# Investigation of Genetic Resistance to Newcastle Disease in Local Chickens in Tanzania using Natural Challenge by Field Velogenic NDV Strains

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## Summary

Genetic resistance to Newcastle disease among three ecotypes of Tanzanian local chickens, Ching'wekwe, Kuchi and Morogoro MEDIUM was investigated by natural challenge with endemic velogenic strains of Newcastle disease virus (vNDV). The study was designed to determine variations in susceptibility and response to NDV among the three ecotypes. Naturally NDV-infected seeders were introduced into flocks of susceptible chickens under a controlled environment. 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 three days and then every 12 hours thereafter until 29 days post-exposure. Date and time of death was recorded, and post-mortem examinations performed for each dead chicken. Lesions on the trachea, proventriculus, intestines, and caecal tonsils were scored for severity ranging from 0 to 3. Linear models were used for survival days, post-exposure growth rate and average lesion score. Preliminary results indicate that post-exposure weight reduction in Ching'wekwe was significantly less than in Morogoro Medium and Kuchi. No significant differences were observed in the lesion scores and survival times among the three ecotypes after exposure. More data is being collected for more comprehensive analysis.
*Keywords: Newcastle disease, innate resistance, natural infection, local chicken ecotypes*

## Introduction

Poor husbandry and diseases limit village scavenging chicken production in Africa, thus significantly compromising income generation and a source of protein to the growing population. According to Kitalyi et al. (1998), Newcastle disease (ND) accounts for more than 80% mortality among village chickens. Preserving the village chicken population by preventing ND, is envisaged to be the first step in improving productivity and commercial value of local chickens in Africa, this is because outbreaks of ND can decimate all unvaccinated chickens in the village. Given the diversity of local chickens, it is of interest to know how the different ecotypes cope with the disease and to select for strains of chickens that can survive NDV infection. Cole and Hutt (1961) provided one of the earliest known associations between animal genetics and disease resistance by showing existence of innate resistance to Newcastle disease, however, limited studies with modern genetic tools have been made. Initial investigations by Msoffe et al. (2001) provided insights on variations in susceptibility to disease among local ecotypes in Tanzania. This study was designed to compare susceptibility and response to NDV of three common ecotypes of chickens namely Ching'wekwe (Ching), Kuchi and Morogoro medium (MoroMed).

## Material and methods

### Animal Population and La Sota NDV Inoculation

Birds used in this study are a sub-population from 3 sequential replicates of a previous experiment in which they were vaccinated with $10^7$EID50 of a lentogenic La Sota NDV strain via the oculo-nasal route. Except for the age of exposure, all replicates underwent exact similar experimental protocol. During the period between the La Sota and the vNDV challenge, anti-NDV antibody titres in sera (20 randomly selected birds/ecotype/rep) were measured to ensure that they were at negligible levels before the vNDV challenge. Table 1 summarizes the bird population structure used in this study.

### Velogenic NDV Challenge (natural challenge)

Birds from the three replicates, along with a random sample of non-vaccinated birds (controls) from each replicate were transferred to a biosecure natural challenge facility and spread over 5 pens. Two sick chickens from the market were confirmed for vNDV infection via RT-qPCR targeting the F-gene of NDV and then transferred to the natural challenge facility to be mixed with 30 "seeder" chickens (non-NDV vaccinated). Seeders with overt clinical signs at 2 days post-exposure were then placed into the 5 pens for the natural challenge trial at a rate of 2 infected birds per 100 experimental birds.

*Table 1. Number of birds (and control birds) used for Velogenic NDV Challenge.*

| Replicate | Ecotype | | |
|---|---|---|---|
| | Ching | Kuchi | MoroMed |
| I | 25 (6) | 18 (7) | 33 (5) |
| II | 37 (2) | 30 (4) | 71 (0) |
| III | 34 (3) | 37 (3) | 56 (5) |

### Data Collection after natural challenge

Mortalities were recorded 3 times per day for the first three days and then 2 times per day thereafter until 29 days post-exposure (dpe), when the experiment was terminated. Body weights were taken at 0, 7, 9, 11, 13, 15, 17, and 25 dpe, from which post-exposure growth rate was calculated by linear regression of weight on dpe. Lesions on trachea, proventriculus, intestines and caecal tonsils were scored for severity as follows: 0, 0.5, 1, 2, or 3.

### Statistical Analysis

Three traits: survival days (SD) post-exposure, average lesion score, and post-exposure growth rate were analysed. All traits were analysed with linear models for the fixed effects of ecotype, rep and pen, as well as all possible interactions. The final models chosen based on the statistical significance ($p < 0.05$) of the effects. Least square (LS) means from these models were used for ecotype comparisons. The effect of vaccinated (treatment) versus unvaccinated birds (control), was investigated by survival curve analysis on birds from our experiment with birds that had not been vaccinated prior to the natural trial. This was the only analysis that included data on the control birds. All models were implemented in R.

## Results & Discussion

### Post-challenge growth rate

Results indicated that there was no significant difference between the ecotypes. There was however a significant effect of replicate, indicating that either the age group, or the time lapse between vaccination and exposure to the velogenic strain had an effect on growth. The youngest and most recently vaccinated birds (rep III) had a positive growth rate, while the other reps had negative growth rates (Figure 1).

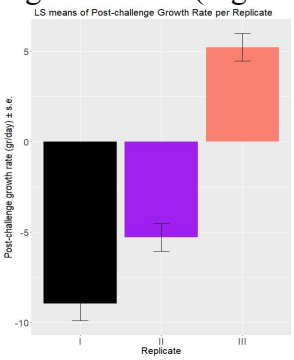


*Figure 1. Least square means of post-challenge growth per replicate*

**Lesion score**

LS means of lesion score in three ecotypes across three replicates is presented in Figure 2a. There was a significant effect of replicate, and suggestive effect ($p< 0.1$) of replicate by ecotype, shown by the difference in the ranking of ecotypes across replicates. The biggest difference between ecotypes was seen in the rep I and II birds.

**Survival days**

LS means of survival days in three ecotypes across three replicates is presented in Figure 2b. There were significant effects of replicate and replicate by ecotype interaction. Ching'wekwe had significantly lower survival days than other two ecotypes only in replicate I.

**Survival rate**

The survival rate of three ecotypes across three replicates in both NDV vaccinated (treatment) and non-vaccinated (control) groups are presented in Figure 3. There was a significant effect of replicate by treatment and treatment by ecotype interaction, therefore, further analyses for each replicate were conducted. In general, as expected, the treatment groups had significantly higher survival rate than the control groups. In replicate I, vaccinated Ching'wekwe birds had significantly lower survival rate than other two ecotypes.

## Conclusions

To our knowledge, this is the first study that natural exposure challenge of vNDV in African local ecotypes was conducted. Preliminary analysis suggest that Ching'wekwe was relatively susceptible, compared to Kuchi and Morogoro Medium based on four phenotypic parameters evaluated, although significance was not observed in all replicates, probably due to limited number of individuals used per ecotype. An additional two replicates on these three ecotypes with the same experiment will be completed and be integrated for more powerful analysis. In addition, all NDV vaccinated birds in this study were genotyped with the Affymetrix 600K SNP panel and associations between genotypes and phenotypic data of post-challenge growth rate, lesion score, survival days and survival rate will be further analysed.

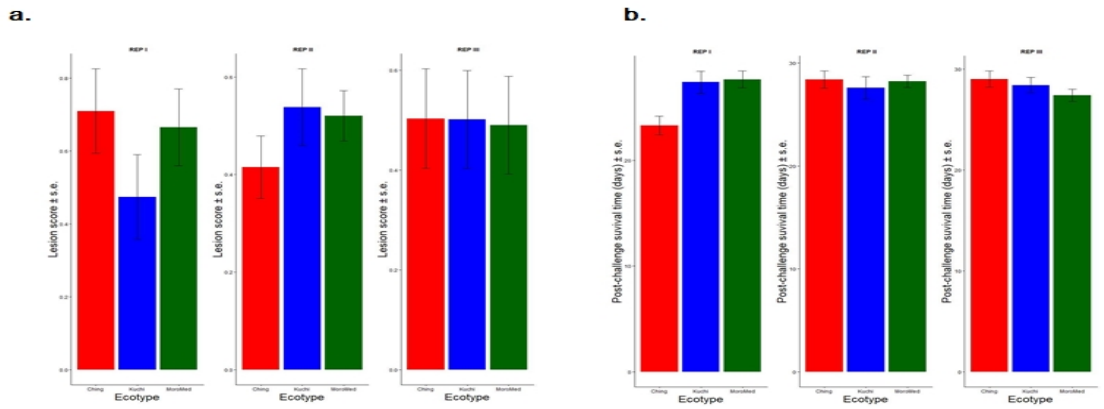


*Figure 2. Least sequare means of lesion score (a) and survival (b) per replicate*

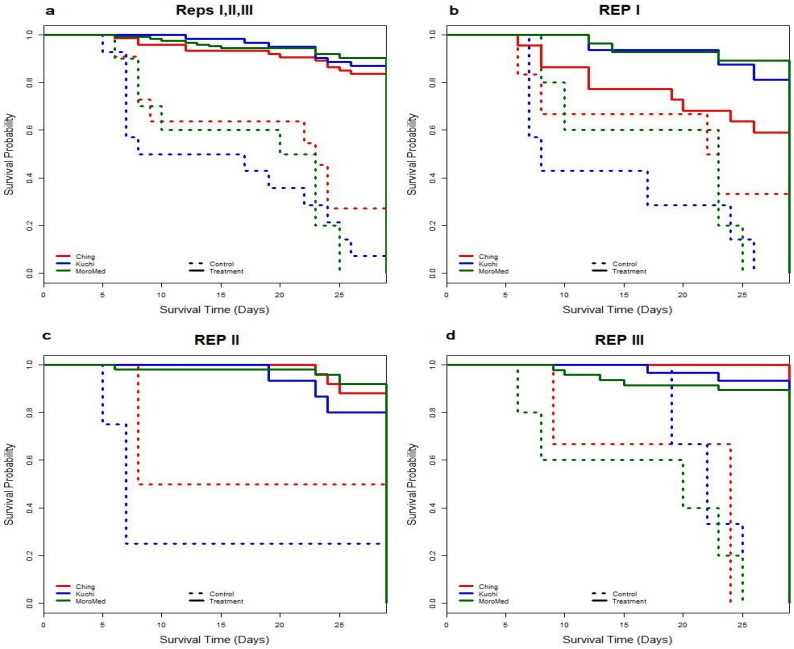


*Figure 4. Survival curves of three ecotypes in three replicates for both NDV vaccinated group (treatment) and non-vaccinated group (control) for combined analysis (a) and individual replicate analysis (b-c).*

## Acknowledgements

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