

Full Length Research Paper

Newcastle disease in layers: Preliminary studies on the stress associated with onset of lay and initiation of clinical disease

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Newcastle disease is endemic in Nigeria and there are reports of frequent outbreaks both in village and commercial poultry population. Of particular importance and interest is the report in layers particularly at the commencement of production up to peak hen day production. This paper reports preliminary investigations on onset of lay and the development of clinical Newcastle disease. It combined complaints as recorded in two Veterinary clinics for a period of three years and field studies in a layer farm where frequent outbreaks of Newcastle disease were known to have occurred. Data collected showed that 38.1% of Newcastle disease cases recorded within the 3-year period in layers were in young layers between the period of first egg lay to the fourth month of production. The remaining 61.9% was observed in older layers above four months of production life. Parameters taken during the field study showed that unprotected young layers were more susceptible to Newcastle disease infection than older ones. We therefore report that onset of lay can be a stress factor that can alter the immune system of young layers and reduce their resistance to Newcastle disease. We also recommend that pullets should be vaccinated against Newcastle disease at point of lay with strategic management targeted at helping the birds achieve maturity to withstand the stress associated with commencement of lay.

Key words: Newcastle disease, immune system, layers, onset of lay, egg production, stress.

INTRODUCTION

Newcastle disease (ND) is a deadly viral disease of poultry all over the world since the time of its first isolation in England in 1926 till today (Banu et al., 2009). It is considered as one of the major economic threats to poultry population because of the high morbidity, which may vary from 90 to 100% in unprotected birds depending on the virulence of the Newcastle disease virus (NDV) exposed (Alexander, 2003). The causative agent, NDV is also known as Avian Paramyxovirus type 1 (APMV-1) and the only member of the genus of *Avulavirus* (Mayo, 2002) in the family Paramyxoviridae. It

is a single stranded, non-segmented enveloped RNA virus with negative polarity (Alexander, 2003). ND is present in many countries of the world and in tropical and subtropical countries, virulent strains of the virus are endemic (Spradbrow, 1990). In most developing countries, ND ranked as the most important poultry disease affecting the poultry population with consequent great economic losses (Aini, 1990; Martin, 1992). In recent years, poultry keeping and production has become a growing and prospective industry in Nigeria. These developments are seriously threatened by outbreaks of acute, contagious and fatal cases of ND. Subclinical cases of ND are also important as they cause problems with significant losses especially in layers. The endemicity of ND in Nigeria leads to high environmental dissemination of the virus and this is enhanced by poor

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husbandry practices (Okwor and Eze, 2010). The presence of free range village poultry, backyard poultry flocks to large scale commercial farming in Nigeria without any legislative, and bio-security measures guiding the operations of these poultry populations have encouraged the endemicity of the disease in many poultry establishments with frequent and sustained outbreaks.

ND is a major problem in laying birds due to huge losses encountered as a result of poor quality eggs, reduction in egg production and mortalities (Alexander, 2003). All the strains of this virus are encountered in this population of poultry with varying degrees of disease severity and losses. Veterinary clinical and field investigations in tropical South-East Nigeria point to the prevalence of severe ND in young layers close to or at peak of production especially if the birds are not protected by vaccination (Okwor et al., 2012). Some factors are known to affect the severity of ND outbreaks. Factors like the strain or pathotype of the virus, host species affected, age of the host, immune status of the host, co-infection with other organisms, route of infection, infective dose of the virus, tropism of the virus, and stress factors are reported to affect the severity of ND infection (Campbell, 1986; Mc Ferran and Mc Craken, 1988; Mahamed et al., 2007). Stress factors from the immediate environment, caging of layers, seasonal changes, extremes of temperatures, transportation or movements, poor nutrition, and management procedures like debeaking, forced-moulting and vaccinations with live attenuated vaccines are known to affect the performance of broilers and layers (Maxwell et al., 1992; Shini, 2003; Marshaly et al., 2004; Arbona et al., 2011). Stress induced immune studies have been conducted in poultry by measuring or utilizing haematological indices such as Heterophil: Lymphocyte (H:L) ratio (Gross and Siegel, 1983) and humoral, immunological or antibody responses (Dohm and Saif, 1983).

This paper reports preliminary investigations on outbreaks of ND in young layers (from onset of lay to peak hen-day production) by comparing some clinical and immunological parameters with that of older layers and cockerels in an attempt to establish whether onset of lay is a predisposing stress factor in clinical ND infection in endemic areas.

MATERIALS AND METHODS

Collected data

Field data collected as complaints and presentations in two Veterinary clinics located at Nsukka, Nsukka Local Govt Area and Obollo-Afor, Udenu Local Government Area of Enugu State, South East Nigeria were used for this study. The cases presented to these two clinics were examined and ND cases in layers were diagnosed and sorted out from other cases in layers for a period of three years. Diagnoses of ND were by clinical examination, post mortem

examination, hemagglutination inhibition (HI) test on serum samples collected and virus isolation studies on cloacal swabs collected. Cases that occurred in young layers (from onset of lay to 4 months into lay) were differentiated from cases occurring in other age groups of layers (layers more than 4 months into lay).

Field investigation

Three separate studies were carried out in a farm with history of outbreaks of ND to investigate the outbreaks of ND in young layers from the onset of lay (16 weeks of age) through to 4 months into production. Natural outbreak of ND was studied in this age group of layers and some parameters including the humoral responses were assessed and recorded.

Experiment 1

Case study in young layers that had ND but were not vaccinated against the disease at point of lay. Three batches of young brown layers in a farm that had outbreaks of ND between the period of first egg drop and 4 months after lay were studied. These were designated as batches A, C and E. Three batches of older layers housed in the same farm and designated as batches B, D and F were also studied. Batches A and B; C and D; and E and F were studied and compared in that order. History showed that these batches of birds were introduced at day old. They were reared on deep litter system with food and water given *ad-libitum*. Newcastle disease vaccine (ND vaccine) Hitchner B1 was administered to the pullets at day old. ND vaccine, La Sota was administered at 3 weeks and repeated at 6 weeks. Infectious bursal disease vaccine, (IBD vaccine) was administered at 2 weeks and booster dose administered at 4 weeks. Fowlpox vaccine (FPV vaccine) was administered at 5 weeks. Prophylactic medications against bacterial and *Eimeria* species infections were administered as the needs arose.

The flocks were monitored and investigated from point of lay (16 weeks) to 4 months into production. Serum samples were collected randomly from the wing veins of 20 birds in each batch at the age of 17 weeks and used for the estimation of antibodies titres against ND using HI test. Parameters like number of birds at first egg drop, hen-day production at outbreak of ND, severity of the disease as indicated by morbidity and mortality, virus isolation from the birds, were recorded and analysed.

Experiment 2

Studies in young layers that were vaccinated at point of lay:

Two batches of birds designated as batches G and H reared in the same farm but vaccinated at point of lay (16 weeks) against ND using ND vaccine, La Sota were studied. The brooding, rearing, vaccination and medication history before point of lay were the same as described for the birds in experiment 1. The same parameters or conditions as mentioned in Experiment 1 were also recorded and analysed.

Experiment 3

Studies in cocks of the same breed which were not vaccinated at 16 weeks:

Two batches of white cockerels of the same breed as the layers designated as batches I and J were reared in the farm. The cocks were not vaccinated at 16 weeks (taking as point of lay for the pullets in this study). At 24 weeks some of the cocks were

Table 1. Number of diagnosed ND cases in young and old layers over a 3-year period.

Period (Year)	No of diagnosed ND cases	No of layers less than 4 months into production and their percentages	No of layers more than 4 months into production and their percentages
1	89	35 (39.3)	54 (60.7)
2	102	45 (44.1)	57 (55.9)
3	95	29 (30.5)	66 (69.5)
Total	286	109 (38.1)	177 (61.9)

sold to reduce cost and 300 birds were left in each group for up to 35 weeks. The brooding, rearing, vaccination and medication history used for the cocks before 16 weeks were the same as those used for the pullets. Moreover, the same conditions or parameters as monitored for the pullets were also monitored in the cocks. Results obtained were recorded.

Hemagglutination Inhibition (HI) Test

The serum samples collected in this study were heat inactivated and used for the estimation of antibodies against ND using the method described by Beard (1989). The highest dilutions of serum causing complete inhibition of agglutination were considered the endpoints. The HI titers were expressed as reciprocal of the highest dilution that showed complete inhibition of agglutination and the geometric mean titre was calculated as described by Villegas and Purchase (1989).

Virus isolation

Cloacal swabs were collected into Brain Heart Infusion Broth (BHI) and were used for the isolation of NDV in embryonated chicken eggs according to the method outlined by OIE (2005).

RESULTS

A total of 286 cases were diagnosed in the two clinics within the 3-year period. Out of this number, 109 (38.1%) cases were diagnosed in young layers of 4 months of production and below (Table 1), while the remaining 177 (61.9%) were diagnosed in layers more than 4 months into production (Table 1). Typical clinical signs and post mortem lesions of ND in the clinical ND cases were seen. Consistent signs recorded included reduced feed and water intake, sudden drop in egg production, laying of discolored and misshaped eggs and occasionally eggs with soft shells, coughing and sneezing, greenish diarrhoea, dehydration, nervous signs of opisthotonus and torticollis at later stages of the infection. Lesions seen include congestion and hemorrhages of the internal organs and muscles.

The result of the investigations on the young layers that were not vaccinated at 16 weeks but had ND outbreak is presented in Table 2. It shows a morbidity of 30.8, 28 and 38.0% and mortality of 4.2, 4.3 and 5.4% in batches A, C,

and E layers, respectively. NDV was isolated in most of the birds sampled; however, no clinical ND was reported or seen in the older layers (Table 2). The Mean HI antibody titres among the birds that had outbreak (A, C, and E) did not differ significantly. The mean titres of the infected layers did not also differ significantly with that of the older layers (B and F) but differed significantly from that of birds in Group D. Table 2 also showed that outbreaks in Groups A, C, and E occurred at or close to peak hen-day production but birds vaccinated at 16 weeks (G and H) did not have outbreak of ND (Table 2) and had higher geometric mean HI titre of 84.4 and 78.8, respectively. The cocks (I and J) showed no outbreak of ND (Table 2), but virus was isolated from 2 and 2.3%, respectively of the birds and they had a low mean HI titre of 21.1 each. The mean HI antibody titre of the cocks (I and J) also did not differ significantly from those of the layers that had clinical ND (A, C and E).

DISCUSSION

These preliminary investigations which combined data collected in the clinics and those of studies carried out in the field points to frequent or common outbreaks of ND among young layers particularly at or near peak hen-day production in ND endemic farms, especially in unvaccinated birds prior to onset of lay. Physiological changes associated with maturity and pregnancy in animals and man, which are mostly hormonal have been shown to affect animals' response to their immediate environment and infection (Foilb et al., 2011). One can therefore suggest that onset of lay on itself can be a stress factor that can induce clinical ND in young layers, which could have ordinarily resisted the infection. Data obtained from the two clinics showed that 38.1% of ND outbreaks in layers occurred among those between the point of lay and 4 months into production. Experiences have shown that under our local tropical conditions, good layers maintain good production cycle that averages 18 months. According to FAO (2003), the production cycle for most good breeds of layers found in both the temperate and tropical zones last for 17 months (72 weeks) under good management practices.

Table 2. Performance, virus isolation and antibody titres of layers.

Batch	No of birds during study	Age at first egg drop	ND outbreak (post lay)	Hen-day production during outbreak (%)	Morbidity during outbreak (%)	Mortality during outbreak (%)	Virus isolation during outbreak (%)	Geometric mean titre
A	924	19 wk 5 days	12 weeks	67	30.8	4.2	88	21.1
B	858	19 wk 1 day	NO	NO	NO	NO	2.0	22.6
C	948	18 wk 4 days	14 weeks	58	28	4.3	80	22.6
D	902	18 wk 5 days	NO	NO	NO	NO	=	32.1
E	964	18 wk	12 weeks	71	38	5.4	94	21.1
F	815	19 wk 1 days	NO	NO	NO	NO	3.1	21.1
G	921	18 wk	NO	NO	NO	NO	=	84.4
H	950	18 wk 4 days	NO	NO	NO	NO	=	78.8
I	1012	=	NO	=	NO	NO	2.0	21.1
J	700	=	NO	=	NO	NO	2.3	21.1

NO = No Outbreak.

Therefore, the 38.1% value of clinical ND obtained for the period of 4 months can be considered to be meaningful when compared with the total production cycle. It has been speculated that reasonable percentage of layers in the South-Eastern Savanna Zone of Nigeria is at high risk of having ND at the period of peak hen-day egg production. Morron (2008) reported that management in layers at the beginning of lay is normally targeted at controlling ovarian functions in females so that maximum life time production can be achieved and at the same time minimizing stress that can predispose the birds to infections. This allows for the maximum prime sequence of egg production and maturation of hens for

sustained egg production. This means that birds that are about to come into lay should be well prepared both nutritionally and otherwise to enable them to cope with the stress associated with initial production process.

Field investigations showed outbreaks of ND in some batches of birds that were not vaccinated at point of lay in the ND endemic farms. However, those groups of layers that were vaccinated at point of lay and the cocks that were reared to maturity but not vaccinated against ND did not show clinical ND. One would therefore be made to conclude that there are anatomical, physiological and hormonal factors/processes associated with maturity and initiation of egg production that could

cause or produce enough stress in non-vaccinated layers at point of lay to compromise their immune status and predispose these groups of birds to ND. This implies that though the cocks were not vaccinated against ND at 16 weeks, they did not come down with clinical ND because they were not exposed to the stress of laying and therefore could not succumb to the infection. As seen in the result, there was no statistical difference in the humoral antibody titres among the unvaccinated layers that had ND and the white cocks that did not have ND. It will be a good practice if birds that are about to come into lay are well prepared both nutritionally and otherwise to enable them to cope with the stress associated

with initial production processes

Stress has been described as adaptive response to challenges in homeostasis (Dohm, 1991) which may include adaptations in the immune responses by the animal (Arbona et al., 2011). It refers to the sum of the physical, mental, emotional and physiological strains or tensions on animals (Mc Ewen, 2003). Therefore a stressor is any stimulus or event that provokes stress responses in an animal and this can be acute or chronic, external or internal. In this condition, we regard onset of lay in birds as a sub acute or chronic internal stimulus that can provoke stress responses in birds. Conditions such as climate, nutritional, social and biological, as well as, experimental elevation of circulating corticosterone in poultry induce a state of stress response associated with increased plasma corticosterone concentration and a number of modifications to metabolic physiology and immunological functions (Cheng and Muir, 2004; Shini et al., 2009), the consequence of which are heavy economic losses due to reduced growth, reduce hen-day production in laying flocks, additional cost in form of production, reduced immunity leading to disease outbreak and mortalities (Ayo and Oladele, 1998; Sahin et al., 2001). We are of the opinion that apart from clinical ND that may be seen in unprotected young layers at the beginning of lay, there also exist subclinical ND cases, which may contribute to these losses including delayed peak hen-day production. While stress is generally regarded as immunosuppressive, the duration, intensity and persistence of the stressors are important distinguishing features of the stress responses (von Hertzen, 2002). The relationship between immune function and disease risk may be greatly influenced by the individual's response to chronic stressors including those that are environmentally induced (Sapolsky, 1994; Arbona, et al., 2011). Prolonged stress responses have been observed to alter an animal's immune function (Mc Ewen, 2007), thus increasing their risk for a wide range of adverse health outcomes. As observed by Miller and O' Callagen, (2002), this increased risk for adverse health outcomes is achieved by suppressing the immune response thus leaving the host vulnerable to opportunistic diseases.

The above assertions on stress would therefore make one conclude that commencement of lay is a prolonged stress factor in young layers that will alter their hormonal system thereby compromising immunity and making them vulnerable to any infection. In this particular investigation, the young layers succumbed to ND while the older layers and cocks resisted the infection. We suggest that the increased hormonal changes associated with maturity leading to heightened hormonal stress in these young layers might be contributing to their susceptibility to ND. Maturity has been reported to triggers the production of gonadotropins, which will eventually lead to the production of ovarian hormones and subsequent ovulation and these transformations throughout maturity

can lead to hormonal stress responses (Foilb et al., 2011). Moreover, the loss of immunoglobulins in laying birds through the egg yolk will lead to rapid decrease in the protective antibodies and predispose the bird to risk of contracting diseases. Though antibody assay was not done at peak hen-day production of lay in this study, we believe that there was significant loss through egg production and this in combination with hormonal stress have contributed in lowering the birds' immunity. However, that is still the need to carry out hematological and immunological studies during the entire period of lay to be able to substantiate this.

The resistance shown by the older layers demonstrated the fact that the birds showed adaptive stress response. Experiments in rats and other animal models showed that prepubertal rats showed heightened hormonal stress reactivity when compared with adults. Their levels of ACTH and corticosterone took twice as long to return to baseline following an acute stressor showing that these responses have distinct developmental profiles (Foilb et al., 2011). Therefore, adult layers were more immunocompetent and showed better adaptations than young layers, though this may be affected by several factors.

CONCLUSIONS AND RECOMMENDATIONS

We conclude by reporting that onset of lay can be a stress factor in the initiation of clinical ND in young layers. Management towards the commencement of lay should be targeted at allowing the birds to mature enough for sustained egg production. Hens must also be vaccinated against ND at point of lay to help them build enough humoral immunity and be able to resist the infection.

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