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Case Report

EFFECT OF POST-INFECTION KOMAROV VACCINATION ON MORTALITIES ASSOCIATED WITH NATURAL OUTBREAK OF VELOGENIC NEWCASTLE DISEASE IN PULLETS

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ABSTRACT
Newcastle Disease is a viral disease caused by avian orthoavulavirus 1. It is an acute, contagious disease that causes severe economic losses within the poultry industry. Asides its negative economic impact on poultry production, the disease also poses a public health threat through mild zoonosis. Vaccination is required to protect birds against exposure to the field strain of the causative virus. Lentogenic Newcastle Disease virus strains such as LaSota, B1, and I_2 are used as live attenuated vaccines which stimulate cell-mediated and mucosal immune responses for the prevention and control of the disease. Other strains such as Komarov and Mukteswar are mesogenic, and are used for booster vaccines after initial vaccination with the lentogenic strains. Post-infection vaccination of birds is a common practise in most endemic countries like Nigeria, with a dearth of reports available in scientific journals. This case study was conducted to investigate the effects of post-infection vaccination on the morbidity and mortality of point of cage pullets exposed to the field strain of viscerotropic velogenic Newcastle disease. The intervention yielded positive results with a significant reduction in mortalities barely 48 hours post-administration of inactivated Komarov vaccine. This provides information on the potential of utilizing post-exposure vaccination in managing natural outbreaks of Newcastle disease in poultry production in order to curb excessive economic loss.

Keywords: Komarov vaccination, mortalities, Newcastle disease, pullets

INTRODUCTION
Newcastle Disease (ND) is an acute, contagious viral disease that causes severe economic losses within the poultry industry. Although primarily an avian disease, Newcastle disease is transmissible to humans. A rare fatal form of ND infection has been described in immunocompromised humans among workers in the veterinary field, poultry production and processing, and poultry vaccine production units (Ul-Rahman *et al*., 2021). Therefore, ND poses zoonotic risk and raises public health concern (Dhar *et al*., 2018; Ul-Rahman *et al*., 2021). The disease is caused by a virus belonging to the Order Mononegavirales, family Paramyxoviridae, genus *Avulavirus*, generally known as Newcastle disease virus, but was recently renamed Avian orthoavulavirus 1 (AOaV-1) by the International Committee on Taxonomy of Viruses (Kuhn *et al*., 2020).

It affects the nervous, reproductive, gastrointestinal and respiratory systems, most often in unvaccinated or poorly vaccinated birds. Clinical signs typical to the most virulent form of ND (Viscerotropic Velogenic form) can be depression, inappetence, watery greenish diarrhoea, and partial or complete cessation of egg production. These clinical signs vary from asymptomatic enteric infections to systemic infections causing 100% mortality (depending on the species of bird, the strain of virus and the host immune status). Gross lesions are also more observed with the virulent form.
Various studies have concluded that ND is endemic in Africa and Asia, where it is a major constraint to commercial and rural poultry production (Conan *et al*., 2012). Birds infected with the disease shed the virus in exhaled air, respiratory discharges, and faeces. Poultry birds get infected from aerosols and also by ingesting feed and water contaminated

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with the virus (Miller *et al*., 2013). Control of ND can be done with vaccination complemented by strict biosecurity measures to prevent introduction of the virus into the poultry farm (Miller *et al*., 2013).

The ND vaccine is required to protect birds against exposure to the field strain of the virus. Lentogenic ND virus strains such as LaSota, B1 and I_2 are used as live attenuated vaccines (Rauw *et al*., 2009) for ND control, stimulating immune response similar to those of the natural infection. Other strains such as Komarov and Mukteswar, which are mesogenic strains, are used as booster (inactivated) vaccines after initial vaccination with lentogenic strains (Roohani *et al*., 2015). Reports of ND outbreaks have occurred in vaccinated flocks (Okoye *et al*., 2001; Okwor *et al*., 2012), which have required re-vaccination even after the appearance of clinical signs, without proper representation in scientific literatures. This study therefore investigated the effects of post ND infection vaccination on the morbidity and mortality of point of cage pullets exposed to the field strain of viscerotropic velogenic ND.

CASE REPORT

CASE HISTORY

On 2 May 2023, there was a complaint of massive mortalities in hundreds among pullets, which became pronounced at 8 weeks of age, by the Farm Manager of a commercial poultry farm located in Badagry, Lagos State, Nigeria. The flock size as at the period of outbreak was 4,030 birds, which had spontaneously reduced to 3,093 at the 14th week when the complaint was made. The daily feed consumption rate was 34.16 grammes per bird. There was record of routine bivalent ND (comprising of LaSota and Infectious Bronchitis H120 strains) vaccination at the 6th week before the onset of the outbreak. Numerous treatment options had been attempted on the farm which all proved abortive.

CLINICAL MANIFESTATIONS

The farm observed persistent severe mortalities since the birds were 8 weeks old. The affected birds displayed reduced activities (dullness), reduction in feed consumption rate, and lethargy. Another clinical sign noticed was greenish-white faecal droppings on the litter floor (Figure I). Fresh carcasses were initially sent to a reputable Diagnostic Laboratory within Lagos State for analyses on 23 March 2023.

LABORATORY INVESTIGATIONS

POSTMORTEM EXAMINATION I

An initial postmortem (PM) examination of the submitted carcasses was conducted by the Diagnostic Centre which revealed the followings; emaciation, whitish necrotic foci on the spleen, congested liver, and enteritis. A tentative diagnosis was given as 'Fowl Typhoid' based on the postmortem findings. Furthermore, bacterial culture was made to determine the antibiotic susceptibility for recommendation purpose on the possible treatment regimen to control the suspected bacterial infection.

Antibiotic Susceptibility Test

The result for the antibiotic susceptibility test (AST) was presented in Table I. Out of the 9 antibiotics tested, only Amoxicillin and Neomycin showed a higher zone of inhibition (15mm Diameter each).

Table I. Antibiotic Sensitivity Results for the pullets.

Sample Tested	Antibiotics	Zones of Inhibition Diameter (mm)	Interpretation
Cloacal Swab	Amoxicillin	15	I
	Colistin	11	R
	Neomycin	15	I
	Gentamicin	12	R
	Tetracycline	0	R
	Doxycycline	0	R
	Ciprofloxacin	0	R
	Trimethoprim	0	R
	Sulphamethoxazole	0	R

N.B.: I = Intermediate, R = Resistance (using CLSI, 2023).

FIELD INVESTIGATION

POSTMORTEM EXAMINATION II

Upon receiving reports from the farm on the increasing mortality of the birds (now at 14 weeks of age), even after the laboratory-based interventions, we collected fresh carcass and conducted another PM examination under sterile conditions in a separated space on the farm. Our PM findings revealed; dehydration, cachexia, prominent keel bone with loss of muscle mass, cloudy air sacs, pneumonic lungs with frothy exudates, haemorrhagic caecal tonsils, enteritis, and underdeveloped ovaries (Figures II-V). Our tentative diagnoses based on the case history and the recent PM findings were Newcastle Disease and Colibacillosis.

CASE MANAGEMENT

In reference to the AST result, a combination of Amoxicillin and Colistin (for synergy), administered orally in water for 5 consecutive days, was recommended by the laboratory for the treatment of the remaining flock as at 26 March 2023. However, mortalities increasingly persisted on the farm in subsequent weeks 9 to 13 (Table II), even after the prompt administration of the recommended antibiotics.

Figure I. Greenish faeces (yellow arrows) on the litter floor.

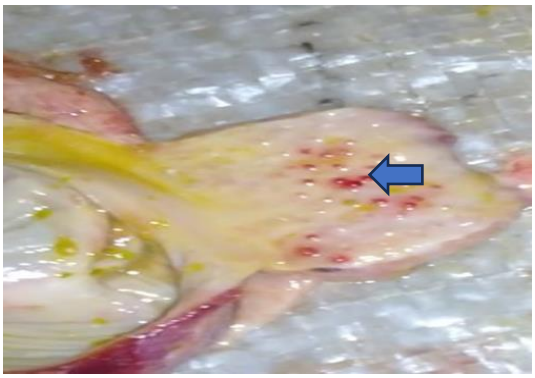

Figure II. Proventriculus showing petechial haemorrhages (blue arrow)

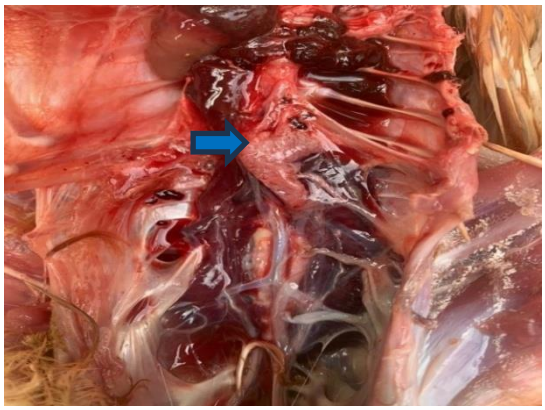

Figure III. Underdeveloped reproductive organ (blue arrow)

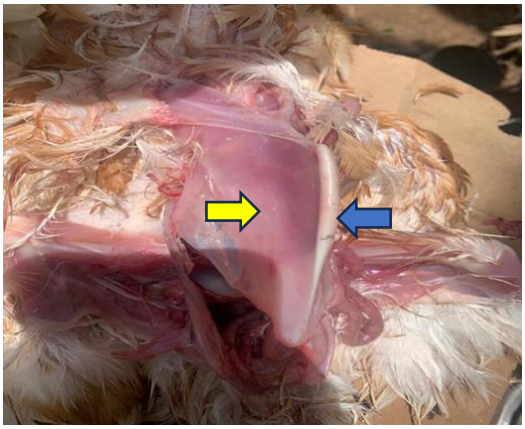

Figure IV. Reduced breast muscle mass (yellow arrow) and prominent keel bone

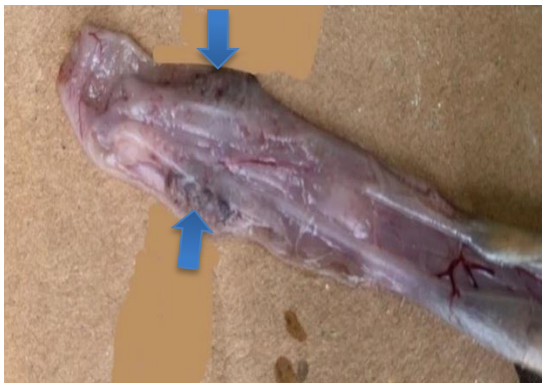

Figure V. Haemorrhages on the caecal tonsils (blue arrows)

Based on the second PM examination, we recommended ND vaccination using oil-emulsion Komarov vaccine, with anti-stress (Vitamin C) also administered. The Komarov vaccine was administered intramuscularly to all the remaining 3093 stock, followed up with antibiotics therapy in subsequent weeks to prevent secondary bacterial infections (Table II). This intervention caused a significant reduction in mortalities between the preceding 13th week (n = 209) and 16th week (n = 43). The difference in mortality rate was observable within

Table II. Showing mortality patterns of the pullets and vaccines administered

Age (Weeks)	Opening Stock	Weekly Mortalities	Closing Stock	Vaccinations	Other Interventions
4	4052	1	4051	-	Enrofloxacin
5	4051	7	4044	-	Vitamins
6	4044	6	4038	ND + IB	Neomycin + Doxycycline
7	4038	8	4030	-	-
8	4030	36	3994	Fowlpox	Amoxycillin + Colistin
9	3994	53	3941	ND + IB	-
10	3941	91	3850	-	Ivermectin
11	3850	324	3526	-	Doxycycline + Gentamycin
12	3526	224	3302	-	Vitamins
13	3302	209	3093	-	Amprolium + Enrofloxacin
14	3093	135	2958	Komarov	Vitamins
15	2958	85	2873	-	Enrofloxacin + Liver Tonic
16	2873	43	2830	ND + IB + EDS	Vitamins
17	2830	33	2797	-	Ivermectin
18	2797	22	2775	-	Liver Tonic
19	2775	31	2744	-	Liver Tonic
20	2744	36	2708	LaSota	Enrofloxacin
21	2708	37	2671	-	Liver Tonic
22	2671	9	2662	-	Liver Tonic
23	2662	8	2654	LaSota	Liver Tonic
24	2654	5	2649	-	Enrofloxacin

N.B.: ND- Newcastle disease; IB- Infectious Bronchitis; EDS- Egg-drop syndrome

48 hours post-administration of the oil-emulsion Komarov vaccine. Besides the significant reduction in mortality level, the egg production of the flock commenced at exactly 21 weeks of age, with observable increase in feed consumption rate from 72.70 grammes per bird, at 20 weeks of age, to 85.00 grammes per bird, at 24 weeks.

Antibiotic treatment was continued in subsequent weeks to control secondary bacterial infections, with routine oil-emulsion multi-valent (LaSota strain of Newcastle disease virus, Massachusetts strain of Infectious Bronchitis virus, and B8/78 strain of Egg Drop Syndrome virus) vaccine administered according to the vaccination schedule of the farm.

DISCUSSION

This case study showed that post-exposure vaccination can be used to manage outbreaks of Newcastle Disease, not only in pullets, but can be translated to other poultry birds. Previous findings by Okwor *et al*. (2012) reported that the post exposure vaccination of chicks against some avian pathogens was often practised in developing countries with varying results probably due to climate change, age, breed of the chicks, severity of the infection at the time of vaccination, immune status of the chicks, or nature of virulence of the pathogen. However, the scientific reports of such interventions are grossly inadequate in most scholarly databases.

Our report also emphasized previous reports that LaSota vaccination may not be sufficient enough to protect against the lesions caused by velogenic ND. Table II even showed that the vaccination of birds with the LaSota strains upon appearance of clinical infection from around Weeks 6 and 9 may have exacerbated the mortality level.

The increased mortality may be due to, among other factors, the combined effects of vaccination stress and the concurrent administration of the live attenuated vaccine which have the potential of reverting to virulence. Even with high circulating antibodies, there are suggestions that virulent ND strains may still replicate in vaccinated flocks, but with the clinical signs subsiding significantly in relationship to the antibody level achieved after re-vaccination (Boven *et al*., 2008; Ezema *et al*., 2009). We were however limited in the quantification of the humoral response in this study.

In conclusion, although it has been shown that post-infection vaccination with inactivated oil-emulsion Komarov vaccine yielded a positive outcome in the management of natural ND outbreak scenario, we opined that vaccination alone does not guarantee maximum poultry protection and production. We therefore recommend that poultry farmers should embrace proper and adequate farm biosecurity, in addition to strict compliance to routine vaccination plans for their birds, to eliminate other confounding factors which may encourage disease outbreaks. There is need for further studies on the immune-stimulating effects of the inactivated vaccines after exposure to field strains of the virus. Farm owners must invest in monitoring the immune status of vaccinated birds (pre- and post-vaccination) in order to ensure effective vaccination and prevent vaccine failure or break.

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