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Feed the Future Innovation Lab for Genomics to Improve Poultry: a holistic approach to improve indigenous chicken production focusing on resilience to Newcastle disease

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SUMMARY

Small-scale poultry production in Africa plays a significant role in alleviating malnutrition and poverty in rural communities. Global climate change and infectious poultry disease such as Newcastle disease (ND) have had tremendous negative impact on poultry production and health due to limited biosecurity, cold chain, and inadequate extension service. Genetic selection for enhanced resistance to ND virus (NDV) offers a promising complementary approach to vaccination and biosecurity in addressing constraints in village production systems. The Feed the Future Innovation Lab for Genomics to Improve Poultry (GIP IL) has led an effort on the identification of genetic markers, genes and signalling pathways associated with enhanced resistance to NDV by conducting NDV challenging experiments in diverse inbred, commercial, and African indigenous chickens. The GIP IL developed a comprehensive genetic selection platform focusing on improved survival time and reduced virus shedding in the face of NDV infection and on enhanced growth rate and egg production. The programme applied the platform for genetic selection and breeding of indigenous chickens through velogenic NDV natural exposure trials. To improve our understanding of the epidemiology of NDV in Africa, we characterised circulating strains of the virus in Ghana and Tanzania and identified NDV risk factors among local chickens. These data contribute to a body of knowledge useful for guiding disease control efforts, informing vaccine strategies, enhancing biosecurity, and contributing to our overall understanding of NDV dynamics. To gauge the demand for genetically improved

KEYWORDS

Backyard; breeding; developing countries; heat stress; immunity; vaccination

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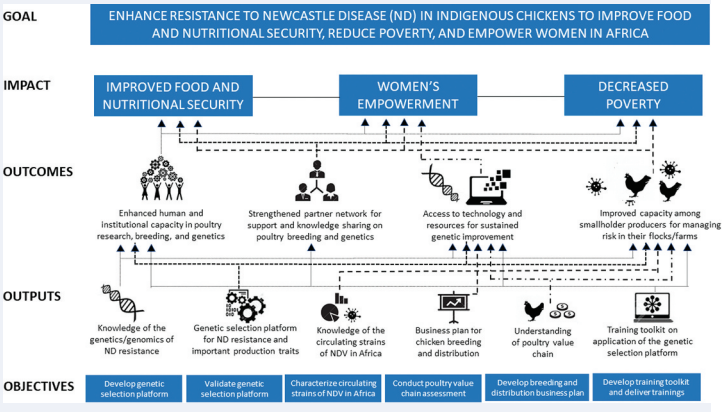
indigenous poultry lines, we undertook assessments of the poultry value chain and conducted choice experiments in Ghana and Tanzania, and the findings suggest that both ND enhanced resistance attribute and other productivity attributes of chicken such as egg production and body weight gain are important for a breeding programme.



Introduction

Small-scale poultry production has tremendous potential for alleviating malnutrition and poverty in rural communities (Iannotti *et al.* 2014; J. K. S. Smith *et al.* 2013). Poultry eggs and meat provide high-quality protein as well as micro- and macronutrients, which are vital for preventing cognitive and growth delays in children (Iannotti *et al.* 2014; Murphy and Allen 2003). Sale of poultry and eggs can also generate household income and support costs of healthcare and childhood education (Sonaiya 2008).

With global climate change, warming temperatures and more extreme climate events are negatively impacting poultry production. Heat stress reduces production and health in poultry (Ayo *et al.* 2011). This is especially impactful in developing countries, where poultry are often raised in management systems without environmentally controlled housing. Much of the poultry that supplies food in low-income countries is located in tropical environments.

Village indigenous poultry flocks in Africa play a significant role in improving food security, poverty alleviation and malnutrition (C. Amoia *et al.* 2021; C. F. Amoia *et al.* 2023). However, Newcastle disease (ND) is the number one health constraint of small-scale chicken production in Africa, causing mortality as high as 100% among village chicken flocks (Awuni 2002; Ekiri *et al.* 2021; Haile *et al.* 2020; Kitalyi 1998; Miguel *et al.* 2013). Biosecurity measures are challenging to put in place in these production systems, where chickens are free-ranging and comingling with other domestic fowl and wild birds, which can serve as reservoirs of circulating ND virus (NDV). On the contrary, producers in high-income countries rely on biosecurity and vaccination to protect their flocks against NDV (Mayers *et al.* 2017). Strains of NDV vary in disease potential, with lento-, meso- and velogenic strains, listed by increasing pathogenicity. Sustainable ND vaccination programmes are difficult to implement in low and middle-income countries,

especially in rural areas with inadequate extension services, limitations to maintaining a cold chain to keep the vaccines viable, and unreliable distribution channels. Furthermore, because ND vaccines do not provide complete protection against virulent strains of the virus (Mayers *et al.* 2017), sub-optimally vaccinated birds can host and transmit velogenic NDV strains to susceptible birds.

Genetic selection for enhanced resistance to NDV offers a promising complementary approach to vaccination and biosecurity in addressing constraints in village production systems. Differences in disease resistance between chickens are in part genetically controlled, allowing for genetic improvement of targeted traits, including innate resistance to the virus and efficacy of host immune response to ND vaccination.

This review paper focuses on the results of collaborative research conducted over a ten-year period as part of a multi-national programme with the goal to improve resistance to NDV and heat stress in indigenous chicken ecotypes of the African continent: the Feed the Future Innovation Lab for Genomics to Improve Poultry (gip.ucdavis.edu). The programme has conducted genome-wide association studies (GWAS) in African indigenous chicken ecotypes by challenging with a low-pathogenic NDV strain and by naturally exposing to high-pathogenic NDV field strains, and also in a widely distributed commercial layer line with a low-pathogenic NDV challenge. In addition, two well-characterised experimental genetic lines were used to identify genes and signalling pathways associated with NDV infection. Furthermore, in order to improve our understanding of the epidemiology of NDV, strains of NDV circulating in both Ghana and Tanzania were characterised. Finally, we undertook assessments of the poultry value chain and choice experiments to gauge the potential demand for genetically improved indigenous poultry lines. These findings lay important groundwork for continued improvements in the ability to better use the natural biodiversity of chickens to provide a nutritious, safe and economical component to the global human food supply.

Genomics research

African chicken ecotypes and response to Newcastle disease virus

Description of ecotypes and sourcing of original breeders

Three local chicken ecotypes were randomly sampled from distinct agroecological zones of Ghana, namely, the Interior Savannah (IS) in the north, the Forest (FO) in the south, and the Coastal Savanna (CS) along the coast in the southeast. The varying climatic conditions of these agroecological zones are described by Kayang *et al.* (2015) One mature bird from households located at least 0.5 km apart was sampled to minimise the chances of including genetically related individuals. The chickens were tagged and maintained at the Livestock and Poultry Research Centre (LIPREC), University of Ghana, Legon, Accra. They were grouped into 25 paternal families per ecotype, with a mating ratio of 1 male to 8 females. These families served as the breeding population that produced offspring for the experiments. Eggs from the breeders were collected daily, labelled and accumulated for up to 10 days at 18 C before incubation. At hatch, the chicks were wing-tagged, weighed and transferred to a bio-secure deep litter facility where they fed on a commercial chick mash with *ad libitum* access to water.

Three Tanzanian free-range local chicken (FRLC) ecotypes namely Kuchi, Ching'wekwe, and Morogoro-medium were randomly sampled from different zones of Tanzania mainland (Mushi *et al.* 2020). These zones have different climatic conditions. Ching'wekwe and Morogoro-medium chickens were sampled from regions in close proximity to the Coastal and Northern zones, whilst Kuchi were sampled from the Lake and Central zones of the country. From the different zones, four different regions were selected from which the chickens were sampled: Tanga, Morogoro, Singida, and Mwanza. A flock of 389 FRLCs (324 females and 65 males) of the three ecotypes were randomly collected from village households in the four regions and were used to establish a breeding parent stock. Each chicken was wing-banded. For each chicken ecotype, a male was placed separately in a pen with 6 to 10 females in a deep litter floor pen. The parent flocks were fed on maize-based layer diets with ad libitum access to water. Routine vaccinations against endemic diseases like Newcastle disease and infectious bursal disease (IBD) were administered. Worm infestations, ectoparasites and coccidiosis were treated/controlled using anthelmintics (piperazine DiHCl®, Kepro, Holland), pesticides (imidacloprid Confidor®, Bayer, Holland), and coccidiostats (Trisulmycine®, Laprovet, France), respectively.

NDV vaccination studies

Immune response to NDV were evaluated for the three Tanzania and the three Ghana ecotypes by challenging ~500 chicks per ecotype with a lentogenic NDV LaSota strain at 28 days of age (Walugembe *et al.* 2019, 2020). All birds were genotyped with a 600K Single Nucleotide Polymorphism (SNP) chip. In addition to growth rate, NDV response traits were measured following infection, including anti-NDV antibody levels [pre-infection and 10 days post-infection (dpi)], and viral load (at 2 and 6 dpi). Genetic parameters were estimated and GWAS analyses were used to identify genomic regions affecting these parameters. Estimates of heritability were moderate to high (0.18–0.55) for all traits, except for viral clearance, for which the heritability estimate was not different from zero for the Tanzania ecotypes. In both the Tanzania and Ghana ecotypes, GWAS revealed QTL in genomic regions with genes that are vital for growth and immune response during NDV challenge, although no QTL overlapped between the two countries. However, the Tanzania GWAS revealed a QTL for viral load at 6 dpi on chromosome 24 that overlapped with the US NDV study in which birds were challenged with NDV under heat stress. This QTL region includes genes related to immune response. The moderate-to-high estimates of heritability suggest that genetic selection can improve host response to lentogenic NDV of local African chicken ecotypes.

Natural NDV exposure studies

Vaccinated birds. To determine whether the heritable immune responses to lentogenic NDV, as described in the previous section, were associated with resistance to velogenic NDV, the chickens from the NDV vaccination studies were naturally exposed to velogenic NDV by introducing naturally NDV-infected seeder chickens into the flocks under a controlled field environment (Muhairwa *et al.* 2018). For each bird, body weights were measured at 0, 7, 9, 11, 13, 15, 17, and 25 days post-exposure. Flock mortality was assessed every 8 hours for the first 3 days and then every 12 hours thereafter until 29 days post-exposure. Lesion scores were recorded on trachea, proventriculus, intestines,

and caecal tonsils for all birds that died during the experiment and also following euthanasia of those that remained at the end of the experiment. Mortality rates and lesion scores were low, indicating a low severity of effects of the velogenic challenge on these previously vaccinated birds, although exposure to the natural infection did not occur until NDV antibodies had waned. In addition, heritabilities of all traits measured during the NDV natural exposure trial were low (≤0.08), from which we concluded that natural exposure of previously vaccinated birds did not result in traits that were amenable to selection.

Naïve challenges. Because of the limited informativeness of natural velogenic NDV exposure trials of previously vaccinated birds, the natural velogenic NDV exposure trials were repeated, but this time with naïve (non-vaccinated) birds from the six ecotypes. Genotyping was conducted with the genotyping-by-sequencing (GBS) panel described in the next section, followed by imputation to 600K. The same traits as described above were recorded. Heritability estimates were low to moderate, ranging from 0.11 for average lesion scores to 0.36 for pre-exposure growth rate. Heritability estimates for survival time following exposure were 0.23 and 0.27 for the Tanzanian and Ghanaian ecotypes, respectively (Walugembe *et al.* 2022). Survival time was genetically negatively correlated with lesion scores and with viral load. These results suggest that response to velogenic NDV of these local chicken ecotypes can be improved by selective breeding. Survival time of naïve birds following natural exposure shows the most promise. Estimates of genetic correlations of traits measured in the velogenic challenge with host response to challenge with a lentogenic vaccine strain (see above) were generally low with high standard errors. Further study is, therefore, needed to evaluate the ability to use response to the vaccine strain to select for improved response to velogenic NDV.

Response to Newcastle disease virus and heat stress in distinct, highly inbred chicken lines: gene expression

Experimental design

Our characterisation of the host response to NDV, with or without heat stress, began by studying immune and physiologic response and gene expression in highly inbred lines that differ in relative resistance to many pathogens (Cheeseman *et al.* 2007; Lakshmanan *et al.* 1997; Pinard-van der Laan *et al.* 2009; Wang *et al.* 2014). Using chicken lines of simplified genetics (inbred) and established phenotypic diversity (relatively disease resistant versus susceptible) facilitated the establishment of reliable experimental models and generation of genetic hypotheses to test. The Fayoumi (M15.2) and the Leghorn (Ghs6) lines have been inbred since 1954 and are over 99.9% inbred (Fleming *et al.* 2016). The Fayoumi line, derived from chickens indigenous to the Fayoum region of Egypt, are relatively resistant to infection with many pathogens and to heat compared to the Leghorn line (Wang *et al.* 2018), which was derived from 1950s layer-type chickens in the U.S (Zhou and Lamont 1999).

From replicate hatches, chicks were divided into groups of equal numbers per family and either placed into local biosafety rooms (NDV challenge treatment only = NDV) or air-shipped to an alternate site and placed the same day into biosafety rooms (heat treatment + NDV challenge = heat + NDV). For the heat + NDV group, at

14 days of age, the experimental group was exposed to 38 C for 4 h, then decreased to 35 C and maintained at this temperature. Control birds of the heat + NDV study, and all birds in the NDV study, were kept at age-appropriate thermoneutral temperatures throughout the study. At 3 weeks of age, both the NDV challenge and the heat + NDV challenge groups received La Sota NDV by the oculo-nasal route and the counterpart nonchallenged (control) group received saline. Approximately one-third of the chickens of each treatment were euthanised for tissue collection at 2, 6, and 10 days post inoculation (dpi) with NDV. The NDV load, viral clearance rates and anti-NDV antibody levels verified the relative resistance of the Fayoumis compared to Leghorns. RNA sequencing was used to characterise the whole transcriptome of multiple tissues of several birds from each experimental and control group, with the goal to use the differential gene expression to identify important genes and pathways involved in response to NDV and/or heat.

Trachea response to NDV or heat + NDV

The trachea, especially its epithelial cells, is one of the first points of virus–host interaction and a major site of viral replication in an NDV infection. After NDV challenge, a major pathway revealed in transcriptome analysis of the tracheal epithelium was that of leukocyte extravasation signalling (Deist, Gallardo, Bunn, Kelly, *et al.* 2017). Ingenuity Pathways Analysis (IPA) predicted the activation of cell mobility and actin cytoskeleton contraction. While this may be a mechanism for immune-activated cells to move to the site of viral infection, actin is also required for NDV to replicate efficiently (El Najjar *et al.* 2014). Thus, actin is a worthwhile target for more detailed analysis.

After NDV challenge combined with heat stress, results suggest that NDV infection elicits proinflammatory processes, and activates pathways including inhibition of cell viability, cell proliferation of lymphocytes, and transactivation of RNA, more rapidly in Fayoumis than in Leghorns. Three candidate genes, *SLC29A, CYP8B1*, and *NFKBIA* were significantly up-regulated in Fayoumis at two dpi (Saelao *et al.* 2021). With time, genetic-line differences diminish, suggesting that the Leghorns may eventually produce an effective immune response to NDV. The different kinetics of host gene expression, however, may underlie the relative differences in response to NDV.

Lung response to NDV or heat + NDV

The mucosal surface of the lung is a major site of infection by NDV. After NDV challenge, relatively few genes were differentially expressed (DE) at any time between lungs of challenged and control birds of both lines, but *ZNFX1*, a sensor that initiates immune responses against RNA virus infection (Wang *et al.* 2019), was DE gene in both lines (Deist, Gallardo, Bunn, Dekkers, *et al.* 2017). In Fayoumis at 10 dpi, however, interaction of NDV challenge and genetic line revealed over 100 DE genes, including *PPIB*, which encodes a protein that binds to lymphocytes. Revealed pathways included protein processing in endoplasmic reticulum, which might relate to antibody synthesis and class switching occurring at this time. Of note, *TNFRSF13B* and *IGJ* were differentially expressed between the genetic lines in both lung and trachea, the two respiratory tissues studied (Deist, Gallardo, Bunn, Kelly, *et al.* 2017)

Following heat and NDV infection, Fayoumi lungs had a larger number of DE genes during the early stages of infection compared to Leghorns. Immune-related DE genes at

two dpi included *IL17REL*, and several genes of the phagosome maturation pathway. By 10 dpi, most DE genes in Fayoumi were downregulated. This phenomenon was similar to results with trachea, lung and Harderian gland, suggesting an overall resolution of the infection and return to homoeostasis in the Fayoumis. Leghorns, in contrast, had very few DE genes at any time, with most involved with metabolic and glucose-related functions. This study also analysed the proteome of the lung, and found little correlation with the transcriptome data, although there were similar pathways and genes enriched with both analyses. The integration of the two global profiling methods identified potential candidate genes and proteins that likely would have been missed with either method alone (Saelao, Wang, Chanthavixay, *et al.* 2018).

Spleen response to NDV

The spleen is one of the most frequently studied secondary immune tissues, due to its discrete anatomical structure and its important role in immune responses. Comparing the NDV challenged birds with their controls, the spleens of Fayoumis showed fewer DE genes than Leghorns. Predicted pathways by IPA analysis, included 'EIF-signaling' in Fayoumi chickens (Zhang *et al.* 2018). Broad-scope studies such as RNAseq, plus careful curation of relevant literature, can lead to a specific hypothesis to test in targeted, complementary studies. To that end, we interrogated the eukaryotic translation initiation factor 2 (*EIF2*) gene family as a likely candidate for control of NDV replication with the hypothesis that expression of these genes would differ in the spleens between the two genetic lines. Of the DE genes, most of the *EIF2* family genes were more lowly expressed in NDV-challenged Fayoumis, suggesting that host protein synthesis down-regulation might be one of the genetic mechanisms used by Fayoumis to reduce viral replication in the spleen. This contrasts with the activation of *EIF2* signalling in the tracheal epithelium (Deist, Gallardo, Bunn, Kelly, *et al.* 2017), indicating the complex nature of this gene family's association with NDV response.

Harderian gland response to NDV or heat + NDV

The Harderian gland is a unique tissue with multiple functions, one of which is local immune response (Deist and Lamont 2018). With an anatomical location near the eye, and a large population of both B and T lymphocytes, the Harderian gland is ideally situated to mount an early response to pathogens that enter the body through the tissues surrounding the eye, including air-borne viruses such as NDV. Comparing NDV challenge with its counterpart control, the Harderian gland of the Fayoumi line had significantly more detectable viral transcripts at two dpi than the Leghorn, but cleared the virus by six dpi, demonstrating the ability to rapidly control the viral replication. Few chicken genes were DE between the challenged and nonchallenged birds. Overall, the Fayoumi was predicted to activate more immune pathways in both NDV-challenged and nonchallenged birds than the Leghorn. This suggests that the Fayoumis have a more readily activated immune system under homoeostatic conditions, a state that may lead to its rapid clearance of the NDV (Deist *et al.* 2018)

In the experiment that combined heat treatment with NDV infection (heat + NDV), the relatively higher resistance of the Fayoumis compared to Leghorns was confirmed by its significantly lower viral load, higher viral clearance, fewer viral transcripts and higher anti-NDV antibody levels (Saelao, Wang, Gallardo, *et al.* 2018). The Fayoumi line had

few DE genes between heat + NDV and control groups at 2 and 6 DPI, but 202 DE genes at 10 dpi. The Leghorn transcriptome showed a different time course, with few DE genes at 2 or 10 dpi, and 167 DE genes at 6 DPI. Pathway analysis identified line-specific signalling pathways, with most of them in the Fayoumi being associated with immune function. Both genetic lines activated immune related pathways including SAPK/JNK and p38 MAPK signalling pathways, but Fayoumis activated them earlier than Leghorns. Thus, both with and without heat stress, there was evidence for the Harderian gland of Fayoumis being better poised to respond quickly to NDV challenge.

Bursa response to heat + NDV

The bursa of Fabricius is a primary lymphoid organ unique to birds and crucial for B cell development. After exposure to heat and NDV, the Leghorn bursa showed down-regulation of cell proliferation, cell cycle and cell division genes, compared to the Fayoumi (Chanthavixay *et al.* 2020). This dampened activity of the bursa may partly explain the lower anti-NDV antibody response of the Leghorn line. The challenged Leghorn also showed greater histone modification levels, enriched in target genes associated with cell cycle and receptor signalling of lymphocytes (Chanthavixay *et al.* 2020). Thus, the Leghorn bursa appears less able to mount an effective immune response to NDV than the Fayoumi.

Liver response to heat + NDV

The liver is a major metabolic organ, which performs many essential biological functions including regulation of most chemical levels in the blood. From 2 to 3 weeks of age, the chicks of the heat + NDV treatment group had been exposed to high ambient temperature but had not yet been inoculated with NDV and thus this period allowed an assessment of the response of the two genetic lines to heat treatment alone. Many blood chemicals were changed in Leghorns at 4 hours after initiation of high heat treatment, disrupting their acid–base balance. Fayoumis maintained a stable acid–base balance, but did have several blood chemicals altered at 6 days post-heat initiation, which might serve as biomarkers for heat stress response (Wang *et al.* 2018). The RNA-seq data showed that Fayoumis had more DE genes than Leghorn birds for both heat, and heat + NDV, treatments. Leghorns appeared to perform metabolic regulation in response to heat stress and NDV infection, while Fayoumis regulated both immune and metabolic functions. Weighted correlation network analysis (WGCNA) indicated that driver genes such as Solute Carrier (SLC) family genes may be important for stabilising acid-base balance and, therefore, maintaining homoeostasis in Fayoumi birds during heat stress (Wang *et al.* 2020).

Hypothalamus response to heat or to heat + NDV

The hypothalamus is an essential endocrine organ, especially important in maintaining physiological homoeostasis including thermoregulation and blood chemistry. After acute heat stress, and after heat stress plus NDV challenge, the hypothalamus had a mild transcriptomic response with few DE genes in either genetic line (J. Smith *et al.* 2022). After acute heat, most enriched pathways in the Leghorn line were metabolic-associated, except for the immune-related peptide antigen assembly with the MHC class I protein complex. Enriched GO terms in the Fayoumi line hypothalamus after acute heat treatment included defence response against virus. In the Leghorn hypothalamus after heat + NDV,

three functional GO terms were enriched in the down-regulated DE genes, all of the metabolic. The DE genes in the Fayoumi after heat + NDV were associated with metabolic and catabolic processes (J. Smith *et al.* 2022).

Breast muscle response to heat + NDV

The breast muscle is the largest muscle in chickens and a highly metabolic tissue. After acute heat treatment, many heat shock protein family genes were upregulated in breast muscle (J. Smith *et al.* 2022). Functional GO terms were similar between the two lines, except for one immune-related GO term: positive regulation of T cell activation in the Fayoumi line. After both heat and NDV, negative regulation of glucocorticoid receptor signalling pathway was enriched by DE genes in the Leghorn line. Thermoregulation from the hypothalamus to downstream organs is modulated by glucocorticoids. The innate immune response was one of the enriched functions in Leghorns. Downregulated Leghorn DE genes enriched metabolic-associated functions. In the Fayoumi line, DE genes were involved in metabolic functions. Two immune-related KEGG pathways were enriched by Fayoumi DE genes in breast muscle, adipocytokine signalling pathway and biosynthesis of antibiotics. The TNF receptor superfamily member 8 (*TNFRSF8*) gene appeared as the top driver gene negatively correlated with the pH levels. As a member of the TNF-receptor superfamily, the *TNFRSF8* gene is expressed by active T and B cells and leads to the activation of NFκB. This gene warrants additional study for its role in control of thermoregulation and NDV response. While both lines responded to heat stress and NDV infection by stimulating metabolic and immune functions, the Fayoumi line had earlier, more active, and specific immune regulation in the breast muscle than the Leghorn line with both treatments.

Response to Newcastle disease virus and heat stress in a commercial egg-laying line: genome-wide associations and candidate genes

In addition to the RNA-seq approach, which primarily focused on gene expression profiling in different biologically relevant tissues from inbred Fayoumi and Leghorn lines with NDV challenge, with or without heat stress, the GIP programme utilised genome-wide association studies (GWAS) in a commercial layer population that is widely distributed in Africa: Hy-Line Brown. The specific goal of this approach was to identify genomic regions and candidate genes associated with viral load, anti-NDV antibody response, heat stress physiological parameters, and body weight. The same experimental design as the RNA-seq study described above was employed, in terms of NDV and heat stress challenges, time of challenges, and sample collection times for measuring viral load, anti-NDV antibody titres, and physiological parameters. In addition, growth rate was measured to evaluate the impact of biotic (NDV) and the combination of biotic and abiotic (heat stress) challenge. In brief, 16 sires with pooled semen and 145 dams were used to generate experimental populations. A total of 540 birds were challenged with the LaSota strain of NDV at Iowa State University, and another 526 birds were challenged with both NDV and heat stress at the University of California, Davis (Rowland, Wolc, *et al.* 2018; Saelao *et al.* 2019).

For immune-related parameters, heat stress treatment decreased viral load with subsequently reduced anti-NDV antibody response (Rowland, Saelao, *et al.* 2018). The

heritability (h^2) of phenotypic traits in the NDV, NDV + Heat, and combined analysis of both experiments are summarised in Table 1. In general, low to medium heritabilities were observed for immune-related parameters and post-challenge growth rate, and medium to high heritabilities were observed for pre-challenge growth rate. Of particular note, environmental factors like heat stress in this study did have an effect on the heritability, in which much lower heritabilities across all measured parameters were found in the NDV + heat treatment trial than in the only NDV treatment trial. This is likely because of the non-genetic control of increased phenotypic variability with the additional stress of heat.

To identify potential genomic regions and candidate genes associated with immune-related parameters, the Axiom 600K chicken SNP array was used to genotype all birds. In general, a limited number of genomic regions affecting immune-related parameters were observed (Table 2; Rowland, Wolc, *et al.* 2018; Saelao *et al.* 2019). There were no overlapped genomic regions for the same parameter between the two treatment trials, which suggests a potentially significant impact of genetics by environment interaction on economically important complex traits. However, we did find one genomic region (24:0.1–1.9Mb) affecting viral load at 6 dpi that was identified in both the combined NDV and heat stress trial in Hy-Line Brown and an NDV trial in Tanzanian indigenous ecotypes (natural heat stress exposure occurred during the trial) (Walugembe *et al.* 2019). These results suggest strong evidence that this genomic region may harbour putative causal variants for viral load. Specifically, two genes (TIRAP and ETS1) in the region are good candidates for further investigation of their biological function on NDV viral replication in poultry.

Furthermore, additional candidate genes were selected based on our GWAS studies and other relevant studies for association analysis with primary focus on SNPs within exons with predicted amino acid sequence change. The results suggest that SLC5A1 was associated with viral load at 6 dpi, IFI27L2 was associated with anti-NDV antibody at 10 dpi,

Table 1. Heritability (h^2) estimates of phenotypic traits in separate trials and combined analysis.

Traits	Both Treatment[a] Groups	NDV[b]	NDV + Heat[c]
Viral load 2 dpi	0.24 (0.06)	0.32 (0.10)	0.17 (0.10)
Viral load 6 dpi	0.09 (0.04)	0.18 (0.10)	0.11 (0.10)
Antibody 10 dpi	0.14 (0.05)	0.24 (0.09)	0.04 (0.06)
Growth rate pre-challenge	0.40 (0.06)	0.46 (0.11)	0.27 (0.09)
Growth rate post-challenge	0.16 (0.05)	0.21 (0.09)	0.11 (0.06)

[a]Referenced from Rowland, Saelao, *et al.* (2018). [b]Referenced from Rowland, Wolc, *et al.* (2018). [c]Referenced from Saelao *et al.* (2019).

Table 2. QTL region positions of suggestively significant SNPs for phenotypic traits in NDV and NDV + heat treated trials.

Trait	Treatment	Number of SNPs	Position (Mb)
Antibody 10 dpi	NDV[a]	1	21:3.5–4.5
Antibody pre-challenge	NDV	3	3:37.7–38.7; 10:2.3–3.3; 10:3.6–4.6
Viral load 2 dpi	NDV + Heat[b]	1	1:6.1–7.1
Viral load 6 dpi	NDV	1	4:52.7–53.7
	NDV + Heat	30	0.1–1.9
Viral clearance	NDV + Heat	7	24:0.1–11
Growth rate	NDV	2	2:26.7–27.7; 10:8.4–9.4
	NDV + Heat	1	2:128.8–129.8

[a]Referenced from Rowland, Wolc, *et al.* (2018). [b]Referenced from Saelao *et al.* (2019).

and HSPA2 and IFRD1 were associated with growth rate post treatment (Rowland, Saelao, *et al.* 2018).

Development of a low-cost genotyping by sequencing SNP panel

The aim here was to develop an economical 5K SNP panel for local Ghanaian and Tanzanian chicken ecotypes using targeted GBS for imputation to higher density. Low-density panel SNPs were selected from haplotype blocks on the 600K SNP panel genotypes, genomic regions on the GWAS studies from the NDV La Sota trials conducted on local ecotypes in Ghana and Tanzania (2.a.2) and on Hy-Line Brown chickens (2.c) in combination with integration of genes and signal pathways associated with enhanced NDV resistance identified through RNA-seq data analysis using two inbred lines (Fayoumi and Leghorn) (2.b), and approximately 100 SNPs from major histocompatibility complex (MHC) regions. A total of 188 birds were genotyped by GBS and an in-house shell script pipeline was utilised to obtain SNP calls. Our in-house pipeline was compared to a standard company pipeline and 600K SNP chip genotypes for the validation. Selected SNPs were evenly distributed across the genome, with at least one SNP in each megabase region. Comparison of the two pipelines revealed a good genotype match for the 5K SNP panel. The 5K GBS panel and SNP calling pipeline are important tools to aid selective breeding in African chicken ecotypes.

Genomic prediction and selection

The results of the natural velogenic NDV challenges lend themselves to genetic improvement of resistance to NDV using genomic selection. To this end, survival time data from the velogenic challenge experiment of naïve birds was used as the training data set to predict breeding values for survival time of the next generation of birds that were generated as selection candidates. The latter were genotyped using the GBS panel and imputed to 600K. Similarly, genomic predictions of other traits were computed on the selection candidates, including growth rates and antibody response from the vaccine trials. Selection aimed to maximise response in survival time, while maintaining growth rates and antibody response. Responses to selection are currently being evaluated.

Characterization of Newcastle disease virus in Africa

NDV is an RNA virus that belongs to the family *Paramyxoviridae* (Rima *et al.* 2019). While the virus has a single serotype, it is quite genetically diverse with at least 22 different genotypes. Newcastle disease viruses are grouped into two classes based on the nucleotide sequence of the fusion (F) protein gene (Dimitrov *et al.* 2016, 2019; Snoeck *et al.* 2013). Class I viruses have only one genotype (I) and consist mainly of lentogenic strains commonly isolated from wild birds worldwide and less frequently found in domestic poultry (Afonso 2021). Class II viruses display a higher level of diversity with at least 21 different genotypes (I-XXI) (Dimitrov *et al.* 2016, 2019) comprising velo-, meso-, and lentogenic strains, which have been found in a wide range of both domestic and wild bird species globally (Afonso 2021).

Most Class II genotypes have been found in Africa with the majority of reports of recently characterised genotypes (XIV-XVIII) originating from regions of the African subcontinent (da Silva *et al.* 2020; Snoeck *et al.* 2013). Although ND is endemic in Africa, there is a paucity of published reports on the distribution and diversity of NDVs circulating across the continent. To investigate genotypes circulating in Tanzania and Ghana, we characterised NDVs obtained from suspect NDV cases among local chickens sampled for studies under the current project as well as for previous NDV studies. Molecular characterisation was performed using a combination of methodologies. First, we utilised the MinION, a third-generation portable sequencing device from Oxford Nanopore Technologies, for on-site sequencing of the NDV F gene hypervariable region of 28 NDVs, 24 from Tanzania and 4 from Ghana, detected among poultry as previously described (da Silva *et al.* 2020). Second, we characterised 4 NDVs detected among local poultry sampled in a live bird market setting in Tanzania using a combination of conventional PCR, Sanger sequencing and Ion Torrent sequencing (Tsaxra *et al.* 2023) at the University of Pretoria, South Africa and Inqaba Biotech (Pretoria, South Africa).

Reference partial and full NDV F gene sequences were used for genotyping and phylogenetic analyses. The NDV sequences were compared with other relevant NDV sequences to the African region deposited in GenBank using the Basic Local Alignment Search Tool (BLAST) and also with isolates of known genotypes (Dimitrov *et al.* 2019). Multiple sequence alignments of the NDV partial F gene were conducted using Geneious Prime and the MAFFT plugin (Katoh and Standley 2013). Phylogenetic trees were constructed using the maximum likelihood method (da Silva *et al.* 2020; Tsaxra *et al.* 2023) with 1000 bootstrap replicates.

The phylogenetic analyses revealed NDV genotypes V, VII and XIII and genotype XVIII among poultry sampled in Tanzania and Ghana, respectively. In Tanzania, all nine genotype V isolates clustered together and with other sequences (Figure 1) that were previously classified as subgenotype Vd (Diel *et al.* 2012). Genotype V has been previously reported in Tanzania (Goraichuk *et al.* 2019; Msoffe *et al.* 2019; Yongolo *et al.* 2011). However, the length of the reported sequences is short, limiting their use in phylogenetic studies with our detected strains. Sequences from NDV strains originally from northern Tanzania (Mbeya) (Yongolo *et al.* 2011), Uganda, and Kenya were included in the analysis and showed clustering with our strains from Morogoro. These findings and results by others (Byarugaba *et al.* 2014; Msoffe *et al.* 2019) suggest uncontrolled border trading of live birds as a potential mechanism for introduction of these now endemic strains into central Tanzania.

Five Tanzania strains were classified as genotype VII.2 (Figure 2), one being the first report of genotype VII in Tanzania (da Silva *et al.* 2020). This strain was isolated in a commercial poultry flock in the Mwanza lake region, the others were detected as part of disease surveillance efforts in the Morogoro live bird market. Considering the distance between Mwanza and Morogoro (~950 kms), these findings could be the result of multiple introductions into the country (Tsaxra *et al.* 2023). NDV genotype VII strains have been detected in neighbouring countries (South Africa, Zimbabwe, Mozambique, and Zambia) suggesting spread of genotype VII isolates across the southeastern region of Africa. Additional surveillance with characterisation of NDV strains in poultry would be necessary for investigating sources of NDV dissemination and genetic diversification across this region.

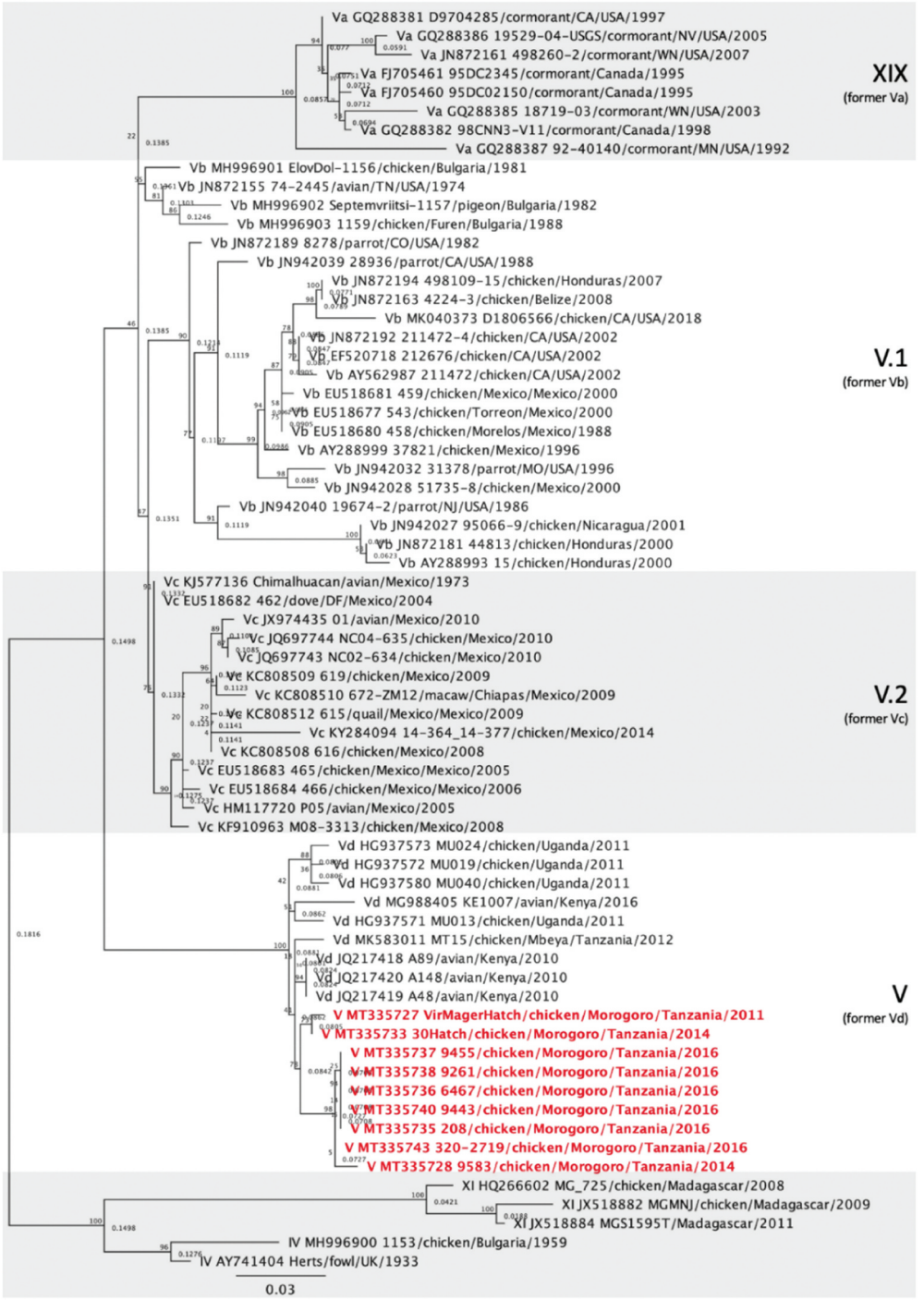


Figure 1. Phylogenetic analysis of NDV genotype V isolates using a 615bp fragment of the fusion gene. Nine of the sequences were collected from diseased chickens in Morogoro, Tanzania (bolded). Maximum likelihood method with 1,000 bootstrap replicates was used for the analysis (modified from source: (da Silva *et al*. 2020)).

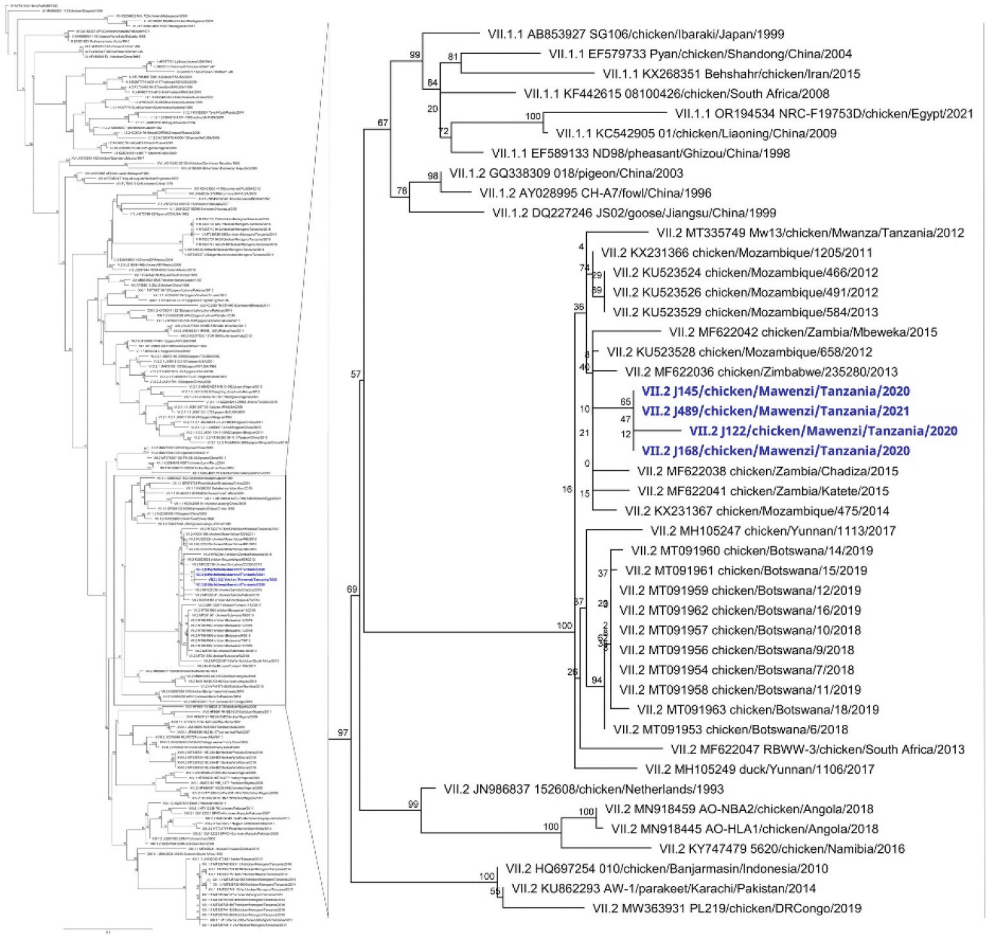


Figure 2. Phylogenetic analysis of NDV genotype VII isolates using a 598bp fragment of the fusion gene. Five of the sequences were collected from sick chickens in Morogoro, Tanzania (bolded). Maximum likelihood method with 1,000 bootstrap replicates was used for the analysis (modified from source: (da Silva *et al*. 2020)).

Most of the sequences detected through our sampling efforts belong to genotype XIII ($n = 13$). The homologies among these strains ranged from 92.5% to 100%, and while most of the sequences were highly homologous, the majority of samples originated from chickens sampled in a live bird market setting in Morogoro, Tanzania. The virus detected in a sample collected from a chicken in Mtwara, Tanzania in the northern region of the country, had a lower homology. According to the characterisation method utilised for this study, the sequences from Tanzania belong to sub genotype XIII1.1, which also encompasses strains detected in Zambia, Burundi, and South Africa (Figure 3).

In Ghana, all 4 studied sequences belonged to genotype XVIII.b (Figure 4). Genotype XVIII NDV strains usually circulate in West Africa (Mauritania, Benin, Mali, Togo, Côte d'Ivoire, and Nigeria) (Samuel *et al*. 2013). The sequences were obtained from samples obtained in Wa and Pokoasi, Ghana (~675 kms apart). The studied sequences share 97.7 to 99.8% homology to each other and are likely variants that originated from a common

Figure 3. Phylogenetic analysis of NDV genotype XIII isolates using a 615bp fragment of the fusion gene. Thirteen of the sequences were collected from sick chickens in Morogoro, Tanzania (bolded) and Mtwara, Tanzania (bolded and yellow). The maximum likelihood method with 1,000 bootstrap replicates was used for the analysis (modified from source: (da Silva *et al*. 2020)).

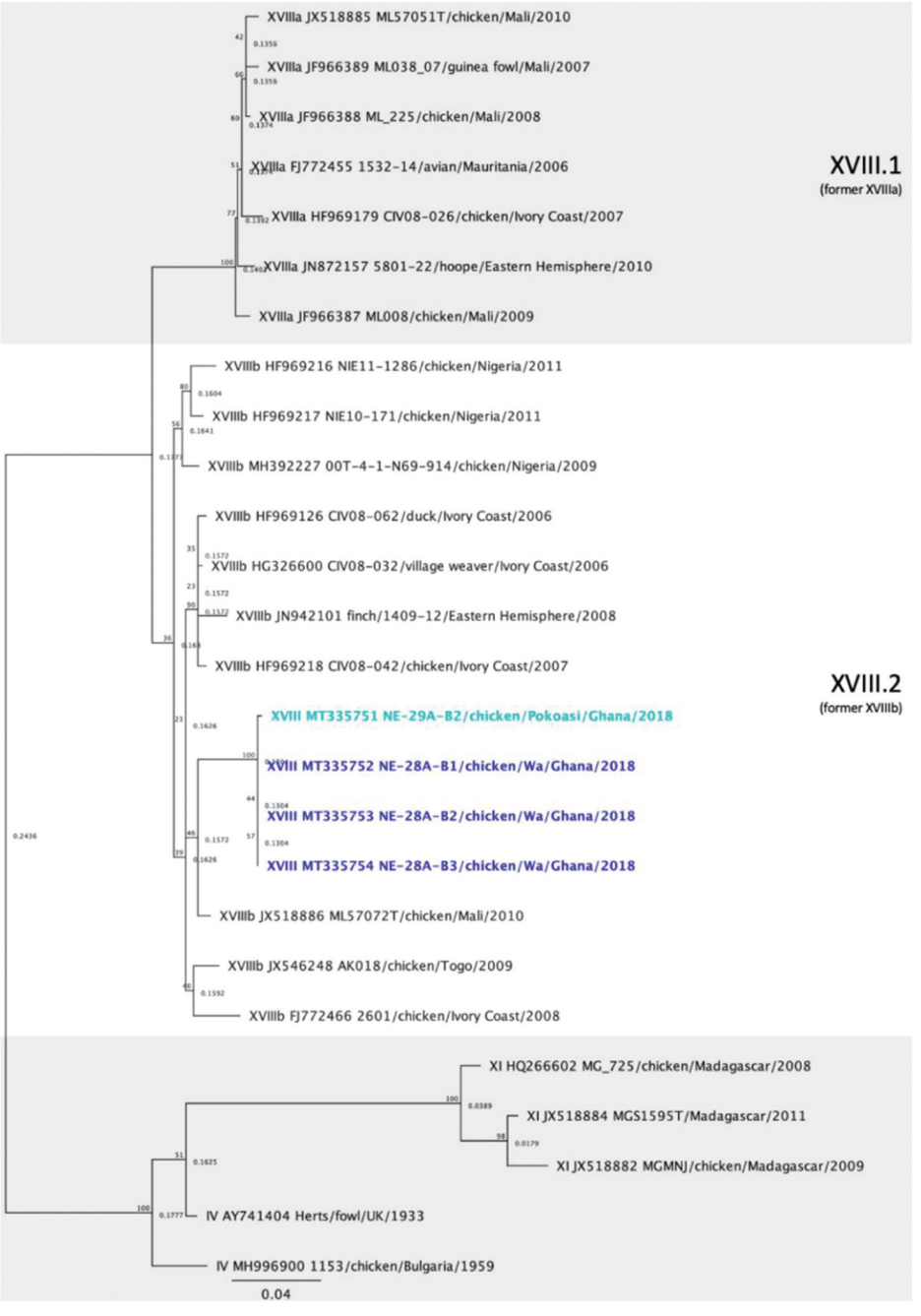


Figure 4. Phylogenetic analysis of NDV genotype XVIII isolates using a 615bp fragment of the fusion gene. Three of the sequences were collected from diseased chickens in Wa, Ghana (bolded) and one from Pokoasi, Ghana (bolded and teal). Maximum likelihood method with 1,000 bootstrap replicates was used (modified from source: (da Silva *et al*. 2020)).

ancestor. The nucleotide difference between these strains and the closest ancestor is ~ 5% suggesting that the Ghana strains are evolving independently from other subgenotype XVIII.b strains detected across West Africa.

Our surveillance and characterisation efforts provide insights into the circulating NDV genotypes in Tanzania and Ghana and underscore the importance of epidemiological surveillance to understand introduction and dissemination of infectious diseases in poultry. In addition, this information can be used in crafting prevention and control programmes in addition to drive policy to protect borders from devastating animal diseases.

Value-chain assessment of improved chicken ecotypes

Poultry health challenges in Central Tanzania and Northern Ghana

The GIP IL implemented poultry value-chain assessments in 2019–2020 in Northern Ghana and Central Tanzania to assess poultry health constraints in the value chain, and value chain actors' preferences for enhanced Newcastle disease resistance traits in local chicken ecotypes. ND was commonly reported as a major disease constraint in these locations, with resulting fatalities particularly impactful on men and women producers and on traders (Enahoro *et al.* 2021). A total of 64 focus group discussions were held, with about 12–15 participants per session. A total of 976 value chain actors participated in the discussions – women made up 45% of all participants.

Common production systems are small-scale semi-intensive and extensive scavenging poultry production systems (Ouma *et al.* 2023). Newcastle disease ranked as the highest cause of morbidity and mortality in chickens. Mortality rates ranged between 21% and 23% in the Dodoma and Singida regions in Tanzania, and 40% in Upper East in Ghana (Tables 3 and 4). The disease occurred mainly during the months coinciding with the dry season in both countries.

Other health challenges among poultry flocks include worm infestation, fowl pox, coryza, and coccidiosis. In most cases poultry diseases were not definitely diagnosed due to shortage of veterinary service providers and lack of laboratory diagnostic facilities. The high flock morbidity due to worm infestation in the Northern region are associated with poor access to veterinary services and lack of knowledge in appropriate husbandry practices, indicated as key constraints by the poultry farmers especially those based in rural locations (Ouma *et al.* 2023). Interventions that enhance farmer access to inputs, and extension services to improve farmer capacities in poultry husbandry, nutrition, and biosecurity will be key for transforming the village poultry systems.

Table 3. Morbidity and mortality rates due to poultry diseases in Tanzania.

	Singida		Dodoma	
Disease	Morbidity rate (%)	Mortality rate (%)	Morbidity rate (%)	Mortality rate (%)
Newcastle (ND)	23.3	22.8	22.5	21.3
Fowl pox	6.0	3.8	3.3	1.5
Coryza	3.0	2.0	3.0	1.8
Coccidiosis	6.5	4.5	3.8	2.8
Colibacillosis	5.0	5.0	1.3	1.3

Source: Ouma *et al.* (2023).

Table 4. Morbidity and mortality rates due to poultry diseases in Ghana.

	Northern		Upper East	
Disease	Morbidity rate (%)	Mortality rate (%)	Morbidity rate (%)	Mortality rate (%)
Newcastle (ND)	3.0	0.3	49.3	40.5
Fowl pox	5.3	2.5	13.8	7.0
Worm infestation	12.5	10.0	0.5	0.3
Chemical poisoning	11.3	11.3	0.0	0.0
Indigestion	2.5	1.8	0.0	0.0
Coccidiosis	0.0	0.0	4.5	1.8

Source: Ouma *et al.* (2023).

The high transaction costs associated with poor infrastructure, long distances and low business potential in rural areas prevent the private sector from investing in these areas where most poultry producers are located. Models with high potential for success in delivery of health care services in the rural communities are those that focus mainly on the delivery of quality veterinary products and services that are affordable, enhance supply of quality drugs and vaccines in rural areas and are tailored to reach poorer producers and more women (Enahoro *et al.* 2021). Community-based approaches and increased use of technology such as mobile phones have much to offer, as do increased engagement and cooperation between, grass root institutions, private sector, and non-government organisations.

Preference for enhanced Newcastle disease resistance traits in local chicken ecotypes

We utilised choice experiment surveys to assess farmer trait preferences for local chicken ecotypes and the ND enhanced resistance attribute in particular. Several attributes identified through farmer focus group discussions and key informant interviews were included in the choice experiment study – the attributes comprised phenotypic, productivity and ND enhanced resistance traits. The attributes and their levels were combined using a fractional factorial design and presented to the farmers using pictorial cards such as in Figure 5.

The choice experiment survey was implemented to 600 chicken farmers in Ghana and Tanzania. Mixed logit model was used to model preference behaviour for the chicken attributes from the choice experiment data and assess potential demand or willingness to pay for the attributes. Results from both Tanzania and Ghana showed high preference and willingness to pay for productivity attributes such as number of eggs produced per clutch and ND enhanced resistance – survival with vaccination during a ND velogenic outbreak with ND vaccination regime (Tables 5 and 6).

For Ghana, phenotypic attributes such as colour of chicken feathers were also highly preferred as these are important during cultural functions. The results show that preference of a ND enhanced resistance attribute is important but breeding programmes need to take into consideration other preferred attributes, particularly the productivity attributes of chicken – egg production and body weight gain – in addition to other phenotypic attributes depending on context.

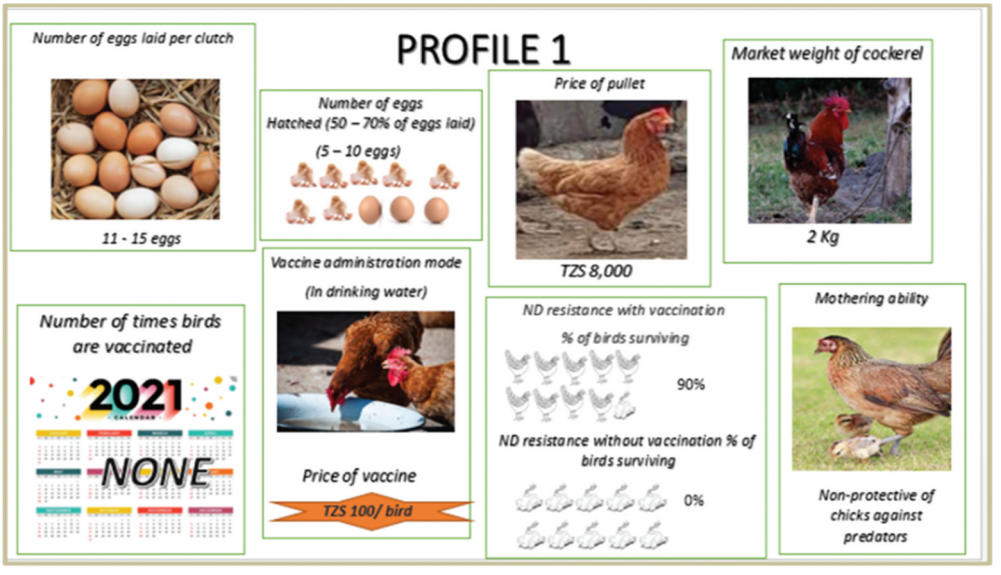


Figure 5. Choice option used to assess farmer trait preferences.

Table 5. Willingness to pay for chicken traits – Tanzania.

Chicken attributes	Willingness to Pay (TZS)	Standard Error
Chicken weight	12774.1***	1904.1
Protective	20052.1***	1037.4
100% survival with vaccination	4157.2***	851.1
16–20 eggs produced per clutch	7906.4***	1238.3
Above 20 eggs produced per clutch	9847.7***	872.3

Exchange rate: 1US$ = 2313 Tanzania Shilling.

Table 6. Willingness to pay for chicken traits – Ghana.

Chicken attributes	Willingness to Pay (GHC)	Standard Error
100% survival with vaccination	0.17392***	0.030
16–20 eggs produced per clutch	1.28456***	0.384
Above 20 eggs produced per clutch	2.83453***	0.481
Weight of mature bird	2.11421***	0.679
White feathers	3.20497***	0.628
Red feathers	2.29742***	0.608

Summary and future directions

Over the past decade, the GIP IL has taken complimentary approaches to improving food security in Africa through genetic improvement of resilience to NDV infection and heat stress in indigenous poultry. The GIP programme has developed an economical genomic selection SNP panel (around 5K SNPs across the chicken genome) for selection and breeding with the major goal of improving the survival time after velogenic NDV infection along with reducing virus shedding, improving vaccine efficacy, egg production and growth rate. The first generation of genomic selection has been conducted (2–5% males and 20% females). Due to the nature of low-medium heritability of the survival time, it is expected that multiple generations of selection are needed in order to observe

a marketable genetic improvement on the survival time in the face of velogenic NDV infection. In addition, natural exposure velogenic NDV challenges on each generation of selection is needed to re-train breeding value prediction models and finally validation of genomic selection panels.

The GIP IL has strengthened capacity, including both institutional and human capacity for poultry genetics and disease research. For the improvements in institutional capacity, we renovated both poultry breeding facilities and animal challenge facilities, and strengthened molecular laboratory equipment capacity in both Sokoine University of Agriculture and the University of Ghana. For human capacity strengthening, a total of 17 graduate students, many research staff, and more than 900 individuals have received training in relevant topics, such as biosecurity, virology, immunology, genetics, experimental design, NDV challenge models, sample collection and processing, husbandry and health management, molecular assays, and data analysis.

Novel NDV strains discovered through the programme have provided new information on the diversity and geographic distribution of circulating strains and risk factors for NDV infection among local poultry. This data contributes to our body of knowledge on the molecular epidemiology of NDV in Africa and strategies for NDV prevention and control. Furthermore, we have also conducted poultry value-chain assessments in Ghana and Tanzania, which provide essential information on the socio-economic factors of uptake and market demand, how an ND resilience enhanced chicken line and its characteristics suit the local production systems and potential economic viability of the improved line, the market segments and collective actors who are important for initial promotion, and business strategies for scaling.

Indigenous chickens have a robust resilience for adaptation to the rough environment of smallholder farmers in the rural area of African countries, such as scavenging systems with limited feed resources. With the escalating climate change leading to weather extremes, the resilience of indigenous chickens will come handy in mitigation some of these effects to the animal health and production. However, our business model analysis has indicated that lower productivities of these ecotypes for egg production and growth rate could be a limiting factor for private sector uptake and scale up. Future programmes could employ a hybrid model by utilising indigenous birds that are genetically improved for survival time and vaccine efficacy as a sire, and using dual-purpose birds as a dam to take advantage of the hybrid offspring's good performance in both egg production and growth rate as well as more hardiness in the local environment compared to commercial broilers and layers.

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Disclosure statement

No potential conflict of interest was reported by the author(s).

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