

**LESIONS AND PREVALENCE OF NEWCASTLE DISEASE IN CHICKEN  
PRESENTED FOR NECROPSY AT FACULTY OF VETERINARY MEDICINE,  
MAKERERE UNIVERSITY.**

**BY**

**AFAYOA MATHIAS  
2007/HD17/9011U**

**DISSERTATION SUBMITTED TO THE GRADUATE SCHOOL IN PARTIAL  
FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF MASTER OF  
SCIENCE IN VETERINARY PATHOLOGY OF MAKERERE UNIVERSITY.**

**MAY 2010**

**DECLARATION**

I Afayoa Mathias declare that this dissertation is my original work and has never been submitted to any university or institution of higher learning for award of any qualification.

Sign .....

Date.....

**Author**

**Afayoa Mathias**

Sign .....

Date.....

**My Supervisor,**

**Professor Ojok Lonzy**

Department of Veterinary Pathology

Faculty of Veterinary Medicine,

Makerere University, Kampala.

## **DEDICATION**

This work is dedicated to my dear parents, Mr. Okuti Jino and Mrs Olikuru Zena Okuti, my brothers and to my lovely children and their mother.

## **ACKNOWLEDGEMENTS**

I acknowledge the parental support and encouragement of Mr and Mrs J. Okuti, and the good will gesture extended to me by my brothers during the long period of academic struggle.

Special thanks to my supervisor Professor Ojok Lonzy for his patience and continuous encouragement and advice to me.

I am grateful to all technicians in the department of Veterinary Pathology for work well done and I sincerely appreciate the support and technical advice from Mr. Musisi Lubowa, the Chief Technologist, Department of Microbiology and Parasitology, Faculty of Veterinary Medicine.

I am grateful to RUFORUM for funding this study and to the Faculty administration for including me among the beneficiaries of the scholarship.

May the almighty God reward each one of you for whatever support you offered to me that enabled me fulfill this task.

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## LIST OF ABBREVIATIONS AND ACRONYMS

ND.....Newcastle disease

IHC.....Immunohistochemistry

DAKO-ARK.....Denmark Animal research kit

*E. coli*.....Escherichia coli

ml.....Micro litre

MAAIF.....Ministry of Agriculture animal industry and fisheries

°C.....Degree celcius

No.....Number

PM.....Post- mortem

Pp.....Page

USA.....United states of America

UK.....United kingdom

OIE.....International organization on epizootics

%.....Percentage

HI.....Haemagglutination inhibition

HN.....Haemagglutination- Neuraminidase

EU.....European Union

RBCs.....Red blood cells

MAB.....Monoclonal antibodies

PAB.....Polyclonal antibodies

FO.....Fusion proteins on paramyxovirus envelope

RT-PCR .....Reverse transcriptase polymerase chain reaction

ELISA.....Enzyme linked immunosorbent assay

RNA.....Ribonucleic acid

CNS.....Central nervous system

AR.....Antigen retrieval

RUFORUM.....Regional Universities Forum

## ABSTRACT

Poultry diseases are among the major constraints of chicken production in Uganda of which Newcastle disease (ND) is still one of the most important devastating diseases of chicken. This study was conducted from October 2008 to March 2010 to identify and describe the lesions due to ND, determine its prevalence and relate the presence of the viral antigen in tissues to the lesions in the various organs of chicken presented for disease diagnosis in Department of Pathology, Faculty of veterinary Medicine, Makerere University. Chickens for necropsy in the study period were received from clients coming from the Central, Eastern and Western regions of Uganda. Necropsy was done on a total of 216 chicken carcasses; samples for histopathology were obtained from various organs and fixed in 10% buffered formalin. The fixed tissue samples were then trimmed and processed for histopathology. Then immunohistochemistry (IHC) was performed on 86 samples of formalin fixed, paraffin embedded tissue sections to confirm ND in which the presence of viral antigens in the target tissues were specifically detected as brown precipitates mainly in mononuclear phagocytic cells. Diagnosis based on clinical and pathological findings showed that coccidiosis was the most common condition encountered (25.0%), followed by ND (23.61%), helminthiasis (12.50%), pasteurelosis (6.02), salmonellosis (3.70%), infectious bursal disease (3.24%) and other non specific conditions constituted 25.93%. Of the 86 samples tentatively diagnosed with NCD and tested for ND using IHC, 44 (51.16%) were found positive for the disease.

In conclusion, Newcastle disease is still among the most prevalent diseases of chicken in the study area. Clinical – pathologic findings provided some bases for ND diagnosis but are less reliable method, therefore, a more sensitive and specific diagnostic tests such as IHC, RT-PCR, HI, *in situ* hybridization and other definitive tests should be used in addition to histopathology to confirm ND, so as to provide accurate and reliable advice to poultry farmers.

## CHAPTER ONE: INTRODUCTION

### 1.1 Background

Uganda's economy is dependent on agricultural production for 29.9% of its GDP of which, the livestock sector alone contributes 17% ([www.ugandainvest.com.18/5/2007](http://www.ugandainvest.com.18/5/2007)). Livestock are important sources of animal protein to the people. They contribute to food security and to socioeconomic well being of many households in the country. There are an estimated 30 million poultry, 1.6 million pigs, 6.6 million goats, 1.1 million sheep and 6 million cattle in Uganda (Statistical abstract and MAAIF Report, 2003).

Despite the above figures, the supply of animal proteins to the population is far inadequate. The limited number of animals available for slaughter, coupled with high cost of meat, has denied many families access to animal proteins. The population of Uganda has significantly increased over the recent years. This calls for a need to increase food production in order to meet the increasing need of the people. With the available arable land remaining constant or even decreasing due to land degradation, and pressure on it from human settlement, the need to cultivate high yielding variety of crops or of rearing faster growing and high producing breeds of animals are called for. Species of animals with short cycles of life would be suited to meet these challenges.

Poultry is one of the short-cycle species of animals, which are more able to increase in numbers within a shorter time than some of the longer-cycle species of animals. Almost every farm family in the rural areas of Uganda keep a few birds. Therefore increasing and improving the production of poultry by encouraging and empowering every farm family to rear poultry would help towards providing food security and elevating their socio-economic status. This will help in the fight against poverty.

In many regions of Uganda, especially Eastern and Northern regions, poultry rearing is considered to be the beginning of livestock production,

(Illango, *et al*, 2003). Poultry production provides a relatively cheaper and quicker means of availing animal protein to the people.

There are several diseases which severely affect poultry production some of the most important diseases include Newcastle Disease (ND), infectious basal disease, fowl cholera, fowl typhoid and other less fatal but economically important diseases.

In Uganda, ND is still the most important devastating poultry disease both in birds reared under intensive systems and in local birds kept under traditional free range system (Ojok, 1993). If this disease is not controlled, poultry farmers and subsistent peasants will continue to experience low poultry productivity, increased economic loses and increased malnutrition.

Newcastle disease virus has been grouped into pathotypes based on clinical signs which are influenced by the strains of the virus.

The gross lesions and the organs affected in poultry are also dependent on the strains and pathotypes of the infecting virus (Alexander, 2003).

The severity of the disease varies depending on the strains of the virus, the species and breed of the birds, the age, and the immune status of the animal, environmental stress, viral dose as well as on the nutritional and management practices. In natural infections the disease may vary from per-acute to unapparent infection. One of the marked properties of ND virus is the variation in the ability of different isolates to cause disease and death in chicken. Several forms of virulence of the disease classified into pathotypes as velogenic, mesogenic and lentogenic have been recognised (Alexander, 2003). The velogenic is the most virulent and together with the highly pathogenic avian influenza (Bird flu) are list A, diseases of the Office of International Epizootes (OIE). The lentogenic pathotype is the least virulent with no clinical signs and pathological lesions. Detection of this form of the disease can only be by virus isolation from the gut or faeces and by the presence of specific antibodies. The mesogenic pathotypes is of moderate virulence and affects mainly the respiratory system.

The velogenic isolate are further divided into a velogenic viscerotropic pathotype which causes mainly diarrhoea and visceral haemorrhages with mortality of up to 100%.

The velogenic neurotrophic pathotype causes predominantly neurological signs.

Although there is no pathognomonic lesion of ND, the presence of haemorrhagic lesions

in the intestine of infected chickens has been used to distinguish velogenic viscerotropic ND from other forms of the disease (Hanson 1980).

## **1.2 Problem statement:**

Newcastle disease (ND) is a highly infectious and devastating disease of poultry that is distributed worldwide. World animal health report (2004) indicates that, the entire five east African countries reported out breaks of the disease along side other thirty African countries.

In Uganda in particular, ND is one of the most important devastating poultry disease that causes great losses to commercial and local poultry farmers (Illango, *et al*, 2003).

The disease can be confused with highly pathogenic avian influenza, fowl cholera, infectious bursitis and infectious laryngotracheitis as they present similar clinical signs (Ojok, 1993).

Post-mortem records in the Department of Veterinary pathology in the previous three years showed that 15- 20% of the cases handled, were tentatively diagnosed as Newcastle disease. However, there was no evidence of confirmatory diagnosis.

In Uganda currently, diagnosis of ND is based mainly on clinical history, postmortem examination and histopathology. All these are tentative diagnostic methods hence less accurate.

Therefore there was need to study and document the prevalence and describe the lesions caused by the disease in various organs of chicken presented for port mortem examination at the faculty and also confirm the diagnosed cases.

## **1.3 Justification**

Correct disease diagnosis, and knowledge about the frequency of occurrence of any disease in an area in a given period (prevalence), is a pre requisite for instituting effective and reliable disease control and prevention measures (Ojok, 1993).

Newcastle disease (ND) as being a major devastating, highly fatal poultry disease in the country, and more so, the velogenic Newcastle disease that belong to list A of poultry diseases of the office of international epizootes needs to be diagnosed accurately and the prevalence established so as to recommend correct prevention and control measures to poultry farmers and subsistent peasants. This would improve poultry productivity, reduce economic losses by poultry farmers and lower malnutrition rates especially among the population who live on marginal diets.

The gross lesions and organs affected in birds infected with ND virus are dependent on the strain and pathotypes of the infecting virus, in addition to the host and other factors that affect the severity of the disease (Saif, *et al*, 2005).The presence of hemorrhagic lesions in the mucosa of proventriculous, caeca, and small intestines of infected chickens has been used to distinguish Velogenic Viscerotropic ND virus from Neurotropic Velogenic ND viruses (Hanson, 1980).

Therefore this particular study was vital as to confirm ND diagnosis, described and documented the lesions in the various organs and established the prevalence of the disease in chicken, basing on the gross lesions, histopathology and Immunohistochemistry technique. The confirmatory diagnosis helps in recommending an appropriate prevention and control measures to the clients.

## **1.4 Objectives**

### **1.4.1 Primary objective**

To determine the prevalence of Newcastle disease and relate the presence of the viral antigen in tissues to the lesions in various organs of chicken presented for necropsy.

### **1.4.2 Specific objectives:**

1. To identify and describe gross and histopathological lesions due to Newcastle disease in parenchymatous organs and other tissues of chickens presented for necropsy.
2. To determine and relate the presence of the ND Viral antigens in tissues to the lesions in organs of the chickens presented and confirm the diagnosis.
3. To determine the prevalence of ND in chicken presented for necropsy at the Faculty of Veterinary medicine, Makerere University from October 2008 to March 2010.

### **1.4.3 Hypothesis**

More than 15% of chicken presented for PM examination at Faculty of Veterinary medicine have lesions in various parenchymatous organs suggestive of Newcastle disease.



## CHAPTER TWO: LITERATURE REVIEW

### 2.1 Distribution and Characteristics of Newcastle disease virus

Newcastle disease (ND) is a highly contagious and fatal disease of domestic poultry, caged birds and wild birds, caused by a haemagglutinating type- 1 avian paramyxovirus belonging to genus rubulavirus. It has a worldwide distribution, although it has been eradicated from some countries. However, the reported chronic occurrence of ND virus infection in psittacine and wild birds (Collins, *et al.*, 1994) raises the possibility of the disease being introduced at any time by wild birds to the local and commercial chickens, even in countries free of the disease. The disease can be confused with highly pathogenic avian influenza, fowl cholera, infectious bursitis and infectious laryngotracheitis which show similar clinical signs (Ojok, 1993).

Newcastle disease virus is enveloped virus that has a negative sense, single stranded genome which codes for RNA directed polymerase, hemagglutinin- neuraminidase protein, fusion protein, matrix protein and other viral proteins (Bruce, *et al*, 1998).

The virus has a wide host range, with 27 of the 50 orders of birds reported to have been infected by ND (Kaleta and Baldauf, 1988).

The primary molecular determinant for pathogenicity of ND is the fusion protein cleavage site amino acid sequence and the ability of specific cellular proteases to cleave the fusion proteins of different pathotypes (Glickman, *et al* 1988).

Methods of diagnosis recommended by OIE and EU council directive (1992), comprise of isolation of ND virus on specific pathogen free embryonated eggs and identification using hemagglutination inhibition test. Reverse transcription polymerase chain reaction (RT-PCR) and immunohistochemistry methods are used in many laboratories in the world for the detection and identification of the virus.

Regardless of the virulence of the strains, pooled samples of different organs are always positive in either test, due to the fact that ND virus strains of different virulence show diversity in tissue predilection in different time, post infection (Krzysztof, *et al*, 2005).

Newcastle disease virus has been grouped into pathotypes based on clinical signs which are influenced by the strains of the virus. The gross lesions and the organs affected in poultry are

also dependent on the strains and pathotypes of the infecting virus (Alexander, 2003). The severity of the disease varies depending on the strains of the virus, the species, breed, the age and the immune status of the birds, environmental stress, viral dose as well as on the nutritional and management practices. In natural infections the disease may vary from per-acute to in-apparent infection.

One of the marked properties of ND virus is the variation in the ability of different isolates to cause disease and death in chicken. Several forms of the disease have been classified into pathotypes as velogenic, mesogenic and lentogenic forms (Alexander, 2003).

The velogenic ND is the most virulent and together with the highly pathogenic avian influenza (Bird flu) are list A diseases of the Office of International Epizootics (OIE). The lentogenic ND is the least virulent with no clinical signs and pathological lesions. Detection of this form of the disease can only be by virus isolation from the gut or faeces and by the presence of specific antibodies. The mesogenic pathotypes is of moderate virulence and affects mainly the respiratory system. The velogenic isolate are further divided into a velogenic Viscerotropic pathotype which causes mainly diarrhoea and visceral haemorrhages with mortality of up to 100% and the velogenic neurotropic pathotype which causes predominantly neurological signs (Saif, *et al.*, 2005). Although there is no pathognomonic lesion of ND, the presence of haemorrhagic lesions in the intestine of infected chickens has been used to distinguished velogenic viscerotropic ND from velogenic neurotropic form (Alexander, 2003). Of importance is the fact that ND virus isolated from a given form of the disease induces that particular form of the disease in the same host species. This fact has great epidemiological, pathological and diagnostic significance and can be used to identify the different pathotypes occurring in the country.

## **2.2 Economic and Public Health Significance**

The global economic impact of velogenic ND is enormous and it certainly surpassed any other poultry disease (Saif, *et al.*, 2005).

In many developing countries, velogenic ND is endemic, and represents an important limiting factor in the development of commercial poultry production and the establishment of trade links. The constant loses from ND severely affect the quality and quantity of food for people on marginal diets (Saif, *et al.*, 2005)

Newcastle disease virus is a human pathogen and clinically presents as eye infection, seen as excessive lacrymation, reddened eyes, oedema of eyelids, conjunctivitis and sub-conjunctival haemorrhages (Chang, 1981).

Human infections with ND virus have usually resulted from direct contact with the virus, such as from splashing infective allantoic fluid into the eye in laboratory accidents, rubbing the eye with contaminated hands, handling infected birds or their carcasses.

## **2.3 Biological properties of paramyxoviruses**

### **2.3.1 Hemagglutination and Neuraminidase activity:**

The ability of ND virus and other paramyxoviruses to agglutinate red blood cells (RBCs) is due to the binding of the hemagglutinin- neuraminidase (H-N) protein to receptors on the surface of the red blood cells. This property and the specific inhibition of agglutination by antisera have proven to be powerful tools in the diagnosis of the disease (Burnet, 1942 and Saif *et al.*, 2005).

Chicken RBCs, usually are used in hemagglutination (HA) tests, but ND virus cause agglutination of all amphibian, reptilian and avian cells.

Winslow and others, (1950) showed that, human, mouse and guinea pig RBCs were agglutinated by all ND virus strains tested, but the ability to agglutinate cattle, goat, sheep, swine and horse cells varied with the strain of ND virus.

The enzyme neuraminidase is part of the hemagglutinin- neuraminidase molecule and is present in all members of the genus rubula virus. The enzyme cause gradual elution of the agglutinated RBCs (Saif, *et al.*, 2005)

### **2.3.2 Antigenicity**

Viral neutralisation or agar gel diffusion techniques have shown minor antigenic variations between different strains and isolates of ND viruses (Gomez, *et al.*, 1974)

Saif and colleagues (2005), pointed out that for all practical purposes, isolates of ND virus can be considered to represent a single antigenically homogeneous group.

Monoclonal antibody (MAB) technology provides a new approach to antigenic differentiation of ND virus strains and isolates.

### **2.3.3 Pathogenicity**

The virulence of ND virus strains varies greatly with the host. Chickens are highly susceptible, but ducks and geese may be infected asymptotically (Saif, *et al*, 2005).

In chickens, the pathogenicity of ND is determined chiefly by the strain of the virus, although the doses, route of administration, age of the chicken and environmental conditions have an effect. In general, the younger the chicken, the more acute the disease however, the breed or genetic stock does not have a significant effect on the susceptibility of the chickens to the disease. Natural routes of infection like nasal, oral and ocular appear to emphasize the respiratory nature of the disease while intramuscular, intravenous and intra cerebral routes appear to enhance the neurological signs (Beard and Easter day, 1967).

Molecular basis of the pathogenicity of ND virus is dependent on the post translation cleavage of fusion protein, FO to F1 and F2, for the progeny virus particles to be infective (Rott and Klenk, 1988). The mechanism controlling the pathogenicity of ND is similar to that described for influenza viruses (Webster and Rott, 1987).

The presence of additional basic amino acids in virulent strain fusion protein means that cleavage can be effected by proteases present in a wide range of cell types in different host tissues and organs (Nagai, *et al* 1976). For lentogenic viruses, cleavage can occur only with proteases recognising a single arginine (Saif, *et al.*, 2005). Lentogenic viruses, therefore replicate only in cells where there are trypsin like enzymes, such as the respiratory and intestinal epithelia, whereas virulent viruses can replicate in cells located in a wide range of tissues and organs, resulting in a fatal systemic infection (Rot, 1979).

## **2.4 Clinical signs**

Beard and Hanson (1984), summarized ND into pathotypes, based on clinical signs in chicken as:

Viscerotropic velogenic ND, also known as Doyle's form in which, clinical signs often begin with listlessness, increased respiration and weakness, prostration and death. Oedema around the eyes and head may occur. Greenish diarrhoea, muscular tremors, torticollis, paralysis of legs and wings and opisthotonus may occur and mortality may reach 90- 100% in fully susceptible flock (Cynthia, *et al.*, 2005).

The neurotropic velogenic form (Beach's form) of ND presents with sudden onset of severe respiration distress, followed by neurologic signs. Egg production falls dramatically but diarrhoea is usually absent. Morbidity may reach 100% and mortality 50 to 90% (Saif, *et al.*, 2005).

Mesogenic strains of ND virus causes respiratory disease with marked drop in egg production and the mortality rate is usually low, while the lentogenic virus strain does not cause disease in adult chickens. In young birds, respiratory disease may occur and death may result from secondary bacterial infection.

## **2.5 Gross lesions**

Diseases cause structural changes in tissues and organs that manifest as lesions. The lesions depend on the extent of tissue damage by the etiologic agent. The study of the lesions helps to explain rationally, the causes of symptoms seen in the diseased animal and to predict the changes that might supervene if treatment is not given (Ganti, 1983).

The gross lesions and organs affected in birds infected with ND virus are dependant on the strain and pathotypes of the infecting virus, in addition to the host and other factors that affect the severity of the disease.

The presence of hemorrhagic lesions in the intestine of infected chickens has been used to distinguish velogenic Viscerotropic ND virus from neurotropic velogenic ND viruses (Hanson, 1980). These hemorrhagic lesions are prominent in the mucosa of proventriculus, caeca, and

small intestines. They appear to result from necrosis of intestinal wall or lymphoid tissue such as caecal tonsils and Peyer's patches.

Generally, gross lesions are not observed in the central nervous system (CNS) of birds infected with ND virus.

Lesions in the respiratory tract may consist of mucosal haemorrhages and marked congestion of trachea (Mc Ferran and Mc Cracken, 1988). Air sacculitis and thickening of air sacs with catarrhal or caseous exudates is often observed in association with secondary bacterial infections (Saif, *et al.*, 2005).

Chicken and turkeys infected in lay with velogenic viruses have egg yolk peritonitis. The ovarian follicles are often flaccid and degenerative. Haemorrhages and discoloration of the reproductive organs occur.

## **2.6 Histopathology lesions**

Several descriptive reports of the literature on the histopathological changes following ND infections are related to virulent pathotypes (Beard and Hanson 1984).

Lesions of CNS include: a non purulent encephalomyelitis with neuronal degeneration, foci of glial cells, perivascular infiltration of lymphocytes and hypertrophy of the endothelial cells. These lesions usually occur in the cerebellum, medulla, mid brain, brain stem and spinal cord but rarely in the cerebrum.

Vascular disturbance leads to congestion, oedema, and haemorrhages in many organs. Hydropic degeneration of tunica media, hyalinization of capillaries and arterioles, thrombosis in small vessels and necrosis of endothelial cells occur.

Regenerative changes found in lymphopoietic system consist of the disappearance of the lymphoid tissue, Hyperplasia of the mononuclear phagocytic cells in various organs, especially the liver and areas of necrosis in the spleen (Saif, *et al.*, 2005).

Focal vacuolation and destruction of lymphocytes may be seen in the cortical area and germinal centres of the spleen and thymus. Marked degeneration of lymphocytes in the medulla of cloacal bursa often observed.

In the intestinal tract, haemorrhages and necrosis of mucosal lymphoid tissue are seen with infections of virulent strains of ND virus.

Respiratory tract lesions include: loss of cilia of the epithelia, congestion, and oedema of the mucosa with dense mononuclear cells infiltration (Saif, *et al.*, 2005).

## **2.7 Diagnosis of Newcastle disease:**

At present, definitive diagnosis of ND is by isolation of the ND virus (Saif, *et al.*, 2005). Other reliable methods of diagnosis include; direct detection of viral antigens by immunohistologic techniques, which offer a rapid method for the specific demonstration of the presence of the virus or viral antigen in organs or tissues.

Immunohistochemical staining on formalin-fixed, paraffin embedded sections has been revolutionized in 1991 by the discovery of heat-mediated retrieval (Antigen Retrieval, AR) of immunoreactivity (Shin *et al.*, 1991).

This method is now widely used and applies to the detection of the overwhelming majority of antigens, with few exceptions for which enzymatic retrieval is required ([www.immunohistochemistry.html](http://www.immunohistochemistry.html)). Nobuko and colleagues in (2007) compared reverse transcription- polymerase chain reaction (RT-PCR) from formalin fixed paraffin embedded tissues to the immunohistochemistry and insitu hybridization assays for detection of ND virus and found that PCR is more effective diagnostic test than others.

Serological tests for ND include, single radial immunodiffusion test, agar gel precipitation and enzyme- linked immunosorbent assays (ELISA) which are semi automated techniques and have become popular as part of flock screening procedures (Synder, *et al.*, 1984).

Good correlation has been reported between ELISA and HI test (Cvelic-Cabrilo *et al.*, 1992). The hemagglutination (HA) and HI tests are not greatly affected by minor changes in the methodology, although Brugh, *et al.*, (1978) stressed the critical nature of the antigen and antiserum incubation period in test standardization.

## CHAPTER THREE: MATERIALS AND METHODS

### 3.1 Study design

This was a descriptive study that involved examination of live birds and carcasses submitted for post-mortem (PM) examination, followed by a thorough systematic necropsy done in the faculty PM unit. Live birds and carcasses of chicken were received from clients for diagnostic purposes in the study period, (October 2008 to March 2010). The clients originated from the Districts of Kampala, Wakiso, Mukono, Jinja, Kyenjojo and Mpigi. Tissue samples were collected and fixed in 10% buffered formalin for histopathology and immunohistochemistry.

### 3.2 Sample size determination

Sample size was determined using a formula described by Wayne and colleagues in (1987) as below.

$$N = \frac{4PQ}{L^2}$$

Where, N is the sample size

P, the estimated prevalence,

L is the allowable error and  $Q = 1 - P$

Assuming that the prevalence of ND in chicken presented for necropsy at the faculty was 15% and the allowable error was 5%. The number of samples collected was calculated as:

$$\begin{aligned} N &= \frac{4 \times 0.1 \times 0.9}{(0.05)^2} \\ &= 204 \end{aligned}$$

Basing on the above formula, samples from 216 carcasses of chickens were obtained for the study.

### 3.3. Data analysis.

Descriptive statistics was used to analyze the data



### 3.4 Methods

Clinical history of each chicken presented for necropsy was obtained from the clients. The information recorded included, each client's and clinician's details such as the name and address of the farmer, location of the farm, the name and address of the clinician. Bio-data of the chickens were also recorded, namely, the breed, age, sex, mortality and morbidity rates including the date when the death or drop in production started.

A thorough systematic post mortem examination was then done on all chickens presented for necropsy during the study period; gross lesions observed were recorded and described basing on the location, severity and distribution of the lesions. Samples for histopathology were obtained from the following organs, liver, spleen, caecal tonsils, lungs, segments of intestines, kidneys, and the brain. The tissue samples were fixed in 10% buffered formalin.

Formalin fixed samples were trimmed and processed in automated tissue processor following the protocol of Histotechnology as described by Edna *et al*, (1994). In brief; the tissue samples were dehydrated by passing them through graded isopropyl alcohol (70%, to 80% to 90% to 96% and 100% absolute alcohol). Alcohol in tissue samples was cleared by xyelene, followed by paraffin infiltration. Samples in solidified paraffin wax were cut into paraffin blocks; each sample was sectioned to 5µm thick, and adhered onto clean glass slide. They were stained with Haematoxylin and Eosin (H & E) and examined with light microscope; lesions seen were recorded and described.

Immunohistochemistry (IHC) on formalin fixed, paraffin embedded sections was the diagnostic method used to confirm Newcastle disease and for studying the viral antigen distribution in the target tissues. Direct labelled Avidin- Biotin complex immunohistochemistry technique on formalin fixed paraffin embedded chicken tissue sections were performed on 86 samples out of the 216 total samples collected in this study. Samples for IHC were taken from all those chickens that had clinical signs and lesions suggestive of ND (51 samples) and those chickens from flocks with history of high mortalities and or rapid drop in production without implicating lesions of any disease (35 samples).

Formalin fixed paraffin embedded blocked samples were sectioned to 4µm thickness and adhered onto clean labelled slides. The samples were then placed in an oven at 54-56°C for 24-

48 hours so as to enhance firm adhesion of the tissue sections onto the slides. This was followed by quenching of the endogenous peroxidase activity by incubating the specimen with peroxidase block (3% hydrogen peroxide) for 5 minutes. The primary antibody was biotinylated by mixing calculated quantities of diluent (PBS), concentrated primary antibody and Biotinylation reagent in a test tube and the solution was incubated for 15 minutes at room temperature. The biotin labelled primary antibody was then applied to the specimen on the slides and incubated for 30 minutes. The specimens were then incubated with Streptavidin- peroxidase followed by reaction with 3, 3 Diaminobenzidine as substrate chromogen. The samples were counter stained with haematoxylin and dipped into ammonium water (2% ammonium solution) for the blue back ground staining and finally mounted.

Chicken tissue from a known ND positive case was used as positive control. Two sections were cut from Paraffin block containing a known ND positive sample, and each section was adhered onto a clean glass slide. On to one positive control section, biotinylated primary antibody was added and on to the other section, buffer was added instead of the primary antibody and the same IHC staining procedure described previously were followed on both slides. The later section served as a negative control while the former was used as positive control. The same procedure of substituting buffer for biotinylated primary antibody was used to run negative control for each test section and all other stages of staining were maintained following the protocol of DAKO ARK kit ([www.histochem.org/archives/ark.pdf](http://www.histochem.org/archives/ark.pdf)). The test sections, positive and negative control sections from each set of slides were stained co-currently as quality assurance measure to evaluate non specific staining and allow better interpretation of specific staining at antigen – antibody sites. The stained slides were air dried and then examined under light microscope.

### **3.5 Challenges faced during this study**

Delayed fund release and inadequate funding for this study which was very frustrating and limited the scope of the research.

Some tissues got washed off between the different stages of IHC staining procedure. This challenge was overcome by ensuring that each sample was sectioned in triplicate, one for control, another for the actual test and the third section was reserved.

Many of the foreign companies were not willing to supply reagents in small quantities and those that responded delayed to deliver them and this led to the prolonged study period.

Potency of the available Newcastle disease vaccines in the market should be assessed as most poultry farmers who presented their birds for necropsy claimed that the birds were vaccinated against Newcastle disease in the previous four to five weeks but were diagnosed with the disease.

Possibility of manufacturing Newcastle disease vaccine using the antigens from local strains of Newcastle disease virus should be studied. The vaccines derived from the local strains may present better option in disease prevention than those from foreign strains of the virus.

## CHAPTER FOUR: RESULTS

Necropsy was done on a total of 216 chicken carcasses, gross and histopathology lesions indicative of various disease conditions were observed and their frequency of occurrence was recorded.

Lesions suggestive of Newcastle disease (ND) was frequently observed in chicken examined in the study period (October 2008 to March 2010). However, Coccidiosis was the most prevalent condition encountered constituting 25% of the cases handled in the study period, followed by ND, 51 cases which were tentatively diagnosed and later confirmed using immunohistochemistry (IHC), constituting 23.6% of the cases examined., pasteurellosis was also common, 13 cases salmonellosis 8 cases, infectious bursal disease, 7 cases

Collibacillosis, nutritional related conditions, clostridial infections, infectious bronchitis, neoplastic conditions and fowl pox were also encountered, but were all grouped under other conditions and these constituted 56 cases hence 25.9%

Special interest was paid to lesions that were suggestive of Newcastle disease in all parenchymatous organs and other tissues, which are the primary target organs/ tissue for Newcastle disease virus. The target organs namely, the spleen, liver, lungs, the brain, proventriculus, caecal tonsils, and kidneys were thoroughly examined and lesions noted. Other non-parenchymatous organs frequently damaged by the virus such as segments of the intestines, especially at the regions of lymphoid tissues (Peyer's patches), combs, wattles and subcutaneous tissues were also examined.

The gross and the histopathology lesions in the various organs and their frequency of occurrence were noted and described as follows.

In 44 (86.27%) carcasses that had lesions suggestive of ND, the caecal tonsils of the affected chicken were grossly swollen as seen in the serosal surface in figure 7 A .On opening the mucosa of the caeco-colic junction, the caecal tonsils ( lymphoid aggregates) were enlarged, haemorrhagic and necrotic, as shown in figure 7(B-1).

Histopathology section of the swollen tonsils revealed haemorrhages, congestion and necrosis in the lymphoid tissue and sloughing off of the necrotic tissue (figure 8C and 8 D respectively)

Thirty two (62.75%) carcasses had spleen lesions. The spleen of the ND affected chicken were swollen, and had grayish - white focal areas of necrosis seen grossly through the serosal surface as shown in figure 3. Microscopic examination indicated that the lymphoid tissues especially the lymphoid follicles of the spleen were necrotic and most of the lymphocytes in the medulla were undergoing degeneration and various nuclear changes that occur in the stages of necrosis were observed, for example, nuclei pyknosis as indicated by arrows in figure.11, (K-1 & K-2).

Twenty four (47.06%) cases had haemorrhages in the mucosa of the proventriculus. The hemorrhages extended underneath of the cornified lining of the ventriculus in four (4) carcasses. Histologically, haemorrhages and congestion were seen in the mucosa and sub-mucosa of the proventriculus (Figure 6). There was degeneration of the lymphocytes in the lymphoid aggregates of the proventriculus.

In the intestines of the affected birds, segmental hemorrhages in the mucosa of the intestines especially in the Peyer's patches were grossly observed in 19 cases, (37.25%). The lymphoid tissues in the affected segments were necrotic histologically.

Nineteen chicken carcasses (37.25%) also had lesions in the upper respiratory tract, characterised by hemorrhages, congestion and in some cases excess mucoid exudate in the mucosa of trachea and bronchi. The pharyngeal tonsils were congested and covered with excessive mucoid exudates. Air sacculitis characterized by thickened and cloudy air sacs were recorded in all these cases. The air sacs were covered by thick caseous exudates making them appear translucent or opaque (cloudy) in more severe and chronic cases (Figure 3).

Forty four (86.27%) chicken carcasses with ND had lung lesions, consisting of lung hemorrhages, congestion, emphysema and edema as seen in histological section shown in figure 13. Mononuclear cells infiltration was most often observed around the bronchioles and bronchi. The predominant cells were the lymphocytes, plasma cells and macrophages.

The liver of the ND affected chicken were grossly congested. Focal areas of hepatic necrosis and wide spread hemorrhages and congestion were observed histologically in 32 cases (62.75%), of these; 15 samples had significant lymphocytes and macrophages infiltration especially around blood vessels in the portal area (figure 15).

Renal hemorrhages and tubule degeneration in association with vascular disturbance lesions in other parenchymatous organs especially liver and spleen were observed in 18 (35.29%) samples. However the renal lesions were non specific as these lesions might have arisen from other septicaemic conditions.

Thirteen chickens (25.49%) that were presented a live had nervous signs such as torticollis, and paresis (figure 2). Apart from congestion, no significant gross lesion was observed in the brain of chicken that had ND. However, histopathological sections of the brain tissue had lesions, characterized by hemorrhages, neuron degeneration and non purulent encephalitis in which mononuclear cells (lymphocytic and macrophages) aggregations occur in the brain tissue (figure 9A and 9B). Sixty percent of the chicken that had brain lesions also had lesions in other parenchymatous organs, especially lung haemorrhages, congestion and edema, air sacculitis and in a few cases with liver and spleen lesions as earlier described.

Subcutaneous edema was observed at the neck region with associated facial edema, (figure 1), swollen combs and wattles and these lesions were noted in only two cases constituting 3.92% ND cases diagnosed. In one of the cases the carcasses had degenerative and atrophic ovarian follicles, air sacculitis (figure 5) and greenish intestinal contents.

Egg yolk peritonitis; that is inflammation of the peritoneal cavity provoked by the presence of yolk material in the coelomic cavity occurred in 11 (21.57%) cases (Figure 4). While nine carcasses of chicken from laying flocks had ovarian atresia and deformed, flaccid or degenerative follicles, (Figure 5). Egg yolk peritonitis was characterised by fibrin or albumin like material that had a cooked appearance in the abdominal cavity.

The serosal surfaces of the intestinal loops and the mesenteric fat were coated by strands of yellowish friable material. Localized to diffuse peritonitis with the associated secondary ascitis was observed in all the 11 cases. Grossly the exudate was yellowish and contained visible yolk material and fibrin strands.

Greenish material was observed throughout the digestive tract in 4 (5.71%) cases. This was associated with evidence of diarrhea such as soiled vent and weight loss. Three birds out of the four that had greenish ingesta had focal areas of necrosis and ulcerations in the intestinal mucosae especially in the areas of lymphoid aggregation (Peyer's patches).

Myocardial congestion and hemorrhages associated with vascular disturbances and other lesions in the visceral organs as earlier described were recorded in 14 (27.45%) cases.

Out of the 86 chicken carcasses including the 51 carcasses that were tentatively diagnosed as ND based on clinical pathologic findings, 44 (51.16%) tested positive for ND using immunohistochemistry. Of the 51 tentatively diagnosed cases, samples from 40 (78.4%) carcasses were confirmed to have ND using IHC. The presence of the viral protein in the positive cases were indicated as brown precipitate at the antigen sites particularly in the target organs, for example, the spleen, liver and lung tissues as shown in figures 13b, 14, and 15b . Proventriculus, caecal tonsil, kidneys and brain also had the ND viral protein. The brown precipitate was frequently observed in monocytes, macrophages, kupffer and dendritic cells in the target organs. In the spleen sections, the viral antigens were stained more intensely and much clearer than in other tissues. However non-specific back ground staining was also noted in some of the immunohistochemical stained slides.

The findings are summarized in tables one, two and three that follows:

**Table 1: summary of the occurrence of common poultry diseases among chicken basing on clinical and pathology findings in 2008 to 2010**

<b>Type of Disease</b>	<b>of positive cases based on Clinico-pathologic diagnosis</b>	<b>Percentage (%)occurrence</b>
coccidiosis	54	25.00
<b>Newcastle disease</b>	<b>51</b>	<b>23.61</b>
Pasteurellosis	13	6.02
Salmonellosis	8	3.70
Gumboro	7	3.24
Helminthiasis	27	12.50
Others	56	25.93
	<b>216</b>	<b>100</b>

**Table 2: Prevalence of ND based on clinical- pathologic findings and IHC**

Clinical- Pathologic diagnosis of ND			IHC diagnosis of ND		
Sample size (n)	.of Positive cases	%	Sample size (n)	.of Positive cases	%
216	51	23.6	86	44	51.16

**Table 3: common gross and histopathological lesions seen in the Newcastle disease cases diagnosed from October 2008 to March 2010**

s/no	Lesions seen in ND cases	No. cases with lesions out of 51 cases diagnosed as ND	Percentage occurrences of such lesions (%)
1	Egg yolk peritonitis	11	21.57
2	Greenish ingester/digester	4	7.84
3	Enlarged haemorrhagic or congested caecal tonsils	44	86.27
4	Haemorrhages in the mucosa of the proventriculus	24	47.06
5	Congestion and haemorrhages in the tracheal mucosa with excess Mucoïd exudates	19	37.25
6	Haemorrhages, congestion and edema in the lungs	44	86.27
7	Mono-nuclear cells infiltration in the lungs	19	37.25
8	Necrotic foci in the spleen with degeneration of the lymphoid follicles	32	62.75
9	Atrophic ovaries and those with deformed follicles	9	17.65
10	Hepatic necrosis and haemorrhages	42	82.35
11	Peri-vascular infiltration of lymphocytes in the liver	15	29.41
12	Renal haemorrhages and tubule degeneration	18	35.29
13	Peri-vascular infiltration of lymphocytes, haemorrhages and neuron degeneration in the brain	13	25.49
14	Myocardial haemorrhages and necrosis	14	27.45
15	Segmental and focal area of haemorrhages in the intestinal mucosae	19	37.25
16	Edema and congestion of the comb and wattles	2	3.92



#### 4.3 Pictorial presentations of the Gross, Histopathology lesions, and Immunohistochemistry diagnosis of ND in chicken.



**Figure 1(A):** Picture of 10 month old chicken.that had Newcastle disease.Note the Facial and peri-orbital Edema (see the arrow). Necropsy showed haemorrhages in the proventriculus and caecal tonsils as indicated in figures 3 and 4.



**Figure 1(B):** Sub cutaneous edema as indicated by arrows in carcass of chicken in figure 1 (A).

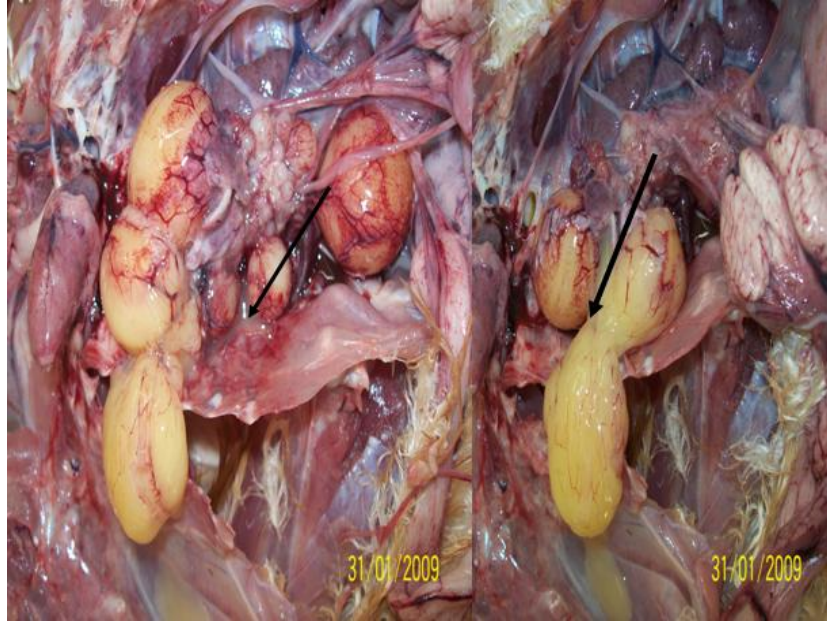


**Figure 2: Torticollis due to CNS infection in chickens.** Bird-1 was five month old broiler cockerel that had air sacculitis (fig: 8) and mononuclear cells aggregation in the brain (figure 7). Bird-2 was a six month old layer pullet; necropsy showed pneumonia and encephalitis with neuron degeneration (fig 6a & b). IHC test on the brain tissue confirmed the presence of ND viral protein in the two chicks.

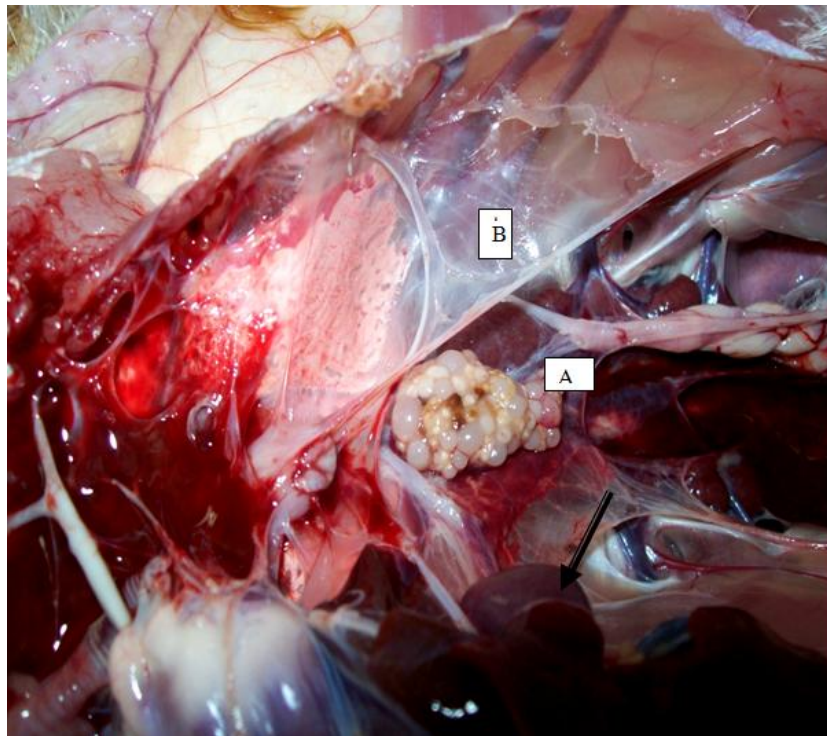


**Figure 3: Visceral lesions in chicken.** Air sacculitis and swollen spleen with white sub-capsular necrotic foci in a broiler chicken. In the flock, 30% of the birds died and histopathology lesions observed included, necrosis of lymphoid follicles in the spleen (Figure 11) and haemorrhages in the lungs (figure 13) and viral protein was detected using IHC.

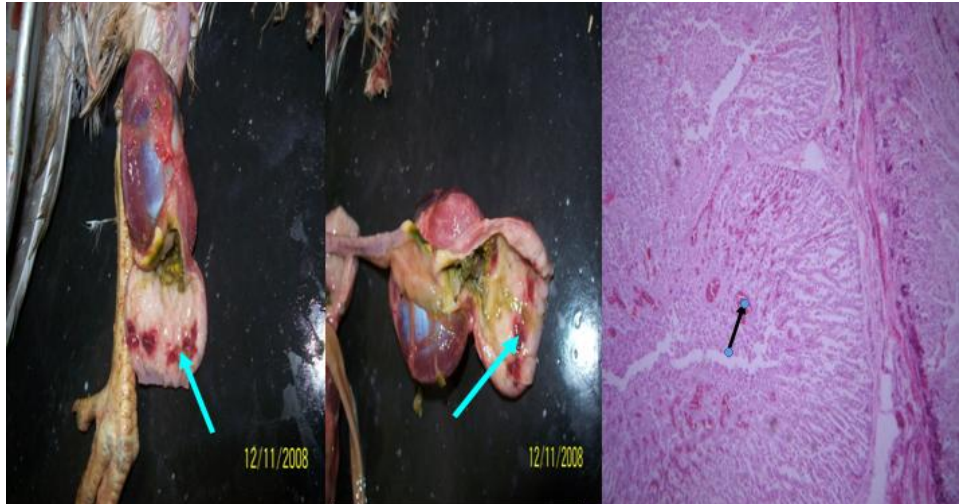




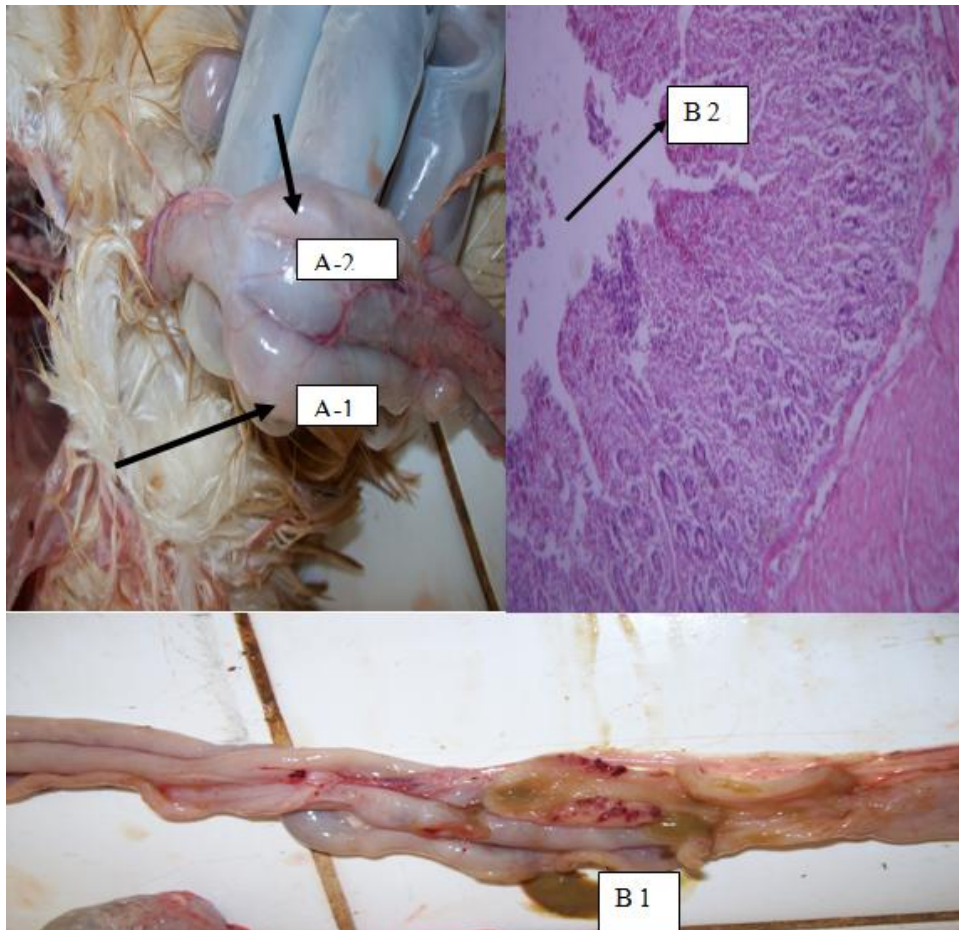
**Figure 4: Visceral lesions in chicken, Ruptured eggs in the peritonea.** This was from two layer chickens from the same flock that had ruptured eggs in the peritoneal cavity leading to egg yolk peritonitis. The spleens had sub capsular grayish foci of necrosis and haemorrhagic caecal tonsils.



**Figure 5: Visceral lesions in chicken.** Atrophic Ovarian follicle (A) and air sacculitis (B) in a laying flock that had ND and other lesions observed in the same carcass included, swollen spleen (arrow) and congested lungs.

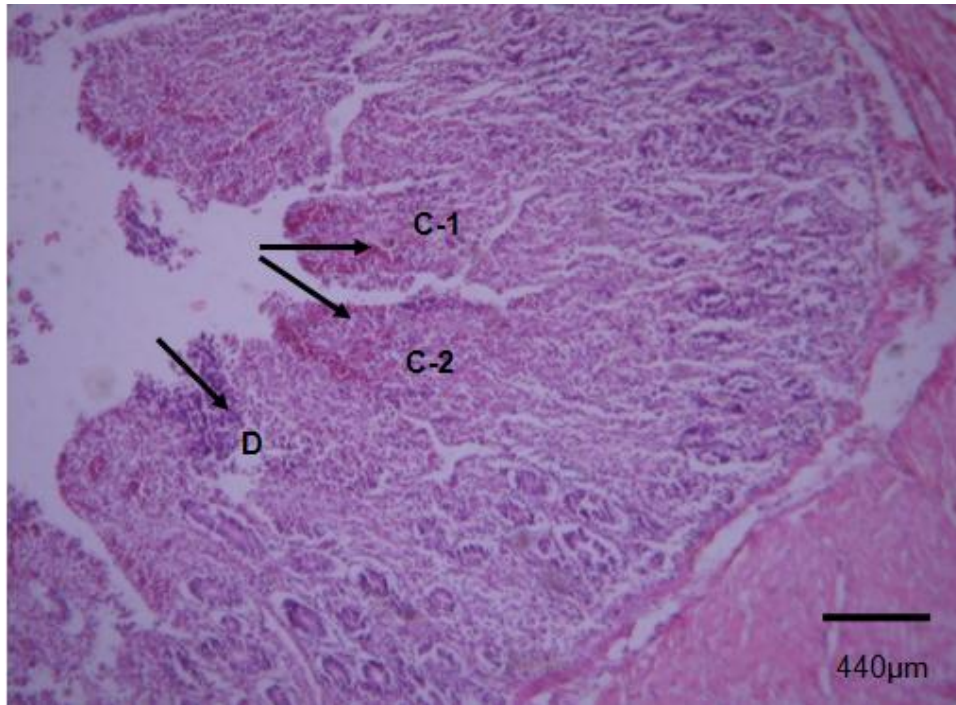


**Figure 6: Visceral lesions of ND in chicken.** Haemorrhages and congestion in the proventriculus of a chicken that had facial edema (figure 14), and haemorrhagic caecal tonsils (figure 5).

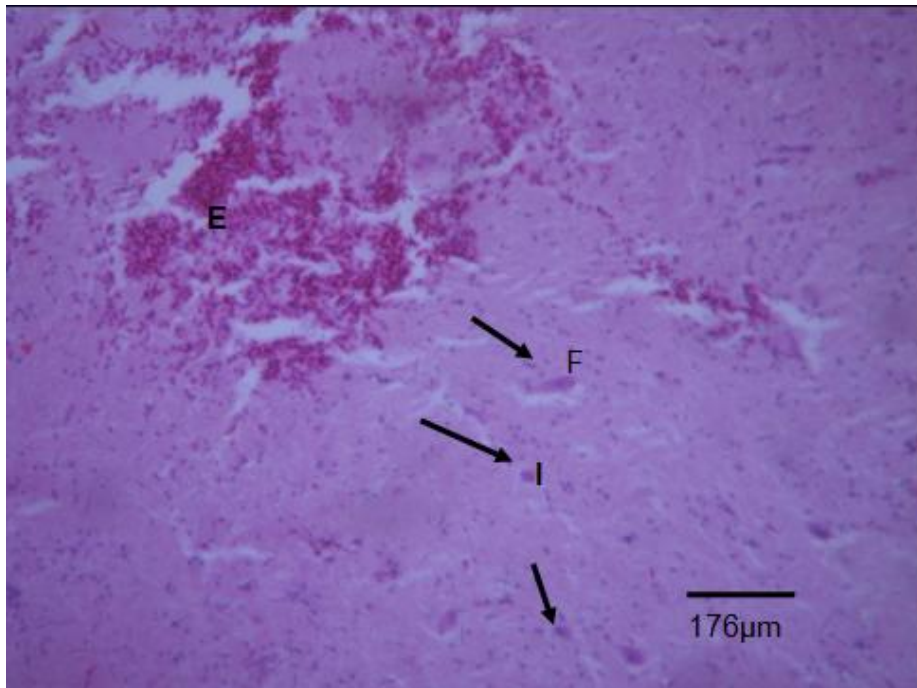


**Figure 7: Visceral lesions of ND in chicken.** Bilateral enlargement (A-1& 2) and haemorrhages in the caecal tonsils (B1& B2) in a layer chicken that had severe egg yolk peritonitis and air sacculitis. ND viral protein was detected in the caecal tonsils using IHC.

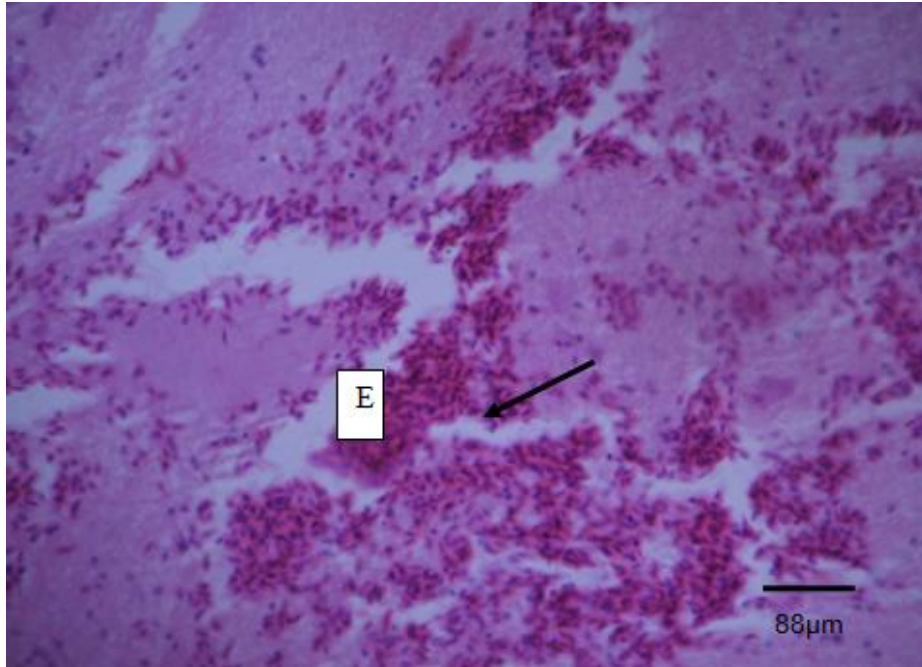




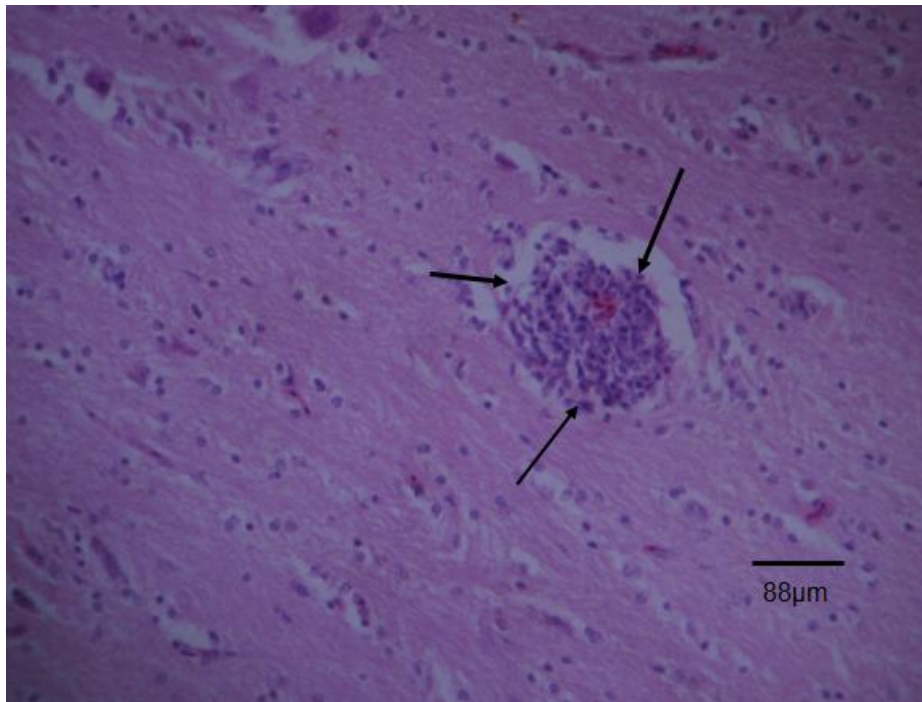
**Figure 8: Histopathology of caecal tonsil in figure 10 A.** Note the haemorrhages and congestion in the mucosa of the tonsils as indicated by the arrows, C-1 and C-2, necrosis and sloughing off of the lymphoid tissues (D).



**Figure 9(a): Brain section of chicken with ND at lower magnification**

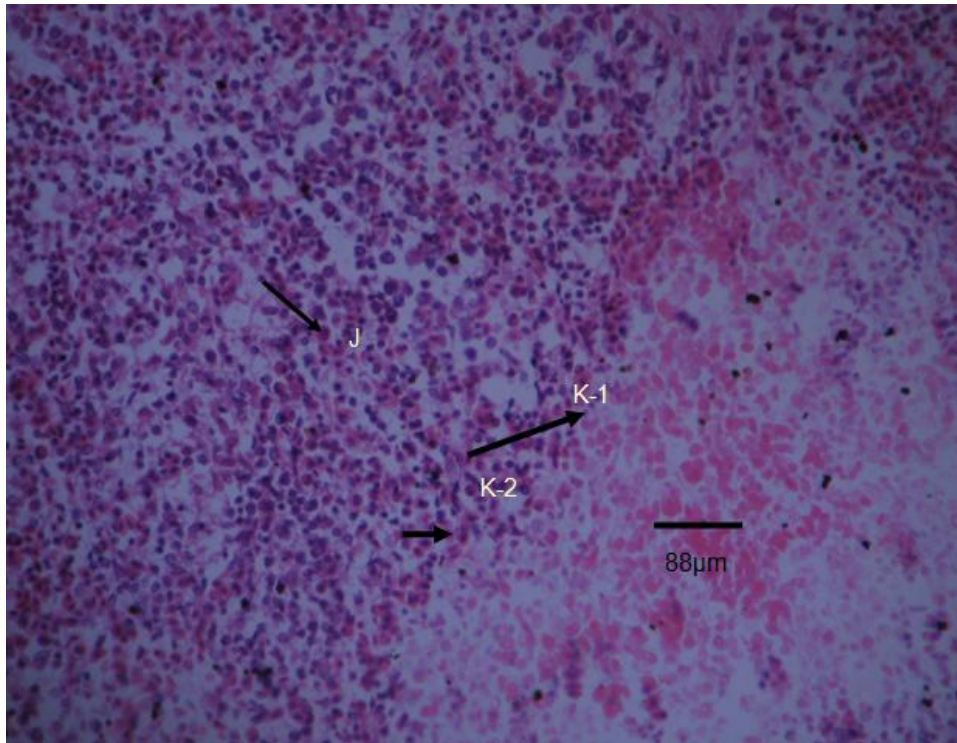


**Figure 9 (b): Section of the Brain at higher magnification.** Note Haemorrhages (E) and neuron degeneration/pykinosis (I) in the brain of a mature chicken that had torticollis, lesions observed in other organs included, air sacculitis and pneumonia.

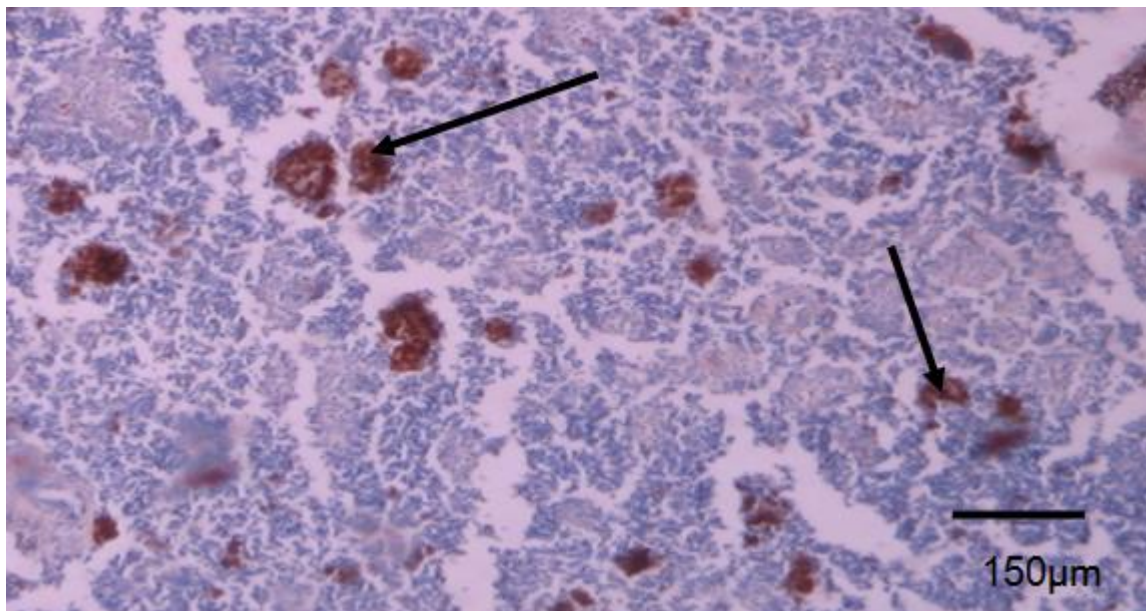


**Figure 10: Encephalitis with mononuclear cells aggregation.** Section of the Brain of a chicken that had torticollis as indicated in the figure 15. The client reported high mortalities (above 60%) in the flock. Note the Encephalitis with mononuclear cells aggregation as indicated by the arrows. IHC test detected ND viral antigens in the brain tissue.

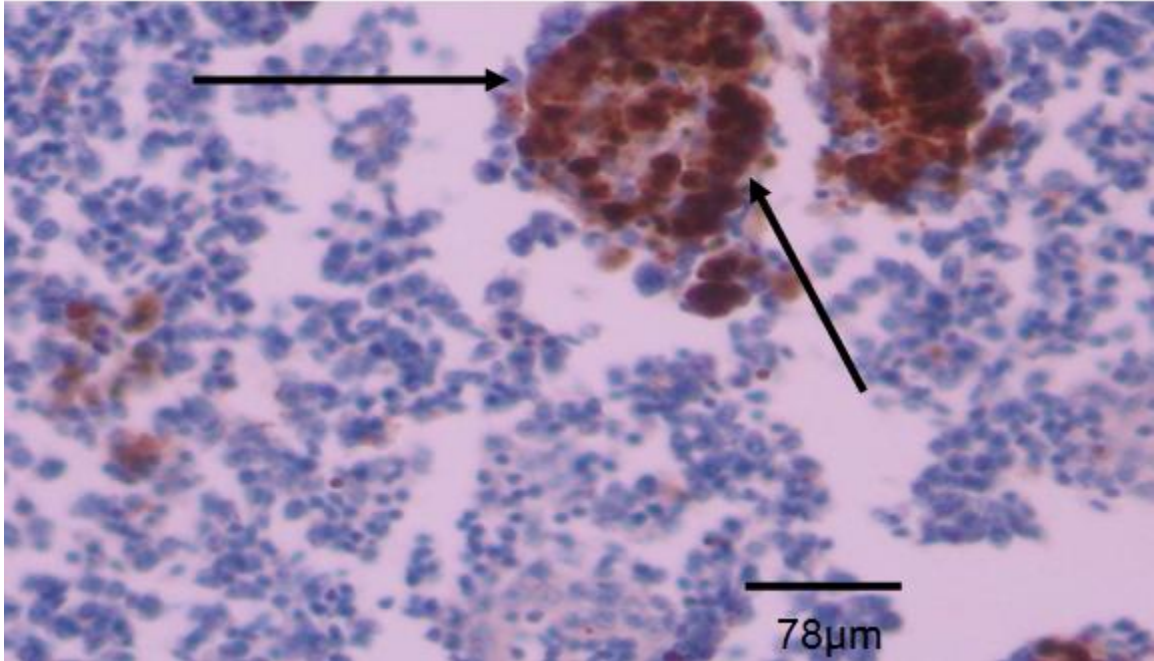




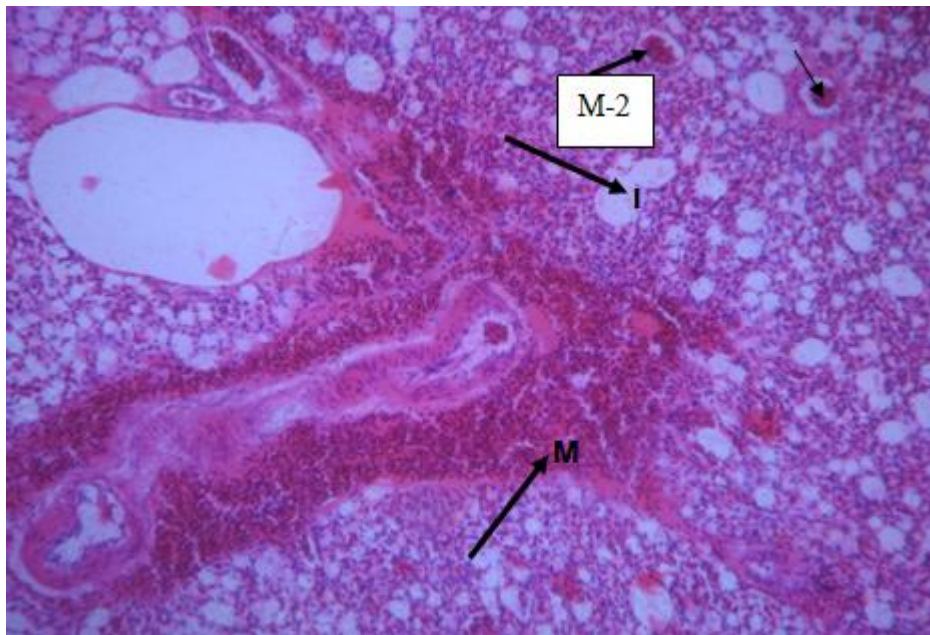
**Figure 11: Histopathology of spleen of a broiler chicken that had ND.** Note, the necrosis and lymphocytes degeneration in the spleen, nuclear Pyknosis (K-I & K-2) and Macrophages infiltration in the spleen tissue (J). Gross lesions noted in the same bird included swollen spleen with necrotic foci in the sub capsular area and air sacculitis as shown in figure 8.



**Figure 12 (A): A Spleen Section IHC stained.** IHC Detection of ND in Spleen Section, of chicken, positive case. Note the arrows pointing to the brown precipitates indicating presence of ND antigens.

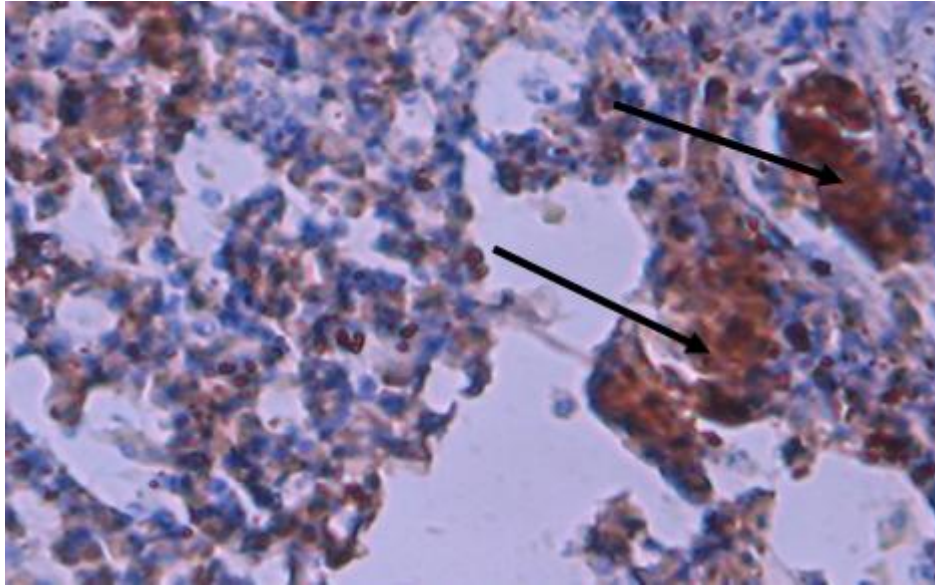


**Figure 12 (B): Spleen section (IHC).** Spleen tissue of chicken stained using Avidin- Biotin Peroxidase IHC technique, Positive case, Note the arrow pointing to the brown precipitates mainly in the macrophages. 12 A was at low magnification (X 10) and 12 B at X 40.

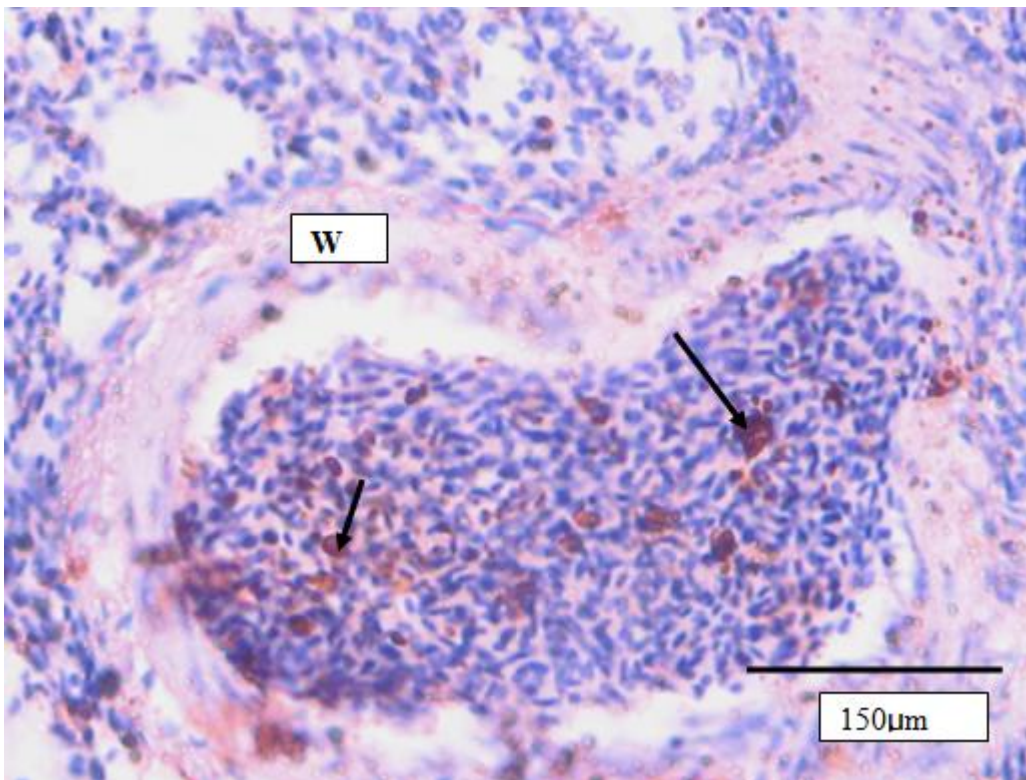


**Figure 13(A): Section of chicken lung tissue showing lesions of ND (H& E stain).** Note Haemorrhages (arrow M), Congestion (arrow M-2) and Emphysema (arrow I). Grossly, the carcass had cloudy air sacs (air sacculitis), lung edema and greenish ingesta.

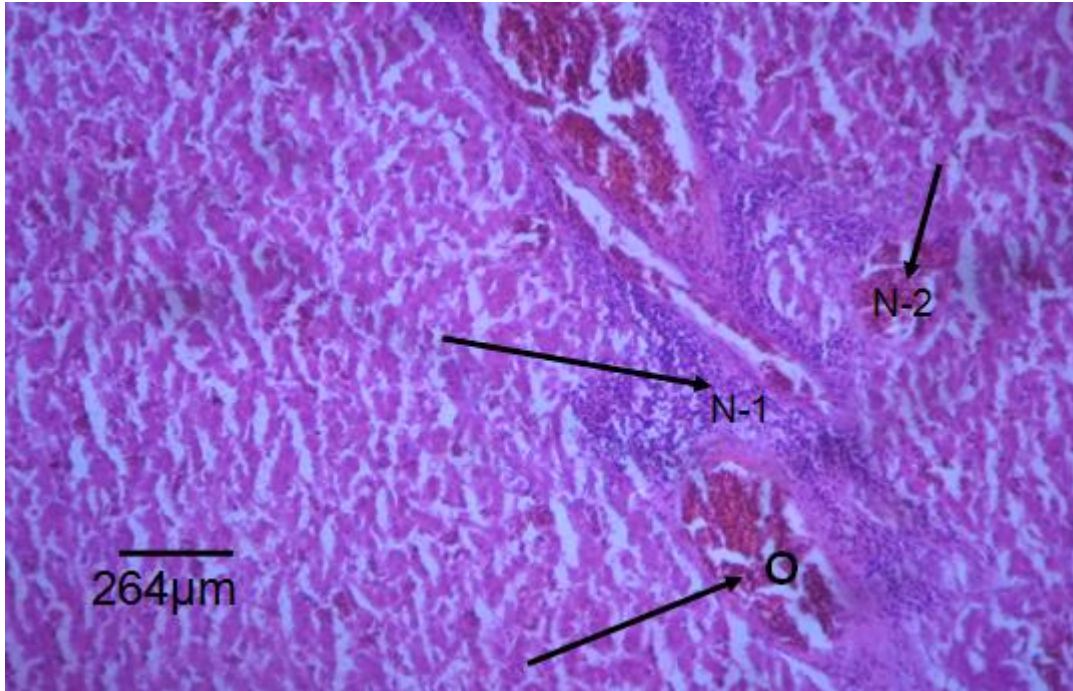




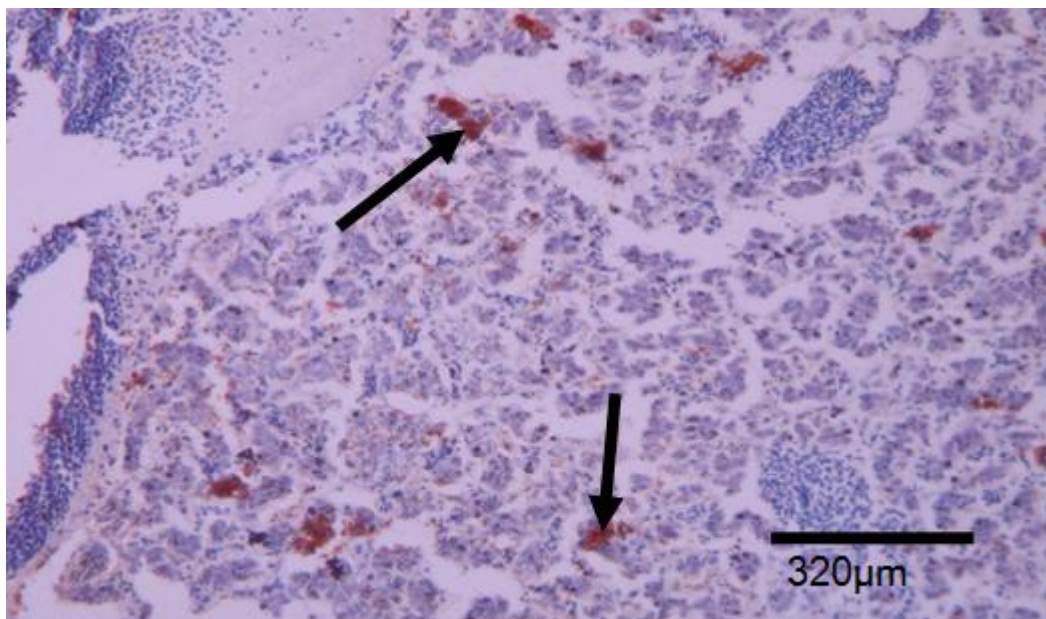
**Figure 13 (B):** Section of chicken lung tissue (IHC stained). Note the brown precipitate in tissues, as indicated by arrows. This was ND positive case.



**Figure 14:** IHC stained section of lung tissue of chicken, positive case for ND. Note: the brown precipitates in monocytes in the congested blood vessels as indicated by the arrows. The wall of the blood vessel is labeled **W**.



**Figure 15 (A), liver section H& E (X10).** Note the peri-vascular lymphocytic infiltration (N-1 & N-2) and congestion (O) in liver of a chicken that had ND.



**Figure 15 (B): Liver section, IHC stained, positive for ND.** Note the brown precipitate in kupffer cells indicating the presence of ND viral protein in the tissue, Avidin - Biotin Peroxidase IHC test was used.

## CHAPTER FIVE: DISCUSSIONS

The wide spread lymphoid tissue necrosis observed in ND cases in this study, particularly in the spleen follicles, Peyer's patches, caecal tonsils and proventriculus was due to ND virus induced apoptosis which is an antiviral mechanism of host defense utilized by eukaryotic cells, to minimize viral replication and reduce damage caused by infection while clearing the invading pathogens. Viral antigen (viral protein) was detected in these lymphoid tissues using immunohistochemistry as shown in the spleen section in figure 12 (A) and 12(B). Susta, *et al* (2009) detected nucleoprotein expressed by the ND virus in various tissues especially at site of viral replication using immunohistochemistry. The presence of the ND viral protein in the tissue was visualized as brown precipitate. The findings in this study therefore concur with what was described by Susta *et al.*, (2009). The swelling of the spleen and caecal tonsils was probably due to the inflammatory response and hyperplasia of lymphocytes in these organs that occurred during viral replication in the lymphoid tissues.

The nervous signs such as torticollis (figure 2) and opisthotonus observed in 13 chickens were due to neurotropic Newcastle disease virus infection. Torticollis in ND occur when the virus affects the cerebellum and brain stem, producing multifocal glial nodules and necrosis of the neurons (<http://www.partnersah.vet.cornell.edu/avian-atlas>). Torticollis, also known as wryneck may result from other conditions that cause contracture of sternocleidomastoid muscle of the neck, causing the head to tilt towards the affected side. The contracture of the neck muscle could result from muscle injury, congenital malformation of the cervical spine or nervous disorder. Torticollis can also occur in some infectious and nutritional related diseases such as, fowl cholera, Marek's disease, vitamin E and selenium deficiency. If it's due to fowl cholera, torticollis result from the infection of the middle ear, meninges and cranial bones by *Pasteurella maltocida* organism (Saif *et al.*, 2005). In Marek's disease torticollis is due to infection of the peripheral nerves of the cervical region, especially the vagus nerve. The affected nerves are usually grossly enlarged and lose their striation, and diffuse or nodular lymphoid tumours may occur in various organs particularly the liver, spleen, gonads, heart, lungs, kidneys proventriculus and muscles. However these neoplastic lesions do not occur in ND virus infection and were not observed in any of the ND cases. Opisthotonus that occur in vitamin E- selenium deficiency is usually associated with encephalomalacia (Saif *et al.*, 2005). All these conditions were differentiated from Newcastle disease by immunohistochemical (IHC) staining of the target tissues to



specifically detect the presence of ND viral protein which does not occur in any other condition apart from ND virus infection.

Nineteen carcasses of chicken diagnosed with ND (37.25%) had lesions in the upper respiratory tract, characterised by hemorrhages, congestion and sometimes excess mucoid exudate in the mucosa of trachea and bronchi. The excessive mucous production in the trachea and pharynx was as a result of the ND virus infection of the epithelia that lead to the hypertrophy of the goblet cells. Mast *et al.*, (2005) described similar changes that occurred in the trachea of chickens infected with ND virus and noted that, the rupture of the hypertrophied goblet cells hence release of excess mucous in the trachea and pharyngeal area peaked 4 days post infection with ND virus and later simple squamous epithelia replaced the usual ciliated glandular cuboidal epithelia of the tracheal mucosa as infection persisted. Air sacculitis was associated with respiratory tract infection and was characterized by thickened and cloudy air sacs. The air sacs were covered by thick caseous exudates making them appear translucent or opaque (cloudy) in more severe and chronic cases (Figure 3). The thick caseous exudate was mainly due to the effect of secondary infection of the respiratory system by pathogens such as *E. coli*, *Pasteurella*, avian *Mycoplasma* and other bacteria species.

The lung lesions consisted of hemorrhages, congestion, emphysema and edema as seen in figure 13. This was as a result of the circulatory disturbance that occurred due to viraemia and secondary bacterial infection. Mononuclear cells infiltration was most often observed around the bronchioles and bronchi. The predominant cells were the lymphocytes, plasma cells and macrophages. Newcastle disease viral protein was detected using IHC in the affected lung tissues as brown precipitates and this was prominent in the monocytes in the congested blood vessels as shown in figure 14.

Renal hemorrhages and tubule degeneration in association with other lesions due to vascular disturbance in the parenchymatous organs particularly in the liver and spleen were non specific, and these lesions could have arisen from other septicaemic conditions. However immunohistochemistry diagnostic test detected ND viral antigens in some renal tissues of chicken carcasses that showed lesions associated with vascular disturbances.

The subcutaneous edema observed in chickens in this study at the neck region (figure 1B) with associated facial edema, (figure 1A), was as a result of the vascular disturbance that frequently

occur in ND virus infection. Similar lesion was described by Banerje, *et al.*, (1992) in a flock of cormorants in which there was excessive mortalities and consistent gross lesions observed were; edema of the eyelids, and peri-vascular tissues, pulmonary edema and congestion, marked splenomegally, hepatic necrosis and wide spread haemorrhages in the visceral organs. Histologically, the principal alterations observed by Banerje, *et al.*, (1992) were; severe lymphocytic meningo-encephalitis and myelitis as well as splenic lymphoid necrosis and haemorrhages. The lesions described by Banerje *et al.*, (1992) were similar to what was recorded and described in this study except myelitis. The lesions were as shown in the figures, 1(B), 11, 12, 13 and 14.

Egg yolk peritonitis; that is inflammation of the peritoneal cavity provoked by the presence of yolk material in the coelomic cavity occurred in 11 (21.57%) carcasses examined. Yolk material by itself induces a mild inflammatory response and may be reabsorbed by the peritoneum. However, egg yolk is excellent growth media for bacteria, hence severe egg yolk peritonitis was enhanced by secondary bacterial infection. Coliform bacteria have been reported as the most common isolates in egg yolk peritonitis (Merck's Veterinary manual, 2005). Lodgment of eggs in the oviduct was probably due to reverse peristalsis induced by breakage of the thin shelled eggs in the oviduct. Peritonitis followed reverse movement of albumen together with the bacteria from the oviduct into the peritoneal cavity through fimbriae resulting into egg yolk peritonitis, (<http://www.avianweb.com/eggyolkperitonitis>).

Immunohistochemical diagnosis found that 51.16% of the chicken tested had ND viral protein. The presence of the viral protein (antigen) in the tissues was indicated as brown precipitate. The viral protein was more frequently detected in the lymphoid tissues particularly, the spleen and caecal tonsils. However liver, Lung, kidney, and, brain tissues also had the viral antigen. The viral proteins were mainly located in the macrophages and monocytes particularly in the lung and spleen tissue, while in the liver tissue the viral protein was more prominent in the hepatocytes and macrophages. The presence of detectable amounts of ND viral antigens in these organs was an indication that the lesions in the organs were associated with ND virus infection. The finding agrees with what Ojok and Brown described in (1996) in their immunohistochemical study of the pathogenesis of virulent viscerotropic ND in chicken, the team reported that ND virus caused severe damage to the lymphoid tissues. They further reported that the greatest

amount of viral antigen detected was in the proventriculus, small intestines, spleen, thymus and eyelid. More so, immunohistochemical labeling was confined to large mononuclear cells.

Newcastle disease diagnosis based on gross lesions, histopathology and clinical history indicated that the prevalence of the disease among the chickens presented for diagnosis in the study period was 23.61% compared to 51.16% based on immunohistochemical diagnosis (table 2). Of the 51 tentatively diagnosed cases, samples from 40 (78.4%) carcasses were confirmed to have ND using IHC. The relatively higher prevalence of ND in the later case was because IHC was able to diagnose per-acute velogenic and lentogenic ND unlike the tentative diagnostic methods. In lentogenic and per-acute forms of the disease, lesions suggestive of ND could not be observed, hence missed by clinical and pathologic diagnosis. Furthermore, the prevalence of the disease in chicken in the study area could have been even higher than what is reported. This is because direct immunohistochemical test was used as confirmatory diagnosis and was reported to have relatively low sensitivity (though highly specific) compared to other definitive diagnostic tests such as RT-PCR indirect IHC, (Edner *et al.*, 1994); hence some of the tissues with low ND viral protein and those where epitope retrieval was not sufficiently effected might have not been detected by IHC. On the other hand some septicaemic conditions such as pasteurelosis and salmonellosis and other similar conditions could have been tentatively diagnosed as ND.

Jose *et al.*, (1999) noted that, a negative result by immunohistochemistry does not completely rule out the presence of a particular infectious agent or its potential significance. Jose *et al.*, (1999) further recommended that, results by immunohistochemistry like those obtained by other diagnostic methods must be supported by clinical and pathologic data.

The light brown uniformly stained background observed in some specimens in this study was due to the non immunological binding of the specific immune sera by hydrophobic and electrostatic forces to certain sites within tissue sections (Seok *et al.*, 2003). The non specific background staining was minimized by the use of normal mouse serum. The mouse immunoglobulin present in the blocking reagent bound residual biotinylation reagent, thus minimising potential interaction with endogenous immunoglobulins. The strict adherence to the protocol also helped in minimizing non specific staining.

## **CHAPTER SIX: CONCLUSIONS, RECOMMENDATIONS AND CHALLENGES**

### **6.1 Conclusion**

Newcastle disease is still among the most endemic and fatal poultry diseases that constraint chicken production in the study area (Districts of Kampala, Wakiso, Mukono, Mpigi, Jinja and Kyenjojo). Despite the availability of vaccines for Newcastle disease, poultry farmers continue to experience losses due to mortalities, drop in production, diagnostic and control costs.

Over 20% of the chicken examined in the study period had Newcastle disease with lesions in the various target organs, this agrees with the positive hypothesis. The lesions observed in parenchymatous organs in ND cases were associated with the presence of the viral protein in the tissues as detected by immunohistochemistry. The presence of the virus in these tissues probably induced cellular damage and apoptosis hence lesion development as described by (Susta *et al*, 2009).

### **6.2 Recommendations**

There is need to carry out confirmatory diagnosis in all poultry cases presented for necropsy so as to provide reliable advice to the clients to enable them make accurate decisions.

Wider area of study should be considered so as to get actual national prevalence of Newcastle disease and its impact on poultry production in Uganda.

Highly sensitive and specific diagnostic facilities establishment should be considered by the University for the Faculty, especially for Departments that deal with disease diagnosis. This will be used as teaching facility for students and provide vital services to the community who regularly demand for such services.

Study should be done to assess the efficacy of the vaccines in the market and effectiveness of poultry vaccination exercise against devastating diseases such as Newcastle disease, infectious basal disease and other fatal diseases.

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