

STRAINS OF *NEWCASTLE DISEASE VIRUS*, CIRCULATING AMONG SICK POULTRY FLOCKS IN NSUKKA, NIGERIA.

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ABSTRACT:

Aim: *Newcastle disease virus* (NDV), detected from sick poultry flocks in Nsukka, Nigeria were characterized.

Materials and methods: *Newcastle disease virus* (NDV) detected from 63 poultry disease cases, characterized by clinical signs and lesions suggestive of Newcastle disease (ND) were confirmed by haemagglutination test with extracts of tissue homogenates of affected chickens. The NDV detected, were characterized by a combination of their ability to agglutinate mammalian erythrocytes and their erythrocyte elution time (EET).

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

Conclusion : We conclude that live vaccines used for prophylaxis may be source of NDV circulating in Nigeria. The NDV circulating in the country could be responsible for some outbreaks of the disease.

Key words: Circulating *Newcastle disease virus* strains; Uncontrolled use of live vaccines; Nsukka- Nigeria.

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 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 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 inactivated 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 adsorbing them onto aluminum hydroxide gel [8].

The *Newcastle disease virus* is classified into 4 pathotypes [6]. Viscerotropic Velogenic Newcastle Disease Viruses (VVND) cause the Dolye's form of ND which is characterized by depression, 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 diarrhea, Odematous swelling of the head, cyanosis of combs, conjunctivitis, inflammation of the shank and toes [9-12]. Nervous signs seen prior to death could manifest as muscular tremor, torticollis,

paralysis of legs and wings, drooping wings and twisting of head and neck. Sometimes mortality may reach up to 100 % in unvaccinated flocks[13]. Birds that survive the infection may develop partial or complete cessation of egg production. Sometimes egg production declines dramatically. 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 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 usually does not cause disease in adults. It 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 the outbreaks to study strains of the *Newcastle disease virus* involved in outbreaks of the disease in the country.

2.MATERIALS AND METHODS.

Faeces were collected from sick chickens in each farm with clinical signs suggestive of ND, in Nsukka Enugu state in South East, Nigeria. Intestinal contents, lungs, liver, trachea and spleen were collected from dead chickens in affected flocks. History of the flocks, including, flock size, management system, rate of egg production, vaccination history, mortality and morbidity rates, and 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, containing 10-20 % of 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]. 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 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(≥ 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.

Of 170 cases with clinical signs suggestive of ND, the virus was confirmed in 63. Fifty three of 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 strain (Table 1).

4.DISCUSION.

It is generally believed that Lentogenic and Mesogenic NDV strains do not cause disease in adult chickens but in the outbreaks, confirmed as Newcastle disease, 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, eggs without yolk 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 adult chickens in Nigeria justify tentative diagnosis of ND, because, other diseases of poultry that have similar clinical signs in chickens are rare in the country. Agglutination of chicken RBC and mammalian RBC (the Lentogenic strains) which were 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 range of mortalities observed in the outbreaks studied, have already

been reported by Morgan and Bryden [9] and 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 may be capable of causing clinical newcastle disease and mortalities in adult chickens as have been reported [9,14]. Before now, it had also been reported that pathotypes of Mesogenic and Lentogenic NDV strains circulating in Nigeria were virulent [18]. We have also observed that some Nigerian NDV isolates characterized by their Intra-cerebral pathogenicity index (ICPI) as Mesogenic strains were heat stable [19] whereas only the Velogenic strains are reported to have haemagglutinin antigen that is heat stable [18].

Since pathogenicity of NDV is a function of the viral heamagglutinin antigen [20-22], heat stability by isolates characterized as Mesogenic NDV strains means that the isolates would be different in pathogenicity from the other isolates also characterized as Mesogenic strains which were heat labile.

These 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

there are minor strains of the virus, different from the three major strains and that these yet to be classified minor strains, are circulating in Nigeria.

Detection of 10 NDV isolates classified as Mesogenic strains from 63 samples in Nigeria is of economic importance, because Mesogenic strains of NDV are reported to cause drop in egg production. Infection from Mesogenic NDV in laying flocks is 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] reported that once NDV ICPI is greater than 0.7, it can cause disease outbreak. If live vaccines are allowed uncontrolled circulation in poultry populations their ICPI could increase beyond 0.7 thus they may 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 in the outbreaks studied (84% Lentogenic, 16 % Mesogenic and zero % Velogenic) reflects these rates of use of the three strains as live vaccines in the country.

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

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**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

Key Note: +: positive

-ve: Negative

L: Lentogenic strain

M: Mesogenic strain

Competing interests:

There is no competing interests. The authors are staff of Universities in Nigeria, employed to teach and research and students who used the experiments for their studies.

Authors` contributions to the research:

Dr. M. C. O. Ezeibe designed and coordinated the experiments. He also, drafted the manuscript while Prof. John Okoye, Dr. Ihuoma E. Ezeala, Dr. Ijeoma J. Mbuko and Mr. Augustine A. Ngene carried out the laboratory researches.