Pathological Characterization in Chickens of a Velogenic Newcastle Disease Virus Isolated from Guinea Fowl

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Key words

Chicken - Cockerel - Newcastle disease virus - Experimental infection - Nigeria.

Summary

A flock of 160 six-week-old Harco cockerels was inoculated intramuscularly with a local Nigerian isolate of velogenic Newcastle disease virus (NDV) isolated from a dead guinea fowl. The birds came down with clinical signs on day 3 postinoculation (PI). The major signs were depression, greenish diarrhea, paralysis, opisthotonus and torticollis. Morbidity was 100% but mortality was 92%. By day 18 PI torticollis was the only sign persisting in some of the birds. The major gross lesions were hemorrhages in the proventricular mucosa, hemorrhagic ulcers in the intestines and transient atrophy of the lymphoid organs. Sections of the organs showed lymphocytic necrosis and depletion of the lymphoid organs, endotheliosis, gliosis and perivascular cuffing of the cerebrum and cerebellum. The above observations showed that the isolate was a viscerotropic velogenic strain. It is suggested that the hemorrhagic ulcers in the intestines could be regarded as diagnostic for viscerotropic velogenic NDV in the absence of epizootiological evidence of avian influenza.

■ INTRODUCTION

The velogenic Newcastle disease (ND) is a major disease problem of poultry birds in Africa and Asia (3, 22). The exotic chickens used in commercial poultry production in these places are routinely vaccinated against ND. Outbreaks of velogenic ND occur nevertheless frequently in these flocks. But the clinical signs and lesions in such outbreaks are often modified by the partial immune status of these birds that do not manifest the full or classical disease. Village or rural poultry chickens constitute 70 to 94% of the total poultry population (22) of these two continents. These data are changing with the latest developments in modern poultry production. Village chickens are not usually vaccinated against ND and other diseases. Consequently, velogenic ND wipes out large populations of these birds in raging seasonal epizootics that occur annually (1, 9). Other diseases such as intestinal parasitism, poor nutrition and immunosuppression, and harsh environmental conditions could exacerbate the severity of the disease in village chickens. Reports of isolation of velogenic ND virus (NDV) from several outbreaks of ND and even from apparently healthy birds have been common (1, 3, 6). But studies of the sequential pathogenesis of velogenic ND under experimentally controlled conditions have been limited. Pathognomonic lesions have not been identified and the disease can be confused with other diseases. This paper describes the systematic pathogenesis of ND produced in chickens with a local Nigerian isolate of the velogenic NDV isolated from guinea fowl. It also attempts to identify specific lesions that could be very useful in the field diagnosis of the disease.

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■ MATERIALS AND METHODS

Chickens

Two hundred and forty Harco cockerels were collected at one day of age. They were not vaccinated against any disease. Brooding and rearing were performed by the deep litter system. Water and feed were supplied *ad libitum.*

Newcastle Disease Virus Inoculum

The velogenic NDV isolate used was the VGF-1 characterized by Echeonwu *et al.* (9). The virus was isolated from a dead guinea fowl in Vom, Plateau State of Nigeria. The inoculum was kindly supplied by G.O.N. Echeonwu of the National Veterinary Research Institute, Vom, Nigeria.

Newcastle Disease Virus Challenge

At six weeks of age the 240 chickens were divided into two groups, one with 160 birds and the other with 80 birds. The inoculum was diluted with phosphate buffered saline (PBS) to give a median embryo lethal dose (ELD50) titer of $10^{6.36}$ per milliliter. Each chicken in the 160 birds' group was inoculated intramuscularly (IM) with 0.2 ml of the inoculum (infected group) while each bird in the other group received 0.2 ml of PBS IM (uninfected group). The two groups were housed in separate locations about half a kilometer apart.

Clinical and Pathological Examinations

Both groups of birds were observed daily for clinical signs. At days 0, 14 and 20 postinoculation (PI), ten chickens were randomly collected in each group and weighed. Three chickens were sacrificed in each group daily from day 3 PI for 12 days, and thereafter at two days' intervals until day 20 PI. The birds along with the dead ones were examined for gross lesions. Samples of the brain, thymus, proventriculus, bursa of Fabricius, kidney, spleen, cecal tonsils, intestine trachea, liver and heart were fixed in 10% formal saline, processed, embedded in paraffin wax and sectioned. They were stained with hematoxylin and eosin and examined under the light microscope.

Serology

Blood samples were collected from ten birds in each group on days 0, 7 and 20 PI. Sera were stored at -20°C for a few weeks. NDV hemagglutination inhibition (HI) antibody quantification was done using the hemagglutination (HA) and HI procedures of Beard (4). The sera were inactivated by heating at 56°C for 30 min in water bath. The antigen used for the HI test was a PBS suspension of LaSota NDV vaccine which had 10 HA units.

Statistical Analysis

The significance of difference between means was statistically analyzed using Student t-test.

■ RESULTS

Clinical Signs

On day 3 PI birds in the infected group came down with dullness, ruffled feathers, drop in feed and water consumption, and droopy wings. Some tucked their heads under their wings. Head shaking, paralysis of the legs and wings, jerking of the head downward and upward and greenish diarrhea appeared on day 4 PI. Morbidity was 100%. Six chickens showed torticollis on day 7 PI and the number increased to ten the next day. Weight loss was significant in the infected chickens on days 14 and 20 PI ($P < 0.5$) (Table I). Mortality was first observed on day 5 PI and 14 birds were involved. Peak mortality occurred on days 6 and 7 PI and involved 30 and 28 birds, respectively. Only two birds died on day 13 PI which was the last day of mortality. Total mortality was 92% excluding the sacrificed birds. Improvement in the clinical signs was observed on day 10 PI. By day 18 PI only torticollis persisted in some birds. The uninfected group presented no clinical signs.

Gross Lesions

Muscles of the breast, leg and thigh were congested. Hemorrhages were observed on the proventricular mucosa while intestines showed catarrhal or hemorrhagic enteritis. The jejunum and ileum often presented sharply-demarcated button-like hemorrhagic ulcers. These ulcers were evident from their serosal surface. On the mucosa they were often covered by a thin greenish layer of necrotic intestinal tissue. The cecal tonsils were swollen, hemorrhagic and often contained cheesy necrotic material. The thymus was severely atrophic (Figure 1). At a certain stage the tissue was no longer detectable. The spleen and the bursa of Fabricius were also atrophic (Figure 2). But the three lymphoid organs regained their normal sizes later. The spleen was mottled with dark spots on the serosal surface. The kidneys were swollen and hemorrhagic while the liver and the heart were congested. The trachea and the lungs showed no lesion. The distribution and persistence of the lesions are shown in Table II. The uninfected birds showed no gross lesion.

Histopathology

In the spleen, mild to moderate lymphocytic necrosis and depletion were observed on day 3 PI. By day 4 PI these lesions were severe especially around sheathed arterioles. The reticular cells around the sheathed arterioles were hypertrophic. The walls of some arteries were markedly thickened. Deposition of fibrin around the sheathed arterioles was observed on days 6, 7 and 10 PI (Figure 3). Germinal centers (GC) increased in number on day 10 PI when evidence of lymphocytic repopulation was first observed. Complete repopulation almost occurred on day 20 PI with the appearance of numerous GCs.

Mild lymphocytic necrosis was observed in some bursal follicles on day 3 PI. The lesion was moderate and plical epithelium hyperplastic on day 4 PI. There was intra- and interfollicular edema. Depletion of the lymphocytes was severe on day 6 PI (Figure 4) and the hyperplastic epithelium showed numerous folds. The follicles were atrophic on day 7 PI. Evidence of lymphocytic repopulation was found in the medulla on day 10 PI and repopulation was almost complete by day 20 PI when the plical epithelium was also normal.

Thymus and cecal tonsils showed mild lymphocytic necrosis on day 3 PI. The lesion was severe by day 4 PI (Figure 5), but by day 20 PI repopulation was nearly complete with increased number of GCs in the cecal tonsils.

Table I

Mean body weight (g), mortality (%) and hemagglutination inhibition (HI) antibody titers of control and infected chickens

Days postinoculation	0		7		14		20	
	Cont.	Inf.	Cont.	Inf.	Cont.	Inf.	Cont.	Inf.
Body weight	356	355	-	-	487	374*	516.5	434*
Mortality	0	0	0	63	0	92	0	92
HI antibody titers (GMT[1])	2.0	1.9	0.7	13.0*	-	-	0.0	588.1*

* Means with asterisks are significantly different from their controls ($P < 0.5$)
1. Geometrical mean titer

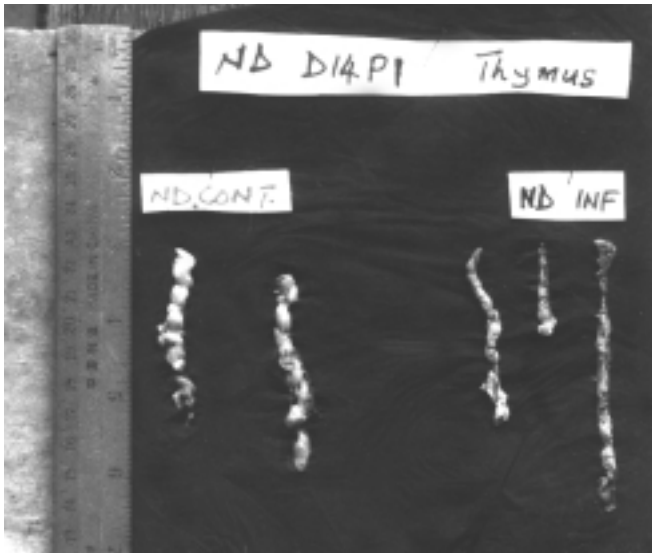

Figure 1: *Atrophy of the thymus in infected chickens on day 14 postinoculation.*

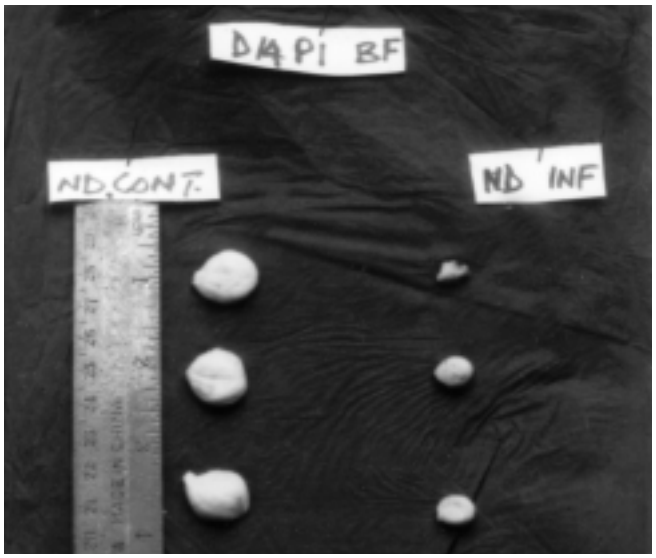

Figure 2: *Atrophy of the bursa in infected chicken on day 14 postinoculation.*

Submucosa edema and ulceration of the mucosa and villi were observed in the intestines on day 4 PI. There was increased ulceration, hemorrhages, congestion and hyperplasia of the goblet and crypt cells on day 6 PI. These persisted up to day 10 PI.

Congestion of the peritubular blood vessels, casts and pyknosis of the tubular epithelial cells (Figure 6) were observed in the kidneys on days 4 to 10 PI. Liver and heart muscles showed congestion and edema up to day 10 PI.

Cerebrum and cerebellum presented submeningeal edema and mild lymphocytic infiltration on day 3 PI. Vacuoles were observed in the gray matter of the cerebellum and the cerebrum. Meningeal edema and lymphocytic infiltration persisted in both organs. Demyelination and degeneration of the Purkinje cells were observed in the cerebellum on day 7 PI. Perivascular cuffing with lymphocytes (Figure 7), edema, congestion, gliosis and endotheliosis (Figure 8) were observed in both organs on day 10 PI. Necrosis of the cerebral parenchyma and markedly thickened arteries were also observed. These lesions persisted up to day 20 PI.

Table II

Frequency and persistence of the gross lesions

Organ	Lesion	Days postinoculation													
		3	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**16**	**18**	**20**
Breast, thigh and leg muscles	Congestion	0[a]/3[b]	2/3	17/17	33/33	31/31	13/13	13/15	11/11	2/2	1/5	1/5	1/3	0/3	0/3	0/3
Proventriculus	Mucosal hemorrhage	1/3	1/3	6/17	10/33	6/31	3/13	2/15	1/11	0/3	0/5	0/5	0/3	0/3	0/3	0/3
Thymus	Atrophy	0/3	2/3	15/17	33/33	31/31	13/13	15/15	11/11	3/3	5/5	5/5	3/3	3/3	1/3	0/3
	Disappearance of the tissue	0/3	0/3	0/17	0/33	0/31	0/13	0/15	11/11	3/3	5/5	5/5	0/3	0/3	0/3	0/3
Bursa	Atrophy	0/3	0/3	4/17	10/33	27/31	13/13	15/15	11/11	3/3	5/5	3/5	3/3	3/3	2/3	0/3
Spleen	Mottling with dark spots	2/3	3/3	14/17	6/33	2/31	1/13	0/15	0/11	0/3	0/5	0/5	0/3	0/3	0/3	0/3
	Atrophy	0/3	0/3	0/17	26/33	28/31	13/13	12/15	11/11	3/3	2/5	1/3	1/3	1/3	1/3	0/3
Kidney	Congestion and enlargement	1/3	2/3	10/17	21/33	30/31	10/13	11/15	7/11	2/3	2/5	1/5	1/3	0/3	0/3	0/3
Intestine	Hemorrhagic ulcer	0/3	1/3	4/17	8/33	7/31	3/13	2/15	1/11	0/3	0/5	0/5	0/3	0/3	0/3	0/3
	Hemorrhagic or catarrhal enteritis	3/3	3/3	14/17	25/33	20/31	5/13	5/15	4/11	3/3	0/5	0/5	0/3	0/3	0/3	0/3
Cecal tonsils	Mucosal hemorrhage	2/3	1/3	6/17	8/33	6/31	2/13	1/15	0/11	0/3	0/5	0/5	0/3	0/3	0/3	0/3

a = Number positive for lesion; b = Total number necropsied

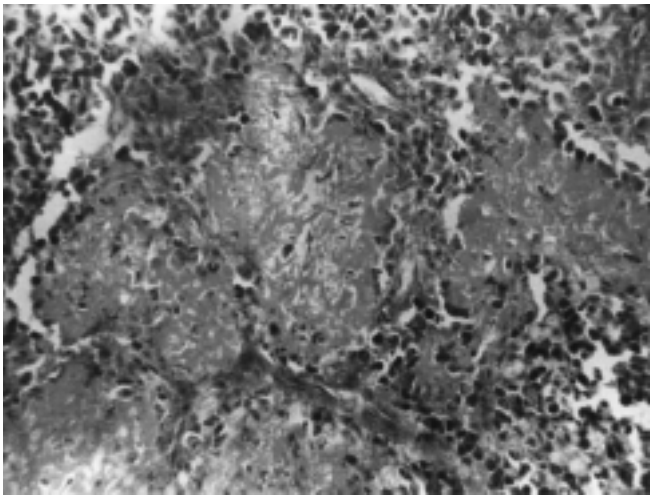

Figure 3: *Spleen showing fibrin deposition around the sheathed arterioles on day 6 postinoculation. (Hematoxylin and eosin x 200)*

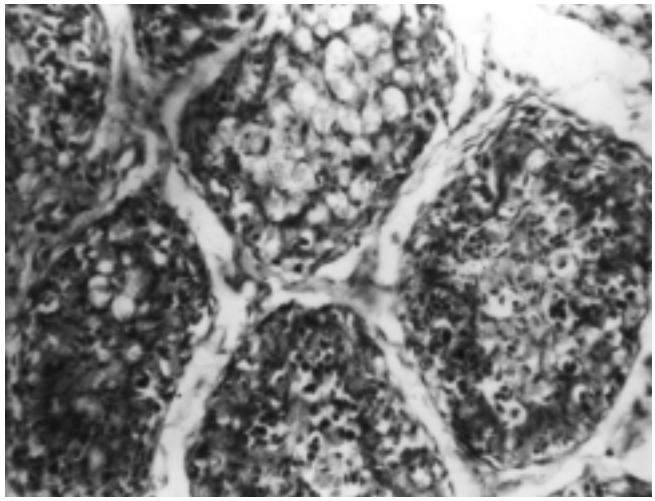

Figure 4: *Bursa showing lymphocytic necrosis, depletion and interfollicular edema on day 6 postinoculation. (Hematoxylin and eosin x 200)*

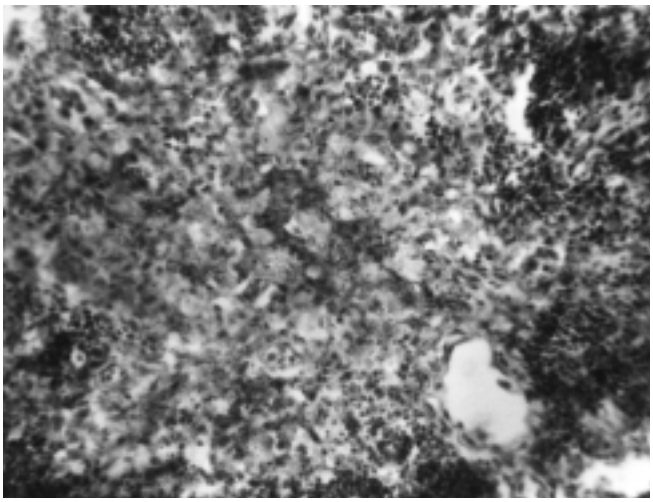

Figure 5: *Thymus showing lymphocytic necrosis, depletion and hyperemia on day 4 postinoculation. (Hematoxylin and eosin x 200)*

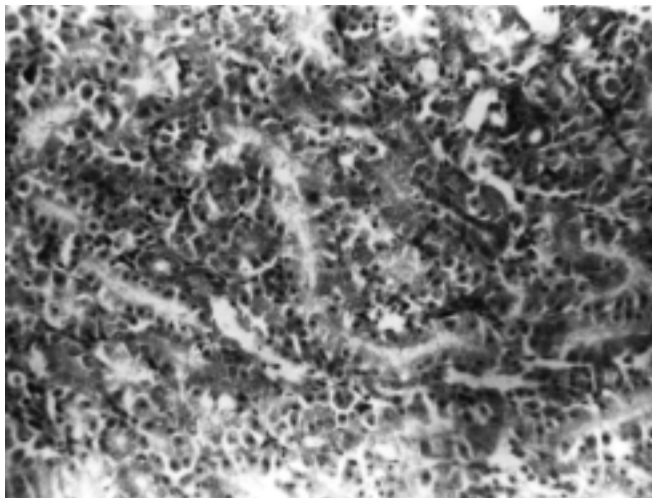

Figure 6: *Kidney showing pyknosis of renal epithelial cells on day 10 postinoculation. (Hematoxylin and eosin x 200)*

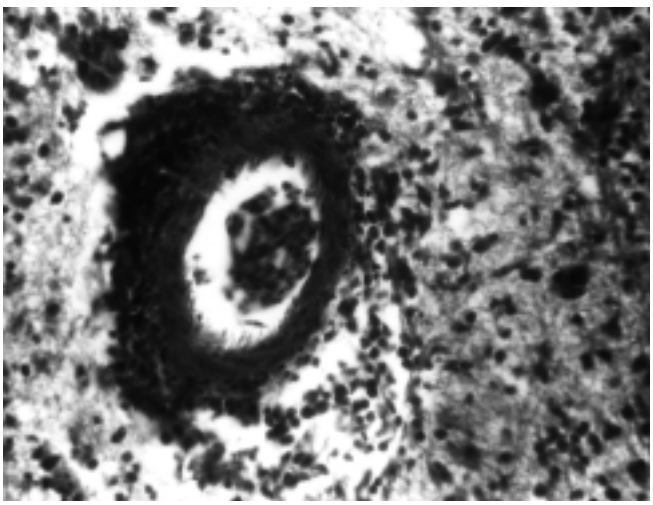

Figure 7: *Cerebrum showing perivascular cuffing with lymphocytes and edema on day 10 postinoculation. (Hematoxylin and eosin x 200)*

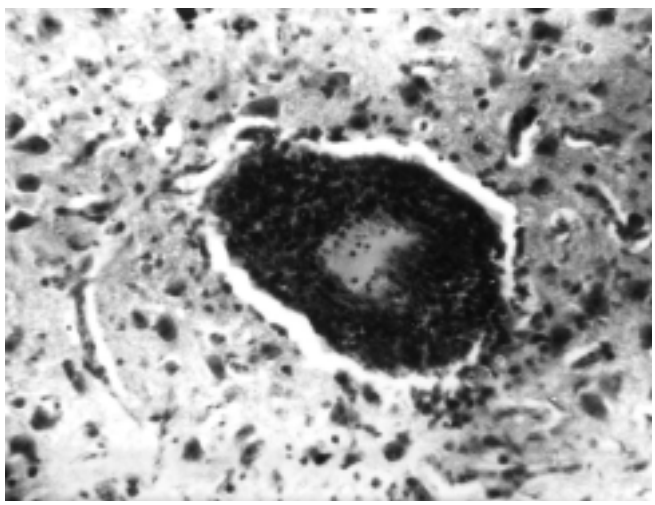

Figure 8: *Cerebrum showing endotheliosis on day 14 postinoculation. (Hematoxylin and eosin x 200)*

Serology

Infected birds showed seroconversion while the uninfected birds did not (Table I).

■ DISCUSSION

The hemorrhages in the proventriculus, intestines and cecal tonsils indicate that the isolate VGF-1 is a velogenic viscerotropic strain of NDV (VVNDV). The nervous signs and lesions were severe, but they affected many less birds than the gastroenteric lesions. The nervous involvement could be due to the IM route used in the inoculation of the virus. Beard and Hanson (5) reported that the IM, intravenous and intracerebral routes appeared to enhance the neurological signs while the natural routes (oral, intraocular and nasal) emphasized respiratory involvement. Most outbreaks of ND in village chickens are transmitted through ingestion of contaminated feed (16). They are characterized by viscerotropic signs such as greenish diarrhea and few nervous signs. The pathotype of the NDV involved in an outbreak appears to be the major factor that determines the form of the disease that is manifested by the birds (2).

The atrophy, lymphocyte depletion in the lymphoid organs and proventricular hemorrhage, observed in this experiment, can make velogenic viscerotropic ND outbreaks in young chickens closely resemble the infectious bursal disease (IBD) in the field. But it should be noted that the enlargement and the massive heterophilic infiltration present in the bursa at the early and acute stages of IBD do not occur in ND. While IBD causes progressive and premature involution of the bursa, ND causes transient atrophy of the organ.

No pathognomonic lesion has been described for ND. But the hemorrhagic button-like ulcers in the intestines do not appear to have been described for any other poultry disease except avian influenza (20). These hemorrhagic-necrotic lesions develop in the lymphoid aggregates of the wall of the intestine. They could be regarded as pathognomonic for ND because severe outbreaks of avian influenza have been rare in the past 20 years (8).

The incubation period in this experiment was three days postinoculation with total mortality of 92%. Hamid *et al.* (11) studied an Indonesian strain of VVNDV and reported incubation periods of 2 to 16 days and 3 to 5 days in 7- and 20-week-old nonimmune chickens, respectively. Mortalities were 85.3 and 100%. Contrary to their observations, our isolate produced neither respiratory signs nor lesions.

The severe clinical signs observed in the present experiment have been described for VVNDV (2, 10, 11, 13). However, Hamid *et al.* (11) did not observe tremor of the head and torticollis contrary to results obtained in this study. The greenish diarrhea and gastrointestinal lesions are in agreement with what has been described for other VVNDV strains (2, 4, 11, 12, 14, 21). Contrary to the present results and those of McFerran and McCracken (15), Hamid *et al.* (11) described gross lesions in the brain of infected chickens. Atrophy of the lymphoid organs has been described for VVNDV infections but reports of such severe thymic atrophy leading to transient disappearance of the organ have been rare (2, 11, 12, 21). The atrophy and the lymphocytic depletion in the thymus, cecal tonsils and spleen were more severe than what is generally observed in IBD (18). However, Hamid *et al.* (11) reported that the bursal lesions were less severe than those of IBD and this is in agreement with the present observations. The acute fibrinoid necrosis of the spleen observed in this experiment has also been described for VVNDV by Riddel (20) and Hamid *et al.* (11). Alexander (2) reported no such lesion. The severe microscopic lesions observed in the brain are not in agreement with the mild changes described by Hamid *et al.* (11). Alexander (2) described no histopathological lesion in the brain while Spradbrow (21) reported that it was difficult to find explanations at the cellular level for the nervous lesions of ND. However, the present observations are in agreement with those of Mayor (17), Riddel (20) and Bhaiyet (7). The severe pyknosis observed in the kidney in our experiment does not appear to have been reported earlier. Lymphocytic repopulation of the lymphoid organs was almost complete by day 20 PI while Hamid *et al.* (11) reported complete repopulation by day 18 PI. The rapid increase of the mean HI antibody titer from 13.0 to 588.1 between days 7 and 20 PI could be due to the advanced repopulation of the organs and the increased number of GCs in the spleen and cecal tonsils by day 20 PI. Payne (19) suggested that antibodies developed in GCs containing memory cells specifically sensitized to the antigen.

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Résumé

Okoye J.O.A., Agu A.O., Chineme C.N., Echeonwu G.O.N. Caractérisation pathologique chez des poulets du virus vélogénique de la maladie de Newcastle isolé chez une pintade

Une bande de 160 coquelets Harco âgés de six semaines ont été inoculés par voie intramusculaire avec un isolat nigérian local de virus vélogénique de la maladie de Newcastle (VMN) obtenu à partir d'une pintade morte. Des signes cliniques de la maladie, principalement la dépression, des diarrhées verdâtres, la paralysie, l'opisthotonos et le torticolis, ont pu être observés sur les volailles trois jours après l'inoculation. Le taux de morbidité a été de 100 p. 100 alors que celui de mortalité a été de 92 p. 100. Au dix-huitième jour après l'inoculation, seul le torticolis a été encore présent chez certaines volailles. Les lésions macroscopiques principales ont été les hémorragies dans les muqueuses proventriculaires, des ulcères hémorragiques des intestins et une atrophie transitoire des organes lymphoïdes. Des coupes d'organes ont révélé des nécroses et des déplétions lymphocytaires des organes lymphoïdes, une endothéliose, une gliose et des manchons périvasculaires du cerveau et du cervelet. Ces observations ont indiqué que l'isolat provenait d'une souche vélogénique viscérotropique. Les auteurs proposent de considérer les ulcères hémorragiques dans les intestins comme des éléments diagnostiques du VMN vélogénique viscérotropique en l'absence de preuve épizootiologique de virus grippal aviaire.

Mot-clés : Poulet - Coquelet - Virus de la maladie de Newcastle - Infection expérimentale - Nigeria.

Resumen

Okoye J.O.A., Agu A.O., Chineme C.N., Echeonwu G.O.N. Caracterización patológica en pollos de un virus rápido de la enfermedad de Newcastle aislado en aves de Guinea

Una parvada de 160 gallos Harco de seis semanas de edad fue inoculada en forma intramuscular con una aislamiento local nigeriano de un virus de la enfermedad de Newcastle (NDV) rápido, aislado a partir de aves muertas. Las aves aparecieron con signos clínicos al día 3 post-inoculación (PI). Los principales signos fueron depresión, diarrea verdosa, parálisis, opistótono y tortícolis. La morbilidad fue de 100%, pero la mortalidad fue de 92%. Al día 18 PI, la tortícolis fue el único signo persistente en algunas de las aves. Las principales lesiones fueron hemorragias en la mucosa pro ventricular, úlceras hemorrágicas en los intestinos y atrofia pasajera de los órganos linfoides. Algunos sectores de los órganos mostraron necrosis linfocitaria y vaciamiento del endotelio de los órganos linfoides, gliosis y pliegues perivasculares del cerebro y cerebelo. Las observaciones anteriores mostraron que el aislamiento fue de una cepa rápida viscerotropica. Se sugiere que las úlceras hemorrágicas en los intestinos podrían utilizarse como diagnostico para los NDV viscerotropicos rápidos en caso de ausencia de evidencia epizootiologica de la influenza aviar.

Palabras clave: Pollo - Gallito - Virus de la enfermedad de Newcastle - Infección experimental - Nigeria.