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Host response to successive challenges with lentogenic and velogenic Newcastle disease virus in local chickens of Ghana

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ABSTRACT Newcastle disease (**ND**) is a highly contagious viral disease that constantly threatens poultry production. The velogenic (highly virulent) form of ND inflicts the most damage and can lead to 100% mortality in unvaccinated village chicken flocks. This study sought to characterize responses of local chickens in Ghana after challenging them with lentogenic and velogenic Newcastle disease virus (**NDV**) strains. At 4 wk of age, chicks were challenged with lentogenic NDV. Traits measured were pre- and post-lentogenic infection growth rates (**GR**), viral load at 2 and 6 d post-lentogenic infection (**DPI**), viral clearance rate and antibody levels at 10 DPI. Subsequently, the chickens were naturally exposed to velogenic NDV (**vNDV**) after anti-NDV antibody titers had waned to levels ≤1:1,700. Body weights and blood samples were again collected for analysis. Finally, chickens were euthanized and lesion scores (**LS**) across tissues were recorded. Post-velogenic exposure GR; antibody levels at 21 and 34 days post-velogenic exposure (**DPE**); LS for trachea, proventriculus, intestines, and cecal tonsils; and average LS across tissues were measured. Variance components and heritabilities were estimated for all traits using univariate animal models. Mean pre- and post-lentogenic NDV infection GRs were 6.26 g/day and 7.93 g/day, respectively, but mean post-velogenic NDV exposure GR was −1.96 g/day. Mean lesion scores ranged from 0.52 (trachea) to 1.33 (intestine), with males having significantly higher ($P < 0.05$) lesion scores compared to females. Heritability estimates for the lentogenic NDV trial traits ranged from moderate (0.23) to high (0.55) whereas those for the vNDV natural exposure trial were very low (≤ 0.08). Therefore, in contrast to the vNDV exposure trial, differences in the traits measured in the lentogenic challenge were more affected by genetics and thus selection for these traits may be more feasible compared to those following vNDV exposure. Our results can form the basis for identifying local chickens with improved resilience in the face of NDV infection for selective breeding to improve productivity.

Key words: local chicken, Newcastle disease, lentogenic, velogenic, phenotypic correlation

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INTRODUCTION

Globally, local chickens are a valuable resource for many rural communities, providing sustenance in the form of meat and eggs, as well as manure for fertilizer and income (Nyongolo et al., 2019). In many areas, local chickens are also reared for cultural and social purposes (Padhi, 2016; Manyelo et al., 2020). In Africa, local chickens are relatively resilient in the face of disease and heat stress and, hence, are more adapted to harsh tropical conditions (Msofe, 2003; Fayeye et al., 2011). Their productivity is, however, very low and hampered by poor husbandry practices, predation, and disease, particularly Newcastle disease (**ND**). In Ghana, ND is the primary cause of death, affecting 80% of local chickens annually and can cause up to 100% mortality in unvaccinated flocks (Aboe et al., 2006; Awuni, 2006; Enahoro et al. 2021).

ND is caused by Newcastle disease virus (**NDV**), commonly known as *Avian Paramyxovirus type 1*

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(APMV 1) or *Avian avulavirus 1* (**AAvV 1**), and currently referred to as *Avian Orthoavulavirus 1* (Amarasinghe et al., 2017; Susta et al., 2018). It will be referred to as NDV hereafter. In terms of pathogenicity, there are 3 main categories of NDV strains, namely, lentogenic, mesogenic, and velogenic that result in different clinical forms of the disease (Amarasinghe et al., 2017). The mesogenic strains are moderately lethal with chickens exhibiting moderate respiratory and nervous signs, whereas the lentogenic strains cause clinically mild or unapparent infections of the respiratory tract (Abdolmaleki et al., 2018). Velogenic NDV (**vNDV**) strains are extremely virulent and cause death within days of infection. Some chickens infected with vNDV show severe clinical signs, including torticollis. However, it is not uncommon to have peracute death without clinical signs. Several NDV vaccines, based on lentogenic NDV strains, have been developed in response to ND (Bello et al., 2018). Unfortunately, there are challenges associated with NDV vaccination coverage in local chickens, especially in rural settings, due to a lack of or ineffectiveness of a cold chain to keep vaccines viable, limited veterinary or extension services, or inadequate access routes to rural areas where the population of local chickens is highest. Studies have also shown that vaccination does not entirely prevent viral shedding of NDV infectious particles (Mariappan et al., 2018). Identification and breeding of chickens with enhanced natural resistance to NDV infection and efficacious response to vaccination could therefore facilitate the control of NDV. This study reports the response of Ghanaian local chickens to a challenge with a lentogenic NDV strain (Walugembe et al., 2020) with subsequent exposure to a velogenic (field) strain of the virus, as part of a larger project to breed local chickens for enhanced resilience in the face of NDV infection.

MATERIALS AND METHODS

Experimental Animals

Mature local chickens were sampled from 3 major agro-ecological zones of Ghana corresponding to 3 ecotypes, namely, Coastal Savannah (**CS**), Forest (**FO**), and Interior Savannah (**IS**). The agro-ecological zones have been described by Kayang et al. (2015). These chickens served as the parental breeding stock for the experimental chickens. The breeders were placed into 25 pens per ecotype, each pen with a mating ratio of 1 male to 8 females. Fertile eggs from each pen were collected and hatched, resulting in chicks from ~25 parental half-sib families per ecotype (511 CS, 518 FO, and 411 IS). All animal procedures were approved by the Intuitional Animal Care and Use Committee at the Council for Scientific and Industrial Research, Ghana (RPN 001/CSIR-IACUC/2014) and the Institutional Animal Care and Use Committee at the University of California, Davis (**IACUC** #17853).

Experimental Design

For the LaSota lentogenic NDV challenge trials, four independent replicates that were hatched at different times were conducted consecutively over a period of 8 mo. For each replicate, chicks were housed in a challenge facility in 11 pens, with approximately 50 chicks per pen (N = 557, 567, 487, 418 for replicates 1, 2, 3, and 4, respectively). The challenge facility was an isolated biosecure facility at the Livestock and Poultry Research Center of the University of Ghana, Legon, Accra, Ghana. Chickens had ad libitum access to feed and water. Body weights were measured at hatch and at 7, 14, 21, and 28 d of age (**DOA**). The chickens were then challenged via oculo-nasal route with 10^7 EID_{50} of a live attenuated type B1 LaSota (lentogenic) NDV strain as described by Walugembe et al. (2020). The lentogenic virus was acquired from the Veterinary Services Directorate, Ministry of Food and Agriculture, Accra, Ghana and replicated in specific pathogen free (**SPF**) eggs (VALO BioMedia GmbH, Germany), following the protocol by Grimes (2002). To determine the infectivity titer, a series of 10-fold serial dilutions were prepared and the end point calculated, following Reed and Muench (1938).

Body weights were further taken at 34 DOA corresponding to 6 days postinfection (**DPI**) and at 38 DOA representing 10 DPI. Pre- and post-infection growth rates were calculated in grams per day using linear regression of weight on age. Tear samples were collected from each chicken at 2 and 6 DPI to measure the viral load (Walugembe et al., 2020). To determine the viral load, RNA was isolated from 50 μL of each tear samples using MagMAX-96 viral RNA isolation kit (Life Technologies, Carlsbad, CA) according to the manufacturer's protocol. Each RNA sample was processed for qPCR using the LSI VetMAX NDV realtime PCR kit (Life Technologies), as described by Rowland et al. (2018). The mean viral RNA was transformed to $\log_{10}$ for downstream analysis. Blood samples were also collected at 0 DPI and 10 DPI for antibody analysis (Walugembe et al., 2020). In brief, to determine anti-NDV antibody levels, 2 μL of each serum sample was diluted with 98 μL of diluent and analysed in duplicate via an ELISA using the IDEXX kit (IDEXX Laboratories, Inc., Westbrook, ME), according to the manufacturer's protocol. The mean sample/positive absorbance ratio (S/P ratio) was then calculated and log transformed to $\log_{10}$. At 38 DOA, chickens were relocated to another facility for the vNDV natural exposure field trials.

In order to compare and determine the genetic basis of immune response among ecotypes to velogenic strains of NDV, a vNDV natural exposure field trial was conducted on the same birds that had previously been challenged with lentogenic NDV. The plan was to initiate the trials after the average anti-NDV antibody levels for each replicate had waned to levels at or below 1:1,000 (3.0 $\log_{10}$) in order to prevent neutralization of the virus. However, following monthly screening of antibody levels using ELISA assays (IDEXX Laboratories, Inc.), the average titer levels, although declining, were still above

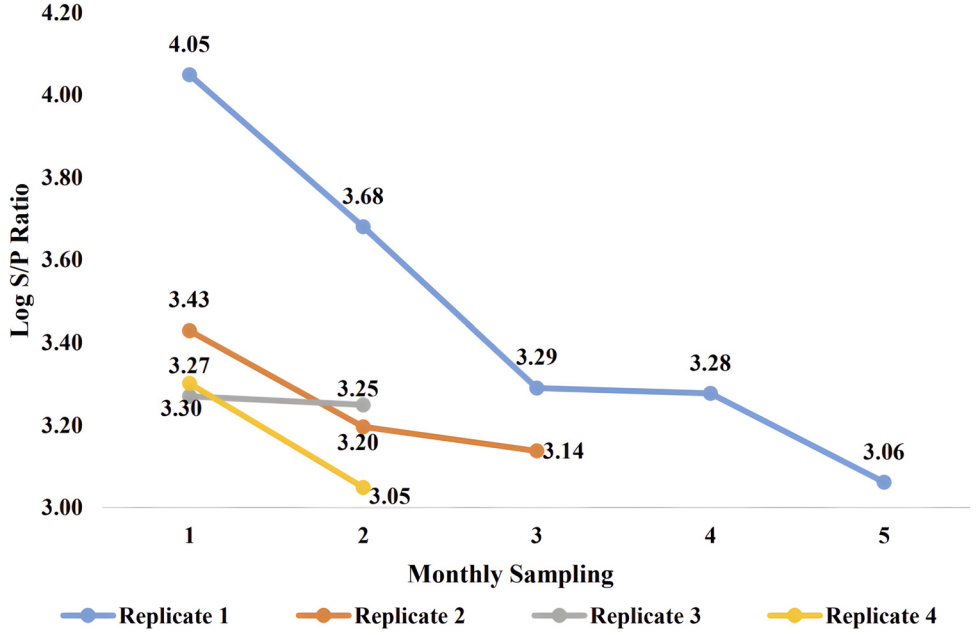

Figure 1. Average monthly antibody titer decline for the four replicates post lentogenic NDV challenge (pre-velogenic NDV exposure). Serum samples were collected from all four replicates post lentogenic trial, over an average of 114 days, for antibody titer analysis until levels dropped to about 3.0 $\log_{10}$ prior to the natural velogenic NDV exposure.

3.0 $\log_{10}$ after an average of 114 d (Figure 1), indicating that the chickens still had some anti-NDV antibodies (Rauw et al., 2009). However, because the chickens were advancing in age, we decided to proceed with the vNDV natural exposure trials (average anti-NDV titers for all 4 replicates was <1,750). The vNDV natural exposure trial was conducted in 2 field trials: trial 1 and trial 2. Trial 1 was carried out on surviving chickens from replicates 1 (n = 487), 2 (n = 447), and 3 (n = 381) of the lentogenic trials, which were 396, 332, and 263 DOA, respectively. At the start of trial 1, surviving chickens from replicate 4 (n = 277) of the lentogenic trial, which were from the latest hatch, still had high levels of anti-NDV antibodies. We therefore had to wait for their antibody levels to wane and include them in trial 2, which started when they were 294 DOA.

The vNDV natural exposure trials were conducted by exposing the chickens to field strains of vNDV circulating in Ghana in a manner that mimics natural exposure and transmission in the field. To initiate these trials, sick chickens exhibiting clinical signs of ND were obtained from local live bird markets or village farms with reported ND outbreaks. Oropharyngeal swabs were obtained from the sick chickens and tested immediately to rule out avian influenza virus and confirm infection with vNDV. The confirmed vNDV infected chickens were then introduced to a flock of 30 naïve chickens (seeder chickens) in a bio-secure enclosure at the Amrahia Dairy Farm for 3 d to increase the number of infected chickens. The seeder chickens, which exhibited clinical signs consistent with ND, were then distributed among the experimental chickens (ratio of 1:50 and 1:16 chickens in trial 1 and trial 2, respectively). To screen the chickens obtained from local live bird markets or village farms for avian influenza virus and NDV, oropharyngeal swabs were obtained and tested immediately to rule out avian influenza virus, using the FluDETECT Avian Influenza Virus Type-A Antigen Test Kit (Zoetis Inc. Kalamazoo, MI), following the manufacturer's protocol. All the samples tested were negative for avian influenza virus (Table 1) and were then used in a qPCR assay to confirm infection with NDV.

Table 1. Avian influenza virus and Newcastle disease virus diagnostic tests.

		Newcastle disease virus	
Sample ID	Avian influenza virus	Matrix gene (M)	Fusion protein gene (F)
Pokuase 1	Negative	Positive	Negative
Pokuase 1	Negative	Positive	Positive
Pokuase 1	Negative	Positive	Positive
Wa 1	Negative	Positive	Positive
Wa 2	Negative	Positive	Positive
Kasoa 1	Negative	Positive	Negative
Kasoa 1	Negative	Positive	Positive
Kumasi 1	Negative	Positive	Negative
Kumasi 2	Negative	Positive	Negative

Only chickens whose samples tested negative for avian influenza and positive for the fusion protein gene of Newcastle disease virus were confirmed to be infected with velogenic Newcastle disease virus and were used in the natural exposure trials.

To confirm the presence of NDV, RNA was isolated as described above and then viral RNA was quantified using the LSI VetMAX NDV real-time PCR kit (Life Technologies), targeting the Matrix (**M**) and Fusion (**F**) genes of NDV in 2 separate qRT-PCR (Spackman et al., 2002; Wise et al., 2004). The M-gene qPCR was performed as described by Rowland et al. (2018). The F-gene qPCR mix was the same as that of the M-gene, except that the primers and probe were replaced with F-gene primers and probe. The following F-gene primers and probe were used: Forward primer 5′ TCCGCAAGATCCAAGGGTCT 3′, Reverse primer 5′ CGCTGTTGCAACCCCAAG 3′ and Probe 5′

(6FAM) AAGCGTTTC TGTCTCCTTCCTCCA-(BHQ1) 3′ (Spackman *et al.*, 2002; Wise *et al.*, 2004). The qPCR was run on a Quantstudio 3 RTqPCR machine (Applied Biosystems by Thermo Fisher Scientific, Marsiling, Singapore), with a reverse transcription step at 45 °C for 10 min, followed by 42 cycles of 95 °C for 5 min, 95 °C for 5 s, and a final extension at 60 °C for 30 s. Chickens whose samples produced amplification for both M and F genes were confirmed to be infected with NDV (Table 1) and were used in the natural exposure trials.

Body weights were recorded on all chickens immediately before exposure to the seeder chickens and then at 3, 7, 11, and 34 days post-exposure (**DPE**) for the vNDV exposure trial 1 and at 6, 13, 21, and 27 DPE for vNDV exposure trial 2. Growth rate was computed by linear regression of weight on days of age. Following exposure, the experimental flock was monitored 3 times a day for mortalities to record the date and time of death. Only 8 chickens died in the velogenic NDV exposure trial, therefore, we did not analyze mortality or survival data. Blood samples were collected at 34 DPE for trial 1 and at 21 DPE for trial 2 to measure antibody levels. The data from these 2 time points were therefore combined as antibody_21 and 34 DPE. The vNDV trial 1 was terminated at 34 DPE and trial 2 at 29 DPE. At termination, all surviving chickens were euthanized, and postmortem examinations were conducted by a veterinarian to score hemorrhagic lesions in the trachea, proventriculus, intestines, and cecal tonsils on a scale ranging from 0 (no lesions) to 4 (extremely severe), as described by Gibson-Corley et al. (2013). Other organs, including the brain, lungs, and spleen were also observed for lesions. Lesion scores were also recorded on chickens that died during the trial.

Statistical Analyses

Best-fit linear mixed models were utilized to analyze the data. Previous admixture analysis based on 32K SNP genotypes of 1,440 chickens revealed that the three Ghanaian ecotypes are not separate breeds, but rather a mixture of 2 ancestral populations (Walugembe et al., 2020). All chickens in the current study had been genotyped for the lentogenic challenge, as described by Walugembe et al. (2020), therefore, population proportions in that study were used as fixed covariates in the models.

To estimate variance components and heritabilities for all traits, we began with univariate animal models that included the fixed effects of replicate, sex, pen, and assay plate (only applicable for the anti-NDV antibody level and viral load), population admixture proportions fitted as covariates, and the random effects of the individual's genetic effect and the effect of its dam or sire. Dams were assigned based on the genomic relationship matrix. The model used was:

$$\boldsymbol{y} = \boldsymbol{X}_1\boldsymbol{b} + \boldsymbol{Z}_{\boldsymbol{a}}\boldsymbol{a} + \boldsymbol{Z}_{\boldsymbol{m}}\boldsymbol{m} + \boldsymbol{e},$$

where $\mathbf{y}$ is the vector of phenotypic measurements; $\mathbf{X_1}$ is the incidence matrix relating the fixed effects and covariates to vector $\mathbf{y}$; $\mathbf{b}$ is the vector of fixed effects and covariates; $\mathbf{Z}_{\boldsymbol{a}}$ is the incidence matrix relating the phenotypic observations to the vector of random chicken genetic effects, $\boldsymbol{a}$, with a genomic relationship matrix to explain the (co)variance among chickens; $\mathbf{Z}_{\boldsymbol{m}}$ is the incidence matrix relating the phenotypic observations to the vector of dam or sire effects, $\boldsymbol{m}$; and $\boldsymbol{e}$ is the vector of random residuals.

The effects of sire, dams, and random residual effects were all assumed to be independent. The significance of fixed effects and covariates for each trait was determined using the REML procedure of the ASREML software version 4.1 (Gilmour et al., 2015). Effects significant at $P < 0.05$ were kept in the model. Significance of the random effects of dam or sire in the models was determined by comparing likelihoods of full and reduced (excluding the random effect being tested) models. Based on this we selected the final model for each trait, and these were used to set up all the pairwise bivariate models to estimate phenotypic and genetic correlations among traits. In addition, we ran bivariate models that only included an overall mean and a random residual. Phenotypic (co)variances were estimated from the bivariate models, from which we calculated phenotypic correlations. Heritability was calculated as a ratio of the estimates of animal genetic variance to phenotypic variance.

RESULTS

Growth Rate

Pre- and post-infection growth rates for the lentogenic NDV (LaSota) trial were similar ($P < 0.05$) for all 4 replicates (Figure 2). Comparatively, the growth rate post-velogenic exposure was significantly lower ($P < 0.05$) than growth rate pre- and post-lentogenic challenge. Replicate had a significant effect on growth rate post-velogenic exposure ($P < 0.05$), with replicate 4 having the highest growth rate. There was also a significant interaction effect of replicate and sex ($P < 0.05$), with males generally having superior post-velogenic exposure growth rate (Table 2).

Viral Load and Viral Clearance Rate in the Lentogenic Challenge

Generally, viral load was higher at 2 DPI than at 6 DPI. There was no significant difference between the FO and CS ecotypes, which had lower viral load and higher viral clearance rates compared to the IS ecotype (Table 3). There was no interaction effect for any of the time points measured. Sex significantly affected viral clearance rate and viral load at 6 DPI, with females clearing the virus faster than males.

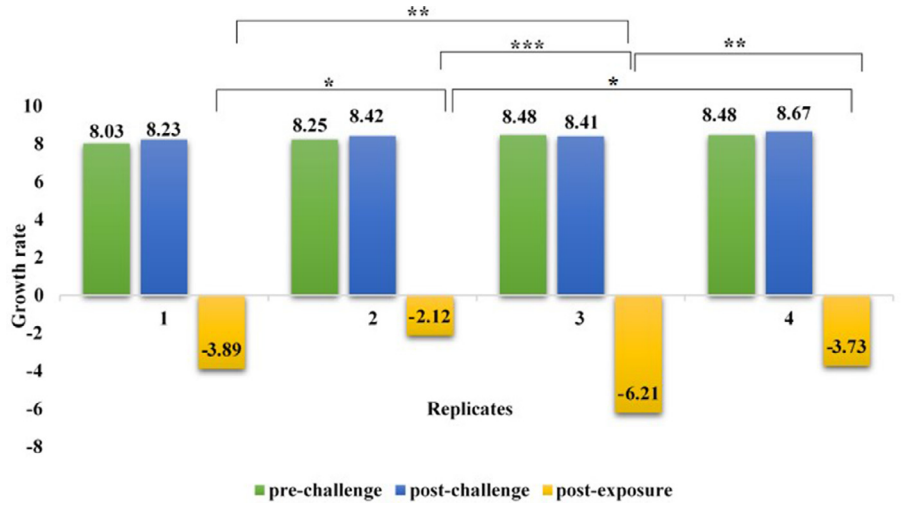

Figure 2. Average growth rate of Ghanaian local chickens during lentogenic NDV challenge and velogenic NDV natural exposure trials per replicate. For the lentogenic NDV challenge, pre-challenge or pre-infection body weights were measured at day of hatch (0) and then at 7, 14, 21, and 28 days of age (DOA) for replicates 1, 2, 3, and 4. Post-challenge or post-infection body weights were also measured for all four replicates at 34 and 38 DOA, which were 6 and 10 days post-infection (DPI) respectively. Pre- and post-infection growth rates (GR) were calculated as grams per day from these weights using linear regression of weight on age. Similarly, for the velogenic NDV exposure trials, GR was calculated from weights at 3, 7, 11, and 34 days post-velogenic exposure (DPE) for trial 1 (chickens from lentogenic trial replicate 1, 2 and 3) and at 6, 13, 21, and 27 DPE for trial 2 (chickens from lentogenic trial replicate 4) * ($P < 0.05$), ** ($P < 0.01$), *** ($P < 0.001$).

Viral load analysis was not conducted for the vNDV trial.

NDV-Specific Antibody Response

Pre-lentogenic challenge antibody levels were all lower than those for 10 DPI (results not shown). Pre-velogenic exposure average log titers were 3.06, 3.14, 3.25, and 3.05 for replicates 1, 2, 3, and 4, respectively (Figure 1). There were ecotype differences in antibody titers for both the lentogenic and velogenic NDV trials, with the Forest ecotype being significantly different ($P < 0.05$) from the 2 other ecotypes (Figure 3). Corresponding antibody levels for the replicates during the lentogenic NDV trial (10 DPI) and velogenic NDV trial (21 and 34 DPE) are shown in Figure 4. Antibody levels were higher during the velogenic NDV trial compared to the lentogenic NDV trial. For the velogenic trial, the mean antibody levels at 21 and 34 DPE was significantly lower ($P < 0.05$) for replicate 4 than replicates 1, 2, and 3 (Table 2).

Velogenic NDV Natural Exposure Lesion Scores

At the end of the vNDV natural exposure trial, 660 (46%) chickens showed prominent lesions in the trachea, proventriculus, intestines, and cecal tonsils whilst 778 (54%) chickens showed no signs of lesions. The cecal tonsils were swollen and haemorrhagic. Apart from lesions observed in these 4 organs, there were hemorrhagic lesions in the brain of three chickens in vNDV trial 1. In trial 2, congestion was also observed in the lungs (n = 4) and spleen (n = 1) of the chickens. Other lesions observed were pericarditis (n = 1) and hemorrhages in the spleen (n = 1) and ovarian follicle (n = 1). Lesions were found in more than 55% of the males used in the study whilst only 36% of the females had lesions. The highest mean lesion score was recorded in the intestine (1.33), with lesions in the trachea (0.52) being the least severe (Figure 5). The average lesion score (**LS**) was significantly higher in males ($P < 0.05$) than females with a very significant interaction effect between sex and replicate ($P < 0.05$) for all the replicates (Table 2). The average lesion scores for replicates 1, 2, 3, and 4 for males and females are shown in Table 4.

Genetic Parameters

Heritabilities were generally low for all the traits measured as shown in Table 5, with average lesion scores and intestinal lesion scores being the least heritable (0.00). The highest heritability estimates were recorded for antibody levels at d 21 and 34 post-velogenic exposure (0.08). There were significant dam effects ($P < 0.05$) for cecal tonsils, average lesion score, and post-velogenic exposure growth rate.

Phenotypic Correlations

Phenotypic correlations of traits measured within the velogenic NDV trial and correlations of traits between the lentogenic and velogenic NDV trials, respectively are shown in Tables 6 and 7. Except for the proventriculus LS, antibody levels at d 21 and 34 DPE were weakly and positively correlated with all traits (Table 6). Post-velogenic exposure GR was also weakly and positively correlated with all traits except cecal tonsils LS (Table 6). However, average LS had strong positive correlations with intestinal LS and cecal tonsil LS but correlated moderately and positively with trachea LS and proventriculus LS (Table 6).

Table 2. Least square means, standard errors and P-values for traits measured in velogenic Newcastle disease virus trials.

		Traits						
Sex	Replicate	Trachea Lesion Score	Proventriculus lesion score	Intestinal lesion score	Cecal tonsils lesion score	Average lesion score	Postexposure Growth Rate (g/day)[1]	Antibody at 21 and 34_DPE[2]
Female		0.08 ± 0.02^{b}	0.08 ± 0.02^{b}	0.79 ± 0.05^{b}	0.53 ± 0.03^{b}	0.46 ± 0.02^{b}	-2.51 ± 0.37	$4.17 \pm 0.0.4$
Male		0.17 ± 0.02^{a}	0.17 ± 0.02^{a}	1.79 ± 0.06^{a}	1.00 ± 0.04^{a}	0.87 ± 0.02^{a}	-1.74 ± 0.20	4.25 ± 0.03
	1	0.51 ± 0.04^{b}	0.17 ± 0.03	1.25 ± 0.06	0.75 ± 0.03	0.67 ± 0.03	-2.83 ± 0.37	4.27 ± 0.04^{a}
	2	0.44 ± 0.04^{bc}	0.10 ± 0.02	1.48 ± 0.08	0.91 ± 0.06	0.74 ± 0.03	-1.12 ± 0.32	4.31 ± 0.03^{a}
	3	0.33 ± 0.04^{c}	0.16 ± 0.03	1.66 ± 0.09	0.92 ± 0.07	0.05 ± 0.04	-1.67 ± 0.27	4.27 ± 0.21^{a}
	4	0.96 ± 0.08^{a}	0.10 ± 0.03	0.99 ± 0.13	0.48 ± 0.07	0.06 ± 0.07	-3.90 ± 0.96	3.55 ± 0.12^{b}
P-value								
Sex		1.91E-09	0.001	2.00E-16	3.53E-15	2.00E-16	>0.05	>0.05
Replicate		0.002	0.119	1.32E-06	2.26E-05	0.016	4.73E-05	<2e-16
Sex x Replicate		>0.05	>0.05	0.002	0.003	0.003	0.013	>0.05

Values are means ± standard errors.
[a,b,c]Means in the same column with superscripts in common are not significantly different.
[1]Growth rate in grams per day after velogenic Newcastle disease virus (NDV) exposure.
[2]Antibody levels at 21 and 34 days post-velogenic NDV exposure.

Average lesion scores correlated positively with pre-lentogenic infection growth rate, antibody levels at 10 DPI and viral load at 2 DPI, but negatively with post-lentogenic infection growth rate, viral load at 6 DPI, and viral clearance (Table 7). Post-velogenic exposure growth rate was negatively correlated with all the lentogenic traits except antibody levels at 10 DPI and viral clearance. There was a positive correlation between antibody levels at 21 and 34 d post-velogenic NDV exposure and all the lentogenic traits with the exception of viral load (Table 7).

DISCUSSION

ND is a very important disease in chickens, with vNDV infection resulting in severe production losses in poultry (Miller and Koch, 2013) if not controlled. Age of infection, sex, immune status, species, breed, co-infections, etc. exacerbate the effects of the disease (Kaleta and Baldauf, 1988; Wakamatsu et al., 2006; Jafari et al., 2019). Affected chickens often exhibit clinical signs and mortality is high with velogenic strains. In the current study, the chickens in the velogenic trial exhibited some clinical signs such as conjunctivitis, droopy wings, torticollis, whitish-greenish diarrhea, and circling. In a study by Mariappan et al. (2018), to investigate an outbreak of vNDV in NDV vaccinated chickens in India, similar clinical signs as in the current study were reported.

Growth Rate

For the lentogenic NDV trial, growth rate post-lentogenic challenge was higher than the pre-lentogenic challenge growth rate. The NDV virus strain used for the inoculation during the lentogenic NDV trial was a less virulent NDV strain and acted as vaccination against subsequent infections. The virus probably did not adversely affect the growth of the chickens, although it elicited an immune response. These results agree with those of Rowland et al. (2018) who challenged chickens with lentogenic NDV and reported higher post-lentogenic challenge growth rates compared to pre-lentogenic challenge growth rate. Contrary to this finding, the NDV strain used in the velogenic NDV trial was virulent, and therefore, could have resulted in the evident weight loss across all the replicates. The loss in weight post-velogenic exposure could be attributed to reduction in feed intake or inability of chickens to feed as a result of the infection. In a study by Li et al. (2020), NDV vaccination induced stress, which resulted in the production of adrenocorticotropic hormone and cortisol which negatively affected growth performance (average daily gain, average daily feed intake, and feed conversion ratio) in broilers. The concentration of these hormones increased with an increasing number of consecutive vaccinations. The chickens in the current study were given only one dose of the LaSota lentogenic NDV vaccine and therefore it did not

Table 3. Effect of sex and ecotype on viral load and viral clearance rate for the lentogenic NDV trial.

		LogV2DPI	LogV6DPi	Viral clearance rate
Sex	Male	4.00 ± 0.00	3.00 ± 0.04[a]	0.25 ± 0.01 [b]
	Female	4.00 ± 0.00	2.83 ± 0.04[b]	0.30 ± 0.01 [a]
	P-value	0.916	<0.001	0.004
Ecotype	Interior Savannah (IS)	4.37 ± 0.00 [a]	3.15 ± 0.05 [a]	0.26 ± 0.01
	Forest (FO)	4.05 ± 0.00 [b]	2.80 ± 0.0.05 [b]	0.28 ± 0.01
	Coastal Savannah (CS)	4.07 ± 0.00 [b]	2.84 ± 0.05 [b]	0.28 ± 0.01
Replicate	*P*-value	<0.001	<0.001	0.382
	1	4.15 ± 0.06 [a]	3.33 ± 0.04 [a]	0.17 ± 0.01 [b]
	2	4.35 ± 0.06 [a]	2.68 ± 0.04 [b]	0.37 ± 0.01 [a]
	3	3.99 ± 0.06 [a b]	2.50 ± 0.04 [c]	0.34 ± 0.01 [a]
	4	3.93 ± 0.06 [b]	3.26 ± 0.04 [a]	0.14 ± 0.01 [b]
	P-value	<0.001	<0.001	<0.001

logV2 DPI = viral load at 2 days post-lentogenic infection.
logV6 DPI = viral load at 6 days post-lentogenic infection.
[abc]For each factor, means in the same column with superscripts in common are not significantly different.

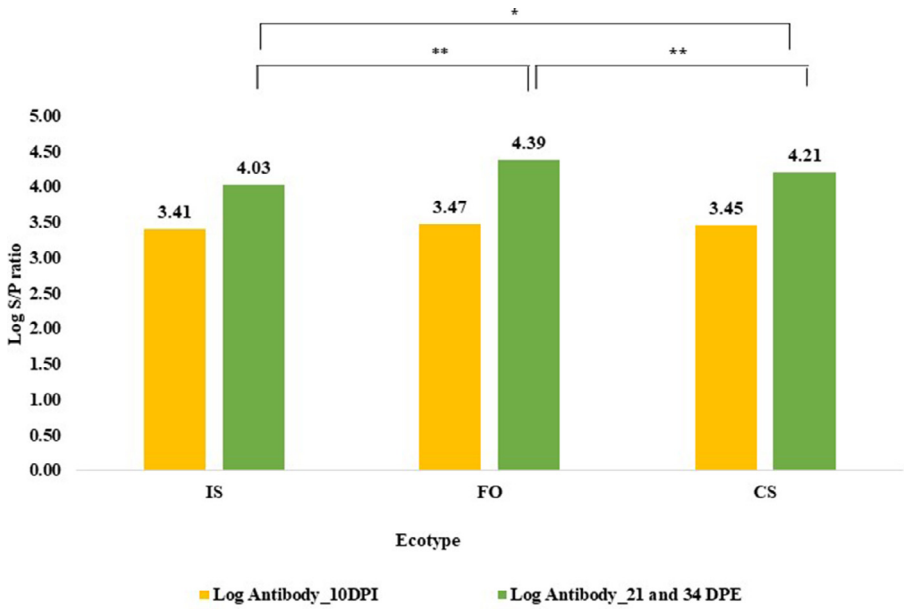

Figure 3. Ecotype mean antibody levels (log S/P ratio) at 10 days post-infection (DPI) for the lentogenic NDV challenge and at 21 and 34 days post-exposure (DPE) for the velogenic NDV natural exposure trials * ($P < 0.05$), ** ($P < 0.01$).

affect growth rate, probably due to low concentrations of these hormones. Conversely, the virulent nature of the velogenic strain could have resulted in increased concentration of these hormones, which reduced growth performance in the chickens. The significant Replicate × Sex interaction was expected as males and females responded to the virus differently and also because of the age differences between chickens in the four replicates. Further, replicate 3 chickens lost more weight compared to those in the other three replicates and also recorded the highest average lesion scores. The chickens in this replicate were younger (263 DOA) than the chickens in replicates 1 (369 DOA), 2 (332 DOA), and 4 (294 DOA) at the time of the velogenic natural exposure trial, and therefore, produced a weaker immune response to the velogenic NDV virus. Time differences in acquiring infection could have also played a role in the variations in growth rate in the current study.

Viral Load and Viral Clearance Rate for the Lentogenic Trial

The lowest viral loads at 2 and 6 DPI were recorded for the Forest ecotype chickens compared to the other ecotypes, which corroborates the antibody level results presented in this study. The Forest ecotype chickens also had the highest antibody levels at all time points in both the lentogenic NDV trial and the velogenic NDV exposure trial, and therefore were able to clear the virus the fastest. Conversely, the Interior Savannah chickens had the highest viral load, evidenced by the lower antibody levels. These results imply that the

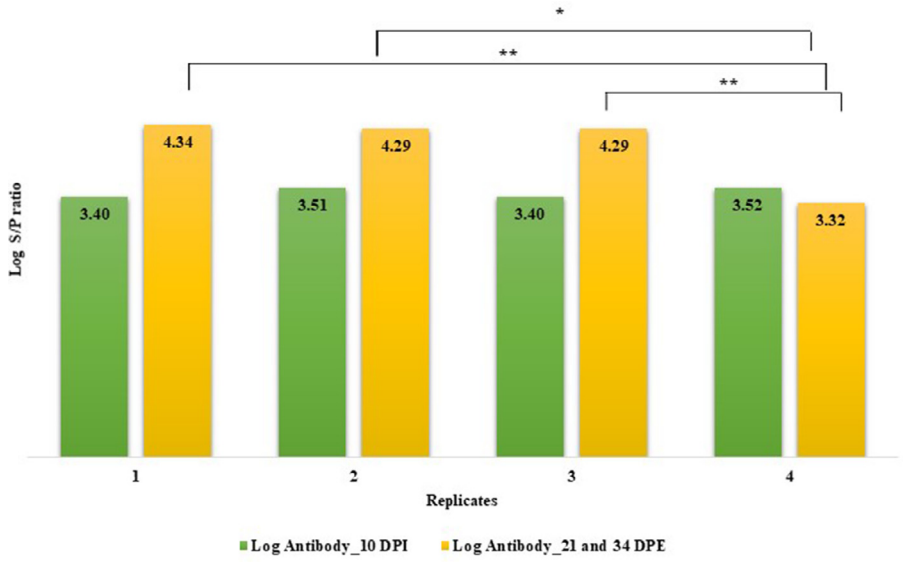

Figure 4. Mean antibody levels (log S/P ratio) at 10 days post-infection (DPI) for the lentogenic NDV challenge and at 21 and 34 days post-exposure (DPE) for the velogenic NDV natural exposure trials, per replicate * ($P < 0.05$), ** ($P < 0.01$).

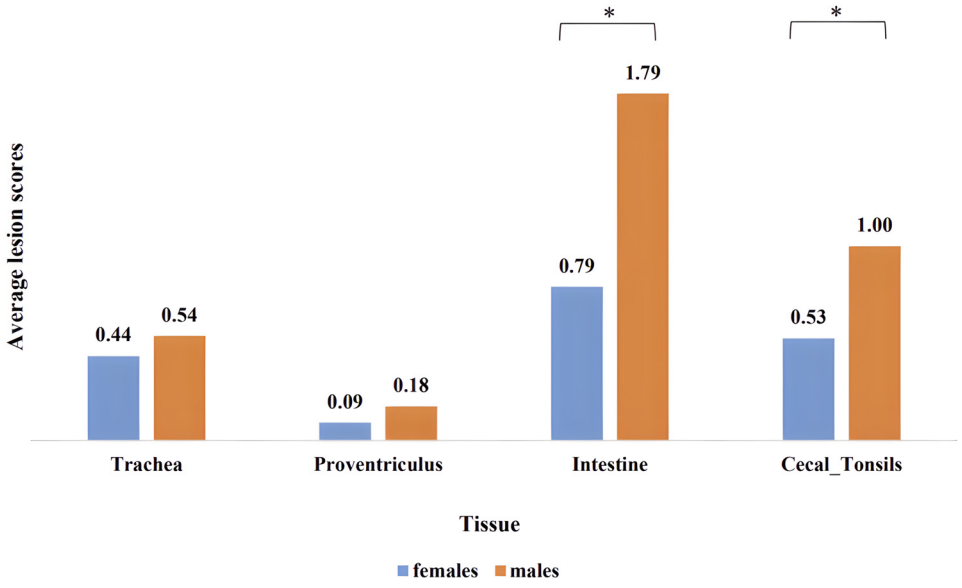

Figure 5. Average lesion scores measured on four organs for males and females in the velogenic NDV natural exposure trial. Lesions were scored on a scale ranging from 0 (no lesions) to 4 (extremely severe) on the intestine, cecal tonsils, proventriculus and the trachea. * ($P < 0.05$).

Forest ecotype is the least susceptible to the virus, followed by the Coastal Savannah ecotype, with the Interior Savannah ecotype being most susceptible. These findings confirm previous reports on variant responses of different chicken ecotypes to NDV. Bobbo et al. (2013) reported differences in immune response to NDV between Nigerian local chicken phenotypes. In a study by Schilling et al. (2019), three main Tanzanian local chicken ecotypes responded to NDV infection differently, with the Ching'wekwe recording the lowest viral load, followed by Kuchi and then Morogoro Medium, which had the highest viral load. Adeyemo et al. (2012) also reported that in Nigeria Yoruba ecotype chickens had higher immune response to NDV, followed by the Fulani ecotype, and then the exotic breed.

Males had the highest viral load in the current study, similar to reports by Boakye et al. (2016) and Appiah et al. (2020) for village chickens in Ghana. These were, however, contrary to findings by Njagi et al. (2010), who reported higher viral load in females in Kenyan village chickens. Sex differences in immune response have been observed to be inconsistent (Lamont et al., 2014). Similar to previous reports, the females had higher antibody response in the current study, which together with a higher viral clearance rate observed in females, could

Table 4. Number of birds and average lesion scores.

Velogenic trial	Lentogenic trial replicate	Sex	Total number	Number with lesions	Average lesion scores
1	1	Female	248	106	0.52
1	1	Male	238	134	0.79
1	2	Female	207	56	0.39
1	2	Male	239	126	0.90
1	3	Female	181	60	0.40
1	3	Male	199	120	0.96
2	4	Female	66	32	0.48
2	4	Male	60	26	0.80

Table 5. Estimates of variance components for traits measured in velogenic Newcastle disease virus trials.

Trait	N	Mean	SD	σ^2d	σ^2a	σ^2p	σ^2e	h^2 (SE)
Trachea lesion score	663	0.52	0.61	0.00	0.00	0.34	0.34	0.02 (0.05)
Proventriculus lesion score	663	0.13	0.33	0.00	0.00	0.11	0.11	0.05 (0.06)
Intestinal lesion score	663	1.33	1.02	0.00	0.00	0.94	0.94	0.00 (0.00)
Cecal tonsils lesion score	659	0.80	0.66	0.02	0.04	0.48	0.45	0.01 (0.05)
Average lesion score	659	0.70	0.43	0.01	0.02	0.19	0.17	0.00 (0.00)
Post-exposure growth rate [1]	618	−1.96	4.86	0.03	0.06	19.03	18.98	0.06 (0.06)
Antibody at 21 and 34 DPE [3]	421	4.22	0.46	0.00	0.00	0.08	0.08	0.08 (0.09)

Abbreviations: N, number of samples; SD, standard deviation; σ^2d, dam variance; σ^2a, additive variance; σ^2p, phenotypic variance; σ^2e, residual variance; h^2, heritability; SE, standard error.
[1]Growth rate after velogenic Newcastle disease virus (NDV) exposure.
[3]Antibody levels at 21 and 34 days post-velogenic NDV exposure.

Table 6. Phenotypic correlations among measured traits within the velogenic Newcastle disease virus trial.

Trait	Trachea lesion score	Proventriculi lesion score	Intestine lesion score	Cecal tonsil lesion score	Average lesion score	Postexposure growth rate
Trachea lesion score						
Proventriculus lesion score	−0.01 (0.04)					
Intestinal lesion score	0.14 (0.04)	0.01 (0.04)				
Cecal tonsils lesion score	0.14 (0.04)	0.10 (0.04)	0.34 (0.03)			
Average lesion score	0.50 (0.03)	0.25 (0.04)	0.80 (0.01)	0.70 (0.02)		
Post-exposure growth rate [1]	0.03 (0.04)	0.02 (0.04)	0.02 (0.04)	−0.01 (0.04)	0.02 (0.04)	
Antibody at 21 and 34 DPE [2]	0.02 (0.05)	−0.01 (0.05)	0.07 (0.05)	0.14 (0.05)	0.10 (0.05)	0.08 (0.06)

Values in parentheses are standard errors.
[1]Growth rate after velogenic Newcastle disease virus (NDV) exposure.
[2]Antibody levels at 21 and 34 days post-velogenic NDV exposure.

Table 7. Phenotypic correlations among the traits between velogenic and lentogenic NDV trials.[1]

Trait	Pre-infection Growth rate	Post-infection Growth rate	Antibody_10 DPI	Viral load_2 DPI	Viral load_6 DPI	Viral clearance
Trachea lesion score	−0.01 (0.04)	−0.09 (0.04)	0.07 (0.04)	−0.03 (0.04)	−0.21 (0.04)	0.08 (0.04)
Proventriculus lesion score	0.07 (0.04)	0.01 (0.04)	−0.01 (0.04)	0.08 (0.04)	−0.19 (0.04)	0.02 (0.04)
Intestinal lesion score	−0.01 (0.04)	0.01 (0.04)	0.02 (0.04)	−0.02 (0.04)	0.06 (0.04)	−0.08 (0.04)
Cecal tonsils lesion score	0.03 (0.04)	−0.02 (0.04)	0.02 (0.04)	0.02 (0.04)	−0.07 (0.04)	0.03 (0.04)
Average lesion score	0.02 (0.04)	−0.04 (0.04)	0.04 (0.04)	0.01 (0.04)	−0.14 (0.04)	−0.01 (0.04)
Post-exposure growth rate	−0.04 (0.04)	−0.02 (0.04)	0.01(0.04)	−0.09 (0.04)	−0.06 (0.04)	0.05 (0.04)
Antibody at 21 and 34 DPE	0.04 (0.05)	0.03 (0.05)	0.04 (0.05)	−0.02 (0.05)	−0.25 (0.04)	0.04 (0.05)

Abbreviations: DPI, days post-lentogenic infection; DPE, days post-velogenic exposure.
[1]Rows are traits measured in the lentogenic NDV trial and columns are traits measured in the velogenic NDV natural exposure trial. Values in parentheses are standard errors.

have resulted in the lower viral load observed for females in the current study.

NDV-Specific Antibody Response

The Forest ecotypes recording higher antibody levels compared to the other ecotypes in both the lentogenic NDV trial and the velogenic NDV trial could be attributed to adaptation. Agbenyegah (2017) also presented preliminary results indicating that Forest ecotype chickens had significantly higher mean log antibody titers (3.68) than the Interior Savannah (3.59) and Coastal Savannah (3.52) ecotypes during the lentogenic NDV trial. The differences between ecotypes were attributed to a possible exposure to constant NDV outbreaks in the

Forest zone. Also, it is possible that the Forest ecotype may have been exposed to more virulent NDV strains compared to the Coastal and Interior Savannah ecotypes and therefore developed an adaptation for higher antibody production to enhance survival during an outbreak. These results here are in line with those of Kemboi et al. (2013) who reported significant ($P < 0.05$) seasonal effects on antibody levels in local chickens of Mbeere District in the Eastern Province of Kenya. In the study, cooler temperatures enhanced antibody levels whilst hotter temperatures reduced the concentration of circulating antibodies in Kenyan local chickens. Given that the Forest zone has a cooler climate compared to the other zones, it is a likely explanation for the chickens in this zone recording the highest antibody response. On the other hand, the Interior Savannah ecotype had the least antibody response and this could be linked to the constant high temperatures the chickens are exposed to in the warmer climate of the Interior Savannah zone, as heat stress is known to suppress antibody levels in poultry (Bartlett and Smith, 2003; Niu et al., 2009).

The chickens generally generated higher antibody levels in the vNDV trial than in the lentogenic NDV trial. Even though the vNDV natural exposure trial had no naïve group to serve as control and therefore all the chickens had some antibodies to NDV, the higher antibody response observed in the vNDV natural exposure trials was not surprising because it is a more virulent strain compared to the lentogenic strain (Borland and Allan, 1980; Otim et al., 2007). Considering that the chickens in this study had received an initial dose of a less virulent strain of NDV in the lentogenic NDV trial, it could have acted as a form of vaccination which primed the chickens against subsequent NDV infections. The chickens therefore had memory B cells and T cells and developed much faster and efficient immunity against vNDV infection with very few mortalities. In addition cell-mediated adaptive immunity may have played a vital role in suppressing the morbidity and mortality across replicates. Rauw et al. (2009) reported that cell-mediated immunity was activated slowly but lasted longer and was stronger in vaccinated chickens that were later infected with a virulent NDV strain. Findings of Kapczynski et al. (2013) indicate that cell-mediated immune response reduced viral shedding and increased NDV viral clearance rate in post-NDV vaccinated chickens. Although viral shedding and viral clearance rate were not measured in the vNDV exposure trial, a higher viral clearance rate with reduced viral shedding could have contributed to the recovery of the chickens post-exposure. For instance, Jafari et al. (2019) reported that all surviving turkeys challenged with velogenic NDV recovered 14 days post-lentogenic infection.

The significantly lower antibody levels for chickens in replicate 4 at 21 DPE than those observed at 34 DPE for replicates 1, 2, and 3 could mean that a different virulent NDV strain was used in this replicate. It is also likely that the antibody titers would have increased with time if the study had been extended. Notably, the chickens were not directly inoculated with the virus and had to rely on the interaction with the sick chickens. It is also possible that different chickens picked up the virus at different times and therefore were at different stages of response to infection. Further, given that the chickens were at different ages (i.e., 369, 332, 263 and 294 DOA, for replicates 1, 2, 3, and 4, respectively) at the time of the vNDV exposure, the ages of the chickens could have also affected antibody levels, with younger chickens producing the lowest antibody response. Resistance to virulent and non-virulent forms of ND infection has been shown to increase with maturity (Lancaster, 1996). This finding agrees with that of Wakamatsu et al. (2006) who reported similar age effects of immune response during a velogenic challenge with turkeys and chickens.

Velogenic NDV Natural Exposure Lesion Scores

Lesions in the intestines and cecal tonsils seemed more prominent and severe compared to the other organs in most of the chickens. However, based on the brain lesions observed in only vNDV trial 1 chickens, it is likely that the strain of vNDV used was neurotropic while that used in trial 2 was viscerotropic. In a study by Mariappan et al. (2018) who also challenged NDV vaccinated chickens with vNDV, gross lesions were prominent in the intestines and proventriculus. Susta et al. (2011) also reported severe lesions in the lymphoid organs (cecal tonsils, thymus, spleen, and bursa) and the intestines for White Leghorn chickens infected with vNDV isolated from Long Bien duck. The cecal tonsils are critical lymphoid organs in chickens as they comprise a majority of the lymph nodules (Kitagawa et al., 1998).

Majority of the chickens (54%) in the current study recorded no lesions at the end of the trial. Absence of lesions have also been reported at 20 DPI in Nigerian chickens infected with vNDV (Okoye et al., 2000). In another vNDV challenge experiment, Igwe et al. (2014) noted the absence of lesions in some selected organs at 21 DPI and attributed it to a possible case of recovery. This possible case of recovery, together with the initial challenge with the lentogenic strain, could have accounted for the majority of the chickens in our study recording no lesions at the end of the trial.

Sex had a significant effect on lesion scores, with females having lower average lesion scores than males. This could imply sexual differences in relation to innate immune response and perhaps resistance. This finding is also in line with the viral clearance rate for the lentogenic trial that shows that females recovered at a faster rate from the virus than males. This is attributed to the possibility of the trait being partially sex-linked as suggested by Bovenhuis et al. (2002). In a study by Deist et al. (2017), large sex effects were found to affect lung transcriptome of NDV challenged chickens. Sex related effects have also been reported in relation to disease and haemoglobin levels in chickens (Leitner et al., 1989; Huff et al.,

1991; Osei-Amponsah et al., 2013). It is interesting to note that male birds had significantly higher lesion scores compared to females yet they had higher post-velogenic exposure growth rates. This is probably due to the higher antibody response observed in males compared to females, which could have accounted for their superior postexposure growth rate despite the higher lesion score in males. More importantly, the majority of the chickens (54%) in the study recorded no lesions at the end of the trial, possibly due to recovery from the disease. Therefore, lesions probably did not have much effect on growth.

Genetic Parameters

Heritabilities observed in the velogenic NDV exposure study were low (0.00−0.08) implying that the variations in the traits measured were probably more due to environmental factors than genetics and therefore selection for these traits in the chickens would not be effective. These heritabilities for the velogenic NDV exposure trial response traits were however lower than those obtained in the lentogenic NDV trial which ranged from moderate (0.23) to high (0.55), as reported by Walugembe et al. (2020) for viral load and pre-infection growth rate, respectively. Rowland et al. (2018) also reported moderate to high heritability estimates, ranging from 0.18 to 0.46 for commercial brown egg laying chickens (Hy-line Brown, Hy-Line International, Dallas Center, IA) in a lentogenic NDV trial. Heritabilities reported here are also lower than the moderate heritabilities in the range of 0.18 to 0.35 reported by Walugembe et al. (2019) for Tanzanian local chickens challenged with lentogenic NDV.

Phenotypic Correlations

Within the velogenic NDV trial, average lesion score correlated strongly and positively with intestinal lesion score and cecal tonsil lesion score, implying that lesions in these organs are especially important in NDV response in chickens. Notably, higher lesion scores were observed in these organs compared to the proventriculus and trachea in the present study. The positive correlation of average lesion scores (velogenic NDV trial) with antibody levels and viral load (lentogenic NDV trial) suggests that the chickens would continue to be sick if the lesions persisted. On the other hand, average lesion scores negatively correlated with post-lentogenic infection growth rate, viral load at 6 DPI, and viral clearance. Thus, chickens that had increased viral clearance and increased post-lentogenic infection growth rates had fewer lesion scores. This is in agreement with findings by Rowland et al. (2018) who reported negative correlations between pathogen response and growth rates and Lwelamira et al. (2009) who also reported an inverse relationship between NDV response and production traits. The positive correlation between antibody levels at 21 and 34 DPE and viral clearance was expected as the antibody levels had started to decline at that time.

CONCLUSIONS

The current study revealed that NDV response and probably resistance may differ in male and female local chickens. Velogenic NDV infection resulted in higher antibody response and adversely affected growth, compared to lentogenic NDV infection. Among the 3 ecotypes, the Forest ecotype seemed to have a stronger antibody response and therefore selection for chickens with enhanced innate resistance to NDV could be considered in this ecotype. There was evidence of phenotypic correlation between some traits measured in the lentogenic NDV challenge and the vNDV natural exposure trial. Although this study also revealed some phenotypic correlation among traits within the vNDV trial, heritabilities were generally very low and therefore selective breeding for these traits may yield very little response. However, selection based on the traits measured in the lentogenic NDV trial may be more appropriate. Further, the variation in clinical signs between velogenic NDV trial 1 and trial 2 implies that different strains of the vNDV could be circulating in the local chicken populations of Ghana. Vaccination for NDV is critical in reducing effects and mortalities due to NDV in local chickens. However, chickens need to be vaccinated multiple times per year to maintain protective titers. Therefore, identification of chickens with enhanced natural resistance to NDV is critical in reducing the impact of NDV on the local poultry industry to improve rural livelihoods in Ghana.

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DISCLOSURES

The authors have nothing to disclose.

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