TITLE

EVALUATION OF FACTORS LIMITING TURKEY PRODUCTION IN ENUGU STATE AND COMPARISON OF PATHOLOGY ASSOCIATED WITH NEWCASTLE DISEASE IN TURKEYS AND CHICKENS.

BY

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CERTIFICATION

OKOROAFOR, Obianuju Nkiruka, a postgraduate student in the Department of Veterinary Medicine and with the registration number PG/Ph.D/08/50241 has satisfactorily completed the requirement for course and research work for the degree of the Doctor of Philosophy in Veterinary Medicine. The work embodied in this thesis is original and has not been submitted in part or full for any degree of this or any other university

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This work is dedicated to my late father **Dr. FREDRICK NWANKWO ANOZIE.**

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CHAPTER THREE

Abstract

The objectives of this study were to evaluate the factors limiting turkey production in Enugu State, Nigeria, determine the sero-prevalence of Newcastle disease (ND) virus infection in turkeys raised in Enugu State and compare the clinical signs and lesions of velogenic ND virus (NDV) in turkeys and chickens.

The study consisted of a cross-sectional survey of turkey producers in the three senatorial zones of Enugu State and an experimental infection of chickens and turkeys with a velogenic NDV (VNDV).

Three Local Government Areas in each of the three senatorial zones, Enugu East, West and North were purposively selected for the survey because of the preponderance of turkeys in the selected areas. A structured questionnaire was used for data collection. Two hundred and fifty copies of the questionnaire were administered to turkey producers in the selected areas. Five hundred and sixty-nine serum samples were randomly collected from unvaccinated turkeys in ninety backyard poultry farms in Enugu East, Enugu West and Enugu North senatorial zones. The sera were analysed for ND antibody titres using haemagglutination inhibition (HI) test.

For the challenge experiment, 120 turkeys and 120 chickens were used. The 120 turkeys and chickens were divided into two groups of sixty vaccinated and sixty unvaccinated. The vaccinated groups were given HB1 (I/O) and La Sota ND vaccines on day 1 and day 21 of age respectively. Inoculated birds were given 0.2 ml ($10^{6.46}$ per ml) of the VNDV (KUDU 113) intramuscularly, at 6 weeks of age. Morbidity and mortality, changes in live body weight and gross lesions were recorded. Antibody response of the birds were carried out by collection of sera

on days 0, 5, 10, 15 and 21 PI. Pack cell volume (PCV), haemoglobin concentration (HbC), red blood cell count (RBC) and white blood cell (WBC, total and differential) were carried out on days 0, 3, 6, 10, 15 and 21 PI. Data generated from survey were subjected to descriptive statistics and Chi-square test, while for the challenge studies, studentsô t test and one way analysis of variance were used. Variant means were analyzed post hoc using the least significant difference method. Significance was accepted at $p < 0.05$.
Turkey production was carried out mainly by adult female in Enugu East (65.3%) and Enugu West (57.7%) and adult males in Enugu North (51.1%) Although most (87.5%) of the respondents in the three senatorial zones had formal education, they were not (48.8%) experienced in turkey production. The major (52.5%) age group involved in the business was 36-50 years old. Turkey production in Enugu State was generally a part-time occupation as respondents were engaged in other primary occupation such as crop farming, trading, civil service etc. The management system adopted by the respondents in the three senatorial zones, Enugu East, Enugu West and Enugu North respectively were mainly (20.4%,55.0%,47.3%) intensive or semi-intensive (59.2%,34.0%,36.8%).

Majority (84.8%) of the respondents in Enugu State keep turkeys in small numbers (1-20) along with local chicken, exotic chicken, guinea fowl, ducks etc. According to majority (91.2%) of the respondent, turkey production was found to be profitable venture based on the cash generated after sale of the turkeys. Newcastle Disease (57.0%), fowl pox (65.0%), fowl typhoid (6.0%), fowl cholera (3.7%), ectoparasitism (1.0%), fracture and nutritional deficiencies (2.6%) were the diseases constantly encountered in turkey production by majority of the respondents and of all the diseases reported by the respondents, fowl pox (65%) and Newcastle Disease (57%) were the major diseases limiting production in the study area.

The major factors limiting turkey production in Enugu State as identified by the respondents in the study area were high cost of feed (86.5%), high disease morbidity and mortality (85.2%), inadequate access to veterinary care (78.80%), unavailability and high cost of poults (74.40%), lack of management skills (63.3%) and lack of capital (61.7%) while minor problems were low reproductive potential (56.9%), theft and predators (52.5%).

Out of the 569 sera collected from the three senatorial zones and tested for Newcastle Disease virus antibody, a total of 186 sera representing 32.7% were positive for Newcastle Disease antibody. Out of the 186 sera, 138 (74.1%) had titres of 8 and above and were considered protected while 48 (25.9%) had titres of less than 8 and considered to be at risk. The mean HI antibody titre in Enugu East senatorial zone against ND was significantly ($P<0.05$) higher than mean HI antibody titres in Enugu West and North senatorial zones. The results showed that these turkeys were exposed to Newcastle Disease Virus.

Following intramuscular inoculation with the KUDU 113, severe depression, whitish -greenish diarrhoea, lethargy, hunched posture, tremors and torticolis were consistent clinical feature observed in unvaccinated turkeys and chickens while mild depression and lethargy were observed in vaccinated chickens and very mild clinical signs in few vaccinated turkeys. Morbidity was 100%, 92%, 22.2% and 4.1% in unvaccinated infected chickens and turkeys, vaccinated inoculated chickens and turkeys respectively, while mortality of 90%, 80%, 13.3% and 0% were recorded for unvaccinated inoculated chickens and turkeys, vaccinated inoculated chickens and turkeys respectively. Reduction in weight was highly significant in both unvaccinated and vaccinated inoculated groups ($p<0.05$). Postmortem examination showed atrophy of the lymphoid organs (thymus, bursa of Fabricius and spleen) and congestion of kidneys in inoculated groups. Haemorrhages on the mucosa of the proventriculus, sharply-demarcated haemorrhagic ulcers in the intestine and haemorrhagic and swollen caecal tonsils

were observed only in inoculated chickens. Congestion of the brain vessels was present in inoculated turkeys.

By day 6 PI all the unvaccinated inoculated chickens had died showing no significant changes in the weights of the thymus. Inoculated vaccinated chickens also showed no significant changes in thymic weights ($p>0.05$). But the gross lesions of the control and inoculated chickens showed clear reduction in sizes of the thymus of both unvaccinated and vaccinated inoculated chickens on days 5 and 6 PI. The only change in the weight of the spleen was significant reduction in vaccinated inoculated chickens on day 5 PI only. The gross lesions showed clear atrophy of the spleen in vaccinated and unvaccinated inoculated chickens on day 10 and 5 PI respectively and in the vaccinated and unvaccinated inoculated turkeys on days 20 and 10 PI respectively. The weights of the bursa were lower ($p<0.05$) in the unvaccinated inoculated chickens on days 3 and 5 PI and days 3, 10, 15 and 21 PI in the unvaccinated inoculated turkeys.

There was a significant increase ($p<0.05$) in antibody titre in both inoculated unvaccinated/ vaccinated cockerels and turkeys. Newcastle Disease virus was detected in brain, intestine and spleen but none detected in the cloacal swabs.

No significant ($p>0.05$) changes were observed in the erythrocytic parameters in chickens while there was a significant decrease ($p<0.05$) in PCV on day 15 PI in unvaccinated turkeys. Significant ($p<0.05$). leucocytosis, heterophilia and lymphopenia were observed in unvaccinated inoculated chickens on day 3PI while days 3, 10 and 21PI for unvaccinated turkeys.

Despite the factors limiting turkey production as outlined by the respondents such as high cost of feed, high disease morbidity and mortality, inadequate access to veterinary care, unavailability and high cost of poults, lack of capital and lack of reasonable degree of management skills, turkey production has great potential in bridging the animal protein supply therefore, poultry farmers should be encouraged by the government to increase their level of production by establishing reliable breeding centres in the South East Nigeria which will ensure regular supply of day old poults, subsidizing the price of feed and drugs, prompt disease control by employment of more veterinarians, provision of animal health care delivery vehicles provision of poultry vaccines, provision and easy access to soft loans. These will boost overall production and increase the standard of living of the farmers..

The seroprevalence of 32.7% shows exposure of the turkeys to Newcastle Disease virus thus the local husbandry practice of keeping different species together, increases the chances of transfer of Newcastle Disease virus across these species, it will be noteworthy to recommend that turkey farmers be encouraged in the study area to keep species separately and vaccinate against Newcastle Disease regularly.

Susceptibility to highly virulent NDV was shown to vary among chickens and turkeys. However, in general, disease among turkeys was less severe, despite virus detection in its various organ (Spleen, Intestine and Brain), than in chickens. Intramuscular inoculation test showed high mortality in chickens with intestinal ulcers and haemorraghic lesions which indicated that the KUDU-113 NDV strain is a velogenic viscerotropic virus.

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CHAPTER ONE

1.0 INTRODUCTION

1.1 Background of the study

The production and consumption of eggs and poultry meat is increasing worldwide. Over the last quarter of a century the consumption of eggs has doubled and chicken meat tripled (Jordan and Pattisons, 1996). Poultry production forms an important component of Nigeriaôs livestock sector. Poultry is the most commonly kept livestock and over 70% of those who keep livestock are reported to keep chickens (Amar Klemesu and Maxwell, 2000). As a provider of employment and income, poultry production constitutes an important form of livelihood for rural and urban dwellers. Poultry farmers who are well spread all over the different ecological zones of this country engage in the production of chicken, eggs, day old chicks and poultry manure for rural, urban and peri-urban populations (Eduvie, 2002).

The poultry population in Nigeria is estimated at 104.3 million comprising 72.4 million chickens,11.8 million ducks,4.7 million guinea fowl,15.2 million pigeons and 0.2 million turkeys (FDLPCS,1992). Poultry sector is a major source of animal protein supply in Nigeria. It is next to ruminants as a source of animal protein supply in Nigeria and accounts for almost 25% of local meat production (Ajala and Alli-Balogun, 2004). According to Food and Agricultural Organization (FAO) report of 1988 cited by Nwanta *et al*. (2012), Nigeria recorded the lowest animal protein intake with an average of 6 g per head per day. The FAO (2010) also estimated that in an average Nigerian meal, animal protein contributes 3% against 12% recommended for healthy living. Among Nigerians, poultry meat and eggs are to some extent still considered luxury food (Adene and Oguntade, 2006). One of the major reasons for the poor intake of animal protein among Nigerians maybe due to inadequate supply of animal products occasioned by low productivity and consequent rise in cost of meat. With the continued rise in the cost of production

of beef, sheep and chicken, which are the primary sources of animal protein in Nigeria, it has become very necessary to explore other efficient and less common but potential sources of animal protein for economic viability (Ajala and Alli-Balogun, 2004).

There are many underrated, but highly promising poultry species such as turkeys, quails and guinea fowl. Turkey (*meleagris gallopavo*) is a type of poultry specie with numerous attributes; Turkey thrives better under arid conditions, tolerates heat better and has higher quality meat (Fisinin and Zlochevskaya, 1989; Yakubu *et al.,* 2013). Smith (1990) reported that carcasses of turkey contained a higher percentage of protein than the carcasses of chicken. Turkeys have also been found to be of considerable economic and social significance in the tradition of Nigerians (Peters *et al.,* 1997).

While the production of other types of poultry species has rapidly increased in recent years (Okoruwa *et al.,*2006) and despite its greater potential than the chicken, very little successes have been recorded in turkey production in the developing countries (Shingari and Sapra 1993; Peters *et al.,* 1997; Perez-Lara *et al*., 2013). In Nigeria, consumers continue to pay high prices for imported turkeys and even for local ones. The reason for the apparent inertia in turkey production appears to be due to high cost of turkey poults, inconsistency in feeding programmes, lack of understanding of its management and production requirements, disease threats and lack of appreciation of its potential in contributing to the protein needs of the consuming public. (Nwagu, 2002; Ojewola *et al*., 2002).

Disease patterns in poultry production are changing with increasing industrialization and intensification of rearing systems. Outbreak of diseases is often due to increased stress the birds are subjected to and the high infectious disease pressure on thousands of birds in a confined space (Jordan and Pattison, 1996). In developing countries, infectious diseases still play a

predominant role. Sainsbury (1992) reported that tropical countries have more problems with infectious diseases due to climatic circumstances.

Diseases have been the major constraint in rearing domestic poultry. Of all the diseases of poultry, velogenic Newcastle disease (VND) constitutes a major problem of poultry in Africa and Asia (Awan *et al*., 1994). Newcastle disease (ND) is one of the most important avian diseases, because of its economic impact on the poultry industry (Alexander *et al*., 1997a). It continues to be a serious economic threat to the poultry industry causing increased morbidity and mortality rates, loss of eggs for both breeding and human consumption (Jungherr *et al.,* 1946; Abdu *et al*., 1992; Philips, 1998). It is a worldwide problem in poultry industry (Lancaster, 1966). It has been the most important disease of chickens, turkey and other poultry species in Nigeria (Ezeokoli *et al.,* 1984), since the first outbreak of the disease that occurred in Ibadan, Nigeria in 1952 (Hill *et al.,*1953).

Newcastle Disease is caused by avian *paramyxovirus.* Nine serogroups of avian *paramyxovirus* have been recognized (APMV1-9). APMV-1 remains the most important pathogen for poultry while others are known to cause diseases in poultry and other types of birds (Alexander, 2003). Newcastle disease virus (NDV) has been grouped into five pathotypes based on clinical signs seen in infected birds. These include velogenic viscerotropic, velogenic neurotrophic, mesogenic, lentogenic and asymtomatic pathotypes (Jordan and Pattison, 1996; OIE, 2005) and it is believed that all these pathotypes of NDV exist in Nigeria (Nawathe *et al.,* 1975; Onunkwo and Momoh, 1980; Adu *et al*., 1985; Ibu *et al.,* 2009). All isolates of NDV in Nigeria so far characterized, belong to the velogenic virulent strains (Majiyagbe and Nawathe, 1981, Adu *et al.,* 1985). Studies of naturally occurring and experimental infections have shown that the velogenic NDV is the commonest pathotype in Nigeria (Onunkwo and Momoh, 1980; Echeonwu *et al*., 1993, Fagbohun *et al*., 2000; Saôidu *et al*., 2004 and Oyekunle *et al*., 2006). It is the major cause of infection in many species of birds both domestic and wild birds. Turkeys are susceptible to VND

virus (VNDV) even though they do not develop severe signs (Fatumbi and Adene, 1979; Gomwalk *et al*., 1985; Adu *et al.,* 1986). Outbreaks of ND occurred in domestic fowl and turkeys in Great Britain in 1997 (Alexander *et al*., 1999) and in Nigeria there are few reports of natural outbreak of ND in turkeys (Saâidu *et al*., 1994).

Currently, turkey (Meleagris gallopavo) production is an aspect of poultry industry whose potentials have not been fully utilized and is one of the most suitable alternative sources of animal protein supply in Nigeria considering the cost of production (Ibe, 1990; Peters *et al*., 1997).

1.2 Statement of the problem

The impact of ND is most notable in commercial and local chickens, due to the high susceptibility of the chickens and the severe consequences of outbreaks on the poultry industry. In fact, it has been argued that ND may represent a bigger drain on the world economy than any other viral disease of animals (Alexander, 2003). Despite constant vaccination polices to prevent outbreaks, ND continues to occur in vaccinated chicken flocks (Alexander, 2003; Senne *et al*., 2004; Ezema *et al*., 2009).

Because turkey production in Nigeria is still at a small holder level, (Ojewola *et al.,* 2002), there are few reports of natural outbreaks of ND in turkeys (Saâidu *et al.,* 1994). The few reports of serologic evidence of NDV infection in turkeys are reports by Saâidu *et al.* (2004) in Zaria with a prevalence rate of 68% and Saidq *et al.* (2011) in Maiduguri which recorded a prevalence of 57.2%. None has been reported in turkeys in south-east, Nigeria.

Most birds other than the domestic chicken are known to be sources of the spread of NDV (Lancaster, 1966; Roy *et al*., 1998). It was reported by Alexander *et al.* (1985a) that the spread of NDV to chickens has occurred in several countries, including Great Britain, where 20 outbreaks

in unvaccinated chickens occurred in 1984 as a result of feed that had been contaminated by faeces of infected birds. In rural Nigeria, like in the South East it is common to find a combination of different poultry species such as chickens, turkeys, Muscovy ducks and pigeons being reared in the same compound (Ibrahim and Abdu, 1992). This encourages cross infection of NDV across species (Abdu *et al*., 1985).

Furthermore, studies on the susceptibility of chickens to ND is well documented unlike in turkeys (Piacenti *et al*., 2006). There is need to study the susceptibility of turkeys to velogenic NDV. This may throw more light on the risk factors to the successful production of turkeys and chickens in the study area.

1.3 Aim of the study

The aim of this study was to determine the susceptibility of turkeys to velogenic NDV. Specifically, the objectives of the study were to;

1. Determine the limiting factors associated with turkey production and assess the significance of Newcastle Disease among other disease problems of turkeys in Enugu State.
2. Determine the sero-prevalence of NDV infection in turkeys raised in Enugu State.
3. Compare the clinical signs, gross and histopathologic lesions of experimental velogenic NDV in turkeys with those of the chickens.
4. Investigate the shedding of virus in the faeces/tissue of turkeys and chickens, experimentally inoculated with VNDV.
5. Compare the antibody response of turkeys and chickens to VNDV infection.
6. Compare the effect of VNDV strain on the blood parameters of turkeys and chickens experimentally inoculated.

1.4 Null Hypothesis

The following hypotheses will guide the study and will be tested at 0.05 level of significance.

1. There are no limiting factors associated with turkey production in Enugu State.

2. ND is not significant among other disease problems of turkeys in Enugu State.
3. Thereôs no significant difference in the susceptibility, pathogenesis and pathology of VND in experimentally inoculated turkeys and chickens?
4. There is no significant difference in the shedding of NDV in faeces/tissues of turkeys compared to that of chickens.
5. There is no significant difference in the antibody response of turkeys and chickens experimentally inoculated with VNDV.
6. There is no significant difference in the haematological changes in turkeys and chickens experimentally inoculated with VNDV.

1.5 Significance of the study

1. The results of the study will give information on the nature of the disease caused by the Nigerian velogenic NDV strain in turkeys. This will be of help in diagnosis of ND in turkeys.
2. The information will help farmers formulate a proper ND vaccination schedule for the turkeys in Nigeria.

1.6 Scope of the study

This study covered farmers who keep chickens and turkeys in Enugu State. The farmers in the study area were used as respondents.

The study centred on gathering information on socio-economic characteristics of turkey producers, production patterns, management practices and prevalent diseases in turkeys, in order to identify the common problems facing turkey production in Enugu State.

The study also determined the sero-prevalence of NDV infection in turkeys raised in backyard poultry farms/households in Enugu State, with the aim of determining the role of turkeys in the epidemiology of ND in both local and exotic chickens.

Experimental infection of turkeys and chickens with VNDV was be carried out in order to characterize clinicopathologic features, determine the persistence of the virus in faeces, monitor the immune response and study the haematologic changes in turkeys and chickens inoculated with VNDV.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Background information on Newcastle Disease

2.2 Synonyms

Avian pneumoencephalitis (Beach, 1944), fowl pest, pseudo-fowl pest, Newcastle fever, pseudovogel-pest, atypische, geflugelpest, pseudo-poultry plague, avian pest, avian distemper, ranikhet disease, Tetelo disease, korean fowl plague.

2.3 Definition of Newcastle Disease

Newcastle disease is globally distributed, regarded throughout the world as one of the most important, highly contagious avian diseases, not only due to the serious disease and high flock mortality that may result from some ND virus (NDV) infections, but also because of the economic impact that may ensue due to trade restrictions and embargoes placed on areas or countries where outbreaks have occurred (Seal *et al*., 2000; Alexander, 2003; Aldous *et al*., 2008). In fact, it has been argued that ND may represent a bigger drain on the world economy than any other animal viral disease (Alexander, 1988, 2003), considering the huge resources committed annually in vaccination against Newcastle Disease. Poultry, as defined by Office international des Epizooties (OIE), includes domestic fowl, turkeys, guinea fowl, ducks, geese, quails, pigeons, pheasants, partridges and ratites that are reared or kept in captivity for breeding, the production of meat or eggs for consumption, or for restocking supplies of game. Virulent ND can cause very severe disease in susceptible birds, with mortality rates exceeding 50 percent (%) in poultry. For this reason ND belongs to the reportable list A disease of the OIE, and within the OIE, terrestrial Animal Health Code for International Trade (OIE, 2005), justifiable trade restrictions may be put in place to prevent the introduction of virulent NDV by live birds, poultry meat and poultry products to countries free from ND. The variable nature of ND strains in terms of virulence for poultry and the different susceptibilities of the different species of birds mean

that for control and trade purposes, ND requires careful definition. The currently proposed OIE definition for reportable ND in poultry adopted at the 67th General Session of the OIE held in Paris in May 1999 was: ôND is defined as an infection of birds caused by a virus of avian paramyxovirus serotype 1 (APMV-1) that meets one of the following criteria for virulence: a) The virus has an intracerebral pathogenicity index (ICPI) in day-old chicks (*Gallus gallus*) of 0.7 or greater; b) Multiple basic amino acids have been demonstrated in the virus (either directly or by deduction) at the C-terminus of F2 protein and phenylalanine at residue 117, which is the N-terminus of F1 protein. The term ómultiple basic amino acidsô refers to at least three arginine or lysine residues 113 to 116. Failure to demonstrate the characteristic pattern of amino acid residues as described above would require characteristic of the isolated virus by an ICIP testõ. Therefore, in many countries the disease remains one of the major problems affecting existing or developing poultry industries. Even in countries where ND may be considered to be controlled, an economic burden is still associated with vaccination and / or maintaining strict biosecurity measures. Newcastle disease is one of few avian zoonotic diseases. Clinically, ND induces conjunctivitis in chickens and humans (Cheville *et al.,* 1972; Hales and Ostler, 1973; Spalatin *et al.,* 1973; Katoh, 1977; Chang, 1981; Alexander, 2003). In the latter report, the eye infections consist of bilateral reddening, excessive lachrymation, edema of the eyelids, conjunctivitis, follicular conjunctivitis and a rise of antibodies against Newcastle Disease virus.

2.4 History of Newcastle Disease

Outbreaks of Newcastle Disease were first reported in poultry in 1926 with the description of a highly pathogentic disease at two geographical sites on different sides of the world, Newcastle-upon-Tyne, England and Island of Java, now part of Indonesia (Kraneveld, 1926; Doyle, 1927). The location of the first outbreak in Britain suggests a possible method of introduction. In 1926 Newcastle ï upon-Tyne was an exceptionally busy port. Doyle (1927) made the point that poultry food was supplemented with offal from Newcastle and, even more categorically, Brown (1965)

stated that the offal came from foreign ships**.** The outbreaks in 1926 were responsible for an entirely different disease signs of pnemoencephalitis and low death rate first described in the 1930s in infected poultry in California (Beach, 1942) was NDV. By 1946 Newcastle Disease virus infection had been identified in 17 states across the USA (Lancaster, 1966). Although inactivated vaccines were used initially to combat Newcastle Disease in the USA, the continued isolation of mild strain led to the concept and development of live vaccine strain. Beaudette *et al.* (1949) selected the Roakin strain after screening 105 Newcastle Disease virus isolate of low virulence. However, this strain proved too virulent for young susceptible birds. Strains B1 (Hitchner and Johnson, 1948; Hitchner, 1975) and La Sota (Goldhaft, 1980) were selected and were to become the most widely used animal vaccines.

2.5 Aetiology of Newcastle Disease

2.5.1 Classification

The causative virus, Newcastle Disease virus, has been classified as a member of the Order *Monoegavirales*, family *Paramyxoviridae,* and subfamily *Paramyxovirinae*. In the current virus taxonomy, ND is caused by Avian *Paramyxovirus* serotype 1 which is together with the other eight APMV serotypes in the genus *Avulavirus* (Mayo 2002; Lamb *et al.,* 2005).

2.5.2 Morphology

Electron microscopic examinations of purified preparations of NDV from infected allantoic fluid of domestic fowl embryos reveal pleomorphic structures. Most of these are rounded and 100-500 nm in diameter. Occasionally, filamentous particles of 100nm in diameter and variable length can be seen. The virion is enveloped with a liquid bilayer membrane derived from the host cell membrane. The ribonucleic acid (RNA) genome contains six major genes that encode the structural proteins (nucleoprotein or nucleocapsid (NP), phosphorprotein (P), matrix (M) protein, fusion (F) protein, hemagglutininï neuraminidase (HN) and polymerase or large (L) protein)

(Millar and Emmerson, 1988), in the order 3 ï NP- P-M-F-HN-L-5 (Chambers *et al*., 1986a; Wilde *et al.,* 1986) as well as two non-structural proteins, W and V. These sequences may be involved in the regulation of NDV replication, transcription and encapsidation of the genomic and antigenomic RNAs (Lamb and Kolakofsky, 1996). Embedded in the envelop are two different glycoproteins, the HN and F proteins, which appear as spikes projecting from the external surface of the membrane when observed under an electron microscope. The HN glycoprotein of NDV possesses haemagglutinatinin (HA) and neuraminidase (NA) activities (Scheid and Choppin, 1974). HA is caused by the adsorption of the virus to specific receptors on red blood cells to form a lattice network between the cells (Kimball, 1990). NA hydrolyses the ketosidic bond between substituted neuraminic acids on host receptors, allowing the membranes to come closer together and permitting the F glycoprotein to make contact with the host membrane, thereby allowing the virus to penetrate the cell surface (Lamb and Kolakofsky, 1996). This region, known as fusion peptide or fusion sequence, is thought to participate directly in the fusion of viral and host cell membranes (Hernandez *et al*., 1996). The F glycoprotein that mediates fusion of the viral and cellular membrane is synthesized as an inactive precursor, F0, (Chambers *et al.,* 1986b; Salih *et al.,* 2000). The precursor is proteolytically cleaved at the peptide bond of residues 116 and 117, to generate two active disulphide-linked polypeptides, F1 and F2 by specific cellular proteases (Gotoh *et al.,* 1992; Ogasawara *et al.,* 1992). Mutagenic experiment using infectious cDNA clone recently confirmed that the cleavability of F0 is a major determinant for virulence (Peters *et al.,* 1999). Studies comparing the deduced amino acid sequence of the F0 precursor of ND strains varying in virulence for chickens showed that virus that are virulent for chickens have the amino acid motif of 112R/K-R-Q-K/R-R-F112 at the cleavage site whereas viruses of low virulence have sequence of 112G/E-K/R-Q-G/E-R-L117 in the same region (Nanthakumar *et al*., 2000; Aldous and Alexander, 2008). These relatively complex proteins interact with each other and are involved in viral infectivity and virulence

(Stone- Hulslander and Morrison, 1997). Either of these proteins can induce protective immunity (Meulemans *et al*., 1986a; Nagy *et al*., 1991). Beneath this lipid membrane is a layer of M protein, which is not only associated with the membrane but also with the N-terminal segment of the HN protein located in its inner surface (Garcia-Sastre *et al.,* 1989). The protein is generally hydrophobic and contains many basic residues, with the location of the protein on the inner surface of the viral envelope (Li *et al*., 1980). The M protein is believed to play an important role in the assembly of the virus by interacting with the NP, the lipid bi-layer and also the regions of the surface glycoproteins that are exposed on the inner surface of the membrane. The viral RNA is located inside the central channel, surrounded by NP subunits (Choppin and compans, 1975) that protect it from nuclease activities. In association with the L and P proteins, the NP protein is thought to be involved in replication and transcription of the viral genome. This herringbone-like structure comprises thousands of NP subunits that are associated tightly with several copies of P and L protein. The P protein in association with L and NP proteins form an active complex involved in genome replication and transcription (Hamaguchi *et al*., 1983, 1985). The non-structural proteins are involved in the replication and pathogenesis of the virus (Mebatsion *et al*., 2001). The L proteins are largest structural protein of NDV. The L and P proteins are involved in viral RNA synthesis (Hamaguchi *et al*.,1983) RNA sequencing studies have shown that sequences may be involved in the regulation of NDV replication, transcription and encapsidation of the genomic and antigenomic RNAs (Lamb and Kolakosky, 1996).

2.6 Biologic properties of the Newcastle Disease virus

2.6.1 Haemagglutination activity

The ability of NDV and other paramyxoviruses to agglutinate red blood cells (RBC) is due to binding of the HN protein to receptors on the surface of the RBCs. The HA activity of NDVs is used for virus identification and measurement of antibody (Alexander, 1997a). This property and

the specific inhibition of agglutination by antisera (Burnet, 1942) have proven to be powerful tools in the diagnosis of the disease. Ito *et al*. (1999) reported that NDVs have different HA activity on erythrocytes from mammals, depending on the avian species from which the viruses were isolated.

2.6.2 Neuraminidase activity

The enzyme NA is also part of the HN molecule or gene which plays an important role in the pathogenesis of paramyxoviruses. An obvious consequence of the possession of this enzyme is the gradual elution of agglutinated RBCs (Ackerman, 1964). It has been postulated that NA removes virus receptors from the host cell based on NA activity on salicylic acid containing receptors which prevents the reattachment of released virus (Nagai, 1993).

2.6.3 Cell fusion and Hemolysis

Cell fusion is basically by the same mechanism stated above. The rigid membrane of the RBCs results in lysis from the virus membrane fusion.

2.7 Replication of Newcastle Disease virus

Since NDV contains NA activity and infects a wide variety of cells containing salicylic acid residues, which serve as receptors for the virus when the RNAs that are required for the synthesis of the viral proteins. Genomic replication then occurs by the synthesis of full-length positive RNA which in turn functions as a template for the production of negative genomic RNA. NDV replication follows the ñrule of sixò (Philips *et al*.,1998; Peters *et al*., 2000), in which the length of the viral genome is an exact multiple of six, and is most probably related to the fact that each NP monomer associate exactly with six nucleotides of the genomic RNA (Egelman *et al*., 1989). The M protein is particularly important for virion assembly followed by enclosure of virus nucleocapsid within the envelope; and release of virus particles which are budded from cell surface.

2.8 Strains and pathotypes of NDV

All strains of NDV are morphologically and serologically indistinguishable but differ in their biological properties, including virulence for chickens and eggs (Alexander and Allan, 1973) as measured by standard techniques (Allan *et al.,* 1973). Mean death time (MDT), pathogenicity for 8 week-old chickens, and the plaque formation activity in chicken embryo fibroblast monolayer, ICPI for day-old chicks and the intravenous pathogenicity index (IVPI) in 6-week-old chickens are used to distinguish the pathotypes. The thermostability of haemagglutinin, the rate of elusion from chicken erythrocyctes, the agglutination of mammalian erythrocytes, are used to distinguish strains within a pathotype. The responsiveness to monoclonal antibodies used for differentination of NDV strains and isolates are used to classify NDV strains (Spalatin *et al.,* 1970; Hanson and Spalatin, 1973; Alexander *et al*., 1997c, 1999; Lomniczi *et al.,* 1998; Werner *et al.,* 1999). Newcastle disease virus strains have also been distinguished on the basis of the clinical signs produced in experimentally infected chickens. Beard and Hanson (1984) defined the following five groups or pathotypes: (1) Doyleôs form (or Velogenic viscerotropic): characterized by acute lethal infections, usually with ulcerative, haemorrhagic lesions in the intestines of dead birds. (2) Beachôs form (or Velogenic neurotropic): characterized by high mortality which follows acute respiratory and neurological diseases characterized by tremors and torticollis, but in which gut lesions are usually absent. (3) Beaduttoôs form or (Mesogenic): clinical signs consist of inapparent respiratory and neurological signs in adult chickens and could be severe in day-old chcks, with low mortality. (4) Hitchnerôs form or (Lentogenic): virus causing inapparent infections in older chickens and very mild infections of the respiratory tract of young chicks. (5) Asymptomatic enteric: an avirulent inapparent intestinal infection in which replication appears to be primarily in the gut. The terms velogenic, mesogenic and lentogenic refer to the MDT in inoculated embryonated eggs (Hanson and Brandly, 1955). The viscerotropic or neurotropic refers to the pathology of the disease produced. The intra-cloacal test was developed to

differentiate velogenic viscerotropic NDV (VVNDV) from other virulent strains (Pearson *et al.,* 1975; Alexander, 1998a). Most pigeon paramyxovirus-1 (PPMV-1) isolates differ from other APMV-1 isolates by having unique monoclonal antibody binding profiles (Alexander *et al.,* 1984 a,b,c; Lana *et al.,* 1988; King, 1996). Those antigenic difference and the difficulty in classifying PPMV-1 isolates by standard NDV pathotyping scheme (that is, by the classic velogenic, mesogenic, and lentogenic criteria which have been more difficult with most other NDV isolates) are the basis for their identification as NDV variants (Alexander, 1997a). However, identification of two antigenic groups among pigeon isolates demonstrates that not all NDV isolates from pigeons are typical of the variant identified as PPMV-1 (Kommers *et al.,* 2001).

2.9.0 Laboratory host systems

Newcastle disease virus can infect and multiply in a range of non-avian (Lancaster, 1966) as well as avian (Kaleta and Bauldauf, 1988) species, following laboratory infection.

2.9.1 Chicken Embryos

Newcastle disease viruses replicate in embryonated chicken eggs preferably from specific pathogen-free sources. Their sensitivity for virus growth, and the high titers to which virus grows in them, make them suitable for virus isolation and propagation. NDV strains and isolates vary in their capacity and time taken to kill chick embryos (Gough *et al.,* 1974). The alantioc cavity is the preferred route of inoculation (Beard and Hanson, 1984).

2.9.2 Cell Cultures

Newcastle disease virus strains can replicate in a wide range of avian cells. Cytopathic effects (CPE) are usually the formation of syncytia with subsequent cell death (Reeve and Poste, 1971). Plaque formation in chick embryo cells is restricted to velogenic and mesogenic virus unless trypsin (Rott, 1985) is added to the overlay.

2.10.0 Pathogenicity

In chickens, the pathogenicity of ND is determined chiefly by the strain of virus, although doses, route of administration, age of the chicken and environmental conditions all have an effect and varies greatly with the host. Chickens are highly susceptible, but ducks and geese may be infected and show few or no clinical signs, even with strains lethal for chickens (Higgins, 1971; Alexander, 2001). The primary molecular determinants for NDV pathogenicity are the F proteins cleavage site and amino acid sequence (Nagai *et al.,* 1976; Glickman *et al.,* 1988) though changes in the structural proteins like the L, F proteins or HN proteins have been reported recently as major determinants of tropism and virulence (Huang *et al.,* 2004; Deleeuw *et al.,* 2005) and the ability of specific cellular proteases to cleave the fusions of different pathotypes (Gotoh *et al.,* 1992; Ogasarawa *et al.,* 1992). The presence of dibasic amino acids in the F proteins sequence allows for systemic spread of velogenic NDV (VNDV), whereas replication of lentogenic NDV is limited to mucosal surfaces of the host (Ogasarawa *et al.,* 1992). The presence of basic amino acids at positions 113, 115 and 116 and phenylalanine at 117 in virulent strains means that cleavage can be affected by protease or proteases present in a wide range of host tissues and organs. However, for lentogenic viruses, cleavage can occur only with proteases recognizing a single arginine, such as trypsin-like enzymes. Lentogenic viruses therefore replicate in areas with trypsin-like enzymes such as the respiratory and intestinal tracts, whereas virulent viruses can replicate in a range of tissues and organs resulting in fatal systemic infections (Rott, 1979). This is also the major factor in diferentiating velogenic and mesogenic NDV from lentogenic NDV isolates in cell culture. All NDV isolates will replicate in chicken embryo kidney cells (king, 1993), presumably because of the presence of a required protease (Ogasarawa *et al*., 1992). However, lentogens must have added proteases for replication in avian fibroblasts or mammalian cell types, whereas mesogenic and velogenic NDV isolates do not have this requirement (Nagai *et al.,* 1976; Kaleta *et al.,* 1980; King, 1993).

2.11 Distribution of Newcastle Disease

An accurate assessment of the distribution of ND throughout the world is difficult to achieve due to the widespread use of live vaccines. However, studies have concluded that ND remains present in many countries of Asia, Africa and America. Western Europe reported marked outbreaks in early 1990s. Phylogenetic studies show that several strains were responsible for these outbreaks and only countries of Oceania have maintained relative freedom from the disease, although serious outbreaks occurred in Australia during 1998-2000 (Kirkland, 2000; Westbury, 2001).

2.11.1 Worldwide situation

Several panzootics of Newcastle Disease have occurred since it was first reported. The first appeared to spread very slowly throughout the world. Hanson, (1972) estimated that it took 16 years to become a true panzootic. The Second World War caused great disruptions to trade and consequently influenced the progression of Newcastle Disease. The beginning of the second panzootic was first recognized at the end of 1960s and within 4 years had reached all areas of the world (Hanson, 1972). The reason for the difference in the rate of spread of the two panzootics is complex. During the 40 years or more separating the start of the two panzootics the commercial poultry industry had been revolutionized in the West and in many of the more developed countries in Asia moving from relatively small privately owned flocks to much larger flocks owned by international companies. Poultry food production had become commercialized, resulting in greater contact between separate farms as food delivery vehicles moved from one to another. Transport had also developed and birds could be moved relatively easily and quickly by air to all parts of the world. This last factor was largely responsible for the huge and growing trade in captive caged birds. Hanson (1972), Francis (1973), Walker *et al.* (1973) and Inskipp and Thomas (1976) were able to link most of the outbreaks occurring in their countries especially USA between 1970 and 1972 to importations of exotic birds. This association in the USA of panzootic virus with caged birds, especially psittacines that appear to be able to excrete virulent

NDV for long periods in the absence of clinical signs (Erickson *et al.,* 1977), gave rise to the theory of a natural reservoir of NDV in psittacine species. Field experience has shown that the viscerotropic virus has an effective method of achieving transport over long distances, traversing ocean and desert barriers with ease, and that it is communicable within flocks, usually infecting all birds (Dawson, 1972). Antigenic and genetic evidence (Alexander *et al*., 1997c; Lomniczi *et al*., 1998) indicate that there was spread of a virulent virus worldwide during the late 1970s. The start and spread of this third panzootic is, presumed to be as a result of the almost universal use of vaccines, since the mid-1970s. Another ND panzootic occurred in the 1980s. There were reports of ND infections in pigeons, probably arising in the Middle East in the late 1970s (Alexander *et al*., 1985b; Kaleta *et al*., 1985), affecting the racing and show pigeons (*Columba livia),* although some spread of the virus to poultry did occur. The world population is enormous and at the end of the 1970s these birds were largely unvaccinated and fully susceptible to infection by NDV. The NDV strain responsible for the pigeons panzootic showed some antigenic variation from other ND viruses, especially using monoclonal antibodies, and as a result its spread around the world could be followed without confusion, with live vaccines or other virulent viruses (Alexander *et al*., 1985a). By 1981 this virus, termed PPMV-1, had reached Europe (Biancifiori and Fiorini, 1983) and by 1985 was a true panzootic. In many countries where outbreaks occurred there was also spread to feral pigeons and doves, presumably as result of contact with infected racing pigeons that failed to return home. In general, this panzootic has proven difficult to bring under control and in several countries it remains probably enzootic in racing and possibly feral pigeons. The biggest challenge of ND is its enzootic presence in developing countries and the effect on village chickens production (Animal and Plant Health inspection Service, United States Department of Agriculture (USDA), 1992; Spradbrow, 1993-94; Awan *et al*., 1994), poultry being an important asset, representing a significant source of protein in the form of eggs and meat in the rural areas.

2.11.2 Incidence of Newcastle Disease in Nigeria

\Newcastle disease was first reported in West Africa in Gambia (Lindley, 1951). According to Hill *et al.* (1953), ND of chickens was reported in Eastern Nigeria during the period of December 1952 to February 1953. The disease was first confirmed by laboratory test at Vom, from outbreaks in Benue province and Ibadan (Hill *et al.,* 1953; Nawathe *et al.,* 1975). Since then ND has become widespread throughout the country, resulting in its endemicity in both village and commercial poultry with frequent severe outbreaks being recorded in highly susceptible poultry flocks (Adu *et al.,* 1986; Orajaka *et al.,* 1999). Studies of naturally occurring and experimental infections have shown that the VNDV is the commonest pathotype in Nigeria. The pathotype has been isolated from captive African grey parrot (*Psittaus erithracus*), by Onunkwo and Momoh (1980) and apparently healthly free-roaming birds by Echeonwu *et al.*(1993). Seroepidemiological studies have been done in cattle egrets and Nigerian laughing doves by Fagbohun *et al*. (2000); local ducks and guinea fowls by Ibu *et al*. (2000) and Maw *et al.* (2003); domestic and semi-domestic birds by Saõidu *et al.* (2004); unvaccinated indigenous chickens by Nwanta *et al.* (2006a) and Oyekunle *et al.* (2006) who from positive findings concluded that NDV was enzootic in Nigeria and that some of these avian species could serve as reservoirs of the virus for commercial chickens. The VNDV strains can infect vaccinated poultry (USDA, 1992) and are the major cause of infection in susceptible commercial and village poultry. Among poultry, chickens are the most susceptible, ducks and geese are the least susceptible and show few or no clinical signs even with strains lethal to chickens are capable of spreading the virus (Higgins, 1971; Spradbrow, 2000; Alexander, 2001; Otim *et al.,* 2006a). As the most important avian disease in Nigeria, ND has been a major limiting factor of increasing poultry production (Fatumbi and Adene, 1979; Majiyagbe and Lamorde, 1997) because it devastates the village poultry, interferes with development of commercial poultry and establishments of trade links. There are about 150 million local chickens which are kept by mainly women and children

(Kitalyi, 1996), providing significant economic and nutritional values. Despite rigorous vaccination programmes, outbreaks of ND are often reported in vaccinated as well as unvaccinated flocks representing commercial and backyard farms in different parts of Nigeria (Nawathe *et al*., 1975; Adu *et al*., 1985; Echeonwu *et al*., 1993). Outbreaks in vaccinated flocks are thought to be due to faulty administration of the vaccines, errors in vaccine production, poor handling and storage, power outages, immunosuppression, poor management of poultry flocks and the presence of intercurrent disease. There is also the possibility of minor antigenic differences between the vaccines and indigenous field strains (Shamaki *et al*., 1989). Ezema *et al*. (2009) reported that ND vaccine induced immunity that prevented clinical disease; the chickens vaccinated against ND became infected by VNDV without developing clinical signs but developed severe lesions. A study of risk factors of ND found salvage sales of chickens in the markets and the presence of wild birds to be important determinants of ND outbreaks in rural chickens in Nigeria (Nwanta, 2003). Other modes of NDV spread to susceptible birds in different parts of the world are applicable in Nigeria, most importantly birds-to-birds contact.

Turkey is one of the sources of animal protein in Nigeria and is the most suitable alternative for small or large scale producers considering the cost of production. Local turkeys are natural foragers and can be kept as scavengers (Peters *et al.,* 1997). National Research Council (1991) reported that turkeys could be reared virtually anywhere and their natural habitat was open forest and wooded areas. Smith (1990) also reported that the carcasses of turkeys contained a higher percentage of protein than the carcasses of chicken. There are very few reports of natural outbreaks of ND in turkeys (Saõidu *et al.,*1994). Serological evidence of ND infection in turkeys was also reported in Zaria, Nigeria by Saõidu *et al.(*2004) with a prevalence rate of 68%, Saidq *et al*.(2011) in a retrospective study on ND cases in Maiduguri,Nigeria reported a prevalence of 57.2%.

2.12 Epidemiology of Newcastle Disease in poultry

Newcastle disease virus has been reported to infect animals other than birds, ranging from reptiles to man (Lancaster, 1966). In a case report, VNDV was isolated from 2 of 29 house mice captured in and around chicken houses (Johnson et al., 1974). Kaleta and Baldauf (1988) reported that natural or experimental NDV infections have been established in birds representing 27 of the 50 orders of the class. They suggested that despite no record of infection of birds in some orders, it is likely that all would be suseceptible to infection, but in addition to different viruses producing marked variation in the severity of disease seen, the same virus may behave differently in different host. They reported a high level of susceptibility in galliformes, psittaciformes, structhioniformes and Columbriformes. This statement is well supported by the fact that these species have been affected in many recent outbreaks of NDV across the world (Jorgensen *et al*., 1998; Kuiken *et al.,* 1998; Schelling *et al*., 1999; Alexander, 2000, 2001). All ages of pheasants are susceptible to ND, and infection with NDV often leads to death, which is preceded by a range of clinical signs (Higgins, 1982). Beer (1976) reported that the first outbreak of ND in free-living pheasant in UK was in 1963. During the extensive ND epizootics in poultry that began in Essex (UK) in 1970, isolations of NDV were made from birds showing clinical signs (Borland, 1972). It was suggested that an outbreak in Denmark, in 1996 was due to virus introduced by infected feral migrating birds (Jorgensen *et al*., 1999). In a further example, pigeons and doves infected with PPMV-1 were proposed as the likely sources of virus causing disease in nearby pheasants, since the viruses isolated from the two sources were quite distinctive and very closely related (Alexander *et al.,* 1997a; Aldous *et al.,* 2004). The virus has also been recovered from commercial and village poultry populations worldwide, passing to poultry from infected feral birds (Alexander *et al.,* 1984a, b; Ezeifeka *et al.,* 1992; Echeonwu *et al.,* 1993; Mathivanan *et al*., 2004). However the factors probably involved in the maintenance of infection are; presence of carrier chickens, constant introduction of susceptible birds, other poultry species, wild birds,

favourable physical environment, movement of live birds and mechanical transport of the virus by humans, especially by vaccination and poultry service crews (Awan *et al*., 1994; Nwanta, 2003; Kinde *et al*., 2005). Virulent NDV isolates have been obtained from captive caged birds (Lancaster, 1977; Senne *et al*., 1983; Kinde *et al*., 2005) and they are associated with the worldwide spread of ND in poultry. Kaleta and Baldauf (1988) speculated that the infections more probably originated at holding stations as a result of enzootic NDV at those stations or spread from nearby poultry such as backyard chicken flocks, and not from enzootic infections in feral birds in countries of origin. Illegal importations were assumed to be responsible for the introduction of the virus. The infected birds either in incubative stage of the infection or as a convalescent carrier is probably the primary means of long distance transport (Lancaster, 1981a). Spread to exotic birds was from contact with infected imported stock. Spread to other species was most probably through contact with infected chickens (Utterback, 1973). A second important source of the virus through international movement has been the illicit transport of fighting cocks in their incubative stage of infection. The most significant outbreaks of NDV in feral birds have been those reported in double-crested cormorants (DCCs, *phalacrocorax auritus*) in North America during the 1990s. Earlier reports of ND in DCCs and related species had been in the late 1940s in Scotland (Blaxland, 1951) and in Quebec in 1975 (Cleary, 1977), and in DCCs in 1990 in Canada in North America (Wobeser *et al.,* 1993). In 1992 the disease re-appeared in free-ranging DCCs in western Canada (Glaser *et al*., 1999), in the latter case spreading to domestic turkeys (Mixson and Pearson, 1992, Heckert, 1993; Meteyer *et al*., 1997). Antigenic and genetic analyses of the viruses suggested that all the 1990 and 1992 viruses were very closely related despite the geographical separation of the host. Pathotype characterization of the cormorant isolates from the epizootic identified the virus as velogenic neurotropic NDV (VNNDV) (Banerjee *et al.,* 1994; Meteyer, *et al.*, 1997). Since these outbreaks covered birds which would follow different migratory routes it seems most probable that initial infection occurred at a

mutual wintering area in Central America. The disease in DCCs was observed again in 1997 and 2002. In both instances virulent Newcastle Disease virus was isolated from dead birds. As before these viruses appeared to be closely related (Kuiken, 1998; Allison *et al*., 2005). Antibodies to Newcastle Disease virus have been detected repeatedly in migratory geese and ducks (Liu *et al.,* 1999; Mai *et al.,* 2004). Newcastle Disease virus isolates of low virulence for chicken have been obtained frequently from migratory feral waterfowl and other aquatic birds. The virus reservoir probably exists in nature (Spalatin and Hanson, 1975). However, outbreaks of Newcastle Disease have occurred in domestic ducks in Hong Kong (Higgins, 1971), geese in China (Liu *et al.,* 2003) with high mortality even in experimental infections (Wan *et al*., 2004). The Newcastle Disease isolates were all determined to be velogenic viruses (Liu *et al., 2003),* revealing that goose-originated NDVs could be readily transmitted to chickens. The movement of migratory birds is a possible mechanism of international transport (Dawson, 1972). Alexander (2000) reviewing ND in ostriches (*Struthio camelus*), pointed out that natural (Placidi and Santucci, 1954; Kauker and Sigert, 1957; Samberg *et al.,* 1989) and experimental (Alwright, 1996) infections have demonstrated the susceptibility of ostriches and other ratites to infection with Newcastle Disease viruses virulent for poultry. Jorgensen *et al.* (1998) reported the isolation of virulent Newcastle Disease virus from ostriches dying while held in quarantine in Denmark in 1995. Cadman *et al.* (1994) carried out a serological survey of nine ostrich farms in Zimbabwe and reported that some were positive for Newcastle Disease virus antibodies. In the late 1970s, an Newcastle Disease virus strain showing some antigenic difference from classical strains appeared in pigeons, causing a frequently fatal disease primarily associated with neurological signs (Alexander *et al.,* 1985a). In Europe it was first reported in racing pigeons in Italy in 1981 (Biancifiori and Fioroni, 1983) and subsequently produced a true panzootic, spreading in racing and show pigeons to all parts of the world (Alexander, 1985a, 1991). Common features of these isolates were that they were antigenically indistinguishable from each other, but different from Newcastle Disease

viruses (APMV-1) isolated in poultry (Meulemans *et al.,* 1986b; Alexander *et al.,* 1993; Collins *et al*., 1993, 1994, 1996). Eleven outbreaks of highly virulent ND for laboratory infected chickens were confirmed in 1997, 1999, and 2000 in Great Britain, in Scandinavian countries in 1996 in commercial poultry (Alexander *et al.,* 1998b). Epidemiolgical investigations indicated that the majority of the outbreaks occurred as a result of secondary spread by human agency from two or more primary infected flocks. The unusual patterns of movement of migratory birds at the end of 1996 and beginning of 1997 suggested that they might have been responsible for primary introduction of the causative virus into Great Britain (Alexander *et al.,* 1998a). Until 1998 Austria had been free of virulent NDV, since the 1932 outbreak (Albiston and Gorrie, 1942), although it had been recognized since 1966 that viruses similar to those placed in the ñasysmptomic entericò pathotype group (Westbury, 1981; Spradbrow, 1988) were present in wild birds and on occasions spreading to commercial poultry flocks. Outbreaks of virulent ND occurred in 1998 and 1999 (Kirkland, 2000), being extremely closely related based on phylogenetic studies of enzootic virus of low virulence suggesting their emergence by mutation. This method for the generation of virulent virus had been suggested earlier for the virulent NDV isolated in Ireland in 1990 (Alexander *et al.,* 1993). This may be a worrying development, not least because of the vast quantities of live vaccines used almost universally.

2.13 Specie susceptibility

NDV is infective for almost all avian species, both domestic and wild. Natural infection has been reported in humans and rodents, and a variety of laboratory animals have been infected experimentally. Infections in non-avian species could spread the disease but the significance of this is not known. However, these animals pose a significant risk because they can act as mechanical vectors of Newcastle Disease.

Chickens

Chickens are highly susceptible to infection with NDV including the pigeon variant of APMV-1 (Kaleta and Baldauf 1988). They are considered to be the most susceptible of domestic poultry species. Most ND outbreaks in chicken are caused by velogenic strains (often referred to as ówirulent ND virusô). Although mesogenic virus strains fall within the OIE classification of ND, very few isolations of mesogenic ND viruses have been made.

Turkeys

Turkeys are susceptible to ND. Outbreaks can occur in turkey flocks but they are usually less severe than those in chickens. Effects on egg production are similar to those in chickens. Some outbreaks have resulted in high mortalities, others in leg paralysis (Saif *et al.*,1997)

Pigeons

Pigeons are susceptible to ND. The pigeon variant of APMV-1 can produce up to 80% morbidity, with nervous signs and diarrhoea being the most notable clinical features (Parnigraphy *et al*.,1993).

Ducks and geese

Ducks are reported to be readily infected with NDV and to be capable of spreading the virus. There are few reports of clinical NDV in ducks, Turkeys can also be infected with the virus, but are apparently not very susceptible to the disease (Saif *et al*., 1997).

Peafowls, guinea fowls, pheasants and quails

All are susceptible to natural NDV infection. Although mortalities have been recorded, infection usually produces only mild disease unless it occurs in quail, which are very susceptible (Heckert, 1993).

Canaries

Canaries are susceptible to NDV infection, which usually produces mild or inapparent disease. However, 20ï 30% mortalities have been recorded in experimental infections in which nervous signs predominated (Kaleta and Baldauf 1988).

Psittacines

They are susceptible to ND (budgerigars are more susceptible than canaries. Nervous signs usually predominate when there is clinical disease.Tropical parrots form a reservoir of virulent ND virus and have been responsible for a number of introductions to the United States. Infected psittacines can excrete virus for at least one year (Kaleta and Baldauf 1988).

Ratites

They are susceptible to infection but are probably fairly resistant to developing clinical signs. In an outbreak in Israel, 13 of 46 ostriches aged 5ï 9 months died with typical nervous signs of ND. The virulent Israel-67 strain of ND virus was isolated (Samberg *et al*., 1989). In 1993, three outbreaks occurred on ostrich farms in South Africa. The mortality rate was low and limited to a particular group or camp (Kaleta and Baldauf 1988).

Wild waterfowl

Wild waterfowl are another reservoir of avirulent ND viruses usually associated with intestinal infection. However, wild waterfowl have been strongly implicated in the spread of outbreaks across Europe. Infections have occurred in cormorants in the United States and Canada over a number of years (Kaleta and Baldauf 1988).

Humans

Humans exposed to ND virus may suffer headache and flu-like symptoms and can develop conjunctivitis, which is usually mild and persists for 1ï 2 days. Occasionally, the conjunctivitis can become quite severe and even lead to some lasting impairment of vision. The incubation period is reported to be 6ï 7 days. Most infections have occurred among laboratory workers who handle the virus in research or vaccine production laboratories. Vaccinators and individuals who eviscerate and process poultry for market may also become infected. Person-to-person transmission of ND virus has not been reported (Alexander, 2001).

Rodents

Rodents harboured ND virus in a 1974 outbreak in California (Johnson, 1974).

2.14 Age susceptibility

Although all ages are susceptible to ND chicks up to 2 weeks old with high levels of maternal antibodies may be less susceptible as implied by Shoyinka (1983), Ezeokoli *et al.* (1985) and Shamaki (1989). Abdu and Garba (1981) reported that the maternal antibodies play a role in protecting chicks against ND. It was reported by Saiôdu *et al.* (2006) that the maternal antibodies in chicks decline to a non-protective level by 2 weeks of age. Halle *et al.* (1999) reported that chicks 3-4 weeks old are at high risk of suffering from ND which may be due to a decline in maternal antibody levels. It was also reported that birds of 9-10 weeks of age were more resistant to ND. This was attributed to the presence of substantial antibody titre due to ND vaccinations at 6 weeks of age (Halle *et al.* 1999).

2.15 Reservoir hosts

A wide range of avian and non-avian species act as reservoirs of NDV and transmit the disease to susceptible birds. In Nigeria, velgenic, mesogenic and lentognic strains of NDVs have been found in wild birds which were considered as sources of infection to susceptible village poultry (Olabode *et al.,*1992). Psittacines can harbor and spread virulent NDV (Awan *et al.,* 1994). Roy *et al*. (1998) reported that NDV isolates obtained from feces of clinically normal seven different species, psittaciformes (Macow parrot; red breasted parakeet, white cockatiel), columbiformes (white dove), phasianiformes (golden pheasant, jungle fowl), and passriformes (white crested laughing thrush) were found to contain VNDV in their feces without showing clinical signs. They could have contaminated the environment thereby serving as reservoirs and a potential source of virulent NDV to other susceptible birds. The duration of virus excretion depends on the pathotypes of NDV and species of birds, virus shedding being usually short in galliformes, but may last many months in psittacines (Lancaster, 1966; Luthgen, 1972, cited in Kaleta and Bauldauf, 1988). Some species (e.g. crane and parrots) may be infected and shed virulent NDV for weeks without showing clinical signs (Erickson *et al.*1977). The Amazon parrot may act as a

carrier and shed virus for longer than one year (Cubas, 1993). Several shared changes within the F protein and M proteins among virulent NDV isolates have been identified (Holland *et al*.,1992). These differences have occurred among virus isolates of different virulent types from a variety of birds with different geographic origins (Collins *et al*., 1994; Seal *et al*., 1998). This further indicates that multiple lineages of virulent NDV are circulating among demostic, pet and wild birds, harboring virulent NDV chronically (Erickson *et al*.,1977; Collins *et al*., 1994; King, 1996). Also, Kommers *et al.* (2002) demonstrated that PPMV-1 causes severe lesions among infected chickens, mostly affecting the heart and brain and concluded that pigeons must be considered seriously as a potential source of NDV infection and disease for commercial poultry flocks. Consequently, highly virulent NDV isolates continue to circulate among birds other than chickens and threaten commercial poultry worldwide (Kommers *et al.,* 2001, 2003b).

2.16 Transmission of Newcastle Disease

Newcastle disease virus replicates in the intestine and may be transmitted by ingestion of contaminated feces or by inhalation of small infective particles produced from dried feces (Alexander, 1997a).

2.16.1 Methods of Infection

Through Eggs

Hens infected with NDV may shed large quantities of virus in feces (Alexander, 1997b). Eggs from infected hens contaminated with virus-laden feces could be a mechanical source of NDV in ahatchery and result in infected chicks upon hatching, or the virus could penetrate the shell during incubation and cause the death of the embryos (Williams and Dillard, 1968). Cracked or broken NDV-infected eggs also could serve as a source of virus for newly hatched chicks. Although the presence of low or high virulent NDV in the reproductive organs following vaccination or infection has been reported (Razewska, 1964), vertical transmission has been

controversial, and definitive evidence is lacking (Lancaster, 1966). However, Chen and Wang (2002) in a study demonstrated that in ovo injection causes the spread of NDV if the eggs are contaminated with NDV; chicks could hatch and die later from virulent NDV infection. This may confirm the possibility of NDV infection through eggs. Although the study is not definitive proof for transovarian transmission, it is important in understanding potential non-conventional ways for ND to spread.

Airborne spread

Winds may carry the virus particularly on feathers and fecal particulates, from to farm, as suggested by field reports. Hugh-Jones *et al.* (1973) were able to detect virus 64 meters but not 165 meters downwind of infected premises. They stressed the importance of relative humidity on the likelihood of airborne spread in epizootics of ND, especially when climatic conditions have been right (McFerran, 1989). Though, in recent years this has not been an issue in reported outbreaks (Zander *et al.,* 1997).

Movement of poultry and the agency of man

Contaminated feces will be a source of infection for suscepitable birds, since the virus can survive for more than 8 weeks in hot dry tropical areas at temperatures of 40 ^{0}C (Warner, 1989) and for 3 months at 20^{0}C to 30^{0}C (Lancaster, 1966). The sale of infected carcasses, with subsequent infection of susceptible birds by the discarded un-boiled giblets, or the movement of day-old chicks and point-of-lay pullets or adult birds, known to have been in contact with the disease (Nwanta, 2003). In an area where the poultry industry is dense, spread will be though the movement of supervisors and other itinerant workers, especially vaccination and poultry service crews, feed lorries, packing station crews and the free movement of sparrows and wild birds. Spread to exotic birds was from contact with infected imported stock (Lancaster, 1975; Panigraphy *et al*., 1993).

2.16.2 The role of free-ranging/semi-captive and wild/feral birds in the transmission of Newcastle Disease Virus

Seroepidemiological and isolation studies have shown that virulent NDV is enzootic in rural poultry populations (Spradbrow, 1993, 1994; Nwanta, 2003; Otim *et al.,* 2004, 2006a). Otim *et al*. (2006a,b) experimentally confirmed that though ducks can be infected with virulent NDV, they do not show clinical signs but are able to transmit NDV to in-contact unvaccinated susceptible chicks. Vaccinated birds appear to show little or no disease signs when infected with NDV, so could be asymptomatic shedders (Capua *et al*., 1994; Ezema, 2009). Aldous *et al.* (2007) reported an outbreak of ND in pheasants in Great Britain where disease signs were recorded at a shooting estate shortly after receiving a batch of imported pheasant chicks and virulent NDV was isolated. It was reported that the spread of NDV to chickens has occurred in several countries (Alexander *et al.*, 1984a and 1985c; Lister *et al*., 1986; Vinoevogel and Duchatel, 1988). Twenty-three outbreaks of ND in unvaccinated commercial chickens occurred in 1984 as a result of feedstuffs that had been contaminated by feces and carcasses of feral pigeons infected with PPMV-1 (Alexander *et al.*, 1984a). The role of wild birds as carriers in the spread of NDV is illustrated by the outbreak in free-living pheasants in Denmark, where in the absence of any traceable contact with diseased poultry or other birds. It was suggested that the outbreak occurred due to virus introduced by infected feral migrating birds (Jorgensen *et al.,* 1999). In a further example, pigeons and doves infected with PPMV-1 were proposed as likely sources of virus causing diseases in nearby pheasants, since the viruses isolated from the two sources were quite distinctive and very closely related (Alexander *et al*., 1997a; Aldous *et al.,* 2004). A virus of APMV serotype 7 usually associated with pigeons and doves has also been isolated from intestinal contents of two 5-month old ostriches (Woolcock *et al.,* 1996). It was concluded that the infection resulted from contact with feral columbine birds. An elevated risk of diseases transfer to semi-domestic hosts could exist where birds are kept in direct or indirect

contact with domestic poultry, due to increased risk of exposure to viruses present in the domestic population (Pearson and Mccain, 1975). Examination of sera from capitive game birds (pheasants and partridges, cattles ergrets, pigeons and Nigerian laughing doves) showed antibodies to infectious bronchits, marble spleen disease, avian rotavirus, pheasant coronavirus and NDV. Gough *et al.* (1990) and Oyekunle *et al.*(2006) speculated that the antibodies to NDV were most likely the result of spread of live ND vaccines used on nearby poultry farms.

2.17 Epizootic and Enzootic Newcastle Disease

In non-immune, intensively managed, commercial poultry, the introduction of a pathogenic strain of NDV in sufficient quantity to infect a bird is in itself a sufficient cause of ND epizootic (Martin, 1992). When the virus is introduced, it spread quickly. There will be few survivors. Epizootic ND is not self-sustaining. The virus will vanish when no susceptible chickens remain (Spradbrow, 2000). Mildly virulent NDV strains are enzootic and circulate on a regular basis in many poultry populations. These viruses are thought to impair clearance of other respiratory pathogens, leading to secondary infections that cause diseases (Ficken *et al.*, 1987a, b; Nakamura *et al.*, 1994). Also virus of low virulence cause respiratory disease in broilers in Australia (Hooper *et al.*, 1999b).

2.18 Incubation period

The incubation period of ND after natural and experimental exposure has been reported to vary from 2-15 days (average 5-6 days). Appearance of signs is variable depending on the infecting virus (Brown *et al*., 1999a). Mishra *et al*. (2001) and Wakamatsu *et al.* (2006) showed that susceptibility to highly virulent NDV varied among the host species which included chickens, turkeys, pigeons and guinea fowls. ND was more severe in younger birds (Nwanta *et al.*, 2006b). Susceptibility and severity also depend on immune status, infection with other organisms, environmental conditions, the route of exposure, and the dose of the virus.

2.19 Clinical signs

Many avian species are susceptible to NDV and the clinical signs of ND vary greatly in morbidly and mortality, ranging from subclinical infection to 100% mortality in a short period of time. Many factors related to the host (species, age, and immune status), virus (pathotype, dosage, and route of infection), co-infection with other organisms, and environmental or social stress can influence the severity and the course of the disease (Kaleta and Baldauf, 1988; Alexander, 1997a). With extremely virulent viruses, susceptible birds may die without showing clinical signs (Alexander, 2003). A death rate of almost 100% can occur in unvaccinated poultry flocks. The incubation period of the peracute type is short (2 to 4 days) and the onset is sudden. Diarrhea is often marked and there may be copious tracheal discharge. The head and wattles may swell with edema and the bird becomes prostrate. Paralysis may be evident but tremors and torticollis are rare unless the disease is prolonged. Death is usually prompt, occurring 1 to 3 days after signs appear. In experimental infections in uvaccinated chickens with the VVND pathotype the disease was marked by depression at day 2 PI, nervous signs, such as birds down on their hocks, head twitches and tremor, periocular edema, 100% mortality by day 5 P1 (Wakamatsu *et al.*, 2006). Brown *et al.* (1999a) reported marked bilaterial conjunctives. In addition, periocular oedema, coma, prostratation, open-mouth breathing, ruffled feathers, dark foci on the combs (interpreted to be foci of necrosis), extensor rigidity and spastic leg paralysis, walking on hocks, head twitching, diarrhea, hunched posture, conjunctivitis were reported (Kommers *et al*., 2002, 2003a, b). Okoye *et al*., (2000) observed dullness, ruffled fearthers, drop in feed and water consumption, and droopy wings on day 3 PI, greenish diarrhea at day 4 PI and nervous signs such as head shaking, paralysis of the legs and wings, jerking of the head downward and upward, torticollis in few birds from day 7 PI. Morbidity was 100% and mortality 92%. ND causes a sudden and drastic drop in egg production and quality, and mortality of 0 to 50% in layers in natural outbreaks (Biswal and Morrill, 1954; Echeonwu *et al*., 1993). The greatest loss among laying

birds frequently results from reduced egg production and impaired eggshell and albumen quality. Egg production returns to normal within 4 to 5 weeks regardless of whether the infection was of natural orgin or a result of vaccination (Quinn *et al*., 1953).

Other species

Following infection with velogenic strains in unvaccinated turkeys, conjunctivitis, periocular oedema, nasal discharge, dyspnea, watery/ bloody droppings, diarrhea, mild to severe depression, in-coordination in few birds, and 100% mortality were reported (Piacenti *et al*., 2006; Wakamatsu *et al*., 2006) Abdu and Saiḉdu (1990) reported circling, paralysis of the legs and wings, profuse yellowish green or white diarrhea, ruffled feathers, depression and dehydration. In guinea fowls, clinical signs were first observed on day 5 PI. These included dullness, depression, anorexia, diarrhea and paralysis of the legs. They exhibited nervous signs such as in-cordination, muscle tremors and trembling of neck at the advanced stage of the disease. Mortality was 52% and 8% in groups infected with chicken and guinea fowl isolates respectively (Mishra *et al*., 2001). In guinea fowl,incubation period was 4 days PI and no mortality was recorded by Agoha *et al.* (1992), in addition, 24.3% moratlity, coughing, sneszing, and complete cessation of egg production were reported in a natural outbreak (Haruna *et al*., 1993). In pigeons, body tremors at day 10PI, 17% mortality in unvaccinated infected was observed (Wakamatsu *et al.,* 2006). In ND epizootic cormorants showed central nervous system dysfunction such as partial or complete paralysis of the limbs, with the legs and the wings held against the body with curled toes, and the knee, tarso-metarsals and digital joints were stiff. The skin and feathers of the leading edges of both carpal joints were abraded, due to leaning on the ground with their wings: other nervous signs included head tremors, ataxia, standing on a foot with toes curled, walking in circles, and apparent blindness (Kuiken *et al.*, 1999).

2.20 Pathology of Newcastle Disease in birds

2.20.1 Gross Lesions

As with clinical signs, the gross lesions and the organs affected in birds infected with NDV are dependent on the strain and pathotype of the infecting virus, in addition to the host and all the other factors that may affect the severity of the disease. No pathognomonic lesions are associated with any form of the disease (Alexander, 1997a, 1998a). The gross and microscopic lesions of the original peracute form was studies by Jungherr *et al.* (1946) cited by Simmons (1967). The descriptions of gross pathological changes of VVNDV infection in chickens have been studied by many workers and are seen in many organs. Congestion in the breast, leg and thigh muscles has been observed (Okoye *et al*., 2000). In the gastrointestinal tract, hemorrhages were observed in the proventricular mucosa, in the mucosa of the junctions of oesophagus-proventriculus and proventriculus-gizzard. The intestines showed catarrhal or haemorrhagic enteritis, while sharply demarcated button-like haemorrhagic ulcers on the jejunum and ileum were observed. The caecal tonsils were swollen, haemorrhagic and often contained cheesy necrotic material (Okoye *et al*., 2000). Petechiation in the cloaca had been observed at days 4 to 5 PI (Brown *et al*., 1999a). Multiple necrotic foci of different sizes and petechial haemorrhages were often observed in the pancreas (Wan *et al.*, 2004). The thymus was severly atropic. At a certain stage the tissue was no longer detectable (Okoye *et al.,* 2000). Atrophy with petechial heamorrages was occasionally observed in the thymus (Kommers *et al.,* 2002). The bursa of Fabricius was atropic after being initially enlarged (Okoye *et al*., 2000). The spleen was initially enlarged, mottled with dark spots on the serosal surface but later atrophic (Okoye *et al.*, 2000; Wakamatsu *et al*., 2006). But the three lymphoid organs regained their normal sizes later (Okoye *et al.*, 2000). Congestion and exudates were observed in the tracheal mucosa on day 6 PI. There were copious amounts of frothy exudates in the bronchi, extending into the congested, oedematous and occasionally consolidate lung tissues (Hamid *et al*., 1991; Echeonwu *et al.*, 1993; Kommers *et al*., 2003a).

Congested liver has been observed (Okoye *et al.*, 2000). A slight serous pericarditis and petechial heamorrage in the epicardium and myocardium from day 5 PI have been reported (Hamid *et al*., 1991). Reticulated kidneys (urate deposits), swollen and heamorrhagic were reported by Okoye *et al*. (2000). The eyes showed oedema, congestion and petechial heamorrhage in the sclera by days 2 to 5 PI (Nakamura *et al*., 2004). Pale bone marrow and necrosis of the comb tips has been observed occasionally (Kommers *et al*., 2002; 2003a, b). The reproductive tract showed congestion and oedema of the oviduct and uterus in laying birds (Rao *et al*., 2002). The carcasses are often emaciated and dehydrated in chronic cases without internal lesions. In a chicken inoculated with neutrophic velogenic Newcastle disease (NVND) pathotype; same lesions as in VVND pathotype were observed in the spleen, proventricular mucosa at days 2 to 5 PI, except opacity of the air sac at day 10 PI (Brown *et al*., 1999a). In chickens inoculated with mesogenic pathotype, the major gross lesions included cloudy air sacs, haemorrhage in the lungs, congestion of the trachea, splenomegaly, atrophy of the thymus and bursa of fabricius, and whitish discolouration of the brain (Bhaiyat *et al*., 1994). Low virulence viruses showed congested trachea (Hooper *et al*., 1999b).

Other Species

The major gross lesions observed in unvaccinated turkeys infected with VVND pathotype included enlarged and mottled spleen, pale bone marrow, multiple foci of hemorrhage in the pancreas and mucosa of the ileum, atrophy of the lymphoid organs (spleen, thymus, and bursa) and cloudy air sacs (Piacenti, *et al*., 2006). Abdu and Saĝidu (1990) reported hemorrhages in the gastrointestinal tract (GIT), caecal tonsils,kidneys and liver enlargement. Congestion of trachea and kidneys were observed. In unvaccinated guinea fowls infected with VVND hemorrhages at the tip of the proventricular glands and in the caecal tonsil were reported (Mishra *et al.*, 2001). But only emaciation with prominent keel bone, empty intestinal tract and distended gall bladder in most keets were reported by Agoha *et al.* (1992). In the cormorants, gross lesions seen were

hemorrhagic conjunctiva of the sclera, nictitating membrane and eyelid. Oedematous brain, with generalized venous congestion, enlarged, mottled and later atrophic spleens. necrotic foci in the pancreas and liver parenchyma. The later were later reduced in size with sharp edges (Kuiken *et al*., 1999).

2.20.2 Histopathology

The histopathology of NDV infections is as varied as the clinical signs and gross lesions and can be greatly affected by the same parameters. In unvaccinated chickens infected with VVND pathotype, ulceration of overlying intestinal epithelium, mucosa, which may be extensive, hemorrhages and hyperplasia of the goblet and crypt cells occurred. Proventriculus showed necrosis of the glandular epithelium, and hemorrhages in the lamina propria (Okoye *et al*., 2000; Kommers *et al.*, 2002). There was necrosis of the lymphoid tissue in caecal tonsils. Also the thymus and bursa of Fabricius had severe lymphoid depletion and necrosis. Lymphocytic necrosis was seen as pyknosis or karyorrhexis while lymphocyte depletion was characterized by fewer lymphocytes than normal, or none. Observed in the bursa of Fabricius was hyperplastic plical epithelium that showed numerous folds, intra inter-follicular oedema, and later follicular atrophy. There was ballooning degeneration in the bursa. Spleens had severe lymphoid depletion and necrosis, often with extensive deposits of fibrin replacing peri-arteriolar lymphoid sheaths, especially around the sheathed arterioles. Reticular cell hyperplasia and increase in number of the germinal centers occurred towards recovery (Okoye *et al.,* 2000; Rao *et al*., 2002). Eye lesions showed severe conjunctivitis, characterized by oedema and cellular infiltration in the lamina propria of the conjunctivae.Thrombi were observed in the capillaries of the lamina propria (Kommers *et al*.,2002; Nakamura *et al.,*2004). Tracheitis, characterized by extensive lymphocyte infiltration, loss of cilia and mucous glands, and squamous metaplasia of surface eptithelium was observed (Hooper *et al.*, 1999a). In the breast, leg and thigh muscles, myofiber necrosis was observed. In the heart, necrosis of cardiac myofibers and accumulations of monoculear infiltrates

were present in the myocardium (Brown *et al.*, 1999a). In other organs histopathologic lesions described were focal areas of necrosis in the pancreas, bone marrow and liver, with lymphoplasmacyic infiltrates in the latter. Some epithelial necrosis of the comb was also reported (Kommers *et al.*, 2003a). In the kidneys congestion of the peri-tubular blood vessels, casts and pyknosis of the tubular epithelial cells occurred (Okoye *et al.*, 2000). In the reproductive tract, degenerative and diffuse, marked necrotic changes were observed in the glandular epithelial cells with accumulation of cellular debrie between folds and fibrinous cellular debrie within the lumen as well. In the infected magnum, the surface epithelial cells showed extensive areas of necrosis and desquamation and marked atrophy of tubular glands, intertubular oedema with scattered infiltration of macrophages (Rao *et al.,* 2002). Brain lesions were characterized by necrosis in the granular layer, and vacuolation in the molecular layer and white matter, demyelination and degeneration of the purkinje cells in the cerebellum. Perivascular cuffing with lymphocytes in cerebrum and cerebellum, congestion, submeningeal edema, endotheliosis and gliosis were observed (Okoye *et al.,* 2000). Focal neuronal degeneration and gliosis in the cerebellar molecular layer, intracytoplasmic inclusion bodies and peripheral chromatolysis were reported by Brown *et al.,* (1999b). In other organs, histopathogic lesions described were focal areas of necrosis in the pancreas, bone marrow and liver, with lymphoplasmacytic infiltrates in the later. Severe epithelial necrosis of the comb was also reported (Kommers *et al.,*2003a). In experimental infection with velogenic isolates in guinea fowls, there were degeneration and necrosis of lymphoid cells in the follicles and around the adenoid sheath in bursa of Fabricius and spleen respectively. The later also showed reticulo-endothelia cell hyperplasisa. There was pulmonary congestion with mild interlobular oedema. Lesions in the brain were characterized by mild endotheliosis and gliosis in the cerebellum (Mishra *et al*., 2001). The presence of intrancular and intracytoplasmic eosinophilic inclusion bodies in epithelial cells of esophageal glands and in the hepatocytes of cuckoo doves has been reported (Shivaprasad *et al*., 1999). In cormorants, same

lesions observed in infected chickens were described in their organs except, in the brain and spinal cord lesions where additionally spongy change due to necrosis and loss of neurons and axons in the cerebellar nuclei, in the brain stem, and in the ventral horn of the grey matter of the lumbar spinal cord were described. Axonal degeneration considered of swelling and fragmentation of axons, and the formation of ellipsoids (peripheral chromotoysis). Eye lesions consisted of suppurative keratitis characterized by diffuse cellular infiltration of the corneal stroma. The chromaffin (medulary) cells of the adrenal gland were infiltrated by few to moderate numbers of mature granulocytes (Kuiken *et al.,* 1999). In unvaccinated chickens inoculated with mesogenic pathotype, lesions of splenic lymphoid hyperplasia, degeneration and necrosis of myofibers and mononuclear inflammatory infiltrates occurred. There were lymphoid depletion, necrosis, increased evidence of apoptosis and mild conjunctivitis. The brain lesions were characterized by severe multifocal lymphoplasmacytic encephalitis with neuronal necrosis, perivascular oedema, perivascular cuffs, endothelial cell hyperplasisa, peripheral chromatolysis, neuronal necrosis with neuronophgia, gliosis, and less often neuropil vacuolation and meningitis (Kommers *et al.,* 2001, 2002). In another report, there was mild heamorrhagic penumoina, catarrhal tracheitis, and diffuse non-suppurative encephalitis characterized by malacia, and demyelination (Bhaiyat *et al*., 1994). In unvaccinated turkeys infected with VVND pathotype; in the lymphoid organs there were severe depletion with necrosis; moderate to severe pancreatic necrosis and myofiber necrosis. In addition, gliosis with perivascular cuffing in the cerebellum and brain stem were described for NVND (Piacenti *et al.*, 2006).

2.21 Immunity to Newcastle Disease infection/ Serology

The immune system in poultry has developed several levels of defense strategies to cope with a wide spectrum of pathogens, and for the variety of vaccines that may be used, often repeatedly, to protect flocks against exposure to virulent organism in the environment. Included are aspects of innate immunity such as physical and chemical barriers that prevent entry of the pathogen, and

adaptive immunity such as cellular and soluble components that are deigned to elimnnate the pathogen once it has gained entry. Although very effective, innate immunity is often not able to fight off the pathogen and prevent disease. At this point adaptive immunity is required to specifically focus defense mechanisms on that particular pathogen resulting not only in the elimination of the pathogen but also as protection in case of a repeat encounter with the same pathogen. It is the ability of adaptive immunity to recognize molecular features of the pathogen using highly specific antigen receptor-antigen interactions that conveys specificity to adaptive immunity and allows it to specifically focus immune activities on the invading pathogen. Additionally, immune mechanism mediated by adaptive immunity are also differentially tailored to effectively deal with pathogens that are in the body fluids and tissues spaces compared with such pathogens that are located inside cells. These broad categories of specialization within adaptive immunity are inherent in anti-body-mediated (humoral) immunity and cell-mediated immunity (CMI) respectively (Davsion, 1996; Abbas *et al*., 2000). When antigens have entered cells (e.g., by endocytic mechanisms; exogenous antigens) or are generated within the cell (e.g., viral proteins), humoral immune mechanisms involving direct antibody-antigen contact are no longer effective in eliminating the antigen through mucosal surfacees (e.g., secretory IgA) and in eliminating pathogens that are in the extrcellular environment of the host (Abbas *et al*., 2000). In this situation, cell-mediated immune mechanisms that lead to the intracellular destruction of the antigen or to the elimination of the host cell are the most promising approaches to antigen elimuination. In poultry, the initial immune response to infection with NDV is cell mediated and may be detectable as early as 2-3 days after infection with live vaccine strains, though it is not protective against challenge with virulent NDV because it does not stimulate a measurable antibody response. The antigen-specific component of CMI is the T cell (Chen *et al*., 1991). T cells that play primarily a regulatory role in adaptive immunity, whether cell-mediated or humoral, are referred to as T helper (Th) cells and typically express CD4 molecules on their

surface (Chen *et al.,*1991). The main effector mechanisms of CMI include activation of cytotoxic cells [natural killer (NK) and cytotoxic T lymphocytes (CTL) to deal with endogenous intracellular antigens. The NK cell plays an important role in the elimination of cells harboring endogenus antigen that is not visible to CTL. (Yawata *et al*., 2002; Yokoyama and Poulgastel, 2003). In poultry, adaptive immunity, including initation of cell-mediated and humoral immune activity, is critically dependent on regulation by T- cells (Arstila *et al*.,1994). This process occurs in the thymus during T-cell development, and leaves the thymus after maturation. Activation of Th cells results in proliferation of the activated Th cells and their differentiation into effector cells or memory cells. Memory cells are important for future encounters with the same antigen. The concept of diseases protection (immunity), which forms the basis of vaccination, is inherent in the development of memory cells during the primary encounter with antigen. During a repeat encounter with the same antigen, the actions of antigen-specific effector cells derived from these memory cells will result in the rapid elimination of the antigen which, in the case of a pathogenic antigen, would mean before the pathogen can cause disease (Abbas *et al.,*2000). During an infection, microorganisms are phagocytosed by the macrophage and are contained within phagolysosome where these exogenous antigens are killed and digested (Abbas *et al.,* 2000). Immunolobulins (Ig) or antibodies secreted by B cells constitute the principal component of humoral immunity. Antibodies are readily detected in the serum or plasma fraction of blood. Exposure of birds to microorganisms stimulates the production of specific antibodies, which, in turn, react with microorgansism and hasten their destruction. One of the three mechanisms by which antibodies contribute to defense against pathogens is: neutralization. Antibodies bind to and neutralize specific pathogens, particularly viruses. Neutralized viruses are unable to attach to surface receptors of target cells and are thus prevented from replication. Chickens have three main classes of immunoglobulin (Ig); IgM, IgA. IgM is found on the surface of most B cells and is the first antibody produced following immunization. B cells use surface Ig to bind to antigens.

As the immune response progresses, the IgM- producing cells stop IgM production and start the production of IgG or IgA. IgG is also the principal antibody produced after secondary immunization and is the predominant Ig class in chickens blood. IgA is the most important Ig involved in mucosal immunity. IgA protects mucosal surfaces against pathogens, particularly viruses, by neutralizing and preventing their attachment to receptors on target cells. This feature may represent a completive advantage in the event of a velogenic viscerotrophic challenge where the virus has been reported to induce massive destruction of intestinal lymphoid areas and extensive ulceration of overlaying intestinal epithelium associated with active viral replication (Brown *et al*., 1999b).

2.21.1 Passive Immunity

Passive immunity is critical for protecting the chicks against infections during early life. In chickens, eggs are the principal mode of transfer of immunity. The developing chick acquires maternal IgG from the yolk sac by absorption across the highly vascularized yolk sac epithelium. IgA and IgM are transferred via the amniotic fluid by swallowing. Peak levels of maternal IgG in the circulation of newly hatched chick are reached around 2 to 3 days of age. Maternally derived antibodies decline linearly in the recipient and become undetectable after 2 to 5 weeks. Systemic humoral immunity represented by neutralizing IgG antibodies against NDV HN and F glycoproteins is a relevant component of the birdôs protection against infection. Immunity against ND is due primarily to antibody or activity directed against the two viral glycoproteins; the HN and F proteins (Meulemans, 1986a).

2.21.2 Assays to measure immunity

Antibody levels

Birds exposed to pathogens develop circulating antibodies that generally persist for several weeks after the antigen has been cleared. Detection of these antibodies is much more than

detecting cellular immunity and a number of serologic assays are available to quantitate antibodies. Some of the commonly used serologic tests include agar gel precipitation test, virus neutralization (VN) test, immunofluoresence test, hemagglutination inhibition (HI) test, and enzyme linked immunosorbent assay (ELISA). Because the VN response appears to parallel the HI response, the later test is frequently used to assess protective response especially after vaccination (Allan *et al*., 1978).

Serologic test

The standard test for poultry is the haemagglutination inhibition (HI) test, which is described in detail in OIE (2004) and America Association of Avian pathogist Manuals. In these documents, it is pointed out that sera from different species (including turkeys) may cause nonspecific agglutination of chicken red blood cells, complicating the test. To eliminate this, it is necessary to pre-adsorb with chickens red blood cells. The HA and HI test are not generally affected by minor changes in the methodology (Alexander, 2003). The HI test is the most commonly used serological method for determining flock immunity to ND. The serum HI-titre is only a reflection of a more complex immune response, consisting of systemic and local immune mechanisms. Besides a systemic antibody system, chickens have a more or less separate local antibody system (Zakay-Rones *et al*., 1971, 1972). The avian upper respiratory and intestinal tracts are provided with lymphoid tissue and plasma cells capable of responding mainly to topical stimulation (Bang and Bang, 1968; Mueller *et al*., 1971). Since the identification of avian secretory IgA, a preponderance of IgA-containing plasma cells was shown to be present in the avian intestinal mucosa, bronchi and oviduct (Lebacq-Verheyden *et al*., 1972). According to Parry and Aitken (1973), the virus neutralizing activities of respiratory tract secretions in the early stage of infection with NDV were mainly associated with presence of IgA, the major Ig class of local secretions. The virus neutralizing activities of local secretory antibodies, particularly IgA, are an indication of their protective role.

2.22 Diagnosis of Newcastle Disease

As a result of the variations in virulence and the lack of characteristic clinical signs, confirmatory diagnosis involves two steps: (i) isolation of the virus from affected birds, in 10-11 days embryonated chicken eggs obtained from a flock free of NDV antibodies; and identification of the virus as NDV using HA and HI tests. Specimens for attempting viral isolation should be selected from case in the early stages of the diseases. The virulent NDV strains are widely distributed in the avian body, and can be isolated from lungs, trachea, spleen, intestine bursa, thymus, kidneys, and brain depending on the main sites of replication of NDV in infected poultry, and from carcasses based on clinical signs prior to death and organs mostly affected. Tissue triturates or media from cloacal and tracheal swab are inoculated into 8-11 day embryonated chicken eggs, and after a variable period of incubation, depending on the virulence of the strain, the virus will be found in the amnionic allantoic fluids, which are then tested for avian erythrocyte agglutinating activity. Subsequently, it is determined if the hemagglutination reaction is inhibited by known ND antiserums (ii) Establishment of the virus as fulfilling a predetermined definition of ND that would distinguish it from vaccine virues or avirulent enzootic NDVs (Alexander, 1988) The NDV isolates are characterized by determining the time necessary to kill chicken embryos and the lesions produced in chickens inoculated with the virus. Early diagnosis of NDV would allow a more effective control of the disease. Direct detection of NDV can also be achieved through immune-histochemistry, in situ hybridization and immune-peroxidase assay which offer a rapid means of identifying NDV antigens (Lockaby *et al*., 1993; Brown *et al.*, 1999b). It can be applied to formalin-fixed, paraffin-embedded tissues, potentially providing a diagnosis even in cases in which fresh tissue or serum is unavailable. However, the methods used most widely are routine serological tests such as virus neutralization and HI assays. Serum or clotted blood sample should also be taken for serology, and unclotted blood for hematology from live clinically affected birds (Talebi *et al.,* 2005). Cloacal or tracheal swabs

should be taken for virus detection, pathogenicity assessment and virus characterization. Tissues are collected based on clinical signs prior to death and organs mostly affected for pathology-based techniques, pathogenicity assessment and virus characterization from recently dead birds. Fresh samples and swabs in transport medium should be forwarded chilled. Once serological evidence have been found, a pathogenicity assessment is done as soon a possible. Different techniques are available including plaque test in chicken embryo fibroblast cultures, mean death time of embryonated chicken eggs, ICPI 1-day-old chickens.

2.23 Differential diagnosis of Newcastle Disease

The clinical signs and course of virulent ND closely resemble those of a number of other avian diseases including, HPAI, diferentiated based on a history of an outbreak within the locality or its environs. Severe necrotic foci, heamorrhages, and cyanonsis of the non feathered skin is common especially, wattles, combs and shanks is common. Also severe respiratory signs are common in HPAI. Infectious laryngotrachetis (ILT), in severe epizootic forms, there would be degeneration, necrosis, and severe hemorrhages into the lumen of the trachea which may result in blood casts, marked dyspnosa, and expectoration of blood stained muscus. Also ILT is characterized by pathognomonic intranuclear inclusion bodies in the respiratory and conjunctiva cells. Infectious bronchitis (IB) is generally less severe than ND. Nervous signs may be greater in IB. The lymphomatosis of the viscera which occurs in Marekôs diseases is not seen in ND. In avian encephalomyelitis (AE), peculiar lesions are i) Gliosis in the nucleus (ii) rotundus and ovoidalis iii) Lymphocystic foci in the muscular wall of the proventriculus, and circumscribed lymphocytic follicles in the pancreas. ND rarely causes an interstial pancreatitis. iii) Central chromatolysis as opposed to peripheral chromatolysis of ND. In encephalomalacia, characteristic histological lesions are severe degenerative and necrotic lesions in the purkinje cells of the brain. In infectious bursal disease, there are characteristic spiking mortality curve and rapid recovery (5to 7 days) from clinical signs, haemorrhages in the mucosa at the juncture of the proventriculus and

gizzard. There is gross enlargement of the brusa of Fabricius due to severe oedema, hyperemia and cell debris. Marked accumulation of heterophils is pathognomonic. In ND, marked accumulation of heterophils is not part of the enlargement. In aspergillosis, the lesions of the respiratory and encephalitic forms comprise caseous nodules and septate fungal hyphae. In acute fowl cholera, the general pronounced hyperemia in vessels of the abdominal viscera and subepicardial and subserosal petechial and ecchymotic haemorrhages are common.

2.24 Control of Newcastle Disease

The control of ND is achieved when vaccination programmes are complemented with biosecurity measures. The success of any control measure is dependent on the nature of the poultry industry Countries with mostly village chicken flocks have far greater control problems than those with mostly large commercial flocks (Nwanta *et al.,* 2006b).

2.24.1 Biosecurity

The term biosecurity encompasses any measure that is employed to prevent the transmission of an infectious organism from one host to another. For ND it is usually taken to mean measures that may be used to prevent the introduction of NDV to flock. To impose the restriction in good biosecurity it is, of course, necessary to understand how the virus is spreads (Alexander, 1988b) and the relative risks each of these presents. Essentially the virus may be spread by any animal that can be infected and any medium that can be contaminated with infective feaces. This results in contact directly or indirectly with susceptible poultry. According to Zander *et al.* (1997), good biosecurity measures involve starting new industry and plentiful supply of land on which to site it. In this way commercial poultry farms and flocks could be well sepreated, hatcheries kept remote from poultry farms. In practice in Nigeria, the poultry industry has been developed with little attention to such planning. Because of high populations and little land, poultry farms and flocks are closely packed together and it seems inevitable that disease will spread rapidly once

introduced. However, this may not necessarily be the case. If measures such as bird proofing houses, food stores and water tanks, minimizing movements on and off the farm; ensuring that all equipment, especially vehicles, is distinfected before access to the site is permitted; ensuring restrictions of movements between different farms for eggs collection, carcass collection, food delivery etc. are limited will result in dramtic reduction in the spread of NDV. Access to the birds should be kept to a minimum. If it is unavoidable then visits by personnel who may have visited other farms such as bleeding, beak trimming or vaccination crews, inseminators and veterinarians must be considered as the most likely method of introduction of ND and regiments of clothing change, equipment disinfection and other basic hygiene controls enforced before access to the birds is allowed. Although many biosecurity measures may often be regarded as costly, laborious and time consuming by those involved, in fact they represent a good investment in future profitability of poultry production. There is need for proper education of poultry farmers on improved management practices.

2.24.2 Vaccines and Vaccination

The objective of vaccination of any animal is to produce an immune response that will prevent disease (Kapczynski and King, 2005; Ezema *et al.,* 2009). Rapid dissemination of ND during the 1960s and 1970s was attributed to increased international trade of commercial poultry and psittacine birds (Francis, 1973; Alexander, 1997a). These events lead to development of both inactivated and live-virus vaccine for control of NDV in poultry (Meulmanns, 1988). It is known that vaccination of poultry provides an excellent means of reducing clinical signs of infection caused by virulent NDV (Alexander, 2003; Senne *et al.,* 2004; Kapczynki and King, 2005). For NDV, with currently available vaccines, solid immunity cannot be achieved in poultry following a single does or even using multiple vaccinations. Despite the fact that following ND vaccination of poultry the protective immune response is transient; vaccination may protect birds from the more serious consequence of NDV infection, virulent epizootic virus may infect, replicate, be

excreted and be present in the tissues and organs of apparently healthy birds (Asplin, 1952; Utterback and Schwartz, 1973; Parede and Young, 1990; Capua *et al.,* 1994; Guittet *et al*., 1993; Ezema *et al.,* 2009), representing a threat in terms of overt disease to unvaccinated suscepitable birds which may come in contact with them. The contact may be either directly, such as by trade in birds, especially for backyard flocks, or indirectly through transfer of infective faeces. However, outbreaks have been reported in vaccinated populations (Burridge *et al.,* 1975; Adu *et al.*, 1985; Alexander, 2003). Yet, prophylactic vaccination in Nigeria is indispensable, considering that ND is enzootic. There is little possibility of enforcing efficient biosecurity measures to prevent spread to commercial poultry and as a consequence there is little alternative to vaccination. The need to vaccinate is the risk of disease occurring. Correctly administered efficacious ND vaccines may prevent death, clinical signs and even egg production problems if enough high antibody titres are achieved. Although inactivated vaccines were used initially to combat ND in the USA, the continued isolation of mild strains led to the concept and development of live vaccine strains. Beaudette *et al*. (1949) selected the Roaikn strain. However, this strain proved too virulent for young susceptible birds and strains BI (Hitchner and Johnson, 1948; Hitchner, 1975) and La Sota (Goldhaft, 1980) were selected to become the most used animal live vaccines. Current vaccination programs for NDV include the use of low-virulent, live-virus and inactivated vaccines designed to control enzootic, low virulent field strains. The goal of current vaccination procedures is to induce protective immunity while producing a minimal antagonistic response in the bird. For the poultry producer, this decreases economic losses at harvest. The mildly virulent (lentogenic) LaSota and Ulster strains (Alexander, 2003) are preferred. Most of the commercially available lentogenic vaccines are able to induce antibodies against NDV. However, systemic humoral immune response measured as the presence of specific NDV antibodies in serum is not enough for protection (Reynolds and Maraqa, 2000a). It has been established that the mucosal immunity represented by immunoglobulin A (IgA)

production plays an important role in the development of protection in chickens vaccinated against ND (Reynolds and Maraqa, 2000b; Seal *et al.*, 2000; Scott, 2004). Antibody production in the mucosa is closely related to viral replication in the target cells; hence the pathogenesis and tissue tropism of the virues used for vaccination is to be considered in order to assess the efficacy of a given live vaccine against a direct challenge (Jayawardane and Spradbrow, 1995). The intestinal tropism of the thermostable ND vaccine and the consequent induction of local immunity is important for protection against VVNDV that cause extensive ulceration of overlying intestinal epithelium (Brown *et al*., 1999b; Nwanta *et al.*, 2006a).Many different types of vaccines have been produced for protection against NDV outbreaks. Protection against ND reportedly involves both humoral and cellular immunity (Meulemans *et al*., 1986b; Reynolds and Maraqa, 2000a,b). Inactivated oil-emuslsion vaccines are generally used for individual injection of birds, which is both laborious and costly. Low-virulence live-virus vaccines have been produced and are usually administered to drinking water or sprayed by aerosol droplet (Bell *et al.*, 1991a, b; Alexander, 1997a). Subunit, recombinant, and DNA vaccines have also been developed and provide various degrees of protection against ND (Boursnell *et al*., 1990a, b; Letellier *et al*., 1991; Heckert *et al.,* 1996; Sakaguchi *et al*., 1996; Peters *et al*., 2001). ND outbreaks are common among growers in endemic areas in Nigeria and hence use of mesogenic vaccine is recommeneded at 8 or 12 weeks of age after priming with a lentogenic ND live vaccine at 14 days of age to provide protection upto the point of lay when another booster dose of vaccine is advised.

2.24.3 Combined Vaccines

The NDV envelope glycoproteins, F and HN play a key role in virus ï cell interactions and virulence of the virus (Nagai, 1993). Both F and HN have been primary targets for anti-viral vaccine development (Sakaguchi *et al.,* 1996). Several studies conducted in specific pathogen-free (SPF) chickens have shown that both F and HN are able to induce protective immune

response (Loke *et al*., 2005). Administration of multiple antigens increases immunogenticity and protection compared with individual antigens (Senne *et al.,* 2004). In the chicken, co-immunisation with recombinant plasmid containing individual HN and F genes has been reported to agument the protective efficacy of chicks following simultaneous expression of N and F genes cloned into a bi-cistronic expression. Although various authors have reported that in SPF chicks immunized with a DNA vaccine, immune response is elicited which protect against virulent NDV challenge (Heckert *et al*., 1996; Sakaguchi *et al*., 1996; Loke *et al*., 2005; Patel *et al*., 2007). But there have been no such reports for their use in chicks with maternal antibodies. The presence of maternal antibodies in commercial chicks neutralizes the immunogenic effects of these DNA vaccines, raising questions about the protective efficacy of DNA vaccines for use with commercial chickens (Rajawat *et al.,*2008). The efficacy of the different treatment combinations using both VG/GA and La Sota strains, demonstrated the feasibility of using a multiple strain vaccine protocol with VG/GA strain for initial vaccination when high challenge is present and field revaccination is scheduled (Perozo *et al.,* 2008).

2.25 Turkey production

Turkey occupies an important position next to chicken, duck, guinea fowl and quail in contributing to the protein needs of our growing population. Turkey production is playing a significant role in augmenting the economic and nutritional status of the population. They form almost 2% of the total poultry population (Anon,2004). They are reared for meat only and its meat is the leanest among other domestic avian species. Turkey farming is very popular in western countries and the major turkey producing countries are United States of America, Canada, Germany, France, Italy, Netherlands and the United Kingdom. The annual per capita consumption of turkey meat in the above mentioned countries ranges from 4-8 kg. Turkey population touched 259 millions in 1992 (Singh,2005). The estimated world turkey meat production in 2004 is 4.94 million tonnes. Turkey (*Meleagris gallopavo)* is a large gallinaceous

bird of the family *Meleagridae* that is native of North America, domesticated in Europe and are now important source of food in many parts of the world. Columbus took specimens to Spain in 1948. Reports on turkey production recorded there in Germany in 1530 and in England by 1541.

2.25.1 Varieties of turkey

Turkeys are not classified into breeds, however seven standard varieties are available, Bronze, White Holland, Bourbon red, Narragansett, Black, Slate, Beltsville small white.

Board breasted bronze:

The basic plumage color is black and not bronze. The females have black breast feathers with white tips, which help in sex determination as early as 12 weeks of age.

Board breasted white:

This is a cross between Board breasted bronze and White Holland with white feathers. This variety was developed at the Cornell University. White plumage turkeys seems to be suitable for Indian-Agro climatic conditions as they have better heat tolerance and also good and clean in appearance after dressing.

Beltsville small white:

This variety was developed at Agricultural University Research Station, Beltsville, USA. It closely resembles the Board breasted white in color and shape but smaller in size. Egg production, fertility and hatchability tend to be higher and broodiness tends to be lower than heavy varieties.

2.25.2 Turkey meat

Turkey meat has nutritional and sensorial properties which make it almost ideal raw material for rational and curative nutrition. People prefer turkey meat because of its leanest nature. The protein, fat, energy value of turkey meat are 24%, 6.6%, 162 calories per 100 gm of meat. Mineral like potassium, calcium, magnesium, iron, selenium, zinc and sodium are present. It is

also rich in essential amino acids and vitamins like niacin, vitamin B6 and B12. It is rich in unsaturated fatty acids and essential fatty acids and low in cholesterol.

2.26 Turkey production in Nigeria problems and prospects

Nigeria is endowed with an impressive array of domestic livestock. The dominant species include chickens (estimated population of 160 million), guinea fowl (8.3 million), ducks (1.7 million), and turkey (0.7 million) (Apantaku *et al.,* 1998). This bountiful animal resource base reflects the availability of unconstrained supply of poultry to bridge the dietary protein gap. The poultry industry in Nigeria is aiming at furnishing the much needed high quality protein to Nigerians through meat and egg supply. The industry is essentially a bicameral production system in which the traditional methods of poultry keeping exist side by side with commercial systems. The growth of turkey industry in Nigeria has risen to 1.5-2.0 million tonnes per year. This fast growth in the industry was made possible by intensification of production and development of large breeds with standard weights ranging from 15-17 kg for male and 8-10 kg for female; some of these come from homestead, (Ogundipe and Dafwang, 1986; Ojewola, 1993). Turkey production in Nigeria has largely remained at the small holder level due to various reasons ranging from management problems to lack of incentives by Government. There is obvious lack of information on specific requirements for turkey production in Nigeria, which may be attributed to low level of research in Nigeria. Moreso, the lack of interest on turkey production was mainly due to the government policy that liberalized turkey importation since 1977. According to Thear and Fraser (1986) imported turkey formed about 60% of the total turkey in Nigeria market, while the rest is supplied by other sources. Nigerians consume about 8.6 g animal protein per day with turkey accounting for about 1.5 g despite its great potentials in the supply of good quality animal protein and high rate of turnover of investment (Oluyemi, 1985; Ojewola 1993; Ojewola *et al.,* 2002). The potential of local poultry cannot be overlooked considering the huge foreign exchange implication of the importation of improved exotic stock (Ibe, 1990) and also genotype-

environment interaction which leads to considerable loss of fitness of the exotic stock (Oluyemi and Oyenuga, 1971). Up till now in Nigeria there is no known discriminatory attitude towards the production and consumption of turkeys according to FGN and UNICEF (1990), Turkey has no consumption problems as 116 million Nigerians are active consumers but then they are very scarce to find. Scarcity of this local variant of turkey could be due partly to the fact that chickens are so familiar and grow so well that there seems to be no reason to consider any other poultry and partly because modern turkeys have been so highly breed for intensive production that the resulting birds are inappropriate for home production. Local turkeys are natural foragers and can be kept as scavengers (Peters *et al.,* 1997). Indigenous turkeys are both functionally and genetically valuable because they contain genetic materials, which may have been lost in the improved gene pool. They possess relic characteristics or genetic variants that are either absent in modern improved stocks or existing in their rare ancestors. These traits may be of commercial value (Adebambo, 2003).

CHAPTER THREE

3.0 STUDIES ON THE FACTORS LIMITING TURKEY PRODUCTION IN ENUGU STATE

3.1 Abstract

The study was to gather relevant information on turkey production and management systems in Enugu State, constriants to turkey productivity and prevalent diseases in turkeys.

The study was conducted in nine local government areas in the three senatorial zones of Enugu State, Nigeria covering 297 turkey producers. A structured questionnaire was adminstered to turkey producers and information on the socio-economic characteristics of turkey producers, production patterns, management practices, prevalent diseases in turkeys and the common problems facing turkey production in Enugu State were identifed and collected.

The finding of the study indicated that turkey production was carried out mainly by adult female in Enugu East (65.3%) and Enugu West (57.7%) and adult males in Enugu North (51.1%) Although most (87.5%) of the respondents in the three senatorial zones had formal education, they were not (48.8%) experienced in turkey production. The major (52.5%) age group involved in the business was 36-50 years old. Turkey production in Enugu State was generally a part-time occupation as respondents were engaged in other primary occupation such as crop farming, trading, civil service etc. The management system adopted by the respondents in the three senatorial zones, Enugu East, Enugu West and Enugu North respectively were mainly (20.4%,55.0%,47.3%) intensive or semi-intensive (59.2%,34.0%,36.8%).

Majority (84.8%) of the respondents in Enugu State keep turkeys in small numbers (1-20) along with local chicken, exotic chicken, guinea fowl, ducks etc. According to majority (91.2%) of the respondent, turkey production was found to be profitable venture based on the cash generated

after sale of the turkeys. Newcastle Disease (57.0%), fowl pox (65.0%), fowl typhoid (6.0%), fowl cholera (3.7%), ectoparasitism (1.0%), fracture and nutritional deficiencies (2.6%) were the diseases constantly encountered in turkey production by majority of the respondents and of all the diseases reported by the respondents, fowl pox (65%) and Newcastle Disease (57%) were the major diseases limiting production in the study area.

The major factors limiting turkey production in Enugu State as identified by the respondents in the study area were high cost of feed (86.5%), high disease morbidity and mortality (85.2%), inadequate access to veterinary care (78.80%), unavailability and high cost of poults (74.40%), lack of management skills (63.3%) and lack of capital (61.7%) while minor problems were low reproductive potential (56.9%), theft and predators (52.5%).

Despite the factors limiting turkey production as outlined by the respondents, turkey production has great potential in bridging the animal protein supply therefore, poultry farmers should be encouraged by government to increase their level of production by establishing reliable breeding centres in the south-east Nigeria which will ensure regular supply of day old poults, subsidizing the price of feed and drugs, prompt disease control by employment of more veterinarians, provision of animal health care delivery vehicles provision of poultry vaccines, provision and easy access to soft loans. These will boost overall production and increase the standard of living of the farmers.

3.2 INTRODUCTION

The growth of turkey industry in Nigeria has risen to 1.5 to 2 million tons per year yet turkey production in Nigeria has largely remained at the small holder level due to various reasons ranging from management problems to lack of incentives by government (Udokainyang, 2001). Nwagu (2002) reported that the apparent inertia in turkey production in Nigeria appears to be due to lack of appreciation of its potential in contributing to the protein needs of the increasing population or lack of knowledge of its management and production requirements. Furthermore, Ojewola *et al*. (2002) reported that high cost of feeding programme as well as lack of knowledge of adequate levels of nutrient requirement for turkeys had kept turkey production in its smallholder level. The potentials of turkeys cannot be overlooked considering the huge foreign exchange implication of the importation of improved exotic stock (Ibe, 1990). Turkey is one of the sources of animal protein in Nigeria and is the most suitable alternative for small or large scale producers considering the cost of production. Local turkeys are natural foragers and can be kept as scavengers (Peters *et al.,* 1997). National Research Council (1991) reported that turkeys can be reared virtually anywhere and that their natural habitat is open forest and wooded area. Smith (1990) also reported that the carcasses of turkey contain a higher percentage of protein than the carcasses of chicken. Turkeys have also been found to be of considerable economic and social significance in the traditional life of Nigerians, in that they are used as gifts during festivals like Christmas and as a sign of appreciation and expression of good will (Peters *et al.,* 1997). Thereôs no known discriminatory attitude toward the consumption of turkeys (Peters *et al.,* 1997).

Inspite of all the attributes of turkey, its production in Nigeria has been low compared to other poultry species. This may be due to unavailability and high cost of poults and feed, low hatchability/ productive potential and diseases (Ajala *et al.,* 2007). Okoli *et al. (*2009) also attributed low turkey production to turkey farmers not fully understanding the management

requirements for turkey production or due to government policy that liberalized turkey importation since 1977 (Mbanasor and Sampson, 2004). According to Thear and Fraser (1986) imported turkey formed about 60% of the total turkeys in Nigerian market while the rest is supplied by other sources. Despite the importance of turkey to human nutrition, research activities on turkey production are still very scanty compared to other poultry species. There is need, to gather information on socio-economic characteristics, production patterns and management practices and prevalent diseases in turkeys, in order to identify the common problems facing turkey production in Enugu State.

3.3 Materials and Methods

3.3.1 Study Area

The study was conducted in Enugu State, the south east of Nigeria. Enugu State is located between latitudes 5^0 56ôN and 70 55ôN and longitudes 6^0 53ô E and 7^0 55ôE (NPC, 2006). .It covers a total land area of about 802,295km^2 and has a population of 2.5 million with a population density of 248 persons per square kilometer (NPC, 2006). It is bounded in the south by Abia and Imo States, in the east by Ebonyi State, in the North-east by Benue State, in the North-west by Kogi State and in the west by Anambra State. Enugu State is made up of 3 senatorial districts and 17 Local Government Areas. The senatorial zones include; Enugu east, west and north senatorial zones (Fig.3.1). Tropical forest and savannah predominate the area, ecologically. The annual rainfall in Enugu State is between 1.5-2.0 meteres.The wet season lasts from April to October while the dry season lasts from October to early April (Ike, 2011). The indigenous people of Enugu state are predominantly Igbo speaking and are involved in two major farm activities, crop and livestock. Cassava and yam are the main crops cultivated and other crops of importance are maize, cocoyam and plantain. The main animal production activity in the state is poultry; other livestock kept include pigs, goats, sheep and occasionally cattle. The

predominant poultry specie kept is chickens (local and exotic). Others are turkeys and ducks.the chicken population is 108,354 while turkey population is 28,985 (Ikepeze,2005). These poultry species are reared intensively, semi-intensively and free roaming (Ike, 2011).

Figure 3.1 Map of Enugu Sate showing the three senatorial zones

Note: Enugu East senatorial zone-Blue ; Enugu west senatorial zone-Pink; Enugu north senatorial zone- yellow

3.3.2 Study Design

The study design used was a cross sectional survey (Hennekens and Bury, 1987).

3.3.3 Study Population

Poultry/turkey farms in Enugu State were used for this study. Poultry/turkey farmers were identified with the help of resident veterinarians who had a good knowledge of the area.

3.3.4 Sample Size, Determination and Sampling Technique

Twenty percent (20 %) of poultry farmers in Enugu State were assumed to keep turkeys. Based on this assumption, a minimum sample size of 250 respondents was determined (Thrushfield, 2005). A multi-stage sampling technique was used for selection of communities in the study area; All the three senatorial zones were selected. At least three Local Government Areas were purposively selected within each senatorial zone where back yard poultry keepers reared turkeys as well. Within each Local Government selected, two communities were purposively selected thereby giving a total of nine local government areas and eighteen communities selected for the study. In each of the selected communities, purposive sampling technique was used to select the households that kept poultry/turkey. Selection of the turkey keepers was based on the willingness of the owners to participate in the study. Participation was encouraged by giving veterinary services to willing farmers.

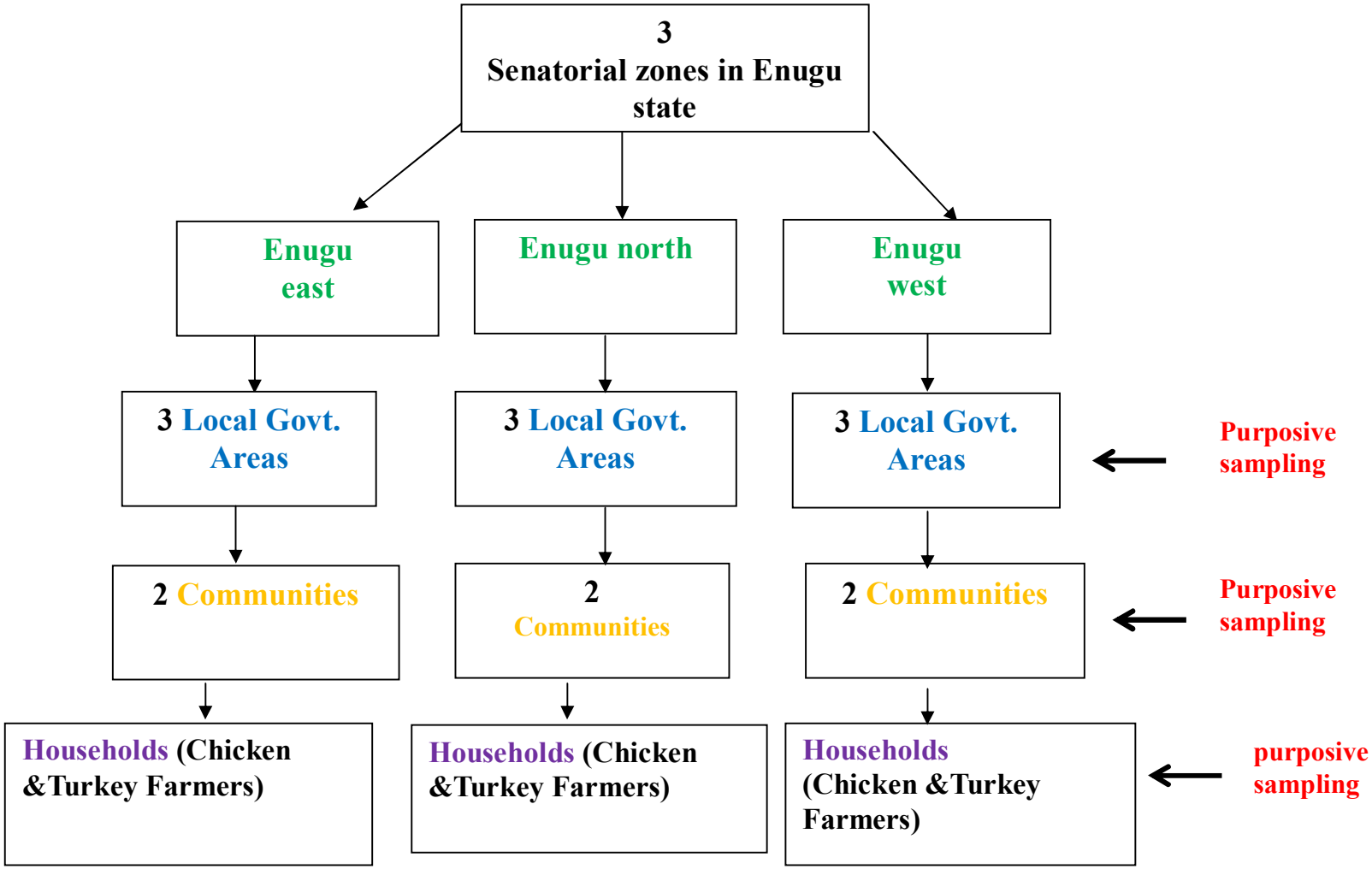
Sample size calculation

Formular - $N = \frac{Z^2 P q}{d^2}$

N= Sample size
P= percentage of subject of interest
Z= 1.96
q = 1-P
d^2 = Precision allowance for error (0.05)

therefore, $N = \frac{1.96^2 \times 20\% \times 1 - 0.02}{0.05^2}$ =245.8 rounding off to 250.

Multi-stage sampling technique



3.3.5 Instrument for Data Collection

The major instrument for data collection in this study was a structured questionnaire. The questionnaire was in 2 parts, A and B. Part A sought information on the demographic characteristics of the respondents. Part B requested information on the production patterns, management practices and prevalent diseases in turkeys in Enugu State.

3.3.6 Validation of Instrument

The research instrument was given to three experts in the Faculty of Veterinary Medicine, each in Veterinary Public Health and Preventive Medicine, Veterinary Medicine, and Veterinary Pathology and Microbiology with a letter requesting them to vet the contents of the questionnaire, the appropriateness with respect to the research objectives and title. After moderation their inputs were included in the final copy produced.

3.3.6 Reliability of Instrument

The reliability of the instrument was determined using Cronbach alpha statistics (Cronbach,1951)). The instrument was administered to thirty poultry/turkey farmers in Ekwulobia and Igbo- ukwu in Aguata Local Government Area in Anambra State for trial testing of the questionnaire. The responses of the farmers were analyzed and a reliability co-efficient of 0.82 was obtained using Cronbach alpha statistics.

3.3.7 Method of Questionnaire Administration

The 300 copies of the questionnaire were administered to willing turkey farmers with the help of veterinary assistants resident in Enugu State. The content of the questionnaire was transmitted in vernacular to farmers who were unable to read and write. 297 copies of the questionnaires correctly filled were returned.

3.3.8 Data Presentation and Statistical Analyses

Data generated from this study were converted to frequencies and percentages to determine frequencies of the responses of turkey farmers to questions on flock size, diseases, management practices and productivity.Chi - Square analyses was used to determine the association between

the farmers experience in poultry production and management system adopted by turkey producers in Enugu State and also to determine the association between farmers level of education and management system adopted by turkey farmers in Enugu State The results are presented in tables.

3.4 RESULTS

Out of the three hundred questionnaires distributed in the three senatorial zones in Enugu State, two hundred and ninety- seven copies (99%) were correctly filled and returned while only three were not returned by the respondents in Enugu west (Table 3.1).

Most of the people keeping turkeys in Enugu east (EE) and Enugu north (EN) were predominantly females (EE: 65.5%, EN: 57.7%) while in Enugu west (EW) senatorial zone more males (51.0%) were in the turkey production business (Table 3.2).

They major (EE: 55.1%, EW: 53.0% and EN: 51.7%) age group involved in the business was 36-50 years old. The major (EE: 93.8%, EW: 73.4% and EN: 76.1%) people involved in turkey rearing were civil servants, farmers and traders. The highest educational qualification for most of them was either degree or secondary school certificates.

Most (EE 40.8%,EN 50.2% and EW 46.8%) of the turkey producers in the 3 senatorial zone in Enugu State had less than 5 years experience in turkey keeping and the commonest flock size was 1-20 turkeys in a farm. The exotic breeds of turkeys kept were very few (EE: 4.1% and EN: 0.5%). Most (EE: 75.5%, EW: 72.3% and EN: 647%) of the turkeys kept were pure local breeds followed by crosses between locals and exotic breeds and were obtained mainly from neighbours and relations (Table 3.3). Most (EE: 81.6%, EW: 76.6% and EN: 73.6%) of the keepers used commercial chicken feeds in feeding their turkeys while a few (EE: 18.4%, EW: 23.4% and EN: 26.4%) compounded their own feed (Table 3.3). The management system was mainly (EE: 20.4%, EW: 55.0% and EN: 47.3%) intensive or (EE: 59.0%, EW: 34.0% and EN: 36.8%) semi-intensive. Most (EE, 61.2%, EN, 53.2% and EW, 80.9%) of the farmers kept turkeys and exotic chickens in the same farm (Table 3.4). Few (EE: 26.3%, EW: 8.5% and EN: 19.9%) kept turkeys only.

Most (91.6%) of the farmers who kept turkeys reared less than 50 chickens (Table 3.5) and they said that their main reason for keeping turkeys was for both consumption and financial income

(Table 3.7). Most (EE: 100%, EW: 91.5% and EN: 93.5%) of them agreed that turkey business is profitable (Table 3.6) but only few (EE: 26.3, EW: 10.9% and EN: 10.4%) believed that they kept turkey because turkeys were resistant to diseases and had high egg production and hatchability (Table 3.7).

From their responses, the factor limiting turkey production in Enugu State were high cost of feed (86.5%), followed by disease morbidity and mortality (85.2%), poor access to veterinary services (78.8%) , unavailability and high cost of poults (75.4%) , lack of management skill (63.3%) (Table 3.8). Turkey pox (65%) and Newcastle Disease (57%) were the main disease problems of turkeys in the three zones (Table 3.9).

Turkey farmers with < 5 years and 5-10 years experience practiced mainly intensive and semi-intensive poultry keeping (Table 3.10). Most of the turkey farmers who had primary to tertiary education kept their turkeys in intensive and semi-intensive systems (Table 3.11).

Table 3.1: **Zonal distribution of backyard turkey owners sampled in Enugu State.**

Senatorial Zone	No of Farmers	No of Respondents
Enugu East	49	49
Enugu West	50	47
Enugu North	201	201
Total	300	297

Table 3.2: Zonal distribution of demographic data of the respondents and flock size of turkey farms sampled in selected communities in the three senatorial zones of Enugu State.

Characteristics	Senatorial Zone		
	Enugu east	Enugu west	Enugu north
	No of Respondent(%)		
Sex			
Male	17(34.7)	25(51.0)	85(42.3)
Female	32(65.3)	22(49.0)	116(57.7)
Age group			
< 20years	0(0)	1(2.0)	2(1.0)
21-35 years	8(16.3)	9(19.0)	78(38.8)
36-50 years	27(55.1)	25(53.0)	104(51.7)
>50 years	14(28.6)	12(26.0)	17(8.5)
Major occupation			
Students	4(8.20)	4(8.5)	31(15.4)
Traders	15(30.6)	8(17.0)	49(24.4)
Farming	8(16.3)	22(46.8)	67(33.3)
Civil servant	13(26.5)	6(12.8)	37(18.4)
House wife	5(10.2)	0(0.0)	10(5.0)
Retirees	0(0.0)	2(4.3)	1(0.5)
Artisans	4(8.20)	5(10.6)	6(3.0)
Educational level			
No formal education	11(22.4)	5(10.6)	21(10.4)
Primary education	12(24.5)	8(17.0)	41(20.4)
Secondary education	10(20.4)	24(51.1)	87(43.3)
Tertiary education	16(32.7)	10(21.3)	52(25.9)
Turkey keeping experience			
< 5years	20(40.8)	22(46.8)	101(50.2)
5-10 years	16(32.7)	18(38.3)	87(43.3)
11-20 years	11(22.4)	7(14.9)	13(6.5)
>20 years	2(4.10)	0(0.0)	0(0.0)
Flock size			
Small (1-20)	46(93.9)	42(89.4)	164(81.6)
Medium (21-40)	3(6.10)	4(8.5)	14(7.0)
Large (above 40)	0(0.0)	1(2.1)	23(6.5)

Table 3.3: Breed distribution, sources of parent stock, feed and mangement practices of turkey producers in selected communities in the three senatorial zones of Enugu State.

Parameters	Senatorial zone		
	Enugu east	Enugu west	Enugu north
		Frequency (%)	
Breed type			
Exotic	2(4.1)	0(0)	1(0.5)
Cross bred	10(20.4)	13(27.7)	70(34.8)
Pure local	37(75.5)	34(72.3)	130(64.7)
Source of parent stock			
Market	12(24.5)	9(19.1)	32(15.9)
Neighbor	25(51.0)	25(53.2)	99(49.3)
Commercial hatcheries	12(24.5)	13(27.7)	70(34.8)
Source of feed			
Self formulated	9(18.4)	11(23.4)	53(26.4)
Commercial feed	40(81.6)	36(76.6)	148(73.6)
Management system			
Intensive	10(20.4)	26(55.0)	95(47.3)
Semi intensive	29(59.2)	16(34.0)	74(36.8)
Extensive	10(24.0)	5(11.0)	32(15.9)

Table 3.4: Zonal distribution of poultry species kept by turkey producers in selected communities in the three senatorial zones of Enugu State

Specie of Poultry	Senatorial zone		
	Enugu east	Enugu west	Enugu north
	No. of responents (%)		
Turkeys only	13(26.5)	4(8.5)	40(19.9)
Turkeys and exotic chickens	30(61.2)	38(80.9)	107(53.2)
Turkeys and local chickens	1(2.0)	3(6.4)	31(15.4)
Turkeys ,exotic and local chickens	3(6.1)	0(0.0)	12(6.0)
Turkeys ,exotic chickens and others	1(2.0)	2(4.2)	7(3.5)
Turkeys , local chickens and others	1(2.0)	0(0.0)	4(2.0)
Total	49(100)	47(100)	201(100)

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Table 3.5: Distribution of Flock size of chicken farmers who keep turkeys in selected

communities in the three senatorial zones of Enugu State

Flock size	No of respondents (%)
< 50	272(91.6)
50-100	14(4.7)
Above 100	11(3.7)
Total	297(100)

Table 3.6: Zonal distribution of turkey farmers responses on the profitability of turkey

production in selected communities in the three senatorial zones of Enugu State

Senatorial zone	Total no. of responses	Responses on profitability	
		Yes (%)	NO(%)
Enugu east	49	49(100)	0(0)
Enugu west	47	43(91.5)	4(8.5)
Enugu north	201	188(93.5)	22(6.5)
Total	**297**	**271**	**26**

Table 3.7: Zonal distribution of respondents on the basis of their major reason for raising

turkeys in selected communities in the three senatorial zones of Enugu State.

Main reason for raising turkey	Senatorial zone		
	Enugu east	Enugu west	Enugu north
	Frequency (%)		
Income/consumption	41(54.0)	39(61.0)	159(79.1)
Turkeys are resistant to diseases	20(26.3)	7(10.9)	21(10.4)
High egg production/hatchability	7(9.2)	7(10.9)	11(5.3)
Simpler feeding system	8(10.5)	11(17.2)	18(8.6)
Total	76(100)	64(100)	209(100)

Table 3.8 Farmers affirmative answers on factors limiting turkey production in selected communities in the three senatorial zones of Enugu State. (n=297)

Factors limiting turkey production	Frequency (%)
High cost of feed	**257 (86.5)**
Disease morbidity and mortality	**253 (85.2)**
Poor access to veterinary service	**231 (78.8)**
Unavailability and high cost of poult	**224 (75.4)**
Lack of management skill	**188 (63.3)**
Lack of capital	**183 (61.7)**
Low reproductive potential of birds	**169 (56.9)**
Theft and predators	**156 (52.5)**

Table 3.9: Zonal distribution of disease prevalence in turkeys in selected communities in the three senatorial zones of Enugu State. (EE; n= 49; EW: n= 47;EN: n= 201)

Disease	Senatorial zones		
	Enugu east	Enugu west	Enugu north
		Frequenc(%)	
Newcastle disease	20(40.8)	18(38.3)	133(66.1)
Turkey pox	38(77.6)	27(57.4)	140(69.6)
Fowl typhoid	1(2.0)	3(6.3)	14(7.0))
Fowl cholera	1(2.0)	2(4.2)	8(4.0)
Ectoparasitism	0(0.0)	1(2.1)	3(1.5)
Fractures/nutritional deficiencies	4(8.2)	3(6.3)	2(1.0)

Table 3.10: Distribution of farmers experience in poultry production and management system adopted by turkey producers in selected communities in the three senatorial zones of Enugu State

Experience in poultry	No of respondents			Total
	Management system			
	Intensive (%)	Semi-intensive (%)	Extensive (%)	
<5 years	75	52	25	152
5-10 years	37	58	13	108
11-20 years	13	14	6	33
Above 20 years	1	2	1	4
Total	**126**	**126**	**45**	**297**

X^2 Calculated value- 10.6 , Table value-12.592, p< 0.05, Chi –square analysis.

Table 3.11: Distribution of farmers level of education and management system adopted by turkey producers in selected communities in the three senatorial zones of Enugu State

Level of education	No. of respondents			Total
	Management system			
	Intensive (%)	Semi-intensive (%)	Extensive (%)	
No education	8	18	7	33
Primary education	27	27	14	68
Secondary education	50	51	18	119
Tertiary education	40	32	5	**77**
Total	**125**	**130**	**42**	**297**

X^2 calculated value- 13.800, Table value -12.592, p< 0.05, Chi-square analysis

3.5 DISCUSSION

The predominance of women in turkey production in Enugu State as observed in this study is in agreement with the report of Brobolt and Odegaard (1999) who said that poultry keeping was the skill of house wives. Reports from Zimbabwe (Oakeley,1999), Bangladesh (Nielsen *et al.*, 2003), Kenya (Okitoi *et al.,* 2007) and Nigeria (Ogunlade and Adebayo, 2009) indicated that females mainly participate in rural poultry production using the extensive method. This finding is not consistent with the report of some researchers in northern and western parts of Nigeria where Ajala *et al.* (2007) and Peters *et al.* (1997), observed that men dominated turkey production business. The disparity recorded in these studies may be related to cultural/religious differences among the people living in the regions.

The age distribution of the majority of the turkey farmers (52.5%) fell within 36-50 years of age. This is within the active working age of citizens in Nigeria. This observation is consistent with the report of Ajala *et al*. (2007) who said that turkey farmers in Kaduna State fell within the age of 31-50 years. The respondents that were above the age of 50 years may likely be retirees from the state and federal civil service while the few population of less than 20 are school children augmenting the family income while 21-35 years of age are young school leavers and graduates looking for a means of livelihood.

A greater proportion of turkey farmers in Enugu State were mainly either traders, farmers or civil servants. This observation is comparable with the findings of Ajala *et al*. (2007) and Peters *et al.* (1997) who reported that civil servants were mainly part time turkey producers. Many people in other occupations were also involved in turkey production as a source of additional income probably

due to the high rate of profitability. Civil servants who were part time turkey producers do so as a way to augment their income base since salaries might be inadequate to meet family needs.

Majority of the respondents in the study area had formal education that was a primary education, a secondary education or tertiary education. This finding is in agreement with the reports of Peters *et al.* (1997) and Ajala *et al.* (2007) where majority of the respondents had at least a secondary education,. Literacy will enable the farmers engage in better management practices such as in the administration of drugs, vaccines and feed. Therefore, the preponderance of part-time educated farmers maybe of assistance to extension officers for easy communication and understanding of extension messages especially for application of newer technology in poultry production. Training has been shown to improve business performance and returns to farmers (Mishra *et al*., 2009). In other words better trained and educated farmers will adopt better technology and management practices that will guarantee success and better returns on investment.

The respondents (EE 40.8%,EN 50.2% and EW 46.8%) in the 3 senatorial zones of the study area had less than 5 years experience in turkey production, which shows that turkey production in Enugu State is a new venture still at a small holder level. This validates the claims of Udokainyang (2001) that turkey production in Nigeria is still at a small holder level despite the numerous attribute of turkeys in providing the protein needs of the public. This development may be attributed to management problems and lack of incentive by government (Udokainyang, 2001). It may also be due to lack of appreciation of the potentials of turkeys in contributing to the protein needs of the public (Ajala *et al.,* 2007) or government policy on liberalization of importation of turkey in Nigeria since 1977 (Thear and Braser, 1986).

Majority (EE: 75.5%, EW: 72.3% and EN: 64.7%) of the farmers in the three senatorial zones of Enugu State kept local breeds of turkeys sourced from open markets, friends and neighbours. Keeping mainly the local breeds of turkeys may be due to the high of cost of foreign poults which could be obtainable in commercial hatcheries. This finding is consistent with the reports of Peters *et al*. (1997) and Ajala *et al.* (2007).

Commercial feed was predominantly used by majority (EE: 81.6%, EW: 76.6% and EN: 73.6%) of the turkey farmers in the three senatorial zones of Enugu State. This agrees with the report of Okoli *et al.* (2009), who said that commercial feed was the poultry farmersô choice. Etuk (2005) observed that farmers were unable to formulate ration for turkeys and relied on rations originally formulated for chickens. However, the findings of this study disagrees with the observation of Peters *et al.* (1997) and Ajala *et al.* (2007) who reported that majority of the respondents resorted to self-formulated and supplemental feed for turkeys.

The 3 systems of management of turkey identified in this study as reported by the respondents were intensive, semi-intensive and extensive which were also reported by Peters *et al.* (1997); Mbanasor and Sampson (2004) and Ajala *et al.* (2007). A greater proportion of the respondents adopted either intensive or semi intensive systems. Although the extensive system is less expensive very few (15.8%) of the respondents adopted the management system. This finding agrees with that of Ajala *et al.* (2007) and Peters *et al*. (1997). The choice of management system maybe due to the level of education and maybe responsible for good performance of turkeys produced in the study area.

The present study showed that turkey farmers keep turkeys along with other species of poultry in small numbers and that such poultry species included exotic, local chickens, ducks, and guinea fowls. This finding agrees with the report of Peters *et al*. (1997) in Ogun State, Nigeria, where a

combination of animal species were kept along with local turkeys. Ibrahim and Abdu (1992) noted that in rural Nigeria it was a common practice to find a combination of different poultry species in the same compound. Lancaster (1966) and Roy *et al. (*1998) reported that birds were known to be sources of spread of ND virus thus keeping different species of birds together could increase the spread of ND virus from one species to another. Only few (EE: 26.3%, EW: 8.5% and EN: 19.9%) of the farmers keep only turkeys. This indicates that turkey farming is a new venture in the study area. This maybe due to lack of understanding of management practices associated with turkey production (Nwagu, 2002).

The diseases reported in the present study included fowl pox, Newcastle disease, fowl typhoid, fowl cholera, ectoparasitism, which were also observed by Ajala *et al*. (2007). Although they reported worm burden to be the major disease limiting production, fowl pox and Newcastle Disease were the major diseases limiting turkey production in this study area. Use of palm oil in the treatment of fowl pox reported by some farmers in the present study was also reported by Peters *et al.* (1997) and Ajala *et al.* (2007).

The major reason for keeping turkey as identified by the respondents (40.0%) in this study was for cash and consumption which were equally observed by other researchers (Peters *et al.*, 1997 and Ajala *et al.,* 2007).

Majority (91.2%) of the farmers responded favorably that turkey keeping business was profitable in the study area, which Ajala *et al*. (2007) also reported among turkey farmers in Kaduna State. The favorable response was mainly based on the huge income generated after sale of turkeys.

The major limiting factors to production as identified by the farmers in the study area were high cost of feed, high disease morbidity and mortality, inadequate access to veterinary care, unavailability and high cost of poults, lack of capital and lack of management skills. These observations are comparable to the findings of Ajala *et al.* (2007). The high cost of feed was also reported by Mbanasor and Sampson (2004) and validates the claims by Oluyemi and Roberts (2000) that the prospects for investments in poultry and turkey production is being hampered by high cost of input especially the cost of feed. The constraint by diseases was also reported by Mbanasor and Sampson (2004) and maybe due to lack of awareness on the proper management practices related to turkey rearing as well as inadequate access to veterinary care in the study area.

There was no association between the farmers experience in poultry production to the choice of management system used by the farmers. This may be due to a lack of knowledge and paucity of information on the management practices peculiar to turkey production.

There was an association between the level of education and the choice of management system used by the farmers. Those farmers with formal education (primary, secondary and tertiary) adopted mainly the intensive and semi-intensive system of management of turkeys. The two main system of management chosen by the farmers will help in controlling entry and spread of disease for better performance in poultry production. This also validates the claims of Mishra *et al*. (2009) that education and training has been shown to improve business performance and better returns for farmers.

3.6 CONCLUSION

The major factors limiting its production as outlined by the respondents include high cost of feed, high disease morbidity and mortality, inadequate access to veterinary care, unavailability and high cost of poults, lack of capital and lack of reasonable degree of management skills. Despite these limitations, turkey production has great potential in bridging the animal protein supply, create employment and enhance the economy. It is therefore necessary that poultry farmers be encouraged to increase their level of production for increased profitability by:

1. Establishment of a reliable breeding centre in the south east Nigeria, to ensure supply of day old poults.
2. Provision of feed or its raw materials, vaccines and drugs by the government to producers at subsidized rate.
3. Prompt disease control measures such as employment of more veterinarians, provision of animal health care delivery vehicles and provision of poultry vaccines by the government.
4. Provision and easy access to soft loans to boost overall production and increase the standard of living of the farmers.
5. Discouraging farmers from keeping different species of poultry and instituting a regular Newcastle disease vaccination programme.
6. Formulating feed to meet the nutritional requirement of turkeys, reduce the cost of production and increase profitability.

CHAPTER FOUR

6.0 SEROLOGICAL SURVEY OF NEWCASTLE DISEASE ANTIBODY IN TURKEYS IN ENUGU STATE

6.1 Abstract

The aim of the study was to determine the seroprevalence of Newcastle Disease in turkeys .The study was carried out in 9 Local Government Areas of the 3 senatorial zones of Enugu State. Blood samples were randomly collected from 20 adult turkeys in each household and a total of 569 serum samples were collected from nine Local Government Areas. The serum samples were screened for antibody against Newcastle Disease using the haemagglutination inhibition (HI) tests.

Out of the 569 sera collected from turkeys in the three senatorial zones and tested for ND virus antibody, a total of 186 sera representing 32.7% were positive.

Out of 186 sera that had antibodies titres positive to ND, 138 (74.1%) had titres Ó 8 and were considered protected from a challenge with Newcastle Disease virus while 48 (25.9%) had titres < 8 and were considered to be at risk when challenged with a virulent strain of Newcastle Disease virus. The mean HI antibody titre (579.41± 98.46) in Enugu East senatorial zone was significantly ($P<0.05$) higher than mean HI antibody titres in (61.89± 11.69) Enugu West and (85.92±28.58) North senatorial zones. The result showed that the turkeys had antibodies against Newcastle Disease virus. Therefore regular vaccination of turkeys against Newcastle Disease should be advised especially in backyard farms where turkeys are reared together with other species of poultry.

4.2 INTRODUCTION

Newcastle disease is the most important viral disease of poultry in the world including developing countries (Nawathe *et al.,* 1975; Adu *et al.,* 1986; Adene, 1990; Sprabrow, 1997). Over 200 species of birds have been reported to be susceptible to natural and experimental infection with ND virus and it seems probable that more are susceptible (Alexander, 1999). A range of hosts like chickens, pigeons, turkeys, partridges, pheasants, doves, sparrows, geese, starlis and other free flying birds are susceptible to the ND virus (Vindevogel *et al.,* 1972). Ducks and geese tend to show few signs even when infected with the most virulent strains of ND virus from chickens (Alexander, 1999). Birds other than the domestic chicken have been known to be sources of spread of ND virus (Lancaster, 1966; Roy *et al*., 1998). It was reported by Alexander *et al.* (1984a) that the spread of ND virus to chickens has occurred in several countries, including Great Britain, where 20 outbreaks in unvaccinated chickens occurred in 1984 as a result of feed that had been contaminated by faeces of infected pigeons.

In rural Nigeria, it is common to find a combination of different poultry species and breeds such as chickens, turkeys, Muscovy ducks and pigeons being reared in the same compound/environment (Ibrahim and Abdu, 1992), Non domesticated species such as turtle doves are also regularly seen around human dwellings where domestic chickens and other birds are reared together (Ibrahim and Abdu, 1992). At present, it is also customary to find ostriches, peacocks, geese and mallard ducks being reared together in the same compound in cities and in some poultry farms (Saõidu *et al.*, 2004). This production system has facilitated introduction and spread of ND virus among poultry species and breeds in Nigeria. Saõidu *et al.* (2004) reported higher NDV antibody titres among galliformes. They suggested that with this high ND titres among galliformes, local husbandry practices in Nigeria may encourage cross infection by ND virus among the different species kept. In Nigeria, ND was

reported in guinea fowls and a highly velogenic strain of ND virus was isolated from apparently healthy ducks (Echeonwu *et al.,* 1993). A suspected outbreak of ND in young ostrich was also reported by Saõidu *et al.* (1994). In Ankara, Indonesia, outbreaks were reported in chickens and turkeys (OIE, 2005), but in Nigeria very few reports are available on natural outbreaks of ND in turkeys (Saõidu *et al.,* 1994).

Serological evidence of ND infection in turkeys was also reported in Zaria, Nigeria by Saõidu *et al*. (2004) with a prevalence rate of 68% and in a retrospective study on ND cases carried out in Maiduguri, Nigeria a prevalence of 57.2%. was recorded by Sadiq *et al.(*2011) and in south eastern Nigeria, Orajaka *et al.* (1999) recorded an ND seroprevalence of 63% in local chickens. In rural Nigeria poultry species are kept together (Ibrahim and Abdu., 1992) and there are higher chances of ND virus transfer across the bird species (Saõidu *et al.,* 2004).

Thereõs limited information on the susceptibility of turkeys to NDV and the role they play in transmitting the virus to other species, it therefore becomes necessary to confirm the presence of ND antibodies in turkeys in other to determine the sero-prevalence of ND in turkeys in Enugu State. The findings of this study alongside with vaccination history against ND may provide information on the role of turkeys in the ND outbreaks commonly seen in both local and exotic chickens in the study area.

4.3 Materials and Methods

4.3.1 Study area

Refer to section 3.3.1

4.3.2 Study design

Refer to section 3.3.2

4.3.3 Study population

Adult turkeys without previous history of ND vaccination will be sampled in the study area.

4.3.4 Sample size and Sampling technique

Saẽdu *et al*. (2004) reported a prevalence of 68% from turkeys sampled in Zaria, Kaduna State Nigeria. Based on this prevalence recorded, a minimum sample size of 350 was determined using the formular described by Thrushfield (2005). The households that keep turkeys were purposively selected; blood samples were collected from 20 adult turkeys per household/farm visited. Where the adult turkeys were less than twenty, all the adult turkeys in the household/farm were sampled

Sample size calculation

Formular - $N = \frac{Z^2 P q}{d^2}$

N= Sample size
P= percentage of subject of interest
Z= 1.96
q = 1-P
d^2 = Precision allowance for error (0.05)

therefore, $N = \frac{1.96^2 \times 68\% \times 1- 0.02}{0.05^2}$ **=334.37 rounding off to 350.**

4.3.5 Blood sampling, serum harvesting and storage

Two mililitres of blood was collected from the wing vein of the selected adult turkeys and discharged into sterile bijou bottles without anticoagulant. The bottles were slanted in a rack after covering with a screw cap for several hours at room temperature and the blood allowed to clot. The clotted blood samples were left in a refrigerator (4 ^{0} C) overnight for the sera to form and were decanted into bijou bottles and stored at -20^0 C until analyzed.

4.3. 6 Haemagglutination test

Determination of the ND antibody titre of sera collected were carried out using Haemagglutination (HA) and Haemagglutination inhibition (HI) tests (OIE,2005).

i) 0.025 ml of PBS was dispensed into each well of a plastic V-bottomed microtitre plate.

ii) 0.025 ml of the virus suspension was placed in the first well.

iii) Twofold dilutions of 0.025 ml volumes of the virus suspension were made across the plate.

iv) A further 0.025 ml of PBS was dispensed into each well.

v) 0.025 ml of 1% (v/v) chicken RBCs was dispensed to each well.

vi) The solution was mixed by tapping the plate gently. The RBCs was allowed to settle for 40 minutes at room temperature,

vii) HA was determined by tilting the plate and observing the presence or absence of tear-shaped streaming of the RBCs. The titre was the highest dilution giving completes HA (no streaming). Total HA was 64. The antigen was converted to 4HA by diluting it 1:15 (64/4= 16) with PBS.

4.3.7 Haemagglutination inhibition test

i) 0.025 ml of PBS was dispensed into each well of a plastic V-bottomed microtitre plate.

II) 0.025 ml of serum was placed into the first well of the plate.

iii) Twofold dilutions of 0.025 ml volumes of the serum were made across the plate.

iv) 4 HAU antigen in 0.025 ml was added to each well and the plate was left for a minimum of 30 minutes at room temperature.

v) 0.025 ml of 1% (v/v) chicken RBCs was added to each well and, after gentle mixing, the RBCs were allowed to settle for 40 minutes at room temperature, when control RBCs settled to a distinct button.

vi) The HI titre was the highest dilution of serum causing complete inhibition of agglutination of the RBCs. The agglutination was assessed by tilting the plates. Only those wells in which the RBCs stream at the same rate as the control wells (containing 0.025 ml RBCs and 0.05 ml PBS only) were considered to show inhibition.

4.3.8 Data presentation and analysis

The Geometric Mean Titre (GMT) was obtained using the Tube method (Villegas and Purchase, 1989). Numbers and percentages of turkeys at risk were calculated based on the turkeys with HI antibody titre >8 were considered positive (OIE, 1996). Individual HI titres between the zones were compared by one way ANOVA and variant means were separated by Duncan multiple range test. Significance was accepted at $p < 0.05$.

4.4 RESULTS

Out of the 569 sera samples collected from the three senatorial zones and tested for ND virus antibody, a total of 186 sera representing 32.7% had antibodies titres positive for ND while a total of 383 serum samples representing 67.3% of the sampled birds were negative for ND antibodies. (Table 4.1).The geometric mean HI antibody titre of 45.3 was calculated for the three senatorial zones (Table 4.1).

Out of 186 sera that had antibodies titres positive for ND, 138 (74.1%) had titres of Ó8 which were presumed protected against Newcastle Disease based on OIE recommendation of 1996, while 48 (25.9%) had HI antibody titres of < 8 which were presumed to be at risk (Table 4.2).

Table 4.3 shows that the mean HI antibody titre in Enugu East senatorial zone against ND was significantly ($p<0.05$) higher than mean HI ND antibody titre level in Enugu West and North senatorial zones.

Table 4.1; Distribution of Newcastle disease antibodies in turkeys sample in selected local government area in Enugu State.

Local Government Area	No of serum samples tested	No of serum positive to ND antibodies (%)	No of serum negative to ND antibodies (%)	Geometric mean titre
Enugu east	110	29(26.4)	81(73.6)	222.9
Enugu north	39	13(33.3)	26(66.7)	294.1
Enugu south	36	18(50.0)	18(50.0)	58.6
Udi	50	19(38.0)	31(62.0)	29.9
Ezeagu	57	11(19.3)	46(80.7)	22.6
Agwu	68	23(33.8)	45(66.2)	36.8
Nsukka	136	40(29.4)	96(70.6)	29.9
Igboetiti	38	19(50.0)	19(50.0)	22.6
Igbo eze	35	14(40.0)	21(60.0)	16.0
	569	186 (32.7)	383(67.3)	45.3

Table 4.2: Zonal distribution of ND antibody titres in turkeys in selected communities in the three senatorial zones of Enugu State

Senatorial zones		Titre	Titres
	No of +ve sera	< 8	≥ 8
		No not protected	No protected
Enugu east	60	7	53
Enugu west	53	17	36
Enugu north	73	24	49
Total	186 (100%)	48 (25.9%)	138 (74.1%)

Note : Individual HI titre of turkeys sampled in the different selected communites in the three senatorial zones of Enugu. ND HI titres Ó8 were considered protective. (OIE, 1996)

Table 4.3: Zonal distribution of mean HI antibody titre against ND in turkeys sampled in selected communities in the three senatorial zones of Enugu State

Zones	Mean HI titre
Enugu east	**579.41± 98.46[a]**
Enugu west	**61.89± 11.69[b]**
Enugu north	**85.92±28.58[b]**

Values with different superscripts within the column differ significantly (p Ò0.05)

4.5 DISCUSSION

The present study revealed ND haemagglutinating antibodies in turkeys sampled in the three senatorial zones of Enugu state. This is an indication that the turkeys were exposed to the Newcastle Disease Virus. Other researchers who carried out ND serologic surveys in chickens in the study area and other parts of Nigeria detected antibodies against ND (Abdu *et al.,* 1985, Ezeokoli *et al.,* 1985, Olabode *et al.,* 1992, Orajaka *et al.,* 1999, Nwanta *et al*., 2003). Saõdu et *al.* (2006) in a serologic survey also detected haemagglutinating antibodies in turkeys sampled in Kaduna State.

All the turkeys sampled had no history of previous vaccination against ND based on information given by the farmers. It is therefore inferred that antibodies detected in these turkey maybe as a result of survival from natural infection of the turkeys by ND virus or subclinical infections. These turkeys that recovered from ND infection could act as reservoirs of the virus (Bell and Mouloudi, 1988; Olabode *et al.,* 1992 and Orajaka *et al.,* 1999).

In this study an ND seroprevalence of 32.7% was recorded in turkeys. This is lower when compared with the 63% recorded in local chickens sampled in the south east, Nigeria by Orajaka *et al*. (1999), 68% and 57.2% recorded in turkeys in Zaria and Maiduguri by Saõdu *et al*. (2004) and Sadiq *et al.* (2011) respectively. These observed regional differences in ND prevalence shows ecological variation in NDV activity and may be a reflection of the impact of environment on the viability of NDV, its spread and epidemiology (Orajaka *et al.,* 1999).

NDV antibodies were widespread in turkeys in the study area. This shows that the local husbandry practices where different species of poultry including turkeys are reared together may have

encouraged cross infection with NDV among the different species especially where ND vaccination is not really practiced as in the case of local chicken production or where chickens are reared extensively in Nigerian villages. Although ND viral isolation was not attempted in this study, detection of antibodies in these turkeys is suggestive of the presence and continuous circulation of NDV among poultry species (Saũdu *et al*.,2004).

The GMT of 45.5 recorded in this study is comparable with the GMT of 26.0 recorded in turkeys by Saũdu *et al.* (2004). The higher geometric mean titre recorded could be as a result of ecologic variation in NDV activity as suggested by Orajaka *et al.* (1999). Such high antibody titres to NDV are more common in galliformes than other species of birds (Arnal and Keyer, 1975).This is because galliformes are the most susceptible to NDV (Munjeri, 1996).

Majority (67.3%) of the turkeys sampled were negative for ND antibody and 25.9% of the turkeys that had ND positive serum but had titers of < 8 were presumed to be at risk and not protected against challenge from a virulent strain of ND, based on the OIE recommendation of 1996and reports of that titres Ó 8 are presumed to be protective against challenge from a virulent strain of ND. Nwanta *et al.* (2006b) also reported that majority of the birds sampled in Kaduna State before ND vaccination were at risk and that the number at risk decreased tremendously after ND vaccination. This suggests that the turkeys at risk observed in this study if vaccinated may be protected. Other researchers such as Aini et al. (1990) and Bell et al. (1990, 1991a, 1991b) reported similar improvement with ND vaccination.

Allan and Gough (1974) and Bell *et al.* (1991a) reported that individual HI titres of Ó 8 were protective against challenge with a virulent strain of ND virus. Okwor (2014) reported that HI titres of Ó16 were protective against challenge with a virulent strain of ND virus. Based on the reports of

Allan and Gough (1974) and Bell *et al.* (1991a), 138 (74.1%) out of the 186 postive serum samples recorded in this study were presumed to be protected against challenge with a virulent strain of the ND virus.

The very high level of antibody titre against ND observed in Enugu East senatorial zone when compared with the other two other zones shows the high density of poultry farms in Enugu east, which includes Enugu urban and envrions. This shows that the epidemiology of the disease among zones varies with highest chances of NDV transmission more in zones where other poultry species are reared with unvaccinated turkeys (Saiôdu *et al.,* 2004).

4.6 CONCLUSION

From the study NDV infection is evident serologically in turkeys, even though the sero prevalence is low. The local husbandry practice of keeping different species of birds together increases the chances of transfer of ND virus across species. Based on the high percentage of birds at risk, turkey farmers should be encouraged in the study area to keep poultry species separately and routinely vaccinate against ND.

CHAPTER FIVE

5.0 COMPARATIVE STUDY OF THE PATHOLOGY AND PATHOGENESIS OF A VELOGENIC NEWCASTLE DISEASE VVIRUS IN CHICKENS AND TURKEYS.

5. 1 Abstract

The susceptibility, clinical signs, lesions, distributions and persistence of a local Velogenic Newcastle disease (ND) virus KUDU-113 were studied in experimental infection in 6 week-old turkeys and chickens. The experimental birds were divided into 2 major groups vaccinated and unvaccinated turkeys and chickens. The vaccinated groups were given HB1 (I/O) ND vaccine at day old and La Sota at day 21 of age. Following inoculation intramuscularly (Thigh) with the ND virus KUDU 113, severe depression, whitish greenish diarrhea, lethargy hunched posture, tremors and torticolis were consistent clinical features observed in unvaccinated turkeys and chickens while mild depression and lethargy were observed in vaccinated chickens and mild clinical signs in one vaccinated turkey. Morbidity rate was 100%, 92%, 22.2% and 4.1% in unvaccinated chickens and unvaccinated turkeys, vaccinated chickens and vaccinated turkeys respectively, while mortality rates were 90% ,80%, 13.3% and 0% in unvaccinated chickens, unvaccinated turkeys, vaccinated chickens and vaccinated turkeys respectively. Reduction in body weight was highly significant ($p<0.05$) in both inoculated unvaccinated and vaccinated turkeys and chickens. Postmortem examination showed atrophy of the thymus, bursa of Fabricius and spleen and congestion of kidneys in inoculated groups. Haemorrhages on the mucosa of the proventriculus, sharply-dermacarted haemoragic ulcers in the intestine and swelling and haemorrhages in the caecal tonsils were observed only in infected chickens. Congestion in the brain vessels was present in infected turkeys. By day 6 post inoculation all the unvaccinated innoculated chickens had died showing no significant changes in the weights of the thymus. Inoculated vaccinated chickens also showed no significant changes in

thymic weights ($p>0.05$). Grossly of the control and infected chickens showed significant reduction in sizes of the thymus of both unvaccinated and vaccinated infected chickens on days 5 and 6 PI. The only change in the weight of the spleen was significant reduction in vaccinated inoculated chickens on day 5 PI only. Grossly there was atrophy of the spleen in vaccinated and unvaccinated infected chickens on day 10 and 5 PI respectively and in the vaccinated and unvaccinated inoculated turkeys on days 20 and 10 PI respectively. The weights of the bursa were lower ($p<0.05$) in the unvaccinated inoculated chickens on days 3 and 5 PI and days 3, 10, 15 and 21 PI in the unvaccinated inoculated turkeys.

The highest ND antibody titre recorded on day 21 PI were considerably higher in inoculated unvaccinated turkeys than the inoculated vaccinated chickens. Virus was detected in brain, intestine and spleen but none detected in clocal swabs.

No significant ($p <0.05$) changes were observed in erythrocytic parameters in chickens while there was a significant decrease ($p <0.05$) in PCV on day 15 PI in unvaccinated turkeys. Leucocytosis, hetrophilia and lymphopenia were Significant ($p<0.05$) in unvaccinated inoculated chickens on day 3PI while days 3, 10 and 21PI for unvaccinated inoculated turkeys.

Susceptibility was shown to vary among chickens and turkeys. However, ND induced by the virus among turkeys was less severe, despite virus detection in spleen, intestine and brain. In chickens.intramuscular inoculation resulted in high mortality in chickens with intestinal ulcers and haemorrhagess which indicated that the KUDU-113 strain is a velogenic viscerotropic virus.

5.2 INTRODUCTION

Newcastle disease is a recurring concern of poultry industries internationally. It is a major problem of poultry in Africa and Asia (Awan *et al*., 1994). Outbreaks caused by virulent strains of Newcastle disease virus (NDV) are referred to as exotic Newcastle disease (END) in the United States and velogenic Newcastle disease (VND) in Nigeria (Onunkwo and Momoh 1980; Piacenti *et al*., 2006). Newcastle Disease Virus is synonymous with avian paramyxovirustype 1. It is a non-segmented, single-stranded, negative-sense enveloped RNA virus, belonging to the *Paramyxoviridae* family, and a member of the genus *Avulavirus* (Lamb *et al*.,2005). ND virus strains are classified into three major pathotypes, which include lentogenic (low virulence), mesogenic (moderate virulence), and velogenic (high virulence) (Hanson and Bradly 1955). In chickens lentogenic strains produce mild or inapparent respiratory infection. Mesogenic strains are associated with low mortality, acute respiratory disease, and neurologic signs in some birds. The velogenic strains are further divided into either neurotropic velogenic NDV (causing respiratory and neurologic signs with high mortality) or viscerotropic velogenic NDV (acute lethal infections with necro-haemorrhagic lesions most obvious in the gastrointestinal tract) (Alexander *et al.,* 1998b; Alexander and Saif, 2003; Huang *et al.*, 2004). The World Organization for Animal Health (OIE 2005), defines Newcastle disease as notifiable. Studies of naturally occurring and experimental infections have shown that the velogenic ND virus is the commonest pathotype in Nigeria and a major cause of infection in many species of birds both domestic and wild birds (Onunkwo and Momoh 1980, Echeonwu *et al.,*1993, Fagbohun *et al.,* 2000, Saõdu *et al.,*2004 and Oyekunle *et al.,* 2006). NDV infects a wide range of avian species, with more than 250 species of birds known to be susceptible to NDV infection (Kaleta *et al*., 1988). Piacenti *et al*. (2006) reported that turkeys infected with velogenic neurotropic and velogenic viscerotropic isolates showed severe depression and neurologic signs. However, in general, the disease among

turkeys was less severe than in chickens, and turkeys could be considered a subclinical carrier for some of the isolates (Piacenti *et al*., 2006).

The pathogenesis and pathology of the disease in chickens has been well documented however, the pathogenesis in turkeys is still not well documented. A proper understanding of the pathogenesis and pathology of the disease in turkeys may provide a useful guide towards instituting a control programme. Determining the level of susceptibility of turkeys to VND infection, persistence of viral discharge in faecal samples will provide basis for the establishment of epidemiological links for the disease for effective control.

5.3 Materials and Methods

5.3.1 Experimental birds

One hundred and twenty (120) day old commercial cross bred turkey and one hundred and twenty (120) day old white harco cockerel from CHI hatchery in Ibadan, Oyo State were used for this study. Broodings were carried out separately under the same environmental conditions. Feed and water were given *ad libitum* and all the birds were kept in a deep litter system in the Department of Veterinary Medicine experimental animal house.

5.3.2 Experimental design

The experimental birds were randomly divided into two groups; Unvaccinated and Vaccinated. Each of group was further replicated into four subgroups of thirty birds each as shown on Table 5.1.

Table 5.1 Experimental design showing the different treatment groups for turkeys and chickens

Specie/sub group	Number of birds	Treatment	Challenge with Kudu virus (30 birds) per group
Chickens			
1	30	Vaccinated, Inoculated (VCHI)	0.2 ml of KUDU 113 virus
2	30	Unvaccinated, Inoculated (UCHI)	0.2 ml of KUDU 113 virus
3	30	Vaccinated, Uninoculated (VCCH)	0.2 ml of PBS
4	30	Unvaccinated, Uninoculated (UCCH)	0.2 ml of PBS
Turkeys			
5	30	Vaccinated, Inoculated (VIT)	0.2 ml of KUDU 113 virus
6	30	Unvaccinated, Inoculated (UIT)	0.2 ml of KUDU 113 virus
7	30	Vaccinated, Uninoculated (VCT)	0.2 ml of PBS
8	30	Unvaccinated, Uninoculsted (UCT)	0.2 ml of PBS

Sub groups 1,2,5,6 serve as inoculated group while 3,4 ,7 and 8 served as uninoculated control.

The vaccinated groups were given 0.05ml each of NVRI Vom Hitchner B1 (I/O) and La Sota ND vaccine intraoccularly at day old and 21 days old respectively. The experimental chickens from both groups were protected against IBD using 0.05ml each of Abic Gumboro vaccine intraoccularly on days 10 and 21 of age. At 6 weeks of age, sub groups 1, 2, 5 and 6 were challenged with Velogenic Newcastle Disease virus.

5.3.3 The Velogenic Newcastle Disease Virus inoculum

The VNDV strain Kuru duck-113(KUDU-113) obtained from NVRI, Vom Nigeria was used. It was isolated from cloacal swab of an apparently healthy duck (Echeonwu *et al.,* 1993). The virus was maintained freeze dried in 0.5 ml ampoules cryopreserved in Virology Department of NVRI, Vom, Nigeria. The inoculum was first reconstituted by adding 0.5 ml of distilled water into the ampoule. The entire content was diluted with 9.5 ml of phosphate buffered saline (PBS) with PH 7.0 to give 1/10 dilutions. Finally, it was diluted with 99.5ml of PBS to make 1:1000 dilution and to give a median ELD of $10^{6.46\ per}$ ml. The entire content in a sterile beaker was surrounded with ice blocks while inoculating.

5.3.4 Newcastle disease virus challenge

At six weeks of age each bird from sub groups 1, 2, 5 and 6, were inoculated intramuscularly (IM) on the thigh muscle with 0.2 ml of the innoculum while sub groups 3, 4, 7 and 8 received 0.2 ml of PBS IM. The inoculated and uninoculated of the vaccinated and unvaccinated groups were housed in deep litter system in separate houses, in different locations and managed by 2 different animal caretakers.

5.3.5 Clinical signs

The birds were observed twice daily for clinical signs. From day 0 to day 21 post infection (PI). Incubation period, mortality morbidity rates and time of complete recovery were recorded.

5.3.6 Changes in body weight

Ten experimental birds in both the vaccinated and unvaccinated group were tagged and average live body weight taken at days 0 , 3, 5, 10, 15 and 21PI.

5.3.7 Gross pathologic examination

Three birds from each group were humanely sacrificed on days 3, 5, 10, 15 and 21 PI. They were necropsied with the dead ones. Necropsies were performed immediately after death by standard protocol (King *et al.,* 2003). Distribution and persistence of the lesions on affected organs were recorded.

5.3.8 Histopathology

Samples of the bursa, spleen and thymus were collected and fixed in 10% formal saline for minimum of 24hr. they were processed, stained and embedded with haenatoxylin and eosin and studied under the light microscope.

5.3.9 Changes in weight of lymphoid organs

Three birds from each group were weighed and humanely sacrificed on days 3, 5, 10, 15, 21 PI. Their lymphoid organs were dissected out and weighed. The lymphoid organ index were obtained by

organ weights in grams by the body weight in greams and multiplying by 1000 as described by Krasselt (1986).

5.3.10 Serology

Two mililitres of blood was collected from ten randomly selected birds in each group using the jugular vein on days 0, 6. 10, 14, 21 PI. The samples were stored overnight at room temperature. Sera were harvested and stored at -20^{0}C till used. The sera were assayed for ND antibody titre using haemagglutination inhibition test as decribed by OIE (2005).

5.3.11 Virus isolation

Virus isolation was carried out using standard procedures (Hanson, 1980; Anon, 2004). This was done to confirm infectivity of inoculum as NDV and its development in the tissues.

5.3.12 Collection of tissue

1 gram each of the spleen, brain and intestine were collected aseptically from three recently dead or moribund birds in the inoculated group and pooled on days 3, 6, 15 and 21 PI. The same samples were collected from birds sacrificed in control groups. The samples were frozen at -20^o C until used for virus isolation in chicken embryonated eggs.

5.3.13 Preparation of the inoculum

Frozen samples of each organ collected were quickly thawed, ground and homogenized in a mortar with sterile sand and 20% (w/v) suspensions prepared in PBS (Ph 7.0) containing antibiotics (Amphotericin B, 1000 units/ml; penicillin G, 2000 units/ml; streptomycin, 2 mg/ml and gentamycin sulphate,50 µg/ ml). The suspensions were centrifuged at 2,000 rpm for 10 minutes in a

refriginated centrifuge , and the supernatant inoculum kept at room temperature for 1 hour prior to inoculation of embryonated eggs.

5.3.14 Collection of cloacal swabs

Cloacal swabs visibly coated with faecal material were collected randomly from six birds in each group on days 0, 3, 6 , 10 ,15 and 21 PI. The clocal swab were placed in a tube containing 1ml of isotonic PBS, PH 7.0-7.4, antibiotics (Amphotericin B,500 units/ml; penicillin G,10000units/ml; streptomycin,10 mg/ml and gentamycin sulphate, 250µg/ml) and stored in -20 ^{0}C till use.

5.3.15 Preparation of the innoculum

The swabs were placed in a tube containing 1ml of isotonic PBS, PH 7.0-7.4, antibiotics (AmphotericinB,500 units/ml; penicillinG,10000units/ml; streptomycin,10 mg/ml and gentamycin sulphate, 250µg/ml) swab suspensions were centrifuged at 3000 rpm for 10 minutes in a refriginated centrifuge (Hawksley, England), and the supernatant kept at room temperature for 1 hour prior to inoculation of embryonated eggs.

5.3.16 Egg inoculation

0.2 ml of the tissue or cloacal swab supernatant was inoculated into the allantoic cavity of each of five embryonated chicken eggs which had been incubated at 37 ^{0}C for 9-11days. Another batch of five eggs served as negative control. The inoculated eggs were sealed and then re-incubated and candled twice daily to remove dead embryo as they arose for a minimum of 96 hours. The eggs containing dead or surviving embryos were chilled at 4 ^{0}C for at least 4 hours prior to spot testing for the presence of haemagglutinating agents by using the HA test recommended by OIE (2005).

5.3.15 Spot-Haemagglutination Test

The spot-HA test was carried out on a clean and dry white tile. Using a single channel adjustable pipette, 10µl of 10% freshly collected and washed RBC were placed on the tile. A loopful of the allantioc fluid from each chilled egg were placed on the RBCs suspension and mixed. The presence of granular-like particles indicated positive agglutination.

5.4 Haematology

5.4.1 Blood sample collection

Two millimeters of blood were collected from the right jugular vein of five bird in each group and decanted into a sample bottle containing 3 mg of ethyleneamine teracetic acid (EDTA), on days 0, 3, 6, 10, 15 and 21 PI. The blood samples were collected in each day of sampling in the morning between 7.00 am and 9.00 am.

5.4.2 Haematologic methods and procedures

The haematologic parameters determined were packed cell volume (PCV), haemoglobin concentration (HbC), red blood cell count (RBC) and white blood cell count (WBC,total and differential). Packed Cell Volume was determined by the microhaematocrit method (Coles, 1986) using a microcapillary tube, microhaematocrit centrifuge and reader (Hawskey, England).The microcapillary tube was nearly filled with blood sample and sealed at one end. It was centrifuged at 10,000 revolutions per minute for 10 minutes using a microhaematocrit centrifuge. The PCV was later read using the microhaematocrit reader.

The HbC was determined by the cyanomethamoglobin method (Kachmar, 1970). 0.02 ml of the blood sample was added to 5 ml of Drabkins haemoglobin reagent in a clean test tube and allowed

for 20 minutes. The absorbance of the mixture was read at 540 nm wavelength against a reagent blank using a spectrophotometer (Hawksley, England). Standards were also prepared and read. The HbC was calculated by multiplying the spectrophotometeric reading with a calibrating factor (14.5) obtained from the absorbance and concentration of the standards.

The Red Blood Cells (RBC) were determined by the haemocytometer method (Schalm *et al.,* 1975) using an improved neubauer counting chamber (Hawksley, England) and avian diluting fluid (Campbell and Coles,1986). 0.02 ml of blood was pipette from the blood sample and added to 4ml of RBC diluting fluid in a clean tube to make a 1:200 dilution of the blood sample. The diluted blood sample was loaded onto a Neubauer counting chamber and all red blood cells in five out of sixteen small squares in the central area of the Neubauer chamber were enumerated (counted) using a light microscope (Hawksley, England) at X40 objective. The number of cells enumerated for each sample was multiplied by 10,000 to obtain the RBC count per microlitre of blood. The laboratory tally counter (Clay Adams, News Jersey) was used for the RBC count.

Total WBC count were carried out by the haemocytometer method using the improved neubauer counting chamber (Hawksley, England) and a special avian WBC diluting fluid composed of aqueous phloxine, propylene glycol and sodium carbonate (Campbell and Coles,1986) . 0.02 ml of blood was mixed with 0.38 ml of the avian WBC diluting fluid in a clean test tube. After mixing the diluted blood was loaded onto a Neubaur counting haemocytometer chamber and the cells were allowed to settle for three minutes. All cells in the four corner squares were counted using a X10 objective of a light microscope. The total number of cells counted was multiplied by 50 to obtain the total WBC count per microlitre of blood. The differential WBC was carried out following the

Leishman technique (Campbell and Coles, 1986). A drop of the well mixed blood was placed on clean grease-free slide and carefully smeared thinly using a coverslip in order to obtain a thin blood smear. The thin smear was air dried and stained by the Leishman technique. Using the longitudinal counting method, a total of 100 cells were examined and cell types were recorded using a differential cell chamber. Results for each cell type were expressed as a percentage of the total WBC count.

5.5 Data analysis

All the data generated were presented in tables. Statistical analysis was done using Statistical Package for Social Sciences (SPSS) version 16 for windows (SPSS Inc, Chicago, Illinois). Data generated from body weight, HI titres and haematology were analyzed by One-way ANOVA, t- tests and variant means were separated by Duncan multiple range test. All tests were performed at a 5% level of probability.

5.6 RESULTS

5.6.1 Clinical signs

Among the inoculated birds clinical signs were first observed in unvaccinated birds and then vaccinated birds (Fig 5.1& 5.2).

Clinical signs were observed in both unvaccinated chickens and turkeys inoculated with VNDV on day 2 PI. In unvaccinated chickens the signs included ruffled feathers with 20% depressed on day 2 PI, by day 3 PI, 76.9% of the chickens were severely depressed, comatose and lethargic, and some were prostrate, with whitish greenish diaorrhea which soiled the vent. There was also reduction in water and feed intake. Some of the birds in the unvaccinated chicken group tucked their heads under their wings, huddled together with hunched posture. By day 4 PI, there was 100% depression with the previous signs prominent in most of the birds. Nervous signs such as jerking of head and paralysis were evident on day 3 PI and by day 5 PI paralysis and ataxia were evident in the remaining 3 chickens. The unvaccinated turkeys showed clinical signs of depression in 10% on day 2 PI, 33% depression by day 3PI and 92% depression by day 4 PI. Other signs included ruffling of feathers with greenish whitish diarrhoea and reduced feed intake was also observed. There was paralysis in 2 turkeys (Fig. 5.2) which was accompanied by jerking of head, tremors and ataxia, recumbency, torticolis and extensor rigidity. By day 5 PI paralysis, ataxia and torticollis were evident, depression rose to 88%. Paralysis, coma and lethargy, and prostration, were consistent features until day 14PI when survivors recovered fully.

Among the vaccinated inoculated birds clinical signs were first observed in chickens and these included ruffled feathers with depression in 13% of the birds on day 3 PI. By day 4 PI, 22% of the chickens were depressed, comatose, and prostate with whitish greenish faeces soiling the vent area.

There was slight reduction in water and feed intake and by days 5 PI and 6 PI, 19% and 16% of the birds respectively were depressed accompanied by head tremors, wing and leg paralysis. There was full recovery of the chickens by day 12 PI. The vaccinated turkeys showed signs of depression in 3.7% of the birds, by day 4PI the clinical signs observed were; head tremors, ruffled feathers, and greenish faeces with no soiling of the vent. There was also a slight reduction in feed and water consumption. The turkeys were fully recovered by day 8 PI. The uninoculated groups for both unvaccinated and vaccinated birds showed no clinical signs.

Reduction in body weight of inoculated birds is shown in Table 5.2. Reduction in weight was significant in the unvaccinated and vaccinated inoculated chickens when compared withn control. On days 3 and 5 PI the mean body weights of chickens in the vaccinated and unvaccinated inoculated group were significantly ($P < 0.05$) lower than their control groups (Table 5.2). On days 10, 15 and 21 PI the mean body weights of vaccinated inoculated chickens were significantly lower ($P < 0.05$) than the control. The chickens in the unvaccinated inoculated chickens did not survive up to day 10 PI.

There was a significant reduction ($p<0.05$) in the body weight of the unvaccinated and vaccinated inoculated turkeys when compared with controls. On days 3-21 PI the mean body weight of the vaccinated and unvaccinated inoculated turkeys were significantly lower ($p<0.05$) than their controls while there was a significant increase ($p<0.05$) in the mean body weight of the vaccinated inoculated turkeys when compared with that of the unvaccinated inoculated and control turkeys.

Mortality (13.3%) was first observed in unvaccinated inoculated chickens on day 3 PI (Table 5.3). Peak mortality occurred on days 4 and 5 PI involving 8 (34.8%) and 12 (80%) chickens respectively. By day 6PI the remaining 3 (100%) chickens were dead. Mortality (10.7%) was first recorded in

unvaccinated inoculated turkeys on day 4PI (Fig. 5.2). Peak mortality occurred on day 5PI involving 7 (28.0%) turkeys. The last mortality was on day 8 PI involving 3 (25.0%) turkeys. In the vaccinated inoculated flock, mortality was only observed in the vaccinated chickens and none in the vaccinated turkeys. Mortality of 3.8% was observed on day 5 PI. Peak mortality occurred on day 7 PI involving 2 (4.1%) chickens. No mortality was recorded for vaccinated turkeys. The total mortality rates were 90% and 80% for unvaccinated chickens and turkeys respectively while total mortality of 13.3% and 0% were recorded for vaccinated chickens and turkeys respectively.

Table 5.2 Mean body weights (g± sem) of unvaccinated, vaccinated chickens and turkeys experimentally inoculated with KUDU 113 virus

Groups	Days Post Inoculation					
	0	3	5	10	15	21
Chickens						
UCHC	473 ± 14.8	465.5±14.8^{b}	526±12.4^{bc}	599.5±19.2^{c}	663.4±13.9^{c}	782.7±10.9^{c}
UCHI	454± 17.9	419.9±8.2^{a}	387.1±21.3^{a}	-	-	-
VCHC	454±17.9	471.6±9.7^{b}	499 ± 9.1^{b}	535.2±15.2^{b}	607.8±9.8^{b}	672.8±23.9^{b}
VCHI	454±17.9	421.5±10.0^{a}	414.2±9.8^{a}	467.5±11.7^{a}	523.7±16.95^{a}	574.6±29.7^{a}
Turkeys						
UCT	589.7±21.8	613.2±13.4^{b}	693.7±31.5^{c}	826.7±11.95^{c}	904.8±37.96^{bc}	1255.7±35.1^{c}
UIT	628.3±17.0	523.1±34.0^{a}	462.8±7.1^{a}	350.5±81.5^{a}	368.2±100.2^{a}	490±135.3^{a}
VCT	619.5±18.4	621.9±20.2^{b}	719.1±30.5^{c}	837.4±19.7^{c}	946±25.9^{c}	1346.1±38.6^{c}
VIT	628±17.2	568.2±13.9^{a}	557.5±12.9^{b}	585.8±10.96^{b}	756.2±2 1.8^{b}	965.7±35.5^{b}

*Different superscripts in a column indicate significant difference between the groups ($p<0.05$). * UCHI- unvaccinated infected cockerel, UCHC- Unvaccinated uninoculated chickens, VCHC- Vaccinated uninoculated chickens, VCHI- Unvaccinated inoculated cockerel UIT- Unvaccinated inoculated turkey, UCT- Unvaccinated uninoculated turkey, VCT Vaccinated uninoculated turkey, VIT Vaccinated inoculated turkey, **SEM** = Standard error means to the mean values of control and inoculated bird, -not done because all the chickens died

Table 5.3 Depression and mortality in unvaccinated and vaccinated chickens and turkeys experimentally inoculated with KUDU 113

Dpi	Unvaccinated chickens		Unvaccinated turkeys		Vaccinated chickens		Vaccinated turkeys	
	Depression	Mortality	Depression	Mortality	Depression	Mortality	Depression	Mortality
1	$0^{A}/30^{B}$ (0)	0	0/30	0	0/30	0	0/30	0
2	6/30 (20)	0	3/30 (10)	0	0/30	0	0/30	0
3	20/26 (76.4)	4 (13.3)	10/30 (33.3)	0	4/30 (13.3)	0	0/30	0
4	15/15 (100)	8 (34.8)	23/25 (92)	3 (10.7)	6/27 (22.2)	0	1/27 (3.8)	0
5	3/3 (100)	12(80)	16/18 (88.8)	7(28.0)	5/26 (19.2)	1 (3.8)	1/27 (3.8)	0
6	0	3(100)	13/15 (86.6)	3(16.6)	4/25 (86)	1(3.8)	1/24 (4.1)	0
7	-	-	7/9 (77.7)	3 (25)	2/20 (10)	2 (9)	1/24 (4.1)	0
8	-	-	5/7 (55.5)	2(22.2)	2/20 (10)	0	1/24 (4.1)	0
9	-	-	4/7 (57.1)	0	1/20 (5)	0	1/24 (4.1)	0
10	-	-	3/5 (60)	0	1/20 (5)	0	0/24	0
11	-	-	3/5 (60)	0	1/17 (5.8)	0	0/21	0
12	-	-	2/5 (40)	0	0/17	0	0/21	0
13	-	-	1/5 (20)	0	0/17	0	0/21	0
14	-	-	1/5 (20)	0	0/17	0	0/21	0
15	-	-	0/2	0	0/17	0	0/21	0
16	-		0/2	0	0/14	0	0/18	0
17	-	-	0/2	0	0/14	0	0/18	0
18	-	-	0/2	0	0/14	0	0/18	0
19	-	-	0/2	0	0/14	0	0/18	0
20	-	-	0/2	0	0/14	0	0/18	0
21	-	-	0/2	0	0/14	0	0/18	0
Total	27(100)	27 (100%)	23 (92%)	18 (60%)	6 (22.2%)	4(13.3%)	1(4.1%)	0

A = Numbers positive for depression.; B = No of birds per group ,it is the number of birds remaining in a group when first and subsequent mortalities were observed,-= not done

5.6.2 Gross lesions

In inoculated unvaccinated chickens at days 3 to 6 PI the muscles of the breast, thigh and legs were congested (Figure 5.3), Proventricular haemorrhage (figure 5.4) persisted for up to day 6PI. Catarrhal or haemorrhagic enteritis occurred in the intestines on day 4PI and gradually progressed to sharply demarcated ulcers on day 5-6PI in the jejunum and ileum (Fig. 5.5). These ulcers were evident from the serosal and mucosal surfaces. On day 4PI the caecal tonsils were swollen, ulcerated and haemorrhagic and often contained cheesy necrotic material. The spleen was enlarged on day 3PI, mottled with dark spots on the serosal surface on days 4-6PI but later became atrophic (Fig. 5.6). Atrophy of the thymus was noticeable on day 3-6 PI (Fig. 5.7) and by day 6 PI was severely atrophic. The bursa of Fabricus was also observed in 4 chickens and was enlarged in one on day 3 PI. The bursa was severely atrophic on day 6PI (Fig 5.8). The kidneys were haemorrhagic and enlarged by day 4- 6 PI (Table 5.4).

In the inoculated vaccinated chickens at days 4 to 15 PI the muscles of the breast, thigh and legs were congested. This lesion was severe on days 6 and 7 PI. Proventricular haemorrage was observed on the mucosal surface on days 6 and 7 PI on both sacrificed and dead vaccinated chickens. Atrophy of the thymus was evident from day 5 PI (Fig. 5.9) and persistent till day 21PI. On day 10 PI, there was disappearance of the organ in one bird. Atrophy of the spleen was observed from day 3 PI and it persisted till day 21 PI (Fig. 5.10) Enlargement of the bursa of Fabricus was observed on 3 PI and subsequently the organ was atrophied in all the birds on day 5 -10 PI (Fig. 5.11). But by day15-21 PI the organ had returned to normal in some of the birds. Catarrhal enteritis was observed in the intestine on day 3PI and progressed to sharply demarcated ulcers by day 7 PI. Caecal tonsils were swollen, ulcerated and haemorraghic by day 3 PI this persisted till day 10 PI. Enlargement and congestion of the kidneys were evident on days 3 and 6 PI.

In the unvaccinated inoculated turkeys at day 3 PI, congestion of the muscles of the breast, thigh and leg was evident (Fig. 5.3) and this persisted till day 15 PI. The carcasses were emaciated and dehydrated, atrophy and mottling of the spleen was observed by day 3 PI (Fig. 5.12). Enlargement of spleen was also evident in one turkeys from days 3-7 PI. Thymus and bursa of Fabricius were severely atrophic from day 3PI and persisted till day 21PI (Fig. 5.13,5 .14). This was observed in both dead and sacrificed turkeys. There was also congestion of the blood vessels of the brain on day 6PI. Proventricular haemorrage was not observed till the end of the experiment. Parboiling of the liver was evident only on days 6 and 7 PI.. Enlargement and congestion of the kidney were observed from day 4PI and persisted till the end of the experiment and the intestines were congested.

In the vaccinated inoculated turkeys congestion of the breast, thigh and leg muscles were observed by day 3PI and it persisted till day 15 PI. Atrophy of the thymus (Fig. 5.15) and bursa of Fabricus (Fig. 5.16) was observed by day 3 PI and persisted till day 21PI. Atrophy of spleen (Fig.5.17) were observed on day 3PI, atrophy persisted till day 20 PI. Enlargement and congestion of the kidneys was observed on day 6 PI. There was no haemorrage on the mucousa of the proventriculus and caecal tonsils. There was also no catarrahal enteritis or sharply demarcated ulcers. The liver was normal till day 21 PI.

Figure 5.1 First sign of mortality in unvaccinated inoculated chickens on day 3 Post inoculation with KUDU 113.

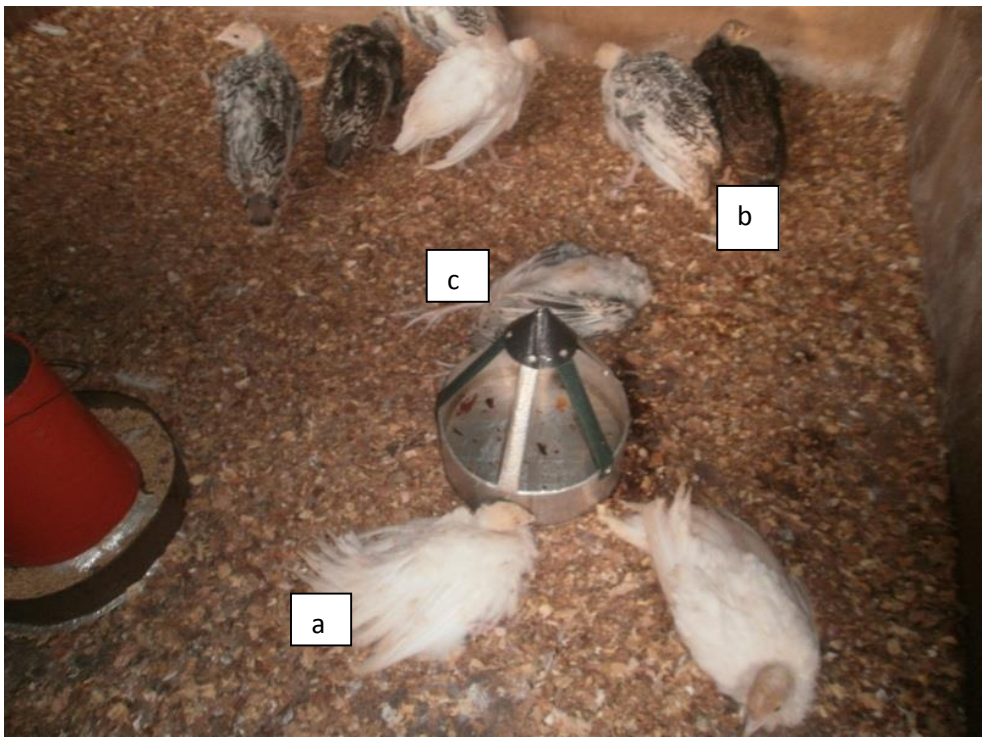

Figure 5.2 a: Paralysis b: depression c: one death among inoculated unvaccinated turkeys on day 4 Post inoculation with KUDU 113.

Chicken **Turkey**

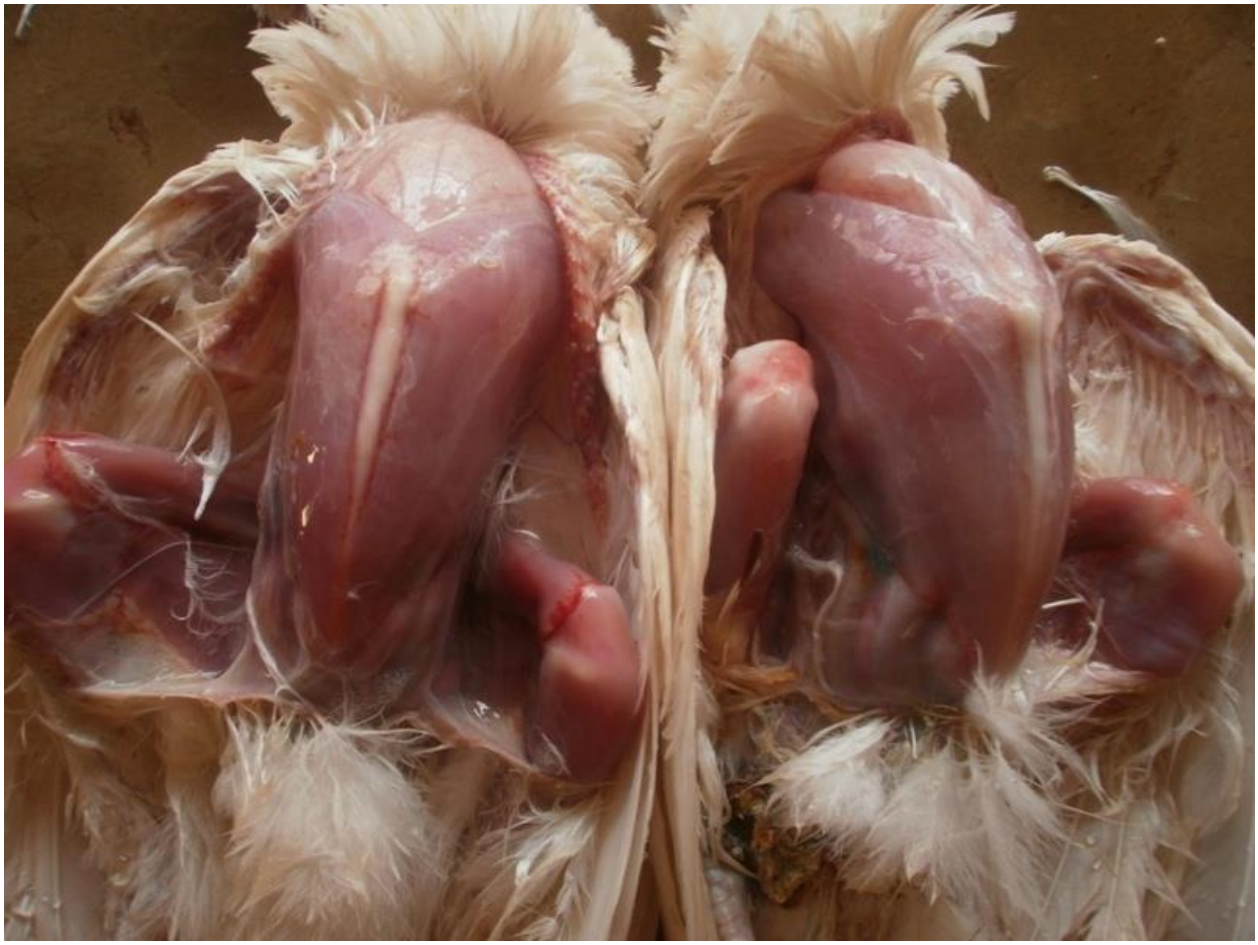

Figure 5.3 Congested breast muscles of unvaccinated inoculated chicken and turkey on day 4 post inoculation with KUDU 113.

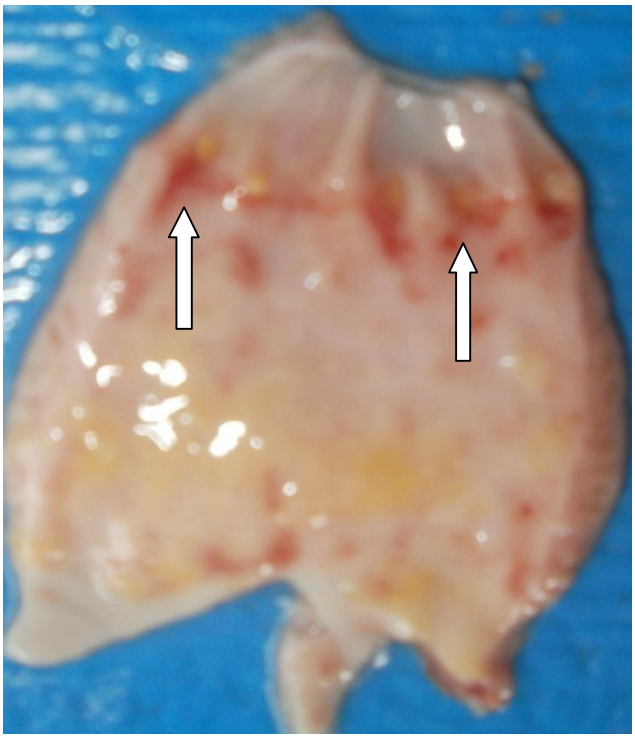

Figure 5.4 Arrows show haemorrhages on the proventriculus of inoculated and dead unvaccinated chicken on day 4 post inoculation with KUDU 113.

Chicken **Turkey**

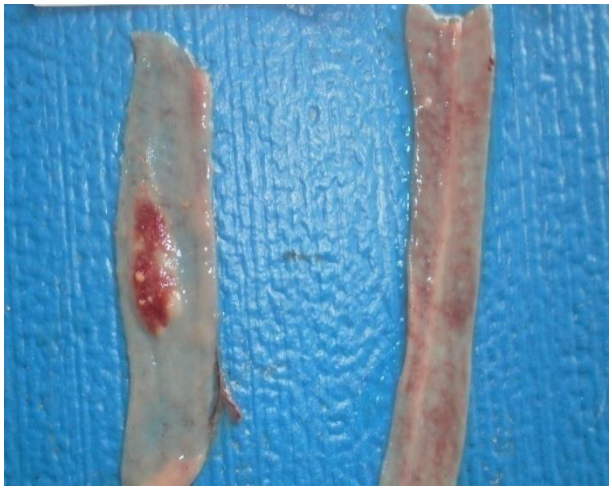

Figure 5.5 Haemorragic intestinal ulcers evident in inoculated chicken and not in inoculated turkey on day 4 post inoculation with KUDU 113.

Control chicken **Inoculated chicken**

Figure 5.6: Atrophy of the spleen of unvaccinated inoculated chicken on day 5 post inoculation with KUDU 113

Control chicken **Inoculated chicken**

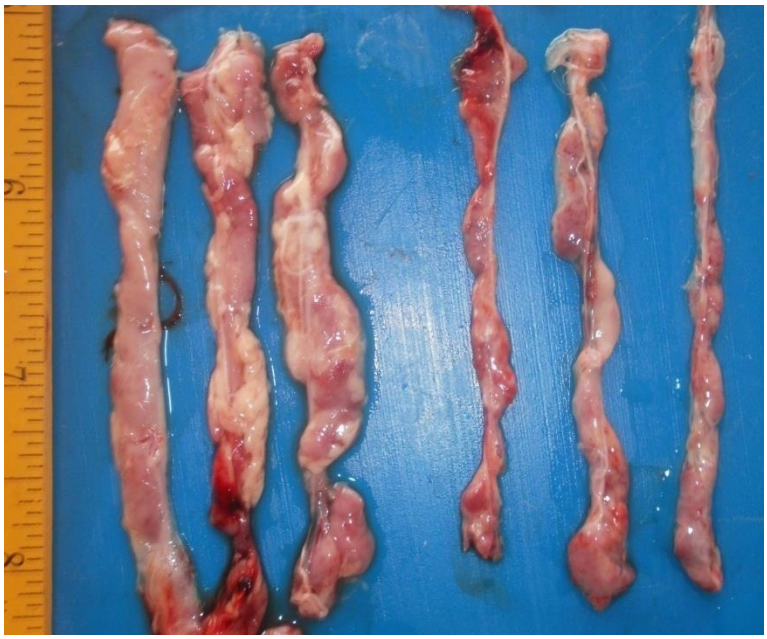

Figure 5.7: Atropy of thymus of unvaccinated inoculated chickens on day 5 posr inoculation with KUDU 113

Control chicken **Inoculated chicken**

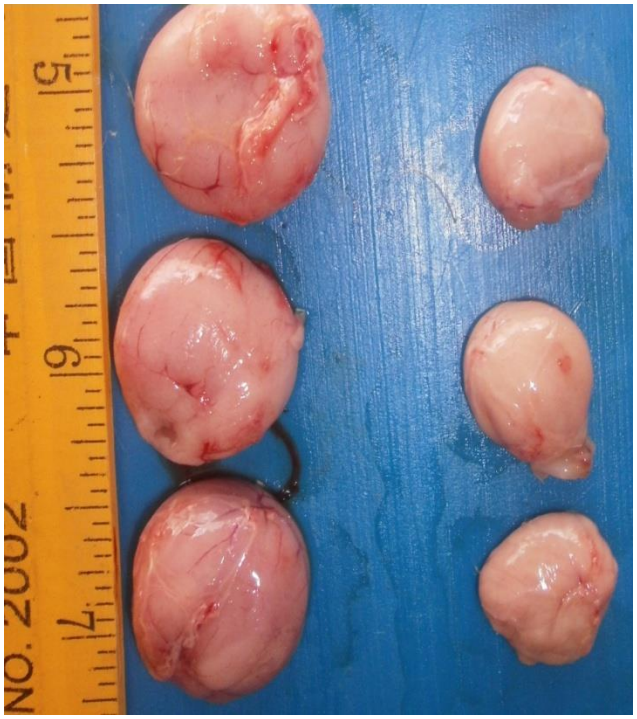

Figure 5.8 Atrophy of the bursae of Fabricus in unvaccinated inoculated chickens day 6 post infection with KUDU 113

Control chicken **Inoculated chicken**

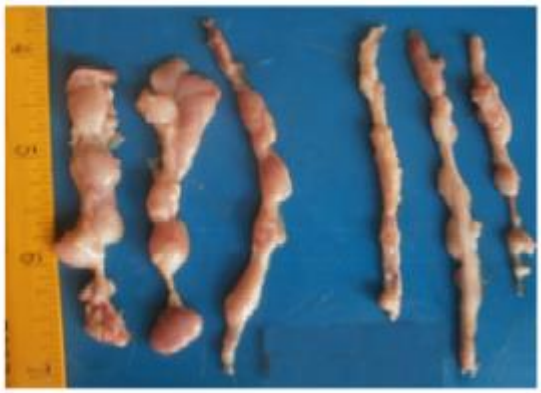

Figure 5.9: Atrophy of the thymus in vaccinated chickens on day 6 post inoculation with KUDU 113

Control chicken **Infected chicken**

Figure 5. 10 Atrophy of the spleen of vaccinated inoculated chicken on day 10 post inoculation with KUDU 113

Control chicken **Inoculated chicken**

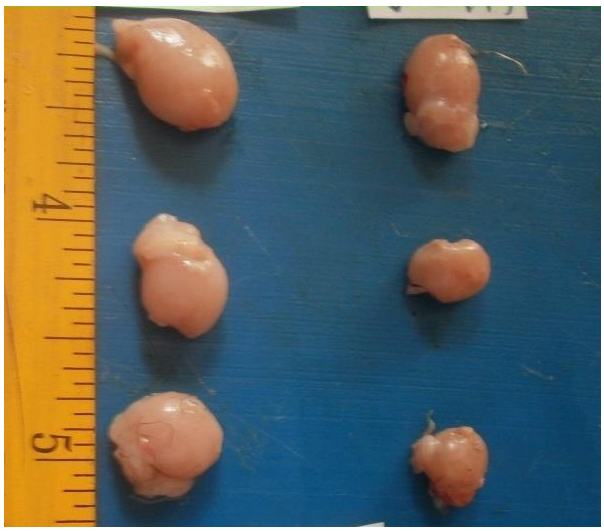

Figure 5.11: Atrophy of the bursae of vaccinated chickens on day 6 post inoculation with KUDU 113 virus.

Control turkey **Infected turkey**

Figure 5.12 Atrophy of the spleen in unvaccinated inoculated turkeys day 10 post inoculation with KUDU 113 virus

Control turkey **Inoculated turkey**

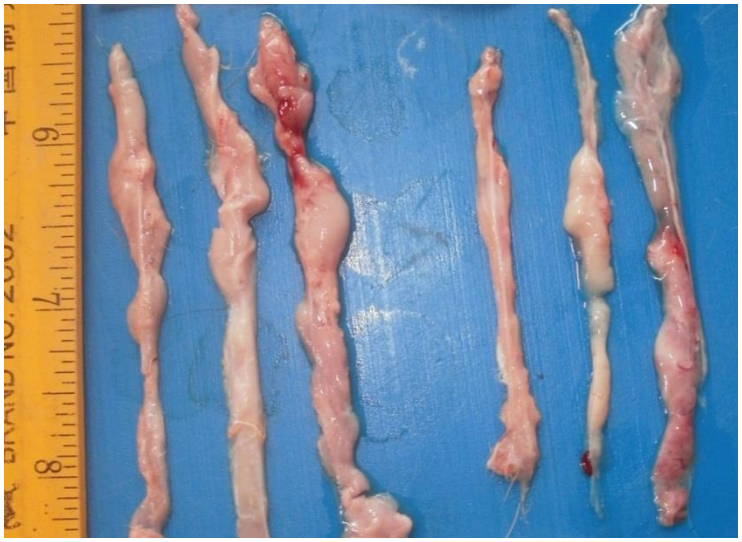

Figure 5.13 Atrophy of thymus of unvaccinated inoculated turkeys on day 5 post inoculation with KUDU 113

Control turkey **Inoculated turkey**

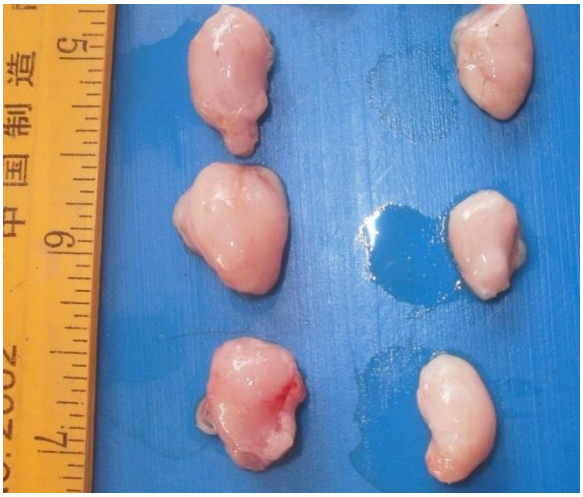

Figure 5.14 Atrophy of the bursa of Fabricus in unvaccinated inoculated turkeys day 5 post inoculation with KUDU 113

Control turkey **Inoculated turkey**

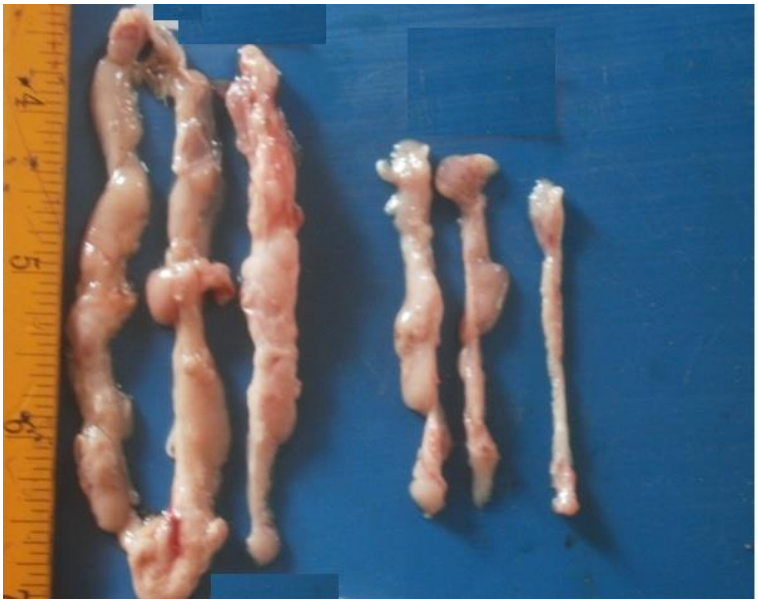

Figure 5.15: Atrophy of the thymus in vaccinated inoculated turkeys on day 6 post Inoculation with KUDU 113.

Control turkey **Inoculated turkey**

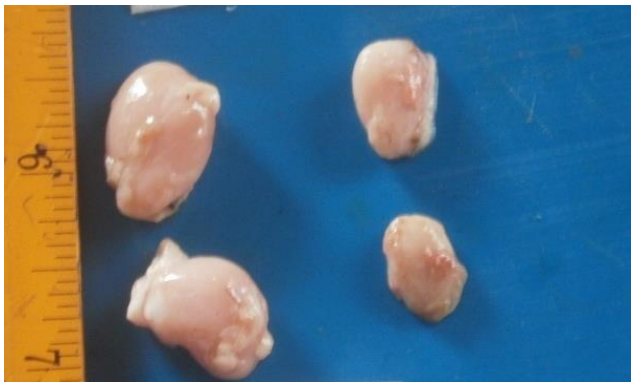

Figure 5.16: Atrophy of the bursa of vaccinated inoculated turkeys on day 6 post inoculation with KUDU 113.

Control turkey **Inoculated turkey**

Figure 5. 17 : Atrophy of the spleen in vaccinated inoculated turkeys on day 20 post inoculated with KUDU 113.

Table 5.4 Distribution of gross lesions in unvaccinated chickens inoculated with the KUDU 113 virus

Organ	Lesion	Days post inoculation			
		3	4	5	6
Breast, thigh Leg muscles	Congestion	7/7	8/8	12/12	3/3
Proventriculus	Mucosal haemorrage	0/7	7/8	12/12	3/3
Thymus	Atrophy	5/7	8/8	12/12	3/3
	Disappearance of the organ	0/7	0/8	0/12	0/3
Bursa of Fabricus	Enlargement	1/7	2/8	0/12	3/3
	Atrophy	4/7	4/8	9/12	0/3
Spleen	Mottling with dark spots	0/7	2/8	0/12	3/3
	Enlargement	6/7	4/8	4/12	2/3
	Atrophy	1/7	4/8	6/12	1/3
Kidneys	Congestion and enlargement	0/7	8/8	10/12	3/3
Intestine	Sharply demarcated ulcers	0/7	7/8	12/12	3/3
Caecal tonsils	Mucosal haemorrhage and enlargement	0/7	7/8	12/12	3/3
Liver	Parboiled	0/7	5/8	8/12	3/3

Table 5.5 Distribution of gross lesions in vaccinated chickens inoculated with the KUDU 113 virus

Organ	Lesion	Days post inoculation						
		3	5	6	7	10	15	21
Breast, thigh leg muscles	Congestion	2/3	1/1	3/4	2/2	1/3	1/3	0/3
Proventriculus	Mucosal haemorrage	0/3	0/1	1/4	1/2	0/3	0/3	0/3
Thymus	Atrophy	0/3	1/1	3/4	2/2	2/3	1/3	1/3
	Disappearance of the organ	0/3	0/1	0/4	0/2	1/3	0/3	0/3
Bursa of Fabricus	Enlargement	1/3	0/1	0/4	0/2	0/3	0/3	0/3
	Atrophy	1/3	1/1	4/4	2/2	2/3	1/3	1/3
Spleen	Mottling with dark spots	1/3	0/1	4/4	0/2	0/3	0/3	0/3
	Enlargement	0/3	0/1	0/4	0/2	0/3	0/3	0/3
	Atrophy	0/3	0.1	3/4	1/2	2/3	2/3	1/3
Kidney	Congestion and enlargement	1/3	0/1	1/4	0/2	0/3	0/3	0/3
Intestine	Sharply demarcated ulcers	2/3	1/1	1/4	1/2	0/3	0/3	0/3
Caecal tonsils	Mucosal haemorrhage and enlargement	1/3	1/1	4/4	1/2	0/3	0/3	0/3
Liver	Parboiled	1/3	0/1	1./4	0/2	0/3	0/3	0/3

Table 5.6 Distribution of gross lesions in unvaccinated turkeys inoculated with the KUDU 113 virus

Organ	Lesion	Days post inoculation								
		3	4	5	6	7	8	10	15	21
Breast, thigh leg muscles	Congestion	2/2	3/3	7/7	6/6	3/3	2/2	2/2	3/3	2/2
Proventriculus	Mucosal haemorrage	0/2	0/3	0/7	0/6	0/3	0/2	0/2	0/3	0/2
Thymus	Atrophy	2/2	3/3	7/7	6/6	3/3	2/2	2/2	1/3	2/2
	Disappearance of the organ	0/2	0/3	0/7	0/6	0/3	0/2	0/2	0/3	0/2
Bursa of Fabricus	Enlargement	0/2	0/3	0/7	0/6	0/3	0/2	0/2	0/3	0/2
	Atrophy	2/2	3/3	7/7	6/6	3/3	2/2	2/2	2/3	2/2
Spleen	Mottling with dark spots	1/2	3/3	4/7	5/6	2/3	1/2	1/2	2/3	1/2
	Enlargement	1/2	1/3	1/7	1/6	1/3	0/2	0/2	0/3	0/2
	Atrophy	0/2	1/3	6/7	2/6	2/3	2/2	2/2	1/3	2/2
Kidneys	Congestion and enlargement	0/2	3/3	7/7	6/6	3/3	2/2	2/2	3/3	2/2
Intestine	Sharply demarcated ulcers	0/2	0/3	0/7	0/6	0/3	0/2	0/2	0/3	0/2
Caecal tonsils	Mucosal haemorrhage and enlargement	0/2	0/3	0/7	0/6	0/3	0/2	0/2	0/3	0/2
Liver	Parboiled	0/2	0/3	0/7	1/6	1/3	0/2	0/2	0/3	0/2

Table 5.7 Distribution of gross lesions in vaccinated turkeys inoculated with the KUDU 113 virus

Organ	Lesion	Days post inoculation				
		3	6	10	15	21
Breast, thigh leg muscles	Congestion	1/3	2/3	2/3	1/3	2/3
Proventriculus	Mucosal haemorrage	0/3	0/3	0/3	0/3	0/3
Thymus	Atrophy	1/3	3/3	3/3	2/3	1/3
	Disappearance of the organ	0/3	0/3	0/3	0/3	0/3
Bursa of Fabricus	Enlargement	0/3	0/3	0/3	0/3	0/3
	Atrophy	3/3	3/3	2/3	2/3	2/3
Spleen	Mottling with dark spots	0/3	0/3	0/3	0/3	0/3
	Enlargement	1/3	0/3	0/3	0/3	0/3
	Atrophy	1/3	0/3	2/3	1/3	0/3
Kidneys	Congestion and enlargement	0/3	1/3	0/3	0/3	0/3
Intestine	Sharply demarcated ulcers	0/3	0/3	0/3	0/3	0/3
Caecal tonsils	Mucosal haemorrhage and enlargement	0/3	0/3	0/3	0/3	0/3
Liver	Parboiled	0/3	0/3	0/3	0/3	0/3

5.6.3 Histopathology

Sections of the bursa (Fig. 5.18), spleen (Fig. 5.19) and thymus (Fig. 5.20) showed severe lymphocytic necrosis and depletion. The spleen in addition showed fibrin deposition around the sheathed arterioles.

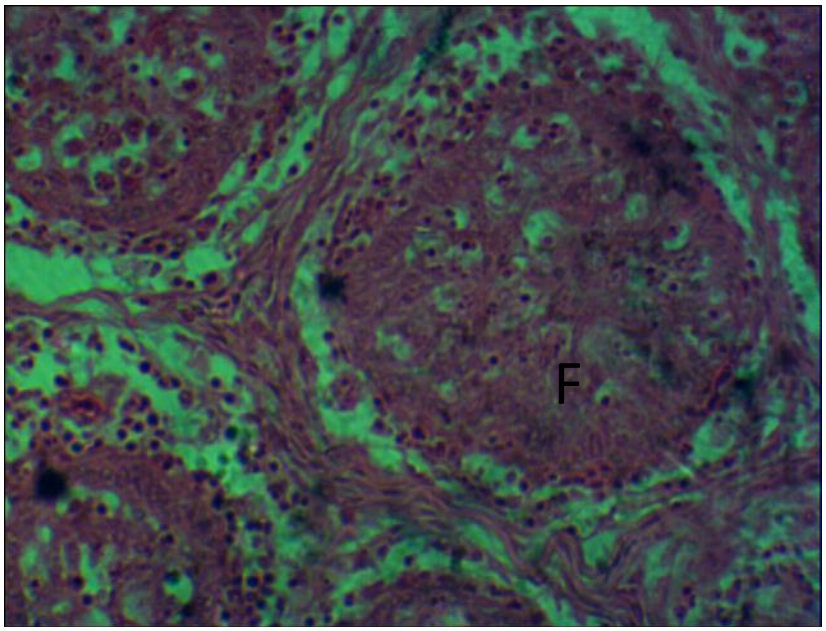

Fig 5.18 : Bursa of unvaccinated inoculated turkey that was sacrificed on day 3 PI showing lymphocytic depletion in the follicle (F). H&E x400

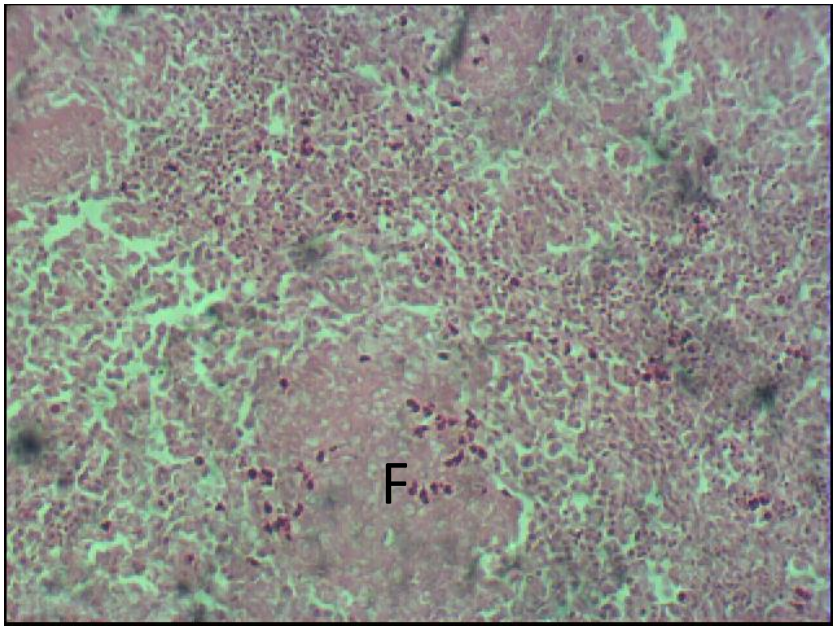

Fig 5.19 Spleen of unvaccinated infected turkey that was sacrificed on day 5 PI showing lymphocytic depletion and fibrin deposition (F) on day 5 PI. H&E x200

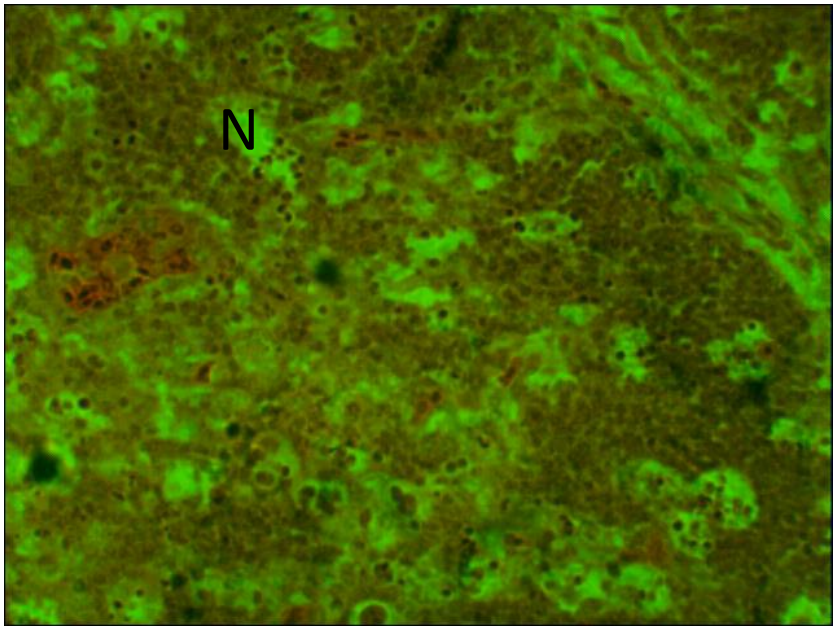

Fig 5.20: Thymus of unvaccinated infected turkey that was sacrificed on day 3 PI showing necrosis and depletion of lymphocytes (N). H&E x 400

5.6.4 Changes in the weight of lymphoid organs

The thymic index for unvaccinated inoculated and vaccinated innoculated chickens were not significantly different when compared with their controls at $p > 0.05$ from days 3-21 PI. There was no significant difference at ($p > 0.05$) between the thymic index of unvaccinated inoculated turkeys when compared with their controls from days 3-21 PI. The thymic index values for vaccinated inoculated turkeys were significantly lower ($p < 0.05$) than the control on days 3-10 PI. However, there was no significant difference at $p > 0.05$ in the thymic index of vaccinated inoculated turkeys and their controls on days 15 and 21 PI.

The mean bursa index of unvaccinated inoculaected chickens was significantly lower than the control at $p < 0.05$ on days 3 and 5 PI. The bursal index of the vaccinated inoculated chickens were not significantly different ($p > 0.05$) from those of their controls on days 3-21 PI. But the bursal index of the vaccinated inoculated chickens were significantly lower ($p < 0.05$) than their controls on days 3 -10 PI. The bursal index of the unvaccinated inoculated turkeys were significantly lower than the controls at $p < 0.05$ on days 3, 10, 15 and 21 PI. The bursal index of the vaccinated inoculated turkeys were significantly lower than the control at $p < 0.05$ on days 3,5 and 10 PI , while the bursae was regaining lost weight from day 15PI.

The splenic index for the unvaccinated inoculated chickens was not significantly different from their contol on days 3 and 5 PI. The splenic index of vaccinated inoculated chickens was significantly lower than the controls ($p < 0.05$) on day 5 PI only. The splenic index for vaccinated inoculated chickens did not differ significantly from those of their controls on days 3, 5, 15 and 21PI. There was no significant difference ($p> 0.05$) in the spleenic index of the unvaccinated turkeys when compared with that of the vaccinated turkeys from days 3-21 PI.

Table 5 8: Changes in the mean thymic index of vaccinated and unvaccinated chickens and turkeys inoculated with KUDU 113 virus

	Days PI				
	3	5	10	15	21
Group	Thymic index 10^{-3}				
UCHC	2.99±0-043	3.12 ± 0.184	-	-	-
UCHI	2.43± 0.179	2.65 ± 0.168	-	-	-
VCHC	5.56 ± 0.179	4.52 ± 1.232	4.31 ± 0.500	6.66± 0.929	5.97±0.541
VCHI	6.09 ± 0.422	2.84 ± 0.082	3.82 ± 0.376	5.32± 1.028	4.96 ±0-302
UCT	2.86± 0.354^{ab}	2.73± 0.432^{ab}	2.66 ± 0.067^{a}	3.28 ± 0.637	2.61 ± 0.233^{a}
UIT	2.23 ± 0.180^{a}	2.82 ± 0.353^{a}	1.89 ±0.567^{a}	2.10 ± 0.173	1.34 ± 0.267^{a}
VCT	3.32 ± 0.110^{b}	3.35 ± 0.423^{b}	4.64 ±0.423^{b}	3.08 ± 0.240	4.01 ± 0.387^{b}
VTI	2.32 ±0.080^{a}	1.70 ± 0.240^{a}	2.33± 0.023^{a}	2.56 ± 0.230	3.67 ± 0.217^{b}

* Different superscripts in a column indicate significant difference between the groups ($p<0.05$). * UCHI- unvaccinated inoculated chickens, UCHC- Unvaccinated uninoculated chickens, VCHC-Vaccinated uninoculated chickens, VCHI- Unvaccinated inoculated chickens, UIT- Unvaccinated inoculated turkeys, UCT- Unvaccinated uninoculated turkeys, VCT Vaccinated uninoculated turkeys, VIT Vaccinated inoculated turkeys, - = not done

Table 5. 9: Changes in the mean bursa index of vaccinated and unvaccinated chickens and turkeys inoculated with KUDU 113 virus

	Days PI				
	3	5	10	15	21
Group	Bursa index 10^{-3}				
UCHC	6.32 ±0.331[c]	4.99 ±0.119[b]	-	-	-
UCHI	3.51± 0.658[b]	2.12 ± 0.467[a]	-	-	-
VCHC	1.42 ± 0.077[a]	3.08 ±0.897[ab]	1.85± 0.153	1.55± 0.155	1.29 ±0.061
VCHI	1.65 ± 0.303[a]	1.65 ± 0.180[a]	1.07 ± 0.275	1.21± 0.167	1.33 ±0.289
UCT	2.77± 0.467[d]	1.47± 0.185[bc]	1.36 ± 0.113[b]	2.28 ± 0.173[b]	1.28 ± 0.233[b]
UIT	2.15 ± 0.100[c]	1.17 ± 0.578[ab]	0.73 ±0.063[a]	1.02 ± 0.080[a]	0..85 ± 0.013[a]
VCT	1.52 ± 0.133[b]	1.54 ± 0.020[c]	1.23 ±0.147[b]	1.30 ± 0.100[a]	1.04 ±0.133[ab]
VIT	1.22 ±0.020[a]	0.96 ± 0.087[a]	0.76± 0.047[a]	1.34 ± 0.027[a]	0.89 ±0.087[ab]

* Different superscripts in a column indicate significant difference between the groups ($p<0.05$). * UCHI- unvaccinated inoculated chickens, UCHC- Unvaccinated uninoculated chickens, VCHC-Vaccinated uninoculated chickens, VCHI- Unvaccinated inoculated chickens, UIT- Unvaccinated inoculated turkeys, UCT- Unvaccinated uninoculated turkeys, VCT Vaccinated uninoculated turkeys, VIT Vaccinated inoculated turkeys, - = not done

Table 5.10: Changes in the mean spleenic index of vaccinated and unvaccinated chickens and turkeys inoculated with KUDU 113 virus

Group	Days PI				
	3	5	10	15	21
	Splenic index 10^{-3}				
UCHC	1.96 ±0.242[ab]	1.00 ±0.413[a]	-	-	-
UCHI	2.52± 0.200[b]	1.75 ± 0.095[ab]	-	-	-
VCHC	1.64 ± 0.247[a]	2.65± 0.497[b]	3.28± 0.064	3.17 ± 0.649	3.19 ±0.896
VCHI	1.91 ± 0.235[ab]	1.26 ± 0.233[a]	1.37 ± 0.033	2.36± 0.325	3.35 ±0.755
UCT	1.75± 0.539	1.03± 0.220	1.36 ± 0.114	1.72 ± 0.378	1.15 ± 0.094
UIT	1.77 ± 0.298	1.00 ± 0.837	1.12 ±0.211	1.60 ± 0.111	1..25 ± 0.156
VCT	1.46 ± 0.151	1.31 ± 0.142	1.23 ±0.234	1.49 ± 0.338	1.48 ±0.266
VIT	1.37 ±0.097	1.25 ± 0.171	1.33± 0.202	1.27 ± 0.156	1.42 ±0.213

* Different superscripts in a column indicate significant difference between the groups ($p<0.05$). * UCHI- unvaccinated inoculated chickens, UCHC- Unvaccinated uninoculated chickens, VCHC-Vaccinated uninoculated chickens, VCHI- Unvaccinated infected chickens, UIT- Unvaccinated inoculated turkeys, UCT- Unvaccinated uninoculated turkeys, VCT Vaccinated uninoculated turkeys,VIT -Vaccinated inoculated turkeys, - = not done.

5.6.5 SEROLOGY

The unvaccinated chickens had no ND antibodies at days 0-5 PI unlike those vaccinated. (Table 5.11). In the vaccinated chickens significant rise ($p< 0.05$) in antibody level was observed in the inoculated group on days 10-21 PI (Table 5.13).

The unvaccinated control turkeys showed no ND antibodies throughout the experiment (Table 5.12). Among the unvaccinated and vaccinated inoculated turkeys, a significant rise ($p< 0.05$) in antibody level was observed in inoculated turkeys on days 5-21 PI.

Table 5.11: Haemagglutination inhibition antibody titre for unvaccinated and vaccinated chickens inoculated with KUDU 113 virus

	Days post inoculation									
	0		5		10		15		21	
	Uninoculated and inoculated groups									
	VCCH	**VICH**	**VCCH**	**VICH**	**VCCH**	**VICH**	**VCCH**	**VICH**	**VCCH**	**VICH**
1	128	128	32	128	128	128	64	512	64	512
2	128	128	128	128	128	128	128	512	64	512
3	128	128	256	256	256	256	256	512	128	1024
4	128	128	256	256	256	512	256	1024	256	1024
5	128	128	512	256	512	1024	512	1024	512	2048
6	128	128	512	256	512	1024	512	1024	512	2048
7	256	256	-	-	-	-	-	-	-	-
8	256	256	-	-	-	-	-	-	--	-
9	256	256	-	-	-	-	-	-	-	-
10	256	256	-	-	-	-	-	-	-	-
GMT	**168.9**	**168.9**	**194.0**	**207.9**	**256.0**	**362.0**	**222,9**	**548.7**	**181.0**	**1024.0**

* VICH- Vaccinated inoculated chickens, UCCH- Unvaccinated uninoculated chickens, VCCH - Vaccinated uninoculated chickenss, UICH- Unvaccinated inoculated chickens , - = HI test not done.

Table 5.12: Haemagglutination inhibition antibody titre for unvaccinated and vaccinated turkeys inoculated with KUDU 113 virus

	Days post inoculation													
	0			5			10			15			21	
	Uninoculated and inoculated groups													
	VCT	VIT	UIT	VCT	VIT	UIT	VCT	VIT	UIT	VCT	VIT	UIT	VCT	VIT
1	16	16	128	32	64	128	64	256	512	32	256	1024	64	256
2	32	32	128	64	64	256	64	256	512	64	256	2048	64	512
3	32	32	128	128	256	256	128	512	1024	64	512	-	64	1024
4	64	64	128	128	256	512	256	512	1024	256	1024	-	128	1024
5	64	64	128	256	512	1024	256	512	1024	256	1024	-	256	2048
6	128	128	128	256	512	-	512	512	-	512	1024	-	256	2048
7	128	128	128	-	-	-	-	-	-	-	-	-	-	-
8	256	256	128	-	-	-	-	-	-	-	-	-	-	-
9	256	256	-	-	-	-	-	-	-	-	-	-	-	-
10	256	256	-	-	-	-	-	-	-	-	-	-	-	-
GMT	**84.4**	**84.4**	**128.0**	**111.4**	**207.9**	**337.8**	**157.6**	**388.0**	**776.0**	**128.0**	**630.3**	**1448.2**	**111.4**	**891.4**

* VIT- Vaccinated inoculated turkeys, VCT ï Vaccinated uninoculated turkeys, UCT- Unvaccinated uninoculated turkeys, UIT- Unvaccinated inoculated turkeys, - = HI test not done.

Table 5.13 The Mean Newcastle Disease Haemagglutination Inhibition titre ± standard error of unvaccinated and vaccinated chickens and turkeys inoculated with KUDU 113 virus.

	Days PI				
Group	**0**	**5**	**10**	**15**	**21**
UCCH	0.00 ±0.000[a]	0.00 ±0.000[a]	0.00 ±0.000[a]	0.00 ±0.000[a]	0.00 ±0.000[a]
UICH	0.00± 0.000[a]	0.00 ± 0.000[a]	-	-	-
VCCH	179.2 ± 20.9[b]	282.7 ±30.5[b]	296.7± 71.4[b]	288.0± 77.1[b]	256.0 ±`85.9[b]
VICH	179.2 ± 20.9[b]	213.3 ± 26.9[b]	512-0 ± 171.7[c]	768.9 ±114.5[c]	864.0 ±111.5[c]
UCT	0.00± 0.00[a]	0.00± 0.00[a]	0.00± 0.00[a]	0.00± 0.00[a]	0.00± 0.00[a]
UIT	0.00± 0.00[a]	128.0 ± 0.00[b]	435.0 ±159.8[b]	819.2 ± 125.4[b]	1040.0 ±16.0[b]
VCT	132.2 ± 31.2[b]	144.0 ± 38.5[b]	213.3 ±69.5[a]	197.0 ± 75.0[a]	138.7 ± 38.5[a]
VIT	132.2 ±31.2[b]	277.0 ± 82.07[c]	426.7± 53.9[b]	682.7 ±157.3[b]	821.3±142.3[b]

* VICH- Vaccinated inoculated chickens, UCCH- Unvaccinated uninoculated chickens, VCCH - Vaccinated uninoculated chickens, UICH- Unvaccinated inoculated chickens VIT- Vaccinated inoculated turkey, UIT- Unvaccinated inoculated turkey, UCT- Unvaccinated uninoculated turkey, VCT- Vaccinated uninoculated turkey , - = HI test not done.

5.6.6 Virus isolation from some organs in chickens and turkeys inoculated with KUDU 113 virus.

The harvested fresh allantoic fluid showed HA activity with washed chicken erythrocytes,thus the KUDU 113 virus was recovered from the spleen, brain and intestine in both unvaccinated and vaccinated inoculated chickens and turkeys from days 3-21 PI (Tables 5.14 and 5.15).

5.6.7 Shedding of virus in faeces of chickens and turkeys infected with KUDU 113 virus.

The harvested fresh allantioic fluid showed no HA activity with washed chicken erythrocytes, thus the KUDU 113 was not recoverd from the faeces of inoculated experimental chcickens and turkey from days 0-21 PI, (Table 5.16).

Table 5.14 Haemaglutination spot-test for unvaccinated, vaccinated chickens inoculated with KUDU 113 virus

Days PI	Group	Spleen	Brain	Intestine
3	**UCCH**	-ve	-ve	-ve
	UICH	+ve	+ve	+ve
	VCCH	-ve	-ve	-ve
	VICH	+ve	+ve	+ve
6	**UCCH**	-ve	-ve	-ve
	UICH	+ve	+ve	+ve
	VCCH	-ve	-ve	-ve
	VICH	+ve	+ve	+ve
10	**VCCH**	-ve	-ve	-ve
	VICH	+ve	+ve	+ve
15	**VCCH**	-ve	-ve	-ve
	VICH	+ve	+ve	+ve
21	**VCCH**	-ve	-ve	-ve
	VICH	+ve	+ve	+ve

* +VE = Positive -VE= Negative

* VICH- Vaccinated inoculated chickens, UCCH- Unvaccinated uninoculated chickens, VCCH - Vaccinated uninoculated chickens, UICH- Unvaccinated inoculated chickens.

Table 5.15 Haemaglutination spot-test for unvaccinated, vaccinated turkeys inoculated with KUDU113 virus

Days PI	Group	Spleen	Brain	Intestine
3	**UCT**	-ve	-ve	-ve
	UIT	+ve	+ve	+ve
	VCT	-ve	-ve	-ve
	VIT	+ve	+ve	+ve
6	**UCT**	-ve	-ve	-ve
	UIT	+ve	+ve	+ve
	VCT	-ve	-ve	-ve
	VIT	+ve	+ve	+ve
10	**UCT**	-ve	-ve	-ve
	UIT	+ve	+ve	+ve
	VCT	-ve	-ve	-ve
	VIT	+ve	+ve	+ve
15	**UCT**	-ve	-ve	-ve
	UIT	+ve	+ve	+ve
	VCT	-ve	-ve	-ve
	VIT	+ve	+ve	+ve
21	**UCT**	-ve	-ve	-ve
	UIT	+ve	+ve	+ve
	VCT	-ve	-ve	-ve
	VIT	+ve	+ve	+ve

* +VE = Positive -VE= Negative

* VIT- Vaccinated inoculated turkeys, UIT- Unvaccinated inoculated turkeys, UCT- Unvaccinated uninoculated turkeys, VCT- Vaccinated uninoculated turkeys.

5.6.8 Haematology

Newcastle Disease infected had no significant effect ($p > 0.05$) on the PCV of inoculated vaccinated and unvaccinated chickens (Table 5.17). But among turkeys significant reduction in PCV was observed only on day 15 PI in inoculated unvaccinated turkey ($p< 0.05$).

There was no significant change in haemaglobin concentration of inoculated chickens ($p> 0.05$) (Table 5.18). Among the turkeys there was no significant difference ($p > 0.05$) between the values of the inoculated and their controls.

Newcastle Disease infection had no significant effect on the RBC count of the chickens ($p > 0.05$) (Table 5.19). Among the turkeys significant reduction ($p < 0.05$) in the RBC count was recorded in the inoculated unvaccinated turkeys only on day 3 PI.

The WBC count was significantly increased in unvaccinated inoculated chickens on day 3 PI only ($p < 0.05$) (Table 5.20). In the turkeys there were significant increases in inoculated unvaccinated turkeys on days 3, 10 and 21 PI ($p <0.05$). Both vaccinated chicken and turkeys showed no significant changes in WBC count in NDV infection.

There was no significant difference ($p>0.05$) in the absolute lymphocyte counts of both the infected chickens and turkeys, when compared with their controls. (Table 5.21)

Absolute heterophil count was significantly higher ($p <0.05$) in the chickens only on 3PI (Table 5.22). There was no significant change in vaccinated chickens. But among the inoculated unvaccinated turkeys NDV produced significant increase in the heterophil count from days 3-21PI {$p<0.05$). The value was significantly higher ($p<0-05$) in the vaccinated inoculated turkeys on day 15 PI only.

No significant change was observed in the monocyte count of inoculated unvaccinated and vaccinated chickens and turkeys when compared with their controls ($p > 0.05$) (Table 5.23).

Newcastle Disease infection also produced no significant change ($p>0.05$) in the eosinophilic count of infected unvaccinated and vaccinated chickens (Table 5.34). But among inoculated turkeys it was only in the unvaccinated turkeys that a significant increase occurred on day 21PI,($p < 0.05$).

The disease also had no significant effect on the basophitl count of inoculated chickens and turkeys (Table 5.25) ($p > 0.05$).

Table 5.16 The mean packed cell volume (%) of vaccinated and unvaccinated chickens and turkeys inoculated with KUDU113 virus

	Chickens				Turkeys			
	Means ± standard error							
	Group							
Days PI	**UCCH**	**UICH**	**VCCH**	**VICH**	**UCT**	**UIT**	**VCT**	**VIT**
0	28.38 ± 0.94	27.38 ± 0.24	27.68 ± 0.72	28.50 ±0.54	30.50 ± 0.61	30.30 ±0.85	31.10 ± 0.51	31.70 ±0.85
3	28.38 ± 0.94	27.38 ± 0.24	27.68 ± 0.72	28.50 ±0.54	30.00 ± 0.20^{ab}	28.63 ± 1.48^{a}	32.00 ± 0.98^{b}	31.50 ± 0.71^{ab}
6	26.50 ± 1.22	-	25.88 ± 1.03	27.38 ±1.43	30.00 ± 1.10	32.25 ±1.13	31.38 ± 1.53	30.75 ± 0.60
10	23.75 ± 0.32	-	24.87 ± 0.63	24.88 ±0.90	30.38 ± 1.09^{b}	28.25 ± 0.43^{b}	32.75 ± 0.60^{a}	32.13 ± 0.43^{ab}
15	25.75 ± 1.11	-	26.00 ± 1.02	29.25 ±1.16	29.50 ± 0.79^{c}	25.25 ± 0.43^{b}	34.88 ± 0.52^{a}	32.38 ± 1.21^{a}
21	28.50 ± 1.04	-	25.00 ± 1.34	27.25 ±1.11	30.75 ± 0.60^{b}	32.75 ± 0.75^{ab}	36.38 ± 1.78^{a}	35.00 ± 0.68^{a}

* Values with different superscripts in a row indicate significant difference ($p<0.05$).

* VICH- Vaccinated inoculated chickens, UCCH- Unvaccinated uninoculated chickens, VCCH - Vaccinated uninoculated chickens, UICH- Unvaccinated inoculated chickens, VIT- Vaccinated inoculated turkeys, UIT- Unvaccinated inoculated turkeys, UCT- Unvaccinated uninoculated turkeys, VCT- Vaccinated uninoculated turkeys, - = not done .

Table 5.17 The mean haemoglobin concentration (g/dl) of vaccinated, unvaccinated cockerels and turkeys inoculated with KUDU113 virus.

	Chickens				Turkeys			
	Means ± standard error							
	Group							
Days PI	**UCCH**	**UICH**	**VCCH**	**VICH**	**UCT**	**UIT**	**VCT**	**VIT**
0	8.89 ± 0.16	8.90 ± 0.19	8.92 ± 0.19	8.79 ±0.23	12.05 ± 0.19	12.21 ± 0.21	12.21 ± 0.10	12.26 ±0.10
3	9.55 ± 0.37	8.75 ± 0.29	8.67 ± 0.25	8.93 ±0.39	9.60 ± 0.40^{b}	9.09 ± 0.49^{b}	11.98 ± 0.52^{a}	11.96 ± 0.96^{a}
6	9.32 ± 0.47^{b}	-	8.15 ± 0.25^{a}	7.82 ± 0.34^{a}	9.84 ± 0.36	10.34 ± 0.28	10.76 ± 0.10	10.73 ± 0.44
10	8.92 ± 0.17	-	9.18 ± 0.32	8.73 ±0.22	11.12 ± 0.34^{b}	10.00 ± 0.62^{ab}	10.34 ± 0.22^{ab}	9.38 ± 0.43^{a}
15	9.25 ± 0.22	-	8.24 ± 0.49	9.09 ±0.19	8.92 ± 0.17^{b}	8.02 ± 0.15^{b}	10.99 ± 0.49^{a}	10.99 ± 0.65^{a}
21	8.60 ± 0.46	-	8.99 ± 0.24	9.25 ±0.43	9.44 ± 0.22^{c}	9.70 ± 0.08^{ac}	11.64 ± 0.69^{b}	11.12 ± 0.45^{ab}

*Values with different superscripts in the rows indicate significant difference ($p<0.05$).

* VICH- Vaccinated inoculated chickens, UCCH- Unvaccinated uninoculated chickens, VCCH - Vaccinated uninoculated chickens, UICH- Unvaccinated inoculated chickens, VIT- Vaccinated inoculated turkeys, UIT- Unvaccinated inoculated turkeys, UCT- Unvaccinated uninoculated turkeys, VCT- Vaccinated uninoculated turkeys, - = not done .

Table 5.18 The mean Red blood cell counts (10^6/µL) of vaccinated, unvaccinated chickens and turkeys inoculated with KUDU113 virus

	Chickens				Turkeys			
	Means ± Standard Error							
	Group							
Days PI	**UCCH**	**UICH**	**VCCH**	**VICH**	**UCT**	**UIT**	**VCT**	**VIT**
0	2.49 ± 0.02	2.53 ± 0.03	2.54 ± 0.03	2.54 ± 0.03	2.38 ± 0.06	2.39 ± 0.04	2.39 ± 0.05	2.43 ± 0.05
3	2.38 ± 0.04	2.32 ± 0.06	2.37 ± 0.07	2.38 ± 0.05	2.37 ± 0.10^{b}	2.25 ± 0.08^{a}	2.51 ± 0.04^{b}	2.45 ± 0.04^{ab}
6	2.79 ± 0.18	-	2.38 ± 0.12	2.38 ± 0.07	2.75 ± 0.08^{b}	2.57 ± 0.06^{ab}	2.35 ± 0.15^{a}	2.31 ± 0.05^{a}
10	2.40 ± 0.08	-	2.39 ± 0.04	2.44 ± 0.03	2.61 ± 0.11	2.38 ± 0.03	2.52 ± 0.12	2.34 ± 0.08
15	2.55 ± 0.10^{b}	-	2.24 ± 0.12^{a}	2.47 ± 0.04^{ab}	2.53 ± 0.07	2.24 ± 0.05	2.56 ± 0.14	2.23 ± 0.39
21	2.65 ± 0.04^{b}	-	2.45 ± 0.03^{a}	2.43 ± 0.05^{a}	2.43 ± 0.09	2.62 ± 0.09	2.46 ± 0.13	2.49 ± 0.09

* Values with different superscripts in a row indicate significant difference between the groups ($p<0.05$).

* VICH- Vaccinated inoculated chickens, UCCH- Unvaccinated uninoculated chickens, VCCH - Vaccinated uninoculated chickens, UICH- Unvaccinated inoculated chickens, VIT- Vaccinated inoculated turkeys, UIT- Unvaccinated inoculated turkeys, UCT- Unvaccinated uninoculated turkeys, VCT- Vaccinated uninoculated turkeys, - = not done .

Table 5.19 The mean White Blood cell counts (10^3/µL) of vaccinated, unvaccinated chickens and turkeys inoculated with KUDU113 virus

	Chickens				Turkeys			
	Means ± standard error							
Days PI	Group							
	UCCH	UICH	VCCH	VICH	UCT	UIT	VCT	VIT
0	13.09 ± 0.20	13.19 ± 0.16	13.17 ± 0.43	13.52±0.52	14.25 ± 0.17	14.53 ± 0.20	14.37 ± 0.44	14.24 ± 0.29
3	13.05 ± 0.25^{a}	20.50 ± 1.32^{b}	13.73 ± 0.56^{a}	12.98±1.02^{a}	13.30 ± 0.43^{b}	18.95 ± 2.18^{a}	14.26 ± 0.95^{b}	16.58 ± 0.59^{ab}
6	12.73 ± 0.92	-	15.88 ± 2.03	14.61 ±0.94	13.53 ± 1.61	14.20 ± 0.71	15.76 ± 1.75	17.11 ± 0.84
10	13.73 ± 1.19	-	14.09 ± 1.48	13.84 ±1.31	12.06 ± 0.54^{a}	26.40 ± 5.05^{b}	14.88 ± 0.67^{a}	14.73 ± 1.37^{a}
15	15.22 ± 1.30	-	12.33 ± 0.79	14.61 ±1.36	17.07 ±1.78^{ab}	20.18 ± 0.91^{b}	14.18 ± 1.36^{a}	16.79 ± 1.69^{ab}
21	16.19 ± 1.75	-	13.54 ± 0.37	13.35 ±0.48	14.38 ± 0.65^{a}	24.73 ± 3.07^{b}	14.33 ± 0.45^{a}	14.24 ± 1.08^{a}

*Values with different superscripts in a row indicate significant difference between the groups ($p<0.05$).

* VICH- Vaccinated inoculated chickens, UCCH- Unvaccinated uninoculated chickens, VCCH - Vaccinated uninoculated chickens, UICH- Unvaccinated inoculated chickens, VIT- Vaccinated inoculated turkeys, UIT- Unvaccinated inoculated turkeys, UCT- Unvaccinated uninoculated turkeys, VCT- Vaccinated uninoculated turkeys, - = not done .

Table 5.20 The mean absolute lymphocyte counts {10^3/µL) of vaccinated, unvaccinated chickens and turkeys inoculated with KUDU113 virus

	Chickens				Turkeys			
	Means ± standard error							
Days PI	**UCCH**	**UICH**	**VCCH**	**VICH**	**UCT**	**UIT**	**VCT**	**VIT**
0	9.96 ± 0.43	9.91 ± 0.23	9.81 ± 0.32	10.72 ± 0.30	9.96 ± 0.37	9.98 ± 0.94	10.41 ± 0.75	10.12 ± 0.53
3	10.47 ±0.29	10.17 ±1.85	10.20 ± 0.56	9.70 ± 1.42	8.41 ± 0.51^{c}	4.30 ± 0.19^{b}	10.61 ± 0.70^{a}	10.92 ± 0.86^{a}
6	9.56 ± 0.82	-	11.38 ± 1.74	11.42 ± 0.48	9.39 ± 1.28^{a}	4.27 ± 0.81^{b}	11.32 ± 0.74^{a}	10.53 ± 1.31^{a}
10	9.04 ± 0.95	-	9.55 ± 0.98	10.25 ± 0.88	7.95 ± 0.56^{b}	6.70 ± 0.78^{b}	10.17 ± 0.59^{a}	10.35 ± 0.94^{a}
15	12.18 ± 1.09^{b}	-	12.18 ± 1.09^{b}	10.89 ± 0.91^{ab}	13.21 ± 1.19^{c}	7.59 ± 1.74^{ab}	10.42 ± 1.38^{bc}	6.45 ± 0.70^{a}
21	12.42 ± 1.36^{b}	-	9.83 ± 0.49^{ab}	9.47 ± 0.24^{a}	10.54 ± 0.39	10.35 ± 0.08	9.73 ± 0.89	10.10 ± 0.90

* Values with different superscripts in a row indicate significant difference between the groups ($p<0.05$).

* VICH- Vaccinated inoculated chickens, UCCH- Unvaccinated uninoculated chickens, VCCH - Vaccinated uninoculated chickens, UICH- Unvaccinated inoculated chickens, VIT- Vaccinated inoculated turkeys, UIT- Unvaccinated inoculated turkeys, UCT- Unvaccinated uninoculated turkeys, VCT- Vaccinated uninoculated turkeys, - = not done .

Table 5.21 The mean absolute heterophil counts (10^3/µL) of vaccinated, unvaccinated chickens and turkeys inoculated with KUDU113 virus

	Chickens				Turkeys			
	Means ± standard error							
	Group							
Days PI	**UCCH**	**UICH**	**VCCH**	**VICH**	**UCT**	**UIT**	**VCT**	**VIT**
0	3.92 ± 0.52	3.26 ± 0.26	2.92 ± 0.26	2.78 ± 0.36	4.08 ± 0.22	4.25 ± 0.35	3.80 ± 0.34	4.00 ± 0.66
3	2.41 ± 0.24^{a}	10.18 ± 0.75^{b}	3.25 ± 0.27^{a}	3.08 ± 0.72^{a}	4.76 ± 0.51^{a}	10.92 ± 2.58^{b}	3.50 ± 0.51^{a}	6.02 ± 0.67^{a}
6	3.08 ± 0.34	-	4.26 ± 0.80	2.82 ± 0.35	4.06 ± 0.47^{b}	9.81 ± 1.12^{a}	4.16 ± 0.92^{b}	6.18 ± 1.72^{ab}
10	2.72 ± 0.31	-	4.14 ± 0.54	3.38 ± 0.47	4.06 ± 0.37^{a}	18.48 ± 3.52^{b}	4.57 ± 0.20^{a}	4.26 ± 0.65^{a}
15	2.78 ± 0.31	-	2.93 ± 0.55	3.54 ± 0.55	3.69 ± 0.65^{b}	12.50 ± 2.70^{a}	3.62 ± 0.22^{b}	10.24 ± 1.07^{a}
21	3.77 ± 0.71	-	3.35 ± 0.17	3.68 ± 0.27	3.74 ± 0.45^{a}	13.07 ± 3.73^{b}	4.50 ± 0.74^{a}	3.89 ± 0.53^{a}

* Values with different superscripts in a row indicate significant difference between the groups ($p<0.05$).

* VICH- Vaccinated inoculated chickens, UCCH- Unvaccinated uninoculated chickens, VCCH - Vaccinated uninoculated chickens, UICH- Unvaccinated inoculated chickens, VIT- Vaccinated inoculated turkeys, UIT- Unvaccinated inoculated turkeys, UCT- Unvaccinated uninoculated turkeys, VCT- Vaccinated uninoculated turkeys, - = not done .

Table 5.22 The mean absolute monocyte count (10^3/µL) of vaccinated, unvaccinated chickens and turkeys inoculated with KUDU113 virus

	Chickens				Turkeys			
	Means ± standard error							
	Group							
Days PI	**UCCH**	**UICH**	**VCCH**	**VICH**	**UCT**	**UIT**	**VCT**	**VIT**
0	0.09 ± 0.06	0.08 ± 0.05	0.16 ± 0.05	0.08 ± 0.05	0.11 ± 0.07	0.04 ± 0.04	0.18 ± 0.07	0.11 ± 0.07
3	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.14 ± 0.06^{b}	0.06 ± 0.04^{ab}	0.07 ± 0.07	0.00 ± 0.00	0.07 ± 0.04	0.00 ± 0.00
6	0.07 ± 0.04	-	0.08 ± 0.08	0.04 ± 0.04	0.04 ± 0.04	0.00 ± 0.00	0.14 ± 0.08	0.04 ± 0.04
10	0.03 ± 0.03	-	0.11 ± 0.06	0.06 ± 0.03	0.03 ± 0.03	0.11 ± 0.11	0.10 ± 0.06	0.03 ± 0.03
15	0.10 ± 0.05	-	0.06 ± 0.03	0.06 ± 0.04	0.11 ± 0.05	0.00 ± 0.00	0.07 ± 0.04	0.05 ± 0.05
21	0.00 ± 0.00^{b}	-	0.10 ± 0.03^{a}	0.07 ± 0.04^{ab}	0.07 ± 0.04^{ab}	0.00 ± 0.00^{b}	0.07 ± 0.04^{ab}	0.18 ± 0.04^{a}

* Values with different superscripts in a row indicate significant difference between the groups ($p<0.05$).

* VICH- Vaccinated inoculated chickens, UCCH- Unvaccinated uninoculated chickens, VCCH - Vaccinated uninoculated chickens, UICH- Unvaccinated inoculated chickens, VIT- Vaccinated inoculated turkeys, UIT- Unvaccinated inoculated turkeys, UCT- Unvaccinated uninoculated turkeys, VCT- Vaccinated uninoculated turkeys, - = not done .

Table 5. 23 The mean eosinophil count (10^3/µL) of vaccinated, unvaccinated chickens and turkeys inoculated with KUDU113 virus

	Chickens				Turkeys			
	Means ± standard error							
	Group							
Days PI	UCCH	UICH	VCCH	VICH	UCT	UIT	VCT	VIT
0	0.12 ± 0.06	0.11 ± 0.05	0.05 ± 0.03	0.03 ± 0.03	0.07 ± 0.04	0.04 ± 0.04	0.07 ± 0.04	0.15 ± 0.06
3	0.13 ± 0.05	0.16 ± 0.05	0.11 ± 0.07	0.12 ± 0.07	0.07 ± 0.04	0.16 ± 0.11	0.03 ± 0.03	0.08 ± 0.05
6	0.03 ± 0.03	-	0.12 ± 0.09	0.11 ± 0.04	0.04 ± 0.04	0.18 ± 0.08	0.20 ± 0.06	0.22 ± 0.04
10	0.09 ± 0.03	-	0.00 ± 0.00	0.07 ± 0.04	0.03 ± 0.03	0.00 ± 0.00	0.04 ± 0.04	0.10 ± 0.06
15	0.10 ± 0.05	-	0.06 ± 0.03	0.10 ± 0.05	0.06 ± 0-.06	0.00 ± 0.00	0.07 ± 0.04	0.03 ± 0.03
21	0.00 ± 0.00^{b}	-	0.07 ± 0.04^{ab}	0.14 ± 0.05^{a}	0.00 ± 0.00^{b}	0.15 ± 0.09^{a}	0.07 ± 0.04^{ab}	0.08 ± 0.04^{ab}

* Values with different superscripts in a row indicate significant difference between the groups ($p<0.05$).

* VICH- Vaccinated inoculated chickens, UCCH- Unvaccinated uninoculated chickens, VCCH - Vaccinated uninoculated chickens, UICH- Unvaccinated inoculated chickens, VIT- Vaccinated inoculated turkeys, UIT- Unvaccinated inoculated turkeys, UCT- Unvaccinated uninoculated turkeys, VCT- Vaccinated uninoculated turkeys, - = not done .

Table 5.24 The mean basophil count of vaccinated, unvaccinated chickens and turkeys inoculated with KUDU113 virus

	Chickens				Turkeys			
	Means ± standard error							
	Group							
Days PI	**UCCH**	**UICH**	**VCCH**	**VICH**	**UCT**	**UIT**	**VCT**	**VIT**
	0.00 ± 0.00	0.00 ± 0.00	0.04 ± 0.04	0.03 ± 0.03	0.00 ± 0.00	0.06 ± 0.06	0.04 ± 0.04	0.00 ± 0.00
6	0.00 ± 0.00	-	0.05 ± 0.05	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
10	0.00 ± 0.00	-	0.03 ± 0.03	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
15	0.00 ± 0.00	-	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
21	0.00 ± 0.00	-	0.00 ± 0.00	0.00 ± 0.00	0.04 ± 0.04	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00

* Values with different superscripts in a row indicate significant difference between the groups ($p<0.05$).

* VICH- Vaccinated inoculated chickens, UCCH- Unvaccinated uninoculated chickens, VCCH - Vaccinated uninoculated chickens, UICH- Unvaccinated inoculated chickens, VIT- Vaccinated inoculated turkeys, UIT- Unvaccinated inoculated turkeys, UCT- Unvaccinated uninoculated turkeys, VCT- Vaccinated uninoculated turkeys, - = not done .

5.7 DISCUSSION

The incubation period (IP) for Newcastle disease from this study was 2 days PI with 100% and 60% mortality in unvaccinated chickens and turkeys respectively while the incubation period was 3 and 4 days PI with 13.3% and 0% mortality for vaccinated chickens and turkeys respectively. The longer IP in the vaccinated chickens and turkeys may be due to ND vaccination which was protective against the clinical disease in chickens. This was also noted by Ezema *et al.* (2009). The lower mortality recorded in the turkeys than chickensô shows the resistant nature of turkeys to Newcastle disease. This validates the claims of Gray (1954), Alexander *et al.* (1999) and Wakamatsu *et al.* (2006); that turkeys were more resistant to virulent ND virus than chickens.

A number of researchers have recorded similar incubation periods for both unvaccinated chicken and turkey. These include Okoye *et al.* (2000), Wan *et al. (*2004), Wakamatsu *et al.* (2006) and Ezema *et al*. 2009). Oladele *et al.* (2005) studied different strain of VNDV and reported an incubation period of 2-3 days in unvaccinated chickens of 4 and 6 weeks old with mortality of 100%, 92%, 100%, 96.9% and 52% respectively. Other researchers also reported IP different from the one recorded in this study, Hamid *et al.* (1991) recorded an incubation period of 2 to 16 days, 3 to 5 days in 7 and 20 weeks old unvaccinated chickens respectively. Incubation period of 4- 6 days has also been reported in unvaccinated chicken by Allan *et al*. (1978), Binta *et al.* (1996), Mishra *et al. (*2000). Piacenti *et al.* (2006) also reported an incubation period of 8-9 and 10 days PI in unvaccinated 4 weeks old commercial turkeys infected with a velogenic viscerotrophic and velogenic neurotrophic strains of ND virus respectively. Faud *et al.* (1975) reported a 4-5 days IP and 82% mortality in 4 week old unvaccinated poults, In vaccinated birds, IP of 3 and 4 days with 13.3% and 0% mortality in chickens and turkeys respectively was recorded. Ezema *et al.* (2009)

reported an IP of 5 days and 0% mortality in vaccinated 6 weeks old chickens challenged with Kudu 113 virus. Hamid *et al.* (1991) also reported an incubation period of 2-16 days with no mortalities in 7 weeks old vaccinated chickens. In vaccinated 1 to 10 months old turkeys Boney *et al*. (1975) reported an absence of clinical signs and mortality The differences in incubation period and mortality recorded could be related to virulence and pathogenicity of the virus strain (Alexander, 1997a), dose of the inoculum used in the various studies (Piacenti *et al*., 2006) host differences such as genetic resistance in response to NDV as previously observed by Alexander, (2000) and Maw *et al*. (2003). The mortalitiy recorded in the vaccinated chickens maybe due to potency of the vaccine and strain of the virus used in this study. Hamid *et al. (*1991) also reported that the birds with antibody titres lower than the protective titre showed signs of depression and anorexia even though mortalities were not observed. However the disease has been reported to occur in both vaccinated and unvaccinated flocks by different researchers (Halle *et al.,*1999; Saõidu *et al.,* 2006).

The clincical signs and 100% mortality by day 6 PI, shown by the chickens are typical signs of infection by velogenic strain of the ND virus. These clinical signs have also been reported in chickens infected with VVNDV by other researchers (Binta *et al.,* 1996; Brown *et al.,* 1999a; Okoye *et al.,* 2000; Oladele *et al.,* 2005; Ezema *et al.,* 2009). The infection in turkeys resulted in a disease syndrome characterized by central nervous system disturbance showing that the virus appears neutrophic in turkeys. Similar observations were made by Piacenti *et al. (*2006) in experimental infection with VNDV in 4 weeks old SPF and 6 weeks old commercial turkey. The disease was marked by unilateral paresis of the leg and wing, head twitch and body tremors. Perhaps neuronal cells in turkeys are more susceptible to viral replication. In contrast, Wakamatsu *et al*. (2006) in an experimental infection with a VNDV pathotype in both SPF and commercial

turkey noted depression, nasal discharge, conjunctivitis, watery and bloody dropping, in SPF turkeys. Faud *et al* (1975) observed an initial respiratory syndrome such as gasping, mouth breathing in turkeys experimentally infected with a VNDV. This was not noted in this study rather a nervous derrangement like paresis, paralysis of extremities was observed. The differences in clinical signs manifested by turkeys when infected with the VNDV could be as a result of the route of infection or tropism of the virus. Beard and Hanson (1984) found that intra muscular or intravenous routes of NDV infection appeared to enhanced neurological signs while natural routes of the infection appeared to emphasize the respiratory nature of the disease (Beard and Easterday 1967). Other signs such as greenish diaorrhea, reduced feed intake, huddling together were also observed in turkeys experimentally infected with a VVNDV (Gillete *et al*., 1974).

The disease had a marked effect on weight. Reduction in weight was more severe in the unvaccinated than the vaccinated experimental birds. This showed that the vaccine was protective against the disease in vaccinated experimental birds. Such reduction in weight was also observed by Okoye et *al.* (2000), Ezema *et al*. (2009) in both vaccinated and unvaccinated chickens infected with VVNDV virus. Okoye *et al.* (2000) reported that the changes in body weight were common occurrence in septicemia or viraemic diseases due to reduction in feed and water consumption.

In the present study both infected vaccinated and unvaccinated chickens and turkeys had similar gross lesions, Haemorrhages in the proventriculus, caecal tonsils and sharply dermacated intestinal ulcers were seen only in the vaccinated and unvaccinated inoculated chickens. Similar lesions were reported by Brown *et al.* (1999b), Okoye *et al.* (2000), Rao *et al.* (2002), Kommers *et al*. (2002, 2003 a,b) and Ezema *et al.* (2009) in both vaccinated and unvaccinated chickens. Wakamatsu *et al.* (2006) and Piacenti *et al.* (2006) reported similar lesions in turkeys.

The most severe and consistent gross lesion seen in unvaccinated chickens and not in turkeys included sharply demarcated haemorrgic intestinal ulcers, haemorrages in the caecal tonsil and proventriculus on days 4 and 5 PI. These were also reported by Beard and Hanson, (1984), Mishra *et al.* (2000). These haemmorraghic lesions in the gastrointestinal tract of the infected chickens have also been used by some researchers to distinguish VVNDV from VNNDV (Hanson and Spalatian, 1973; Wan *et al.,* 2004). The extensive ulceration of overlying intestinal epithelium maybe due to active viral replication in lymphoid organs. In comparison mucosal haemorrhages of the proventricular, caecal tonsils and intestinal ulcers were common lesions in vaccinated and unvaccinated chickens, but were not observed in both vaccinated and unvaccinated turkeys. Researchers like Wakamatsu *et al*. (2006), Piacenti *et al.*(2006) also did not report this lesions in turkeys. Hanson and Spalatian (1973) reported that lesions of ND were rarely produced in the digestive tract of any specie of birds other than chicken. Useh *et al*. (2005) reported that the high erythrocyte surface salic acid concentration in the indigenous Nigerian poultry species could be responsible for their resistance to infectious diseases whose aetiologic agents produce neuraminidases. The pathotype of the NDV involved in an outbreak appears to be a major factor that determines the form of the disease that is manifested by birds (Alexander,1991). The study shows that an NDV strain that shows typical signs and lesions of VVNDV in chickens can appear neutrophic in turkeys. The frequency and severity of the lesion in each organ were probably related to tissue tropism and host species. The extensive congestion of the brain blood vessels in turkeys correlated with the severity of the nervous signs contrary to observations of Mcferran and Mcracken, (1988) who reported that gross lesions are not observed in the CNS of birds infected With NDV regardless of the pathotype. Igwe (2008) reported severe gross congestion of the brain of guinea fowl.

Comparing the sizes of the thymus, bursa and spleen in the photographs taken on day 5 PI, Histopathologic results also show massive loss of lymphocytes in the three lymphoid organs. This means that atrophy was well established in the three organs in infected birds when compared with their controls in both unvaccinated and vaccinated chickens and turkeys. This clearly shows that the ND virus causes depletion of the cells of the lymphoid organs leading to the reduction in size of the organs. This agrees with the findings of Okoye *et al*. (2000) and Ezema *et al.* (2009) who reported atrophy of the lymphoid organs in chickens. Wakamatsu *et al.* (2006) also reported atrophy of the lymphoid organs in turkeys experimentally inoculated with the NDV. By day 6 PI all the unvaccinated inoculated chickens had died showing no significant changes in the weights of the thymus. Inoculated vaccinated chickens also showed no significant changes in thymic weights ($p>0.05$). But the gross photographs of the thymus in control and infected chickens showed clear reduction in sizes of the thymus of both unvaccinated and vaccinated inoculated chickens on days 5 and 6 PI. The only change in the weight of the spleen was significant reduction in vaccinated inoculated chickens on day 5 PI only. The gross photographs showed clear atrophy of the spleen in vaccinated and unvaccinated inoculated chickens on days 10 and 5 PI respectively and in the vaccinated and unvaccinated inoculated turkeys on days 20 and 10 PI respectively. These observations show that gross visual inspection is a better method of assessing atrophy in avian tissues. After all atrophy is reduction in size and not weight. The weights of the bursa were lower ($p<0.05$) in the unvaccinated infected chickens on days 3 and 5 PI and days 3, 10, 15 and 21 PI in the unvaccinated inoculated turkeys. These results are not in agreement with the report of Ruwaan *et al.* (2012) who recorded increases in the thymic and bursal weights and no significant difference in the relative weights of the spleen of vaccinated cocks inoculated with VNDV. The differences could be related to the dose of the virus, the potency of vaccine used in both studies as wells as time

in the study when the organ weight or size was taken. The thymus and bursae are known to produce antibody in chickens thus, the decrease in weights and sizes of the lymphoid organs recorded in this study is an indication that the VNDV used in this study may also cause immunosuppression (Reynold and Arapa, 2000a).

Maternal antibody can be detected in serum of birds of up to 3 weeks of age. The choice of 6 weeks of age for infection was made in this experiment to ensure that maternal antibody did not interfere with the susceptibility or the severity of the disease in the birds. The unvaccinated chickens and uninfected turkeys had no antibody detected till the end of the experiment showing that they were not exposed to the virus. Following inoculation by intramuscular route, the virus elicited a good serological response. The same response was recorded by Okoye et *al. (*2000), Saĝdu *et al.* (2006), Mishra *et al.* (2009), Ruwaan *et al,(*2009) in chickens, Boney *et al, (*1975) and Piacenti *et al*. (2006) in turkeys. Higher titres were observed in chickens than turkeys because Newcastle disease disease was more severe in chickens than turkeys. But the antibody response was more rapid in turkeys. Igwe *et* al. (2013) also recorded higher immune response in chickens than guinea fowls. Spanoghe *et al.* (1977) suggested that the higher immune response is indicative of a higher antigenic stimulation as a result of infection and virus multiplication. Antibody must have developed in germinal centres containing memory cells specifically sensitized by an antigen as suggested by Payne, (1971). Both infected unvaccinated and vaccinated turkeys as well as the vaccinated chickens displayed the highest HI antibody response on days 15 and 21 PI. Igwe *et al.* (2013) also recorded highest antibody responses in chickens on days 15 and 21PI. In contrast Oladele *et al.*(2005) detected the highest HI titres by day 4PI in chickens experimentally infected with KUDU 113 strains. These variations maybe due to differences in the immune status of the chickens.

Virus isolation was used to determine tissue distribution and persistence of NDV infections in chickens in this study. Identification of the infected organ and estimation of the duration of virus excretion are of value in the diagnosis of viral diseases and in studies on viral pathogenesis (Lancaster, 1981; Westbury *et al*.1984). Viral replication was characterized by the presence of virus in the same sites of damage among the affected tissues. The virus was distributed in various organs by day 3 to 21PI in both unvaccinated and vaccinated chickens and turkeys. Similar multisystemic viral distribution has been described with the VVNDV isolates and reference strains (Brown *et al*., 1999; Mishra *et al.,* 2000). The present study detected virus from brain, intestine and spleen of both unvaccinated and vaccinated infected chickens and turkeys. This contradicts the observations of Igwe (2008) who did not isolate the virus from the intestine of infected chicken and guinea fowl. Also, the study recovered the virus from the intestine, brain and spleen of both the unvaccinated and vaccinated chickens and turkeys uptill day 21 PI. This disagrees with the report of Igwe *et al*. (2013) who could not recover the virus by day 21 PI in experimental VNDV infection in guinea fowl. These disparities could be related to the NDV having more affinity to some organs of the body than others.

The virus was not isolated from the cloacal of the infected birds. This finding contradicts the reports of some other researchers. Alexander and Parson (1986) reported that vaccinated birds excreted virus on day 3PI, occasionally birds excreted virus on day 5PI and not after day 7PI. Okwor (2014) reported that in chickens both vaccinated and inoculated with the VNDV, the virus was shed for 2 to 7 days PI / vaccination. Wakamatsu *et al. (*2006) observed shedding of virus in faeces day 14PI in commercial turkeys, day 5 PI in SPF turkeys infected with velogenic NDV. Gillette *et al.* (1974) also reported that unvaccinated turkeys infected with a VVNDV continued to shed ND virus from intestinal tract for up to day 46 PI while the vaccinated turkeys shed virus for up to 53 days PI. The

inability of this present study to recover the virus from the faeces could be due to poor storage facilities before transportation to the laboratory for isolation studies.

There was no significant change in the PCV for the unvaccinated and vaccinated chickens. This couldbe due to the acute nature of the infection in the unvaccinated chickens and the ability of the vaccine to prevent destruction of the RBC in the vaccinated groups. Similar observation was reported by Useh *et al*. (2005), Igwe *et al.* (2013) in 6 weeks unvaccinated chickens and guinea fowls experimentally infected with VVNDV. Ruwaan *et al*. (2009) also reported no significant decrease in the mean PCV throughout the 6 weeks period of observation in vaccinated chickens. Significant decrease in PCV and RBC counts on days 3 and 15 PI in unvaccinated turkeys was reported by Ayodele *et al*. (2013) in unvaccinated chickens and turkeys. Oladele *et al*. (2005) also noted a decrease in the PCV in unvaccinated chickens on day 2 PI. This persisted till day 11 PI. The decrease in PCV signifies anemia which occurs in birds infected with VNDV as a result of destruction of the RBC by the virus (Caldron *et al*., 2005; Ruwaan *et al*., 2009) causing excess bile production and greenish diarrhoea.

A significantly higher WBC count, heterophilia and lymphopenia were recorded in unvaccinated chickens and turkeys. This finding is consistent with the reports of Igwe *et al*. (2013), in 6 weeks old chickens and guinea fowls and Galando-munir *et al.* (2001) in their study with VNDV in which they reported heterophilia and lymphopenia 72 hrs PI. The high WBC count is due to high heterophil count in birds with NDV infection. This demonstrates the marked reactivity of the white cell of poultry species to NDV infection (Coles, 1986). Leucocytosis is usually due to heterophilia and usually relates to the magnitudes or severity of the inflammatory process (Campbell,1994).

Heterophilia is frequently observed in conjuction with tissue damage induced by inflammation or viral infection including NDV (Hawkey *et al.,* 1984; Latimer *et al.,* 1999). As the bone marrow responds to tissue demand for heterophils, leucocytosis and heterophilia intensify Corticosteriod induced heterophilia is observed in diseased birds and as a result of corticosterone release from adrenal cortex. Heterophilia and concurrent lymphopenia are observed in diseased birds. (Hawkey *et al,* 1983)

Lymphopenia observed in this study has been reported by other researchers such as Caldron *et al.* (2005), Igwe *et al*. (2013). Lymphopenia of acute infections has a complex origin and mechanism which include endogenous corticosterone release with temporary lymphocytes redistribution and temporary trapping of recirculating lymphocytes within lymphoid tissues to promote antigen contact and direct destruction of lymphoid tissue especially during viral infection. A sequential study in chickens after VNDV infections was made by Lam (1996) who detected virus induced apotosis principally in mononuclear cells macrophages and lymphocytes of the peripheral blood. Lymphopenia is common in acute inflammatory response because inflammatory mediators stimulate heterophil and lymphocyte migration from the blood and lymphoid tissues to the inflammation. (Jain,1993).

The lack of significant difference observed in Monocytes, eosinophils and basophils values in both infected species in this study is consistent with previous reports that these leucocytes are not involved in viral immunology and appear in low numbers in circulation except in conditions of allergy and parasitism respectively (Coles,1986; Galindo-Munir *et al*., 2001)

5.8 CONCLUSION

The study demonstrates the highly pathogenic nature of the local Nigerian strain of VNDV, KUDU 113 and the variations in pathogenicity between chicken and turkey. Turkeys were less severely affected than chickens. It is conclusive that the strain of NDV obtained from duck after intramuscular route of exposure is velogenic and viscerotropic for unvaccinated chickens with mild respiratory signs and high mortality while it is neurotrophic for turkeys with moderate mortality.

In the vaccinated chickens, infection with VNDV showed moderate clinical signs and mortality. In the vaccinated turkeys showed no clinical signs and no mortality, eventhough post mortem lesions of the disease were prominent in both species. Turkeys do not show haemorragic lesions of VVNDV in the gastro intestinal tract as seen in chickens.

Haematologic findings in both vaccinated and unvaccinated chickens and turkeys showed that leukocytosis, heterophilia and lymphopenia were common in cases of infection with the VNDV.

Rural turkey farmers currently do not vaccinae their turkeys against any disease including ND. This work shows that turkeys are quite susceptible to ND therefore farmers who keep turkeys are taking great risk by not vaccinating their birds.

This work has also shown that nit is more accurate to use visual inspection to measure atrophy than to use weights.

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Appendix

QUESTIONAIRE ON THE COMMON DISEASE PROBLEMS ENCOUNTERED IN TURKEY PRODUCTION IN COMMERCIAL FARMS AND BACKYARD POULTRY FARMS IN ENUGU STATE.

A, INTRODUCTION

1. Name of farmé é é é é é é é é é é é é é é é é é é é
2. Name of the farmeré é é é é é é é é é é é é .
3. Address/Location of Farmé é é é é é é é é é é é é
4. Age: below 20 ( ) 20-35 ( ) 36-50 ( ) above 50 ( )
5. Occupation:----------
6. Level of Education: None ( )
   Primary Level ( )
   Secondary Level ( )
   First Degree ( )
   Postgraduate Degree ( )
7. For how long have you been in poultry production?
   < 5 years ( )
   5-10 years ( )
   10-20 years ( )
   Above 20 years ( )

B.FLOCK SIZE AND GENERAL POULTRY MANAGEMENT

1. What species and number of birds do you keep in your farm?

SPECIES	NUMBER
Broilers	
Pullets	
Turkeys	
Guinea Fowl	
Ducks	
Quails	
Others (please specify)é é é é é é é é é é é é é é é é é é	

2. Do you keep birds of varying age and sizes together?
   Yes ( )
   No ( )
3. Do you raise birds in batches (All in/All out) ?
   Yes ( )
   No ( )
4. How do you house your birds?
   Deep Litter ( )
   Battery Cages ( )
   Both 1&2 ( )
   Free Range
   Others (Specify)
5. What is your source of feed to the birds?
   Commercial feed ( )
   Self Made Feed ( )
   Kitchen wastes ( )
   Occasional feeding with grains etc ( )

C. TURKEY PRODUCTION

1. For how long have you been rearing turkey?
   <5 years( )
   5-10 Years ( )
   10-20 Years ( )
   above 20years ( )
2. How many turkeys do you have in your farm?
   <50 ( )
   50-100 ( )
   Above 100 ( )
3. At what age do you start keeping turkey?
   Day old ( )
   Growers (weeks) ( )
   Adult (month) ( )
4. How do you source your Turkey; day old, grower or adult?
   Commercial hatcheries ( )
   Locally hatched eggs (neighbours) ( )

Open market ( )

5. How do you keep your Turkey?
   Intensive ( )
   Semi Intensive ( )
   Free Range ( )
6. Do you keep turkey with other species of birds in your farm?
   Yes ( )
   No ( )
7. If Question 6 is No , Do you keep all ages of turkey in the same pen ?
   Yes ( )
   No ( )
8. What is your source of feed for your turkey?
   Commercial feed ( )
   Self made feed ( )
   Kitchen waste ( )

9. How often do you feed your turkey?
   Adlibitum ( )
   Once daily ( )
   Twice Daily ( )
   Occasionally, 1-3 times a week ( )
   Roam for their food ( )
10. Do you consider Turkey farming profitable?
   Yes ( )
   No ( )

10a If yes in question 10 above, what in your opinion makes turkey farming profitable?é é

10b. If no in question 10 above ,what in your opinion makes turkey farming unprofitable?..

11. What problems generally have you encountered in turkey rearing? *you can tick more than one response.*
   Disease ( )
   High Cost of Poult ( )
   High Cost of Feed ( )
   High poult mortality ( )
   Lack of reasonable degree of management skill ( )
   Lack of capital ( )
   Lack of market ( )
   Others specify é ..

D.TURKEY HEALTH

1.Do you have any qualified health personnel taking care of you turkey?
Yes ( )
No ( )

2.If YES, which of these take care of the turkey/birds in your farm?.
Animal health Attendant ( )
Livestock attendant ( )
Government Veterinary doctor ( )
Private Veterinary Doctor ( )

3.What disease problems have you encountered in your turkeys?
Newcastle disease ( )
Fowl typhoid ( )
Fowl Cholera ( )
Turkey pox ( )
Pullorum disease ( )
Leg deformities due to nutritional deficiencies,stunting etc ( )

4.Did the outbreak spread to other birds in your farm ?
Yes ( )
No ( )

5.How many chickens and turkeys did you lose during the outbreak ?

Specie	No of deaths
Chickens	
Turkeys	
Others specifyé é é é é é é é é é é é é é é .	

6.Do you have a vaccination program for turkey?
Yes ( )
No ( )

7.Before your turkeys attained the adult stage , which of these vaccines do you normally administer in your farm and at what age?

Vaccine	Age
I.O	
LaSota	
Fowl Pox	
Others specifyé é é é é é é é é é é é é é é é	

8.When last did you vaccinate your turkey? Cant remember ( ) less than 3months( )3-6 Months, Above 6months( )

9. How do you source your vaccine?
Government Vet Teaching Hospital ( )
Private Vet Clinic ( )
Private marketer ( )
NVRI,Vom ( )
Others please specify ( )

10. How do you administer the vaccine?

Call a vet ( )
Call an Animal health attendant ( )
Self administer ( )
Give farm attendant to administer ( )

11. How is your vaccine stored before administration
    In cooler without ice
    In cooler with ice
    In refrigerator
    In clay pot
    Other specify----------

BIOSECURITY MEASURES

1. Do you have other Poultry farms around you?
   Yes( )
   No ( )
2. Do you often pay visits to other Farms?
   Yes ( )
   No ( )
3. Do your workers /yourself cooperate well with your neighbors that you borrow or lend farm items from each other?
   Yes ( )
   No ( )
4. How far is your farm from a major road?
   Very close-less than 500m
   Close- Ikm
   Far- above 1km
   Very far-more than 5km
5. Do your workers who attend to both chickens and turkey at the same time?
   Yes ( )
   No ( )

Thanks a lot and God bless

Antibody titre against NDV in turkeys raised in backyard farms in Enugu east senatorial zone of Enugu state

Enugu east L.G.A

Farms	Sample size	No of positive samples	No of negative samples
1	5	4	1
2	6	2	4
3	2	1	1
4	3	2	1
5	2	0	2
6	6	2	2
7	13	8	5
8	3	0	2
9	11	0	11
10	11	5	6
11	3	0	3
12	3	1	2
13	5	0	5
14	3	2	1
15	2	0	2
16	15	0	15
17	4	0	4
18	5	3	2
19	6	0	6
20	2	0	2
Total	118	30	88

Enugu north L.G.A

Farm	Sample size	No of positive samples	No of negative samples
1	15	1	14
2	7	5	2
3	9	4	5
4	8	3	5
Total	39	13	26

Enugu south L.G.A

Farm	Sample size	No of positive samples	No of negative samples
1	6	4	2
2	6	1	5
3	6	2	4
4	4	3	1
5	5	2	3
6	2	0	2
7	7	6	1
	36	18	18

Antibody titre against NDV in turkeys raised in backyard farms in Enugu north senatorial zone of Enugu state

Nsukka L.G.A

Farm	Sample size	No of positive samples	No of negative samples
1	5	5	0
2	10	7	3
3	10	6	4
4	6	0	6
5	2	0	2
6	3	0	3
7	2	0	2
8	2	0	2
9	2	0	2
10	7	0	7
11	1	0	1
12	7	0	7
13	2	0	2
14	7	0	7
15	6	0	6
16	7	0	7
17	3	0	3
18	3	0	3
19	2	0	2
20	4	0	4
21	2	0	2
22	6	3	3

23	**6**	**4**	**2**
24	**4**	**3**	**1**
25	**10**	**3**	**7**
26	**8**	**5**	**3**
27	**6**	**2**	**4**
28	**2**	**2**	**0**
Total	**136**	**40**	**96**

Igbo etiti L.G.A

Farm	**Sample size**	**No of positive samples**	**No of negative samples**
1	**7**	**4**	**3**
2	**6**	**3**	**3**
3	**6**	**3**	**3**
4	**2**	**0**	**2**
5	**9**	**4**	**5**
6	**2**	**2**	**0**
7	**1**	**0**	**1**
8	**2**	**2**	**0**
9	**3**	**3**	**0**
	38	**21**	**17**

Antibody titre against NDV in turkeys raised in backyard farms in Enugu west senatorial zone of Enugu state

Udi L.G.A

Farm	Sample size	No of positive samples	No of negative samples
1	10	0	10
2	20	7	13
3	10	5	5
4	7	7	0
5	3	0	3
	50	19	31

Ezeagu L.G.A

Farm	Sample size	No of positive samples	No of negative samples
1	10	0	10
2	10	0	10
3	5	5	0
4	7	3	4
5	10	3	7
6	7	0	7
7	8	0	8
	56	11	45

Agwu L.G.A

Farm	Sample size	No of positive samples	No of negative samples
1	6	1	5
2	5	3	2
3	5	2	3
4	8	3	5
5	5	2	3
6	5	0	5
7	20	10	10
8	6	2	4
9	8	0	8
Total	68	23	45

NDV HI titre of turkeys raised in backyard farms in Enugu east senatorial zone of Enugu state

Enugu east L.G.A

Farms	Sample size	HI titre
1	5	10,10,10,10,0
2	6	10,9,0,0,0,0
3	2	7,0
4	3	11,0,0
5	2	0,0
6	6	8,2,0,0,0,0,0,0,0,0,
7	13	11,11,11,9,11,11,11,11,0,0,0,0,0
8	3	0,0,0
9	11	0,0,0,0,0,0,0,0,0,0,0
10	11	5,7,6,2,5,0,0,0,0,0,0
11	3	0,0,0
12	3	7,0,0,
13	5	0,0,0,0,0
14	3	6,6,0
15	2	0,0
16	15	0,0,0,0,0,0,0,0,0,0,0,0,0,0,0
17	4	0,0,0,0
18	5	8,3,7
19	6	0,0,0,0,0,0
20	2	0,0

Enugu north L.G.A

Farms	Sample size	HI titre
1	15	8,0,0,0,0,0,0,0,0,0,0,0,0,0,0
2	7	10,10,11,11,11,0,0
3	9	5,10,4,4,0,0,0,0,0,
4	8	5,9,9,0,0,0,0,0

Enugu south L.G.A

Farms	Sample size	HI titre
1	6	6,6,6,2,0,0
2	6	6,0,0,0,0,0
3	6	6,4,0,0,0,0,
4	4	3,7,6,0
5	5	6,5,0,0,0
6	2	0,0
7	7	6,8,6,6,7,6,0

NDV HI titre of turkeys raised in backyard farms in Enugu north senatorial zone of Enugu state

Nsukka L.G.A

Farms	Sample size	HI titre
1	5	6,6,8,7,5
2	10	5,8,7,5,6,6,7,0,0,0
3	10	5,4,7,8,6,5,0,0,0,0
4	6	0,0,0,0,0,0
5	2	0,0
6	3	0,0,0
7	2	0,0
8	2	0,0
9	2	0,0
10	7	0,0,0,0,0,0,0
11	1	0
12	7	0,0,0,0,0,0,0
13	2	0,0
14	7	0,0,0,0,0,0,0
15	6	0,0,0,0,0,0
16	7	0,0,0,0,0,0,0,0
17	3	0,0,0
18	3	0,0,0
19	2	0,0
20	4	0,0,0,0
21	2	0,0
22	6	11,9,7,0,0,0
23	6	7,5,3,2,0,0
24	4	6,6,2,0
25	10	2,3,2,0,0,0,0,0,0,0
26	8	3,3,2,2,2,4,0,0
27	6	4,4,0,0,0,0

28	**2**	**6,2**

Igbo etiti L.G.A

Farms	Sample size	HI titre
1	**7**	**7,5,3,8,0,0,0**
2	**6**	**6,5,4,0,0,0**
3	**6**	**4,6,3,0,0,0**
4	**2**	**0,0**
5	**9**	**4,7,0,0,0,0,0,0,0**
6	**2**	**7,5**
7	**1**	**0**
8	**2**	**5,3**
9	**3**	**6,4,7**

NDV HI titre of turkeys raised in backyard farms in Enugu west senatorial zone of Enugu state

Udi L.G.A

Farms	Sample size	HI titre
1	10	0,0,0,0,0,0,0,0,0,0
2	20	6,6,6,6,7,7,4,0,0,0,0,0,0,0,0,0,0,0,0,0
3	10	2,4,3,6,6,0,0,0,0,0
4	7	3,6,7,5,2,6,8
5	3	0,0,0

Ezeagu L.G.A

Farms	Sample size	HI titre
1	10	0,0,0,0,0,0,0,0,0,0
2	10	0,0,0,0,0,0,0,0,0,0
3	5	4,3,7,6,4
4	7	0,0,3,2,0,6,0
5	10	0,0,0,7,5,6,0,0,0,0
6	7	0,0,0,0,0,0,0,0
7	8	0,0,0,0,0,0,0,0

Agwu L.G.A

Farms	Sample size	HI titre
1	6	6,0,0,0,0,0
2	5	6,3,5,0,0,0,0,0,0,0,0,0,0,0,0
3	5	6,4,0,0,0
4	8	3,7,6,0,0,0,0,0
5	5	6,3,0,0,0
6	5	0,0,0,0,0

7 8 9	20 6 8	3,3,6,6,8,7,9,6,4,5,0,0,0,0,0,0,0,0,0 6,6,0,0,0,0. 0,0,0,0,0,0,0,0

Farmers and researcher in an interview at Awgu, Enugu West senatorial zone.

Bleeding process in one of the households vsited.

Decanting turkey blood in a sterile tube .

Researcher in the post mortem room in the Department of Veterinary Pathology and Microbiology, University of Nigeria,Nsukka

Virus isolation experiment in the Virology Laboratory, NVRI ,Vom, Plateau State.