

## AN OUTBREAK OF NEWCASTLE DISEASE IN A SMALL COMMERCIAL BROILER FARM IN NSUKKA, ENUGU STATE, NIGERIA: A CASE REPORT

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### ABSTRACT

This is a case report of Newcastle disease (ND) in 6 weeks old broiler chickens with flock size of 94 birds (vaccinated). The client presented history of continuous deaths from 4 weeks and 4 days old. The clinical signs observed in this case study were 17 mortalities (18%), depression, respiratory distress (whistling sound), weakness, and diarrhoea (greenish watery faeces). Post-mortem investigation revealed haemorrhagic caecal tonsils, slightly enlarged and friable liver with areas of discolouration, pin point haemorrhages on pro-ventricular glands, hydropericardium and congestion of the pectoral muscle and trachea. Histopathology examination of the lymphoid organs revealed severe necrosis of lymphocytes. Tracheal washing and oropharyngeal swab were collected for virus isolation and were inoculated into the allantoic cavity of 11 day-old specific pathogens free (SPF) chicken embryo. Three days' post inoculation, isolates were harvested from the inoculated eggs and tested for haemagglutination property using Haemagglutination test. The embryo showed severe haemorrhages and congestion. High HA titres were detected in the allantoic fluid. Using the harvested allantoic fluid as NDV antisera, Haemagglutination inhibition (HI) test was done. The test showed reaction between the causative organism and NDV antisera confirming that the birds were suffering from ND. The remaining apparently healthy birds were re-vaccinated using NDV LaSota strain, followed by administration of a vitamins-mineral supplement for management. The farmer was advised to procure vaccines at reputable veterinary outlets and always apply strict biosecurity measures including isolating sick birds, improving ventilation, and reducing overcrowding.

**Keywords:** *Broilers, haemagglutination, mortality, Newcastle disease, virus isolation*

### INTRODUCTION

Newcastle disease (ND) is a highly infectious and often fatal viral illness that impacts a broad spectrum of both domestic and wild species including chickens, turkeys, geese, ducks, pheasants, partridges and guinea fowl (Dimitrov, 2023). The disease is a major obstacle to the growth of poultry farming, leading to substantial economic losses (Alexander & Senne, 2008; Etriwati *et al.*, 2017). The causative organism, Newcastle disease virus (NDV), is a non-segmented, single stranded, negative-sense RNA virus belonging to the Paramyxovirinae subfamily and Paramyxoviridae family (Ewies *et al.*, 2017).

Based on its ability to cause disease, NDV is categorized into four pathotypes: velogenic, mesogenic, lentogenic and asymptomatic forms. Velogenic pathotypes are further classified as viscerotropic (causing visceral lesions) or neurotropic (causing nervous lesions) (El-Morshidy *et al.*, 2021). The velogenic viscerotropic NDV pathotype has been reported in all parts of Nigeria (Baba *et al.*, 1995) and has resulted to significant harm. Manifested signs of NDV such as depression, diarrhoea, prostration, oedema of the head and wattles, poor egg performance of laying birds, nervous signs (such as paralysis and torticollis) and respiratory signs have been reported (McFerran & McCracken, 1988).

In Nigeria, velogenic viscerotropic NDV is widespread and results in significant financial harm (Ganar *et al.*, 2014).

The severity of NDV's pathogenicity is influenced by several factors, including the specific viral strain, the bird's age and immune status, the viral dose, and the route of entry (Hussein *et al.*, 2019). Natural infection typically occurs through nasal, oral, and ocular routes, with the respiratory system being the primary target site for NDV (Kabiraj *et al.*, 2020). In young chicks, virulent strains of NDV can cause sudden death without clear clinical signs. However, in older chickens, the disease may be prolonged, presenting with distinct clinical signs such as respiratory distress and nervous system involvement (Luna, 1968). The World Organization of Animal Health (2023) classified Newcastle disease as a list of reportable disease because it is highly contagious and associated with high mortalities in susceptible birds. Many countries rely on consistent administration of safe and potent vaccines to keep the disease under control (Miller *et al.*, 2007; Okwor *et al.*, 2016).

The diagnosis of ND outbreak is mainly by case history, clinical signs, post mortem and histopathology lesions. In some cases, these diagnostic techniques may be insufficient to definitively diagnosis ND because of some other diseases such as avian influenza that share similar clinical signs and lesions. The use of virology techniques such as complement fixation test, virus neutralization test, haemagglutination test, haemagglutination inhibition test and virus isolation gives a definitive diagnosis of ND. These virology techniques are not commonly utilized due to some challenges such as high level of expertise and availability of materials for the procedure. In this case report, ND was diagnosed in the farm using haemagglutination test, haemagglutination inhibition test and isolation of virus from inoculated embryonated chicken eggs; in addition to clinical signs, gross and histopathology lesions.

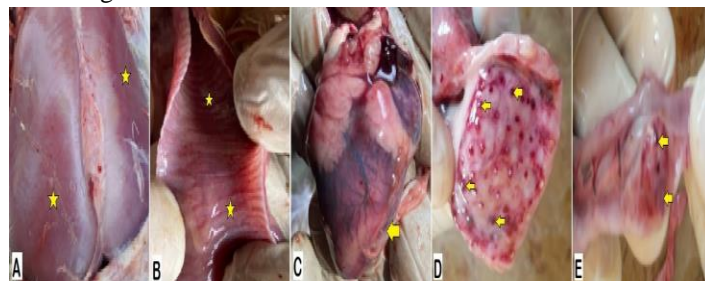
#### CASE HISTORY AND PHYSICAL EXAMINATION

The farmer reported history of continuous death of the birds from four weeks of age. The flock size was 94 broiler chickens that were 6 weeks of age. History also revealed that the birds were fed on commercial feed (Happy Chicken®). The farmer recorded 17 deaths within 10 days prior to reporting. The flock was vaccinated with Hitchner B1 at one-day-old and La Sota at 14 days old. They were previously treated with Doxygen® (Doxycycline and Gentamycin) by the farmer, but the situation did not improve.

On visit to the farm, the birds were observed to be very weak and huddling together, greenish faeces, reduced feed intake, moribund and about 5% mortality on that day. One moribund bird and two carcasses were collected for post mortem examination and further virology tests.

#### POST MORTEM EXAMINATION

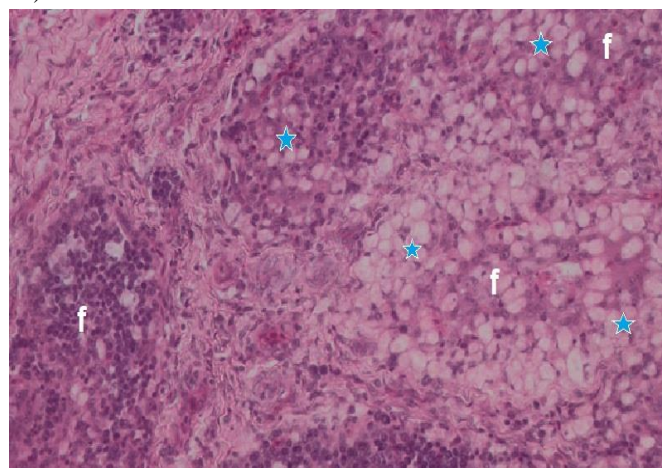
At necropsy, congested pectoral muscle (Figure IA), marked congestion of trachea (Figure IB), hydropericardium (Figure IC), pinpoint haemorrhages on the tips of glands in the proventriculus (Figure ID) and haemorrhagic caecal tonsils (Figure IE) were evident, the body weight of the dead bird was 2.7kg.



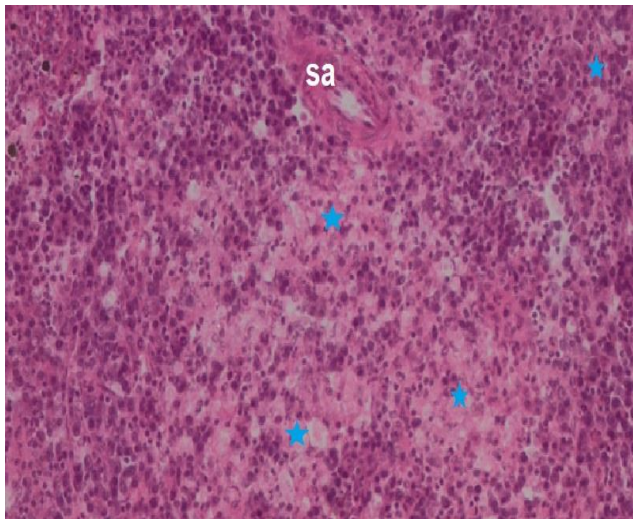
**Figure I: Post-mortem lesions of chicken diseased with Newcastle disease. A: Congested pectoral muscle (star showing areas of congestion); B: Congested trachea (star indicating congested areas); C: Hydropericardium (arrow pointing to excess fluid in the pericardium); D: Pinpoint haemorrhages on the tips of glands in the proventriculus (arrow pointing to the haemorrhages/red spots on the gland); E: Haemorrhagic caecal tonsil (arrow pointing to the haemorrhages on the gland).**

#### HISTOPATHOLOGY EXAMINATION

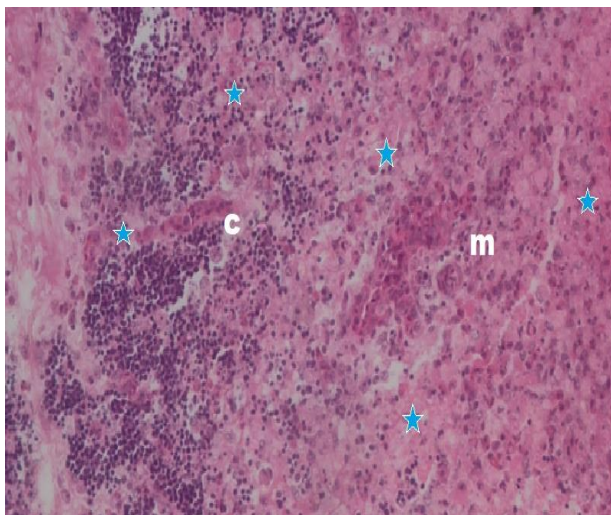
From the post mortem examination, samples were obtained from the lymphoid organs – bursa of Fabricius, spleen and thymus. The samples were fixed in 10% buffered formal saline for histopathology and stained using haematoxylin and eosin (H&E) stain. The processed tissues were viewed under light microscope and images were captured using Axiolab 5 Ziess bright-field light microscope. Results indicated severe depletion of lymphocytes in sections of the bursa of Fabricius (Figure II), spleen (Figure III) and thymus (Figure IV)



**Figure II: Photomicrograph of the bursa of Fabricius showing severe depletion of lymphocytes (star) in the bursa follicles (f). H&E stain. X400 magnification**



**Figure III: Photomicrograph of the spleen showing severe depletion of lymphocytes (star) in the white pulp. H&E stain. X400 magnification. sa – sheathed artery**



**Figure IV: Photomicrograph of the thymus showing severe depletion of lymphocytes (star) in the cortex (c) and medulla (m). H&E stain. X400 magnification**

#### DIAGNOSIS

Tentative Diagnosis was Newcastle disease, while avian influenza and Fowl cholera were listed as Differential Diagnoses.

#### CONFIRMATION OF DIAGNOSIS

The tracheal washings and oropharyngeal swab were taken to the Virology Lab of the Department of Veterinary Microbiology and Immunology, University of Nigeria, Nsukka for virus isolation and serology. Virus isolation was done by inoculating samples (tracheal washings and oropharyngeal swab), each into the allantoic cavity of five samples of 11-days-old specific pathogen free (SPF) embryonated chicken egg according to OIE (2012).

Allantoic fluid was harvested 4 days' post sample inoculation and the embryo examined for possible defects. The allantoic fluid was tested for the presence of haemagglutinating virus using haemagglutination test as described by OIE (2012). High HA titres values of 64 and 128 was recorded for the tracheal washing and oropharyngeal swab, respectively.

Newcastle disease was confirmed by subjecting the isolate to haemagglutination inhibition test (HI) using NDV antisera as known antibody. Inhibition of haemagglutination by the viral isolate was observed.

The inoculated embryo showed virus-induced gross lesions such as oedematous swelling, diffuse congestion, and marked haemorrhages throughout the body of the harvested chicken embryos (Figure V).



**Figure V: Embryo showing congestion and subcutaneous haemorrhages on the neck region. Orange arrow - embryo inoculated with the tracheal washing sample. Blue arrow - embryo inoculated with the oropharyngeal swab sample.**

#### MANAGEMENT

Vitamix® (Vitamins, Amino acid and electrolytes) was administered in drinking water at 1g per 4 liters of drinking water for 5 days as an anti-stress and immune booster. The active birds were re-vaccinated using NDV (LaSota). No antibiotics were recommended. The farmer reported great improvement after the visit as the mortality stopped and he was able to sell off the healthy chickens.

#### DISCUSSION

Poultry disease diagnosis is made mostly in the field by reviewing the clinical histories, clinical signs and by pathognomonic pathological lesions during necropsy.

Newcastle disease virus is not pathognomonic and therefore these criteria cannot be used alone for diagnosis of Newcastle disease. Laboratory confirmation using procedures such as virus isolation, serology and molecular techniques is required for diagnosis of Newcastle disease. In this study, we made the diagnosis based on clinical histories, pathological findings, virus isolation and serology for confirmation. The client reported that 17 birds died within ten days. Recorded clinical signs were depression, respiratory distress (whistling sound), weakness, and diarrhoea (greenish watery faeces) (Eze *et al.*, 2014). Necropsy was performed to examine and record gross and microscopic findings. Observed gross lesions were severe congestion of the pectoral muscle and trachea, hydropericardium, haemorrhagic spots on the tips of proventricular glands, and severely haemorrhagic caecal tonsils. Microscopically, there was severe depletion of lymphocytes due to degeneration and necrosis of the lymphocytes in the lymphoid organs. These lesions are consistent with previous findings (Vegad, 2007; Dhaygude *et al.*, 2017; Zegeye *et al.*, 2022). Other diseases known to cause high mortality, lethargy and respiratory signs, such as highly pathogenic avian influenza were ruled out by inoculating the allantoic cavity of 11-day-old SPF embryonated chicken eggs with preparations from oropharyngeal swab or tracheal washings. The harvested embryo was edematous (swollen) with severe congestion and haemorrhages, an outcome also reported by Qosimah *et al.* (2018).

Newcastle disease virus was confirmed by recovery of a haemagglutinating virus whose agglutination activity was inhibited using NDV antiserum. These were conducted in accordance with the guidelines listed in OIE terrestrial manual (2012) for NDV diagnosis.

For the management, no antibiotics was recommended because withdrawal period of 2 weeks may not be allowed before commencement of sales considering the age and weight of the birds. This is important because it could lead to antimicrobial resistance in humans after consuming the healthy carcass due to transfer of antibiotic deposits. This lack of adherence to withdrawal time for drugs used in animals is a major concern particularly due to antibiotic residues in livestock products like milk and meat consumed by humans (Tufa *et al.*, 2018). These sub-inhibitory or sub-lethal concentrations of antibiotics present in food can enter the human body when consumed, either in raw or cooked form. Microbes within the human body are exposed to these concentrations, which can stimulate the development of various resistance mechanisms against the antibiotics present in the food (Stanton *et al.*, 2022).

The improvement is attributed to the fact that the antibiotic (Doxxygen®) that was administered before the visit must have cleared any secondary bacterial infections complicating the health status of the birds (reason for not conducting bacterial isolation). The re-vaccination provided humoral immunity against NDV, preventing infection of the apparently uninfected birds. This improvement after vaccination could explain a possible earlier vaccination failure or the possibility that the earlier vaccination had waned due to poor vaccine administration procedure. In combination with vaccination (using LaSota strain vaccine), immune booster (vitamins mineral supplements: Vitamix®) may have boosted immunity of the birds against stress as recommended by Sanda *et al.* (2015) and Muhammadamin & Qubih (2010) that using immune boosters will enhance immune response against ND in immunosuppressed birds.

### RECOMMENDATION

The farmer was advised to cull the very weak ones and dispose the carcasses properly to prevent further spread. He was also advised to maintain strict biosecurity measures, improving ventilation, reducing overcrowding and always procure his vaccines and drugs from authorized and reputable veterinary outlets.

### CONFLICT OF INTEREST

The authors declare that there is not conflict of interests regarding publication of this paper.

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