ASSOCIATION OF Mx GENE WITH NEWCASTLE DISEASE AND THE GENETIC DIVERSITY IN NIGERIAN INDIGENOUS CHICKENS AND THEIR CROSSES WITH NAPRI$_X$

BY

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NOVEMBER, 2018

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B. Agric (ABU) 2012 (M.Sc./AGRIC/44204/2012-2013)

A DISSERTATION SUBMITTED TO THE SCHOOL OF POSTGRADUTE STUDIES, AHMADU BELLO UNIVERSITY, ZARIA
IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF MASTER OF SCIENCE DEGREE IN ANIMAL SCIENCE

DEPARTMENT OF ANIMAL SCIENCE,
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NOVEMBER, 2018

DECLARATION

I declare that the work in this Dissertation entitled: “Association of Mx Gene with Newcastle Disease and the Genetic Diversity in Nigerianindigenous Chickensandtheir crosses with $NAPRI_x$” has been carried out by me in the Department of Animal Science of Ahmadu Bello University under the supervision ofProfessor G. N. Akpa (late) and Dr. M. Kabir. The information obtained from literature has been duly acknowledged in the text and a list of references provided. No part of this Dissertationwas previously presented for the award of another degree and/or diploma by me or anyone else at this or any other institution.

_____ _____

Mohammed GanDahiru Date

CERTIFICATION

This dissertation entitled: ASSOCIATION OF Mx GENE WITH NEWCASTLE DISEASE AND GENETIC DIVERSITY OF NIGERIAN INDIGENOUS CHICKENS AND THEIR CROSSES WITH NAPRI$_X$ byMohammed GaniDahiru meets the regulations governing the award of the degree of Master of Science in Animal Science of Ahmadu Bello University Zaria, and is approved for its contribution to knowledge and literary presentation.

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DEDICATION

This work is dedicated to the memory of my late mother, Malama Halima Anace and to the entire Muslim ummah.

ACKNOWLEDGEMENTS

All praises and gratitude to Almighty Allah, the most Knowledgeable, the wisest, the creator and sustainer of the universe. May the peace and blessing of Allah be upon his messenger and Prophet Muhammad (SAW), the members of his household and all those who follow him on the path of righteousness.

I will like to express my heartfelt appreciation and thanks to my Supervisory team,Professor G.N Akpa (late) and Dr. M. Kabir for finding time to go through my work and offer valuable advice. I must mention the untiring effort they put to see this work through to completion. Their guidance and assistance have been very invaluable.

I am also very grateful to the Head of Department and the entire staff of the Department of Animal Science. In particular, mention must be made of DrsRabiu Hassan, SB. Abdu and Professor Zaharraddin for their moral contributions to this work. I must also mention my friends and colleagues in the faculty, MalamLukmanJunaid, Ibrahim Taofeeq and Abdulkareem Guru of CPPR who all helped with the statistical Analysis. May Allah reward them all.

I will also like to thank Dr. Waziri and Dr. M.Babashani of the Faculty of Veterinary Medicine for their valuable contributions. I will also like to thank Dr. Y.Y. Pala of the Department of public health, Avian Influenza Laboratory for his tenacity in guiding me through the Molecular aspect of this work.

Finally, I must not forget my family members, especially my mother who passed away while I was compiling this work. May Allah forgive and reward her for all her efforts in seeing me through school since the death of my father. My sisters, Anti Kulu, Mrs, Amina, Zainab, Rukayya and Fatima were very encouraging. Mention must be made of Baba Sheikh, Meenu, Sulaiman, and all of you out there too numerous to mention. My friends and colleagues in

Mando Kaduna, Ibrahim kailani, Usman, Bashir Sani, Dr. Balogun, MalamYakasai, Dr. Ndams, Malam Mustapha, Jacob Jafiya, I thank you all. I reserve for special mention the love of my life, Rukayya, my children Hannatu and Uthman for standing by me and serving as a source of comfort. May Allah bless you. Mention must be made of Professor U.S. Abdullahi and AlhajiAdamu IdrisKuta. I remain eternally grateful to you.

ABSTRACT

An experiment was conducted using a total of one hundred and ten indigenous chickens of three genotypes and their crosses, comprising of thirty from each genotype and twenty from the indigenous breeds' crosses with NAPRIx. The experiment was to determine the susceptibility or otherwise of the various genotypes and their crosses to Newcastle disease and to determine whether the Mx gene is involved in any resistance. The genetic diversity of the various genotypes was also assessed. The birds were inoculated with the Newcastle disease virus KUDU-113 strain. They were bled on days 0, 3, 7, 14, 21, 28 and the collected blood samples were used for molecular analysis in the laboratory while data from the molecular work was analyzed using the R statistical package. Geometric mean titre of Newcastle disease anti body was highest (4.7) in Normal feathered(NF) and lowest (3.1) in the naked neck (NN) while the NAPRIx × NF had the highest titre of 1024 on day 28. Mortality and morbidity results showed that morbidity was 100% in all genotypes and mortality was highest in NF (87.5%) and lowest in NN (45%). The ELISA results showed that the naked neck (NN) had the highest percentage of antigen (21%) while the NAPRIx×FF had the least (4%). The Association of the Mx gene genotype with resistance to Newcastle disease showed that the frequency of the resistant A allele was higher than the G allele for susceptibility. Analysis of genetic diversity showed that the number of alleles ranged from 2-3 for NF; Polymorphism information content (PIC) ranged from 0.637 to 0.976. The allele number for FF ranged from 2-3 with PIC mean of 0.981. Expected (He) and observed (Ho) heterozygosity were 0.601 and 0.765 respectively. Allele number range from 2-3 for NN, mean PIC was 0.825 while Ho and He were 0.627 and 0.513, respectively. The allele number ranged from 2-3 for all crosses. Mean PIC for NAPRIx × NF was 0.838, Ho and He 0.569 and 0.520, respectively. Mean PIC for NAPRIx × NN was0.948 while Ho and He were

0.608 and 0.575, respectively. Mean PIC forNAPRIx×FF was 0.967 while Ho and He was 0.706 and 0.592, respectively. Analysis of molecular variance (AMOVA) showed an estimated variation of 0.142 among the populations and 0.042 among individuals while variation within individuals accounted for 98% of the total variation. The paired wise population matrix of Nei's genetic distance showed that the longest distance of 0.409 was observed between FF andNAPRIx ×NF while the shortest distance (0.177) was between FF and NAPRIx×NN.Dendogram analysis also shows that the studied chicken populations formed five clusters of genetic dissimilarity. Based on this finding, it can be concluded that the indigenous chicken populations have the ability to resist Newcastle disease and that the Mx gene is involved in that resistance and that the naked neck was more resistant to the disease. It is therefore, recommended that crosses involving the indigenous chicken and other Broiler strains should be carried out with the normal feathered. Further experiments should also be carried out, involving the naked neck and its crosses to determine to what extent the Mx gene is involved.

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CHAPTER ONE

1.0 INTRODUCTION

1.1 Background of the Study

Nigeria is the most populous country in Africa and is home to about 172 million domestic poultry (FAOSTAT, 2011).The livestock sector is vital to the socio-economic development of Nigeria and contributes about 9-10% of Agricultural GDP(FAO, 2006). Consequently, livestock represents an important source of high quality animal protein, providing about 36.5 percent of the total protein intake of Nigerians. It is one of the highest investments in Agriculture with net worth of ₦250 billion.The poultry sub-sector is the most commercialized of all Nigeria's agricultural sub-sectors (AICP, 2007). Smallholder poultry production makes use of local or indigenous genetic resources, which differ from commercially bred poultry in several respects:

The birds are adapted to a harsh environment where resources are often limited and where challenges imposed by climatic conditions, pathogens and predators are severe.

They are often utilized for several purposes simultaneously, and therefore may outperform specialized commercial breeds when scored for multipurpose productivity.Native chicken constitutes 80% of the poultry birds in Nigeria and are sources of high quality protein, reserved for times of celebrations and a good source of income for rural families (Abubakar*et al*., 2008).

Newcastle disease (NCD) is a major problem of poultry in many parts of the world (Alexander and Senne 2008; Munir*et al*., 2012; Luo*et al*., 2013). The causative agent of the disease is Newcastle Disease virus (NDV) which is the prototype virus of the avian Paramyxovirus type 1 in the family Paramyxoviridae (Lamb *et al*., 2005; Alexander and Senne, 2008). The Paramyxoviruses isolated from avian species have been classified into 9 serotypes designated as APMV-1 to APMV-9 (Alexander, 2003). While the disease is enzootic in Africa and Asia (Mathivanan*et al*., 2004), it is exotic in Europe and North America. Despite the advances made

so far in the control of the disease by vaccination and bio security, devastating outbreaks of NCD still occur in many parts of the world (Capua *et al*., 2002). This is because there are many factors that make the control of NCD very difficult. NDV has a very wide host range, affecting at least 241 avian species (Alexander and Senne, 2008). Newcastle disease virus (NDV) has been classified into velogenic, mesogenic and lentogenic strains on the basis of their pathogenesis and virulence. Chickens with Newcastle disease have severe neurological and respiratory signs and show decreased egg quality and production (Islam and Nishibori, 2009).

The Myxovirus (Mx) gene codes for a protein that has direct antiviral activity and inhibits a wide range of viruses by blocking an early stage of the viral replication cycle. The chicken Mx protein has been reported to exhibit antiviral activity against the influenza virus and the vesicular stomatitic virus (Ko*et al*., 2002). Mx plays a major role in IFN-induced host defence. Extensive research about resistance genes in poultry have been undertaken, the major included histocompatibility complex MHC genes, which relates with the resistance to diseases such as the Marek's disease and Rous sarcoma (Niikura*et al*., 2004; Nikolich*et al*., 2004; Xu*et al*., 2007). Mx proteins are key components of the innate antiviral state induced in many species such as human, mouse and chicken among other organisms (Haller *et al*., 2007) and belongs to the super family of GTPases (Luan *et al*., 2010).

Genetic diversity within a livestock species is reflected in the range of types and breeds that exist and in the variation that is present within each genotype. It has been shown that differences among breeds substantially exceed those within breed, suggesting that geneticvariation among breeds is a major component of the readily accessible livestock diversity.Losses of unique types and breeds compromise access to their unique genes and gene combinations, (Adebambo, 2003).If genetic diversity is to decline, then selection of stock for commercially desired traits

will beunproductive for developing improved breeds. Given this prospect then,maintenance of genetic diversity must be given a major consideration in Nigeria's livestock improvement programmes in this millennium (Adebambo, 2003).Ohwojakpor*et al*. (2012) recommended a further study of the level of genetic diversity in different populations so as to bring about improvement in the performance of chicken in the developing countries.

1.2 Justification

There is no effective treatment for Newcastle disease; however, the use of prophylactic vaccines and maintenance of strict bio-security measures can reduce the likelihood of outbreaks. Thus, the ability of chickens to mount an antibody response to NDV plays a key role in controlling Newcastle disease outbreaks, while understanding the molecular basis of immune response to NDV is important for the control of avian Newcastle disease. The antibody response to the same virus differs between chicken breeds , and selection for an antibody response may improve disease resistance in chickens (Luo*et al*., 2013). Despite the advances made in the diagnosis of and vaccination for Newcastle disease since it was first described in 1926, the disease continues to negatively impact on poultry producers by infecting birds worldwide (Alexander *et al*., 2012). From 2006 to 2009, the most widespread animal diseases in terms of the number of countries affected, were rabies, Newcastle disease (NCD) and Bovine tuberculosis (Anonymous, 2011). Newcastle Disease ranked as the fourth most important disease in terms of the number of livestock units lost for poultry species, behind highly pathogenic avian influenza, infectious bronchitis, and lowly pathogenic avian influenza (Kapczynski*et al*., 2012; Anonymous, 2011).

Genetic resistance to a disease is an attractive solution because it is reliable, long lasting, and environmentally sound. Also, chicken lines selected for Mareks Disease (MD) resistance have been shown to have greater vicinal immunity and higher egg production than susceptible lines (Liu *et al*., 2003).

This study was therefore designed to test the following hypothesis:

H0: That the Mx gene is not involved in immunity in Nigerian indigenous chicken populations and their crosses against Newcastle disease.

HA: That the Mx gene is involved in immunity in Nigerianindigenous chicken populations and their crosses against Newcastle disease.

1.3 Objectives of this study is to determine

1. The host response to NCD infection in different Nigerian indigenous chicken Populations and their crosses
2. The genetic basis for NCD host relationship between Nigerian indigenous Chicken Populations and their crosses
3. The various genetic diversity in the Nigerian indigenous chicken populations and their crosses

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Nigerian Indigenous chicken

The Nigerian indigenous chicken as small bodied, poor feed converters and as such slow growing and poor meat animals (Ajayi 2010). The high ambient temperature obtainable in the tropical environment coupled with poor feeding and bad management practices can lead to stress and thus affect the performance of the birds.

The indigenous chicken has been classified based on some criteria. One of the bases of classification is in the line of feather and plumage (whether normal or frizzled). The normal feathered type is the most common of all types of indigenous chicken in Nigeria. It is found in all ecotypes and is further addressed as the Yoruba ecotype, Fulani ecotype etc. based on location (Ajayi 2010). The Nigerian indigenous chicken is also classified on the basis of body form (long legged, dwarf or naked neck) and also on colour (white, brown black or mottled). It was agreed by many researchers that the Nigerian chicken is a light breed, often with single comb and that black and brown plumage, laced with various colours such as mottling are common (Adebambo, 2005).

Another line of classification is based on the occurrence of some major genes such as naked neck gene (Na), dwarf gene (D) and the frizzle feathered gene (F) (Ikeobi*et al*., 1996).Certain major genes have been found potentially useful to the tropical production environment either because of their direct effect on production or because of their indirect effect on quantitative trait loci. Among these major genes with indirect effects are the feather distribution (Naked neck, Na) and feather structure (Frizzle, F) genes; both naked neck and frizzle genes have been associated with increased heat resistance (Ibe 1998). Garces*et al*. (2001) reported the superiority of individual with frizzle and naked neck genes both singly and in combination over individuals with normal

feathering for body weight and egg traits. The local birds seen in villages may have been crossed with exotic cocks in earlier years through the cockerel exchange programme amongst others, but such gene may have been dispersed and lost in the population because of unplanned breeding programme and absence of selection (Njue*et al*., 2002).The naked neck gene is incompletely dominant with Na/na birds showing an isolated tuft of feathers on the ventral side of the neck above the crop, while Na/Na birds either lack this tuft or it is reduced to just a few pin feathers or small feathers. The resultingbare skin becomes reddish, particularly in males as they approach sexual maturity (Somes 1990; Lyimo*et al*., 2013). The reduction in feather coverage in naked neck birds permits convectional heat loss from the animal surface, thereby leading to improved thermo-regulation under the prevailing conditions. Mohammed *et al*. (2005) reported the superiority of naked-neck indigenous chickens over two other Sudanese local fowls in terms of live weight. Similarly, Merat (1990) reported that in high temperatures near 30°C or higher, naked neck birds had a better laying rate, mean egg weight, egg shell strength and carcass yield than normal feathered birds. This could also be attributed to the divergence in the genetic makeup of the chickens, uncontrolled breeding and environmental extremes(BeigiNassiri*et al*., 2007; Hassen*et al*., 2009).

Musa *et al*. (2015) reported higher body weight in Normal feathered at twenty weeks in the purebreds (1083.0 ± 22.74) while in the main crosses, the frizzled-naked had a higher body weight at the same age (1173.2 ± 27.73).In terms of reproductive performance, Idowu (2016) reported better performance in a cross betweenNAPRIx× FF in number of eggs set, percentage fertility and hatchability (19.00, 82.60 and 11.60), respectively. Idowu (2016) also reported a significant higher body weight in NAPRIx NF (1469.62), shank length (8.21), thigh length (13.20) and a significant higher body length in NAPRIx NN (74.91). Muhammad (2015)

reported significant higher value of 27.00 for breast girth and (9.62) for tibia length in Naked neck at twenty weeks of age.

2.2 Newcastle Disease

Newcastle disease is the most important disease of local poultry in Nigeria (Echeonwu*et al*., 2008) since the first documented outbreak of the disease in Nigeria in 1952 (Hill *et al*., 1953) around Ibadan area. The disease strikes all year affecting all ages and sex (Alexander 2003, Haque 2010; Iram*et al*., 2013) but is most devastating during the cold harmattan period (between November- January) causing huge economic losses to both local and exotic birds under intensive system of management. Newcastle disease has a wide host range, including approximately 241 species of birds (Madadger*et al*., 2013). The disease is characterized by respiratory, reproductive, gastrointestinal and nervous systems impairment (Nanthakumar*et al*., 2000;Tiwari*et al*., 2004). Most commonly affected species include chickens, turkeys, ducks, pigeons (Zhang *et al*., 2011), guinea fowl, Japanese quails and many wild birds of all ages (Nanthakumar*et al*., 2000).

In many developing countries of the world, village poultry serves as a source of income generation and are greatly utilized during festivities. In such countries and many others, Newcastle disease continue to have negative effects on their means of livelihood (Mohamed *et al*., 2011) sometimes wiping out flocks entirely and with attendant decrease in egg production (Choi *et al*., 2010). As a result of the level of devastating effects of Newcastle disease on poultry, it is included in the "LISTED" agents (reportable disease) by the office International des Epizooties (OIE) as reported by Aldous and Alexander, (2001). Also, any outbreak of Newcastle disease must be reported (Cao *et al*., 2013) where certain criteria of virulence is met (Cattoli*et al*., 2011;Munir*et al*., 2012).

2.2.1 Etiology

Taxonomically, NDV belongs to the order Mononegavirales, Family Paramyxovirinae (Cattoli*et al*., 2011). The subfamily is divided into five genera: Morbillivirus, Respirovirus, Henipavirus, Rubulavirus and Avulavirus (Miller *et al*., 2009). All the avian Paramixoviruses (APMVs) belong to the genus Avulavirus. The virus exists in 10 serotypes; APMV-1 to 10 (Waheed et *al*., 2013) and all virulent isolates belong to serotype 1 (APMV-1) which is synonymous with NDV (Miller *et al*., 2009; Cattoli*et al*., 2011).

2.2.2 Epidemiology

The very first outbreak of Newcastle disease occurred in 1926 in Java, Southeast Asia and in Newcastle upon-Tyne, England (Seal *et al*., 1995, Arifin*et al*., 2011). The second outbreak (panzootic) began in the Middle East in the late 1960s and spread to other countries till 1973. The third panzootic caused by neutrotropic form which is termed as pigeon Paramyxovirus type 1 appeared in the Middle East in the late 1970s. Europe is the next continent to be hit in 1981 before spreading rapidly throughout the globe (Mase*et al*., 2002).

2.2.3 Pathogenesis

The incubation period from the time of infection to development of disease varies from 2 to 15 days depending on several factors (Saif*et al*., 2008, Alexender and Senne. 2008). The pathogenicity of the virus, host species and age, host immune status, secondary infections, stress, environmental conditions, the amount of virus transmitted, and the route of transmission can all play a role in determining the severity of disease and the length of incubation. Disease severity has led to classification of NDV isolates under three distinct pathotypes (Seal *et al*., 2000, DiNapoli*et al*., 2009, Tan *et al*., 2009). Infection of lentogenic NDV (LoNDV) isolates can range from non-apparent to mild respiratory or gastrointestinal disease in adult chickens. When

replication is limited to the gastrointestinal tract, the infection is often classified as asymptomatic enteric due to lack of respiratory symptoms. Young susceptible birds may develop a more serious respiratory disease that can lead to death due to increased susceptibility to secondary infection. LoNDV are categorized as lentogenic NDV and are commonly used as sources for vaccine production. Mesogenic (mNDV) isolates are considered of intermediate virulence. Infection is typically systemic and can lead to development of a non-fatal respiratory disease. Drop in egg production can be seen in layers infected with mNDV. Rarely symptoms of the nervous system can develop, but mortality is usually low following infection. Pigeon paramyxovirus isolates usually fall in the mNDV classification due to their intermediate virulence and neurologic symptoms.

Highly virulent velogenic (vNDV) viruses are also systemic and can cause high morbidity and mortality. Velogenicviscerotropic ND (vvND) causes acute infection of the gastrointestinal mucosa resulting in hemorrhagic lesions and death (Alexander and Senne2008, OIE 2008). Clinical signs may begin with weakness, increased rate of breathing, listlessness, and prostration. During course of infection, green diarrhoea, muscular tremors, and paralysis of the extremities may be apparent. Oedema may be seen on the head especially around the eyes. In highly susceptible flocks, mortality can be as high as 100%.

2.2.4 Diagnosis

Due to its clinical resemblance to highly pathogenic avian influenza (AI), rapid and accurate diagnosis becomes very important (Khan *et al*., 2010). Clinical diagnosis based on history, signs and lesions may establish a strong index of suspicion but the laboratory confirmations must be carried out. Haemagglutination and haemaggluttination inhibition tests, virus neutralization test, Enzyme-linked immunosorbent assay (ELISA), plaque neutralization test and reverse-

transcriptase polymerase chain reaction (RT-PCR) can be used for confirmation of the NDV (Chaka *et al*., 2013). Using modern technologies, new diagnostic techniques are being developed for identification and differentiation of NDV strains (Rezaeianzadeh*et al*., 2011).Various authors have reported on the seroprevalence in various environments. In Kaduna, (17%), Zamfara (32.5%) and Bauchi states (56.3%).(Nwankiti *et al.,B* 2010, Jibril*et al*., 2014; Abraham-O *et al*., 2014; Amos and Chibuogwu, 2015).

2.2.5 Prevention and control

Control of Newcastle in Nigeria is basically by vaccination. Currently, live and inactivated vaccines are used all over the world (Shim *et al*., 2011;Xiao *et al*., 2013). According to Cho *et al* (2008) administration of the vaccine is through various routes and schedules right from the point of hatching until they assume maturity. Killed virus oil emulsions are usually administered parentally prior to the onset of egg production. According to Chukwudi*et al* (2012), vaccinated birds are not totally protected from virus replication and shedding although they are prevented from clinical disease. As a result, prophylactic vaccination is not employed in developed countries (OIE, 2012) and there is wide use of vaccines on commercial flocks (Munir*et al* 2012). Anti NDV antibody titres of flocks are continuously been monitored and flocks are revaccinated to maintain the protective antibody titres. Oil based vaccines are being used on breeders and layers prior to the onset of egg production for long term immunity (Nadeem*et al*., 2004). Antibody titres of breeders flock is useful in maintaining the antibody titres of the progeny which protect chicks from Newcastle disease during the first week of life. Despite extensive vaccination, outbreaks of the disease are still common (Shabbir*et al*., 2012). In order to overcome such outbreaks, poultry producers are using various combinations of live and killed vaccines in their flock.

Bio-security measures have been proven to prevent to some limit, the occurrence of Newcastle disease in flocks. In commercial flocks, contacts with domestic poultry should be prevented. Wild birds and animals should be kept far away from commercial flocks. Measures of bio-security should include bird proof pens, feed and water supplies, limiting visits of non-personnel or unauthorized persons, disinfecting vehicles and equipment to the farm. Insect pests and mice should also be controlled (Shabbir*et al*., 2012).

2.3Myxo virus Gene

The Mx protein confers resistance activity to Ortho-myxovirus infection and has been found in many organisms, including yeast (Rothman *et al*., 1990) and vertebrates ranging from fish to humans (Plant and Thune, 2004). As an interferon (IFN)-associated protein, it contains conserved tripartite guanosine tri- phosphate binding sites and a Leu zipper (Pitossi*et al*., 1993). Various Mx proteins differ widely with respect to intracellular distribution and biological activities (Bernasconi*et al*., 1995). Isolation of cDNA and an IFN-inducible promoter of the Mx gene have been reported in the chicken (Bernasconi*et al*., 1995). The chicken Mx protein is a predominantly cytoplasmic form and consists of 705 amino acids (Bernasconi*et al*., 1995).Ko*et al*. (2002) reported polymorphisms and differential antiviral activities of the chicken Mx gene in 15 breeds in which a specific amino substitution at position 631 (Ser to Asn) was found to determine differential antiviral activities. The protein has activity only when G takes the place of A at position 2032 point with the resultant change of 631th amino acid changes from Serine to Asparagine (Seyame*et al*., 2006). The substitution also showed skewed allele frequencies in different chicken populations, with a much higher frequency of the favourable allele A in native breeds than in highly selected lines(Li*et al*., 2006). The amino acid variation of Asn (allele A) at position 631 was found to be specific to positive antiviral Mx/resistance,while that of Ser (allele G) was specific to negative Mx/susceptibility (Sulandari *et al* 2009;Pagala *et al.*, 2013).The

research on Mx gene in Chicken was first started in 1995 by Bernasconi*et al*. (1995) as cited in Tian*et al*. (2010) who got the full-length Chicken Mx gene from the IFN (interferon) induced Leghorn fibroblasts by RT-PCR, encoding a total of 705 amino acids. The Chicken Mx gene contains 14 exons and its translations codons are located in the second exon (Schumacker*et al*., 1994; Sulandari *et al* 2009).

2.4Genetic Diversity

Genetic diversity within a livestock species is reflected in the range of types and breeds thatexist and in the variation that is present within each genotype. It has been shown that differences among breeds substantially exceed those within breed, suggesting that genetic variation among breeds is a major component of the readily accessible livestock diversity.Losses of unique types and breeds compromise access to their unique genes and gene combinations(Adebambo, 2003).

The presence of a breed with desired characteristics in place, and with the controlling gene athigh frequency, greatly enhances the efficiency of improvement process. The recombination off favourable genes within major commercial stocks under intensive selection has alloweddevelopment of animals with productive capacities that greatly exceed those of their ancestors.

The store of genetic diversity within commercial breeds has similarly permitted promptresponses to changes in societal demands (Adebambo 2003).If genetic diversity is to decline, then selection of stock for commercially desired traits will beunproductive for developing improved breeds.Technological advances in animal breeding andreproductive control have the potential to change this situation. Given this prospect then,maintenance of genetic diversity must be given a major consideration in Nigeria's livestockimprovement programmes in this millennium.

Temporary but significant reductions in population sizes are referred to as population bottlenecks whose effects can vary depending on both the size to which the population is reduced and the

duration of the bottleneck. Limited population size can lead to a loss of genetic variation and subsequent loss of evolutionary potential. A general result as drift proceeds in small populations is a deficiency of heterozygotes. This is also a common result when there has been inbreeding. In fact genetic drift and inbreeding are related phenomena. One effect of inbreeding is to increase the frequency of homozygotes,and thus, necessarily decreases the frequency of the heterezygotes(Reed and Richard 2003; Ojango *et al*., 2011).

A wide diversity of indigenous chicken breeds in the tropics could form the basis forGenetic improvement and diversification to produce more productive breeds adapted to specific environments and requirements (Horst, 1989). Therefore, the estimation ofGenetic diversity of the local chickens should be carried out to support conservation strategies and utilizations of their performance values. There is a growing recognition that preservation of local chicken breeds is not only important to ensure the livelihoods of poor farmers who depend on these breeds, but their conservation should be regarded as a national policy, as locally adapted chicken genetic resources could become future assets in breeding programs. Decision making in conservation requires specification of model parameters such as diversity, breed values, extinction probabilities and conservation potentials (Simianer, 2005). Beside phenotypic characterization, assessment of genetic characterization of local breeds is a prerequisite for this purpose (Hassen*et al*., 2009). Efforts should be made to preserve the important and unique characteristics that Nigerian local chicken genetic resources possess (Adebambo, 2003).The various genetic distances parameters are given in table 2.1 as reported by several authors. There are wide variations in the values which could be due to several factors which may include sample size, geographical locations, types and sources of markers and also sources of experimental

birds. For any marker to be categorized as informative, it must have a PIC value above 0.50 Olasunkanmi (2010).

Table 2.1:Genetic distances measures of various indigenous chicken populations

Na	Ne	He	Ho	PIC	Sources
6.05	-		0.77	0.71	Hassen*et al*. (2009)
9.04	3.13	0.65	0.60	0.57	Keambou*et al*. (2014)
6.09	-	0.63	6.00	-	Ngo Thi Kim Cuc (2010)
5.70	-	0.62	0.65	-	Lyimo*et al*. (2013)
3.4	2.56	0.56	0.73	0.49	BeigiNassiri*et al*. (2007)
5.6	3.36	0.68	-	0.61	Rajkumar*et al*. (2008)
4.7	-	0.61	0.316	0.55	Abebe*et al*. (2015)
-	3.40	0.67	0.581	0.66	Wei *et al*. (2013)
5.6-9.9	5.0-7.2	0.8-0.86	0.5-0.58	0.76-0.82	Ohwojakpor*et al*. (2012)
-	-	-	0.2-0.64	-	Granevitze*et al*. (2007)
0.40-0.60	-	-	-		Berthouly *et al*. (2009)

pNa= Number of alleles, Ne= effective number of alleles, Ho observed heterozygosity, He= expected heterozygosity, PIC=Polymorphism information content

Table 2.2: Analysis of molecular variance

Sources of error	Sum of Squares	Variation	% Variation	Source
Between pops	142.075	0.45848	4.8133	Lyimo*et al.* (2013)
Within pops	2575.917	9.06672	95.1867	
Total	2717.992	9.52520	100	
Sources of error	Sum of Squares	Variation	% Variation	Source
Among pops	160.8	0.3621	4.27214	Keambou*et al.* (2014)
Among ind	2840.539	0.91252	10.36227	
Within ind	2331.500	7.51741	85.366	
Total	5332.849	8.80614	100	
Sources of error	Sum of Squares	Variation	% Variation	Source
Between groups	165.96	0.04	0.57	Bohani*et al.* (2016)
Between pops	151.01	0.74	10.51	
Within pops	2605.26	6.26	88.92	
Total	2922.24	7.06	100	

Pops= populations, ind= individuals

2.5Kudu-113 Virus

The complete genome length of KUDU-113 is 15,192 nucleotide (nt).It was isolated from domestic duck in Nigeria in 1992 and it contains six genes in the order 3=-NP-P-M-F-HN-L-5=, with coding sequence lengths of 1,470 nt, 1,188 nt, 1,095 nt, 1,662 nt, 1,716 nt, and 6,615 nt, respectively. Phylogenetic analysis classified the isolate as a member of class II, genotype XVII. The strain KUDU-113 contains three basic amino acid residues between positions 113 and 116 of the fusion protein cleavage site and a phenylalanine at position 117 (113RQKR2F117). Such a motif in the deduced amino acid sequence of the cleavage site is specific for virulent NDV isolates. Although a genotype XVII genome from West Africa (2008/Mali/ML007/08) was previously published in GenBank (JF966385), this sequence is incomplete and genetic data are missing at both genome termini. Availability of complete genome sequences will facilitate further studies of NDV genetic diversity(Shittu*et al*., 2016).

CHAPTER THREE

3.0 MATERIALSAND METHODS

3.1 Location of the Experiment

This study was conducted at the Department of Animal of Animal Science Research Farm, Faculty of Agriculture, Ahmadu Bello University, Zaria. Animal Science Research farm is located atlatitude11°09′06′Nandlongitude7°38′55′E,atanaltitudeof706mabovesealevel in Northern Guinea savannah of Nigeria. The relative humidity is very high during the wet season and could reach about 70-80% but falls to about 15-20% during the dry season (Ovimaps, 2014).

3.2 Description/Management of Experimental Birds

A total of 110 sexually matured chickens were used for this experiment. Thirty birds each from the three indigenous genotypes (15 each of males and females), namely Naked neck, frizzle feathered and normal feathered were obtained from the local markets in Zaria and its environs while 20 of the crosses between the various genotypes and a broiler strain (NAPRIx) from National Animal Production Research Institute (NAPRI) were obtained from the Experimental farm, Department of Animal Science, Ahmadu Bello University Zaria. The birds were kept together in a deep litter system and birds were fed a grower diet from commercial feed mill containing 14% crude protein and 2850 kcal metabolizable energy and water was provided *ad libitum*(NRC, 1994).

3.3 Procedure/Methodology

3.3.1 Challenge test

Kudu-113 strain of the Newcastle disease virus used in this experiment was obtained from the National Veterinary Research Institute (N.V.R.I), Vom. A totalof 45chickens were divided into 6 groups according to their genotype. The Newcastle disease virus was diluted with Phosphate Buffered Saline (PBS) of pH 7.2 at the ratio of 1:10 to give virus lethal Dose fifty (LD50) of

106.5ml-1 while 0.2 ml of the solution was used in challenging the birds. The birds were challenged intra nasally and were monitored daily for clinical signs, morbidity and mortality for 28 days post infection. Post mortem examination was carried out on chickens that died.

3.3.2Blood sample collection

3.3.2.1Serum samples

The experimental chickens were bled on days 0, 3, 7, 14, 21 and 28 post infections (Wakamatsu*et al*., 2006). Blood sample (4 ml) was collected from each challenged bird through the wing vein using sterile 21G needle into sterile vacutainer tubesearly in the morning between 6-7am (for all blood sample collections). The collected samples were kept at room temperature to clot. Serum samples were harvested after centrifuging the vacutainer tubes at 1500 rpm for 10 minutes, into sterile cryotubes and kept at -20°C until tested for Haemagglutination Inhibition test.

3.4.1 Red blood cells of chicken

Blood sample (2ml) was drawn from 46 birds onto 1 ml of Alsevers solution (anticoagulant) into a sterile syringe. The RBC solution was washed 3 times first with Alsevers solution and subsequently with Phosphate Buffered Saline (PBS) with a pH 7.4 and centrifuged at 1000 rpm for 5 minutes. A 1% suspension of the RBC in PBS was prepared for use in Haemagglutination (HA) and Haemagglutination inhibition (HI) tests as described by Allan and Gough (1974).

3.4.2 Haemagglutination test (HA)

Phosphate Buffered Saline (PBS) 0.025 ml was dispensed into each well of a microtitre plate and 0.025 ml of antigen was dispensed into the first well. For accurate determination of the HA content, serial double dilutions, i.e. 1/2, 1/4, 1/8, 1/16, was used.Two fold dilutions of 0.025 ml of the antigen suspension across the plate was made, 0.025 ml of 1% (v/v) chicken RBCs to each

well was also dispensed.The plate was gently tapped for proper mixing, and then allowed the RBCs to settle for about 15-20 minutes at room temperature. HA was determined by tilting the plate and observing the presence or absence of tear-shaped streaming of the RBCs. The titration reading the highest dilution gives complete HA (no streaming); this represents 1 HA unit (HAU) and can be calculated accurately from the initial range of dilutions (OIE, 2008).

3.4.3 Haemagglutination inhibition test (HI)

PBS (0.025 ml) was dispensed into each well of a microtitre plate and 0.025 ml of serum was added into the first well of the plate. Two fold serial dilution of 0.025 ml volumes of the serum across the plate was made4 HAU of antigen in 0.025 ml was added to each well and left for 20minutes at room temperature.0.025 ml of 1%(v/v) chicken RBCs was added to each well and gently mixed and left to settle for 15-20 minutes at room temperature. The HI titre is the highest dilution of serum causing complete inhibition of 4 HAU of antigen. The agglutination was assessed by tilting the plates. Only wells in which the RBCs stream at the same rate as the control wells (containing 0.25 ml RBCs and 0.05 ml PBS only) would be considered to show inhibition.

The validity of the result was assessed against a negative control serum, gave a titre >1/4 (>22 or >log 22 when expressed as the reciprocal), and a positive control serum for which the titre should be within one dilution of the known titre. The HI titre was regarded as being positive if there is inhibition at a serum dilution of 1/16 (24 or log 24 when expressed as the reciprocal) or more against 4 HAU of antigen (OIE, 2008).

3.4.4 Elisa (The enzyme-linked immunosorbent assay)

The Elisa was performed using an IDEXX® kit according to the following procedure;

1 ml of diluent was added to dilution tubes 2 µl of test serum was then added to a dilution tube. Negative controls were added to plate: 0.1 ml to wells A1 and A2. Positive control was then added to plate: 0.1 ml to wells A3 and A4 and then incubated for 30 minutes. It was then washed with distilled water.100 µl of conjugate was added to all wells of the test plate and Incubated again for 30 minutes and washed with distilled water. 100 µl of TMB substrate was added to each well and Incubated for 15 minutes. 100 µl of stop solution was then added to each well.

The status of a sample is evaluated by the sample to positive ratio (S/P ratio):

$$\text{S/P ratio} = \frac{\text{Sample mean (mean of optical absorbance)} - \text{negative control mean}}{\text{Positive control mean} - \text{negative control mean}}$$

3.5 Mx Gene Genotyping

Specific primers (NE2, FUL and NE) were used to amplify the Mx gene. Restriction fragment Length Polymorphism (RFLP) method was used to determine the genotypes of the Mx gene. PCR products of the Mx gene were cut by Hpy 81 restriction enzyme that cuts at 631 sites (Sironi*et al*., 2010).The PCR products were separated by electrophoresis 2% agarose gel.

3.5.1 DNA extraction

Blood sample (4 ml) from the brachial vein in the wing (from 50 birds) was collected in plain tubes and stored at -20°C until analysis. DNA was extracted from the blood samples using a modified DNA purification kit (Gentra System PUREGENE DNA).

3.5.2 PCR Amplification

Seventeen (17) primers recommended by the FAO for genetic diversity (FAO, 2011) were used for this study. The primers were obtained from INQABA® of South Africa. The composition of the PCR reaction mixture with final volume of 10 µl in each PCR tube containing 1.5 µl DNA template, 2 µl Taq master mix (enzyme), 0.6µl of primer (0.3 µl each of reverse and forward form) and 5.9 µl of double distilled water. The PCR was carried out in the PCR Hybaid Express

system (PE 9600) under the following conditions: an initial denaturation step for 1 minute at 94°C, followed by 35 cycles of denaturation for 10 minutes at 94°C; annealing for 45 seconds at optimized primer annealing temperature (58-60 °C), extension for 60 seconds at 72°C for 600 seconds. Following this, 4 µl of PCR products were loaded onto a 12% polyacrylamide gel with PBR322 DNA MSPI as ladder and electrophoreses was carried out in a double cassette plates. Gels were stained with intercalating agent-ethidium bromide and visualization of samples was done under UV-trans-illuminator and photographed with a digital camera

.

Table 3.1: Primers used forGenetic diversity studies

Marker	Chromosome location	Primer Sequence (5' to 3') forward (F) and Reverse	Annealing Temp(C)	Allele Size Range (Bp)	Gene Bank Accession No.
ADL0112	10	F=GGCTTAAGCTGACCCATTAT R=ATCTCAAATGTAATGCGTGC	58	120 – 140	G01725
ADL0278	8	F=CCAGCAGTCTACCTTCCTAT R=TGTCATCCAAGAACAGTGTG	60	110-130	G01698
LEI0094	4	F=GATCTCACCAGTATGAGCTGC R=TCTCACACTGTAACACAGTGC	60	235-277	X83246
LEI0234	2	F=ATGCATCAGATTGGTATTCAA R=CGTGGCTGTGAACAAATATG	60	210-254	Z94837
MCW0020	1	F=TCTTCTTTGACATGAATTGGCA R=GCAAGGAAGATTTTGTACAAAATC	60	177-187	L40055
MCW0034	2	F=TGCACGCACTTACATACTTAGAGA R=TGTCCTTCCAATTACATTCATGGG	60	214-248	L43674
MCW0104	13	F=TAGCACAACTCAAGCTGTGAG R=AGACTTGCACAGCTGTGTACC	60	182-234	L43640
ADL0268	1	F=CTCCACCCCTCTCAGAACTA R=CAACTTCCCATCTACCTACT	60	102-116	G01688
MCW0206	2	F=CTTGACAGTGATGCATTAAATG R=ACATCTAGAATTGACTGTTCAC	60	221-249	AF030579
LEI0166	3	F=CTCCTGCCCTTAGCTACGCA R=TATCCCCTGGCTGGGAGTTT	60	354-370	X85531
MCW0295	4	F=ATCACTACAGAACACCCTCTC R=TATGTATGCACGCAGATATCC	60	88-106	G32052
MCW0183	7	F=ATCCCAGTGTCGAGTATCCGA R=TGAGATTTACTGGAGCCTGCC	58	296-326	G31974
MCW0067	10	F=GCACTACTGTGTGCTGCAGTTT R=GAGATGTAGTTGCCACATTCCGAC	60	176-186	G31945
MCW0330	17	F=TGGACCTCATCAGTCTGACAG R=AATGTTCTCATAGAGTTCCTGC	60	256-300	G32085
G32085	1	F=GTTGTTCAAAAGAAGATGCATG R=TTGCATTAACTGGGCACTTTC	60	205-225	G32016
MCW0111	1	F=GCTCCATGTGAAGTGGTTTA R=ATGTCCACTTGTCAATGATG	60	96-120	L48909
MCW0103	3	F=AACTGCGTTGAGAGTGAATGC	64	266-270	G31956

Table 3.2: Primers used for Mx Gene

Marker	Chromosome location	Primer Sequence (5' to 3') forward (F) and Reverse	Annealing Temp(C)	Allele Size Range (Bp)	Gene Bank Accession No.
NE2		F=CCAGAATGCATCAGAG GTGA R=TCCTTTCCATGCATT GTCTG	60	671-690	
FUL		F=ATAGAGCAAGCCAGAAGAACAGCAG R=GCTTTGACA AGGGTAGGCATATCAG	60	1453-1434 113-137	
NE		F=CCTTCAGCCTGTTTTTCTCCTTTTAGGAA R=CAGAGGAATCTGATTGCTCAGGCGTGTA	60	2432-2408	

3.6 Data Collection and Analysis

Blood samples were collected early in the morning and taken to the avian influenza laboratory in the Faculty of Veterinary medicine, Ahmadu Bello University, Zaria for Analysis. Data generated from HI and serological test were calculated using simple percentages and the geometric mean titre of Newcastle disease antibodies was calculated using the formula according to (CDC, 1988). Xgeo = n √x1, x2 …….xn. The Geometric mean of a sample of n positive observations is defined as the nth root of the product of the n numbers. Therefore, the geometric mean was calculated as X geo = antilog10 (1/n ∑log10x1) where x1 = the reciprocal of dilution.Observed bands in the Electrophoregramm were analysed and scored using the image LabTM. Observed allele frequencies were computed using the excel macros and the allele frequencies was used in the determination of both within and between-population genetic variation.

Genetic variation was determined as the Average observed and expected heterozygosity and was calculated using GenAlEx6.5 (Dieringer and Schlötterer, 2003). Analysis of molecular variance, AMOVA (Excoffier*et al*., 1992) was carried out using the GenAlEx 6.5(Peakall and Smouse, 2012) and was performed to quantify further the extent of population differentiation and the distribution of genetic variation in the sample populations.

A genetic distance measure was computed using the GenAlEx 6.5. Phylogenetic trees were constructed from (Nei, 1973) standard genetic distance (DS) and Cavalli-Sforza and Edwards genetic distance measure (DA) generated using the Unpaired Group with Arithmetic Mean (UPGMA) methods in the GENEPOP version 3.4 (Raymond and Rousset, 1955). The reliability of the constructed trees was examined according to (Felsenstein, 1985).

CHAPTER FOUR

4.0 RESULTS

4.1 Host response to inoculation with KUDU-113 virus of Newcastle disease

Table 4.1 shows the Geometric mean titre (GMT) of NDV Antibodies of three genotypes of Nigerian indigenous chickens and their crosses with NAPRIx. In the indigenous purebreds, the NN had a superior performance by developing antibodies geometrically (3.1-512) from Day 0 to day 28, followed by NF (4.7-128) in 28 days and the least in performance was FF (4.1-71). In the crossbreds, the best performance was inNAPRIx×NF (0-1024) which developed antibodies from 0 on Day 0 to 1024 on Day 28, followed by NAPRIx×FF (0-181) and the least was NAPRIx×NN (0-76).

Table 4.1: Geometric mean titre (GMT) of NDV HI Antibodies of Nigerian indigenous chickens and theircrosses

Genotype	GMT OF HI Antibody					
	day 0	day 3	day 7	day 14	day 21	day 28
NF	4.7	9.1	38	45.2	98.0	128
FF	4.1	5.2	17	31.0	57.0	71.0
NN	3.1	4.6	9.8	38.0	49.5	512
NAPRIx×NF	0.0	172	362	90.3	512	1024
NAPRIx×FF	0.0	17	182	57.0	79.0	181
NAPRIx×NN	0.0	65	71	167	76.0	76.0

NF= Normal feathered, FF= frizzle feathered, NN= Naked neck, NAPRIx×NF= cross with NF, NAPRIx×FF= cross with FF, NAPRIx×NN= cross with NN

4.2 ELISA results of Nigerian indigenous chickens and their crosses with NAPRIx challenged with KUDU-113 virus

The results of ELISA (Enzyme-Linked Immunosorbent Assay) of three genotypes of Nigerian indigenous genotypes and their crosses with NAPRIx is shown in Figure 4.1.The highest titre (seroprevalence) was recorded in NN (21%) while the least was in NAPRIx × FF with 4%. Overall, the NN had a higher titre (21%), FF (20%) and NF (16%) in the indigenous breeds. In the crosses with NAPRIx, NF× NAPRIx had a higher titre of 20%, while NN× NAPRIx and FF× NAPRIx had 19% and 4% respectively.

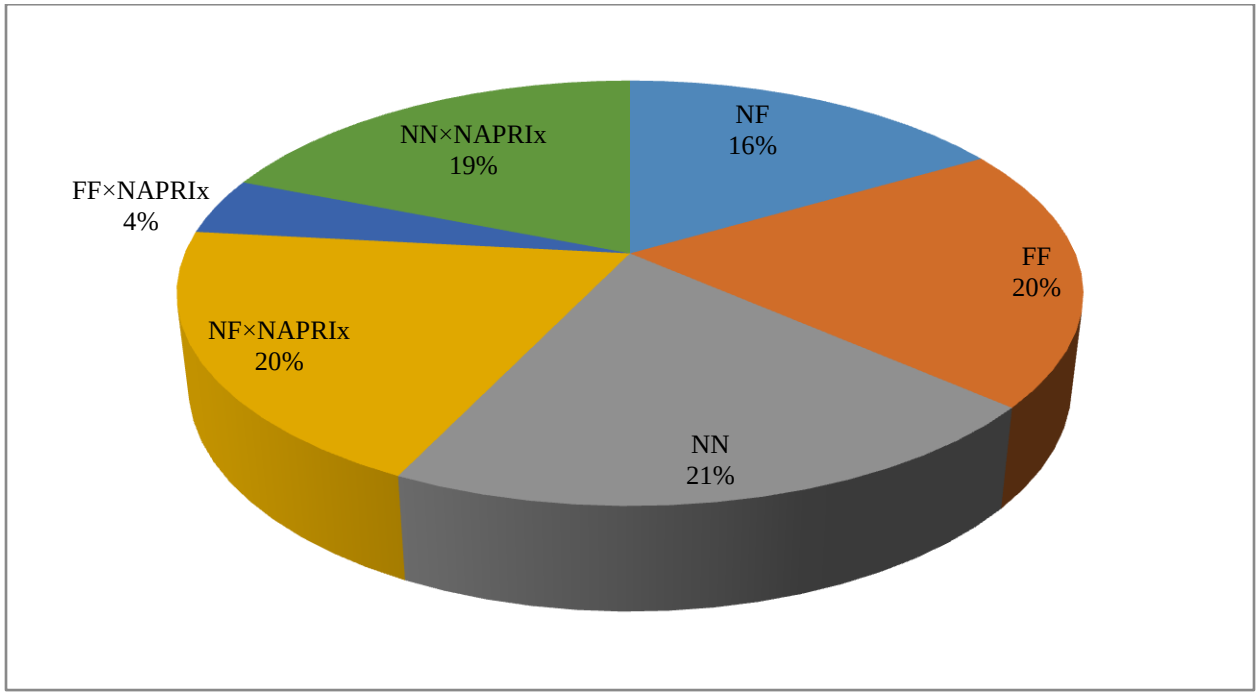

Fig 4.1 ELISA Results Of Nigerian Indigenous chickens and their crosses with NAPRIx. NF=Normal feathered, FF= Frizzled feathered, NN=Naked neck,NAPRIx×NF= cross with NF, NAPRIx×FF= cross with FF, NAPRIx×NN= cross with NN, ELISA= enzyme-linked immunosorbent assay

4.3 Mortality and Morbidity of Three Genotypes of Nigeria's Indigenous Chickens and their crosses

Table 4.2 shows the result of mortality and morbidity of the challenged birds. The morbidity in all genotypes challenged with KUDU-113 NDV was 100%. The onset of clinical signs was earlier (3 days) in the NF and FF. Onset of mortality in days was earlier in the FF (7) and the rate of mortality was highest in in NF (87.5%). The disease also lasted for 27 days in the NF.

Crossing of the local chickens with NAPRIx exhibited similar onset of clinical signs at 4 days after infection with 100% morbidity in the three genotypes. The earliest onset of mortality was for NAPRIx×FF (10 days) with high mortality of 75% within 15 days duration of the disease. The longest onset of mortality was for NAPRIx×NN (15 days) with 50% mortality in 18 days duration of the disease. The cross-breds, NAPRIx×NF was intermediate in terms of onset of mortality (13 days), mortality rate (50%) and duration of the disease (16 days).

Table 4.2: Results of Mortality and morbidity among three Genotypes of Nigerian indigenous Chicken and their Crosses Challenged with Kudu 113 NDV.

Parameters	Genotypes					
	NF	FF	NN	NAPRIx×NF	NAPRIx×FF	NAPRIx×NN
Sample size	9	9	9	6	6	6
Onset of Clinical signs(days)	3.00	3.00	4.00	4.00	4.00	4.00
Onset of Mortality (days)	11.0	7.00	16.0	13.0	10.0	15.0
Morbidity (%)	100	100	100	100	100	100
Mortality (%)	87.5	50.0	45.0	50.0	75.0	50.0
Duration of the disease (days)	27.0	20.0	18.0	16.0	15.0	18.0

NF: normal feathered, FF: frizzle feathered, NN: naked neck,NAPRIx × NF = cross with NF, NAPRIx × FF= crosss with FF, NAPRIx × NN= cross with NN

4.4Mx gene Genotypes found in Nigerian Indigenous Chickens and their crosses

The Association of Mx gene Genotype with resistance to Newcastle disease in Nigerian indigenous chicken and their crosses is presented in table 4.3. The frequency of the A Allele was higher than the G allele in all Genotypes except in NAPRIx×NN which had 0.45. The highest frequency obtained for the A allele was 0.65 in NF and NN.

Table 4.3:Association of Mx gene Genotypes in Nigerian indigenous chickens and their crosses with NAPRIx

		Genotype Frequency			Gene Frequency	
Chicken type	No	AA	AG	GG	A	G
NF	10.0	0.40	0.50	0.10	0.65	0.35
FF	10.0	0.30	0.40	0.30	0.50	0.50
NN	10.0	0.50	0.30	0.20	0.65	0.35
NAPRIx×NF	6.00	0.33	0.50	0.17	0.58	0.42
NAPRIx×FF	6.00	0.33	0.60	0.07	0.63	0.37
NAPRIx×NN	6.00	0.20	0.50	0.30	0.45	0.55

NF=normal feathered, FF=frizzled feathered, NN=naked neck,NAPRIx × NF = cross with NF, NAPRIx × FF= crosss with FF, NAPRIx × NN= cross with NN

4.5 Average measures of genetic diversity of Nigerian indigenous chickens

Table 4.4shows the number of alleles (Na), Effective Number of alleles (Ne), observed (Ho) and Expected (He) heterozygosity, Polymorphism information content (PIC) of Nigerian indigenous chicken populations.

The number of alleles for NF ranged between 2 and 3 with a mean of 2.941 and all markers used gave positive results. Effective number of allele also ranged from 2 to 3 with a mean of 2.492. The value for PIC ranged from 0.637 to 1.099 with a mean of 0.976. The Ho and He ranged from 0.33 to 1 with a mean of 0.75 and 0.44 to 1 with a mean of 0.59, respectively.

The Allele number for FF ranged from 2-3 and all seventeen (17) markers gave positive result. The values of PIC ranged from 0.637 to 1.011 with a mean of 0.981. The Ho and He ranged from 0.444 to 1 and 0.333 to 1 with a mean of 0.765 and 0.601, respectively.

Allele number for NN ranged from 2-3 and all seventeen (17) primers gave positive results. Values for PIC ranged from 0.637 to 1.099 with a mean of 0.825. The value for Ho and He ranged from 0.444 to 0.667 and 0.33 to 1 with a mean of 0.627 and 0.513, respectively.

Table 4.4: Average measures ofgenetic diversity of 17 primers loci in three Nigerian indigenous chicken populations

	NF					FF					NN				
	Na	Ne	He	Ho	PIC	Na	Ne	He	Ho	PIC	Na	Ne	He	Ho	PIC
ADL0112	3	3.00	0.67	1.00	1.09	3	2.57	0.67	0.611	1.01	3	2.00	0.67	0.50	0.87
ADL278	3	2.57	0.66	0.67	1.01	3	2.57	0.67	0.611	1.01	3	2.00	0.67	0.50	0.87
LEI0094	3	2.57	0.66	0.67	1.01	3	3.00	0.67	0.667	1.09	3	2.57	1.00	0.61	1.01
LEI0234	3	2.57	0.66	0.67	1.01	3	2.57	1.00	0.611	1.01	2	1.39	0.33	0.28	0.45
MCW0020	3	2.00	0.50	0.67	0.87	3	3.00	0.67	0.667	1.09	2	1.39	0.33	0.28	0.45
MCW0034	3	2.57	0.66	1.00	1.01	3	2.57	0.67	0.611	1.01	2	1.80	0.00	0.44	0.64
MCW0104	2	1.80	0.44	0.67	0.64	3	2.57	0.33	0.611	1.01	3	2.57	1.00	0.61	1.01
ADL0268	3	3.00	0.67	1.00	1.09	3	2.57	1.00	0.611	1.01	3	3.00	0.67	0.67	1.09
MCW0206	3	2.57	0.61	0.67	1.01	3	2.57	1.00	0.611	1.01	3	2.57	1.00	0.61	1.01
LEI0166	3	2.57	0.61	1.00	1.01	3	2.57	1.00	0.611	1.01	2	1.80	0.67	0.44	0.64
MCW0295	3	3.00	0.67	1.00	1.09	3	2.57	0.00	0.611	1.01	3	3.00	1.00	0.67	1.09
MCW0183	3	3.00	0.67	0.67	1.09	2	1.80	0.67	0.444	0.64	3	2.57	1.00	0.61	1.01
MCW0067	3	3.00	0.66	0.67	1.01	2	2.00	1.00	0.500	0.69	2	1.80	0.67	0.44	0.64
MCWO330	3	2.57	0.66	0.33	1.01	3	2.57	0.33	0.611	1.01	3	2.00	0.67	0.50	0.87
G2085	3	2.00	0.50	0.67	0.87	3	2.57	0.67	0.611	1.01	2	1.80	0.00	0.44	0.64
MCW0111	3	2.00	0.50	0.67	0.87	3	2.57	1.00	0.611	1.01	3	3.00	1.00	0.67	1.09
MCW0103	3	2.00	0.50	0.67	0.87	3	2.57	0.61	1.000	1.01	2	1.80	0.00	0.44	0.64
Means	2.94	2.49	0.59	0.75	0.98	2.88	2.54	0.60	0.77	0.98	2.59	2.18	0.51	0.63	0.83

Na: allele numbers and their frequencies, Ne: effective number of allele, Ho and He: observed and expected heterozygosity, PIC: polymorphism Information content , NAPRIx × NF = cross with NF, NAPRIx × FF= crosss with FF, NAPRIx × NN= cross with NN

4.6 Average measures of genetic diversity of the crosses of Nigerian indigenous chicken populations

The Number of alleles (Na), Effective Number of alleles (Ne), observed (Ho) and Expected (He) heterozygosity, Polymorphism information content (PIC) of crosses of Nigerian indigenous chickens is shown in table 4.5.

Allele number for NAPRIx×NFranged from 2-3 with a mean of 2.647. The PIC has values ranging from 0.451 to 1.099 with a mean of 0.838. The Ho and He had values ranged from 0.333 to 1 and 0.278 to 0.667 with average means of 0.569 and 0.520, respectively.

The number of effective alleles for NAPRIx×NNranged from 2-3 with an average value of 2.882. The PIC values ranged from 0.451 and 1.099 with an average value of 0.948. The Ho and He values ranged from 0.278 to 0.667 and 0.333 to 1 with average values of 0.608 and 0.575, respectively.

Number of alleles for NAPRIx×FFranged from 2-3 with a mean of 2.882. The PIC values ranged from 0.637 to 1 with a mean of 0.967. The Ho and He values ranged from 0.444 to 0.667 and 0.333 to 1 with means of 0.706 and 0.592 respectively.

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Table 4.5: Average measures of genetic diversity of 17 Primers loci in Crosses of Nigerian indigenous chicken populations with NAPRIx

	NAPRIx×NF					NAPRIx×FF					NAPRIx×NN				
Markers	Na	Ne	He	Ho	PIC	Na	Ne	He	Ho	PIC	Na	Ne	He	Ho	PIC
ADL0112	3	2.57	0.66	1.00	1.01	2	2.00	0.33	0.5	0.69	3	2	0.67	0.50	0.868
ADL278	3	2.57	0.66	0.67	1.01	3	2.57	0.67	0.61	1.01	3	2.57	1.00	0.61	1.011
LEI0094	2	1.80	0.44	0.00	0.64	3	2.57	0.67	0.61	1.01	2	2.00	1.00	0.50	0.693
LEI0234	3	3.00	0.67	0.67	1.09	3	2.57	0.67	0.61	1.01	3	2.57	0.67	0.61	1.011
MCW0020	2	2.00	0.50	1.00	0.69	3	2.57	1.00	0.61	1.01	3	2.57	0.67	0.61	1.011
MCW0034	3	2.57	0.66	0.67	1.01	2	2.57	0.33	0.27	0.45	3	2.57	1.00	0.61	1.011
MCW0104	3	2.57	0.66	0.67	1.01	3	1.39	0.33	0.61	1.01	3	2.57	0.67	0.61	1.011
ADL0268	2	2.00	0.50	0.33	0.69	3	2.57	1.00	0.61	1.01	3	2.57	1.00	0.61	1.011
MCW0206	2	1.39	0.28	0.33	0.45	3	2.57	0.67	0.61	1.01	2	1.80	0.00	0.44	0.637
LEI0166	3	2.00	0.50	0.67	0.87	3	2.57	0.67	0.61	1.01	3	2.57	1.00	0.61	1.011
MCW0295	3	2.00	0.50	0.67	0.87	3	2.00	0.67	0.50	0.87	3	2.57	1.00	0.61	1.011
MCW0183	2	2.00	0.50	0.33	0.65	3	2.57	0.33	0.61	1.01	3	2.57	0.67	0.61	1.011
MCW0067	2	1.39	0.28	0.33	0.45	3	2.57	1.00	0.61	1.01	3	2.57	0.33	0.61	1.011
MCWO330	3	2.57	0.66	0.67	1.01	3	3.00	0.00	0.67	1.10	3	3.00	0.67	0.67	1.099
G2085	3	2.57	0.66	0.67	1.01	3	2.57	0.33	0.61	1.01	3	2.57	0.33	0.61	1.011
MCW0103	3	2.00	0.50	0.67	0.87	3	2.57	1.00	0.61	1.01	3	2.57	1.00	0.61	1.011
MCW0111	3	2.00	0.50	0.33	0.87	3	2.00	0.67	0.50	0.87	3	2.57	0.33	0.61	1.011
Means	2.65	2.18	0.52	0.57	0.84	2.88	2.43	0.58	0.61	0.95	2.88	2.48	0.59	0.71	0000

Na: allele numbers and their frequencies, Ne: effective number of allele, Ho and He: observed and expected heterozygosity, PIC: polymorphism Information content, NAPRIx × NF = cross with NF, NAPRIx × FF= crosss with FF, NAPRIx × NN= cross with NN

4.7 Analysis of molecular variance (AMOVA) of Nigerian indigenous chicken and their crosses with NAPRIx.

Table 4.7 shows the Analysis of molecular variance (AMOVA) of Nigerian indigenous chickens and their crosses. The estimated variation among the populations is 0.142, 0.042 among individuals and 10.778 within individuals while total variation was 10.961. Variation within individuals accounted for 98% of the total variation.

Table 4.6: Analysis of Molecular Variance (AMOVA) of Nigerian indigenous Chicken and their crosses with NAPRIx

Source	Df	SS	MS	Estimated Variance	%
Among Pops	5	58.556	11.711	0.142	1
Among Ind	12	130.333	10.861	0.042	1
Within Ind	18	194	10.778	10.778	98
Total	35	382.889	10.961	10.961	100

Df= degree of freedom, SS= sum of squares, MS= mean square, pops= populations, Ind= individual

4.8 Pairwise population matrix of Nei genetic distance of Nigerian indigenous chickens and their crosses with NAPRIx.

The pairwise population matrix of Nei genetic distance of Nigerian indigenous chickens and their crosses is given in table 4.7.The longest distance of 0.409 was obtained between FF and NAPRIx×NF while the shortest distance of 0.177 was obtained between FF and NAPRIx×NN.

Table 4.7: Pairwise Population Matrix of Nei Genetic Distance of Nigerian indigenous Chickens and their Crosses with NAPRIx

NF	FF	NN	NAPRIx×NF	NAPRIx×FF	NAPRIx×NN	
						NF
0.205						FF
0.276	0.213					NN
0.264	0.409	0.358				NAPRIx×NF
0.212	0.226	0.361	0.251			NAPRIx×FF
0.247	0.177	0.220	0.294	0.228		NAPRIx×NN

NF= Normal Feathered, FF= Frizzle feathered, NN= Naked neck, , NAPRIx × NF = cross with NF, NAPRIx × FF= crosss with FF, NAPRIx × NN= cross with NN

Plate 4.1 showed the pattern of primers for the Mx gene in Nigerian indigenous chickens and their crosses with NAPRIx. The bands that are visible are indicating the presence of the Mx gene in the studied populations.

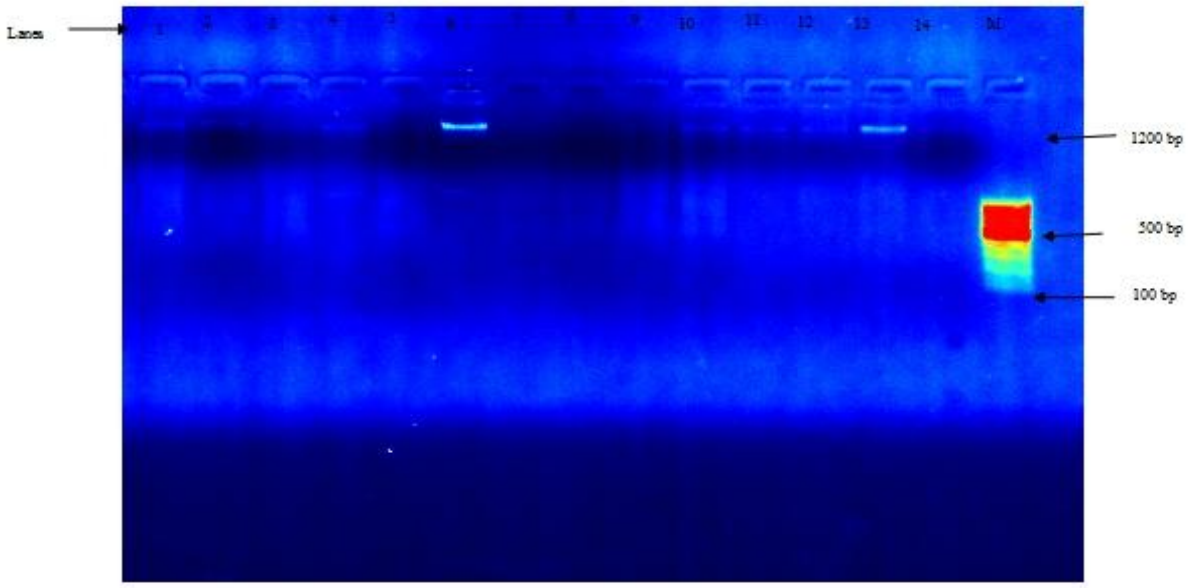

Plate 4.1: PCR Amplicons Lane 1-14, (M is 1000 base pair ladder)

Figure 4.2is a bi-dimensional graph showing the distribution of Genotypes of Nigerian indigenous chickens and their crosses. The result showed close genetic ancestry between the various genotypes and their crosses. They all clustered within a single coordinate except two of the NF. The clustering of majority of the breeds is indication of close genetic ancestry between the populations.

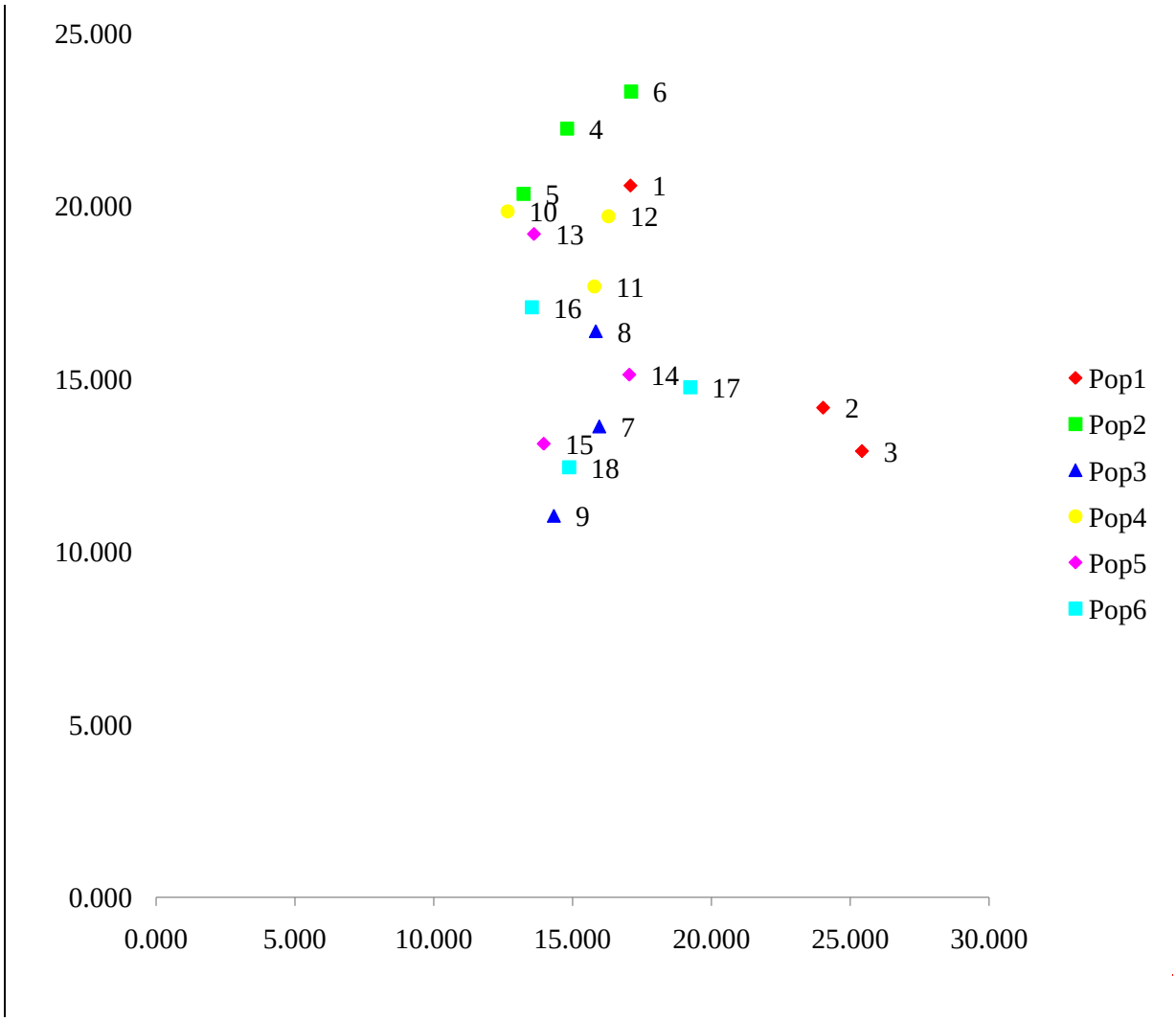

Figure 4.2: A bi-dimensional graph showing the distribution of Genotypes in Nigerian indigenous chickens and their crosses. Pop 1= NF, Pop 2= FF, Pop 3= NN, Pop 4= NAPRIx×NF, Pop 5= NAPRIx×FF, Pop 6= NAPRIx× NN

Figure 4.3 represents a dendrograme showing the cluster analysis of Nigerian indigenous chickens and their crosses with NAPRIx. The studied populations formed two major clusters of genetic dissimilarity. The NF forms a distinct cluster while the remaining genotypes formed the second cluster. Two sub- clusters were formed from the second cluster with NAPRIx× FF, NN and NAPRIx× NF forming one while NAPRIx× NN and FF formed the second sub- cluster.

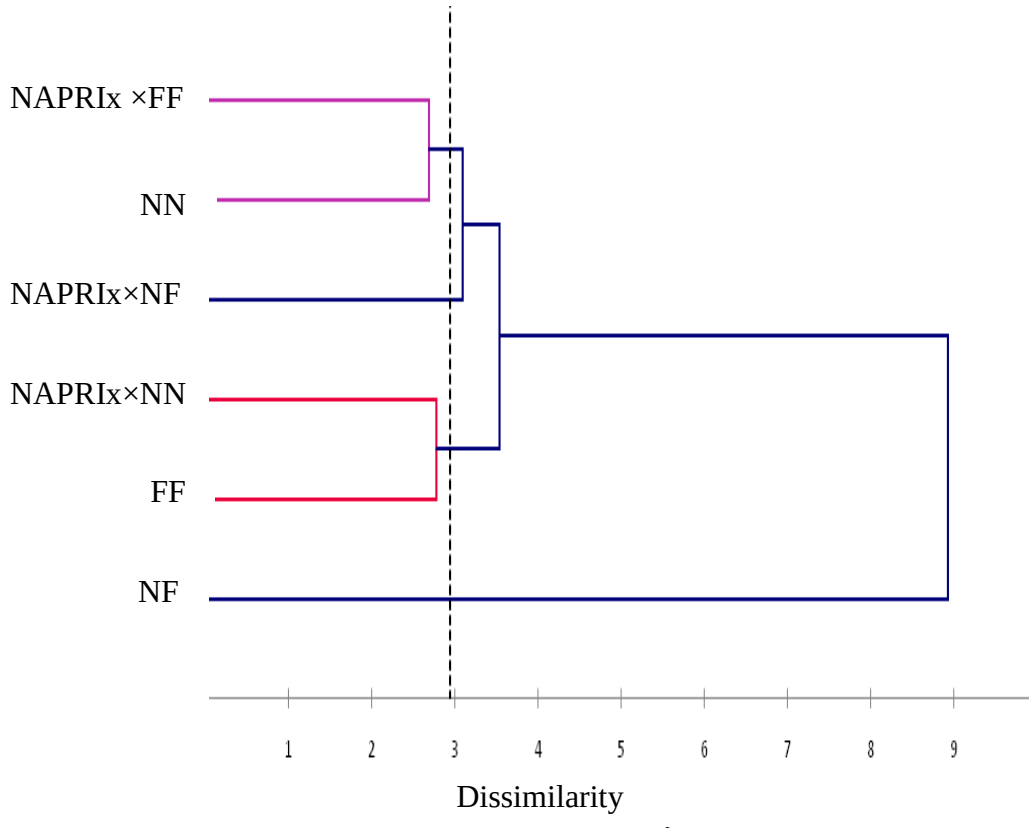

Figure 4.3: A dendrograme showing the cluster analysis of Nigerian indigenous chickens and their crosses.

CHAPTER FIVE

5.0 DISCUSSION

5.1 The Geometric mean titre (GMT) of Nigerian indigenous chickens and their crosses showed that all indigenous genotypes started developing antibodies against Newcastle disease right from day 0.

The implication of this high titreis that the experimental birds used for this experiment were already showing exposure to the virus, as the birds were obtained from the open market where birds scavenge for food (Abdu *et al*., 2004). The crosses on the other hand did not show any titre from day 0 because the experimental birds were kept in deep litter system with scheduled vaccination and proper medications. This is in agreement with the findings of Bobbo*et al*. (2013) in three phenotypes of local chickens in Adamawa state. There was an increase in the geometric mean titre in all genotypes on day 28 which corresponds with the period when the birds developed highest titre.

The seroprevalencerate shown for HI and Elisa for the indigenous chickens were lower than the rates reported by Nwankiti*et al* (2010) who reported a seroprevalence rate of 53.6% for birds in local markets within the Abuja. Also, a prevalence rate of 35.8% was reported by Jibril*et al*. (2014) in Gusau markets of Zamfara State. These reports are all much higher than the mean obtained in this report (16.7%). The differences could be attributed to sampling methods and season in which the research was conducted,and indeed due to differences in location.

5.2 Mx gene and its Association with Newcastle disease in Nigerian indigenous Chicken populations and their Crosses.

From the results of the Association of Mx gene and Newcastle disease, the frequency of the A allele for resistance was found to be in higher frequency in the Naked Neck and Normal feathered, thus making them to be the more resistant group. The implicating of the A allele is in agreement with the reports of Sulandari*et al*. (2009) who reported a frequency of the A allele to

be 62.73% while the G allele had 37.27%.Pagala*et al*. (2013) in Tolaki Chicken of Indonesia also reported the frequency of the A allele to be 0.74 (74%) and 0.26 (26) for susceptible G allele. This is also in agreement with the assertion that when G takes the place of A at position 2032 point with the resultant change of 631th amino acid from Serine to Asparagine, it shows skewed allele frequencies in different chicken populations, with a much higher frequency of the favourable allele A in native breeds than in highly selected lines (Li *et al*., 2006).

5.3 Genetic Diversity

5.3.1. Number of allele (Na), observed (Ho) and Expected Heterozygosity (He), and polymorphism information content (PIC) of Normal Feathered indigenous chickens of Nigeria.

The result from the study of Normal feathered Chicken in Nigeria using 17 set microsatellite markers showed that the mean Ho (0.745) is higher than the mean He (0.588) which is in agreement with the report of BeigiNassiri*et al* (2007) in Mazandaran Native chicken of Iran who reported values of 0.7331 and 0.5579 for Ho and He respectively. But the report is not in agreement with the report of Lyimo*et al*. (2013) in Tanzanian ecotypes who reported lower values. Heterozygosity is a measure of genetic variation within a population. High heterozygosity values for a breed may be due to long-term natural selection for adaptation, to the mixed Nature of the breeds or to historic mixing of strains of different populations. A low level of Heterozygosity may be due to isolation with the subsequent loss of unexploited genetic Potential (Ojango*et al*., 2011). The observed values where Ho is greater than He shows anincrease in heterozygosity in the studied population.The low allele number in this report (2.941) agreed with the range of 2.30-6.72 as reported by Granevitze*et al*. (2007) in Vietnamese local chicken and also that of Berthouly (2009) as cited in Granevitze*et al* (2007) who gave corresponding values for Red jungle foul, Chinese and commercial breeds as 0.60, 0.47 and 0.40 respectively. The low allele number also shows decreased heterozygosity. The PIC in this report

(0.976) is higher than those reported by Hassen*et al*. (2009) in Northwest Ethiopia (0.59-0.78) and (0.4939) by BeigiNassiri*et al*. (2007) in Mazandaran Native chicken in Iran. The values show that the markers were informative enough and could be used for genetic diversity studies. The differences in values in this report could be attributed to the differences in sampling methods, markers used and differences in geographical locations.

5.3.2. Number of allele (Na), observed (Ho) and Expected Heterozygosity (He), and polymorphism information content (PIC) of Frizzled feathered chicken of Nigeria.

The results from the study of Frizzled feathered chicken using 17 primers shows that the He (0.601) is lower than the Ho (0.765).The He is lower than the Ho which also indicates increased heterozygosity. The heterozygosity values showed that the FF may have become adapted as a result of long term natural selection and also because of the fact that the birds might not be pure arising from random mating since the experimental birds were obtained from the open market. The results are in agreement with the report ofBeigiNassiri*et al*. (2007) in Mazandaran native chicken in Iran and that by Wei *et al*. (2013) in a study on Xuen-feng black bone native chicken (0.6285) with 23 loci. Lower values were reported by Ohwojakpor*et al*. (2012) in chicken populations of South-south Nigeria (0.5353). Differences in heterozygosity could be adduced to differences in sample sizes, location, sources of markers and experimental chickens. The values for PIC obtained were very informative (0.891) which is way higher than the 0.5 required before a marker can be considered to be informative (Olasukanmi, 2010). The results are in the range reported by Ohwojakpor*et al*. (2012) who reported an average of 0.8010 in chicken populations of South-south Nigeria but are not in agreement with the reports of BeigiNassiri*et al*. (2007) who reported 0.4939 in Mazandaran Native Chicken in Iran and also the report of Keambou*et al*. (2014) who also reported a lower value of 0.57 in Cameroon indigenous chicken ecotypes.

5.3.3. Number of allele (Na), observed (Ho) and Expected Heterozygosity (He), and polymorphism information content (PIC) of naked neck chicken of Nigeria.

The results from a study of naked chicken using 17 markers shows that Ho (0.627) was greater than He (0.513) showing a decrease in heterozygosity in the studied population. The values in this report are in agreement with the reports of Keambou*et al*. (2014) who reported an average of 0.61 and that of Lyimo (2013) who also reported a value of 0.62 in Tanzania. The PIC in this report (0.825) shows that the markers are highly informative enough and can be used for genetic diversity studies. The values are slightly higher than the ones reported by Hassen*et al*. (2009) in Northwest Ethiopia (0.78) but are not in agreement with those reported by BeigiNassiri (2007) and Keambou*et al*. (2014) who reported 0.4939 and 0.57, respectively. Differences could be attributed to sources of markers, methods of samplings and location of experimental birds.

5.3.4. Genetic Distance

The results of the genetic distance of Nigerian indigenous chicken and their crosses shows that the longest distance obtained (0.409) was between FF and NF and this could be attributed to the differences in genotype coupled with the fact that the crosses contain genes other than those found in indigenous breeds. The shortest distance obtained (0.177) between the FF andNAPRIx×NN could probably be to the fact that the two different genotypes could be closer owing to the fact that the indigenous birds used were obtained from the open market and could therefore contain genes other than what the phenotype shows as a result of uncontrolled breeding. The close genetic distance between all the genotypes indicates a common genetic ancestry. In the indigenous genotypes, the closeness could be attributed to the fact that they live in the same geographical location.

5.3.5. Analysis of Molecular variance

The total estimated variation among population (0.142) showed that the level of variation is low whichindicates that they are virtually almost homozygote or due to low population size which is a driving factor for genetic bottlenecks, random genetic drift, inbreeding and artificial selection or due to method and facilities used (Ojango*et al*. 2011) in sampling. The result of variation among population (0.142) is not in agreement with keambou*et al*. (2014) who reported a higher value of 4.27214.Lyimo*et al*. (2013) and Bohani*et al*. (2016) reported variations of 4.8133 and 10.51 of between populations respectively which do not agree with this report. Variation within individuals accounted for 98% of the variation in the studied populations. The differencescould be attributed to inbreeding, which leads to homozygosity.

5.3.6. Cluster Analysis

The Dendograme formed four clusters at various degrees of similarities based on Nei's genetic distance in the studied population of Nigerian indigenous Chickens and their Crosses.All clusters formed shows that the studied chicken populations have close genetic relationships. This finding is in agreement with the report of Ohwojakpor*et al*. (2012) who reported similar genetic similarities in chicken populations of south-south Nigeria.Results from cluster analysis has clearly indicated and validated the assertion that the Nigerian indigenous chickens have close ancestry and since a single strain was used in the crossing with indigenous genotypes, close genetic distances should naturally be expected.

CHAPTER SIX

6.0 SUMMARY, CONCLUSIONS AND RECCOMENDATION

6.1 Summary

From the results of Geometric mean titre of Nigerian indigenous genotypes and their crosses with NAPRIx, the NN developed the highest titre from 3.1 to 512 in 28 days while the FF had the lowest (71) in the indigenous breeds. In the cross breds, NAPRIx ×NF had the highest antibody of 1024 on day 28, while NAPRIx × NN had the lowest antibody titre of 76 on day 28 in the cross breds. The result from ELISA shows that the NN had the highest prevalence rate of 21% while the NAPRIx × FF had the lowest rate of 4%. Result of mortality and morbidity shows that there was 100% morbidity in all genotypes. Onset of clinical signs was earliest (3) days in NF and FF while onset of mortality was on day 7 in FF and on day 15 inNAPRIx × NN. Mortality was highest in (87.5%) in NF and (45%) in NN. Duration of the disease (in days) was highest in NF (27)and lowest in NAPRIx × FF (15) days. From the results of extracted and genotyped DNA,the various frequencies of the various alleles showed that the resistantA allele has a higher frequency of 0.58 while the susceptible G allele had a frequency of 0.42.Genetic diversity was also analyzed for the various breeds. Mean Na was 2.94, 2.88 and 2.59 in NF, FF and NN, respectively. Mean Ne was 2.49, 2.54 and 2.18 in NF, FF and NN, respectively. Mean He and Howas found to be 0.59 and 0.75, 0.60 and 0.77 and 0.51 and 0.63 in NF, FF and NN, respectively. The meanPIC values for the markers used were 0.98, 0.98 and 0.83 for NF, FF and NN, respectively. In the crosses with NAPRIx, mean Na was 2.65, 2.88 and 2.88 for NAPRIx× NF, NAPRIx×FF and NAPRIx× NN, respectively. Mean Ne was 2.18, 2.43 and 2.48 in NAPRIx× NF,NAPRIx ×FF and NAPRIx× NN, respectively. He and Ho were 0.52 and 0.57, 0.58 and 0.61 and 0.59 and 0.71 in NAPRIx× NF, NAPRIx ×FF and NAPRIx× NN, respectively. The PIC values for the markers used were 0.84, 0.95 and 0.97 for NAPRIx× NF, NAPRIx ×FF

and NAPRIx× NN, respectively. Analysis of molecular variance (AMOVA) shows that the estimated variation among the populations is 0.142, 0.042 among individuals and 10.778 within individuals while Total variation was 10.961. Variation within individuals accounted for 98% of the total variation. The pairwise population matrix of Nei genetic distance of the studied genotypes shows that the longest distance of 0.409 was obtained between FF and NAPRIx×NF while the shortest distance of 0.177 was obtained between FF and NAPRIx×NN. Cluster analysis shows that the studied genotypes all clustered in one coordinate except two from NF genotype. From the dendograme, the studied genotypes formed five clusters of genetic dissimilarity. The first cluster was formed between NAPRIx FF × NN at 3. The second cluster was formed between NN × FF also at position 3. The third cluster was formed between the first cluster and NAPRIx ×NF at position 3.6. The fourth cluster joined all except the NF at position 4 while the last cluster joined the fourth cluster and NF at a position 9 of dissimilarity.

6.2Conclusion

1. The results reveal that the indigenous chicken populations are hardy, and that the naked neck showed better resistance to inoculation with KUDU-113 virus. It had the least mortality (45%), higher onset of mortality in days (20) and a higher prevalence rate of 21% from ELISA results.
2. Association of Mx gene genotypes reveals that the frequency of the resistant A allele is higher in the indigenous breeds (0.65, 0.5 and 0.65 in NF, NN and FF), respectively. It is also higher in the crosses while the G allele for susceptibility was found to be generally lower (0.35, 0.5 and 0.35 in NF, NN and FF), respectively.
3. There is a close genetic relationship amongst the studied genotypes with consistent clusters formed and a common ancestry was formed. Markers used were highly polymorphic (PIC>0.5) for all the indigenous chickens and their crosses with NAPRIx.

6.3 Recommendation

1. The frizzle feathered should be selected for production and crosses with other strains of broilers due to its higher resistance to NCD.
2. Controlled breeding measures should be put in place to preserve the unique genotypes of the Nigerian indigenous chickens
3. Further studies using more advanced technology to examine the extent of the involvement of Mx gene in resistance to NCD.

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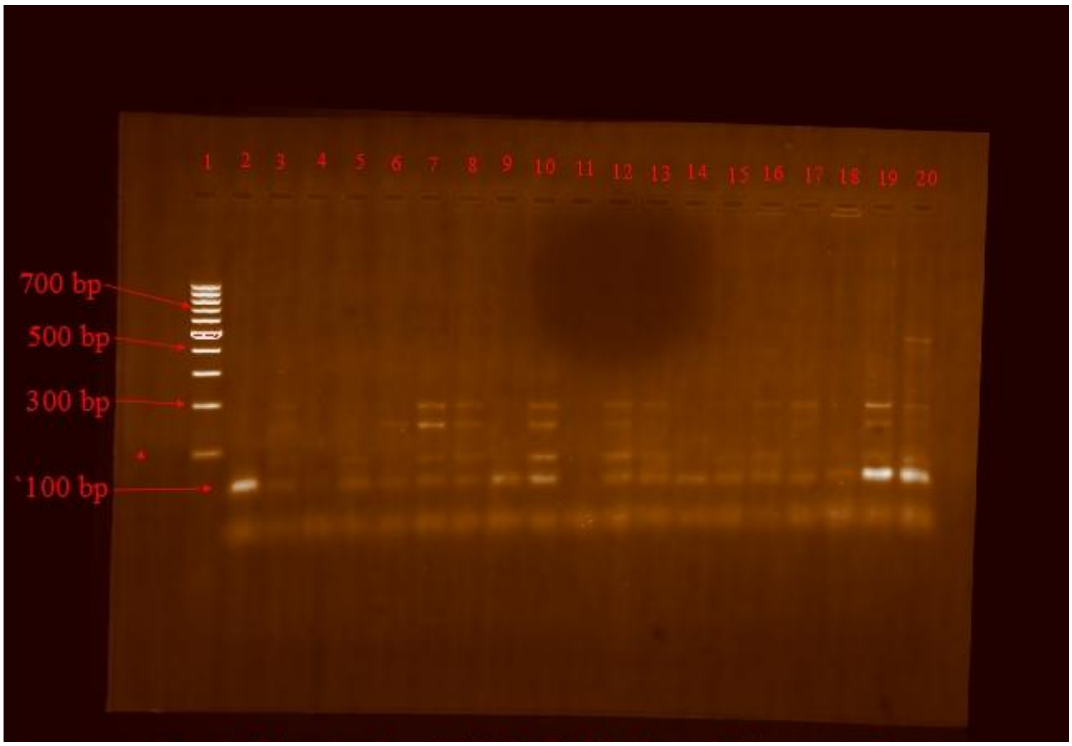
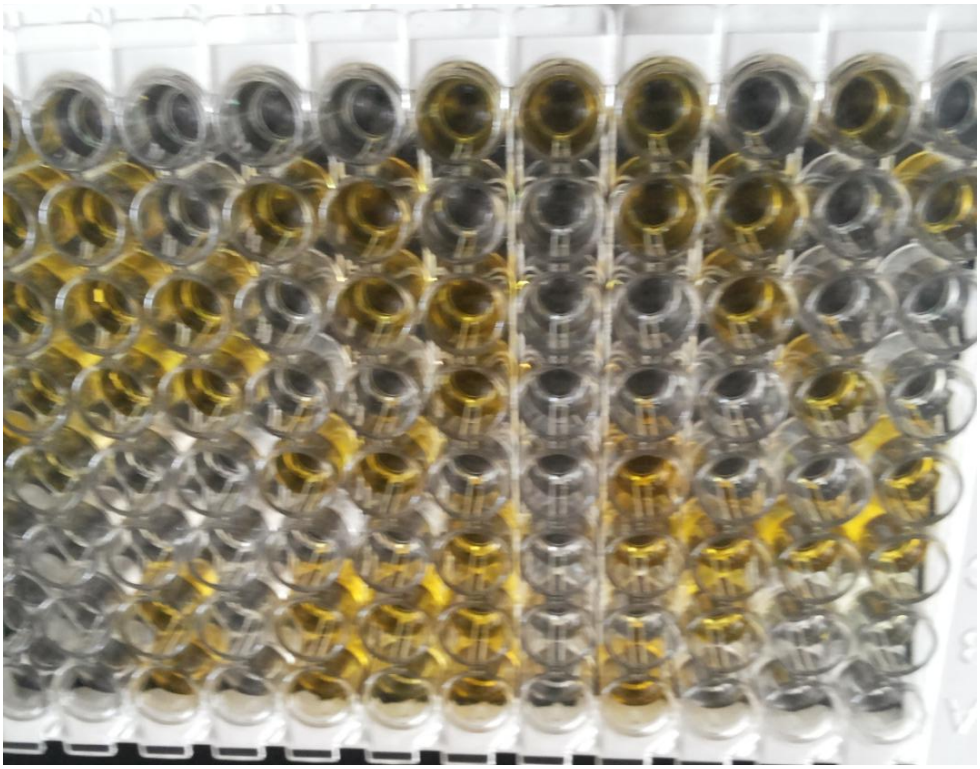
APPENDIX



Appendix I: Genetic diversity; lane 1 100 bp ladder, lanes 2-20 = PCR amplicons

Appendix II: results of HI test showing tear shape drop of positive samples

Appendix III: An ELISA reader



Appendix IV: An ELISA Plate