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Full Length Research

Sequential effects of experimental Newcastle disease virus on performance and severity of clinical manifestation of the disease in three indigenous Nigerian genotypes of chickens

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ABSTRACT: Newcastle disease has been identified as a killer viral infection associated with high case fatality in poultry. This study was conducted to evaluate the sequential effects of experimental Newcastle disease on the performance and severity of clinical manifestation of the disease in three indigenous Nigerian genotypes of chickens. A total of 180 day-old Nigerian indigenous chicks consisting of 60 Naked necks (NN), 60 Frizzle feathers (FF) and 60 Normal feathers (NF) (45 experimental and 15 control per genotype) were used for the study. These chicks had no symptoms of Newcastle disease at the commencement of the experiment. Birds were fed on starter ration (20% CP and 2800 Kcal ME/kg) from day-old to 8th week of age, and growers ration (16% CP and 2750 Kcal ME/kg) from the 9th to 16th week. Fifteen (15) vials of lyophilised Newcastle live virus Kudu 113 strain were obtained from National Veterinary Research Institute, Vom Plateau State, and each vial was diluted with 2 ml of sterile phosphate buffer saline (pH 7.2). Each experimental genotype was inoculated through intra-crop injection with 0.2 ml of the virus in the 5th week. The control group was inoculated 0.2 ml of normal saline. Post infection (PI) observable clinical signs were assessed and live weight changes were determined. Patent Newcastle Disease infection manifested clinical signs included greenish watery diarrhoea, insomnines, ruffled feathers, sneezing and coughing, wherein all of these obvious clinical signs manifested in 90, 31 and 25% of infected NN, NF and FF respectively by day 14 PI. The mortality pattern by day 42 was 94% NF, 100% FF and 79% NN. The study concluded that live-weight changes in the naked neck subsequently increased by day 35 and 42 post infection and the NF had the same sequential result. The study recommended that the NN genotype should be raised in places that are not endemic to Newcastle disease.

Keywords: Clinical manifestation, genotypes, indigenous chickens, Newcastle virus, performance.

INTRODUCTION

Newcastle virus is a highly contagious poultry disease that spreads very fast in a wide range, infecting a large range of poultry species of all levels (Alexander, 2000). Viral infection is a major constraint of avian production in developing societies (Abdu *et al.,* 1986). It has been recorded that the spread of the virus in Nigeria is over 200 to 250 cases per year for all the various species of poultry (Adu *et al.,* 1986).

The Newcastle disease virus (NDV) belongs to the order *Mononegavirales* in the family *Paramyxoviridae,* and genus *Avula virus* with a diverse spread range and genetic diversity, having different virulence within the strains of birds (Amarasinghe *et al.,* 2018).

The symptoms/clinical signs of Newcastle disease virus range from acute virus with 100% mortality to subclinical virus/disease with no lesion (Cattoli *et al.,* 2011). It is characterized by the sudden onset of symptoms/clinical signs such as inappetence, hoarse chirps (in chicks), nasal

discharges, diarrhoea, droopy wings and tail feathers, dullness, dyspnoea or gasping and facial swelling after an incubation period of 2 to 15 days (Sa'idu *et al.,* 2006).

From the period when the first outbreaks occurred in 1926, four panzootics of Newcastle Disease is been documented (Alexander, 2001), and it is possible for another panzootics to occur (Anzaku *et al.,* 2014). Interns of the symptoms or the clinical signs in the infected chickens, five (5) pathotypes of Newcastle Disease Virus isolates have been observed or identified, and they are: mesogenic, viscerotropic velogenic, lentogenic, neurotropic velogenic and asymptomatic. Pathogenicity for birds varies in different dimensions, from asymptomatic (no apparent disease) to severe. In extreme cases, its manifestation as the respiratory and/or neurological disease culminating in 100% mortality of infected herds as well as the flock in the case of the velogenic strain that is very effective.

The survey of the economic possibility or its impact due to Newcastle Disease Virus is not just limited to high rate of mortality recorded from the spread of the virus and the measures taken for the control of the virus (Aldous and Alexander, 2008; Alexander, 2001). The purpose of this research was to determine the sequential effects of the experimental Newcastle Disease Virus on the performance and severity of clinical manifestation of the disease in three indigenous Nigerian genotypes of chickens.

MATERIALS AND METHODS

Experimental site

The experiment was carried out at the College of Veterinary Medicine, the Federal University of Agriculture, Abeokuta located within Odeda Local Government Area of Ogun State, a place where poultry has never been previously raised or reared. It has a prevailing tropical climate with a mean annual rainfall of about 1037 mm. The mean temperature ranges from 28°C in December to 36°C in February with a yearly average of 34°C. Relative humidity ranges from 60% in January to 94% in August with a yearly average of about 82% (Google earth). The Federal University of Agriculture, Abeokuta is located on latitude 7°10'N and longitude 3°21'E in Ogun State, Nigeria (Google earth).

Screening of eggs

To be sure that the chicks inherited immunity to Newcastle Disease Virus from their dam, three eggs from individual strains or genotypes were collected randomly to test if they were carriers of the Newcastle Disease Virus. The individual eggs were taken to the laboratory and the yolk of the egg was dropped inside a Petri dish, and 5 ml syringe was inserted into the middle of the yolk and 1 ml of it was taken into a PBS solution. 1 ml of Phosphate buffer solution (PBS) was taken into bottles and 1 ml of the yolk was added and mixed together, after that, the mixture was centrifuged at 2000 rpm for 10 minutes, the supernatant was then removed and was used for Haemagglutination inhibition test according to the standard method (Allan and Gough, 1974).

Experimental birds and management

The experimental eggs were gotten through artificial insemination of birds on the farm, and the eggs collected were screened with Haemagglutination inhibition to Newcastle disease and after which it was confirmed negative, the eggs were taken to the hatchery of the Animal Breeding and Genetics Department (FUNAAB) for setting. The eggs were labelled with the genotype of their dam for easy identification. After which eggs were arranged into a try for setting. The arranged eggs were candled on the 18th day of setting and hatched chicks were retrieved on the 21st day of setting after which the chicks were all raised under the same management.

Rearing and feeding of the day-old chickens

All chicks were brooded differently according to genotypes in cages and after 4 weeks of age, they were separated based on experimental and control. An acclimatisation period of another one week was allowed. An intensive management system was used to rear the chickens, and water and feed supply *ad-libitum*. The controls were similarly housed in the same vicinity but about 50 to 65 meters away from the infected birds. The birds were fed a starter ration (20% CP and 2800 Kcal ME/kg) from day old to the 8th week of age, and a growers ration (16% CP and 2750 Kcal ME/kg) from the 9th to 16th week of age.

Experimental birds grouping

A total of 180-day old Nigerian local chicks (Normal feather, Naked neck and Frizzle feather) were randomly allocated into three groups based on genotypes containing 60 chicks each, where 45 were for the experimental group and 15 were raised as control respectively, under the same environmental condition.

Newcastle disease virus inoculation and challenge of chickens

Fifteen (15) vials of lyophilised live Newcastle virus Kudu 113 strain were purchased from the National Veterinary Research Institute, Vom, Plateau State, and each vial was

diluted with 2 ml of sterile phosphate buffer saline (pH 7.2). Each chicken was inoculated by crop-intra-crop injection with 0.2 ml of the virus at the 5th week of age. Chickens in the control group were inoculated 0.2 ml of normal saline.

Clinical and performance examination

Initial body weights were taken before the commencement of the experiment and this was done on weekly basis. All the birds both experimental and control were observed twice daily for clinical manifestations and mortality up to 4 weeks post-infection. The presentation of clinical signs were the modifications of the system used by Ogie *et al.* (2012), with (-) indicating no clinical signs; (+) indicates drowsiness and occasional closure of the eyes while (++) indicates closure of the eyes and reluctance to move. Dead chickens were examined for gross lesions (Post mortem), and samples of the spleen, liver, brain, lung, caecum, intestine and kidney were fixed in 10% buffered formalin, processed, embedded in paraffin wax and sectioned. They were stained with haematoxylin and eosin (H and E) and were examined under the light microscope at the magnification of x200.

Mortality (%)

Percentage mortality: this was calculated as the ratio of the number of dead birds to the total number of birds per replicate, expressed as a percentage.

$$\text{Mortality (\%)} = \frac{\text{number of dead birds per replicate}}{\text{Total number of birds stocked per replicate}}$$

Statistical analysis

Data collected were subjected to a one-way analysis of variance using the appropriate least squares method with the genetic group as the main source of variation. Significant means were separated using Duncan's multiple range tests of the Statistical Analysis Software (SAS, 1999).

RESULTS

Table 1 showed body weight changes following experimental Newcastle virus infection on three genotypes of Nigerian indigenous chickens and their control. Day 7 post-infection showed no significant ($p>0.05$) difference in live weight of the normal feather (222.29±17.83), frizzle feather (223.91±29.37) and naked neck (242.62±36.29) when compared with the weight of the genotypes before the infection, although there was a decrease in live-weight in all the genotypes.

However, by day 14 post-infection there was a significant ($p<0.05$) decline in the live weight of normal feather (189.57±12.96) and frizzle feather (194.23±16.53) while in the naked neck (225.24±36.08) there was a decrease though was not significant ($p>0.05$) when compared with other genotypes.

By day 21 and 28 post-infection there was a decline in the live weight in the naked neck while in frizzle feather, 100% mortality was recorded by day 18 post-infection. In the normal feather, the live weight decreased by day 21 post-infection and subsequently increased by days 28 and 35, while in the naked neck the live weight subsequently increased by days 35 and 42 post-infection.

Table 2 revealed the percentage weight losses after experimental Newcastle virus infection in three genotypes of Nigerian indigenous chickens. In the normal feather, 32% of the weight was lost by day 14 while the frizzle feather and naked neck birds lost 20 and 17% respectively. Subsequently, normal feather live weight decreased by 35 and 17% on days 28 and 35 respectively while naked neck weight decreased by 65, 46 and 40% on days 28, 35 and 42 respectively.

Table 3 revealed the case fatality following Newcastle virus infection in three genotypes of Nigerian indigenous chickens. By day 7, 90% of the infected naked necks did not show obvious clinical signs associated with the infection while 31% and 25% also did not show clinical signs of Newcastle virus in normal feather and frizzle feather birds respectively. By day 14, 100% of infected frizzle feathers had developed clinical signs but for normal feathers, only 72% had started showing clinical signs of the virus infection while in the naked neck 71% started showing clinical signs.

Table 4 shows the mortality pattern of three genotypes of Nigerian indigenous chickens following the experimental Newcastle virus infection. The mortality pattern was 34.5% in normal feathers, 59% in frizzle feathers and 0% in the naked neck by day 7. Mortality further increased by 43, 31 and 10% by day 14 in normal feather, frizzle feather and naked neck respectively. By day 18, 100% mortality was recorded in frizzle feathers, while by day 35, 67% (2/3) and 70% (7/10) of normal feather and naked neck respectively. Similarly, by day 42, 100% (9/9) of naked neck and 100% (2/2) of normal feathers survived the infection.

Organs of Nigerian indigenous chickens infected by the Newcastle virus were examined by post-mortem examination of the liver which revealed the appearance of swollen segments as labelled in Plate 1. Plate 2 shows the congestion and presence of pulmonary edema in the lungs due to the infection. Plate 3 shows the appearance of petechial haemorrhages, having tiny pinpoint red marks that are important signs of asphyxia caused by the virus. Plate 4 revealed the presence of dark red and mottled segments as appeared on the heart of the Nigerian local chickens due to the infection. Post-mortem examination of the gizzard revealed appearance of haemorrhages in different parts as shown in plate 5.

Table 1. Live body weight (g) changes of three Nigerian indigenous chickens after experimental Newcastle virus infection.

Genotype	Average weight before infection	Days post infection					
		7	14	21	28	35	42
				Within genotype			
Normal feather							
Infected = 35	230.83±25.48	222.29±17.83[a]	189.57±12.96[b]	182.31±20.57[b]	186.67±32.14[b]	205.00±7.07[b]	-
Control = 15	207.50±10.55	220.00±13.65[a]	233.75±13.33[a]	250.83±19.86[a]	275.42±24.25[a]	293.33±34.72[a]	294.31±24.01[a]
Frizzle feather							
Infected = 32	231.8±32.92	223.91±29.37[a]	194.23±16.53[b]	-	-	-	-
Control = 15	206.07±30.77	211.79±26.21[a]	222.86±30.68[a]	238.93±25.13	260.00±28.82	283.21±29.59	297.86±44.59
Naked neck							
Infected = 42	245.83±40.75	242.62±36.29[a]	225.24±36.08[a]	196.90±32.92[b]	177.17±32.98[b]	196.11±13.86[b]	202.22±24.63[b]
Control = 15	205.67±19.35	222.67±27.31[b]	230.00±28.34[a]	249.87±30.39[a]	258.33±28.64[a]	287.00±29.38[a]	303.33±35.28[a]
				Between genotype			
Normal feather (Infected = 35)	230.83±25.48	222.29±17.83[a]	189.57±12.96[b]	182.31±20.57[b]	186.67±32.14[b]	205.00±7.07[b]	-
Frizzle feather (Infected = 32)	231.8±32.92	223.91±29.37[a]	194.23±16.53[b]	-	-	-	-
Naked neck (Infected = 42)	245.83±40.75	242.62±36.29[a]	225.24±36.08[a]	196.90±32.92[b]	177.17±32.98[b]	196.11±13.86[b]	202.22±24.63[b]

Subscript a, b on same column with different superscript differ significantly (*P<0.05*) from each other.

Table 2. Differences in mean live weight loss in three Nigerian indigenous chickens after experimental Newcastle virus infection.

Genotype	Weight loss by days post infection					
	7	14	21	28	35	42
Normal feather	8%	32%	39%	35%	17%	0
Frizzle feather	7%	29%	0	0	0	0
Naked neck	3%	17%	45%	65%	46%	40%

NF: Normal feather, FF: Frizzle feather, NN: Naked neck.

DISCUSSION

Newcastle virus is a disease that affects poultry globally and its enormous economic benefits, are regarded as a very significant pathogen (Yonash *et al.,* 2001). The disease is able to wipe out a large number of poultry species with various categories of clinical manifestations in different ages of birds. In the present study, there were no losses of weight or mortality in the unchallenged chickens. Clinically, challenged chickens were observed to show vivid depression, reluctance to move,

Table 3. Case fatality of three indigenous Nigerian chicken post experimental Newcastle disease infections.

Breeds of Nigerian local chicken	Case fatality																	
	7 days PI			14 days PI			21 days PI			28 days PI			35 days PI			42 days PI		
	NCS	MOB	MOT	NCS	MOB	MOT	NCS	MOB	MOT	NCS	MOB	MOT	NCS	MOB	MOT	NCS	MOB	MOT
Normal feather =35	31% 11/35	34.5% 12/35	34.5% 12/35	22% 5/23	35% 8/23	43% 10/23	0%	23% 3/13	77% 10/13	0%	100% 3/3	0%	67% 2/3	33% 1/3	0%	100% 2/2	-	-
Frizzle feather=32	22% 7/32	20% 6/32	59% 19/32	0%	69% 9/13	31% 4/13	0%	0%	100% 9/9	0%	-	-	-	-	-	-	-	-
Naked neck=42	90% 38/42	10% 4/42	0%	71% 30/42	19% 8/42	10% 4/42	17% 6/38	49% 17/38	34% 12/38	0%	43% 10/23	57% 13/23	70% 7/10	20% 2/10	10% 1/10	100% 9/9	-	-

NCS: No clinical signs observed, MOB: Morbidity, MOT: Mortality, NF: Normal feather, FF: Frizzle feather, NN: Naked neck.

Table 4. Mortality of three indigenous Nigerian chicken post experimental Newcastle disease infections.

Genotypes	Mortality pattern							
	7 days PI	14 days PI	21 days PI	28 days PI	35 days PI	42 days PI	% Mortality	% Survival
Normal feather (Genotype = 35)	34.5%	43%	77%	0%	0%	0%	94%	6%, 2/35
Frizzle feather (Genotype = 32)	59%	31%	100%	0%	0%	0%	100%	0%
Naked neck (Genotype = 42)	0%	16%	34%	57%	0%	0%	79%, 33/42	11%, 9/42

NF: Normal feather, FF: Frizzle feather, NN: Naked neck, PI: Post infections.

huddling, greenish yellow diarrhoea/droppings, resting on the beak, dropping wings, lack of appetite, oedema, mocoid discharge from the mouth, pecking of floor/base, gasping and making of crack sound before death. Post-mortem examination of dead birds revealed petechiation of the serous mucosa, haemorrhage and necrosis of the mucosa surface, congestion of the lungs and mucoid exudates in the respiratory tract as well as opacity and thickening of the air sac membrane. The beginning of clinical signs on the third and fourth day post infection recorded in this study is in agreement with the findings of Msoffe *et al.* (2002) and Fayeye *et al.* (2006). The variable mortality rates obtained among the three genotypes tend to agree with Akinoluwa *et al.* (2012) who obtained 40, 30 and 70% for Yoruba frizzle, Yoruba naked neck and Yoruba smooth feathered respectively. The relatively higher mortality observed in this study for frizzle feather chickens was higher compared to other genotypes and it does not agree with the earlier report by Akinoluwa *et al.* (2012).

The least mortality observed among the naked neck chickens is in harmony with El-Safty *et al.* (2006) who reported that naked neck have a better ability to secret Acute Phase Protein (APP) by liver cells, which gives protection to the birds against infection or any invasion. The 100% mortality observed among the frizzle feathered chickens in the crop-intra-crop route of inoculation, which is further confirmed by GMT of Haemagglutination antibodies and failure to develop protective immunity in the 21st day of infection is in agreement with OIE (2012) that antibody titre less than Log_2 22 may not be protective and this is probably be the reason why the genotype had highest mortality. The high mortality reported in the frizzled feather genotype was also similar to the report by Bratt and Clavell (1972) when chickens

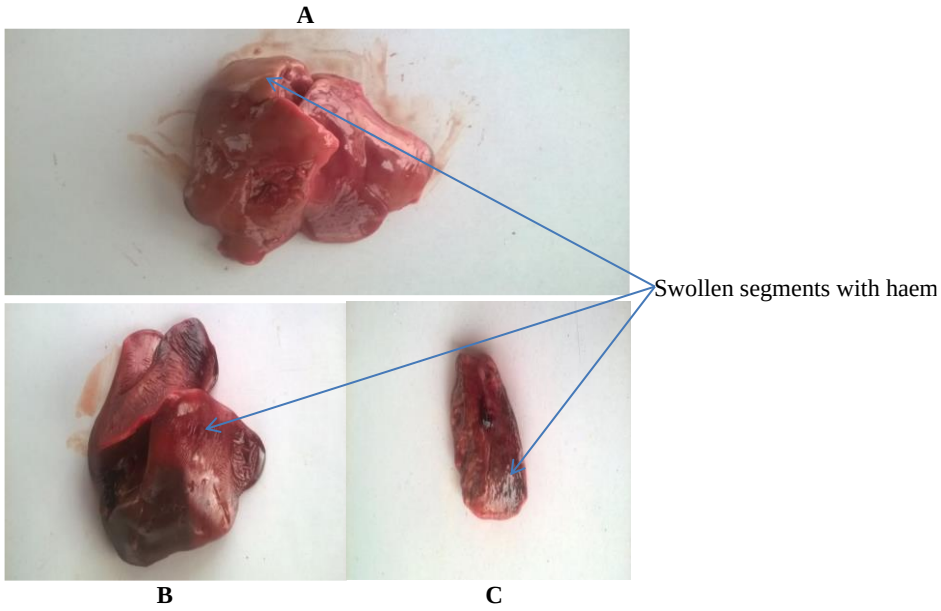

Plate 1. The Liver of the three Nigerian indigenous chickens infected by Newcastle disease **(A:** Normal feather, **B:** Frizzle feather**, C:** Naked neck).

Plate 2. The Lungs of the three Nigerian indigenous chickens infected by Newcastle disease **(A:** Normal feather, **B:** Frizzle feather, **C:** Naked neck).

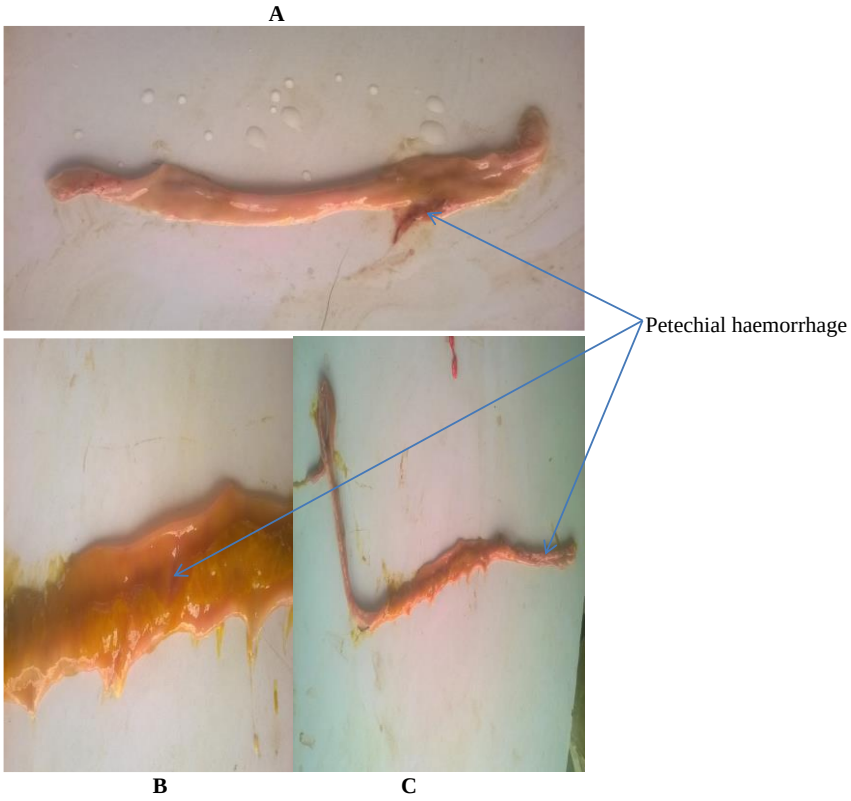

Plate 3. The small intestine of the three Nigerian indigenous chickens infected by Newcastle disease (**A:** Normal feather, **B:** Frizzle feather, **C:** Naked neck).

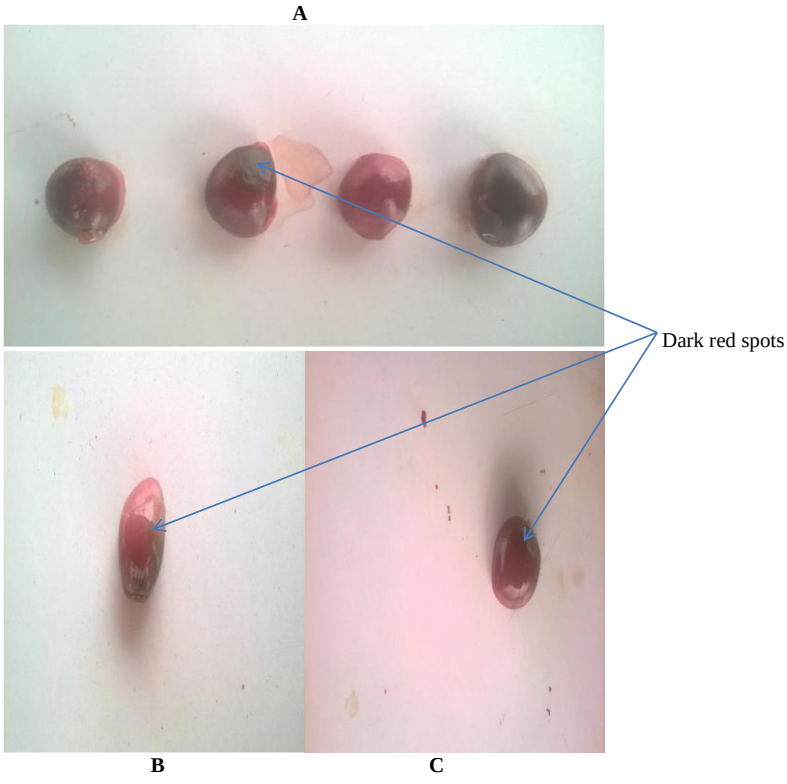

Plate 4. Heart of the three Nigerian indigenous chickens infected by Newcastle disease **(A:** Normal feather, **B:** Frizzle feather, **C:** Naked neck).

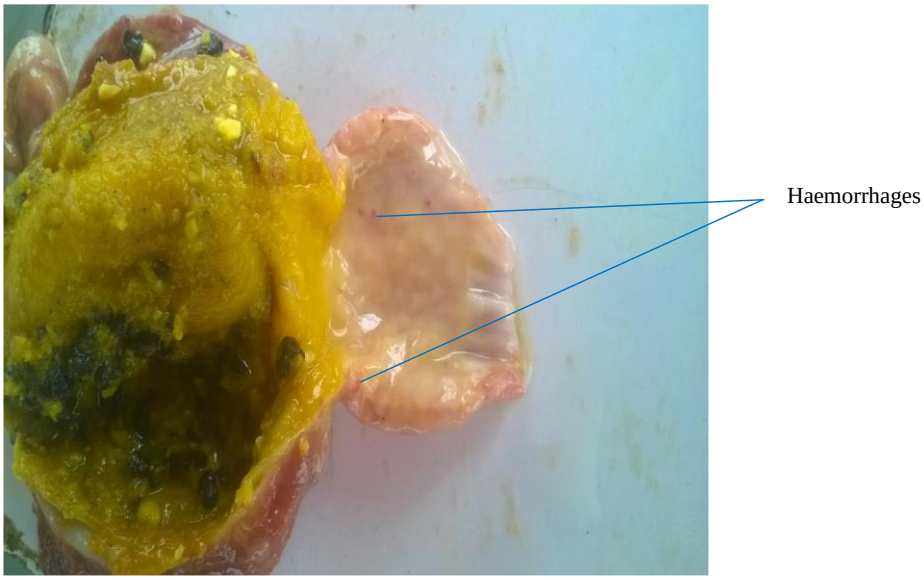

Plate 5. The Gizzard of a chicken infected by Newcastle disease (red spots).

were challenged with the viscerotropic velogenic strain of Newcastle disease virus.

The incubation periods of the virus in three genotypes fell within the range of 2 to 14 days which was in line with the findings of Bratt and Clavell (1972), who reported incubation period of challenged local chickens, to fall within the range of 2 to 15 days. The early stage of morbidity and death in this study suggested that the challenged virus used was highly virulent.

The onset of morbidity and clinical signs of infected birds in this study was similar to that observed by Oladele *et al.* (2005) on exotic chickens (Shaver Brown) infected with a local Nigerian strain of velogenic Newcastle disease virus. Msoffe *et al.* (2002) observed onset of morbidity for Newcastle disease at day 3, onset of mortality at day 5 after inoculation/infection, and mortality rate between day 5 and 7 of 95% in their study on 4 Tanzanian ecotype chickens and this findings is in line with the present research and that of the observations of Alexander (1997), who reported the lack of breed or genetically resistance to Newcastle virus. Hassan *et al.* (2004) however observed that the Mandarah local chickens emerged as a resistant breed with only 20% mortality compared with three other Egyptian chicken ecotypes which had 85-100% mortality which is in agreement with the present study where the NN genotype had the lowest percentage mortality of 20%.

Conclusion

From the results obtained from this study, it could be concluded that:

1. The live-weight changes in the naked neck subsequently increased by day 35 and 42 post-infection and that of the normal feather had the same sequential result.
2. The results revealed that 79, 94 and 100% mortality were obtained for naked neck, normal feather and frizzle feather respectively.
3. For rapid control measures to be adhered to, tracing the different strains in circulation is the first step in achieving the targeted goals. The present method of vaccination in Nigeria and its environment needs to be reviewed to be able to accommodate all the strains of birds.

Recommendations

1. The present study revealed that unvaccinated Nigerian indigenous chicken genotypes possess no natural resistance against the Newcastle disease virus. Vaccination against Newcastle disease is therefore recommended to curb the losses to this disease in Nigerian indigenous chickens.
2. The naked neck Nigerian indigenous chicken had a better ability to secret Acute Phase Protein (APP) by liver cells, which gave the chickens' protection against infection or any invasion.
3. Naked neck chickens can be used to facilitate genetic improvement against Newcastle disease.
4. There is a need for an effective survey of the rural farmers on Newcastle Disease and for the measures in which we can tackle the virus in Nigeria and its environs.

CONFLICTS OF INTEREST

The author declared no conflict of interest.

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