Genetic parameters and genomic regions associated with growth rate and response to Newcastle disease in local chicken ecotypes in Ghana and Tanzania

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Summary

Local chicken breeds play an important role in the livelihoods of people in both rural and urban areas of Africa. One of the main constraints to the poultry sector in many sub-Saharan countries is disease, with Newcastle disease (ND) being the most important. Because vaccination does not adequately control ND, selective breeding offers an effective complement, provided there is genetic variation in resistance, tolerance and/or response to ND. We investigated this topic by challenging 6 local ecotypes from Ghana and Tanzania with a lentogenic (vaccine) strain of Newcastle disease virus (NDV), then measuring growth, anti-NDV antibody levels, and viral load from hatch to 38 days of age. We estimated variance components and performed a genome-wide association study using ~2800 birds genotyped with the 600K Affymetrix chicken genotyping array. Heritabilities were moderate to high (0.14 – 0.55) for all the traits studied, which indicates that selection to improve these breeds for resistance to ND can be feasible. GWAS also revealed several genomic regions that explained ≥0.5% of the genetic variance, including a candidate gene region for antibody response on GGA1. We conclude that all traits investigated in this study appear to be highly polygenic in nature. Future studies will characterize differences between the breeds/ecotypes, determine if large breed-specific quantitative trait loci can be identified, and evaluate the response of the same birds to endemic, velogenic NDV strains.
Keywords: Newcastle disease, local chicken ecotypes, disease resistance, immune response.

Introduction

Local chicken ecotypes play an important role in the livelihoods of people in both the rural and urban areas of Africa. In Ghana, they account for about 70% of the national poultry population (FAO, 2014), and in Tanzania, local chickens supply all the chicken and eggs consumed in rural areas, and 20% of that consumed in urban areas (FAO, 2009). They are mostly reared by small-holder farmers who depend on them for meat, eggs and as a source of income.

One of the main constraints to the poultry sector in many sub-Saharan countries is disease, with Newcastle disease (ND) being the most important, both in Tanzania and Ghana

(Msoffe, 2002; Atuahene et al., 2010). Vaccination is not an adequate means of controlling ND in rural Africa because of cost, lack of a "cool chain", poor husbandry practices, instability of vaccines, and difficulty in correctly administering vaccines. Selective breeding would be an effective complement to vaccination, if genetic variation in resistance, tolerance and/or response to ND exists in the population. Phenotypic measurements that can give an indication of chicken immune responses to NDV are the amount of anti-NDV antibodies produced by the chicken after NDV infection, the rate at which chickens are able to clear the virus from their body or prevent the virus from replicating, and the chickens' ability to continue being productive when infected.

In this study, local chickens from three Ghanaian ecological zones (Coastal Savannah, Forest, and Interior Savannah) and three Tanzanian local ecotypes (Ching'wekwe, Kuchi and Morogoro medium), were challenged with a high-titered LaSota lentogenic NDV strain, with the aim of estimating genetic parameters and identifying genomic regions associated with productivity and response to NDV challenge. Results from the study could then be used to breed robust local breeds enhancing genetic resistance to ND and subsequently improving the local poultry production system.

Materials and Methods

Populations

The same experimental design was used in Ghana and Tanzania. Adult birds of six ecotypes (3 per country) were sourced from local farmers across the country and served as the breeding stocks for the experimental populations. To create the experimental flocks, multiple sires were each mated to several dams but each dam was only mated to one sire. Fertile eggs were collected, incubated and hatched. The chicks were transferred to a separate, bio-secure facility where the NDV challenge was performed.

NDV challenge

Each challenge experiment (henceforth referred to as a replicate) ran from hatch to 38 days of age (doa). All birds were raised under the same conditions, with access to feed and water *ad libitum.* At 27 doa, blood samples were collected and ELISA was used to quantify maternal antibody levels and ensure that they were at negligible levels. At 28 doa, birds were challenged via oculo-nasal route with 10^7EID50 of LaSota lentogenic NDV strain. At 2 and 6 days post-infection (dpi), tear samples were collected from which viral load was measured using RT-qPCR. At 10 dpi, blood samples were collected and ELISA was used to quantify NDV antibody levels in the sera. Body weights were recorded at hatch, 7, 14, 21, 28, 34 and 38 doa. Pre- and post-infection growth rates were calculated from these by linear regression of weight on the applicable doa. Four replicates were conducted in Ghana, yielding data on 1941 birds, and five replicates were conducted in Tanzania, yielding data on 1904 birds. However, complete data was not available for all birds.

Genotype data

DNA samples (from blood) were extracted from all birds to determine genotypes for markers across the genome using the 600K Affymetrix chicken genotyping array. SNP quality control was performed using the following criteria within ecotype: call rate ≥95%, minor allele frequency ≥0.01, Hardy-Weinberg Equilibrium p-value ≥10e-6, and several SNP cluster

quality checks. SNPs shared by ecotypes within country were used for further analyses. This resulted in 346,067 SNPs for ecotypes in Ghana and 373,497 SNPs for those in Tanzania. We also checked that the sample call rate was ≥95% for all birds.

Statistical Analyses

The traits studied were pre- and post-infection growth rate, log-transformed viral load at 2 and 6 dpi, and log-transformed anti-NDV antibody titers at 10 dpi. Genetic parameters were estimated using animal models implemented in ASReml4. We corrected for the random effect of dam, and systematic effects of ecotype, replicate, and sex, and for ELISA plate and RT-qPCR plate as applicable. Genetic effects were captured using a genomic relationship matrix. To identify genomic regions associated with the traits, we performed a genome-wide association study (GWAS) for each trait, using GenSel. In addition to fitting SNP effects as random effects, the GWAS model included the same fixed effects as used for estimation of genetic parameters, with estimates of additive genetic and residual variances obtained from the ASReml analyses set as priors. Missing genotypes were replaced with the ecotype means. We used Bayes-B analysis with π=0.999. For traits where dam variance was ≥1%, we pre-corrected for dam effects before running GWAS. The GWAS resulted in estimates of the percent of genetic variance explained by SNPs in 1-Mb windows across the genome.

Results and Discussion

Genetic parameters

Number of records, heritabilities and dam variances are given in Table 1. Heritabilities for all traits were moderate to high, indicating a good potential for improving these traits with selective breeding. For the Ghanaian population, viral load at 2dpi had a higher heritability than that at 6dpi. We do not yet have data on viral load at 2dpi in the Tanzanian population. Heritabilities were generally higher for Ghana than for Tanzania. In addition, all heritabilities were higher for these local ecotypes than in US-based commercial layer lines that underwent a very similar experiment (Rowland *et al.,* 2017).

Table 1. Number of records, heritabilities and ratio of dam/phenotypic variance.

Trait	Ghana			Tanzania		
	N	h^2 (s.e)	/	N	h^2 (s.e)	/
Pre-infection GR	1436	0.55 (0.08)	0.07	1392	0.43 (0.05)	0
Post-infection GR	1401	0.41(0.08)	0	1359	0.29 (0.05)	0
Log_{10}Antibody titer	1425	0.23 (0.07)	0.03	1394	0.14 (0.06)	0.03
Log_{10}Viral load, 2dpi	549	0.49 (0.15)	0.03	0	-	-
Log_{10}Viral load, 6dpi	333	0.26 (0.20)	0.01	529	0.22 (0.13)	0.006

N: number of records. s.e: standard error. : dam variance. : phenotypic variance. GR: growth rate. dpi: days post-infection.

GWAS

The GWAS revealed several genomic regions associated with the traits studied. A full list of 1-Mb windows that explained ≥ 0.5% of the total genetic variance (TGV) per trait is in Supplementary Table 1. For Ghana, there were 13 regions on 10 chromosomes that together explained 13.3% of TGV for pre-infection growth rate, 4 regions on 4 chromosomes explaining 4% of TGV for post-infection growth rate, 7 regions on 6 chromosomes

explaining 8.3% of TGV for antibody titer at 10dpi, and 9 regions on 6 chromosomes explaining 11.2% of TGV for viral load at 2dpi, of which one region on GGA13 explained 4.4% of TGV. There were no regions that explained ≥ 0.5% of TGV for viral load at 6dpi.

For Tanzania, there were 9 regions on 7 chromosomes that together explained 7.3% of TGV for pre-infection growth rate, and 4 regions on 3 chromosomes explaining 2.8% of TGV for antibody titer at 10dpi, of which one, at around 100Mb on GGA1, has been reported as a candidate region for antibody response (Chenglong et al., 2013). For viral load at 6dpi, 4 regions on 4 chromosomes together explained 2.3% of TGV. There were no regions that explained ≥ 0.5% of TGV for post-infection growth rate.

We did not find genomic associations near the major histocompatibility complex region on GGA16 for either country.

Conclusions

As one of the major efforts of the US Agency for International Development (USAID) Feed the Future Innovation Lab for Genomics to Improve Poultry, the aim of this study was to identify genetic markers or genomic regions associated with resistance to NDV infection and with growth traits in local chicken ecotypes in both Ghana and Tanzania. Results showed that heritabilities were moderate to high for all the traits studied, which indicates that selection to improve these breeds for resistance to NDV is feasible. GWAS also revealed several genomic regions that explained ≥ 0.5% of the genetic variance; however, we conclude that all traits investigated in this study appear to be highly polygenic in nature. Future studies will focus on identifying whether there are significant differences between the ecotypes and if any large ecotype-specific quantitative trait loci can be identified, so genetic markers can be used for genomic selection and breeding for more NDV resistant local chickens. We will also evaluate the response of the same birds to endemic velogenic NDV strains.

Acknowledgements

This study was funded by the USAID Feed the Future Innovation Lab for Genomics to Improve Poultry.

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Supplementary Material

Table 2. List of 1-Mb windows that explained ≥ 0.5% of total genetic variance.

Ghana[2]							
Pre-infection GR		Post-infection GR		Log_{10}Antibody titer		Log_{10}Viral load, 2dpi	
Chr_MbW	%TGV	Chr_MbW	%TGV	Chr_MbW	%TGV	Chr_MbW	%TGV
1_8	0.79	1_58	0.82	**1_165**	**1.45**	1_53	0.51
1_171	0.82	**2_33**	**1.93**	2_67	0.66	**1_192**	**1.07**
1_169	**2.16**	11_5	0.58	2_33	0.81	**1_147**	**1.11**
2_47	**1.23**	Z_66	0.71	**4_88**	**1.24**	**3_31**	**1.05**
11_19	**1.24**			7_15	0.77	**9_12**	**1.27**
14_0	0.76			**17_7**	**2.51**	13_4	0.56
15_7	0.65			21_5	0.81	**13_8**	**4.44**
15_8	**2.72**					15_9	0.62
17_2	0.61					28_0	0.55
18_6	0.6						
21_4	0.57						
27_2	0.51						
Z_51	0.6						

Tanzania[3,4]					
Pre-infection GR		Log_{10}Antibody titer		Log_{10}Viral load, 6dpi	
Chr_MbW	%TGV	Chr_MbW	%TGV	Chr_MbW	%TGV
1_140	0.55	1_10	0.92	1_14	0.66
2_74	0.71	9_13	0.58	3_86	0.57
3_65	0.63	9_14	0.71	6_19	0.57
3_43	**1.04**	11_18	0.56	Z_79	0.53
12_11	0.52				
14_13	0.5				
14_3	0.67				
15_4	0.99				
20_0	**1.71**				

Chr_MbW: Chromosome number_megabase window according to *G. gallus* genome build 5. TGV: total genetic variance. GR: growth rate. dpi: days post-infection.

[1]Windows that explained ≥1% TGV are in **bold** font.

[2] No windows explained ≥0.5% TGV in viral load at 6dpi for the Ghana data.

[3] No windows explained ≥0.5% TGV in post-challenge growth rate for the Tanzania data.

[4]No data available for Tanzania viral load at 2dpi.