

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 : We conclude that it is necessary to investigate reversion of live vaccines to virulence as a possible source of NDV outbreaks in Nigeria and also test Lentogenic and Mesogenic NDV pathotypes circulating in the country for pathogenicity.

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 Avulavirus [5]. Transmission of NDV occurs through aerosol and by contact between healthy birds and sick ones or through formites [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]. It has also been reported that natural infection of NDV leads to the respiratory form of ND while infection by intramuscular (I/m) and by intravenous (I/v) route lead 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.

Hitchner B₁, F strain, V strain and lasota all Lentogenic NDV strains and the Mesogenic strains such as Roakin, Mukteswar and Kamorrov [7] are used in Nigeria as live vaccines. However Velogenic NDV stains used as vaccines in the country are inactivated vaccines [8].

48

49

50 The *Newcastle disease virus* is classified into 4 pathotypes [6]. Viscerotropic
51 Velogenic Newcastle Disease Viruses (VVND) cause the Dolye's form of ND
52 which is characterized by depression, prostration with many birds dying within a
53 few days. The principal signs in this form are respiratory and nervous signs. An
54 infected bird may show increased respiratory distress which include gasping,
55 cough and tracheal rales or sneezing. Some birds develop profuse greenish,
56 whitish or greenish- yellow diarrhea. There could also be Odematous swelling of
57 head, cyanosis of combs and conjunctivitis [9-12]. Nervous signs seen prior to
58 death could manifest as muscular tremor, torticollis, paralysis of legs and wings,
59 drooping wings and twisting of head and neck. Mortality may reach 100% in
60 unvaccinated flocks[13]. Birds that survive ND infection may develop partial or
61 complete cessation of egg production. Affected hens could lay shellless or thin
62 soft shelled eggs with watery albumen. Abnormal colour of eggs is also common.
63 Sudden death without symptoms is possible.

64 Neurotropic Velogenic Newcastle Disease Viruses (NVNDV) cause Beach's form
65 of ND which is characterized by acute respiratory and nervous signs. Egg

production falls dramatically but diarrhea is usually absent. Morbidity may reach 100% while mortality may be about 50% in adult birds and 90% in chicks.

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

Lentogenic NDVs cause Hitchner's form of the infection which is believed not to cause disease in adults. Infection from this strain is reported to be sub-clinical with mild respiratory signs, temporary loss of appetite, drop in egg production with negligible mortality unless concurrent infections are present [9].

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

2.MATERIALS AND METHODS.

Feces were collected from one sick chicken from each poultry flock that had clinical signs suggestive of ND in Nsukka Enugu state in South East, Nigeria while

84 from dead chickens intestinal contents, lungs, liver, trachea and spleen of one
85 chicken were collected. History of the flocks including flock size, management
86 system, rate of egg production, vaccination history, mortality and morbidity rates
87 and clinical signs were recorded.

88 Tissues from each chicken were pooled together and blended using mortar and
89 pestle. One gram of feces or of the organs was homogenized in PBS containing
90 10-20% of antibiotics and antifungals. The suspension was left for 2 hours at
91 room temperature and then centrifuged at 1,000g for 10 minutes. The
92 supernatants were then stored at -20°C until used.

93 Supernatants of feces of sick chicken or of organs of the dead ones were tested
94 for *Newcastle disease virus* by haemagglutination(HA) test as described by
95 Johnson [15]. Those that gave agglutination of chicken RBC which was inhibited
96 by a known NDV antiserum were subjected to agglutination of mammalian
97 erythrocyte test and to determination of erythrocyte elution time (EET).

98 To determine their EET, the NDV positive samples were used for HA test with
99 chicken RBC at a controlled temperature (37°C). Following agglutination each
100 setup was observed until elution of the RBCs occurred and the time interval
101 between agglutination and elution was recorded as EET of the sample. Then a

combination of results of the agglutination of mammalian erythrocytes test and the EET was used to characterize the viral samples. Any sample that failed to agglutinate sheep erythrocytes and had long EET (≥ 1 hour) was classified as a Velogenic strain. Any that failed to agglutinate the sheep RBC but had EET of less than one hour was taken as a Mesogenic strain while those that agglutinated the sheep RBC were classified as Lentogenic NDV strains.

3.RESULTS AND DISCUSSION.

3.1. Results:

Clinical signs observed in the flocks of chickens included paralysis of wings, raised feathers, tucking the head into the wings, huddling together, drop in egg production, white soft shelled eggs, small sized eggs, greenish- yellow diarrhea and mortality.

Of 170 chickens sampled from 170 poultry flocks with clinical signs suggestive of Newcastle disease, 63 were confirmed positive for Newcastle disease. Fifty three (84%) of the 63 NDV detected, agglutinated sheep red blood cells(Lentogenic NDV) while 10 (16%) that could not agglutinate the mammalian RBCs had EET less

than one hour (Mesogenic NDV) . None of the NDV detected had long EET of one hour and above to qualify as Velogenic NDV strain (Table 1).

3.2.Discussion:

Since samples used for the study were collected from clinical cases their qualification as Lentogenic and Mesogenic strains suggests that some Nigerian Lentogenic NDV and Mesogenic NDV pathotypes may cause disease in chickens.

Morgan *et al* [9] reported that Lentogenic and Mesogenic NDV strains do not cause disease in adult birds and that their infections are subclinical with mild respiratory signs and drop in egg production but in this study a flock of adult chickens that manifested clinical signs of ND yielded Mesogenic NDV . From another flock of sick layers Lentogenic NDV was detected. The two flocks also had mortalities of 30% and 10% respectively. These observations suggest that some Nigerian Lentogenic and Mesogenic NDV pathotypes may produce clinical Newcastle disease and may cause mortalities.

135 Detection of Mesogenic NDV from chickens in Nigeria is of economic importance
136 because the Mesogenic strains cause drop in egg production. Their infections in
137 laying flocks are usually characterized by mild respiratory signs and drop in egg
138 production that may last for several weeks [14]. Because the disease is mild
139 farmers may not suspect ND. Affected chickens may be feeding well but produce
140 few eggs, thus leading to great economic losses.

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

146

147 Commercially available vaccines for Newcastle disease in Nigeria include live
148 Lentogenic strains such as Hitchner B₁, lasota, F strain and V₄. Live Mesogenic
149 vaccine used in the country is mainly the Kamorrov. Velogenic ND strains used as
150 vaccines are inactivated [17]. Results of this survey which revealed that
151 Lentogenic strains of the NDV were detected in 84% of ND outbreaks in Nigeria
152 and that the Mesogenic strains were involved in 16% of the outbreaks while the

Velogenic strain was not present in any of the outbreaks suggest that live vaccines used in the country could be sources of the infections. The live vaccines may have reverted to virulence due to uncontrolled passages in chickens. Prevalent rates of the three NDV strains (84%,16% and zero %) in the outbreaks studied also appear to reflect rate of their usage as live vaccines.

Nawathe *et al* [18] had reported that pathotypes of Mesogenic and Lentogenic NDV strains circulating in Nigeria are virulent. We have also observed that some Mesogenic NDV pathotypes isolated in Nigeria have heat stable haemagglutinins. Since pathogenicity of NDV and their haemagglutination reactions, including heat stability, are functions of their surface antigens [19-21] the earlier observations and results of this study suggest that Lentogenic and Mesogenic NDV pathotypes circulating in Nigeria could cause outbreaks of the disease in adult chickens .

CONCLUSION : We therefore suggest that pathogenicity of Nigerian isolates of Lentogenic and Mesogenic *Newcastle disease virus* strains be investigated.

170

171 REFERENCES

- 172 1.Jordan, FTW, Pattison. **Poultry Disease** Fourth ed, 1999, 136 – 156
- 173 2. Arnall L, Keymr IF. Bird Diseases: **An introduction to the study of birds in**
174 **health and disease.**
- 175
- 176 3.Alexander, DJ. Newcastle disease virus Pathotypes. **Avian pathol**, 1975,
177 3: 269 – 278
- 178 4.Spradbrow, PB. Production of thermostable Newcastle disease vaccine in
179 developing countries. **Press vet med**, 19882: 157 – 159.
- 180 5. Gordon RF.. Newcastle Disease. In *Poultry Disease*. The English Language
181 Book Society, 1977. **Bailliere Tindal**: London.
- 182 6.Alexander, DJ. Newcastle disease and other avian paramyxoviridac
183 infections. In **Dease of poultry, tenth ed**, 1997, Iowa State University press,
184 Iowa USA.
- 185 7.Alexander, DJ. The epidemiology and control of avian influenza and
186 Newcastle disease. **J. Comp pathol**, 1995, 112 105 – 126.
- 187 8.Desmukh, SG. Newcastle Disease prevention and control. In article on
188 **Biosecurity and disease control in the Indian poultry industry**, 2010.
- 189 9.Morgan –capner, p., Bryden, AS. Newcastle disease in zoonoses, 1998.
190 **Simpson Oxford University Press**. Bath Presss, Avon.
- 191 10.MIshra, S., Katan, JM., Sah, RL, Verma, KC., Misha, JP. Studies on
192 pathogenicity of *Newcastledisease virus* isolated in guinea fowl, **Trop**
193 **animal health prod**, 2000, 33: 313 – 1320.

- 194 11.Okoye, JOA., Agu, AO, Chineme, CN, Echeowu, GON. Pathological
195 characterization in chickens of a velogenic *Newcastle disease virus* isolated
196 from guinea fowl, **Revue d' Elevage et de Medicine veterianaries de pay**
197 **TropIcauz**, 2000, 53, 325 – 330.
- 198 12.Ezema, WS., Okoye, JOA., Nwanta, JA. Losota Vaccination may not
199 protect against the lesions of velogenic Newcastle disease in chickens. **Trop.**
200 **Animal health prod**, 2009, 41:477 – 484
- 201 13. Office International des Epizootics **Manual of Diagnostic Tests and Vaccines**
202 **for Terrestrial Animals**, 2004.
- 203 14.Gopalakrishna RAO. **A comprehensive textbook on poultry pathology**
204 first ed, 2000, 41 – 47
- 205 15.Johnson, RH. Serological procedures for the study of feline panleukopenia
206 **J. Am. Vet. Med. Assoc.**, 1971, 156: 876 – 884.
- 207 16.Bennejean, G. Vaccination of day-old chicks against Newcastle disease
208 using inactivated oil adjuvant vaccine and/or live vaccine. **Avian Pathol**,
209 1988, 7:15 – 27.
- 210 17.Survashe, BD, Desmukh SG. Newcastle disease control in: Article on
211 **Biosecurity and disease control in the Indian poultry industry**, 2010 .
- 212 18.Nawathe, DR., Majiyagbe, KA, Ayoda, SO. Characterization of
213 Newcastle disease isolates from Nigeria. **Bill. Int. Off. Epiz**, 1975, 11: 1091
214 – 1105.
- 215 19.Nagai, Y., Klerk, HD., Root, R. Proteolytic cleavage of the viral
216 glycoproteins and its significance for virulence of the *Newcastle disease virus*
217 **.Virol**, 1976, 72:494 – 508.
- 218 20.Collins, MS, Bashiruddin, JB., Alexander, DJ. Deduced amino acid
219 sequences at the fusion protein cleavage site of *Newcastle disease viruses*:
220 showing variation in antigenecity and pathogenicity. **Archieves of virology**,
221 1976, 128:363 – 370.

21. Peeters, BPH, De leeuw, O, Koch, G., Gielken, ALJ. Rescue of *Newcastle Disease Virus* from cloned DNA: evidence that cleavability of fusion protein is a major determinant for virulence. **J. Virol.**, 1999, 73 :5001 – 5009.

Table 1: Strains of *Newcastle Disease Virus* involved in outbreaks of the disease in Nsukka, Enugu State, Nigeria

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
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

- 234 Key Note: +: positive
- 235 -ve: Negative
- 236 L: Lentogenic strain
- 237 M: Mesogenic strain
- 238
- 239