therefore conclude that reversion, of viruses used as vaccines, to virulence may be source of some NDV infections in the country and that Lentogenic and Mesogenic NDV pathotypes circulating in Nigeria are virulent.

1.INTRODUCTION.

Newcastle Disease (ND) is a contagious viral disease of poultry that affects many of the avian species, including, chickens, turkeys, geese, ducks, pheasants, guinea fowls, ostriches, emus and rhea [1]. All ages and classes of these species are affected but young ones are more susceptible [2]. ND has been reported in most

parts of the world [3,4]. The disease is caused by , *Newcastle Disease Virus*, an RNA virus of the genus paramyxovirus [5]. Transmission of NDV occurs through aerosol and by contact between healthy birds and sick ones or through fomites [1].

Incubation period of Newcastle disease is reported to be four to five days when infection occurs naturally but when the virus was inoculated by intramuscular injection, the chicks were dead in 48 hours [6]. He also reported that natural infection of NDV leads to the respiratory form of ND while infection by intramuscular (I/m) or by intravenous (I/v) route leads to nervous form of the disease.

Vaccination and biosecurity measures are main methods of control of ND in Nigeria. Both Killed and live vaccines are used in the country. Commercially available Newcastle disease vaccines in Nigeria include live and inactivated vaccines.

Live ND vaccines are lentogenic strains (Hitchner B₁, the F strain, the V strain and lasota) and the Mesogenic strains such as Roakin, Mukteswar, Kamorrov strains [7]. Before Velogenic NDV strains are used as vaccines, they are inactivated by treating them with BPL and formalin or by absorbing them onto aluminum hydroxide gel [8].

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52 The *Newcastle disease virus* is classified into 4 pathotypes [6]. Viscerotropic
 53 Velogenic Newcastle Disease Viruses (VVND) cause the Dolye's form of ND
 54 which is characterized by depression, prostration with many birds dying within a
 55 few days. The principal signs in this form are respiratory and nervous signs. An
 56 infected bird may show increased respiratory distress, which include gasping,
 57 cough and tracheal rales or sneezing. Some birds develop profuse bright greenish
 58 or whitish diarrhoea, greenish yellow diarrhea, Odematous swelling of the head,
 59 cyanosis of combs, conjunctivitis, inflammation of the shank and toes [9-12].
 60 Nervous signs seen prior to death could manifest as muscular tremor, torticollis,
 61 paralysis of legs and wings, drooping wings and twisting of head and neck.
 62 Sometimes mortality may reach up 100% in unvaccinated flocks[13]. Birds that
 63 survive the infection may develop partial or complete cessation of egg
 64 production. Sometimes egg production declines dramatically, there could be shell

65 less or thin soft shelled eggs with watery albumen. Abnormal colour of eggs is also
66 common. Sudden death with few or no symptoms could also be observed.

67 Neurotropic Velogenic Newcastle Disease Viruses (NVNDV) cause Beach's form
68 of ND which is characterized by acute respiratory and nervous signs. Egg
69 production falls dramatically but diarrhea is usually absent. Morbidity may reach
70 100% while mortality may be about 50% in adult birds and 90% in chicks 

71 Mesogenic Newcastle Disease Viruses (MNDV) cause Beaudett's ND which is a
72 less virulent form, characterized by respiratory signs and low mortality. In adult
73 birds, there may be marked drop in egg production that may last for several
74 weeks [14]. Mortality rate may be about 10%.

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76 Lentogenic NDVs cause Hitchner's form of the infection which usually does not
77 cause disease in adults. It is commonly sub-clinical with mild respiratory signs,
78 temporary loss of appetite, drop in egg production with negligible mortality
79 unless concurrent disease is present [9].

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81 Frequent occurrence of Newcastle disease in Nigeria, despite wide vaccination
82 coverage, made it necessary to investigate the outbreaks to study strains of the
83 *Newcastle disease virus* involved in outbreaks of the disease in the country.

84 **2.MATERIALS AND METHODS.**

85 Faeces were collected from sick chickens in each farm with clinical signs
86 suggestive of ND, in Nsukka Enugu state in South East, Nigeria. Intestinal
87 contents, lungs, liver, trachea and spleen were collected from dead chickens in
88 affected flocks. History of the flocks, including, flock size, management system,
89 rate of egg production, vaccination history, mortality and morbidity rates, and
90 clinical signs were recorded.

91 Tissues from each chicken were pooled together and blended using mortar and
92 pestle. One gram of faeces or of the organs was homogenized in PBS, containing
93 10-20% of antibiotics and antifungals. The suspension was left for 2 hours at
94 room temperature and then centrifuged at 1,000g for 10 minutes. The
95 supernatants were then stored at -20°C, until used.

96 Supernatants of faeces or of organs of sick chickens were tested for *Newcastle*
97 *disease virus* by haemagglutination(HA) test, as described by Johnson [15]. Those
98 that gave agglutination of chicken RBC which was inhibited by a known NDV

99 antiserum were subjected to, agglutination of mammalian erythrocyte test and to
100 determination of erythrocyte elution time (EET).

101 To determine their EET, the NDV positive samples were used for HA test with
102 chicken RBC at a controlled temperature (37°C). Following agglutination, each
103 setup was observed until elution of the RBCs occurred and the time interval
104 between agglutination and elution was recorded as EET of the sample. Then a
105 combination of results of the agglutination of mammalian erythrocytes test and
106 the EET was used to characterize the viral samples. Any sample that failed to
107 agglutinate sheep erythrocytes and had long EET (≥ 1 hour) was classified as a
108 Velogenic strain. Any that failed to agglutinate the sheep RBC but had EET of
109 less than one hour was taken as a Mesogenic strain while those that agglutinated
110 the sheep RBC were classified as Lentogenic NDV strains.

111 3.RESULTS AND DISCUSSION.

112 1. Results:

113 Of 170 disease outbreaks in poultry flocks with clinical signs suggestive of ND,
114 the virus was confirmed in 63. Fifty three of the ND outbreaks were due to

infection of Lentogenic NDV (84%) while 10 were caused by Mesogenic strains of the virus (16%). None of the outbreaks yielded Velogenic NDV strain (Table 1).

3.2.Discussion:

Since samples used for the present study were collected from clinical cases in Nsukka and yet they qualified to be classified as Lentogenic and Mesogenic strains, it means that some Nigerian isolates of Lentogenic NDV and Mesogenic NDV cause clinical ND in chickens.

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Morgan *et al* [9] reported that Lentogenic and Mesogenic NDV do not cause disease in adult birds and that their infection is subclinical, with mild respiratory signs and drop in egg production but in the outbreaks studied, Mesogenic NDV was detected in a flock of sick layers. In another flock of layers, Lentogenic NDV was detected. Clinical signs including, paralysis of wings, raised feathers, tucking in of head into the wings, huddling together at corners, drop in egg production, white soft shelled eggs or small eggs without yolk and greenish- yellow diarrhea were observed in the two flocks. Morality rates in the two flocks, were 30%. and

132 10% respectively. Results therefore, suggest that Nigerian Lentogenic and
 133 Mesogenic NDV strains produce clinical newcastle disease and lead to
 134 rtalitiess.

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136 Detection of 10 Mesogenic NDV from the 63 samples in Nigeria is of economic
 137 importance, because Mesogenic strains of NDV are reported to cause drop in egg
 138 production. Infection from Mesogenic NDV in laying flocks is usually,
 139 characterized by mild respiratory signs and marked drop in egg production that
 140 may last for several weeks [14]. Because disease caused by infection of the
 141 Mesogenic strains is mild, the farmer may not suspect ND. The chickens may still
 142 be feeding well but produce few eggs, thus leading to great economic losses.

143 In Nigeria live vaccines are widely used to control ND but **Bennejean** [16] had
 144 reported that once NDV has intracerebral pathogenicity index (ICPI) greater
 145 than 0.7, it can cause disease outbreak. So, even if the live vaccines have ICPI
 146 lower than 0.7 initially, because they are allowed to passage in the country's
 147 poultry population uncontrolled, they could revert to virulence after some time.

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149 Commercially available vaccines for Newcastle disease in Nigeria include, live
 150 Lentogenic strains such as, Hitchner B₁, lasota, F strain and V₄. Live Mesogenic
 151 vaccine used in the country is mainly the Kamorrov. Velogenic ND strains used as
 152 vaccines are inactivated [17].

153 Results of this survey which revealed that 84% of ND outbreaks in Nigeria are
 154 due to infection by strains of Lentogenic NDV and that 16% are due to
 155 Mesogenic strains while the Velogenic strains were not involved in the outbreaks,
 156 suggest that live vaccines being used in the country may be sources of the v
 157 infections. The vaccines may have reverted to virulence due to long passages in
 158 chickens. Prevalence rates of the three NDV strains (84%,16% and zero %) in the
 159 outbreaks studied, also appear to reflect rate of usage of each virus strain, as live
 160 vaccines, in the country.

161 Nawathe *et al* [18] had reported that pathotypes of Mesogenic and Lentogenic
 162 NDV strains circulating in Nigeria are virulent. We have also observed that isolates
 163 of these virus strains in Nigeria have haemagglutinins that are heat stable. It has
 164 also been reported that pathogenicity of NDV is a function of the heamagglutinin
 165 antigen [19]. So, these earlier observations appear to support the results of

166 this study which have revealed that clinical ND in Nigerian poultry flocks resulted
167 from Lentogenic and Mesogenic NDV infections.

168 **CONCLUSIONS** : Results of the study, suggest that the high incidence of
169 Newcastle disease in Nigeria may be due to reversion to virulence by the virus
170 strains used as live vaccines. They also confirm earlier reports, that pathotypes of
171 Lentogenic and Mesogenic *Newcastle disease virus* strains in Nigeria, cause
172 outbreaks of the disease.

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235 Table 1: Strains of *Newcastle Disease Virus* involved in outbreaks of the

236 disease in Nsukka, Enugu State, Nigeria



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Samples	HA of Mammalian RBC	Erythrocyte Elution Time (Mins)	Strain
1	+	8	L
2	+	5	L
3	+	5	L
4	+	5	L
5	+	5	L
6	+	5	L
7	+	5	L
8	+	3	L
9	+	3	L
10	+	3	L
11	+	5	L
12	+	3	L
13	+	3	L
14	+	3	L
15	+	8	L
16	-ve	2	M
17	-ve	2	M
18	+	2	L
19	+	2	L

UNDER PEER REVIEW

20	+	2	L
21	-ve	2	M
22	-ve	2	M
23	-ve	2	M
24	+	3	L
25	+	2	L
26	+	2	L
27	+	2	L
28	-ve	4	M
29	+	2	L
30	+	2	L
31	+	2	L
32	+	2	L
33	+	2	L
34	+	2	L
35	+	2	L
36	-ve	2	M
37	-ve	2	M
38	-ve	2	M
39	+	2	L
			
40	+	2	L
41	+	10	L
42	+	2	L

43	+	2	L
44	+	50	L
45	+	4	L
46	+	20	L
47	+	8	L
48	+	4	L
49	+	4	L
50	+	8	L
51	+	4	L
52	+	8	L
53	+	2	L
54	+	8	L
55	+	8	L
56	+	8	L
57	+	4	L
58	+	2	L
59	-ve	2	M
60	+	2	L
61	+	2	L
62	+	2	L
63	+	2	L

238 Key Note: +: positive

239 -ve: Negative

240 L: Lentogenic strain

241 M: Mesogenic strain

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