



## Strains of *Newcastle disease Virus*, Circulating among Sick Poultry Flocks in Nsukka-Nigeria

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### Authors' contributions

This work was carried out in collaboration between all authors. Author MCOE designed the experiments and drafted the manuscript while authors JAOC, IEE, IJM and AAN conducted the field research and the laboratory experiments. All authors read and approved the final manuscript.

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### ABSTRACT

**Aim:** To study prevalence of the different strains of *Newcastle disease virus* (NDV) circulating among sick poultry flocks, NDV detected from disease outbreaks in Nsukka-Nigeria were characterized into the three main NDV strains.

**Materials and Methods:** NDV was detected from 63 cases of poultry disease that manifested clinical signs and lesions suggestive of *Newcastle disease* (ND), by the haemagglutination test. The detected NDV samples were characterized by a combination of their ability to agglutinate mammalian erythrocytes and their erythrocyte elution time (EET).

**Results:** Fifty three out of 63 (84%) NDV samples detected from the ND cases belonged to Lentogenic strains while 10 (16%) were Mesogenic strains. None was of the Velogenic strains.

**Conclusion:** Rates of circulation of Lentogenic, Mesogenic and Velogenic NDV strains among sick poultry flocks in Nsukka- Nigeria suggest that the practice of using live NDV vaccines for prophylaxis may be an important factor in epizootiology of *Newcastle disease* in the country.

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## 1. INTRODUCTION

*Newcastle Disease* (ND) is a contagious viral disease of poultry. It 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 Alexander [6] reported that when the virus was inoculated by intramuscular injection, the chicks were dead in 48 hours. He also reported that natural infection of NDV leads to 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 inactivated and live vaccines are used in the country. Live ND vaccines used in Nigeria are lentogenic strains (Hitchner B<sub>1</sub>, the F strain, the V strain and lasota) and the Mesogenic strain, including Roakin, Mukteswar, Kamorrov [7]. Before Velogenic NDV strains are used as vaccines, they are inactivated by treating them with BPL and formalin or by adsorbing them onto aluminum hydroxide gel [8].

The *Newcastle disease virus* is classified into 4 pathotypes [6]. Viscerotropic Velogenic *Newcastle Disease Viruses* (VVND) cause Dolye's form of ND which is characterized by depression and prostration with many birds dying within a few days. The principal signs in this form are respiratory and nervous signs. An infected bird may show increased respiratory distress, which include gasping, cough and tracheal rales or sneezing. Some birds develop profuse bright greenish or whitish diarrhoea, greenish yellow diarrhoea, oedematous swelling of the head, cyanosis of combs, conjunctivitis and inflammation of the shank and toes [9-12]. Nervous signs seen prior to death include muscular tremor, torticollis, paralysis of legs and

wings, drooping wings and twisting of head and neck. Sometimes mortality may reach up 100% in unvaccinated flocks [13]. Birds that survive the infection may develop partial or complete cessation of egg production. There could be shell-less or thin soft shelled eggs with watery albumen. Abnormal colour of eggs is also common. Sudden death with few or no symptoms could also be observed.

Neurotropic Velogenic *Newcastle Disease Viruses* (NVNDV) cause Beach's form of ND which is characterized by acute respiratory and nervous signs. Egg production falls dramatically but diarrhoea 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 usually does not cause disease in adults. In adults the infection is commonly sub-clinical with mild respiratory signs, temporary loss of appetite, drop in egg production with negligible mortality unless concurrent disease is present [9].

Frequent occurrence of *Newcastle disease* in Nigeria, despite wide vaccination coverage, made it necessary to investigate outbreaks of poultry diseases, to study strains of *Newcastle disease virus* circulating among sick poultry in the country.

## 2. MATERIALS AND METHODS

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

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

Supernatants of faeces or of organs of sick chickens were tested for *Newcastle disease virus* by haemagglutination (HA) test as described by Johnson [15-17]. Those that gave agglutination of chicken RBC which was inhibited by a known NDV antiserum were subjected to agglutination of mammalian erythrocyte test and to determination of their erythrocyte elution time (EET).

To determine their EET, the NDV positive samples were used for HA test with chicken RBC at a controlled temperature (37°C). Following agglutination, each setup was observed until elution of the RBCs occurred and the time interval 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 ( $\geq 1$  hour) was classified as a Velogenic strain. Any that failed to agglutinate sheep RBC but had EET of less than one hour was taken as a Mesogenic strain while those that agglutinated sheep RBC were classified as Lentogenic NDV strains.

### 3. RESULTS

Of 170 sick chickens with clinical signs suggestive of ND, the virus was confirmed in 63. Fifty three of the NDV detected from the outbreaks were of Lentogenic NDV (84%) while 10 were of the Mesogenic strains of the virus (16%). None was of the Velogenic NDV strains (Table 1).

### 4. DISCUSSION

It is generally believed that Lentogenic and Mesogenic NDV strains do not cause disease in adult chickens 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, huddling together, drop in egg production, white soft shelled eggs, reduction in egg size and greenish-

yellow diarrhea were observed in the sick flocks. Mortality rates of 30% and 10% were also recorded in the two outbreaks.

Observation of these clinical signs in chickens in Nigeria justify tentative diagnosis of ND, because, other diseases of poultry that have similar clinical signs are rare in the country. Agglutination of RBCs which was inhibited by known *Newcastle disease* positive serum is all that is required to confirm the outbreaks as ND.

That Lentogenic and Mesogenic NDV strains are capable of causing the clinical signs and rates of mortality observed in the outbreaks, have already been reported by Morgan and Bryden [9] and by

Gopalakrishna [14]. Since the disease-outbreaks yielded Lentogenic and Mesogenic NDV strains, it suggests that isolates of Mesogenic and Lentogenic NDV strains circulating in Nigeria include those reported to be capable of causing clinical *Newcastle disease* and mortalities in adult chickens [9,14]. Before now, it had already been observed that pathotypes of Mesogenic and Lentogenic NDV strains circulating in Nigeria are virulent [18]. We have also reported that some Nigerian NDV isolates characterized by their Intra-cerebral pathogenicity index (ICPI) as Mesogenic strains were heat stable [19] whereas it has been the belief that only Velogenic NDV strains have heat stable haemagglutinin antigen [18].

Since pathogenicity of NDV strains is a function of their haemagglutinin antigen [20-22], heat stable Mesogenic NDV isolates would likely be more pathogenic than the heat labile isolates. So, heat stable Mesogenic NDV strains may be responsible for the clinical signs and mortality recorded in adult chickens in the outbreaks studied.

The earlier observations and results of present study, suggest that some ND outbreaks in Nigerian poultry flocks may result from isolates of NDV characterized as Lentogenic and Mesogenic NDV strains. It is also possible that variants of the Lentogenic NDV and of the Mesogenic NDV exist and that these yet to be classified variants, are circulating in Nigeria.

Detection of 10 NDV samples classified as Mesogenic strains from 63 samples in Nigeria is of economic importance, because Mesogenic strains of NDV cause drop in egg production. Infection from Mesogenic NDV in laying flocks is

**Table 1. Strains of Newcastle disease Virus involved in disease outbreaks in Nsukka-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

Key Note: +: positive; -ve: Negative; L: Lentogenic strain; M: Mesogenic strain

usually characterized by marked drop in egg production [14]. The chickens may be feeding well but produce few eggs, thus leading to great economic losses.

In Nigeria there is uncontrolled use of live ND vaccines but Bennejean [16] has reported that all that is required of NDV to be able to cause disease outbreaks in infected chicken flocks is for its ICPI to be greater than 0.7. If viruses (live vaccines) are allowed free passages in cells of their normal hosts (chickens) as is currently the case with NDV in Nigeria, their ICPI could increase with each passage. When this happens, live vaccines could revert to virulence.

Commercially available vaccines for *Newcastle disease* in Nigeria include live Lentogenic strains and live Mesogenic strains, whereas Velogenic ND strains used as vaccines are inactivated [17]. Prevalence of the three main NDV strains as circulating viruses in the outbreaks studied (84% Lentogenic, 16% Mesogenic and zero % Velogenic) reflects rates of use of the three strains as live vaccines in the country.

## 5. CONCLUSION

Reversion of live vaccines to virulence due to long passages in chickens may be cause of some outbreaks of ND in Nigeria.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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