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Experimental intraocular infection of exotic cockerels with field strain of velogenic Newcastle disease virus in Nigeria

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A B S T R A C T

Experimental intraocular (conjunctival) infection of exotic cockerels with a new field strain of viscerotropic velogenic Newcastle Disease Virus (NDV) was conducted to explore the concurrence of some pathological changes with humoral immune responses. After the NDV infection of 4-week-old cockerels, pathologic changes and antibody responses were observed. The clinical signs observed after the artificial inoculation included inappetence, depression, diarrhea, dyspnea, wing and leg paralysis, torticollis and weight loss. Morbidity due to the NDV was 100%, but mortality was 80% by day 18-21 post-infection. Early hyperthermia followed by terminal hypothermia, decreased packed cell volume (PCV), and 231.4 folds peak-antibody response were observed. Necrotic and/or inflammatory lesions were present in the proventriculus, intestine, liver, spleen, kidney and brain. Neurologic and digestive tract perturbations occurred in 10% and 85% of cases, respectively. The disease consistently caused stunted growth, decreased PCV, and necro-inflammatroy lesions concurrent with antibody response, suggesting probable involvement of immune-mediated mechanisms and cell membrane desialylation by viral neuraminidase in the pathogenesis.

Keywords

Chickens, intraocular infection, Newcastle disease, Nigerian NDV strain, velogenic virus, viscerotropic virus

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INTRODUCTION

Newcastle disease (ND), an acute infectious disease of poultry caused by avian paramyxovirus serotype 1 is endemic in Nigeria (Saidu et al., 2006; Ibu et al., 2009; Musa et al., 2010). Strains of Newcastle Disease Virus (NDV) have been reported to be isolated from apparently healthy ducks, chickens, feral doves, pigeons and migratory wild birds (Ojeh and Okoro, 1992; Echeonwu et al., 1993; Meseko and Moses, 2012), and from ND outbreaks in vaccinated and unvaccinated exotic and local chickens, and characterized to be mostly velogenic strains causing visceral lesions mainly in the proventriculus and intestinal tract (Echeonwu et al., 1993). Sometimes, velogenic NDV may be neurotropic in which case lesions appear in the respiratory and nervous systems (Agoha et al., 1992; Igwe et al., 2014).

Factors affecting the severity of NDV infections include tissue tropism of the virus, inoculum and route of infection, viral strain involved and host factors such as age, species and immune status (Ezeokoli et al., 1990). Susceptibility to infection and severity of infection are higher in chickens than ducks (Eze et al., 2014b) and in exotic than local chickens (Halle et al., 1999). Experimental infections with local strains of the velogenic NDV through oral, aerosolized (occulanasal) and intramuscular routes have been reported in chickens (Oladele et al., 2005; Bobbo et al., 2013; Igwe et al., 2014) where the infections produced viscerotropic and neurotropic lesions.

Newer clusters of the local NDV strains from Nigeria are emerging and are posing a problem in the control of NDV infections by vaccination (Solomon et al., 2011). In this report, we describe the pathological responses of exotic cockerels after intraocular infection with a new

Nigerian field strain of viscerotropic velogenic NDV of chicken origin with reference to clinical signs, body weight and packed cell volume changes, antibody response and lesions in concurrence with pathogenic mechanisms associated with NDV-host interactions.

MARERIALS AND METHODS

Experimental chickens: Thirty unvaccinated day-old cockerels used for the experiment were obtained from Obasanjo Farms in Ota-Ogun State, Nigeria, and were raised by standard methods to 4 weeks of age for the experiment. The cockerels were kept in locally constructed cages (1.5 m^2) in an animal house and were fed commercial feeds (Vital feeds®, GCOML, Jos, Nigeria), and water was also supplied *ad-libitum*. Infectious bursal disease (IBD) vaccine, obtained from National Veterinary Research institute (NVRI) Jos, Nigeria, was administered orally to each of the experimental cockerels at 2 weeks of age. The experimental cockerels used in this study were handled according to the standard procedures approved by the Faculty of Veterinary Medicine Ethics and Research Committee.

Newcastle disease virus: A vial of lyophilized NDV field strain was obtained from Virology Unit of the National Veterinary Research Institute (NVRI) Vom, Nigeria. The strain was of chicken origin, and was characterized as viscerotropic and velogenic. The lyophilized virus was reconstituted with 1.5 mL sterile phosphate buffered saline (pH 7.2) to obtain virus EID_{50} of $10^{8.5}$/mL and subsequently diluted to $10^{6.5}$/mL to be used to infect birds through intraocular route with 0.05 mL in each eye.

Experimental design: Twenty experimental birds were infected with NDV at 4-week of age while 10 were left as uninfected controls. The infected and uninfected birds were housed in different locations to avoid contact between the groups, and biosecurity measures were ensured to avoid extraneous contaminant infections. The body weights, rectal temperatures, clinical signs, packed cell volumes (PCV), hemagglutination inhibition (HI) antibody titers and lesions were monitored in the birds.

Physical evaluation: The body weight was estimated using a weighing balance (Camry Premium®, China). Rectal temperature was measured with a digital thermometer (Hartmann® Digital Thermometer, Heidenheim, Germany).

Determination of Packed Cell Volume (PCV): PCV was determined by microhematocrit technique (Tvedten, 2010) using heparinized hematocrit capillary tubes filled with blood directly from the jugular vein.

Determination of hemagglutination inhibition (HI) antibody titer: Blood (3 mL) was collected from each bird from the jugular vein, allowed to clot and the serum was harvested into cryotubes. One percent red blood cell (RBC) working suspension was prepared using blood from ND-free chickens for the HI test and lyophilized ND vaccine LaSota virus was used as the antigen. Hemagglutination (HA) test was carried out to determine the titer and a working dilution of antigen.

Antibody titer to NDV was determined using a modification of HI test, as described by El-Yuguda et al. (2009). HI test was carried out after the frozen sera were thawed to room temperature. A two-fold serial dilution of 25 µL of neat test serum was made in a microtiter plate and 25 µL of 4 hemagglutination units (HAU) of NDV antigen was added to each well except the last well (serum control). Finally, 25 µL of 1% suspension of chicken RBC was added to all the wells and plate incubated at room temperature (35-38°C) for 45 min. Positive samples were identified by the formation of button at the bottom of each well.

Necropsy: Carcasses were subjected to detailed postmortem examination following the procedure described by Majó and Dolz (2011).

Histopathology: The tissues fixed in 10 % buffered formalin were processed, embedded in paraffin and cut at about 4μm thickness. The sections were stained according to standard procedure (Akpavie, 2004).

Statistical Analysis: The HI titers were summarized as frequencies of titers in infected and non-infected groups. Geometric mean titer (GMT) of HI antibody and standard deviation (SD) were calculated as follows (CDC, 1988; Singha, 1992) with the reciprocal antibody titer (X) and its frequency (f) and total number of birds testing positive (n):

$$\text{GMT} = \text{Antilog}\left(\frac{1}{n}\sum f \log_{10} X\right)$$

$$\text{SD} = \text{Antilog}\sqrt{\frac{1}{n-1}\sum\left[f\left(\log_{10} X - \overline{\log_{10} X}\right)^2\right]}$$

Other data were summarized as mean±SD. Differences between mean values of infected and non-infected groups were assessed by two-tailed Student's t-test. Analyses were carried out using computer software (GraphPad Instat, 2003 version, www.graphpad.com).

RESULTS AND DISCUSSION

Apparent clinical signs began to manifest on day 4 post-infection (PI), with initial morbidity of 9 out of 20 (45%). The clinical signs were inappetence, droopiness, depression from day 4 PI and greenish diarrhea from day 5 PI. One out of 20 (5%) manifested signs of sneezing on day 5 PI, wing and leg paralysis on day 6 PI, while 2 out of 20 (10%) showed signs of rales and torticollis on days 7 and 14 PI, respectively. Clinical signs of malaise (depression and droopiness) and disturbance of the digestive system (inappetence and diarrhea) occurred more frequently (17/20; 85%) than nervous signs (paralysis, 1-2/20; 5-10%) and respiratory signs (sneezing and rales, 1-2/20; 5-10%) (P<0.05). Morbidity was 100% including stunting and other clinical signs. The mortality started on day 5 PI (1/20; 5%), and most of the deaths (15/20; 75%) occurred on days 5-10 PI. A cumulative mortality rate of 80% was recorded from days 18-21 PI. The survival rate was 20%, and surviving birds were alive up to maturity, as did the control birds. The incubation period after infection was short (4 days) and agreed with previous reports where similar clinical signs occurred at comparable time with associated viral isolation and shedding (Brown et al., 1999; Susta et al., 2011). NDV had been reported to cause noticeable edematous and necrotic lesions in the eyelid after intraocular viral inoculation due to inflammation at the site of inoculation (Brown et al., 1999), but no swelling was noticed around the eyes in this study.

The mean live body weights (MBW) of infected and uninfected birds are presented in **Table 1**. Uninfected birds (control) had significant (P<0.05) increases in MBW of 59.6%, 147.0% and 181.9% on days 7, 14 and 21 PI, respectively, when compared to MBW on day 0 PI. However, MBW of the infected birds did not show any significant (P>0.05) increase from MBW on day 0 pi; instead, there was significant (P<0.05) decrease in MBW (7.4%) on day 7 PI compared to MBWs on day 0. No weight gain occurred during infection and the control birds were 1.7-2.3 times heavier than infected ones. Although the infected cockerels that survived the infection after day 21 PI did not show other clinical signs, they remained stunted having failed to gain weight compared to the control. The absence of body weight gain or loss of body weight was a feature of the velogenic NDV infection that was frequently reported (Oladele et al., 2008a; Ezema et al., 2009; Kapczynski et al., 2013; Igwe et al., 2013, 2014; Eze et al., 2014a,b). Birds vaccinated with LaSota vaccine lose weight after velogenic NDV infection, even if they show no other clinical sign (Ezema et al., 2009). When the infection occurs early in life with recovery from clinical signs after live virus vaccination, birds grow slowly and are usually stunted (Dai et al., 2014). Similarly, the birds that recovered from this infection remained underweight and stunted.

Table 1. Body weights (g) of exotic commercial cockerels experimentally infected with velogenic Newcastle disease virus compared to the non-infected group.

Days PI	Control	Infected
0	188.0±16.9^{a} (n=10)	216.0±24.8^{a} (n=20)
7	300.0±55.4^{a} (n=10)	179.1±37.3^{b} (n=11)
14	465.0±74.1^{a} (n=10)	200.0±38.1^{b} (n=5)
21	530.0±25.8^{a} (n=10)	250.0±24.5^{b} (n=4)

PI= post infection; a,b, Mean±Standard deviation with different superscript alphabets are significantly (P<0.05) different.

Table 2. Rectal temperature (°C) of exotic commercial cockerels experimentally infected (IN) with velogenic Newcastle disease virus compared to the non-infected (NI) group.

Days PI	Control	Infected
0	41.46±0.08^{a} (41.30-41.60) n=10	41.49±0.09^{a} (41.40-41.60) n=20
2	41.50±0.07^{a} (41.40-41.60) n=10	41.75±0.14^{b} (41.50-42.0) n=20
4	41.52±0.08^{a} (41.40-41.60) n=10	42.57±0.39^{b} (41.70-43.0) n=20
7	41.62±0.10^{a} (41.50-41.80) n=10	38.86±4.80^{a} (32.0-42.50) n=10
14	41.51±0.07^{a} (41.40-41.60) n=10	40.14±3.63^{a} (32.0-41.90) n=7
21	41.50±0.07^{a} (41.40-41.60) n=10	41.70±0.18^{a} (41.50-41.90) n=4

PI= post infection; a, b, Means±Standard deviations with different superscripts are significant (P<0.05). Range in parenthesis n, Number of birds

The mean rectal temperatures (RT) of the infected and control cockerels are presented in **Table 2**. The increase in the mean RT due to infection was first detected on day 2 PI. The mean values were significantly (P<0.05) higher in the infected than the uninfected (control) on days 2-4 PI. Some birds (n=5/20; 25%) in the infected group had RT up to 43°C on day 4 PI. On day 7 PI, some cockerels in the infected group were hypothermic with about 3 out of 10 (30%) having RT of 32°C, and on day 14 PI, hypothermia was recorded in 2 out of 7

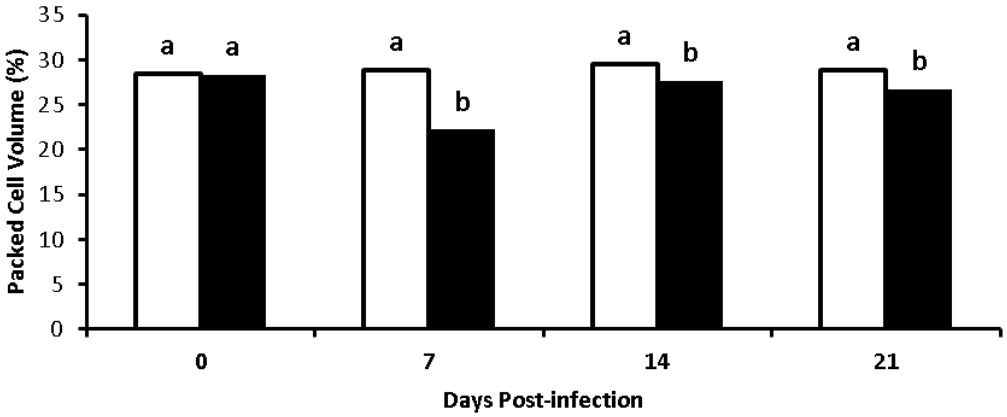

Figure 1. Packed cell volume (%) of exotic commercial cockerels infected (■) with velogenic Newcastle disease virus compared with the non-infected (□) group. Different letters on the bars indicate significant (P<0.05) differences on various days post-infection.

Table 3. Geometric mean titer (GMT) of hemagglutination inhibition antibody in exotic commercial cockerels experimentally infected with velogenic Newcastle disease virus compared with the non-infected.

Days P I	Control	Infected
0	2.4±1.6^{a} (n=10)	1.7±1.2^{a} (n=15)
7	2.0±1.3^{a} (n=9)	75.0±2.1^{d} (n=9)
14	6.5±2.1^{b} (n=10)	1,504.0±3.2^{e} (n=4)
21	32.0±1.9^{c} (n=7)	1,218.0±3.2^{f} (n=4)

PI= post infection; a-f, Means±Standard deviations with different superscripts along the same columns and rows are significantly (P<0.05) different.

Table 4. Frequencies of occurrence of gross lesions in commercial cockerels experimentally infected with velogenic Newcastle virus in the population involved.

Organs affected	Lesions	Number (%) affected
Lung	Congestion, edema	2/16 (12.5)^{b}
Spleen	Congestion, petechiae and enlargement	1/16 (6.3)^{b}
Cecal tonsils	Ecchymoses and necrosis	9/16 (56.3)^{a}
Proventriculus	Ecchymotic and petechial hemorrhages on glands	9/16 (56.3)^{a}
Intestine	Hemorrhages	5/16 (31.3)^{ab}
Liver	Congestion, pale streaked areas	3/16 (18.8)^{ab}
Kidney	Congestion, enlargement, urate deposit in ureters	5/16 (31.3)^{ab}

a,b, values with different superscripts vary significantly (P<0.05).

(28.6%) cockerels. On day 21 PI, no bird was hypothermic in the infected group and the mean RTs were within control value. The hyperthermia (increased RT) which occurred 2-4 days PI coincided with the period when cytokine expression was expected to be strong and would contribute to inducing fever (Ackerman, 2012; Rasoli et al., 2014). Increased RT occurred on days 1-13 PI in chickens infected with velogenic NDV and HI antibody titer increased on days 3-9 PI with positive correlation of RT with antibody titer (Oladele et al., 2005). In this study, antibody response was not estimated before day 7 PI and we could not determine whether RT correlated with antibody response, but the report of Oladele et al. (2005) supported the contention that the elevation of RT was associated with immune response to NDV. Hyperthermia was reported in NDV infections, which were subsequently followed by hypothermia in the period preceding death of affected birds (Kapczynski et al., 2013). Some of the infected birds, in this study, had reduced RT compared to the reference lower limit (41.3^{o}C) for control birds, indicating a tendency to hypothermic state which could be associated with cardiovascular insufficiency and emerging circulatory failure observed in shock. Hypovolaemic shock might have arisen from fluid loss due to profuse diarrhea observed in the disease. The existence of electrolyte and acid-base imbalances, probably, could negatively affect cardiac function and contribute to cardiogenic shock which was suggested by reports of altered electrocardiogram and apoptosis of cardiac myocytes in chickens infected with velogenic NDV (Lam, 1996).

The mean PCV of the infected and non-infected groups are presented in **Figure 1**. The PCV of the infected group showed significant (P<0.05) decrease on days 7, 14, and 21 PI when compared with the mean value of day 0 and mean values of non-infected groups on the various days PI. Decreased PCV in infected birds indicated that erythrocytes were being eliminated from

the circulation and similar observations were reported in birds challenged by virulent live NDV infection or vaccination (Oladele et al., 2002, 2006, 2008a, 2008b). In other studies, no significant variation occurred in the PCV of chickens after infection with velogenic NDV (Rwuaan et al., 2009; Igwe et al., 2013). The PCV variation during infection depends on the NDV strain and pathogenic characteristics. The NDV strain that reduced PCV values were reported to express neuraminidase activity and therefore increased serum neuraminidase activity and serum free sialic acid concentration and decreased erythrocyte surface sialic acid concentration in infected birds (Oladele et al., 2002, 2006, 2008a). Sialic acid cleavage from the surface of erythrocytes exposes receptor sites for macrophage surface binding and subsequent phagocytosis of erythrocytes. Desialylation of erythrocyte membrane results in exposure of naïve antigenic sites for adaptive antibody response which leads to elimination of erythrocytes carrying antibodies or immune complexes on their membrane surfaces by macrophages. Activation and expansion of splenic macrophages by NDV infection increases nitric oxide production because of expression of inducible nitric oxide synthetase (Kapczynski et al., 2013). Apoptosis of erythrocytes may be driven by cytokines, nitric oxide and hyperthermia after which the erythrocytes are phagocytosed because of membrane surface alteration by lipid leaflet scrambling and phosphatidylserine exposure (Lang et al., 2012).

GMT of HI antibody in NDV infected and control groups of cockerels are presented in **Table 3**. On day 0, GMTs of the infected and uninfected birds were comparable. The control group had significant (P<0.05) increases in GMT on days 14 and 21 PI when compared to value on day 0. The infected cockerels had significant (P<0.05) increases in GMT than the control group on days 7, 14 and 21 PI. There was a significant (P<0.05) decline in the GMT on day 21 PI compared with the value on day 14 PI in the infected group indicating that the peak titer occurred on day 14 PI. GMT of infected birds when compared with those of control birds increased by 37.5, 231.4 and 38.0 folds on days 7, 14 and 21 PI, respectively. The infected birds were not vaccinated and had negligible antibody titer that could inhibit viral replication. Before infection, there was low antibody titer from maternal transfer and non-infected birds showed marginal seroconversion from possible environmental exposure to endemic NDV strain.

The gross lesions in infected bird were moderate congestion and edema of the lungs. Liver showed moderate to marked congestion with some pale streaked areas. The splenic lesions included moderate swelling, paleness and multifocal petechial hemorrhages. Kidneys had marked congestion with urate deposit in the ureters. Proventriculus showed moderate to marked glandular petechial and ecchymotic hemorrhages with few necrotic areas. Intestinal lesions were characterized by marked widespread ecchymotic hemorhages on the mucosae, and focal ulcers covered with diphtheritic membrane. Cecal tonsils were raised, hemorrhagic and necrotic. The frequencies of occurrence of gross lesions in the organs are summarized in **Table 4**. Lesions occurred most frequently in the proventriculus and cecal tonsils. There was no significant variation (P>0.05) in the frequencies of lesion in the intestines, kidneys, lungs, liver and spleen. The frequencies were significantly (P<0.05) lower in the lungs and spleens than the proventriculus and cecal tonsils. No gross lesion was observed in the brain. Microscopic lesions in infected birds included multifocal neuronal degeneration with satellitosis in the cerebral cortex; pulmonary congestion and thickening of the interbronchiolar septae by edema fluid; congestion of hepatic sinusoids with perivascular lymphocytic infiltration in portal triads and marked peri-portal hepatocellular necrosis; mild to marked lymphocyte depopulation of the cecal tonsils and splenic white pulps; marked renal tubular necrosis, deposition of proteinaceous materials in renal intratubular lumen, glomerular basement membranes and capsular spaces; proventricular multifocal congestion and epithelial necrosis and diffuse hemorrhages into the intestinal mucosae. The most consistent gross lesions occurred in the digestive tract, particularly the proventriculus and cecal tonsils, but the virus had the capacity to cause some damage to most visceral organs. It was presumed that after the infection had established at the inoculation site by viral replication in mucosal epithelial cells and submucosal lymphoid tissues, the virus had spread to other locations through the blood during viremia.

Although the velogenic NDV was mainly viscerotropic, it manifested a minor neurotropic predilection in chickens preceded by respiratory distress due to pulmonary congestion and edema. Birds inoculated via the intraocular (conjunctival) route had minimal detection of the virus in the brain, lungs and trachea (Brown et al., 1999). The viral spread to the brain could not have been from the blood after viremia because of the blood-brain barrier, but it was presumed that

hypoxic tendency elicited by drop in PCV, respiratory difficulty associated with rales and hyperthermia might have weakened the barrier to allow sipping of viral particles into brain tissues (Natah et al., 2009). It was also anticipated that vascular damage by cytotoxic mechanisms, apoptosis and autophagy (Sun et al., 2014) might have facilitated the infection of the central nervous system. Neuronal necrosis with satellitosis ought to have induced the torticollis as reported, but non-suppurative menigoencephalitis and encephalitis were often reported in neurotropic velogenic NDV infections (Agoha et al., 1992; Brown et al., 1999; Ecco et al., 2011).

Humoral immune (HI antibody) response to NDV was associated with hyperthermia, decreased PCV, stunted growth with lack of weight gain, tissue inflammatory reactions and necrosis in affected organs. These pathological features of the infection were arising from pathogenic mechanisms in ND initiated by interaction of the virus with host cells (Brown et al., 1999). NDV elicits innate and adaptive immune responses in the host and ND pathogenesis seems to be mediated by the biologic effects of these responses (Ecco et al. 2011; Rue et al. 2011; Hu et al., 2012; Kapczynski et al., 2013; Rasoli et al., 2014). Virulent NDV stimulates robust production of cytokines (interferons, interleukins, and tumor necrosis factor), chemokines and nitric oxide by host cells which have been suggested to be deleterious and could be responsible for pathological effects of the disease (Rue et al., 2011; Kapczynski et al., 2013). The cytokines engage in antiviral activities and promote adaptive immune responses, but could trigger adverse effects which include tissue necrosis and inflammation, fever, anorexia, weight loss and depression (Ackerman, 2012). Apoptosis of lymphocytes induced by NDV or mediated by cytokines has been reported in peripheral blood and lymphoid tissues and partly contributes to lymphocyte depopulation in infected birds (Lam and Vasconcelos, 1994; Harrison et al., 2011). The serum HI antibodies (IgM, IgY and IgA) were reported to be produced from day 6 PI reaching a peak at 21-28 days PI (Jeurissen et al., 2000; Al-Garib et al., 2003), but our infected birds had peak mean antibody titer on day 14 PI. The antibodies bind and neutralize the viral particles in order to prevent viral shedding and infection of new cells (Al-Garib et al., 2003). As the antibody titer increased with progression of period of infection, the capacity of the NDV-infected bird to eliminate the virus was expected to increase and the formation of immune complexes would interact with host's cells and complement proteins, increase macrophage, lymphocyte and heterophil recruitment in affected organs and stimulate production of chemical mediators which cause inflammatory responses and necrosis. Virulent velogenic NDV was reported to elicit robust cell-mediated immune response even earlier than humoral immune response, commencing about 2-3 days PI with magnitude and duration of response being enhanced by increasing virulence (Reynolds and Maraqa, 2000). Although cell-mediated immunity probably contributes to decreasing viral shedding by direct killing of infected cells, necrosis of cells from dysregulation of such immune response may play a role in inducing some necrotic and inflammatory lesions (Kapczynski et al., 2013).

The reason for the loss of muscle mass and inability to add muscle mass during NDV infection may usually not be considered beyond apparent anorexia, inadequate feed intake and nutrient loss due to enteropathy and nephropathy which have been observed in this study. The role of pro-inflammatory cytokines and alterations in protein, energy and lipid metabolism in the weight gain deficit is yet to be studied, but these factors are associated with diseases eliciting acute-phase responses and certain cytokine expressions (Kotler, 2000; Delano and Moldawer, 2006). Metabolic acidosis probably caused by diarrhea and renal insufficiency due to the lesions in the kidney may be responsible for degradation of essential amino acids and proteins (Kotler, 2000). Hepatocellular necrosis was observed in the study and it was likely contributing to the metabolic derangement leading to loss of body weight. Inability to gain optimal body weight after recovery from infection could be associated with delayed inflammatory healing and slow decay of circulating catabolic cytokines.

Overall mortality due to the infection was 80%, but 75% of the mortality occurred on days 5-10 PI before the peak of the HI antibody response on day 14 PI. The period of mortality coincided with intense virus-host interaction to produce antibodies and other immune molecules, which probably contributed to the pathogenesis of the disease. The humoral response was paramount in resolution of the disease process as mortality abated and birds developed tremendous antibody titer with an increase of 231.4 fold to eliminate viral infectivity and build resistance to infection. It seemed that the degree of antibody response at peak response had a role in the survivability of the birds as no mortality occurred from day 18 PI. Recovery from infection afterwards was guaranteed by sustained protective antibody titer with the likelihood that antiviral cytokines were also in circulation. Early

expression of interferon gamma (IFN-γ) was reported to have a significant protective role against the effects of highly virulent NDV infections in chickens (Susta et al., 2013).

CONCLUSION

Nigerian strain of viscerotropic velogenic Newcaste disease virus (NDV) used to infect young naïve chickens through intraocular route can cause a disease that has a minor neurotropic component, but can be associated mainly with viscerotropic lesions, hyperthermia, lack of body weight gain, decreased PCV, and intense HI antibody response. Cell membrane desialylation by viral neuraminidase during viremia and immune-mediated cytotoxicity and inflammation concurrent with the antibody response in the virus-host interaction seem to be the pathogenic basis of the disease. Recovery from disease may involve neutralization of virus, inflammatory resolution and decay ofpro-inflammatory immune molecules.

CONFLICT OF INTERESTS

The authors declare that they have no conflict of interests.

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