PREVALENCE AND CLINICO-PATHOLOGICAL MANIFESTATIONS OF AVIAN LEUCOSIS IN CHICKEN IN NAIROBI AND SURROUNDING COUNTIES

A thesis submitted in partial fulfillment of the requirements for a Master of Science Degree in Veterinary Pathology and Diagnostics, University of Nairobi

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DECLARATION

This thesis is my original work and it has not been presented for award of a degree in any other university.

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DEDICATION

This work is dedicated to my family and friends. Special gratitude goes to my grandparents, Mr. Isaya Martin Miheso and Mrs. Rose Minaywa Miheso.

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LIST OF ABBREVIATIONS AND ACRONYMS

AL Avian Leucosis

ALV Avian Leucosis Virus

ALV-J Avian Leucosis Virus Subgroup J

ALVs Avian Leucosis viruses

ALSV Avian leucosis/sarcoma virus group

ATVs Acutely Transforming Viruses

Br. Broilers

C.L Commercial Layers

CVL Central Veterinary Investigation Laboratories

DNA Deoxyribonucleic acid

DVS Director of Veterinary Services

ELISA Enzyme Linked Immunosorbent Assay

EU Elisa Unit

EUs Elisa Units

FAO Food and Agriculture Organisation of the United Nations

H&E Hematoxylin and eosin stain

HPAI Highly Pathogenic Avian Influenza

IgG Immunoglobulin G

In. Indigenous chicken

KNBS Kenya National Bureau of Statistics

Ksh. Kenyan Shillings

LTR Long Terminal Repeat

LL Lymphoid Leucosis

m Metres

MD Marek's Disease

MDV Marek's Disease Virus

ml Millilitre

mm Millimetres

MOLD Ministry of Livestock Development

NaOH Sodium Hydroxide

nm Nanometres

No. Number

NVSL National Veterinary Services Laboratories

OIE Office International des Epizooties (World Organization for Animal Health)

PCR Polymerase Chain Reaction

p27 Avian Leucosis Group Specific Antigen

PHPT Department of Public Health, Pharmacology and Toxicology, Faculty of Veterinary Medicine, University of Nairobi

RE Reticuloendotheliosis

REV Reticuloendotheliosis virus group

RNA Ribonucleic acid

RT-PCR Reverse Transcriptase Polymerase Chain Reaction

Sp Sample to positive ratio

RSV Rous Sarcoma Virus

µl Microlitres

VPMP Department of Veterinary Pathology, Microbiology and Parasitology, Faculty of Veterinary Medicine, University of Nairobi

ABSTRACT

Avian leucosis (AL) is an important disease causing high morbidity, mortality and reduction in chicken production. There is scanty information in Kenya on the status of the disease in chicken to enable its prevention and control. Objectives of this study were to determine the prevalence of AL in chicken in the year 2003 to 2012; determine its seroprevalence; and the clinical and pathological manifestations of the disease in sero-positive and negative chicken in Nairobi and surrounding counties.

Determination of AL prevalence, involved retrospective retrieval of post mortem records from January 2003 to December 2012 in the Department of Veterinary Pathology, Microbiology and Parasitology, Faculty of Veterinary Medicine, University of Nairobi (VPMP) and Central Veterinary Investigation Laboratories (CVL). Seroprevalence determination involved blood sampling from 385 birds comprising indigenous (180), broilers (103) and layer chicken (102) from Nairobi and surrounding counties. The blood was tested for AL p27 antigen using an enzyme linked immunosorbent assay (ELISA). To determine clinical and pathological manifestations; ten AL suspect farms were visited, questionnaire administered, clinical examinations undertaken, and fifty birds acquired for serology, post mortem and histopathological examination done at VPMP.

A total of 3,721 chicken cases were examined in the period of 2003 to 2012. Of these, 5.05% (188/3721) were diagnosed with AL. There has been a rising trend in the number of AL cases over the period. Of these, commercial layers comprised 92.5% (174/188) (8-52 weeks old), broilers 3.7% (7/188) (3-36 weeks old), indigenous chicken 1.1% (2/188) (age not indicated) and 2.7% (5/188) chicken whose type and age were not indicated. Kiambu County was the main

source of these birds with 56.4% (106/188), followed by Nairobi County 27.1% (51/188), Machakos County 10.1% (19/188) and Kajiado County with 6.4% (12/188). Majority of these cases were reported in the rainy season (April-June; October-December) 53.2% (100/188) while the dry season (January-March; July-September) recorded 46.8% (88/188).

In seroprevalence determination, one hundred and five (58.33%) indigenous chicken (12-96 weeks old), nineteen (18.63%) commercial layer chicken (8-65 weeks old) and seven (6.8%) broilers (6-10 weeks old) were positive for avian leucosis virus (ALV) p27 antigen. Among the sampled birds, Kajiado County had the highest seroprevalence rate of 77.78% (35/45), followed by Nairobi County 52.94% (27/51), Machakos County 45.35% (39/86) and Kiambu County 14.78% (30/203).

Of the ten farms visited, sixteen (32%) out of fifty birds from six farms tested positive to ALV p27 antigen. Main clinical manifestations were whitish or brownish diarrhoea, anorexia, unthriftiness, paralysis, dullness and ruffled feathers. Common pathological lesions observed were emaciated carcasses, renomegaly, hepatomegaly with nodular liver lesions, enteritis, cardiomegaly and congestion of visceral organs (spleens, lungs and kidneys). Main histopathological manifestations comprised lymphocytic and lymphoblastic infiltrations with occasional mitotic figures in various organs (spleens, livers, kidneys, lungs, ovaries and sciatic nerves). There was correlation between seropositivity and observed clinical, pathological and histopathological manifestations of unthriftiness; enteritis, renomegaly and cardiomegaly; and lymphocytic infiltration in various organs respectively.

In conclusion; AL is prevalent among poultry flocks in Nairobi and surrounding counties and has been increasing with time, with over 30% of sampled birds testing positive. Majority of seropositive chicken were indigenous. Some clinical and pathological manifestations had a

significant correlation among seropositive birds. This study recommends effective screening of imported birds to prevent entry of infected birds into the country, culling of infected birds with proper disposal and standard biosecurity measures in hatcheries and farms to prevent primary exposure.

1.0 CHAPTER ONE: INTRODUCTION

Kenya has an estimated domestic poultry population of 31 million birds, out of which 81 per cent (%) are free-ranging indigenous chicken, while 19% are commercial layers and broilers (KNBS, 2010). Annually, the country produces about 20 tonnes of poultry meat worth Kenyan shillings (Ksh.) 3.5 billion and 1.3 billion eggs worth Ksh. 9.7 billion (MOLD, 2010). Poultry keeping is one of the most popular livestock enterprises in Kenya due to its low capital investment and small space requirements. About 65% of Kenyan households keep chicken; whereby on average, about 12 chicken are kept by each household (Omiti and Okuthe, 2009). The poultry industry comprises of both smallholder and large scale poultry producers under commercial hybrid or indigenous poultry production system (Omiti and Okuthe, 2009).

The commercial hybrid poultry production system is mainly market oriented, relying on exotic layer or broiler parent and grandparent stock which are imported into the country by breeding companies. These birds are reared under intensive production (Okello *et al*., 2010). Breeding companies and hatcheries have a moderate to high sanitary and biosecurity level unlike individual/small scale farmers who have various flaws in maintaining an effective disease control program (Nyaga, 2007a).

Indigenous poultry production system is the dominant production system in Kenya, which is concentrated in rural and peri-urban areas such as Nairobi and other major towns in the country. This production system is characterized by unconfined birds that roam and scavenge around homesteads and they often interact with wild birds; and domestic and wild animals (Nyaga, 2007a). Breeding stock is rarely replaced and inbreeding is very common (Mburu, 1994), with

replacement stocks mainly originating from hatching own chicks, purchasing from local markets, from neighbours or given as gifts. Disease risks are very high in this type of production system due to minimal or no biosecurity measures (King'ori *et al*., 2010).

The poultry sector in Kenya is faced by various challenges, which include diseases, weak support services (market information, financial and technical services); lack of quality feedstuffs, capital limitations and cyclical fluctuations in production and prices (Omiti and Okuthe, 2009). Diseases have been identified as the major constraints to poultry production in the country (Okuthe, 1999; Okitoi *et al.*, 2006; Mungube *et al.*, 2007; King'ori *et al.*, 2010; Njagi *et al*., 2010; Magothe *et al.*, 2012; Okeno *et al.*, 2012).

Poultry diseases have been reported in the country, and they vary both spatially and temporally; with very few structured epidemiological studies carried out. However, few studies have been conducted, but cannot be extrapolated to represent the disease picture of the whole country. Other sources of information are usually obtained from government annual reports which are mainly based on tentative diagnosis from field reports (DVS, 2001- 2002).

The major diseases causing losses in poultry in Kenya comprise viral (Newcastle disease, fowl pox, infectious bursal disease); bacterial (fowl typhoid, colibacillosis, chronic respiratory disease); parasitic (coccidiosis and helminthosis); and neoplastic (avian leucosis and Marek's disease) diseases (Nyaga, 2007a; Njagi *et al*., 2010; Shepelo and Maingi, 2014). Most of these diseases are treatable or controlled via vaccinations; unlike avian leucosis which has no specific treatment or vaccine.

Avian leucosis, Marek's diseas and reticuloendotheliosis (RE) are diseases of great economic importance (Payne and Venugopal, 2000). Among these oncogenic diseases, avian leucosis is an insidious, but important disease of chicken (Fadly, 1990). Avian leucosis disease outbreaks have

been reported worldwide (Lupiani *et al*. 2006; Payne and Nair, 2012) and are a cause of serious economic losses to the poultry industry in terms of tumour associated deaths (Payne and Nair, 2012), reduced productivity and immunosuppression (Gao *et al.,* 2014).

Avian leucosis expresses itself both in terms of clinical and pathological presentations. Clinical presentations include inappetence, abnormal feathering, depression, paleness of comb and wattles, loss of weight, reduced egg production, paralysis and death (Latif and Khalafalla, 2005). Pathological presentations include gross enlargement of the liver, spleen, kidney and other organs coupled with miliary, diffuse or nodular tumour foci, in the bursa of Fabricius. Microscopically, these lesions often consist of coalescing foci of extravascular uniform lymphoblasts (Payne and Venugopal, 2000).

Despite all these negative impacts of the disease and global spread, there is paucity of information on the disease status in the country. The aim of this study was therefore to investigate the prevalence, clinical and pathological presentations and presence of ALV p27 antigen among chicken flocks in Nairobi and surrounding counties.

1.1 Hypothesis

There is a rising prevalence of AL in chicken in Nairobi and surrounding counties which manifests distinctly clinically and at necropsy.

1.2 Objectives

1.2.1 Overall objective

- To determine the prevalence, clinical and pathological manifestations of AL in chicken in Nairobi and surrounding counties.

1.2.2 Specific objectives

1. Determine the prevalence of AL in chicken in Nairobi and surrounding counties in the year 2003 to 2012.
2. Determine the seroprevalence of AL in chicken in Nairobi and surrounding counties.
3. Determine the clinical and pathological manifestations of AL in sero-positive and negative chicken.

1.3 Justification

Confirmatory diagnosis of a disease and knowledge about its prevalence is a very important tool for instituting effective and reliable disease control strategies and prevention measures (Ojok, 1993). Despite AL disease outbreaks having been reported worldwide and being a major contributor to serious economic losses in the poultry industry; there is very little documented information on avian leucosis in Kenya. Moreover, the country lacks structured studies and information on disease prevalence for the many poultry diseases in all the production systems.

The extent of control on importation/movement of poultry and its products, into and within the country is negligible or non-existent; and pose a significant threat in occurrence of disease outbreaks in the country. There is widespread legal and illegal movement of poultry and poultry products into and within the country (Mulinge *et al.,* 2008). This happens despite various clauses stated in the Animal Diseases Act, Cap 364. Moreover, enforcement of rules and regulations governing the movement of poultry and poultry products and dealing with infected birds is very weak (Mulinge *et al.,* 2008).

This is a hindrance to implementation of effective disease control measures. Available information is very scanty and hence affects planning of disease control strategies in the country (Omiti and Okuthe, 2009). No data on prevalence of AL in Kenya exists; despite most poultry

studies in the country having mainly focused on diseases such as Newcastle disease, infectious bursal disease, fowl pox, helminthosis and coccidiosis among others.

This study therefore aims at ascertaining the presence of avian leucosis virus p27 antigen in chicken in Nairobi and surrounding counties, prevalence and clinico-pathological manifestations. Nairobi was historically associated with poultry keeping in addition to a large number of peri-urban farms in areas such as Ruai, Mwiki, Embakasi, Kangemi among others due to proximity to the market. Moreover, being the capital city of Kenya, it serves as an epicentre of poultry and by-products market, thus surrounding counties are likely to keep chicken in relation to Nairobi market.

Results from this study will give insights into the prevalence of the disease within Nairobi and surrounding counties. This information will help in instituting control measures, which are useful when making objective recommendations on disease control strategies to improve the health and productivity of chicken in the country. This will contribute in attaining food security as stated in the Kenya vision 2030 and the sustainable development goals, whose aim is to eradicate extreme poverty and hunger.

2.0 CHAPTER TWO: LITERATURE REVIEW

2.1 General overview

Neoplastic conditions in poultry are mainly associated with oncogenic viruses, primarily retroviruses and/or herperviruses. Retroviral induced tumours are caused by members of the avian leucosis/sarcoma virus group (ALSV) or by the RE virus group.

Neoplastic conditions induced by the ALSV group involve the lymphopoietic, erythropoietic and myelopoietic systems. Lymphoid leucosis (LL) is the commonest form of leucosis, caused by the ALSV group of viruses known as ALVs. It is a lymphoproliferative disease of chicken, affecting primarily the bursa of Fabricius and other visceral organs (Ewert and de Boer, 1988). However, myeloid leucosis is increasingly more prevalent (Tomar and Saxena, 2007).

Other types of neoplasms of haemopoietic origin, which can also be seen in ALV-infected chicken, include erythroblastosis, myeloblastosis, myelocytomatosis and related neoplasms such as nephroblastoma. Among these, myelocytomatosis is frequently caused by avian leucosis virus subgroup J (ALV-J) especially in meat type chicken (Payne and Venugopal, 2000).

The non-neoplastic conditions induced by ALSV include hepatitis, myocarditis, anaemia, wasting diseases, arthritis, osteopetrosis and glomerulonephritis (Fadly and Nair, 2008). Most chicken flocks are affected by subclinical infections due to ALSV which cause depressive egg and meat production; reduced fertility and hatchability; poor quality of eggs and meat; delayed sexual maturity and non specific mortalities in infected flocks (Fadly and Nair, 2008). Literature cites a worldwide distribution of ALVs among poultry flocks (Payne and Nair, 2012). However, there is scant information on ALVs status among the Kenyan flocks, more so the indigenous chicken; and any clinical or pathological presentations associated with the disease in the locality.

2.2 Avian leucosis sarcoma virus group

In 1908, Vilhelm Ellermann and Oluf Bang demonstrated that chicken leucosis was caused by a virus. In 1911, Peyton Rous demonstrated a cell free transmission of sarcoma in chicken (Dorner and Coffin, 1986). Members of the avian leucosis/sarcoma virus group (ALSV) or ALVs are RNA viruses which belong to the genus *Alpharetrovirus*; subfamily *Orthoretrovirinae* in the family *Retroviridae*. Avian leucosis viruses are classified into ten subgroups A, B, C, D, E ,F ,G ,H , I and J based on interactions between virus-specific cell receptors, viral envelope glycoprotein, virus neutralization test and host range (Payne, 1998; Cheng *et al.*, 2010).

Subgroups A, B, C, D and J are oncogenic and exogenous (transmitted as infectious virus particles) ALVs, while subgroup E is endogenous (vertically transmitted). The other four subgroups, namely, F, G, H and I, are endogenous ALVs and occur in quails, partridges and pheasants (Payne, 1992).

Subgroups A and B have mostly been associated with LL and less commonly with erythroid leucosis in layers, while ALV subgroup J (ALV-J), is mainly associated with myeloid leucosis in broilers (Payne *et al*., 1991) and it was first isolated from the Dorking fowl in the early 1990s (Zhang *et al*., 2006).

Like other retroviruses, ALV, is able to mutate highly and recombine between subgroups, resulting in new recombinant ALVs (Gingerich *et al.*, 2002). This wide range of genetic and antigenic variations among isolates (Wu *et al*., 2010); together with an efficient way of transmission have made eradication efforts in this group of retroviruses difficult (Wu *et al*., 2010).

2.3 Epidemiology of avian leucosis virus

2.3.1 Occurrence of avian leucosis virus

Outbreaks of AL have been reported worldwide (Payne and Nair, 2012) and are a major cause of serious economic losses to the poultry industry in terms of reduced growth, uneven flock growth rates, immunosuppression and predisposition to bacterial diseases (Bagust *et al.,* 2004). Avian leucosis viruses are prevalent in several breeding flocks (Payne and Nair, 2012) although subgroups A, B and J are the most common ALVs occurring in commercial poultry flocks worldwide (Gao *et al.,* 2014). Subgroups C and D have rarely been reported while subgroup E is the ubiquitous endogenous leucosis virus of low pathogenicity (Adkins *et al.,* 2001). Subgroup A and J have also been isolated from broiler hybrids (Liu *et al.,* 2011). Outbreaks of subgroup J have recently been reported to affect parent and commercial layer chicken, and indigenous Chinese chicken flocks (Gao *et al*., 2014). Cases of subgroup J outbreaks among broiler breeding flocks have also been reported in several European countries (Payne, 1998), America, Asia (Sun and Cui, 2007), Africa (Egypt) (Aly, 2000) and Australia (Bagust *et al.,* 2004). The ALV p27 antigen has been detected among exotic commercial layers and broilers; and the indigenous chicken of Nigeria (Sani *et al.,* 2011; 2012). In Sudan, multiple ALV subgroups have been detected among broiler parent flocks (Latif and Khalafalla, 2005). In Kenya, there is no current documented evidence on the occurrence of the disease in the country, apart from antibodies which were detected in the wildfowl, domestic chicken and man (Morgan, 1967).

2.3.2 Transmission of avian leucosis virus

Exogenous ALVs can be both horizontally and vertically transmitted in chicken, (Hatai *et al.,* 2008) while the endogenous ones are transmitted through Mendelian inheritance (Silva *et al.,*

2007). Infection among most chicken occurs through close contact with infected birds (Payne and Nair, 2012).

Exogenous ALV-infected chicken shed virus into the egg albumen, vaginal and cloacal secretions, and congenitally transmit virus to the next generation. However, because of the relatively short life of the virus, infection does not spread readily from infected birds to uninfected birds in indirect contact (in separate houses or cages). However, contact exposure at hatch has been shown to be an effective method of spread of ALV-J among broiler breeder chicken (FadIy and Smith, 1999; Witter *et al*., 2000) and can be prevented by rearing them in small different groups (Witter and Fadly, 2001). Airborne ALV-J transmission has also been reported which can be detected from the feather tips of infected chicken (Sung *et al.,* 2002; Zavala *et al.,* 2002).

Venereal transmission by endogenous ALVs to embryos from vireamic semen and oviducts has also been demonstrated (Tsukamoto *et al.,* 1991; Smith and Fadly, 1994). However, certain strains of ALVs, coupled with immunosuppression, or presence of genes for endogenous virus can potentiate the frequency of ALV shedding with a significant increase in congenital transmission in chicken infected with the virus after hatching (Crittenden *et al.,* 1987; Fadly, 1987).

Poultry vaccine (s) contamination has also been reported as a way of ALVs transmission. Poultry and other live virus vaccines produced in embryos from infected breeder hens and tissue cultures prepared from such, embryos may harbour some of these retroviruses. These serve as potential sources of ALV contamination of poultry and other live virus vaccines, like the vaccine against MD which is produced from such ingredients (Fadly *et al*., 2006; Barbosa *et al*., 2008).

The presence of ALV as a contaminant may be due to spontaneous emergence of ALVs through recombination, which is a possibility that could result in flock infection and vaccine contamination. This mechanism has been suggested as playing part to the emergence of viruses such as ALV-J (Benson *et al.*, 1998).

2.4 Pathogenesis of avian leucosis

Avian leucosis/sarcoma viruses induce various leucoses affecting the erythroid, lymphoid and myeloid series of hematopoietic cells, and a number of neoplasms, including those affecting cells of the mesenchyme, kidney, ovary, testis, liver, pancreas and the nervous system (Payne and Fadly, 1997; Cheng *et al*., 2010).

The major mechanism of ALV pathogenesis is that of activation of oncogene by proviral insertion gene deregulation, which results in formation of tumours after a chronic infection (Yang *et al.,* 2007; Feng *et al.,* 2011). Two types of ALVs have been recognized; slowly and acutely transforming. Acutely transforming viruses (ATVs) can induce neoplastic transformation (*in vivo* or *in vitro*) within a few days or weeks; and cause various types of acute leukemia (leucosis) or solid tumours (usually sarcomas) (Yang *et al.,* 2007; Fadly and Nair, 2008; Feng *et al.,* 2011).

Acutely transforming viruses have acquired certain oncogenes through transduction of their host oncogenes, which occurs during viral replication (Yang *et al.,* 2007). Fifteen of these oncogenes have been described (Rasheed, 1995); and often lead to deletions within the structural genes of the virus such that the oncogene-carrying virion is replication defective and needs a replication competent helper virus to counter the genetic defect. These 15 oncogenes are divided into four main groups, namely; growth factors, growth factor receptors, nuclear factors and signal

transducers. These gene products, uncontrolled by normal regulatory processes, usually alter cell pathways concerned mostly with differentiation and cell growth (Rasheed, 1995).

Slowly transforming viruses do not carry viral oncogenes; and usually induce tumours by a "promoter insertion" or a related mechanism that activates a cellular oncogene resulting in neoplastic transformation and tumour development over several weeks or months (Kung and Liu, 1997; Feng *et al.,* 2011). Slowly transforming viruses have structural genes, *gag*, which encodes the internal structure of proteins in the virion; *pol*, encoding the RNA dependent DNA polymerase (reverse transcriptase); and *env*, encoding the viral envelope (Fadly and Nair, 2008). They are termed slowly transforming because the tumours induced are manifested later in life after infection of the chicken (Yang *et al.,* 2007). These viruses induce LL and erythroid leucosis by insertional mutagenesis in which ALV genome with its long terminal repeat (LTR) region will be genetically integrated upstream, downstream or within a cellular proto-oncogene of the host. The cellular proto-oncogene becomes activated by the enhancer sequence of the LTR, thus results in the abnormal expression of the oncogene leading to neoplasia. Lymphoid leucosis is the commonest neoplasm induced by slowly transforming ALVs (Kung and Maihle, 1987; Yang *et al.,* 2007).

Erythroid leucosis (erythroblastosis) is a rare type of infection caused by slowly transforming ALVs, in which the *c-erbB* gene in an erythroid cell is activated (Fung *et al.,* 1983; Payne and Nair, 2012). This leads to replacement of the bone marrow, mostly by erythroblasts which proliferate, thus leading to development of a leukemia, and eventual enlargement of various organs such as the liver and spleen due to accumulations of intravascular erythroblasts (Fadly and Nair, 2008).

Myeloid leucosis (myeloblastosis and myelocytomatosis) is not common, but sporadically occurs in adult birds (Payne and Fadly, 1997). The myeloid cells become transformed, leading to severe leukemia, and the intravascular and extravascular myeloid cells infiltrate into the liver, spleen and other organs (Fadly and Nair, 2008).

An ALV, designated as HPRS-103, has been reported to induce myelocytic myeloid leucosis, but lacks a viral oncogene (Fadly and Nair, 2008; Payne and Nair, 2012). It has been isolated from meat-type chicken in which it is quite prevalent (Payne and Nair, 2012). This virus causes a late onset of disease and is assumed to induce myeloid leucosis by insertional mutagenesis. The cellular oncogene activated is not yet known (Feng *et al.,* 2011). Other tumours induced by the various strains of ALVs include myxosarcoma, histiocytic sarcoma, osteosarcoma, chondrosarcoma, haemangioma, various types of renal tumours, mesothelioma, hepatocarcinoma, granulosa cell tumour and pancreatic carcinoma. The viral and cellular oncogenes involved in these tumours have not yet been elucidated (Payne and Fadly, 1997; Feng *et al*., 2011).

2.5 Clinical signs and pathological lesions of avian leucosis

2.5.1 Clinical signs

Clinical signs of AL are mostly non specific and include inappetance, abnormal feathering, paleness of comb and wattles, weight loss, diarrhoea, depression, paralysis and death (Latif and Khalafalla, 2005). Erythroblastosis and myeloblastosis type are characterized by haemorrhage from feather follicles and eye orbit which leads to blindness (Fadly and Nair, 2008).

Pressure on the sciatic nerve exerted by renal tumours may cause paralysis (Fadly and Nair, 2008). Osteopetrosis mainly affects the long bones of the limbs which results in thickening of the

diaphyseal or metaphyseal regions. These birds become stunted, pale and will most often limp while walking (Payne and Fadly, 2003).

Myelocytomatosis and skeletal myelocytomas often cause protuberances on the head, thorax and shanks. Myelocytomas occurring in the orbit of the eye may cause haemorrhages and blindness. Haemangiomas may at times affect the skin, appearing as blister-like lesions, which may rupture leading to bleeding, and sometimes lead to death of the affected chicken. Moreover, hemangiomas cause immunosuppression and weight loss (Lin *et al*., 2013). Subclinical infection of AL virus can adversely affect egg production in laying chicken in terms of fewer eggs, prolonged sexual maturity, smaller sized eggs and weaker shells (Spencer *et al*., 2000). Infected hens may have a lower fertility and hatchability (Lin *et al*., 2013). Onset of clinical signs may lead to a rapid course, and death of the birds occurs within a few weeks. However, some affected birds usually die without showing any obvious signs (Fadly and Nair, 2008).

2.5.2 Pathological lesions

2.5.2.1 Lymphoid leucosis

Lymphoid leucosis occurs in chicken of about 4 months of age and older. Grossly, tumours are usually visible and may involve organs such as the liver, spleen, bursa of Fabricius, kidney, lung, gonad, heart, bone marrow and mesentery. These tumours are usually soft, smooth and glistening; and their cut surface appears grayish to creamy white occasionally with areas of necrosis. Neoplastic growths may be nodular, miliary, diffuse or both. Nodular form of lymphoid tumours may vary from 0.5-5 cm in diameter and may occur singly or in large numbers. They are usually spherical but may be flattened when they are close to the organ surface. The miliary form, which mostly occurs in the liver, consists of numerous small nodules less than 2 mm (millimetres) in diameter uniformly distributed throughout the hepatic parenchyma. The diffuse

form causes uniform organ enlargement, which is slightly grayish in colour, and friable. Occasionally, the liver is firm, fibrous and almost gritty (Fadly and Nair, 2008).

Microscopically, tumours are focal and multicentric in origin with coalescing foci. Liver nodules are usually surrounded by a band of fibroblast-like cells that are remnants of sinusoidal endothelial cells (Fadly and Nair, 2008). The bursa may have a follicular-like pattern of tumuor growth. Tumours consist of aggregates of lymphoblasts that have a poorly defined cytoplasmic membrane, basophilic cytoplasm and a vesicular nucleus in which there are margination and clumping of the chromatin and one or more conspicuous acidophilic nucleoli (Payne, 1992).

The cytoplasm of most tumour cells contains a large amount of RNA, which stains red with methyl green pyronin, indicating immature and rapidly dividing cells (Fadly and Nair, 2008).

The tumour cells have B-cell antigen markers, produce and carry IgM on their surface (Fadly and Nair, 2008). Vacuoles are found infrequently in lymphoid cells of birds with LL, but some viral particles have been observed budding from the plasma membranes of lymphoblasts (Fadly and Nair, 2008).

2.5.2.2 Erythroblastosis

Usually occurs in birds between 3 and 6 months of age. The liver and kidney are moderately swollen, and the spleen greatly enlarged. Enlarged organs are usually cherry red to dark mahogany in colour, and are soft and friable. The marrow is usually hyperplastic and red in colour. Petechial haemorrhages occur in various organs, muscles, subcutis and viscera. Thrombosis, infarction and rupture of the liver or spleen may be observed. Pulmonary oedema, hydropericardium and a fibrinous clot on the liver may occur in addition to atrophy of the spleen (Fadly and Nair, 2008).

In cases of severe anaemia, blood is usually watery and light red and takes time to clot. Acute cases may show no gross apparent changes, although blood appears dark red (Fadly and Nair, 2008).

Microscopically, the marrow, in early cases, reveals bloody sinusoids filled with rapidly proliferating erythroblasts; while in advanced cases, sheets of homogeneous erythroblasts with small islands of myelopoietic activity and little or no adipose tissue are seen. With concurrent anaemia, erythropoietic cells may be reduced (Fadly and Nair, 2008).

Alterations in visceral organs are primarily due to haemostasis, resulting in the accumulation of erythroblasts in the blood sinusoids and capillaries. The liver sinusoids, splenic red pulp, bone marrow and sinusoids of organs such as the lungs, kidneys are filled with proliferating erythroblasts (Fadly and Nair, 2008). The erythroblast cell primarily involved. It has a large round nucleus, with very fine chromatin, a large amount of basophilic cytoplasm, a perinuclear halo, vacuoles and occasionally fine granules. The cell is irregular and often has pseudopodia (Fadly and Nair, 2008).

Stained blood smears reveal erythroblasts which vary in maturity from the early erythroblast, which is the dominant cell, to the various stages of polychrome erythrocytes. More mature cells often appear early in the course of the disease (Fadly and Nair, 2008). Thrombocytic series of cells may be increased in number and immaturity, while immature cells of the myelocytic series appear in the peripheral circulation; occasionally, they are as prominent as the erythroblasts. Mixed erythroblastosis and myelocytomatosis may also occur (Fadly and Nair, 2008).

Neoplastic erythroblasts are mostly indistinguishable from corresponding cells in the normal bird, except that viral particles may be present in extracellular spaces and within vacuoles inside cells. There is a great increase in erythroblasts' membrane activity, with vacuolization of the

cytoplasm and budding of viral particles from the cell membrane; with occasionally aberrant structures (Fadly and Nair, 2008).

2.5.2.3 Myeloblastosis

This is uncommon and usually occurs in adult chicken. It is characterized by liver enlargement, firmness, with diffuse grayish tumour infiltrates, which give a mottled or granular appearance. Spleen and kidneys are also diffusely infiltrated and moderately enlarged. The bone marrow is replaced by a solid, yellowish-gray tumour cell infiltration. Severe leukemia occurs, with predominant myeloblast cells which form a thick buffy coat and usually accompanied by anaemia and thrombocytopaenia (Fadly and Nair, 2008).

Microscopically**,** parenchymatous organs, mainly the liver depict massive accumulation of myeloblasts with a variable amount of promyelocytes. The spleen has proliferating cells which accumulate in the red pulp. In the bone marrow, myeloblastic activity is confined to extrasinusoidal areas (Fadly and Nair, 2008).

Leukemic blood smears have myeloblasts, which are large cells with slightly basophilic clear cytoplasm and a large nucleus containing 1-4 acidophilic nucleoli, and do not stain prominently; with presence of promyelocytes and myelocytes. Promyelocytes and myelocytes have specific granulation, which in the early forms is primarily basophilic. This usually may result in a secondary anaemia, with the presence of polychrome erythrocytes and reticulocytes (Fadly and Nair, 2008).

2.5.2.4 Myelocytomatosis

It is grossly characterized by neoplasms which occur on bone surfaces, in association with the periosteum and near cartilage, although other tissues/organs can also be affected. Myelocytomas can also develop at the costochondral junctions of ribs, inner sternum, pelvis and cartilaginous

bones of the mandible and nares. Flat bones of the skull are also commonly involved. Tumours can also occur in the oral cavity, trachea, and in and around the eye (Pope *et al*., 1999). Tumours are usually nodular and multiple, with a soft, friable consistency and of creamy colour. Infections caused by ALV-J, myelocytomatous infiltration will cause enlargement of various organs like the liver and spleen (Williams *et al.,* 2004). Myelocytic leukemia can also occur (Fadly and Nair, 2008).

Microscopically, neoplasms consist of well differentiated masses of myelocytes, which have a large nucleus, which is vesicular, eccentrically located and has a distinct nucleolus. The cytoplasm is tightly packed with acidophilic granules, which are usually spherical. Areas with less well differentiated myelocytes are common within the myelocytomas, and areas of undifferentiated cells, which may be stem cells of the myelocyte-monocyte series, can also be found. The liver has accumulations of neoplastic myelocytes which occur around blood vessels and in the parenchyma. Tumour cells in the spleen are present in the red pulp. The extrasinusoidal myelopoietic areas of the bone marrow are greatly enlarged by uniform neoplastic myelocytes (Fadly and Nair, 2008).

Naturally occurring disease is usually aleukemic, however, myelocytomatosis induced by ALV-J is frequently accompanied by a marked leukemia of myeloid cells. Laboratory strains of myelocytomatosis-inducing virus, such as MC29, can also result in leukemia. Ultrastructural features of myelocytoma cells may vary from well-differentiated myelocytes to those of undifferentiated, non granulated myeloid cells (Fadly and Nair, 2008).

2.5.2.5 Haemangioma

Hemangiomas are vascular tumors which are characterized by abnormal endothelial cell growth from capillary blood vessels. They cause bleeding in chicken and sometimes leading to death (Lin *et al*., 2013).

Haemangiomas affect chicken of various ages, and grossly, these tumours occur in the skin or in visceral organs. They usually appear as blood-filled cystic masses (blood blisters) or more solid tumours, consisting of distended blood-filled spaces lined by endothelium or as more cellular, proliferative lesions (Campbell, 1969). They are usually multiple and may rupture, causing fatal haemorrhage (Lin *et al*., 2013).

Microscopically**,** the cavernous form is characterized by distention of blood spaces with thin walls composed of endothelial cells. Capillary haemangiomas are solid masses in which the endothelium may proliferate into dense masses (haemangioendothelioma), leaving mere clefts for blood channels; which develop into a lattice with capillary spaces; or grow into collagen-supported cords with larger interspersed blood spaces (Fadly and Nair, 2008).

2.5.2.6 Nephroma and nephroblastoma

Two types of renal tumours occur; nephroblastomas (Wilms' tumour), adenomas and/or carcinomas. Grossly, nephroblastomas may vary from small, pinkish gray nodules embedded in the kidney parenchyma to large, yellowish gray lobulated masses that replace most of the kidney tissue. These tumours are pedunculated and connected to the kidney by a thin fibrous vascular stalk. Large tumours are cystic and may involve one or both kidneys. Adenomas and carcinomas vary in size and appearance, similar to nephroblastomas. They are multiple and cystic.

Microscopically, nephroblastomas are characterized by neoplastic proliferation of both epithelial and mesenchymal elements. Epithelial structures may vary from enlarged tubules with

invaginated epithelium and malformed glomeruli; through irregular masses of distorted tubules; to groups of large, irregular, cuboidal, undifferentiated cells with little tubular organization. The growth may be embedded in a loose mesenchymal or sarcomatous stroma. There may be areas of keratinizing stratified squamous epithelial structures (epithelial pearls), cartilage or bone (Fadly and Nair, 2008).

Microscopically, adenomatous or carcinomatous growths vary greatly. In tubular adenocarcinomas, primitive abnormal glomeruli frequently occur in large numbers among abnormal tubules. Papillary cyst adenocarcinomas are frequent. Solid carcinomas at times have evidence of renal tubules development (Mladenov *et al.,* 1967). A trabecular fibrous tissue stroma may separate masses of epithelial tumour tissue. Epithelial nephronic elements of nephroblastomas induced by strain BAI-A of ALV, cytoplasmic aberrant structures are occasionally seen in large or small aggregates (Beard, 1963).

2.5.2.7 Fibrosarcoma and other connective tissue tumours

Malignant and benign connective tissue tumours occur naturally, both in young and mature chicken and transmission occurs by cell-free filtrates. These tumours include; fibromas and fibrosarcomas, myxomas and myxosarcomas, histiocytic sarcomas, osteomas, osteosarcomas, chondromas and chondrosarcomas. Benign tumours grow slowly, are localized and non infiltrative. The malignant forms grow rapidly, eventually infiltrating surrounding tissue and may often metastasize. Fibromas arise as firm fibrous lumps attached to the skin, subcutaneous tissues, muscles and at times other organs; fibrosarcomas have a softer consistency. They may lead to skin ulceration (Payne *et al*., 1992).

Myxomas and myxosarcomas are softer in consistency. They occur mainly in the skin and muscles. Histiocytic sarcomas are usually firm and fleshy, mainly occurring in the viscera.

Osteomas and osteosarcomas are uncommon, occurring as hard tumours; and may arise from the periosteum of bones. Chondromas and chondrosarcomas rarely occur. However, they occur in cartilaginous areas within fibrosarcomas and myxosarcomas. Ganglioneurosarcoma has also been associated with ALV-J infection (Goodwin *et al*., 1998).

Microscopically, fibromas usually consist of mature fibroblasts, which are interspersed with collagen fibers arranged in wavy parallel bands or whorls. Slowly growing tumours are more differentiated and have more collagen and fewer cells than those which grow rapidly. Some fibromas have oedematous areas. In case of necrosis, ulceration or any secondary infection occurring, various inflammatory and necrotic alterations may be seen in the tumour. Inflammation may be prominent, such that the tumour may be easily confused with a granuloma.

Fibrosarcomas are characterized by aggressive and destructive growth, and immaturity of constituent cells. There is a large number of irregular and hyperchromic fibroblasts and mitosis. These tumours have less collagen compared to fibromas, which is concentrated in and near irregular septa that subdivide the tumour. Rapidly growing tumours may often have necrotic areas. Subgroup ALV-J infection may be associated with multiple undifferentiated pulmonary sarcomas (Hafner *et al*., 1998).

Myxomas often consist of spindle shaped cells surrounded by a homogeneous, slightly basophilic, mucinous matrix. The malignant form (myxosarcoma) is characterized by less abundant mucinous matrix, and immature fibroblasts are more numerous than those occurring in myxomas.

Histiocytic sarcomas originate from the monocyte and macrophage. The cellular constituents are highly variable within and between tumours; and are associated with infection by ALV-J (Hafner *et al.,* 1996; Arshad *et al*., 1997). Their cells may be spindle shaped, occurring in bundles as in

fibrosarcomas; stellate reticulum producing elements; and/or large phagocytic cells or macrophages. Stem cell derived tumours of the myelomonocytic lineage may also be considered to be histiocytic sarcomas. Endotheliomas induced by MH2 and MC29 strains may also be tumours of this lineage (Enrietto *et al.*, 1983).

Primary tumours predominantly have spindle shaped cells; whereas in metastatic foci, there are numerous primitive histiocytic forms. Osteomas are structurally similar to bone except that they lack inner histologic details. They consist of a homogenous acidophilic matrix of osseomucin containing collections of osteoblasts at irregular intervals. Cellular infiltrative growths of osteosarcomas usually invade and destroy surrounding tissues. Their cells are spindle shaped, ovoid, or polyhedral, and many are in mitosis. They have a prominent nuclei, basophilic cytoplasm and multinucleated giant cells. These cells grow rapidly, with some areas differentiating to produce osseomucin.

Chondromas have typical unique structure, appearing in groups of two or more chondrocytes lying in a matrix of chondromucin. Chondrosarcomas, have a considerable cellular variation, ranging from immature to fully mature chondrocyte (Fadly and Nair, 2008).

Ultrastructurally, tumour cells are similar to the cells in culture after infection with Rous sarcoma virus (RSV) and may be characterized by numerous pseudopodia and pronounced vaculolation of the cytoplasm, which may contain viral particles (Fadly and Nair, 2008).

2.5.2.8 Osteopetrosis

Grossly, changes occur in the diaphysis of the tibia and/or tarsometatarsus; and other long bones and bones of the shoulder girdle, pelvis and ribs but not the digits. Lesions occur bilaterally symmetrical; appearing as distinct pale yellow foci against the gray-white translucent normal bone. Periosteum is usually thickened, and the abnormal bone is spongy and easy to cut. The

lesion is commonly circumferential and advances to the metaphysis, giving the bone a fusiform-like appearance (Fadly and Nair, 2008). Occasionally, the lesion is focal or eccentric with varying severity from slight exostosis to extensive asymmetrical enlargement with obliteration of the bone marrow cavity. Long-standing cases have thickened periosteum; and when removed, porous irregular surface of hard osteopetrotic bone is revealed (Fadly and Nair, 2008).

Early stages are characterized by slight splenomegaly. However, later on, splenic atrophy occurs as well as premature bursal and thymic atrophy. Lymphoid leucosis may occur in individual birds with osteopetrosis (Fadly and Nair, 2008).

Microscopically, the thickened periosteum is as a result of increased number and size of basophilic osteoblasts. There is increased number of osteoclasts per tibia, but the density of osteoclasts is decreased (Hayward and Neel, 1981). Spongy bone converges centripetally towards the shaft centre. There is increased size and irregularity of haversian canals, as well as increased size, number, and alteratered position, of lacunae. Osteocytes are numerous, large and eosinophilic. The new bone is fibrous and basophilic (Fadly and Nair, 2008).

The blood picture is aleukemic, with secondary anaemia. Active erythropoiesis occurs in the remaining bone marrow and in focal areas of the liver. Viruses causing osteopetrosis may induce an aplastic anaemia (Price and Smith, 1981). Ultrastructurally, viral particles bud transiently from osteoblasts and osteocytes but not from osteoclasts (Fadly and Nair, 2008).

2.5.2.9 Other types of tumours

Epithelial tumours can be caused by ALV, but are uncommon. They are observed during experimental infections with acutely transforming viruses, although some have occurred in natural and experimental infections with ALV-J. Strains BAI-A (Fadly and Nair, 2008) and HPRS-103 (Payne *et al*., 1992) of ALV have induced the granulosa cell tumours and carcinomas

of the ovary. Testicular seminoma has been reported in a bird innoculated with strain MH2 (Beard, 1980). Strains of MC29, MH2, and HPRS-103 ALVs; may induce adenocarcinomas of the pancrease (Payne *et al*., 1992). Squamous cell carcinomas have been reported in chicks infected with MC29 and MH2 strains (Beard, 1980). Strains of MC29 and MH2 have induced hepatocarcinomas (Lapis, 1979; Beard, 1980). Another epithelial tumour induced by ALV-J includes cholangioma (Payne *et al.*, 1992). Strains MC29 and HPRS-103 of ALV may induce mesotheliomas (Fadly and Nair, 2008).

2.6 Differential diagnosis of avian leucosis

2.6.1 Marek's disease and reticuloendotheliosis

Avian leucosis must be differentiated from MD and RE, however, this is sometimes difficult in field situations as the clinical signs and gross pathologic lesions of these diseases overlap (Davidson, 2001).

An important difference between LL and MD is that, in LL, gross lymphomas occur in the bursa of Fabricius, and the tumour has an intrafollicular origin and proliferation pattern (**Table 1**). In MD, where the bursa is involved, the lymphoproliferation is diffuse interfollicular and less apparent grossly. The peripheral nerve lesions are present in MD but not in LL (OIE, 2010).

Reticuloendotheliosis rarely occurs in field situations, and whenever it occurs, it presents with similar clinical signs, gross and histopathological lesions as AL (Witter and Fadly, 2003).

There have been reported cases of mixed infections of these viruses (Cui *et al*., 2009) which probably, may alter the natural pattern of these diseases hence therefore resulting in different clinical signs and lesions. Thus, mixed infections may at times cause difficulties in tumour diagnosis (Fadly and Nair, 2008).

Co-infection with some of these viruses may often complicate diagnosis of myeloid tumours. Thus in chicken with multiple tumour virus infections, it is important to base diagnoses on tumour-specific criteria rather than on virological tests (Witter *et al*., 2005).

Table 1: Features used in differentiating Marek's disease, lymphoid leucosis and reticuloendotheliosis

Features	**Marek's Disease**	**Lymphoid leucosis**	**Reticuloendotheliosis**
Clinical signs	Frequently paralysis (wings and legs)	Non specific	Non specific
Incidence	Frequently above 5% in unvaccinated flocks	Rarely above 5%	Rare
Microscopic lesions			
Neural involvement	Yes	Yes	Infrequent
Liver tumours	Often perivascular	Focal or diffuse	Focal
Bursa of Fabricius	Interfollicular tumours and/or atrophy of follicles	Intrafollicular tumour	Intrafollicular tumour
Central nervous system involvement	Yes	No	No
Lymphoid proliferation in skin and feather follicles	Yes	No	No
Tumour cytology	Pleomorphic lymphoid cells including lymphobasts, small to large lymphocytes and reticulum cells	Lymphoblasts	Lymphoblasts
Neoplastic lymphoid cell involved	T-lymphocyte	B-lymphocyte	B-cell
Age of onset	4-6 weeks or older	16 weeks	10 weeks

Source: OIE, 2010

Key

%-Percentage

2.7 Diagnosis of avian leucosis

Diagnosis based on clinical manifestations of ALSV, might be unreliable as most strains do not produce visible morphologic changes (Tomar and Saxena, 2007). Pathological diagnosis is based on the presence of nodular tumours predominantly in the bursa of Fabricius and other organs. Microscopically, these are composed of a homogenous population of immature lymphoblasts (Fadly and Nair, 2008).

Conventional diagnostic procedures of ALVs mainly consist of isolation and identification of the virus (Fadly and Witter, 1998); both direct and indirect biological, molecular and serological assays (Tomar and Saxena, 2007). Direct biological assays measure p27 antigen either in fluid or in tissue samples by immunological tests like ELISA, complement fixation test and radioimmunoassay or immunocytochemical staining procedures like immunofluorescence test, immunoperoxidase test, anti- peroxidase assay and protein A-gold assay, respectively. Among these, ELISA for direct assay of p27 antigen is the commonest test (Tomar and Saxena, 2007).

Enzyme linked immunosorbent assay has been developed to detect ALV specific group antigen and can be applied to a variety of samples, which include feather pulp, cloacal swabs, sera, leukocytes, albumen, comb tissue and meconium (Clark and Dougherty, 1980). Due to the fact that both endogenous and exogenous ALVs share the group specific antigen (p27), direct biological assays based on detection of p27 cannot be relied upon to differentiate between these two groups of viruses in samples such as serum (Payne *et al*., 1993). Hence therefore, a combination of virus isolation and ELISA test is the standard procedure for differentiating between endogenous and exogenous ALVs (Fadly *et al*., 1989).

Indirect biological assays detect presence of exogenous and endogenous ASLV infections by employing tests such as phenotypic mixing (Okazaki *et al*., 1975), resistance inducing factor (Rubin, 1960) and non-producer cell activation assay (Rispens *et al*., 1970).

Among the serological assays, the most specific method for detecting neutralizing antibodies against gp85 envelope antigens of ALV is the virus neutralization test (Mizuno *et al*., 1970). Antibody to ALV is measured by its reaction with RSV, and virus neutralization is determined by reduced number of foci induced by RSV (Fadly and Payne, 2003). Microneutralization test using ALV as indicator virus has also been used (Fadly and Witter, 1998). Virus neutralization is determined by an ELISA on culture fluids, whereby positive ELISA indicates no antibody, whereas a negative ELISA indicates neutralization of ALV and antibody presence (Tomar and Saxena, 2007).

Direct molecular assays; usually analyze the 'pol' coded reverse transcriptase enzyme, which is essential in multiplication of all retroviruses including ALVs (Gallo, 1973). Reverse transcription polymerase chain reaction techniques (RT-PCR) have been developed to detect and distinguish between endogenous and exogenous ALVs, and are more sensitive for the detection of ALV than ELISA (Pham *et al.*, 1999; Bagust *et al*., 2004). However, such molecular assays are expensive and unsuitable for field conditions; and serological assays only help identification of viral envelope subgroups (Tomar and Saxena, 2007).

2.8 Prevention and control of avian leucosis

The eradication of ALV from primary breeding flocks, though difficult to achieve, is the most effective means for controlling ALV infection in chicken (Fadly *et al*., 1981; Wang *et al.*, 2007). Currently, instituted programs for control of AL infections in breeding flocks are based on

selective breeding and elimination of dams that test positive for the virus (Fadly and Smith, 1999; Qin *et al.,* 2013).

Developing commercial chicken strains free of endogenous retroviruses (Bacon *et al*., 2004) is possible through transgenesis using primordial germ cells (van de Lavoir *et al*., 2006).

There have been recent suggestions that the unusually high rate of horizontal transmission (Fadly and Nair, 2008; Qin *et al.,* 2013) and high frequency of antigenic and molecular variation (Silva *et al*., 2007; Venugopal *et al*., 1998) of ALV-J may interfere with success of eradication programs of ALV-J among broiler breeder flocks; thus development of commercial chicken strains free of endogenous retroviruses may be beneficial in its eradication (Bacon *et al*., 2004).

Biosecurity is the first defence against any disease and should be applied to any scheme aiming at controlling and eradicating ALVs. Vaccination is on the trial stage, although congenitally infected chicks are immunologically tolerant and, thus, cannot be immunized, even if a suitable vaccine was available (Tomar and Saxena, 2007).

However, control of ALV infection mainly depends on early detection and removal of virus shedding birds so as to reduce spread of congenital and contact infection in other birds (Qin *et al.,* 2013). Eradication of ALV infection will solely depend on breaking vertical transmission of virus cycle from the dam to progeny (Tomar and Saxena, 2007).

3.0 CHAPTER THREE: MATERIALS AND METHODS

3.1 Study area

The study was carried out in Nairobi and surrounding counties, which included areas of Kiambu, Machakos and Kajiado Counties. Nairobi County is the capital city of Kenya and occupies an area of approximately 696 square kilometres. It lies between 01° 17´S latitude and 36° 48´E longitude (**Figure 1**). Nairobi has two main agroecological zones; lower highland with an altitude of between 1820 m (metres) and 2070 m above sea level and the other upper midland with an altitude of between 1200 m and 1820 m above sea level. There are two rainfall seasons in these areas. The long rainfall season is in the months of April to June, while the short rainfall season is in the months of October to December. The estimated annual rainfall is a maximum of 765 mm (millimetres) and a minimum of 36 mm. Machakos County borders Nairobi to the East, Kiambu County is adjacent to the northern border of Nairobi County, while Kajiado County borders Nairobi to the South (Wikipedia, 2015).

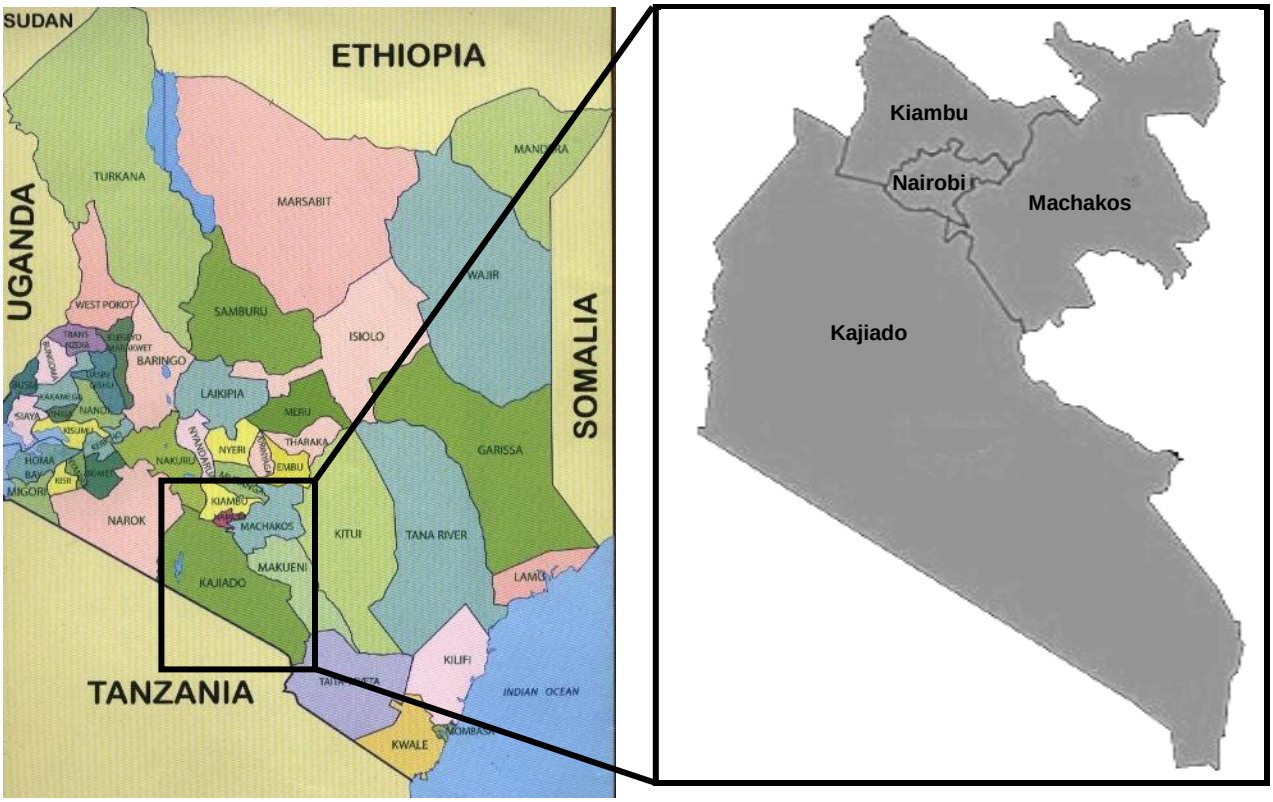

Figure 1: Map of Kenya showing Nairobi and surrounding counties

(Source: KNBS, 2014; Geographic Information Systems version 2.4.)

3.2 Study design

The study was carried out both retrospectively (post mortem records' for a period of 10 years, 2003-2012) and prospectively.

3.2.1 Retrospective study

Post mortem records of necropsies that were conducted on chicken presented to VPMP and CVL were analyzed. All cases which originated from Nairobi and surrounding counties were examined.

The archival post mortem records were stored in hard copy files. Post mortems done in each of the years had been recorded in a separate file and record sheets for each year. Diagnoses in the post mortem records were taken as the final diagnosis on the respective cases.

January 2003 to December 2012 post mortem records were manually retrieved to access the relevant data on AL. Data regarding clinical history, clinical manifestations, pathological lesions and source hatchery of the chicken presented was collected, and recorded into data collection sheets. This was then entered, stored and analyzed using Microsoft Excel (Microsoft Corporation) and statistical package, Stata 2011. ®

The carcasses of chicken brought for post mortem examinations at VPMP or CVL as well as the sacrificed chicken were obtained from smallholder individual farmers and large commercial poultry producing farms in Nairobi and its peri-urban areas of Kiambu, Machakos and Kajiado Counties. All the cases that had details of post mortem findings and poultry identification biodata were included in the study.

3.2.2 Prospective study

3.2.2.1 Seroprevalence study

Minimum sample size for the study was calculated as described by Martin *et al*. (1987);

$$n = \frac{z^2 . p.q}{e^2}$$

$$n = \frac{(1.96)^2 * 0.5 * 0.5}{0.05^2} \qquad n = 384$$

Where n = minimum sample size, p = prevalence rate (0.5), q= (1-p), e= precision error rate (0.05), z= 1.96

The percentage prevalence caused by this disease in chicken was calculated by dividing the total number of chicken that were diagnosed with AL by the overall number of chicken that were sampled and multiplying the answer by 100. This was done as follows:

$$\%\text{prevalence of AL} = \frac{\text{Total number of chicken diagnosed with AL}}{\text{Total number of chicken sampled}}$$

Seroprevalence study involved a convenience surveillance using an antigen capture (ac) ELISA kit. Lists of the flock/farm/abattoir establishments in the randomly selected county wards were obtained from the local veterinary or agricultural offices. These chicken establishments were randomly selected and a total of 385 (proportional to population size by county) (**Table 2**) blood samples randomly collected from 180 indigenous chicken (12-96 weeks old), 103 exotic broiler chicken (6-10 weeks old) and 102 exotic commercial layer chicken (8-65 weeks old) in the study

area. A total of 51, 203, 45 and 86 birds were sampled from Nairobi, Kiambu, Kajiado and Machakos Counties respectively; proportional to population size of these birds.

Table 2: Chicken population figures in Nairobi and surrounding counties and sampled tally (KNBS, 2014)

County	**Population size**		**Number sampled**		**Total sampled**
	Indigenous chicken	Commercial chicken	Indigenous chicken	Commercial chicken	
Nairobi	279,397	342,788	23	28	51
Kiambu	801,070	1,683,565	65	138	203
Kajiado	267,913	276291	22	23	45
Machakos	862,592	182,952	70	16	86
Total					385

3.2.2.2 Clinical and pathological examinations

Based on chicken post mortem examinations carried out at VPMP and CVL, ten farms; Nairobi (2 farms), Kiambu (5 farms), Kajiado (1 farm) and Machakos (2 farms) were identified and investigated from whose cases were diagnosed with AL. Diagnosis was based on flock histories, clinical and gross pathological lesions of AL as well as histopathology. The identified farms were then visited and upon arrival, a questionnaire (**Appendix 1**) was administered to the appropriate personnel present.

Fifty chicken (forty nine exotic commercial layers and one indigenous chicken) were randomly sampled from the ten identified farms (Appendix 2) whereby physical examination of the birds was done at farm level before transporting them to VPMP for blood sample collection and necropsy. The sampling of chicken lasted for five months, starting from February to June, 2014.

3.2.2.2.1 Mortality rates of chicken in identified farms

The number of chicken that died from onset of the disease in each farm was recorded (as reported by farmer in the questionnaire administered). This number was divided by the total number of chicken on the farm before onset of the disease and multiplied by 100 to get the mortality rate (s).

3.2.2.2.2 Examination and isolation of chicken for sample collection

Clinical examinations were done in the randomly selected chicken at the farm, in the farms visited. The observations were recorded in a clinical score card and observation sheet (**Appendix 1**). These were then transported to VPMP poultry diagnostic clinic using well ventilated carrier cages. Personal protective clothing such as disposable gloves, laboratory coats and gumboots were used; and subsequently cleaned and disinfected using Omnicide® (Glutaraldehyde and

coco-benzyl-dimethyl ammonium-chloride, Cooper-K Brands, Kenya), after visiting each farm/and before visiting other farm (s).

3.2.2.2.3 Post mortem examination

From each farm visited, five live chicken were randomly sampled and purchased. After external examination, the chicken were humanely killed by dislocation of the atlanto-occipital joint, followed by severing of the carotid arteries and jugular veins using a scalpel blade. Each carcass was subjected to a routine post mortem examination as described by Charlton (2006). Lesions were recorded (**Appendix 3**) with special attention to various changes in organ size, colour and transactional appearances. Two peripheral nerves were also grossly examined and processed for histopathology to rule out other diseases such as MD (Fatumbi and Adene, 1984). Samples of the liver, spleen, kidneys, proventriculus, heart, sciatic nerves and other organs grossly affected by tumours or not were collected and fixed in 10% neutral buffered formalin for histopathologic examination. The carcasses were then disposed off in a secured departmental disposal pit after proper disinfection of all in contact surfaces and materials during the post mortem using Omnicide®(Glutaraldehyde and coco-benzyl-dimethyl ammonium-chloride, Cooper-K Brands, Kenya). Photographs of the lesions were taken using a digital camera (Sony Cyber-shot DSC-W810 Digital Camera, 20.1 Mega Pixel W Series 6X Optical Zoom, Magnifications 40X, 100X, 400X, Sony Asia Pacific®) and transferred into a computer and labelled appropriately.

3.2.2.2.4 Tissue processing for histological examination

Tissue samples from each bird were fixed in 10% buffered neutral formalin for 48 hours. The tissues for histology were then processed using the paraffin wax method according to Carson and Hladik (2009) as briefly described here.

The tissues were trimmed to a thickness of 3 mm and labelled with a tag. They were dehydrated in graded concentrations of alcohol (70%, 80%, 90%, 95% and 100%) at one hour intervals. They were cleared with xylene for two hours, infiltrated with molten paraffin wax at 60ºC for three hours and embedded in paper boats with molten wax to a solid cast, fixed onto a wooden block using a hot searing spatula. The specimens were cut to 5µm thickness using a microtome, floated on a water bath at 50º C to flatten it out, mounted on a labelled grease free, clean glass microscope slide and dried in an oven at 60ºC for one hour. They were dewaxed in three changes of xylene, each for five minutes. Tissues were rehydrated in descending grades of alcohol to 90-80-70-50% and then put into distilled water for five minutes in each stage. Tissue sections were stained using haematoxylin and eosin. These were dehydrated using ascending concentrations of alcohol from 50-70-80-90%and cleared using two stages of xylene for 5 minutes in each stage. A cover slip was applied using Destrene 80 dibutylphthalate and xylene (DPX) as a mountant and left to dry before examination.

The histological sections were studied using a light microscope at X40, X100 and X400 magnifications. Lesions were recorded according to the organ affected, tissue involved and type of lesion. Photomicrographs of the lesions were captured using a digital camera (Digital Resolution Camera, Magnifications 40X, 100X, 400X, LG Innotek®), transferred to a computer and labelled appropriately.

3.2.2.3 Blood collection and serological diagnosis

3.2.2.3.1 Collection and processing of blood

Blood samples from birds in the seroprevalence (385) and prospective (50) study were collected from the brachial vein. Two millilitres of blood were aseptically collected from the brachial vein of each chicken using sterile 23 gauge hypodermic needles and syringes, and put into universal

bottles (without anticoagulant). Serum was separated from the blood and extracted using the standard procedure (OIE, 2008). Sera was then dispensed into serum vials, labeled using an indelible marker and stored frozen at -20^{0}C (NVSL, 2006) until tested.

3.2.2.4 Enzyme linked immunosorbent assay

Extracted sera were tested using a colorimetric sandwich ELISA for the presence of ALV group specific antigen (p27) as described by the manufacturer (AffiniTech, 2009). The AffiniTech ALV p27 antigen ELISA test kit included: Antigen Wells (12 X 8 strips wells coated with α-ALV), Sample diluent (red buffer with protein stabilizers), Wash solution, Positive control, Negative control, Conjugate (α-p27 IgG alkaline phosphatase), Substrate (ρ -Nitrophenyl phosphatase), and a Stop solution (3.0 M NaOH). The intensity of the colour produced from the ELISA test was measured photometrically at 405 nm primary wavelength and 630 nm secondary wavelength using an ELISA reader (MR-96A Microplate Reader®, Mindray Medical International Limited, Shenzhen, China). The ELISA was carried out at the Department of Public Health, Pharmacology and Toxicology, University of Nairobi (PHPT).

The sample diluent was prepared for use by adding 15 µl (microlitres) of deionized water to 5 µl of the sample diluents, while the wash solution was prepared for use by adding 475 ml of deionized water to 25 ml of the wash solution (AffiniTech, 2009). Other reagents (Positive control, Negative control, Conjugate and Stop solution) were supplied ready for use. The ELISA units (EUs) for all samples tested on the AffiniTech ALV Antigen Detection

Test Kit was calculated using the formula below (AffiniTech, 2009):

The Positive control value was set at 100 ELISA Units (EUs).

$$\frac{\text{Average Absorbance (Test Sample)-Average Absorbance (Negative)}}{\text{Average Absorbance (Positive)-Average Absorbance (Negative)}} = Sp$$

Sp * (100) = ELISA Unit (s)

Key

Sp-Sample to Positive Ratio

The ELISA unit values obtained were interpreted as recommended by the manufacturer and as modified by Emikpe *et al.* (2007). Enzyme-linked immunosorbent assay units (EUs) less than 10 were considered negative, while EUs greater than 10 were considered positive for ALV p27 antigen. Enzyme-linked immunosorbent assay units (EUs) of 10-24 were considered weakly positive, EUs values of 25-75 were considered moderately positive, while EUs greater than 75 were considered strongly positive for ALV p27 antigen. Enzyme-linked immunosorbent assay unit values obtained indicate the level of antigens in a sample tested, with higher readings indicating higher antigen titres and vice versa (Emikpe *et al*., 2007)

3.3 Data analysis

The data collected was coded and put into Microsoft Office Excel 2007 and exported to the statistical package, Stata 2011® for descriptive statistics. The distribution pattern of AL in those 10 years was computed in Microsoft excel, analyzed using proportion and simple percentage methods.

Pearson's correlation was used to determine the relation between detecting ALV antigen and the clinical and pathological manifestations.

Chi square was used to determine the significant difference between detecting ALV antigen and the different types of chicken sampled in the study.

4.0 CHAPTER FOUR: RESULTS

4.1 Retrospective study

4.1.1 Prevalence, clinical and pathological manifestations of avian leucosis in chicken in the period 2003 to 2012

A total of 3,721 (947; 2774) chicken cases from Nairobi and surrounding counties were submitted to VPMP and CVL respectively for post mortem examinations and/or laboratory analysis, between the years January 2003 and December 2012. Of these, 188 (5.05%) chicken cases were diagnosed with AL (**Appendix 5**). One hundred and eighty three (183) cases were post mortem, 4 cases were cloacal swabs; and one (1) case was a tissue fixed in 10% neutral buffered formalin.

Commercial layers comprised 92.5% (174/188) (8-52 weeks old), broilers 3.7% (7/188) (3-36 weeks old), indigenous chicken 1.1% (2/188) (age not indicated) and 2.7% (5/188) chicken whose type and age were not indicated.

Clinical presentations of chicken diagnosed with AL were analyzed (**Figure 2**). The common presenting clinical signs recorded were diarrhoea at 32.61% (60/184), death 32.07% (59/184), weakness 28.26% (52/184) and anorexia 25% (46/184). The least observed signs were blindness, delayed laying, cannibalism, skin swellings, mouth and nasal discharges that were observed in 0.54% (1/184). Other observed signs include dullness (23.37% (43/184), paralysis 6.52% (12/184), weight loss 5.98% (11/184), drowsiness 5.43% (10/184), drooping wings 3.80%(7/184), inappetence 3.80% (7/184), lameness 3.80% (7/184), ruffled feathers 3.80% (7/184), torticollis 3.8% (7/184), reduced egg production 2.72% (5/184), stunted growth 2.17% (4/184) and snoring 1.09% (2/184).

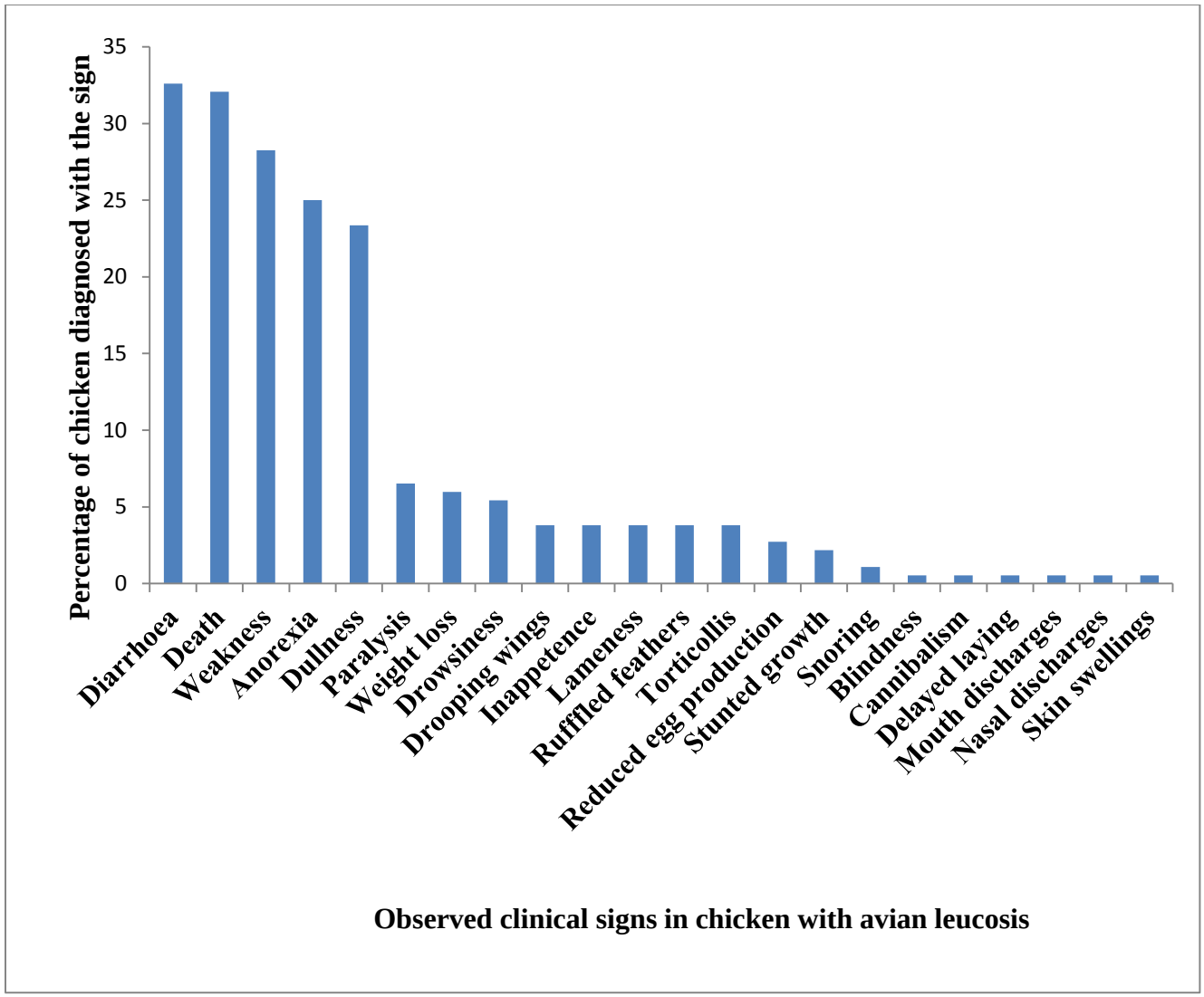

Figure 2: Type and percentage of clinical signs in chicken diagnosed with avian leucosis in the period 2003 to 2012 in Nairobi and surrounding counties

Gross lesions observed on post mortem examination (**Figure 3**) comprised of organomegaly mainly of livers 57.61% (106/184), spleens 49.46% (91/184) and kidneys 28.26% (52/184). Many carcasses were emaciated 50% (92/184). The least observed post mortem lesions were epicardial grayish foci 2.72% (5/184), cyctic ovaries (2.72%) (5/184) and enlarged sciatic nerves (2.72%) (5/184). Other gross lesions observed include nodular liver lesions 27.72% (51/184), enteritis 20.11% (37/184), cardiomegaly17.93% (33/184), nodular spleen lesions 13.59% (25/184), nodular intestinal lesions 9.78% (18/184), nodular kidney lesions 9.24% (17/184), enlarged proventiculi 8.70% (16/184), regressed ovaries 8.70% (16/184), peritonitis 7.61% (14/184), pneumonia 6.52% (12/184), enlarged bursae 5.98% (11/184), nodular skin lesions 4.89% (9/184), congestion of visceral organs 4.35% (8/184) and air sacculitis 3.80% (7/184). Livers, kidneys, hearts, spleens, lungs and proventriculi had focal to diffuse neoplastic lesions in 7.61% (14/184) cases that were confirmed on histopathology. Mononuclear cell (mainly lymphocytes) infiltrations with mitosis were observed in these organs.

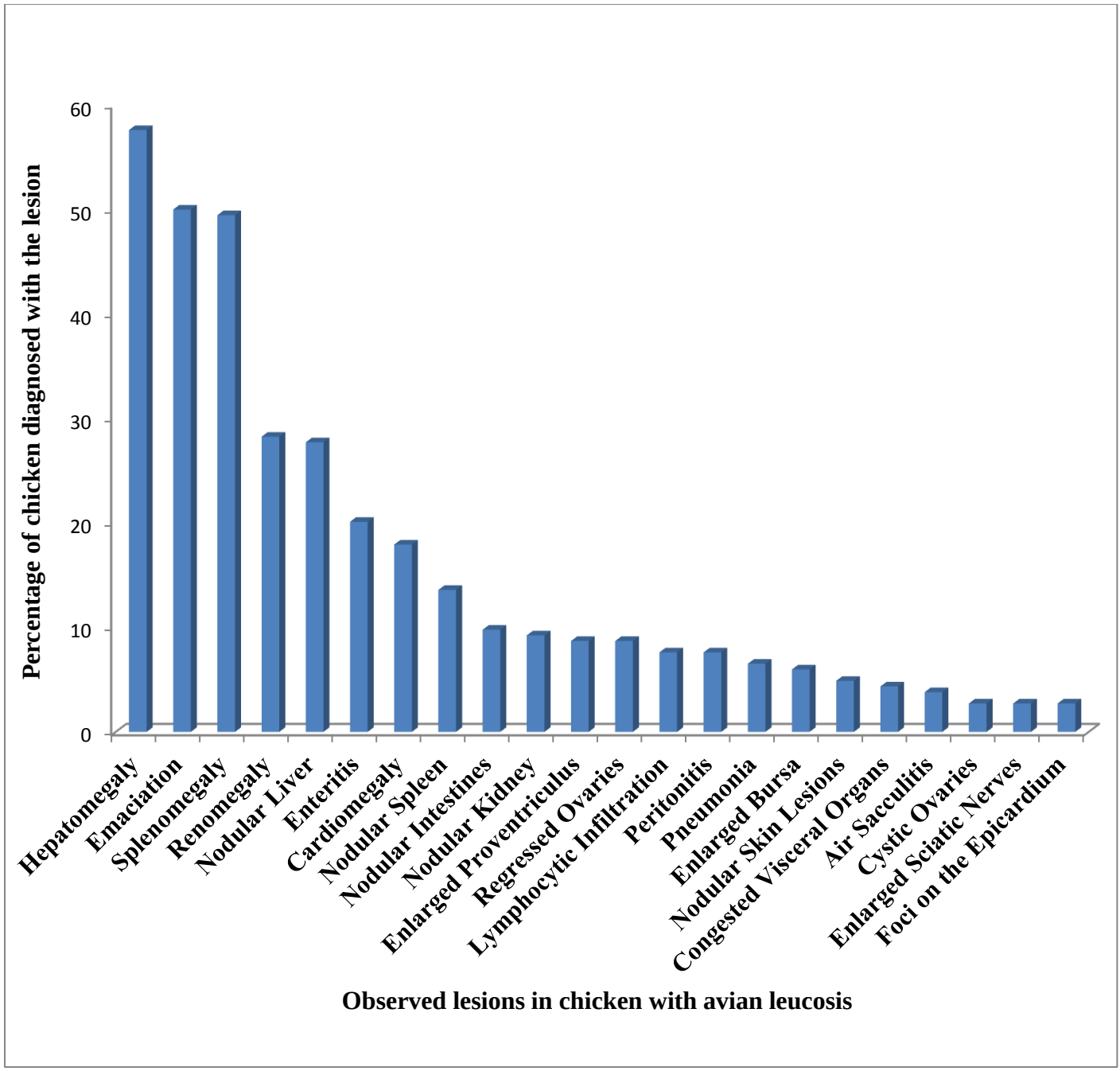

Figure 3: Types and percentage of lesions observed in avian leucosis chicken in the period 2003 to 2012 in Nairobi and surrounding counties (Source: VPMP, CVL)

4.1.2 Yearly occurrence of avian leucosis in chicken

There has been a steady rise in the number of AL cases with the year 2012 recording the highest occurrence of 13.25% (42/317) unlike in 2003 with 1.35% (3/222) cases. The year 2005 had least number of AL cases of 0.29% (1/346). The prevalence rate of AL from 2003 to 2009 was 1.35% (3/222), 2.14% (5/234), 0.29% (1/346), 1.15% (7/611), 3.77% (16/424), 5.07% (19/375) and 1.80% (6/334), respectively. This was low compared to the last 3 years of the study; 2010 10.53% (38/361), 2011 10.26% (51/497) and 2012 13.25% (42/317), which had high cases ranging from 38 to 51 of each year as shown in **figure 4** ($p < 0.05$).

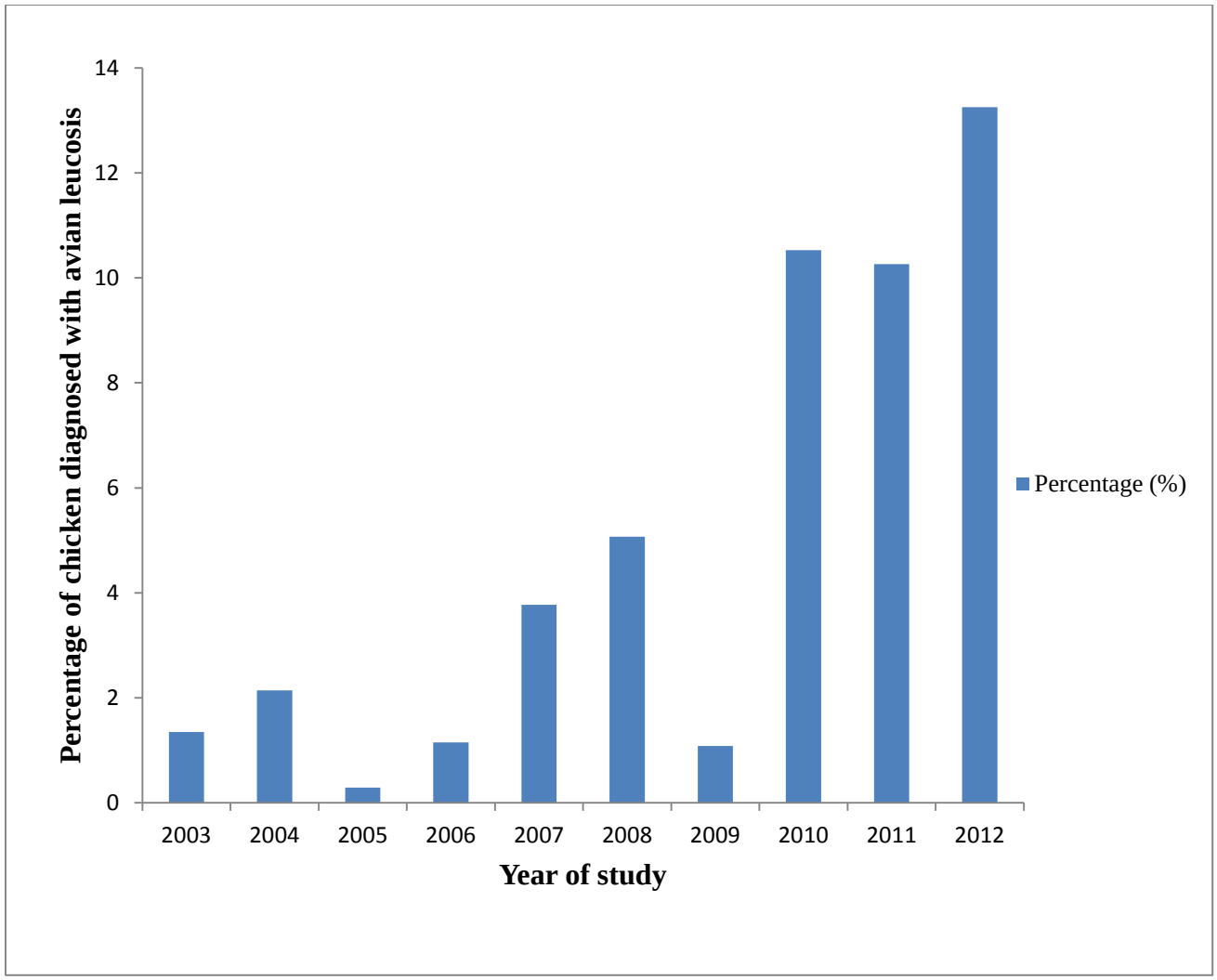

Figure 4: Percentage yearly occurrence of avian leucosis in chicken in the period 2003 to 2012 in Nairobi and surrounding counties (Source: VPMP, CVL)

4.1.3 Prevalence of avian leucosis in different counties and hatcheries

Kiambu County was the main source of these birds with 56.4% (106/188), followed by Nairobi County 27.1% (51/188), Machakos County 10.1% (19/188) and Kajiado County with 6.4% (12/188) (**Figure 5**). Majority of these cases were reported in the rainy season (April-June; October-December) 53.2% (100/188) while the dry season (January-March; July-September) recorded 46.8% (88/188).

Most chicken with AL had originated from seven hatcheries serving the region; but the source of 33.51% (63/188) of positive AL birds could not be established (**Figure 6**). These hatcheries are located within Nairobi, Kiambu, Kajiado, Machakos and Nakuru Counties. **Figure 6** also demonstrates various hatchery trends from where the chicken were sourced from between the year 2003 to 2012. Hatcheries with highest number of cases reported from were A-21.81% (41/188); B-19.68% (37/188); C-14.89% (28/188); D-7.98% (15/188); and E-1.06% (2/188). Least cases were reported from F and G both at 0.53% (1/188).

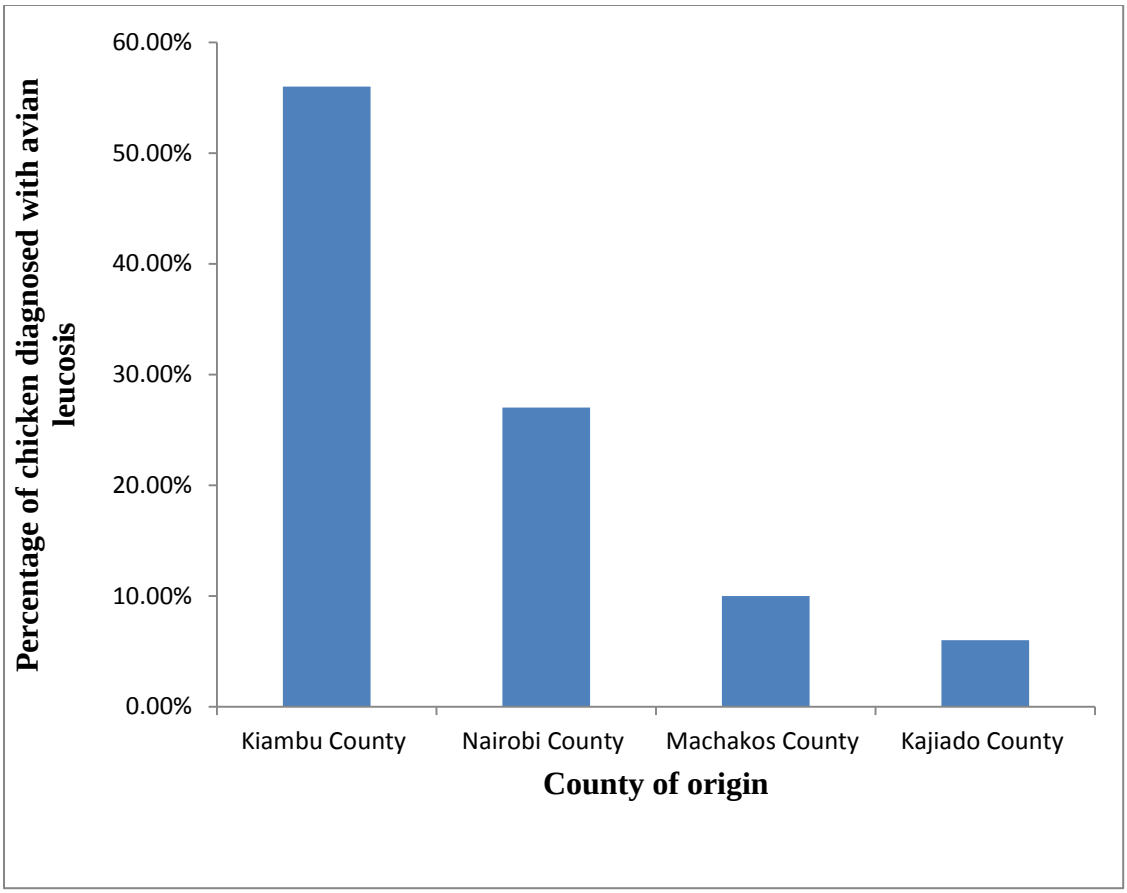

Figure 5: Percentage distribution of chicken diagnosed with avian leucosis in Nairobi and surrounding counties in the period 2003 to 2012 (Source: VPMP, CVL)

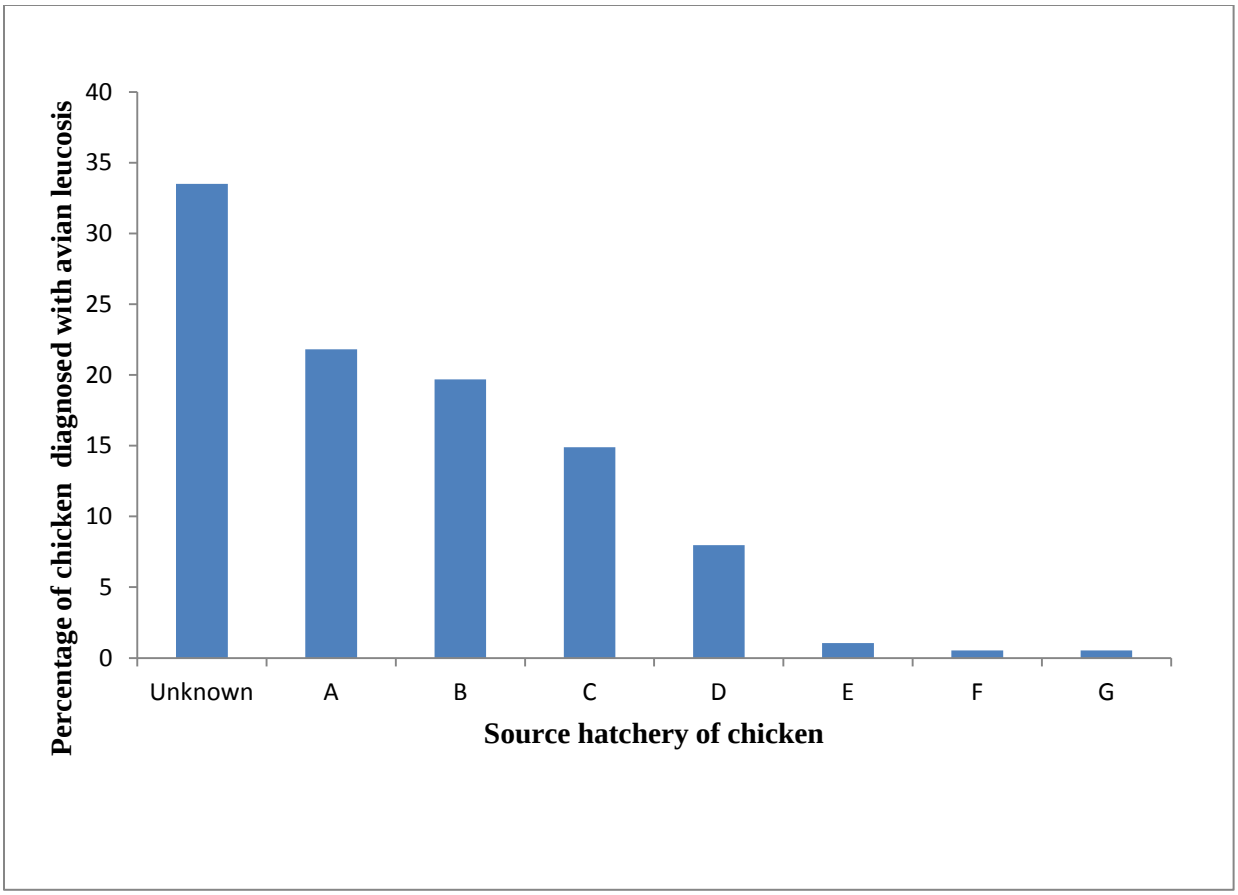

Figure 6: Occurrence of avian leucosis in chicken sourced from various hatcheries (A-G) from 2003 to 2012 in Nairobi and surrounding counties (Source: VPMP, CVL)

4.2 Prospective study

4.2.1 Seroprevalence of avian leucosis

Of the 385 sera samples tested, 131 (34.02%) were positive to ALV p27 antigen (**Table 3**). Nineteen (18.63%) of 102 commercial layers' sera tested positive, while 7 (6.8%) of 103 broilers birds' sera were positive to ALV p27 antigen. A total of 105 (58.33%) of 180 indigenous chicken sera were positive for the ALV p27 antigen. Of the 19 commercial layer sera that were positive to ALV p27 antigen; 11 (57.90%) were weakly positive with EUs ranging from 10% to 25%, 4 (21.05%) sera samples were moderately positive with EUs ranging from 28% to 73%, while 4 (21.05%) sera samples were strongly positive with EUs ranging from 80% to 99% (**Figure 7**).

Among the 7 broiler chicken sera that were positive, 6 (85.71%) were weakly positive with EUs ranging from 10% to 23%, while 1 (14.29%) serum sample was moderately positive with an EU reading of 40%. Eighty eight (83.81%) of 105 indigenous chicken sera were weakly positive with EUs ranging from 10% to 25%, 15 (14.29%) serum samples were moderately positive with EUs ranging from 26% to 37%, while 2 (1.90%) sera samples were strongly positive with EU values of 91% and 93% (**Figure 7**).

Among the sampled birds, Kajiado County had the highest prevalence rate of 77.78% (35/45), followed by Nairobi County 52.94% (27/51), Machakos County 45.35% (39/86) and lastly Kiambu County 14.78% (30/203) (**Figure 8**). There was a significant difference ($p < 0.05$) between detecting ALV antigen in chicken and the types of chicken sampled in the study.

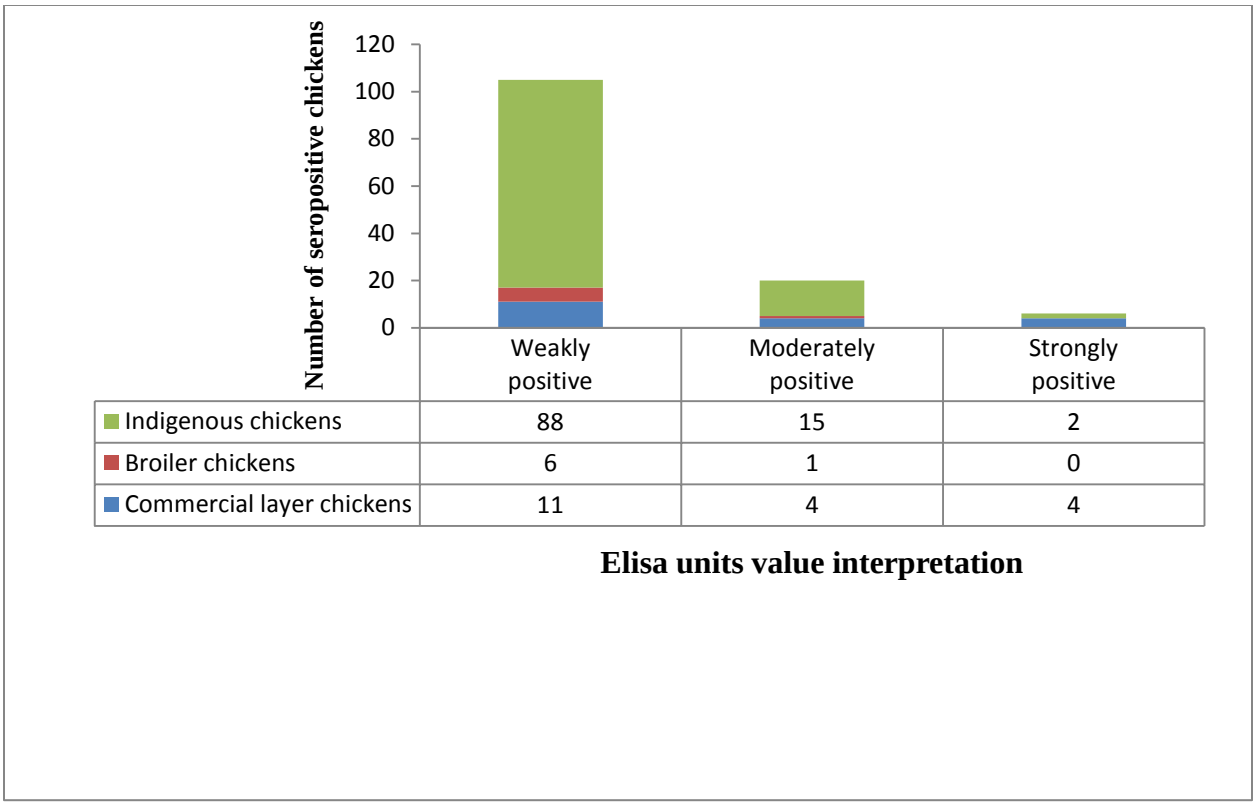

Figure 7: Enzyme linked immunosorbent assay unit values in seropositive chicken in Nairobi and surrounding counties

Table 3: Seroprevalence of avian leucosis virus antigen in layer, broiler and indigenous chicken in Nairobi and surrounding counties

Type of chicken	**Number of sera tested**	**Number of sera positive**	**Percentage (%) of sera positive**
Commercial layer chicken	102	19	18.63
Broiler chicken	103	**7**	6.80
Indigenous chicken	180	105	58.33
Total	385	131	34.03

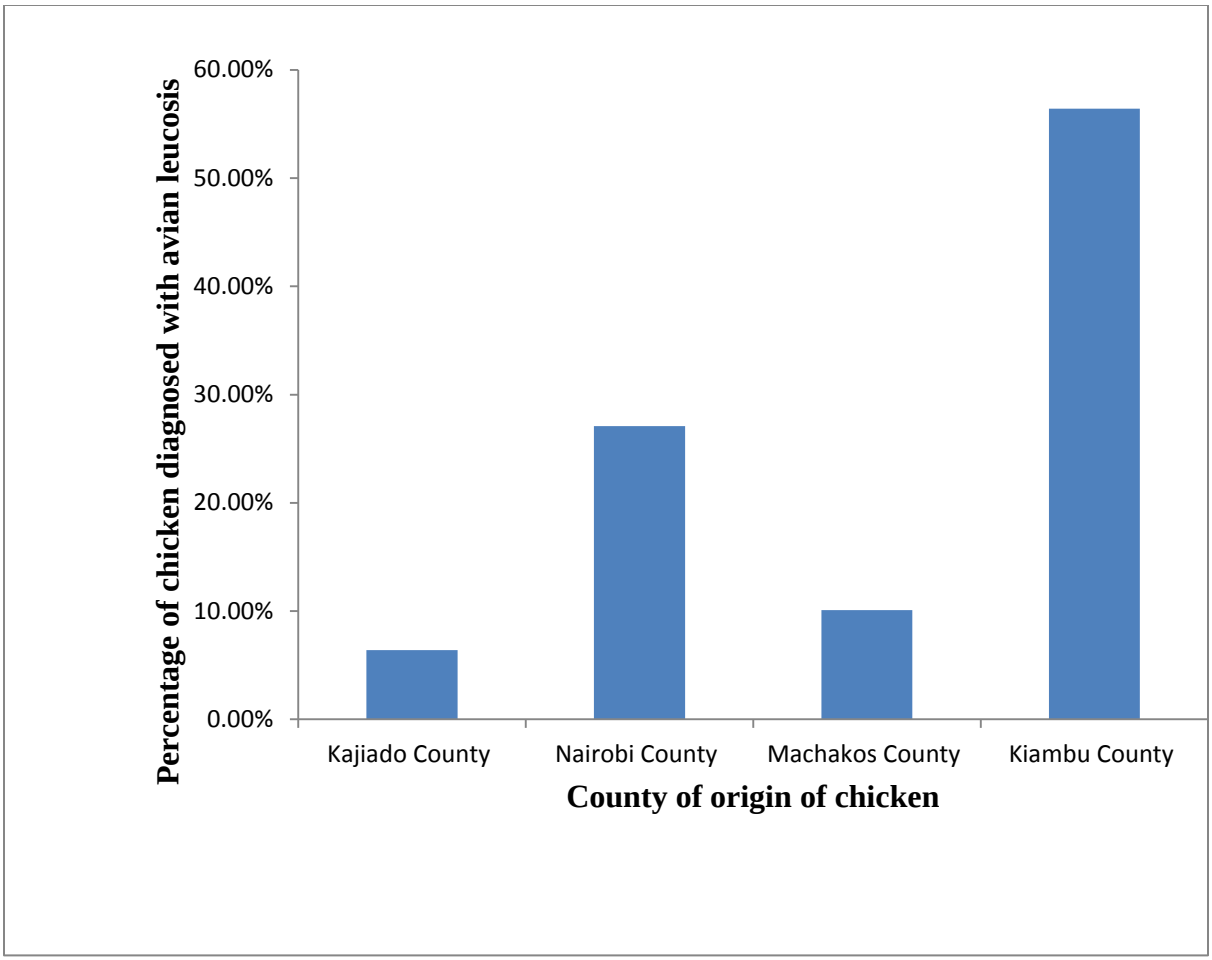

Figure 8: A graph showing counties in Nairobi and surrounding counties from where chicken diagnosed with avian leucosis originated in the period of February to June 2014

4.2.2 Clinical, pathological manifestations and hatchery of origin of birds that had avian leucosis

4.2.2.1 Clinical manifestations

A total of ten farms (5 in Kiambu County, 2 in Nairobi County, 2 in Machakos County and one in Kajiado County) were visited during prospective study. A total of 49 commercial layer chicken and 1 indigenous chicken were examined for clinical presentations indicative of AL with most birds showing combined clinical presentations (**Table 4**). Whitish or brownish diarrhoea (observed in 19 chicken) (**Figure 9**) was the most commonly observed clinical manifestation of AL in birds from eight (8/10) farms except birds from two farms (IV and X) didn't have the sign. Anorexia (observed in 18 chicken) was observed in birds from seven (7/10) farms (III, IV, V, VII, VIII, IX and X). Unthriftiness (observed in 13 chicken) was observed in birds from six (6/10) farms (I, V, VI, VII, VIII and X) while paralysis was observed (in 13 chicken) (**Figure 10**) in birds from five (5/10) farms (I, II, IV, VII and X). Dullness (observed in 10 chicken) was observed in birds from five (5/10) farms (III, IV, VII, VIII and X) and ruffled feathers (observed in 8 chicken) was observed in birds from four (4/10) farms (V, VI, VIII and X). Drowsiness (observed in 2 chicken) was observed in birds from two (2/10) farms (VIII and X). Skin swellings were observed in one (1) chicken from a single farm (VII), cannibalism (observed in 1 chicken) observed in farm (IX) and torticollis (observed in 3 chicken) observed in farm I.

Clinical signs observed in birds from farms whose chicken sera was seropositive to ALV p27 antigen were whitish or brownish diarrhoea (in farms I, II, III, V, VI and VIII), unthriftiness (in farms I, V, VI and VII), paralysis (in farms I, II and VII), dullness (in farm III and VII), ruffled feathers (in farm V and VI), anorexia (in farm V and VII), torticollis (farm I) and skin swellings

(farm VII). There was a correlation between seropositivity and observation of unthriftiness among the birds ($p < 0.05$).

Table 4: Clinical signs observed in chicken on suspect farms harbouring avian leucosis virus antigen in Nairobi and surrounding counties for the period of February to June 2014

Clinical sign (s) observed in chicken	Farms with sick birds and signs shown									
	Farm I	Farm II	Farm III	Farm IV	Farm V	Farm VI	Farm VII	Farm VIII	Farm IX	Farm X
Whitish or brownish diarrhoea	+	+	+	–	+	+	+	+	+	–
Unthriftiness	+	–	–	–	+	+	+	+	–	+
Paralysis	+	+	–	+	–	–	+	+	–	+
Skin swellings	–	–	–	–	–	–	+	–	–	–
Cannibalism	–	–	–	–	–	–	–	–	+	–
Ruffled feathers	–	–	–	–	+	+	–	+	–	+
Anorexia	–	–	+	+	+	–	+	+	+	+
Dullness	–	–	+	+	–	–	+	+	–	+
Torticollis	+	–	–	–	–	–	–	–	–	–
Drowsiness	–	–	–	–	–	–	–	+	–	+

Key

+ Positive

-Negative

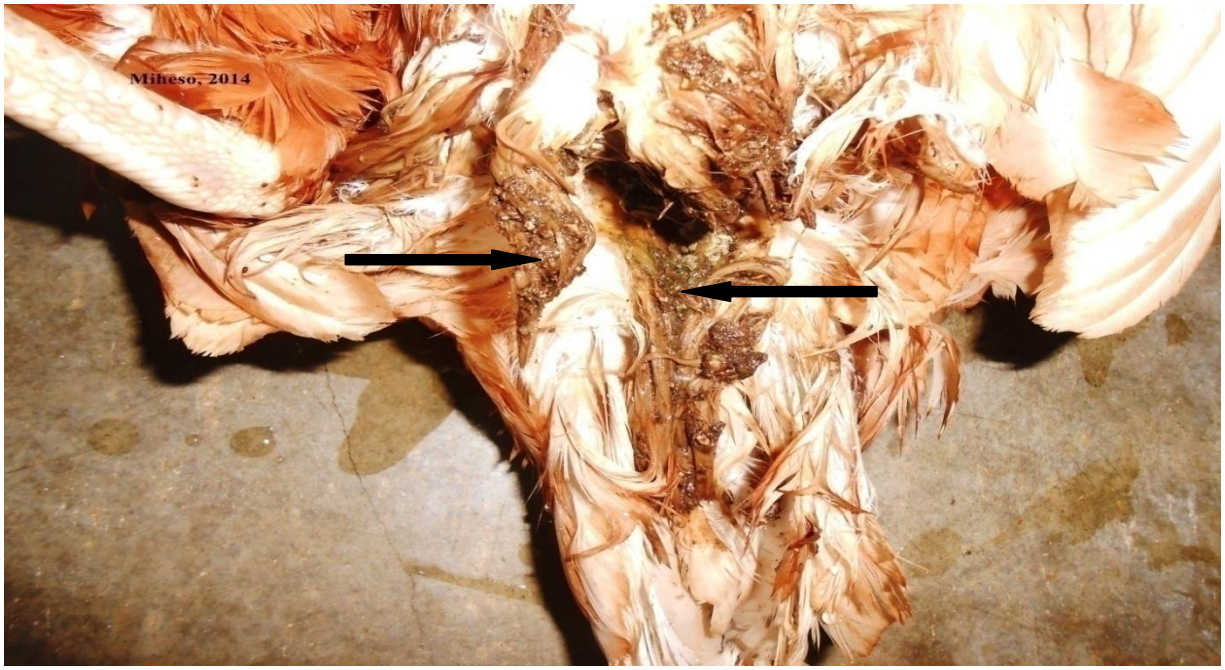

Figure 9: Brownish diarrhoea matting of the cloacal region (arrows) in a commercial layer chicken, Case number B06/2014, obtained from farm VI

Figure 10: Recumbent commercial layer chicken, Case number C01/2014, (18 weeks old) from farm I with wing paralysis (black arrow), and diarrhoea (white arrow)

4.2.2.2 Pathological presentations of chicken suffering from avian leucosis

4.2.2.2.1 Gross lesions

Forty nine commercial layer chicken and one (1) indigenous chicken were examined at post mortem. Gross lesions observed in 50 chicken from ten farms (5 in Kiambu County, 2 in Nairobi County, 2 in Machakos County and one in Kajiado County) (**Table 5**). Most birds had mixed lesions with multiple organs affected.

Twelve emaciated chicken carcasses (**Figure 11**) were observed in all the farms. Kidney enlargements was observed in 11 chicken (in farms I, II, IV, V, VI, VII, IX and X) (**Figure 12**) and spleen congestion was observed in nine chicken (from farm I, II, III, IV, VI, VII, VIII and X); in birds from eight farms. Lesions that were observed in chicken from seven farms include multifocal grayish nodular lesions on the liver (observed in 10 chicken) that were extending into the liver parenchyma that measured 0.5 to 1 cm in diameter (in farm I, II, III, IV, VI, VII and IX) (F**igures 13, 14** and **15**), hepatomegaly was observed in 13 chicken (from farm I, III, IV, V, VI, VII and IX), kidney congestion was observed in seven chicken (from farm II, III, IV, VI, VII, VIII and X) and enteritis was observed in seven chicken (from farm I, II, III, V, VI, VII and VIII). Lung congestion was observed in birds from six farms (observed in six chicken) (from farm II, III, IV, V, VII and X). Lesions that were observed in birds on five farms include thickened proventricular wall observed in five chicken (from farm I, II, III, VI and X) and cardiomegaly which was observed in six chicken (from farm I, II, III, V and VII).

Birds in four farms had distention of the proventriculus which was observed in five chicken (from farm I, II, IV and VII) (**Figure 16**), liver congestion was observed in four chicken (from farm II, III, VII and X), flabby heart was observed in 4 chicken (from V, VI, VII and IX) and adhesion of visceral organs was observed in three chicken (from farm VI, VII, VIII and X). In

three farms birds had enlargement of the bursa of Fabricius that was observed in three chicken (from farm IV, V and VII) and enlarged thigh muscles which were oedematous was observed in three chicken (from farm I, IV and VI). Nodular spleen lesions were observed in chicken in two farms (observed in 3 chicken) (from farm II and VII).

Some lesions were observed in birds from one farm and included ascites (farm VIII), enlargement of the sciatic nerves (farm X), haemorrhagic ovules (farm V), haemorrhagic intestines (farm V), nodular intestinal lesions (farm V) and regressed ovarian follicles (farm V).

Birds from seropositive six farms (I, II, III, V, VI and VII) had gross lesions namely emaciated carcasses, nodular liver lesions and enteritis. Hepatomegaly (I, III, V, VI and VII), renomegaly (I, II, V, VI and VII) and spleen congestion (I, II, III, VI and VII) were observed in birds from five farms. Congested kidneys (II, III, VI and VII) and thickened proventricular walls (I, II, III and VI) were observed in birds from four farms; while splenomegaly (I, II and V), nodular spleen lesions (I, II and VII), enlarged proventriculus (I, II and VII), congested liver (II, III and VII), congested lungs (II, V and VII) and cardiomegaly (I, III and VI) were observed in three farms. Flabby hearts (VI and VII), adhesion of visceral organs (VI and VII) and enlarged thigh muscles (I and VI) were observed in birds from two farms. Lesions observed in birds from one farm included nodular intestinal lesions (V), haemorrhagic intestines (V), haemorrhagic ovules (V) and enlarged bursa of Fabricius (VII).

There was a correlation between seropositivity and occurrence of enteritis ($p=0.0426$), renomegaly ($p=0.0321$), lymphocytic infiltration in various organs ($p=0.0062$) and cardiomegaly ($p=0.0047$) ($p < 0.05$).

Table 5: Gross post mortem lesions observed in chicken on suspect farms harbouring avian leucosis virus antigen in Nairobi and surrounding counties for the period February-June 2014

Post mortem lesion (s) observed in chicken	Farms with sick birds and lesions observed									
	Farm I	Farm II	Farm III	Farm IV	Farm V	Farm VI	Farm VII	Farm VIII	Farm IX	Farm X
Emaciated carcass	+	+	+	+	+	+	+	+	+	+
Hepatomegaly	+	_	+	+	+	+	+	_	+	_
Nodular liver lesions	+	+	+	+	_	+	+	_	+	_
Spenomegaly	+	+	_	+	+	_	_	_	+	_
Nodular spleen lesions	_	+	_	_	_	_	+	_	_	_
Renomegaly	+	+	_	+	+	+	+	_	+	+
Enlarged proventriculus	+	+	_	+	_	_	+	_	_	_
Thickened proventricular wall	+	+	+	_	_	+	_	_	_	+
Congested liver	_	+	+	_	_	_	+	_	_	+
Congested spleen	+	+	+	+	_	+	+	+	_	+
Congested kidneys	_	+	+	+	_	+	+	+	_	+
Congested lungs	_	+	+	+	+	_	+	_	_	+
Cardiomegaly	+	+	+	_	+	_	_	+	_	_
Flabby heart	_	_	_	_	+	+	+	_	+	_
Regressed ovarian follicles	_	_	_	_	+	_	_	_	_	_
Nodular lesions on the intestines	_	_	_	_	+	_	_	_	–	_
Enteritis	+	+	+	_	+	+	+	+	_	_
Haemorrhagic intestines	_	_	_	_	+	_	_	_	_	_

Table 5 (Continues)

Post mortem lesion (s) observed in chicken	**Farms with sick birds and lesions observed**									
	Farm I	**Farm II**	**Farm III**	**Farm IV**	**Farm V**	**Farm VI**	**Farm VII**	**Farm VIII**	**Farm IX**	**Farm X**
Haemorrhagic Ovules	–	–	–	–	+	–	–	–	–	–
Enlarged thigh muscles	+	–	–	+	+			–	–	–
Adhesion of visceral organs	–	–	–	–	–	+	+	+	–	+
Enlarged sciatic nerve	–	–	–	–	–	–	–	–	–	+
Ascites	–	–	–	–	–	–	–	+	–	–

Key

+ Positive

-Negative

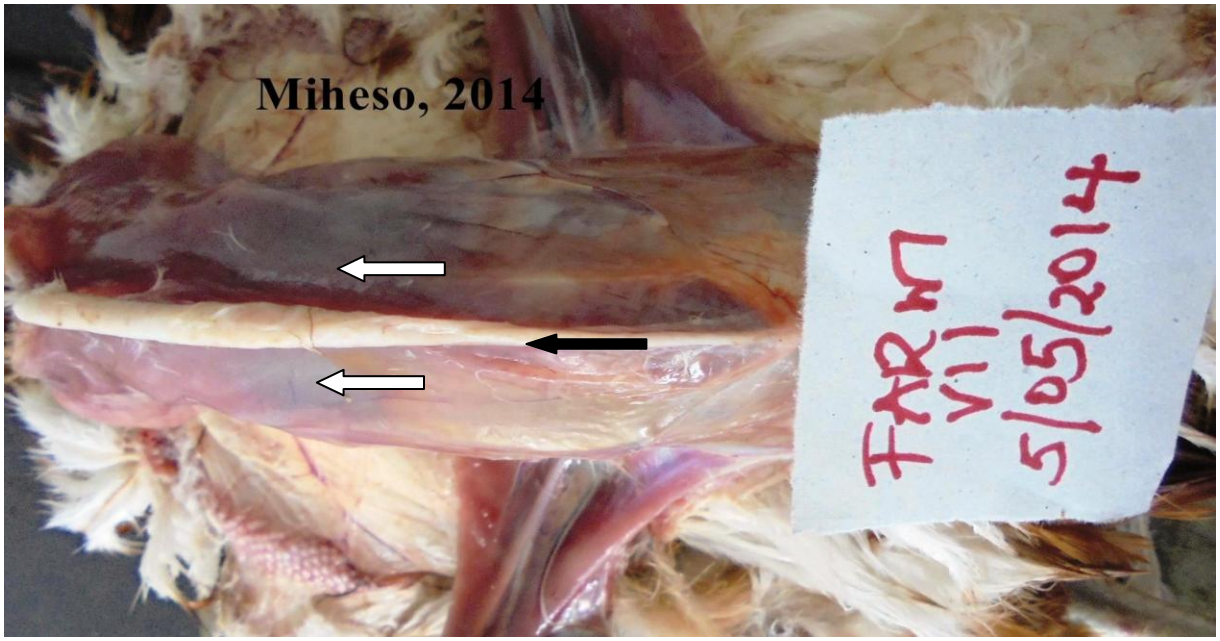

Figure 11: Emaciated carcass of a commercial layer chicken, Case number C07/2014, obtained from Farm VII, showing a prominent keel (black arrow) and poor breast muscle cover (white arrows)

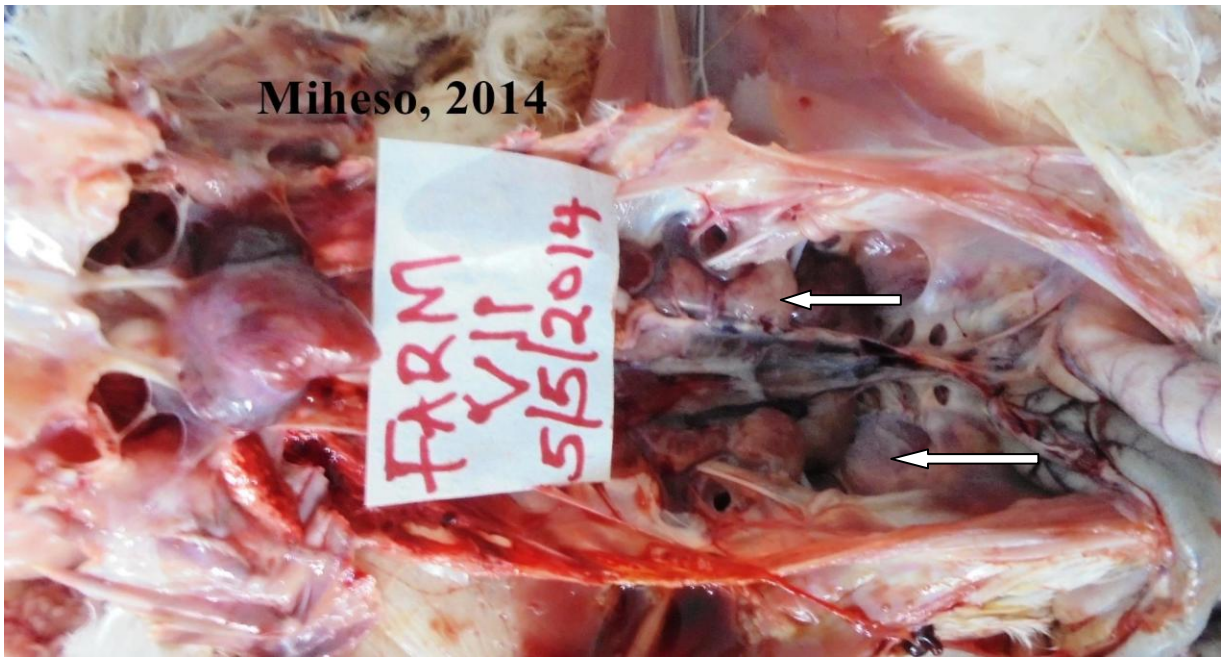

Figure 12: Enlarged kidneys (arrows) in a commercial layer chicken, Case number E07/2014, obtained from farm VII

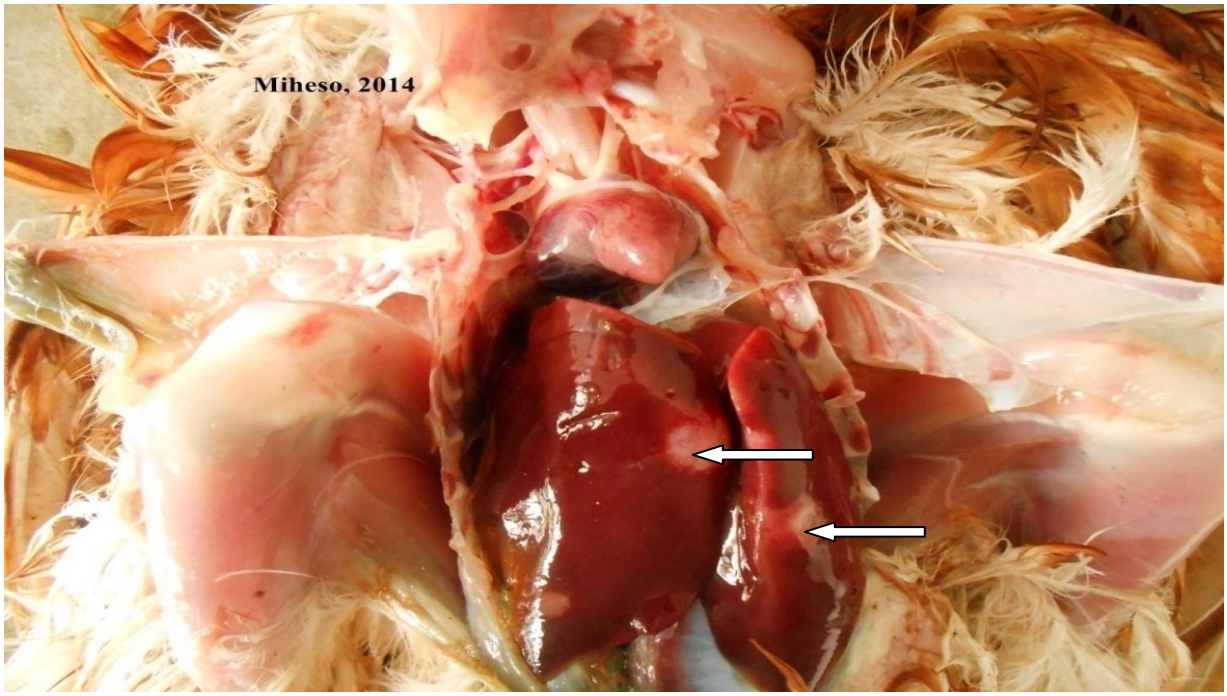

Figure 13: Nodular lesions on the liver (arrows) of a commercial layer chicken, Case number A01/2014, obtained from farm I

Figure 14: Multiple variable sized focal nodular liver lesions (arrows) in an indigenous chicken, Case number E06/2014, obtained from Farm VI

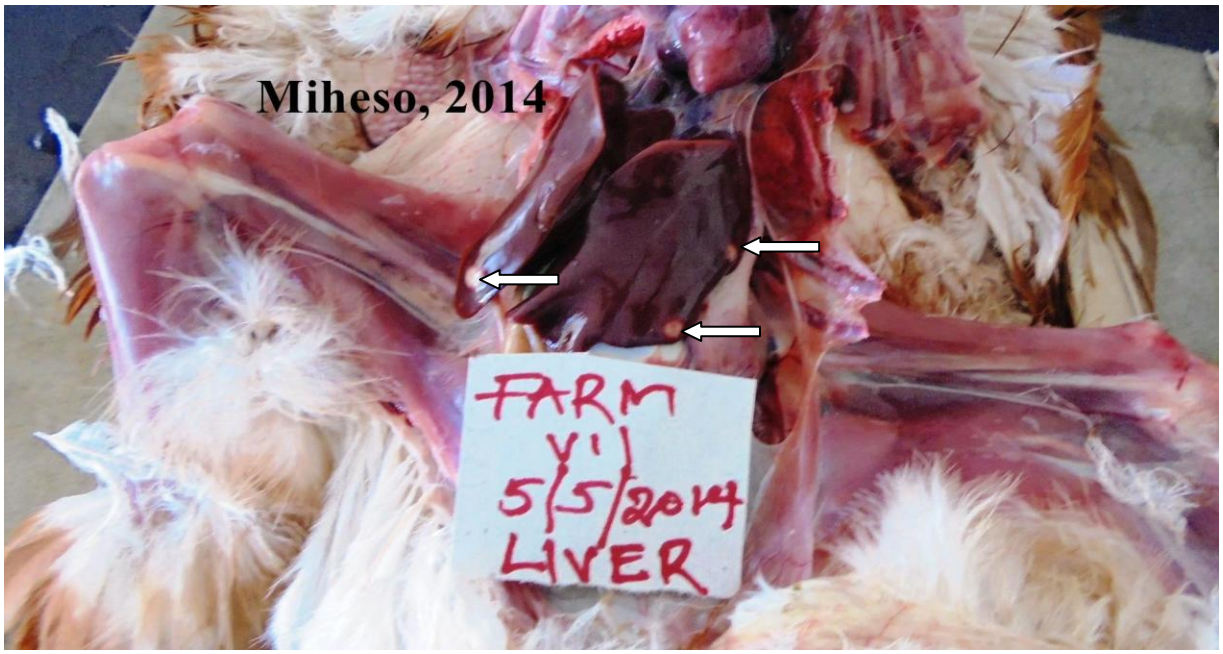

Figure 15: Nodular lesions on the liver (arrows) in a commercial layer chicken, Case number C07/2014, obtained from farm VII

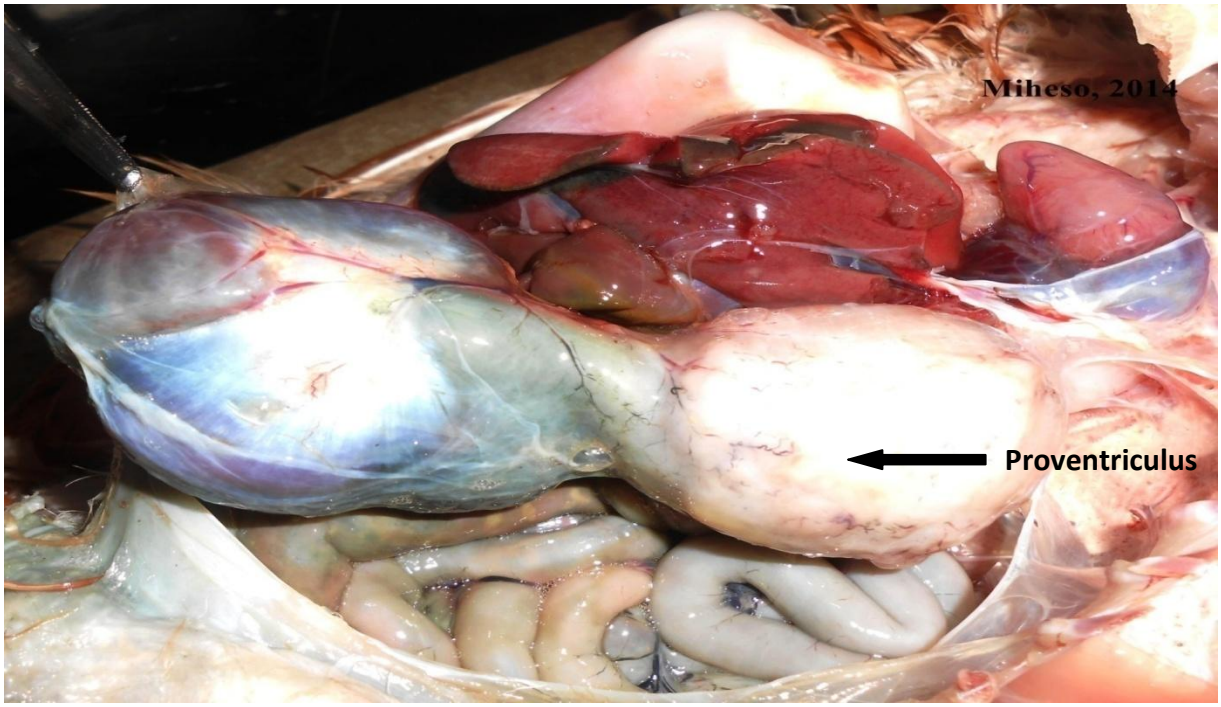

Figure 16: Distended proventriculus (arrow) in a commercial layer chicken, Case number B01/2014, obtained from Farm I

4.2.2.2.2 Histopathological manifestations

Three hundred and four tissue samples harvested from the 50 chicken were examined at histopathology. Lesions encountered in the organs were as follows:

4.2.2.2.2.1 Liver

Examination of the liver tissues from chicken whose serum tested positive to ALV p27 antigen had pleomorphic population of cells, predominantly lymphoblastic cells and a few mature lymphocytes. Heavy infiltrations of lymphoblastic cells were observed in the liver with focal areas of coagulative necrosis of hepatocytes (**Figure 17**). Slight infiltration of lymphocytes, macrophages and heterophils were observed around portal triads of the liver. Liver tissues sampled from seronegative birds in farms II and X had similar lesions.

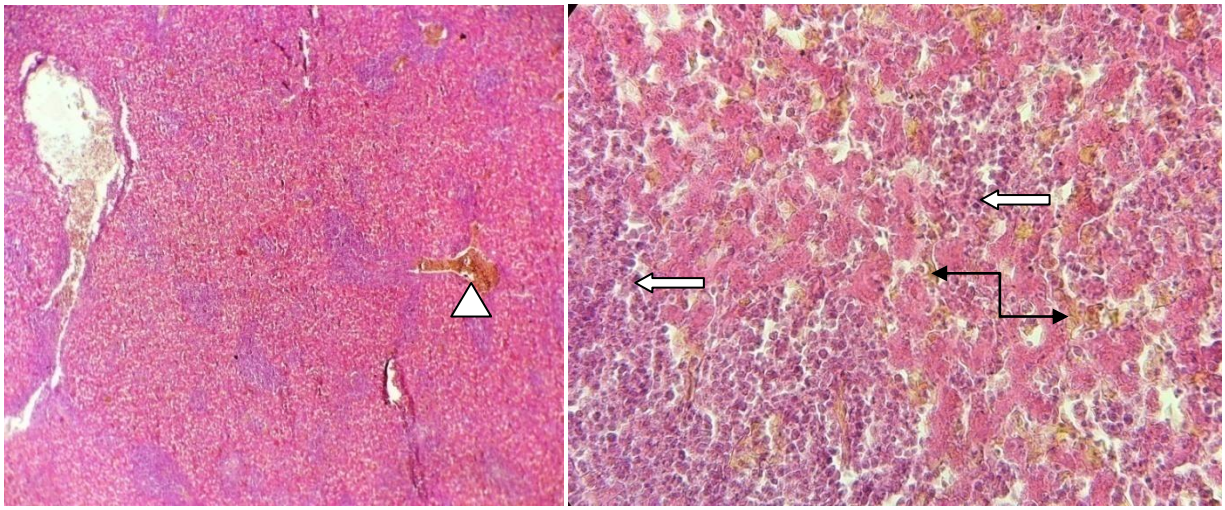

Figure 17: Histological section of a chicken liver, Case number B06/2014, from farm VI showing focal lymphocyte and lymphoblast cellular infiltration (block arrows); congestion (arrow heads), and haemosiderosis in sinusoids (double arrow) (Figure on the left 40X, right 400X H/E)

4.2.2.2.2.2 Spleen

Spleen tissues from seropositive and negative birds to ALV p27 antigen had varied light to heavy lymphoblastic and lymphocytic infiltrations. Occasionally, some spleens had areas of necrosis and inflammatory cellular infiltrations. Spleen from seronegative birds in farms II and X had similar lesions.

4.2.2.2.2.3 Kidney

Kidneys from seropositive birds to ALV p27 antigen had few focal areas of lymphoblastic cell infiltrations predominantly in the cortex, with some cells having mitotic figures (**Figure 18**). However, some kidneys had blood vessel congestion and haemorrhages with inflammatory cells predominantly heterophils. Kidney tissues sampled from seronegative birds from farm II and X had similar lesions.

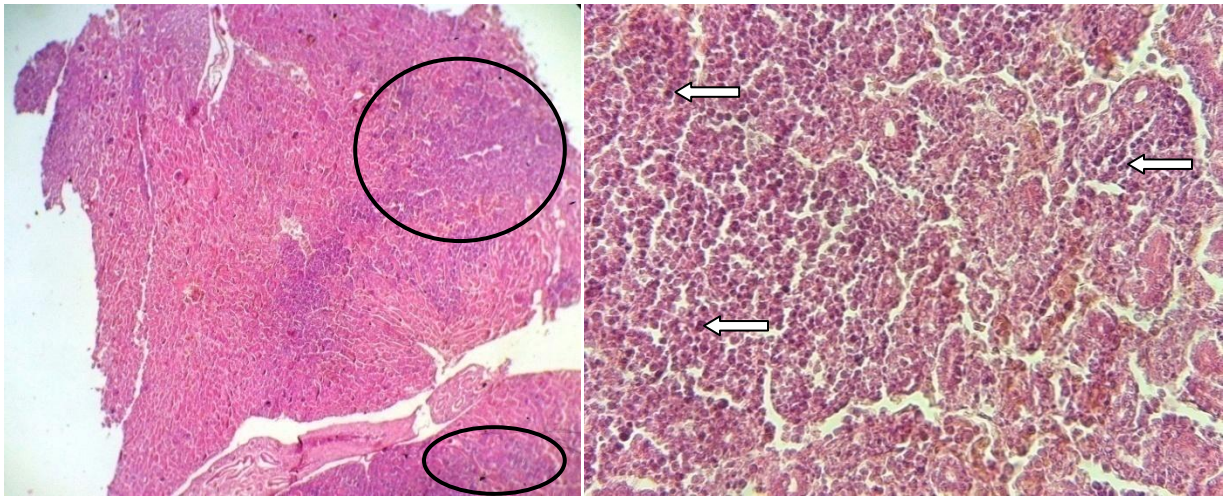

Figure 18: Histological section of a chicken kidney showing lymphocyte and lymphoblast cellular infiltration (black circles) with some cells in mitosis (block arrows) (Figure on the left 40X, right 400X H/E), Case number A01/2014, from farm I

4.2.2.2.2.4 Heart

Heart tissues from seropositive birds to ALV p27 antigen had slight to heavy lymphoblastic and lymphocytic infiltrations within and between cardiac muscle fibres; with eventual destruction of myofibres in some of the birds (**Figure 19**). There were also areas with macrophages and heterophil infiltrations in some of the heart sections.

Hearts sampled from sampled from seronegative birds from farm II and X had similar lesions.

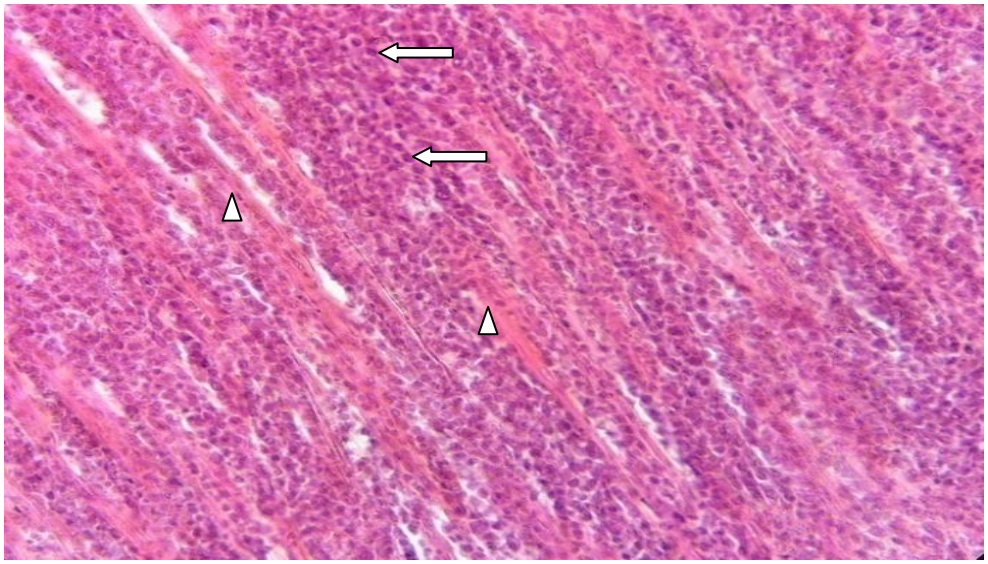

Figure 19: Histological section of a heart from a chicken, Case number C01/2014, in farm I showing mixed population of lymphocyte and lymphoblast cellular infiltration (block arrows) with destruction of muscle fibres (arrow heads) (400X H/E)

4.2.2.2.2.5 Proventriculus

Proventriculi tissues from seropositive birds to ALV p27 antigen had varied slight to heavy lymphoblastic cell infiltrations, predominantly on the epithelial lining and mucosal layers, with some of the cells depicting mitotic figures. Some of the proventriculi also had areas undergoing degeneration and inflammatory cell infiltration. Proventriculi sampled from seronegative birds from farm II and X had similar lesions.

4.2.2.2.2.6 Sciatic nerve

Sciatic nerve tissues from seropositive birds to ALV p27 antigen had no observable cellular reaction, except those from farm III, which had a heavy infiltration with both lymphocytic and lymphoblastic cells (**Figure 20**). The sciatic nerves from seronegative birds had no cellular reaction except in farm X, which had heavy lymphocytic and lymphoblastic infiltrations with some showing mitosis and oedema.

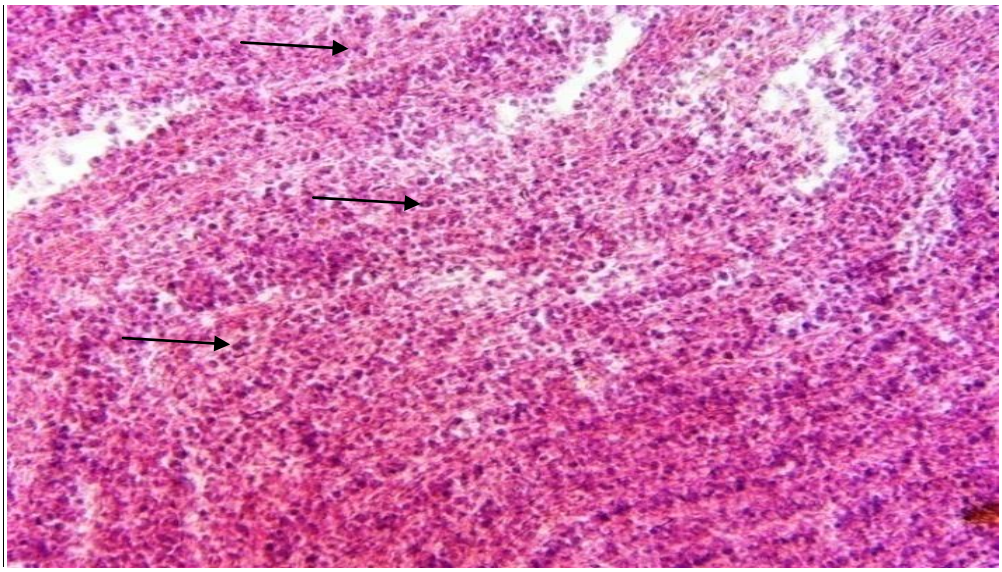

Figure 20: Histological section of a nerve from a chicken , Case number C03/2014, in farm III showing heavy infiltration by lymphocytes and lymphoblasts (arrows) (400X H/E)

4.2.2.2.2.7 Other tissues/organs

Other tissues/organs sampled from seropositive birds to ALV p27 antigen included the lungs (**Figure 21**), ovary (**Figure 22**) and the intestines; which had masses of lymphocytic and lymphoblastic cells which infiltrated the mentioned organs. There were areas of congested blood vessels noted in the lungs.

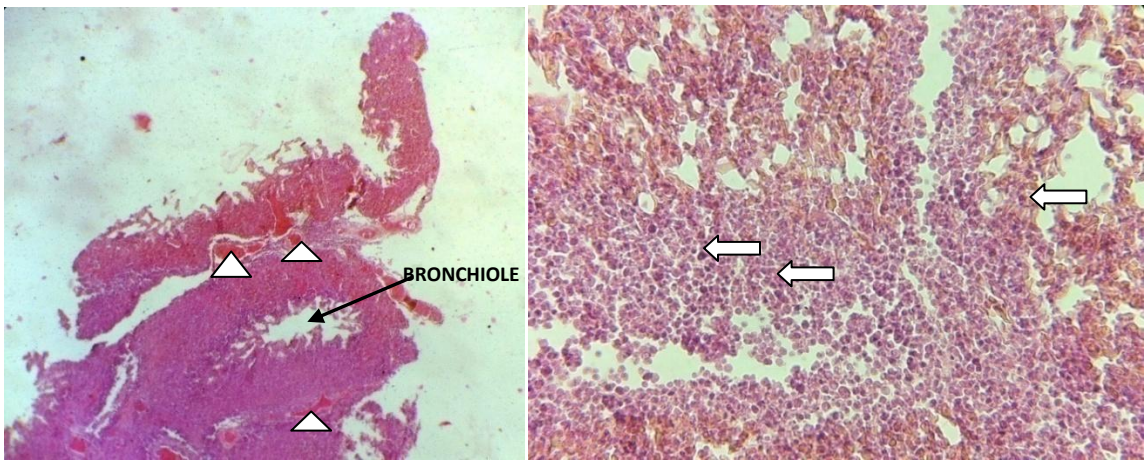

Figure 21: Histological section of a chicken lung, Case number B02/2014, from farm II showing congestion (arrow heads) and lymphocytic infiltration (block arrows) which had replaced most of the tissue (Figure on the left 40X, right 400X H/E)

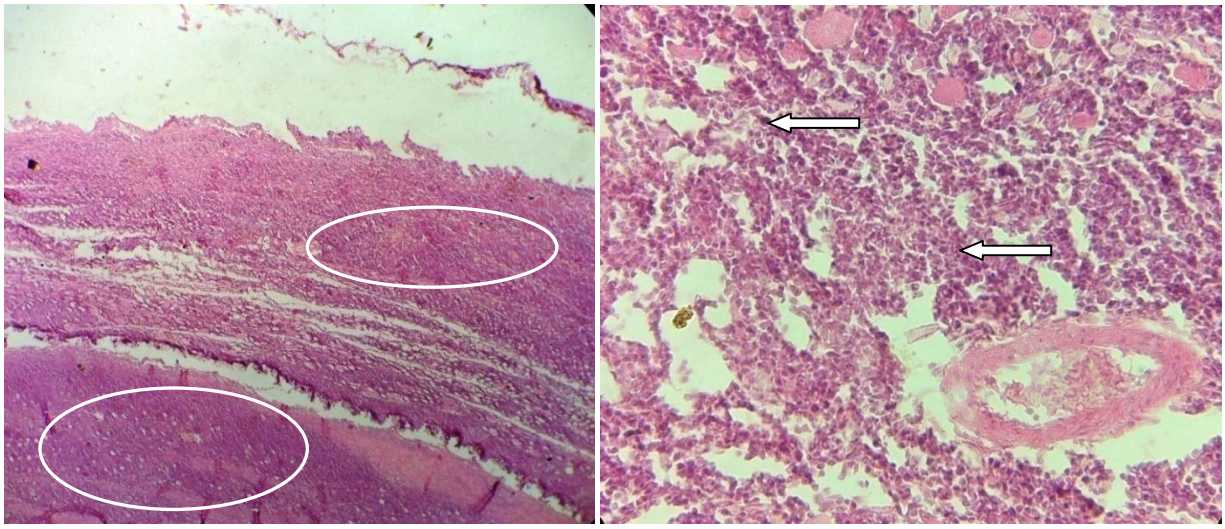

Figure 22: Histological section of a chicken ovary, Case number A05/2014, from farm V showing lymphocyte and lymphoblast cellular infiltration (white ovals; block arrows) (Figure on the left 40X, right 400X H/E)

4.2.2.3 Seroprevalence rates in chicken in farms and hatchery source of the birds

Fifty sera samples from ten farms were tested for ALV p27 antigen, out of which 16 (32%) were positive (**Table 6**) with EUs ranging from 10% to 80%. Among the ten farms, those that had chicken with positive sera were farm I, II, III, V, VI and VII. Corresponding EUs from seropositive birds were; 10%, 14%, 19% and 23% from four birds in farm I; 12% and 14% from two birds in farm II; 67% from one bird in farm III; 11% and 16% from two birds in farm V; 10%, 17% and 84% from three birds in farm VI; and 19%, 28%, 42% and 80% from four birds in farm VII. Avian leucosis virus p27 antigen was not detected in farms IV, VIII, IX and X.

Eleven (11/16) (68.75%) sera samples were obtained from commercial layers' chicken in farms I (10%, 14%, 19% and 23%), II (12% and 14%), V (11% and 16%), VI (10% and 17%) and VII (19%) that were weak positives with EU values ranging from 10% to 23%. Three (3/16) sera samples (18.75%) obtained from commercial layer chicken tested moderately positive from farm VII (28% and 42%); and farm III (67%). Two (2/16) (12.5%) chicken sera samples were strongly positive to ALV p27 antigen, of which one sample was from a commercial layer in farm VII which had an EU value of 80%, while the other one was from an indigenous chicken that had an EU value of 84%.

Four different hatcheries (A, B, C and H) supplied the ten farms with commercial layers and broilers, while the indigenous chicken in farm VI were sourced from an individual farmer hatching own eggs. Out of the ten farms, those with positive chicken sera were from farm I (hatchery B), farm II (hatchery H), farm III (hatchery B), farm V (hatchery A), farm VI (hatchery B) and farm VII (hatchery B). Hatchery B supplied birds to four farms, while hatchery A and H supplied birds to one farm each. Farms that tested negative to ALV p27 antigen were farm IV

supplied chicks by hatchery B, farm VIII supplied chicks by hatchery A, farm IX supplied chicks by hatchery C and farm X with birds from hatchery B.

Table 6: Hatcheries that supplied avian leucosis virus antigen seropositive and negative chicken sampled in farms in Nairobi and surrounding counties

Farms	**Source hatchery**	**Location (county)**	**Percentage/Number of sero-positive**	**Number of sero-negative**
Farm I	B	Kiambu	80% (4/5)	1
Farm II	H	Nairobi	40% (2/5)	3
Farm III	B	Nairobi	20% (1/5)	4
Farm IV	B	Kiambu	0% (0/5)	5
Farm V	A	Machakos	40% (2/5)	3
Farm VI	B	Kiambu	60% (3/5)	2
Farm VII	B	Machakos	80% (4/5)	1
Farm VIII	A	Kiambu	0% (0/5)	5
Farm IX	C	Kiambu	0% (0/5)	5
Farm X	B	Kajiado	0% (0/5)	5

4.2.2.4 Mortality rates of chicken suffering from avian leucosis in different farms

Reported chicken mortality rates as captured in the questionnaires among the ten farms ranged from 3.57% to 50% (**Table 7**). The highest mortality rates were reported in farm VI (50%), while farm X (3.57%) reported lowest mortalities. Most farms kept commercial layers, except farms VI and VII which also had indigenous and broiler chicken respectively on the same farms.

Commercial layers reported higher mortalities compared to indigenous and broiler chicken. Farm VI reported a mortality rate of 50% among commercial layers compared to 20% of indigenous chicken on the same farm. There was only one farm (VII) which had broilers whose mortality rate was at 10%, while commercial layers on the same farm had a mortality rate of 42.86%.

Chicken mortalities from farms which had seropositive chicken varied from 20% (III), 25% (V), 33.33% (II), 42.86% (VII), 43% (I) and 50% (VI). Farms that tested negative to ALV p27 antigen reported mortalities ranging from 3.57% (X), 19.64% (IV), 30% (IX) and 40% (VIII). The average mortality rate among commercial layers from ALV seropositive farms were much higher (35.70%), compared to seronegative birds (23.30%).

Table 7: Mortality rates reported in commercial layer, broiler and indingenous chicken, their ages and flock sizes in avian leucosis virus antigen seropositive and negative farms in Nairobi and surrounding counties

Farms	Chicken age in weeks	Flock size before disease onset	No. of birds at the time of sampling	No. of birds dead since disease onset	Percentage mortality rate
Farm I	18	1,000 (C.L)	570	430	43
Farm II	32	300 (C.L)	200	100	33.33
Farm III	24	500 (C.L)	400	100	20
Farm IV	14	560 (C.L)	450	110	19.64
Farm V	24	2,000 (C.L)	1,500	500	25
Farm VI	16 12	1,000 (C.L) 50 (In.)	500 40	500 10	50 20
Farm VII	24 7	700 (C.L) 500 (Br.)	400 450	300 50	42.86 10
Farm VIII	40	1,000 (C.L)	600	400	40
Farm IX	52	1,000 (C.L)	700	300	30
Farm X	29	2,800(C.L)	2,700	100	3.57

Key

C.L-Commercial layers Br.-Broilers

In.-Indigenous chicken No.-Number

5.0 CHAPTER FIVE: DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

5.1 Discussion

5.1.1 Prevalence of avian leucosis in chicken between the years 2003 to 2012

There has been a steady rise in the number of AL cases being diagnosed within Nairobi and surrounding counties from the year 2003 to 2012 more so in the last 3 years (2010-2012). This could be attributed partly to the method of diagnosis used, whereby in earlier years (2003-2009), clinico-pathological manifestations was used, compared to the latter 3 years (2010-2012) of the study, when ELISA diagnostic kit was in additional used in diagnosis of AL at CVL. The ALV ELISA is reported to have 99.2% sensitivity and 100% specificity (Silva *et al*., 2007), thus probably it was able to detect a higher number of cases during 2010-2012 period, unlike previously.

The year 2005 recorded the least number of AL cases that were reported during that period of study (0.29%). This could have probably been attributed to the Highly Pathogenic Avian Influenza (HPAI) scare initiated by media reports and compounded by HPAI outbreaks in Sudan (Omiti and Okuthe, 2009). This had adverse impacts on poultry production and trade, resulting in farmers reducing their flock sizes, preventative culling of birds (Kimani *et al.*, 2006), and resulting in fewer AL cases being reported that year.

Most cases diagnosed were commercial layers, compared to broilers and indigenous chicken with Kiambu County as the main source of these birds while Kajiado County had the least. This could be attributed to susceptibility and population of commercial birds in these regions where Kiambu County has the highest population of birds, and vice versa for Kajiado County (KNBS, 2014). However, proximity to diagnostic facilities could also play a role, with VPMP and

adjacent CVL in Nairobi County serving as the main referall diagnostic institutions in the region bordering neighbouring Kiambu County (KNBS, 2014).

Majority of AL cases were reported in the rainy season, unlike the dry season. This could be attributed to low ambient temperatures which is a serious stressful factor to birds and may have devastating effects on an already compromised birds' immune system due to AL infection.

5.1.2 Seroprevalence of avian leucosis in chicken

Results of this study showed that indigenous chicken had AL prevalence of 58.33%, as reported in other parts of the world among these birds. Sani *et al*. (2011) reported a high prevalence of 60% among Nigerian indigenous chicken while Mohammadi *et al*. (2008) reported a prevalence rate of 76% in Iran.

Prevalence among the exotic commercial layers was 18.63% similar to 18.33% reported by Sani *et al*. (2012) in commercial layers in Zaria and its environs in Nigeria. A prevalence of 6.8% in broiler chicken was found in this study which is slightly higher than 4.7% and 1.25% in Nigeria reported by Sani *et al*. (2011) and Olabode *et al*. (2009), respectively; and 3.33% in Iran by Mohammadi *et al*. (2008). The high prevalence rate in indigenous chicken compared to other chicken can be attributed to the free range management system which exposes them more, to infectious agents than other chicken raised under the intensive system of management (Bebora *et al.,* 2005).

Age is also a factor that contributed to higher prevalence rates among indigenous chicken (12-96 weeks old) compared to both commercial layers (8-65 weeks) and broilers (6-10 weeks); and commercial layers compared to broilers. Higher prevalence rate could probably been due to longer exposure to the virus in older birds, which allows ample time for viral multiplication and establishment in the birds. These findings agree with Wu *et al*. (2010) who reported higher ALV

detection rates in old chicken. Kenyan indigenous chicken could be potential reservoirs and sources of contamination of the environment and spread of ALV and need to be targeted in control of the disease. Avian leucosis infection in broilers could probably be due to vertical transmission, unlike indigenous and commercial layers whose infection could mainly be both vertical and horizontal transmission, and this could be attributed to age and life span of these birds.

Among ALV positive birds; broilers had 85.71% weak and 14.29% moderate; commercial layers had 57.90% weak and 21.05% moderate; and indigenous had 83.81% weak and 14.29% moderately positive birds. This is comparable to Sani *et al*. (2011) who reported 92.86% weak and 7.14% moderate in broilers; and weak positives in commercial layers (Sani *et al*., 2012).

In this study, higher levels (strongly positive) of p27 antigen levels were found in both commercial layers (80% to 99%) and indigenous chicken (91% to 93%) unlike reports by Sani *et al*. (2011, 2012). However, Emikpe *et al*. (2007) found 18.75% of sera from indigenous chicken in Nigeria being strongly positive to ALV p27 antigen. Higher than 25% EU levels found among the commercial layers and indigenous chicken in this study, could be an indication of repeated exposure to ALVs. This repeated exposure can either be through contact exposure with congenitally infected hatch mates or contaminated environment/formites (Aden, 1983). Wide range of antigen titre levels got from this study (10%-99%), may also suggest differences in exposure time spans and exposure dose of the ALVs (Emikpe *et al*., 2007).

5.1.3 Clinical and pathological manifestations, hatchery of bird origin and mortality rates of avian leucosis in chicken

5.1.3.1 Clinical and pathological manifestations

The clinical manifestations of the birds from prospective study were non-specific. Moreover, retrospective study also revealed a non-specific clinical presentation of the disease and therefore such presentations could be confused with several other disease conditions (Davidson, 2001; OIE, 2010).

Nervous signs recorded in the study included paralysis and torticollis. Leg paralysis has previously been reported by other researchers (Latif and Khalafalla, 2005), which was attributed to the pressure exerted on the sciatic nerves due to enlargement of neoplastic organs such as kidneys. Torticollis has also been reported in ALV mixed infections with subgroup A and B (Cho *et al*., 1968). Torticollis may also result from other conditions that cause or lead to contracture of sternocleidomastoid muscle of the neck, causing head tilt on the affected side. This contracture could be as a result of muscle injury, congenital cervical spine malformation or nervous disorders (Saif *et al.,* 2008). Some infectious conditions (fowl cholera and MD) and nutritional diseases (vitamin E and selenium deficiency) may also lead to torticollis. Nutritional deficiency could also be attributed to anorexia which was also recorded clinically. Fowl cholera induced torticollis results due to infection of the middle ear, meninges and cranial bones by *Pasteurella multocida* (Saif *et al.,* 2008; Mbuthia *et al*., 2011). Mareks disease induced torticollis occurs due to infection of the peripheral nerves of the cervical region, more so the vagus nerve (Saif *et al.,* 2008; OIE, 2010).

This study however noted a correlation between seropositivity and observation of unthriftiness, previously not reported.

Gross lesions observed among 16 chicken whose sera tested positive to ALV p27 antigen were non pathognomonic and an almost similar picture was also noted in the retrospective study. Murphy *et al*., (1999) also found that gross lesions of AL are non pathognomonic and non-specific and there is a possibility of overlapping with several other diseases.

Organomegaly of various organs such as the liver, spleen and kidneys are possible lesions seen in diseases like fowl typhoid (Garcia *et al*., 2010); while congestion of the lungs, liver, spleen and kidneys which were recorded in this study, are possible gross lesions seen in many other septicaemic diseases (Sani, 2012). Nodular lesions observed in various organs like the intestines, livers and spleens, and eventual enlargement of the affected organs can also be associated with avian tuberculosis (Tell *et al.*, 2001; OIE, 2008) and other tumourous conditions (Saif *et al*., 2008).

Histopathological examination of various organs and tissues revealed varied lymphoblastic and lymphocytic infiltrations undergoing mitosis in various organs such as the liver, spleen, proventriculus and the kidney. Occasionally, some of the spleens had areas of necrosis and inflammatory cell infiltrations, while some livers had focal areas of necrosis of hepatocytes, leading to destruction of normal anatomic features due to massive lymphocyte infiltrations. Some tissues like the liver had slight infiltration of lymphocytes, macrophages and heterophils more so around portal triads. Similar findings have been reported by Sani, (2012). Mononuclear cell infiltrations have also been described in Newcastle disease (Saif *et al.*, 2008). There was necrosis of hepatocytes and similar features have been described in LL by Cooper *et al.* (1968); and to some extent myelocytomatosis as described by Mladenov *et al.* (1967).

The sciatic nerves had no cellular reaction except in farms III and X, which had heavy lymphocytic and lymphoblastic infiltrations; with those from farm X additionally showing mitosis and oedema.

Lesions observed in a seropositive chicken from farm III whose sciatic nerves had heavy lymphocytic and lymphoblastic infiltrations could suggest a possible co-infection with probably MD or RE. Although the farms in this study were only screened for AL, the microscopic lesions of MD observed in this study were probably a result of mixed AL and MD infection. Detection of mixed infection can also be supported by cellular infiltration of pleomorphic cells instead of homogenous cells in single infection (Sani, 2012)

Enlarged peripheral nerves with lymphocytic infiltration were historically associated with MD (Pappenheimer *et al.*, 1926). However, such lesions in chicken are not necessarily pathognomonic for MD. Enlargement of peripheral nerves can also be induced by REV (Witter *et al.*, 1970b). Both REV and subgroup J avian leucosis virus can induce lymphoid infiltrations in peripheral nerves (Witter *et al.*, 1970b; Payne *et al.*, 1991). Another syndrome designated as idiopathic polyneuritis which was described in specific pathogen free laboratory chicken by Biggs *et al.* (1982) showed enlarged peripheral nerves with nerve lesions such as demyelination, oedema and scattered lymphocytic infiltration with lymphocytes and plasma cells.

Tumours in MD are less apparent, grossly diffuse and interfollicular in location with occasional bursal involvement. Moreover, peripheral nerve lesions are not a feature in AL as unlike in MD and the affected nerves are usually grossly enlarged and with loss of striations (OIE, 2010). There have been reported cases of mixed infections of these viruses (Cui *et al.*, 2009) which probably, may alter the natural pattern of these diseases, hence therefore resulting in different

clinical signs and lesions. Mixed infections may at times cause difficulties in tumour diagnosis thus influencing its gross and histological lesions (Fadly and Nair, 2008).

Co-infection with some of these viruses may often complicate diagnosis of AL. Thus in chicken with multiple tumour virus infections, it is important to base diagnoses on tumour-specific criteria (such as cell type markers or LTR insertions near c-myc) rather than on virological tests (Witter *et al*., 2005). Co-infection of AL with other diseases has been reported to be prevalent in other parts of the world like China (Fenton *et al*., 2005; Zhang *et al*., 2008; Cheng *et al.*, 2011), by immunosuppressive or oncogenic viruses such as REV (Cheng *et al.*, 2011; Cui *et al*., 2009; Ongor and Bulut, 2011), MD and chicken anaemia virus (Lütticken, 1997; Qin *et al*., 2010; Williams and Sellers, 2012). Hence this study cannot exclusively rule out possibility of co-infection due to non specificity of signs and lesions.

Davidson and Boreinstein (1999) reported that the outcome of an avian oncogenic disease might be altered by co-infection with other avian oncogenic viruses thus this may also explain non specific disease outcome in terms of clinical and pathological manifestations found in this study.

It is worthy noting that this study reports a new finding of correlation between AL seropositivity and occurrence of enteritis, renomegaly, cardiomegaly and lymphocytic infiltration in organs of various birds.

5.1.3.2 Hatchery origin of the birds

Both seronegative and seropositive birds emanated from the same hatcheries. However, hatcheries A, B and C were recorded in both retrospective and prospective study, which clearly indicates that AL could also be partly attributed to the source hatchery of chicken. This could also be attributed to the production capacities of individual hatcheries (Nyaga, 2007b; Okello *et al*., 2010). Moreover, the recorded hatcheries, some of them being major day old chick producers

in the country, is an indication of the presence of the disease in the country, possibly indicating the origin of disease spread. However, more research is required to elucidate this. There is also an emerging trend of individual farmers hatching their own eggs from incubators in the country, as depicted in farm VI, which could also serve as a potential source of infection if unregulated. This happens despite various clauses stated in the Animal Diseases Act, Cap 364 which restricts such, unless approved by the DVS.

5.1.3.3 Chicken mortality rates

High percentage mortalities among seropositive flocks could probably be attributed to direct AL deaths and immunosuppressive effects of ALVs (Spackman *et al*., 2003) with birds more likely to succumb to secondary infections. Such high mortalities have previously been reported by Cox *et al*. (2004), Latif and Khalafalla (2005) who reported 40% mortality among broilers; while Sani *et al.* (2012) reported an average mortality rate of 20.8% among exotic commercial layers, whose range in farms sampled varied from 6.25 to 37.5%.

Commercial layer chicken in farm VI reported the highest overall mortality rate (50%), while that of indigenous chicken on the same farm was much lower (20%). This is despite EU value in the sampled indigenous bird being much higher (84%) due to repeated exposure to the virus compared to the exotic commercial layer counterparts (EU value range of 10%-17%). This may signify less mortality rates among the ALV infected indigenous birds compared to their exotic commercial counterparts. Maybe indigenous birds are hardier, not succumbing easily to the AL infection. There could also be a possibility of indigenous chicken posing as reservoirs of the virus hence contaminating the environment and infecting their exotic commercial layer chicken on the same farm. JinKai *et al*. (2014) also reported reduced susceptibility of ALV among older Chinese indigenous chicken.

5.2 Conclusions

The study showed that:

1. There is a rising prevalence of AL in chicken from the year 2003 to 2012.
2. There is high seroprevalence rate of ALVs among chicken flocks in Nairobi and surrounding counties. The p27 ALV antigen detected was due to a natural infection as there was no history of vaccination due to ALV among the sampled birds, moreover, no vaccine against the disease is commercially available yet.
3. Clinical manifestations, gross and histopathological lesions observed for ALVs are non pathognomonic and may often overlap. Thus diagnosis of AL based on clinical and pathological manifestations may be difficult, necessitating employing other tests such as immunological and molecular techniques.
4. There was correlation between seropositivity and observed clinical, pathological and histopathological manifestations of unthriftiness, enteritis, renomegaly and cardiomegaly; and lymphocytic infiltration in various organs respectively.

5.3 Recommendations

The study therefore recommends that:

1. There should be an established effective screening of all imported birds to prevent entry of infected birds into this country.

2. There should be culling of infected birds with proper disposal, and standard biosecurity measures be enhanced in hatcheries and farms to prevent primary exposure**.**

6.0 CHAPTER SIX: REFERENCES

Aden, D.F. (1983): Serological survey of Marek's disease in exotic and local chicken in Nigeria. *Tropical Veterinarian,* **1**:138-140.

Adkins, H.B., Blacklow S.C. and Young J.A. (2001): Two functionally distinct forms of a retroviral receptor explain the nonreciprocal receptor interference among subgroups B, D and E avian leukosis viruses. *Journal of Virology,* **75:**3520–3526.

AffiniTech Limited (2009): Avian leucosis virus antigen test kit, Product leaflet, version 4, Bentonville, USA.

Aly, M.M. (2000): Isolation of a subgroup J-like avian leukosis virus associated with myeloid leukosis in meat-type chicken in Egypt. Proceedings of International Symposium on ALV-J and Other Avian Retroviruse, Rauischholzhauzen, Germany, pp 165-176.

Arshad, S. S., Bland, A. P., Hacker, S. M. and Payne, L. N. (1997): A low incidence of histiocytic sarcomatosis associated with infection of chicken with the HPRS-103 strain of subgroup J avian leukosis virus. *Avian Diseases,* **41**:947-56.

Bacon, L. D., Fulton J. E., and Kulkarni G. B. (2004): Methods for evaluating and developing commercial chicken strains free of endogenous subgroup E avian leukosis virus. *Avian Pathology,* **33**:233–243.

Bagust, T.J., Fenton S.P. and Reddy M.R. (2004): Detection of subgroup J avian leukosis virus infection in Australian meat-type chicken. *Australian Veterinary Journal,* **82**:701–706.

Barbosa, T., Zavala, G. and Cheng, S. (2008): Molecular characterization of three recombinant isolates of avian leukosis virus obtained from contaminated Marek's disease vaccine. *Avian Diseases,* **52**: 245-252.

Beard, J.W. (1963): Avian virus growths and their etiologic agents. *Advances in Cancer Research*, **7**:1–127.

Beard, J. W. (1980): Biology of avian oncornaviruses, pp. 55–87. In G. Klein (edition), Viral Oncology. Raven Press, New York.

Bebora, L. C., Mbuthia, P. G., Macharia, J. M., Mwaniki, G., Njagi, L. W. and Nyaga, P.N. (2005): Appraisal of Indigenous chicken's potential in egg production. *The Kenya Veterinarian,* **29**: 10-13.

Benson, S. J., Ruis, B. L., Fadly A. M. and Conklin K. F. (1998): The unique envelope gene of the subgroup J avian leukosis virus derives from ev/J proviruses, a novel family of avian endogenous viruses. *Journal of Virology,* **72**:10157–10164.

Biggs, P. M., Shilleto, R. F. W., Lawn, A. M. and Cooper, D. M. (1982): Idiopathic polyneuritis in SPF chicken. *Avian Pathology,* **11**: 163-178.

Campbell, J. G. (1969): Tumours of epithelial tissues. Tumours of the fowl. London: William Heinemann Medical Books Limited, pp. 125-223.

Carson, F. L. and Hladik, C. (2009): Histotechnology: A Self-Instructional Text American Society for Clinical Pathology Press. *Hong Kong*, 361.

Charlton, B.R. (2006): Necropsy of the fowl; In: A.J Bermudez, M. Boulianne, D.A Halvolson, J.S Schrader, L.J Newman, J.E Sander and P.S Wakennel (edition) Avian disease manual. 6th edition, American Association of Avian pathologists, pp. 232-233.

Cheng, Z., Liu J., Cui Z. and Zhang L. (2010): Tumors associated with avian leukosis virus subgroup J in layer hens of during 2007 to 2009 in China. *Journal of Veterinary Medical Science,* **72**:1027–1033.

Cheng, Z., Zhang, H., Wang, G., Liu, Q., Liu, J., Guo, H. and Zhou, E. (2011): Investigations of avian leukosis virus subgroup J and reticuloendotheliosis virus infections in broiler breeders in China. *Israeli Journal of Veterinary Medicine*, **66**: 34-42.

Cho, B. R., Kenzy, S. G. and Kim, U. H. (1968): Case report: mixed infections with subgroups A and B avian leukosis viruses in commercial white leghorn chicken. *Avian Diseases,* 585-593.

Clark, D. P. and Dougherty R. M. (1980): Detection of avian oncovirus groupspecific antigens by the enzyme- linked immunosorbent assay. *Journal of General Virology,* **47**:283- 291.

Cooper, M. D., Payne, L. N., Dent, P. B., Burmester, B. R. and R. A. Good, (1968): Pathogenesis of avian lymphoid leukosis. Histogenesis. *Journal of National Cancer Institute,* **41**:373–378.

Cox, N.A., Wilson, J.L., Musgrove, M.T., Buhr, R.J., Sander J.E. and Hudson, B.P. (2004): Positive relationship of the avian leukosis-J strain virus to the detection of *C*ampylobacter in the digestive tract and semen of broiler breeder roosters. *Journal of Applied Poultry Research,* **13**: 44–47

Crittenden, L. B., McMahon, S., Halpern, M. S. and Fadly, A. M. (1987): Embryonic infection with the endogenous avian leukosis virus Rous-associated virus-0 alters responses to exogenous avian leukosis virus infection. *Journal of Virology*, **61**:722-725.

Cui, Z., Sun, S., Zhang, Z. and Meng, S. (2009): Simultaneous endemic infections with subgroup J avian leukosis virus and reticuloendotheliosis virus in commercial and local breeds of chicken. *Avian Pathology*, **38**:443-448.

Davidson, I. and Borenstein, R. (1999): Multiple infections of chicken and turkeys with avian oncogenic viruses: prevalence and molecular analysis. *Acta Virologica,* **43**: 136-142.

Davidson, I. *(*2001): Differential diagnosis of avian oncogenic viruses. http://www.poultrymed.com. Accessed on 6th October, 2014.

Department of Veterinary Services, (DVS) (2001 - 2002): Annual report for the Department of Veterinary Services, Kenya.

Dorner, A. J. and Coffin J. M. (1986): Determinants for receptor interaction and cell killing on the avian retrovirus glycoprotein gp85. *Cell*, **45**:365-374.

Emikpe, B. O., Oladele, O. A., Oluwayelu, D. O., Adene, D. F., Ohore, O. G. and Bolarinwa, A. B. (2007): Detection of avian leukosis virus P27 antigen in Nigeria indigenous chicken. *Journal of Animal and Veterinary Advances*, **6**: 36-38.

Enrietto, P. I., Hayman, M. J., Ramsay, G. M., Wyke, J. A. and Payne, L. N. (1983): Altered pathogenicity of avian myelocytomatosis (MC29) viruses with mutations in the v-myc gene. *Virology*, **124**:164-172.

Ewert, D. L. and de Boer G. F. (1988): Avian lymphoid leukosis: mechanisms of lymphomagenesis. *Advances in Veterinary Science and Comparative Medicine*, **32**:37-55.

Fadly, A. M. (1987): Differential diagnosis of lymphoid leukosis. In G. F de Boer (edition). Avian Leukosis. Martinus Nijhoff: Boston, MA. pp.197– 211.

Fadly, A., Davison, T. F., Payne, L. N. and Howes, K. (1989): Avian leukosis virus infection and shedding in brown leghorn chicken treated with corticosterone or exposed to various stressors. *Avian Pathology,* **18**:283-298.

Fadly, A. M. (1990): Leukosis and sarcomas. In: Purchase HG, Arp LH, Domermuth CH, Pearson JE (eds), Isolation and Identification of Avian Pathogens, 3rd edition. Kendall/Hunt Publishing Dubuque, I. A. pp. 135-142.

Fadly, A.M. (2003): Neoplastic diseases. In: Y.M. Saif (Editor–in–Chief); H.J. Barnes; J.R. Glisson; A.M.Fadly; L.R.McDougald and D.E.Swayne (Associate Editors): Diseases of poultry. 11th. ed., Iowa State Press, A Blackwell Publishing Company, Iowa, pp. 405–407.

Fadly, A. M., Okazaki W. and Witter R. L. (1981): Hatchery-related contact transmission and short- term small-group-rearing as related to lymphoid-leukosis-virus-eradication programs. *Avian Diseases,* **25**:667–677.

Fadly, A.M. and Witter, R.L. (1998): In: A Laboratory Manual for the Isolation and Identification of Avian Pathogens. (Editions) J.R., Glisson, D.J., Jackwood, J.E., Pearson, W.M., Reed and D.E., Swayne. 4th edition pp. 185-196. Kennett Square, Pennsylvania : American Assoc. Avian Pathologists.

FadIy, A. M. and Smith, E. I. (1999): Isolation and some characteristics of a subgroup J-like avian leukosis virus associated with myeloid leukosis in meat-type chicken in the United States. *Avian Diseases,* **43**:391-400.

Fadly, A.M. and Payne, L.N. (2003): Lymphoproliferative disease of turkeys. In *Diseases of Poultry,* (Edition) Y.M., Saif, H.J., Bernes, J.R., Glisson, A.M., Fadly, L.R., Mcdougald and D.E., Swayne, 11th edition. pp. 465-516, Iowa State Press.

Fadly, A., Robert S., Henry H., Arun P. and Carolyn D. (2006): Isolation and characterization of an adventitious avian leukosis virus isolated from commercial Marek's disease vaccines. *Avian Diseases,* **50**:380–385.

Fadly, A.M. and Nair, V. (2008): Leukosis/sarcoma group. In Y.M. Saif, A.M. Fadly, J.R. Glisson, L.R. McDougald, L.K. Nolan and D.E. Swayne (Eds.). *In Diseases of Poultry*, 12th edition (pp. 514-568). Ames, IA: Blackwell Publishing.

Fatumbi, O.O. and Adene D.F. (1984): A ten year prevalence study of Marek's disease and avian leukosis at Ibadan, Nigeria. *Acta Veterinaria Brno,* **55**:49-53.

Feng, S. Z., Cao, W. S. and Liao, M. (2011): The PI3K/Akt pathway is involved in early infection of some exogenous avian leukosis viruses. *Journal of General Virology*, **92**: 1688-1697.

Fenton, S.P., Reddy, M.R., and Bagust, T.J. (2005): Single and concurrent avian leukosis virus infections with avian leukosis virus-J and avian leukosis virus A in Australian meattype chicken. *Avian Pathology,* **34**: 48-54.

Fung, Y. K., Lewis W. G., Crittenden L. B. and Kung H. J. (1983): Activation of the cellular oncogene c-erbB by LTR insertion: molecular basis for induction of erythroblastosis by avian leukosis virus. *Cell,* **33**:357–368.

Gao, Q., Yun, B., Wang, Q., Jiang, L., Zhu, H., Gao, Y. and Gao, Y. (2014): Development and Application of a Multiplex PCR Method for Rapid Differential Detection of Subgroup A, B and J Avian Leukosis Viruses. *Journal of Clinical Microbiology*, **52**: 37-44.

Gallo, R. (1973): Reverse transcriptase, the DNA polymerase of oncogenic RNA viruses. *Nature,* **234**:194-198.

Garcia, K. O., Santana, A. M., Freitas Neto, O. C., Simplício, M. G., Alessi, A. C., Berchieri Júnior, A. and Fagliari, J. J. (2010): Experimental infection of commercial layers using a *Salmonella enterica* sorovar *gallinarum* strain: Blood serum components and histopathological changes. *Brazil Journal of Veterinary Pathology,* **3**: 111-117.

Gingerich, E., Porter, R. E., Lupiani, B. and Fadly, A. M. (2002): Diagnosis of myeloid leukosis induced by recombinant avian leukosis virus in commercial white leghorn egg laying flock. *Avian Diseases,* **46**: 745-748.

Goodwin, M. A., Hafner, S., Bounous, D. I. and Brown, J. (1998): Myeloid leukosis" is a misnomer: multiple and diverse tumor morphotypes are found in broiler breeder chicken that are infected with subgroup J avian leukosis/sarcoma viruses (ALV-J). Proceedings of American Association of Avian Pathologists-American Veterinary Medical Association, Poultry Medicine Section, Baltimore, MD.pp. 189.

Hafner, S., Goodwin, M. A., Smith, E. J., Bounous, D. I., Puette, M., Kelley, L. C., Langbeinrich, K. A. and Fadly, A. M. (1996): Multicentric histiocytosis in young chicken. Gross and light microscopic pathology. *Avian Diseases,* **40**:202-209.

Hafner, S., Goodwin, M. A., Smith, E. J., Fadly, A. and Kelley, L. C. (1998): Pulmonary sarcomas in a young chicken. *Avian Diseases,* **42**:824-828.

Hatai, H., Ochiai K., Murakami M., Imanishi S., Tomioka Y., Toyoda T., Ohashi K. and Umemura T. (2008): Prevalence of fowl glioma-inducing virus in chicken of zoological gardens in Japan and nucleotide variation in the *env* gene. *Journal of Veterinary Medical Science,* **70**:469–474.

Hayward, W. S. and Neel B. G. (1981): Retroviral gene expression. *Current Topics in Microbiology and Immunology,* **19**:217–276.

JingKai, X., Qing, Y., YuSheng, L., YuanYuan, J. and JiaQian, C. (2014): Isolation and identification of avian leukosis virus in Shouguang chicken. *Research Opinions in Animal and Veterinary Sciences*, **4**: 462-466.

Kenya National Bereau of Statistics (KNBS) (2010): Population and housing census final report.

Kenya National Bereau of Statistics (KNBS) (2014): Kenya facts and figures 2014, http://www.knbs.or.ke/index.php?option=com_content&view=article&id=260:kenya-facts-and-figures-2014&catid=82:news&Itemid=593

Kimani, T., Obwayo M. and Muthui L. (2006): "Avian Flu Threat: Socio-Economic Assessment of the Impacts on Poultry-Related Livelihoods in Selected Districts in Kenya." Pan-African Program for the Control of Epizoonotics (PACE), Nairobi.

King'ori, A. M., Tuitoek, J. K., Muiruri, H. K. and Wachira, A. M. (2010): Effect of dietary crude protein levels on egg production, hatchability and post-hatch offspring performance of indigenous chicken. *International Journal of Poultry Science*, **9**: 324-329.

Kung, H. J. and Maihle N. J. (1987): Molecular basis of oncogenesis by non-acute avian retroviruses, p. 77–100. In G. F. De Boer (edition), Avian Leukosis. Martinus Nijhoff, Boston.

Kung, H. J. and Liu J. L. (1997): Retroviral Oncogenesis, pp. 235–266. In N. Nathanson (edition), Viral Pathogenesis. Lippincott- Raven Publishers, Philadelphia.

Lapis, K. (1979): Histology and ultrastructural aspects of virusinduced primary liver cancer and transplantable hepatomas of viral origin in chicken. *Journal of Toxicology and Enviromental Health*, **5**:469–501.

Latif, M. M. and Khalafalla, A. I. (2005): Detection by PCR of multiple subgroups of avian leukosis virus in broilers in the Sudan. *Journal of Animal and Veterinary Advances*, **4**: 407-413.

Lin, Y., Xia, J., Zhao, Y., Wang, F., Yu, S., Zou, N. and Huang, Y. (2013): Reproduction of hemangioma by infection with subgroup J avian leukosis virus: the vertical transmission is more hazardous than the horizontal way. *Virology Journal*, **10**: 97.

Liu, S., Wang, B., Zhang, Z., Wang, J., Sun, S. and Cui Z. (2011): The separation of subgroup A and J ALV in soft tissue sarcomas of "817" broiler hybrids. *Chinese Journal of Animal and Veterinary Sciences*, **3:**012.

Lupiani, B., Pandiri, A.R., Mays J., Hunt H.D. and Fadly A.M. (2006): Molecular and biological characterization of a naturally occurring recombinant subgroup B avian leukosis virus with a subgroup J-like long terminal repeat. *Avian Diseases*, **50**: 572-578.

Lütticken, D. (1997): Viral diseases of the immune system and strategies to control infectious bursal disease by vaccination. *Acta Veterinaria Hungarica*, **45**: 239-249.

Magothe, T. M., Okeno, T. O., Muhuyi, W. B. and Kahi, A. K. (2012): Indigenous chicken production in Kenya: I. Current status. *World's Poultry Science Journal*, **68**: 119-132.

Martin, S.W., Meek, A.H. and Willeberg, P. (1987): Veterinary Epidemiology. Principles and Methods. Iowa State University Press, Ames, p. 32.

Mburu, B.M. (1994): The role of research in poultry production and development. In: proceedings of the first workshop on poultry research priority setting held at Kenvash Hotel-Naivasha, 18th-19th October.

Mbuthia, P.G., Njagi, L.W., Nyaga, P.N., Bebora, L.C., Minga, U., Christen J.P. and Olsen J.E. (2011): Time-Course investigation of infection with a low virulent Pasteurella

multocida strain in normal and immune-suppressed 12 week old free range chickens. *Avian Pathology*, **40**: 629-637.

Ministry of Livestock and Fisheries Development (MOLD) (2010): Various Animal Production Annual Reports.

Mizuno, Y., Yuasa, N., Hihara, H., Ishizaki, R. and Shimizu, T. (1970): Neutralizing antibody against avian leukosis virus in abnormal chicken sera. *National Institute of Animal Health Quarterly,* **60**: 1-5.

Mladenov, Z., Heine U., Beard D. and Beard J. W. (1967): Strain MC29 avian leukosis virus. Myelocytoma, endothelioma, and renal growths: pathomorphological and ultrastructural aspects. *Journal of National Cancer Institute,* **38**:251–285.

Mohammadi, A., Masoudian K. and Bozorgchami B. (2008): Detection of avian leukosis virus (ALV) in albumen of Shiraz commercial and local layer flocks using ELISA and RT-PCR. *Iranian Journal of Veterinary Research,* **9**:24.

Morgan, H. R. (1967): Antibodies for Rous sarcoma virus (Bryan) in fowl, animal, and human populations of East Africa. II. Antibodies in domestic chicken, wildfowl, primates, and man in Kenya, and antibodies for Burkitt lymphoma cells in man. *Journal of the National Cancer Institute,* **39**: 1229-1234.

Mulinge, W., Wachira, A., Ngotho, R., Kimani, T., Maingi, P., Murithi, F., Wamae, L. and Maina, O. (2008): Potential socio-economic impacts of avian influenza and a compensation strategy for its control in Kenya. An unpublished report submitted to FAO and the Department of Veterinary Services, Kenya.

Mungube, E.O., Bauni S.M., Tenhagen, B.A., Wamae L.W., Nzioka S.M., Muhammed L. and Nginyi, J.M. (2007): Prevalence of parasites of the local scavenging chicken in a

selected semi-arid zone of Eastern Kenya. *Tropical Animal Health and Production,* **40**:101-109.

Murphy, F.A., Paul, J., Gibbs, E., Horznek, M. C. and Studdert, M. J. (1999): Veterinary Virology, 3rd edition, New York Academic Press. pp. 364-389.

National Veterinary Services Laboratories (NVSL) (2006): Procedures for collection and submission of specimens. National Veterinary Services Laboratories, Ames, Iowa, USA. http://www.aphis.usda.gov/vs/nvsl/html/shipping/html. Accessed on 21/07/2013, at 12.13 pm.

Njagi, L.W., Nyaga, P.N., Mbuthia, P.G., Bebora, L.C., Michieka, J.N., Kibe, J.K. and Minga, U. M. (2010): Prevalence of Newcastle disease virus in village indigenous chicken in varied ago-ecological zones in Kenya. *Livestock Research for Rural Development Journal,* **22:** 95.

Nyaga, P. (2007a): Good biosecurity practices in small scale commercial and scavenging production systems in Kenya. An analysis of the poultry sector in Kenya. Food and Agriculture Organization of the United Nations (FAO), Rome, pp. 93.

Nyaga, P.N. (2007b): The structure, marketing and importance of the commercial and village poultry industry: An analysis of the poultry sector in Kenya. Food and Agriculture Organization of the United Nations.

Ojok, L. (1993): Diseases as important factor affecting increased poultry production in Uganda. *Tropenlandwirt,* **94**: 37–44.

Okazaki, W., Purchase, H. G. and Burmester, B. R. (1975): Phenotypic mixing test to detect and assay avian leukosis viruses. *Avian Diseases,* 311-317.

Okello, J. J., Gitonga, Z., Mutune, J., Okello, R. M., Afande, M. and Rich, K. M. (2010): Value chain analysis of the Kenyan poultry industry: The case of Kiambu, Kilifi, Vihiga, and Nakuru Districts. http://www.ifpri.org/publication/value-chain-analysis-kenyan-poultry-industry.

Okeno, T. O., Kahi, A. K. and Peters, K. J. (2012): ** Characterization of indigenous chicken production systems in Kenya. *Tropical animal health and production*, **44: 601-608.

Okitoi, L.O., Udo, H.M.J. and Jong, D. (2006): Evaluation of low– input interventions for improved productivity of indigenous chicken in western Kenya. *Agricultura Tropica et Subtropica*, **39**: 3.

Okuthe, S.O. (1999): Participatory epidemiology assessment of livestock production constraints in the Western Kenya Highlands. (Unpublished PhD Thesis, University of Reading. Reading).

Olabode, H. O. K., Jwander, L. D., Moses, G. D., Ighodalo, E. and Egbaidomeh, S. A. (2009): Prevalance of avian leukosis and mareks disease in Ilorin, Kwara state, Nigeria. *Nigerian Veterinary Journal*, **30*:*** 64-68.

Omiti, J. M. and Okuthe, S. O. (2009): An Overview of the Poultry Sector and Status of Highly Pathogenic Avian Influenza (HPAI) in Kenya: Background Paper. International Food Policy Research Institute (IFPRI).

Ongor, H. and Bulut, H. (2011): PCR based evidence of reticuloendotheliosis virus infection in chicken from Turkey. *Pakistan Veterinary Journal*, **31**: 360-362.

**Pappenheimer, A. M., Dunn L. C. and Cone V. (1926): ** A study of fowl paralysis (neuro-lymphomatosis gallinarum). *Bulletin 143 Storrs Agricultural Experiment Station,* pp.187–289.

Payne, L. N., Brown, S. R., Bumstead, N., Howes, K., Frazier, J. A. and Thouless, M. E. (1991): A novel subgroup of exogenous avian leukosis virus in chicken. *Journal of General Virology,* **72**:801-807.

Payne, L. N. (1992): Biology of avian retroviruses. *The retroviridae,* **1**: 299-404.

Payne, L. N., Gillespie, A. M. and Howes, K. (1992): Myeloid leukaemogenicity and transmission of the HPRS-103 strain of avian leukosis virus. *Leukemia,* **6**:1167-1176.

Payne, L. N., Gillespie, A. M. and Howes, K. (1993): Unsuitability of chicken sera for detection of exogenous ALV by the group-specific antigen ELISA. *Veterinary Record,* **132**:555- 557.

Payne, L. N. and Fadly, A. M. (1997): Leukosis/Sarcoma Group. In: Diseases of Poultry, 10th ed. B. Calnek, J. Barnes, C. Beard, L. McDougald, and M. Saif, editions. Iowa State University Press, Ames, Iowa. pp. 414-466.

Payne L.N. (1998). Retrovirus-induced disease in poultry. *Poultry Science,* **77**:1204-1212.

Payne, L. N. and Venugopal, K. (2000): Neoplastic diseases: Marek's disease, avian leukosis and reticuloendotheliosis. *Revue scientifique et technique* (International Office of Epizootics), **19:** 544-564.

Payne, L. N. and Fadly, A. M. (2003): Leukosis/sarcoma Group. *In:* Calneck, B. W., Barnes, H. J., Beard, C. W., McDougald, C. R. and Saif, Y. X. (Edition), Diseases of Poultry, 11th Edition, Iowa State University Press, Ames, Iowa. pp. 465-519.

Payne L.N. and Nair V. (2012): The long view: 40 years of avian leukosis research. *Avian Pathology,* **41**: 11-19.

Pham, T. D., Spencer, J. L., Traina V. L., Dorge, M. D. A., G. R. F. and Johnson, E. S. (1999): Detection of exogenous and endogenous avian leukosis virus in commercial eggs

using reverse transcription and polymerase chain reaction assay. *Avian Pathology*, **28**:385-392.

Price, J. A. and Smith R. E. (1981): Influence of bursectomy on bone growth and anemia induced by avian osteopetrosis viruses. *Cancer Research*, **41**:752–759.

Pope, C. R., Odor E. M. and Salem, M. (1999): "Unusual eye lesions associated with ALV-J virus." Proceedings of Western Poultry Disease Conference.

Qin, L.T., Gao Y.L., Pan W., Deng X.Y., Sun F.F., Li K., Qi X.L., Gao H.L., Liu C.N. and Wang X.M., (2010): Investigation of co-infection of ALV-J with REV, MDV and CAV in layer chicken flocks in some regions of China. *Chinese Journal of Preventive Veterinary Medicine*, **32**: 90-93.

Qin, L., Gao, Y., Ni, W., Sun, M., Wang, Y., Yin, C. and Wang, X. (2013): Development and application of real-time PCR for detection of subgroup J avian leukosis virus. *Journal of Clinical Microbiology*, **51**: 149-154.

Rasheed, S. (1995): Retroviruses and oncogenes. *In:* Levy, J. (edition), *The Retoviridae*, **4**: 293-408. Plenum Press, New York.

Rispens, B. H., Long, P. A., Okazaki, W. and Burmester, B. R. (1970): The NP activation test for assay of avian leukosis/sarcoma viruses. *Avian Diseases*, 738-751.

Rubin, H. (1960): A virus in chick embryos which induces resistance in vitro to infection with Rous sarcoma virus. *Proceedings of the National Academy of Sciences of the United States of America*, **46:**1105.

Saif, Y.M., Fadly, A.M., Glisson, J.R., McDougald, L.R., Nolan, L.K. and Swayne, D.E. (2008): Diseases of Poultry 12th Edition, pp. 619-971.

Sani, N.A (2012): Pathological and serological study of avian leukosis of chicken in Funtua, Zaria and Kaduna, Nigeria, Doctoral dissertation.

Sani, N.A., Oladele, S.B., Raji, M.A. and Ibrahim, N.D.G. (2011): Seroprevalence of avian leukosis virus antigen using ELISA technique in exotic broilers and Nigerian local chicken in Zaria, Nigeria. *Veterinary World,* **4**: 345-348.

Sani, N. A., Oladele, S. B., Raji, M. A. and Ibrahim, N. D. G. (2012): Seroprevalence of avian leukosis virus antigen using ELISA technique in commercial exotic-layer chicken in Zaria and its environs. *African Journal of Microbiology Research,* **6**: 4438-4442.

Shepelo, G. P. and Maingi, N. (2014): Major causes of poultry mortality in Nairobi and its environs established from autopsies. *Kenya Veterinarian,* **38:** 32-42.

Silva, R. F., Fadly, A. M. and Taylor, S. P. (2007): Development of a polymerase chain reaction to differentiate avian leukosis virus (ALV) subgroups: Detection of an ALV contaminant in commercial Marek's disease vaccines. *Avian Diseases,* **51**:663–667.

Smith, E. J. and Fadly, A. M. (1994): Male-mediated venereal transmission of endogenous avian leukosis virus. *Poultry Science,* **73**:488–494.

Spackman, E., Pope, C.R., Cloud, S.S. and Rosenberger, J.K. (2003): The effects of avian leukosis virus subgroup J on broiler chicken performance and response to vaccination. *Avian Diseases,* **47**: 618-626.

Spencer, J. L., Chan, M. and Nandin-Davis, S. (2000): Relationship between egg size and subgroup J avian leukosis virus in eggs from broiler breeders. *Avian PathoIogy,* **29**:617-622.

Sung, H.W., Kim, J.H., Reddy, S. and Fadly, A.M. (2002): Isolation of subgroup J avian leukosis virus in Korea. *Journal of Veterinary Science,* **3**: 71-74.

Sun, S. and Cui, Z. (2007): Epidemiological and pathological studies of subgroup J avian leukosis virus infections in Chinese local "yellow" chicken. *Avian Pathology*, **36**:221–226.

Tell, L. A., Woods L. and Cromie, R. L. (2001): Tuberculosis in birds. *Review Science Technical, Office of International Epizootics*, **20**: 180–203.

Tomar, A. and Saxena, V. K. (2007): Avian leukosis/sarcoma virus infections-status and socio-economic impact revisited. *Indian Journal of Poultry Science*, **42***:*231-238.

Tsukamoto, K., Hasebe, M., Kakita, S., Hihara, H. and Kono, Y. (1991): Identification and characterization of hens transmitting avian leukosis virus (ALV) to their embryos by ELISAs for detecting infectious ALV, ALV antigens and antibodies to ALV. *Journal of Veterinary Medical Science*, **53**:859-864.

van de Lavoir, M. C., Diamond, J. H., Leighton, P. A., Mather- Love, C., Heyer, B. S., Bradshaw, R., Kerchner, A., Hooi, L. T., Gessaro, T. M., Swanberg, S. E., Delany, M. E. and Etches, R. J. (2006): Germline transmission of genetically modified primordial germ cells. *Nature*, **441**:766–769.

Venugopal, K., Smith, L. M., Howes, K. and Payne, L. N. (1998): Antigenic variants of J subgroup avian leukosis virus: sequence analysis reveals multiple changes in the env gene. *Journal of General Virology*, **79**: 757-766.

Wang, C.H., Chen, H.J. and Chang, A.C.H. (2007): Detection of subgroup J avian leukosis virus with monoclonal antibodies to the N-terminus of envelope protein gp85. *Taiwan Veterinary Journal*, **33**: 144-151.

Wikipedia (2015): Nairobi County. Accessed from http://en.wikipedia.org/wiki/Nairobi_County on 20th February, 2015 at 9.43 AM.

Williams, S. M., Fitzgerald, S. D., Reed, W. M., Lee, L. F. and Fadly, A. M. (2004): Tissue tropism and bursal transformation ability of subgroup J avian leukosis virus in White Leghorn chicken. *Avian Diseases,* **48**:921-927.

Williams, S.M. and Sellers, H.S. (2012): Response of White Leghorn chicken to infection with avian leukosis virus subgroup J and infectious bursal disease virus. *Avian Diseases,* **56**: 2-**6.**

Witter, R. L., Purchase, H. G., and Burgoyne, G. H. (1970b): Peripheral Nerve Lesions Similar to Those of Marek'ps Disease in Chicken Inoculated With Reticuloendotheliosis Virus. *Journal of the National Cancer Institute,* **45**: 567-577.

Witter, R. L., Bacon, L. D., Hunt, H. D., Silva, R. E. and Fadly, A. M., (2000): Avian leukosis virus subgroup J infection profiles in broiler breeder chicken: association with virus transmission to progeny. *Avian Diseases*, **44**:913-931.

Witter, R. L. and Fadly, A. M., (2001): Reduction of horizontal transmission of avian leukosis virus subgroup J in broiler breeder chicken hatched and reared in small groups. *Avian Pathology,* **30**:641-654.

Witter, R. L. and Fadly. A. M. (2003): Reticuloendotheliosis virus. Diseases of Poultry, 11th ed. Y. M. Saif, H. J. Barnes, J. R. Glisson, A. M. Fadly, L. R. McDougald, and D. E. Swayne. Iowa State University Press, Ames, pp. 517–536.

Witter, R.L., Gimeno, I.M. and Fadly, A.M. (2005): Differential Diagnosis of Lymphoid and Myeloid Tumors in the Chicken AAAP Slide Study Set 27 (Electronic media, pp. 1-49). Athens, GA: American Association of Avian Pathologists.

World Organization for Animal Health (2008): Avian tuberculosis. http://www.oie.int/fileadmin/Home/eng/Health_Standards/tahm/2.03.06_AVIAN_TB. Accessed on 9th September, 2014, 3.28 pm.

World Organization for Animal Health (2010): Marek's disease. Accessed from http://www.oie.int/eng/normes/mmanual/A_00103. Accessed on 9/9/2014, 3.30pm**.**

Wu X., Qian K., Qin A., Shen H., Wang P., Jin W. and Eltahir Y.M. (2010): Recombinant avian leukosis viruses of subgroup J isolated from field infected commercial layer chicken with hemangioma and myeloid leukosis possess an insertion in the E element. *Veterinary Research Communications,* **34**: 619–632.

Yang, F., Xian, R. R., Li, Y., Polony, T. S. and Beemon, K. L. (2007): Telomerase reverse transcriptase expression elevated by avian leukosis virus integration in B cell lymphomas. Proceedings of the National Academy of Sciences of theUnited States of America **104,** 18952–18957.

Zavala, G., Jackwood M. W. and Hilt A. D. (2002): Polymerase chain reaction for detection of avian leukosis virus subgroup J in feather pulp. *Avian Diseases,* **46**:971–978.

Zhang, L.J., Liu, J., Cheng, Z.Q., Wang, G.H., Diao, X.G. and Zhu, G. (2006): Detecting avian leukosis virus subgroup J using tissue chip and immunohistochemical technology. *Chinese Journal of Preventive Veterinary Medicine,* **4**:427-430.

Zhang, H., Bacon, L.D. and Fadly, A.M. (2008): Development of an endogenous virus-free line of chicken susceptible to all subgroups of avian leukosis virus. *Avian Diseases,* **52**: 412-418.

7.0 APPENDICES

APPENDIX 1: CLINICAL SCORE CARD AND OBSERVATION SHEET

A) BACKGROUND INFORMATION

1. Name of the farm/homestead/establishment……..……..

2. County.....County Ward.....Village.....

3. Name of person interviewed (respondent).....Sex 1=male 0=female

4. Respondent's age group: (1) up to 30 years (2) >30-60 years (3) over 60 years

5. Type and number of chicken (s) kept on the farm?

   i) Indigenous……

   ii) Commercial layer…….

   iii) Commercial Broilers……

6. Where did you source your chicken/chick stock? (1) Purchase from hatchery (2) Purchase from local markets (3) others (specify)……..

   (i) If (1), from which hatchery? …….....

7. Age (weeks)…….. Sex …… Type…….

8. Total number of chicken …… Number sick ….Number dead since disease onset……

B) CLINICAL MANIFESTATIONS

Demeanor Active…… Dull……

Feeding YES ……NO……

Locomotion Dragging…… Paralyzed (specify)……

Coughing/snoring YES ……..NO…….....

Eye/nasal discharges ……..

Perineum/Vent (clean/soiled) (Yes) …. …….NO……...

Colour of diarhoea/matting……..

Body surface swelling (s)……..

Nervous signs (specify)…….

Any other clinical signs observed…….

APPENDIX 1 (CONTINUES)

……

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Remarks……

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APPENDIX 2: FINDINGS FROM THE BIRDS IN THE PROSPECTIVE CLINICAL AND PATHOLOGICAL STUDY

Farm Number	County	Case Number	Type of chicken	Clinical signs (summary)	Gross Lesions (summary)	Histopathology Findings (summary)	Elisa Unit Values	Remarks
I	Kiambu	AO1/2014	C.L	Brownish diarrhoea, torticollis, weakness, unthriftiness,and leg paralysis	Emaciation, Nodular liver lesions, renomegaly, cardiomegaly,	Lymphocytic and lymphoblastic infiltrations in the liver and kidney with mitotic figures	10%	Weakly positive
I	Kiambu	B01/2014	C.L	Torticollis, weakness	Emaciation, enlarged proventriculus, thickened proventricular wall, enlarged thigh muscles	Lymphocytic and lymphoblastic infiltrations in the liver, kidney, proventriculus;	23%	Weakly positive
I	Kiambu	C01/2014	C.L	Brownish diarrhoea, weakness, unthriftiness, and leg paralysis	Fair body score, cardiomegaly, hepatomegaly, renomegaly, congested spleen	Heavy lymphocytic and lymphoblastic infiltrations in the liver, kidney, heart, and proventriculus; with destruction of myofibres of heart	14%	Weakly positive
I	Kiambu	D01/2014	C.L	Brownish diarrhoea, torticollis, weakness	Emaciation, nodular liver lesions, splenomegaly, spleen congestion	Lymphocytic and lymphoblastic infiltrations in the liver, kidney, heart, and proventriculus	8%	Negative
I	Kiambu	E01/2014	C.L	Brownish diarrhoea, unthriftiness, weakness, and leg paralysis	Emaciation, hepatomegaly, enteritis,	Lymphocytic and lymphoblastic infiltrations in the liver and proventriculus	19%	Weakly positive
II	Nairobi	AO2/2014	C.L	No clinical signs noted	Congested liver, congested spleen, congested kidneys, congested lungs	Inflammatory cell infiltrations in the liver; with slight lymphocytic infiltrations	14%	Weakly positive
II	Nairobi	B02/2014	C.L	Brownish diarrhea	Renomegaly, cardiomegaly, nodular spleen lesions, emaciation	Lymphocytic and lymphoblastic infiltrations in the lungs, spleen and kidney	12%	Weakly positive
II	Nairobi	C02/2014	C.L	No clinical signs noted	Splenomegaly, enlarged proventriculus, thickened proventricular wall	Inflammatory cell infiltrations in the liver, proventriculus; with slight lymphocytic infiltrations	5%	Negative

APPENDIX 2 (CONTINUES)

II	Nairobi	D02/2014	C.L	Brownish diarrhea	Splenomegaly, enteritis	Inflammatory cell infiltrations in the kidney; with slight lymphocytic infiltrations, congestion of renal arteries	9%	Negative
II	Nairobi	E02/2014	C.L	Leg paralysis	Nodular liver lesions, nodular spleen lesions	Mature lymphocytic infiltrations and mitosis	2%	Negative
III	Kiambu	AO3/2014	C.L	Anorexia, dullness	Congested liver, congested kidneys, congested spleen, congested lungs	Inflammatory cell infiltrations and congested blood vessels in the kidney, spleen, and lungs	4%	Negative
III	Kiambu	B03/2014	C.L	Brownish diarrhea	Enteritis	Inflammatory cell infiltrations in the kidney, and intestines	6%	Negative
III	Kiambu	C03/2014	C.L	Anorexia, dullness	Hepatomegaly, cardiomegaly, emaciation	Lymphocytic and lymphoblastic infiltrations in the liver, and nerves	67%	Moderately positive
III	Kiambu	D03/2014	C.L	Anorexia	Nodular liver lesions	Congestion of blood vessels in the liver with slight inflammatory infiltration and occasional necrosis	3%	Negative
III	Kiambu	E03/2014	C.L	Brownish diarrhoea, dullness	Hepatomegaly, thickened proventricular wall	Inflammatory cell infiltrations in the liver and proventriculus	6%	Negative
IV	Kiambu	AO4/2014	C.L	Anorexia	Emaciation, renomegaly	Inflammatory cell infiltrations in the liver; with slight mature lymphocytic infiltrations	2%	Negative
IV	Kiambu	B04/2014	C.L	Leg paralysis	Splenomegaly, enlarged proventriculus	Inflammatory cell infiltrations in the proventriculus and spleen	7%	Negative
IV	Kiambu	C04/2014	C.L	Anorexia, dullness	Nodular liver lesions	Inflammatory cell infiltrations in various organs/tissues	8%	Negative
IV	Kiambu	D04/2014	C.L	Leg paralysis	Hepatomegaly, enlarged proventriculus, congested spleen, congested kidneys, congested lungs, enlarged thigh muscles	Slight inflammatory cell infiltrations in the lungs and skeletal muscle	5%	Negative
IV	Kiambu	E04/2014	C.L	Anorexia, dullness	Splenomegaly, enlarged bursa	Inflammatory cell infiltrations in kidney	8%	Negative
V	Machakos	AO5/2014	C.L	Unthriftiness	Splenomegaly, cardiomegaly, haemorrhagic ovules	Necrosis and inflammatory cell infiltrations in the liver and spleen; Lymphocytic and lymphoblastic infiltrations in the liver, kidney, and ovary	11%	Weakly positive

APPENDIX 2 (CONTINUES)

V	Machakos	B05/2014	C.L	Brownish diarrhoea, unthriftiness	Hepatomegaly, congested lungs	Predominantly inflammatory cell infiltrations in organs/tissues	3%	Negative
V	Machakos	C05/2014	C.L	Anorexia, ruffled feathers	No significant gross lesions	No significant lesions	6%	Negative
V	Machakos	D05/2014	C.L	Brownish diarrhea	Renomegaly, haemorrhagic intestines with nodular lesions, enteritis	Predominantly inflammatory cell infiltrations in sampled organs/tissues	6%	Negative
V	Machakos	E05/2014	C.L	Anorexia, unthriftiness	Emaciation, hepatomegaly, flabby heart, enlarged bursa	Predominantly inflammatory cell infiltrations in organs/tissues; with necrosis of hepatocytes	9%	Negative
VI	Kiambu	AO6/2014	C.L	Brownish diarrhoea, ruffled feathers	Flabby heart, enlarged thigh muscles	Inflammatory cell infiltrations in the skeletal muscle; Lymphocytic and lymphoblastic infiltrations in the liver, kidney, hepatitis	17%	Weakly positive
VI	Kiambu	B06/2014	C.L	Brownish diarrhoea, unthriftiness	Hepatomegaly, nodular liver lesions	Inflammatory cell infiltrations in organs/tissues, Lymphocytic and lymphoblastic infiltrations in the liver, kidney	10%	Weakly positive
VI	Kiambu	C06/2014	C.L	Ruffled feathers	Congested spleen, adhesion of visceral organs	Predominantly inflammatory cell infiltrations in the kidney	5%	Negative
VI	Kiambu	D06/2014	C.L	Brownish diarrhea	Nodular liver lesions, enteritis, congested kidneys	Predominantly inflammatory cell infiltrations in the liver, with slight lymphoblastic infiltrations	7%	Negative
VI	Kiambu	E06/2014	In.	Unthriftiness, ruffled feathers	Emaciation, hepatomegaly, multifocal nodular liver lesions, thickened proventricular wall, renomegaly	Necrosis and inflammatory cell infiltrations in the liver and spleen; Lymphocytic and lymphoblastic infiltrations in the liver, kidney	84%	Strongly positive
VII	Machakos	AO7/2014	C.L	Anorexia, dullness	Hepatomegaly, flabby heart, enteritis, adhesion of visceral organs, enlarged bursa	Congested blood vessels in the portal area; haemosiderosis	9%	Negative

APPENDIX 2 (CONTINUES)

VII	Machakos	B07/2014	C.L	Whitish diarrhoea, dullness, leg paralysis	Congested liver, congested spleen, congested kidneys, congested lungs	Lymphocytic and lymphoblastic infiltrations in the liver, kidney, and inflammatory cell infiltrating the liver	19%	Weakly positive
VII	Machakos	C07/2014	C.L	Anorexia, unthriftiness, skin swellings	Emaciation, nodular liver lesions, renomegaly, enlarged proventriculus, nodular spleen lesions	Lymphocytic and lymphoblastic infiltrations in the liver, kidney, necrosis and inflammatory cell infiltrations in the liver and spleen	80%	Strongly positive
VII	Machakos	D07/2014	C.L	Whitish diarrhoea, dullness, unthriftiness, leg paralysis	Hepatomegaly	Inflammatory cell infiltrations in the liver; with slight lymphocytic infiltrations	42%	Moderately positive
VII	Machakos	E07/2014	C.L	Anorexia, unthriftiness	Renomegaly, cardiomegaly	Lymphocytic and lymphoblastic infiltrations in the liver, kidney, hepatitis	28%	Moderately positive
VIII	Kiambu	AO8/2014	C.L	leg paralysis, ruffled feathers	Adhesion of visceral organs with fibrin deposition	Inflammatory cell infiltrations in organs/tissues	1%	Negative
VIII	Kiambu	B08/2014	C.L	Anorexia, ruffled feathers	Ascites	Slight inflammatory cell infiltrations in the kidneys	5%	Negative
VIII	Kiambu	C08/2014	C.L	Whitish diarrhoea, drowsiness	Emaciation, congested spleen, congested kidneys, enteritis	Slight inflammatory cell infiltrations in the liver, heart, and kidney	2%	Negative
VIII	Kiambu	D08/2014	C.L	Unthriftiness, leg paralysis	No significant gross lesions	Inflammatory cell infiltrations in the liver; with slight lymphocytic infiltrations	3%	Negative
VIII	Kiambu	E08/2014	C.L	Anorexia	No significant lesions	No significant lesions	1%	Negative
IX	Kiambu	AO9/2014	C.L	Brownish diarrhoea, cannibalism	No significant lesions	Inflammatory cell infiltrations in the liver and skin	6%	Negative
IX	Kiambu	B09/2014	C.L	Brownish diarrhea	Nodular liver lesions	Inflammatory cell infiltrations in the liver	7%	Negative
IX	Kiambu	C09/2014	C.L	Anorexia	Hepatomegaly, flabby heart	Inflammatory cell infiltrations in the heart and liver	4%	Negative
IX	Kiambu	D09/2014	C.L	Anorexia	Emaciation, splenomegaly	Inflammatory cell infiltrations in the kidney, and heart	1%	Negative
IX	Kiambu	E09/2014	C.L	Brownish diarrhea	Hepatomegaly, renomegaly	Inflammatory cell infiltrations in organs/tissues	2%	Negative

APPENDIX 2 (CONTINUES)

X	Kiambu	A10/2014	C.L	Drowsiness, leg paralysis, ruffled feathers	Enlarged sciatic nerves	Lymphocytic and lymphoblastic infiltrations in the liver, kidney,	3%	Negative
X	Kiambu	B10/2014	C.L	Brownish diarrhoea, unthriftiness	Congested liver, congested lungs	Lymphocytic and lymphoblastic infiltrations in the liver, kidney, and nerves	5%	Negative
X	Kiambu	C10/2014	C.L	Anorexia, dullness, leg paralysis	Renomegaly, thickened proventricular wall, adhesion of visceral organs	Mature lymphocytic infiltrations and mitosis in the liver, infiltration of macrophages and heterophils in kidney	2%	Negative
X	Kiambu	D10/2014	C.L	Anorexia, dullness, ruffled feathers	Enlarged sciatic nerves	Lymphocytic and lymphoblastic infiltrations in the liver, kidney, nerves, oedema-like nerves	4%	Negative
X	Kiambu	E10/2014	C.L	Anorexia, dullness, unthriftiness, leg paralysis	Emaciated, congested kidneys renomegaly, congested spleen	Inflammatory cell infiltrations in organs/tissues	5%	Negative

Key:

C.L-Commercial Layers

In.-Indigenous Chicken

APPENDIX 3: POST MORTEM LESIONS RECORDING SHEET

Case Number……..

Date…….

Post Mortem Lesions

External Observation Remarks**……**

…….

Body condition score: Good…….…… Fair……..…….. Poor……

Carcass: Colour……..….Dehydration……Others……..

Liver: Size…….Colour……...Others……..

Nodular Liver lesions…….…… Others……....

Spleen: Size……..……Colour……..Others……..

Nodular spleen Lesions…….……Others…….. …

Heart: Size……Colour……..Others……..

Proventriculus: Size……Colour……...Others……..

Kidneys: Size……Colour…….Others……..

Nodular Kidney Lesions……Others……

Sciatic Nerves: Size……Oedema……Loss of Striations……

Sampled Tissues/Organs for Histopathology (Tick where appropriate)……

Liver……

Spleen……..

Heart……

Proventriculus…….

Kidney…….

Sciatic Nerves……..

Any Other Organ/Tissue Sampled…….**.....**

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APPENDIX 3 (CONTINUES)

Any other lesions/comments…….....**.....**

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Post mortem diagnosis……

APPENDIX 4: ELISA UNIT READINGS INTERPRETATION

Plate 1	1	2	3	4	5	6	7	8	9	10	11	12
A	Blank	4	4.6	4.7	4.5	2	4.3	4.4	1	20.4	3	4
B	NC	1.8	1.5	43	0.6	5	0.6	2	2.5	0.6	1	99
C	NC	2.6	1.9	5.5	4.5	2.5	14.5	3	3.7	7.9	1	73
D	PC	8.7	3.4	3	2.4	7	3	2	3.1	4	2	28
E	PC	3.6	6.6	3	3	4	3	2	15.1	3.3	5.8	3
F	12.9	3.4	7	2.5	4	8.8	2.7	2	3	3	2.8	29.3
G	2.8	10	15.4	4.3	11.8	3	2	6	4	2	4	81
H	80	17.6	3.4	5.3	8	3	2.8	2.5	12.4	2	4	3

Plate 2	1	2	3	4	5	6	7	8	9	10	11	12
A	Blank	79	8	9	5.3	1	3.4	8	4	6	9	6
B	NC	15.2	8.6	4.5	4.5	8.2	1.7	1.5	6	3	4	6
C	NC	4	4	5	4	9	9	9	40	5	7	4
D	PC	19	6.2	5.5	6	8.1	4	8	5.4	8	7	7.2
E	PC	8	8	19.8	16	9	5	2	2	5	8	8
F	8.9	8	7	12	6	5	2	6.4	6.2	2	3.1	1.2
G	23	6	4	6	5	4	6	1	1	2	5	7.3
H	10	6.6	3	4.7	23	4	7	5	3.3	6	6	5.4

APPENDIX 4 (CONTINUES)

Plate 3	1	2	3	4	5	6	7	8	9	10	11	12
A	Blank	9.1	1.9	2	8	10.1	1.7	28	16.6	4	11	27.1
B	NC	6	9	5.2	24.6	6.8	10.9	14.1	3	12	5.8	11
C	NC	6.6	5.8	3	6	11.6	2	5.8	14.7	26.5	12	12.4
D	PC	7.2	8.9	3.3	13.5	30	11	21.7	6	23.6	24	1.3
E	PC	8	7	2.4	23	8.9	13.8	1.7	17.2	16.7	8.6	35.7
F	4.1	2.9	4.5	28	24	13.4	23	23.5	6	33	24	5.8
G	8	4.1	15.4	5.6	6.6	34.5	22.7	4.6	13.6	6	10	35.2
H	7.5	16.5	2.2	27	22	21.3	21.5	22	20	19	21.6	11

Plate 4	1	2	3	4	5	6	7	8	9	10	11	12
A	Blank	26	25	15.5	4	30	2	2	4	13.6	4.2	18.7
B	NC	8.6	5	1	16.2	5.7	19.8	22	20	3.1	19	3.2
C	NC	30	22	15.6	9	7.5	4	8.2	23	1.5	21	5
D	PC	7	7	4.8	13	4	10.4	3.5	12.2	2.1	6.8	21.2
E	PC	16	12	21	5.7	17	15	5	91	6.8	20.5	18
F	5.6	93	2	4.7	37	2.2	11	2.1	5	18.8	8.1	18
G	15.3	1.5	16	13	7.3	4	5	8.3	24	6	17	8
H	2	13	17.5	1.6	14	6	23.4	2	22	14.5	4	14.3

APPENDIX 4 (CONTINUES)

Plate 5	1	2	3	4	5	6	7	8	9	10	11	12
A	Blank	20	19.5	6.7	12	6	6	9	3	5		
B	NC	14.8	6.3	7	5	2	6	19	1	2		
C	NC	3	15.5	10	9	7	9	80	6	4		
D	PC	13.5	5	23	2	8	17	42	7	5		
E	PC	5.8	22.8	14	4	5	10	28	4			
F	13.2	36.6	6.7	8	6	8	5	1	1			
G	13.6	4	18.5	19	67	11	7	5	2			
H	14	24.5	15	14	3	3	84	2	3			

Key

PC-Positive control

NC-Negative control

APPENDIX 5: DIAGNOSED CASES OF AVIAN LEUCOSIS BETWEEN THE YEAR 2003-2012 (RETROSPECTIVE STUDY) (Source: VPMP; CVL)

No.	**Path. Case No.**	**Date**	**Type**	**Age in Weeks**	**No. in the flock**	**No. dead**	**Hatchery**	**C/S**	**Lesions**	**Diagnosis (ses)**
1	DV7/03	20/2/03	Layer	12	304	2	C	Death	Hepatomegaly, splenomegaly and enlarged proventriculus.	AL
2	DV16/03	24/6/03	Layer	16	200	50	-	Weight loss, dullness, death	Pneumonia, pericarditis, congestion, hepatomegaly, splenomegaly, enteritis, peritonitis and enlarged bursa.	AL
3	DV26/03	8/7/03	Layer	20	1000	200	-	Death	Emaciation, hepatomegaly,splenomegaly,renomegaly , thickened proventricular walls, and haemorrhagic intestines	AL
4	A10/04	26/1/04	Layer	-	-	-	A	Diarrhoea	Hepatomegaly, splenomegaly and enlarged nodular intestines.	AL
5	A47/04	16/3/04	Layer	-	-	-	-	-	Hepatomegaly, splenomegaly, and renomegaly	AL
6	DV13/04	17/4/04	Layer	40	204	24	-	Torticollis, diarrhoea	Emaciation, enteritis and splenomegaly.	AL
7	DV19/04	17/6/04	Layer	16	500	30	-	Dullness, weakness	Skin tumours, congestion of viscera	AL
8	DV68/04	21/12/04	Layer	-	300	4	-	Weakness, anorexia	Emaciation, pale and enlarged kidneys.	AL
9	A53/05	2/3/05	Layer	-	-	-	-	-	Nodular lesions on the liver.	AL
10	A329/06	8/5/06	Layer	-	-	-	A	-	Hepatomegaly, congested kidneys.	AL
11	A283/06	4/6/06	Layer	-	-	-	-	Weakness, inappetence, death	Hepatomegaly, splenomegaly, and renomegaly.	AL
12	A400/06	7/6/06	Layer	-	-	-	-	Dullness, anorexia, diarrhea	Hepatomegaly, renomegaly.	AL

APPENDIX 5 (CONTINUES)

13	DV24/06	16/6/06	Layers	18	200	20	-	Anorexia, weakness	Hepatomegaly, renomegaly, splenomegaly, cystic ovaries	AL
14	A452/06	27/6/06	Layers	20	-	-	C	Weakness, diarrhoea, inappetence, death	Hepatomegaly, splenomegaly, renomegaly; with lymphocytic infiltrations in the liver.	AL
15	DV28/06	3/10/06	Layer	19	200	20	-	Death	Emaciation, hepatomegaly, splenomegaly, swollen proventriculus.	AL
16	A668/06	11/12/06	Layer	16	-	-	A	-	Hepatomegaly, splenomegaly.	AL
17	A32/07	8/2/07	Layer	22	500	200	A	Weakness and dullness	Congestion of the liver, heart, and lungs. Enlarged sciatic nerve.	AL
18	A55/07	22/2/07	Layer	24	500	200	-	Diarrhoea, weakness, and death	Nodular growth on intestinal wall, splenomegaly, enlarged sciatic nerve.	AL
19	A76/07	12/3/07	Layer	16	300	30	-	Drowsiness, inappetence, and death	Hepatomegaly, splenomegaly with whitish nodules.	AL
20	A105/07	2/4/07	Layer	28	700	100	-	-	Emaciation, hepatomegaly, splenomegaly, haemorrhagic enteritis, nodular /cystic ovaries.	AL
21	A108/07	1/4/07	Layer	16	400	25	A	Diarrhoea, ruffled feathers, and death	Hepatomegaly, splenomegaly; with nodular lesions on the kidneys.	AL
22	A120/07	13/04/07	Layer	20	600	3	A	Anorexia, drowsiness, and paralysis	Hepatomegaly, nodular lesions on the heart and kidney.	AL
23	A121/07	16/4/07	Layer	14	600	-	A	Drooping wings, inappetence, and death	Hepatomegaly, splenomegaly	AL
24	A174/07	6/6/07	Layer	16	2000	670	A	Diarrhoea and torticollis	-	AL

APPENDIX 5 (CONTINUES)

25	PU26/07	3/7/07	Layer	15	585	1	-	Diarrhoea	Splenomegaly, pericarditis, enlarged and congested kidneys.	AL
26	A199/07	9/7/07	Layer	32	1100	600	A	Diarrhoea	Hepatomegaly with white nodular lesions and regressed ovaries.	AL
27	A205/07	13/7/07	-	-	-	-	-	Sudden death	Hepatomegaly, air sacculitis, lymphocytic infiltration of the lungs, liver, and kidneys.	AL
28	A210/07	16/7/07	Layer	16	700	100	C	Dullness, torticollis	Hepatomegaly, splenomegaly, nodules on the heart.	AL
29	A246/07	23/08/07	Layer	20	200	2	D	Sudden death	Emaciation, hepatomegaly, splenomegaly, regressed ovaries.	AL
30	A259/07	10/9/07	Layer	16	900	5	D	-	Hepatomegaly, splenomegaly, regressed ovaries	AL
31	A260/07	10/9/07	Layer	20	200	10	D	Sudden death	Emaciation, hepatomegaly, splenomegaly and pneumonia.	AL
32	A346/07	6/12/07	Layer	19	600	36	D	Diarrhoea and death	Hepatomegaly, splenomegaly, renomegaly. Hypertrophy of proventricular mucosa.	AL
33	A32/08	26/2/08	Layer	20	1600	50	C	Diarrhoea, anorexia	Hepatomegaly, Splenomegaly, enlarged haemorrhagic proventriculus	AL
34	A33/08	27/2/08	Layer	-	10	-	-	Drowsiness, weakness, death	Emaciation, cardiomegaly, hepatomegaly, splenomegaly, enlarged bursa. Nodular lesions on the liver.	AL
35	A100/08	12/5/08	Layer	-	500	150	-	Sudden death	Emaciation, hepatomegaly, splenomegaly with white nodular lesions	AL
36	DV21/08	12/5/08	Layer	11	-	-	-	-	Emaciation, splenomegaly, hepatomegaly, diffuse whitish foci in the liver, haemorrhagic enteritis. Histopathological Findings; Infiltrating cells comprised of large lymphocytes and mitotic figures.	AL
37	A102/08	19/5/08	Layer	20	500	28	A	Bloody diarrhoea, inappetence	-	AL
38	A105/08	19/5/08	Layer	14	1000	200	A	Brownish diarrhoea	Emaciation, hepatomegaly, splenomegaly, regressed ovaries	AL

APPENDIX 5 (CONTINUES)

39	A115/08	3/6/08	Layer	24	100	25	B	Weakness, diarrhoea	-	AL
40	A117/08	3/6/08	Layer	20	1,000	200	A	Brownish diarrhoea	Emaciation, splenomegaly, pneumonia, regressed ovaries.	AL
41	A119/08	9/6/08	Layer	20	1200	100	-	Lameness, bloody diarrhoea, sudden death	Emaciation, hepatomegaly, splenomegaly, regressed ovaries	AL
42	A126/08	17/6/08	Layer	16	2300	110	D	Weakness, anorexia	Hepatomegaly, splenomegaly, renomegaly	AL
43	A140/08	30/6/08	Layer	14	800	5	A	-	Emaciation, splenomegaly, hepatomegaly,nodular lesions on the kidney	AL
44	A143/08	2/7/08	Layer	18	1000	400	A	Cannibalism, brownish diarrhoea, ruffled feathers	Emaciation, hepatomegaly with whitish nodules	AL
45	A148/08	8/7/08	Layer	22	1000	520	D	Ruffled feathers, whitish diarrhoea	Emaciation, hepatomegaly with white nodular lesions on the heart	AL
46	A183/08	11/8/08	Broiler	7	2500	330	C	Weakness	Swollen joints enlarged sciatic nerves	AL
47	DV55/08	16/9/08	Layer	17	300	40	-	Anorexia, weakness, death	Emaciation, enteritis and proventriculitis.	AL
48	DV58/08	13/10/08	Layer	-	1250	400	-	Weakness, diarrhoea, death	Tumourous masses in the kidneys, liver and spleen	AL
49	DV60/08	15/10/08	Layer	16			-	-	-	AL
50	A273/08	18/12/08	Layer	-	500	52	A	-	Emaciation, liver with white foci	AL
51	A213/08	25/9/08	Layer	-	1000	100	D	Stunted growth rate, diarrhoea	Emacaition, hepatomegaly, splenomegaly	AL
52	A15/09	30/1/09	Layer	16	1200	100	A	Ruffled feathers	Emaciation, hepatomegaly, splenomegaly	AL

APPENDIX 5 (CONTINUES)

53	EXDV28/09	5/2/09	Layer	26	135	8	-	Torticollis, diarrhoea, anorexia	Splenomegaly, hepatomegaly	AL
54	DV42/09	9/4/09	Layer	20	600	100	-	Drowsiness, death	Hepatomegaly, enlarged proventriculus	AL
55	A112/09	29/5/09	LAYER	12	600	22	A	Weakness, brownish diarrhoea	Emaciation, splenomegaly, nodular lesion on the spleen	AL
56	A129/09	23/6/09	Layer	-	200	25	A	Diarrhoea, death	Hepatomegaly, renomegally, consolidated lungs	AL
57	A255/09	8/12/09	Layer	36	1020	200	A	Weakness, anorexia, torticollis	Hepatomegaly, mucoid enteritis	AL
58	A10/10	25/1/10	Layer	28	700	18	A	Dullness, anorexia, diarrhoea	Mucoid enteritis, congestion of the liver	AL
59	A17/10	28/1/10	Layer	18	700	69	A	Dullness, diarrhoea	Hepatomegaly with whitish nodules, splenomegaly, regressed ovaries.	AL
60	A19/10	1/2/10	Layer	44	150	51	C	Weakness, reduced egg production, death	Emaciation, hepatomegaly, splenomegaly, enlarged sciatic nerve.	AL
61	A25/10	5/2/10	Layer	12	-	-	A	Weakness, anorexia	Emaciation, hepatomegaly, splenomegaly, and renomegaly.	AL
62	DV11/10	16/2/10	Layer	15	458	20	-	Bloody diarrhoea	Lymphocytic foci on the liver, proventriculus, gizzard, messentry, spleen; Lymphocytic infiltration in the liver.	AL
63	A30/10	18/2/10	Layer	12	-	-	A	Dullness	Emaciation, hepatomegaly, renomegaly, and splenomegaly.	AL
64	A35/10	22/2/10	Layer	12	500	30	A	Dullness, ruffled feathers	Emaciation, hepatomegaly with nodular lesions.	AL
65	A44/10	26/2/10	Layer	8	200	60	-	-	Haemorrhagic intestinal mucosa, emaciation.	AL
66	A49/10	3/3/10	Layer	12	500	3	A	Dullness, death	Nodular lesions on the spleen, regressed ovaries, cloudy air sacs.	AL

APPENDIX 5 (CONTINUES)

67	DV20/10	30/3/10	Layer	16	500	100	-	Yellowish diarrhoea, lameness	Emaciation, nodular masses on the thigh muscles, subcutis, ventriculus, proventriculus, bursa, and intestinal serosa; Liver with uniform tumour cells arranged in chords with mitotic figures, infiltration of tumour cells (undergoing mitosis) ventriculus and proventriculus.	AL
68	A71/10	6/4/10	Layer	14	600	30	D	Diarrhoea, anorexia	Splenomegaly, hepatomegaly.	AL
69	A75/10	8/4/10	Layer	29	400	100	D	-	Renomegaly with whitish nodules, nodular lesions on the liver, regressed ovaries	AL
70	A81/10	14/4/10	Layer	16	500	30	A	-	Emaciation, hepatomegaly	AL
71	A87/10	4/5/10	Layer	28	100	5	B	Drowsiness, reduced egg production	Hepatomegaly, splenomegaly.	AL
72	A104/10	10/5/10	Layer	8	2500	20	B	Dullness, ruffled feathers	Hepatomegaly with nodular lesions.	AL
73	A105/10	10/5/10	Layer	-	270	4	B	-	Hepatomegaly, nodular lesions on the kidney.	AL
74	DV29/10	31/5/10	Layer	16	2100	15	-	Sudden death	Emaciation, nodular masses on the proventriculus, intestines, liver, enlargement of liver, kidney; air sacculitis	AL
75	A134/10	2/6/10	Layer	28	6000	70	A	-	Emaciation, hepatomegaly, renomegaly with whitish nodules.	AL
76	DV32/10	22/6/10	Layer	16	1000	30	-	Dullness, weakness, anorexia	Lymphocytic infiltration in organs like the liver, heart, and lungs.	AL
77	DV34/10	22/6/10	Layer	8	1000	5	-	Sudden death	Lymphocytic infiltration in the visceral organs such as heart and lungs.	AL
78	A154/10	2/7/10	Layer	24	293	6	B	Weakness, dullness, anorexia	Emaciation, hepatomegaly, renomegaly, splenomegaly	AL

APPENDIX 5 (CONTINUES)

79	A170/10	19/7/10	Layer	28	550	20	C	Anorexia, dullness, diarrhoea	Emaciation, renomegaly, hepatomegaly.	AL
80	A173/10	21/7/10	Layer	40	200	5	B	Anorexia, diarrhoea	Emaciation, Enlarged kidneys, mass on the intestinal wall.	AL
81	DV39/10	21/7/10	Layer	14	2000	80	-	Paralysis, death	Splenomegaly, hepatomegaly and enlarged kidney.	AL
82	A179/10	27/7/10	Layer	24	1000	20	A	Drowsiness, whitish diarrhoea	Emaciation, hepatomegaly, splenomegaly	AL
83	A182/10	28/7/10	Layer	36	750	300	C	Diarrhoea, weight loss, drooping wings	Emaciation, congested lungs, hepatomegaly, splenomegaly	AL
84	A192/10	11/8/10	Layer	16	2600	100	B	Drooping wings, inappetence	Emaciation, renomegaly, haemorrhagic intestines	AL
85	A195/10	13/8/10	Layer	30	2050	38	B	Anorexia, diarrhoea, weakness, death	Peritonitis, fibrin covered liver	AL
86	A196/10	14/8/10	Indigenous	-	12	1	-	-	Emaciation, hepatomegaly and splenomegaly	AL
87	A232/10	29/10/10	Layer	-	350	100	A	Dullness, weight loss	Renomegaly, hepatomegaly, emaciation	AL
88	A263/10	14/10/10	Layer	12	500	20	B	Diarrhoea, drooping wings	Emaciation, hepatomegaly, renomegaly with nodular lesions, cardiomegaly	AL
89	A294/10	23/11/10	Layer	44	750	150	C	-	Emaciation, cardiomegaly, enlarged haemorrhagic kidneys, enteritis	AL
90	A297/10	26/11/10	Layer	18	800	130	C	Weakness, anorexia	Hydropericardium, splenomegaly, haemorrhagic kidneys	AL
91	A307/10	14/12/10	Layer	24	3000	100	C	Torticollis	Cardiomegaly	AL
92	A310/10	16/12/10	Layer	24	350	30	B	Torticollis, diarrhoea	Cardiomegaly, renomegaly, thickened intestinal wall	AL
93	A311/10	16/12/10	Layer	12	11500	12	B	Diarrhoea, anorexia	Emaciation, cardiomegaly, renomegaly, hepatomegaly,	AL

APPENDIX 5 (CONTINUES)

94	A312/10	16/12/10	Layer	16	2500	60	C	Weakness, death	Emaciation, haemorrhagic intestines	AL
95	A313/10	17/12/10	Layer	-	1500	200	C	Anorexia, sudden death	Emaciation, splenomegaly, cardiomegaly, renomegaly, haemorrhagic ovules	AL
96	A17/11	19/1/11	Layer	-	300	-	C	Diarrhoea, anorexia, reduced egg production	Emaciation	AL
97	A51/11	9/2/11	Layer	28	320	2	B	Sudden death	Haemorrhagic and enlarged kidneys, haemorrhagic lungs	AL
98	A77/11	7/3/11	Layer	11	400	5	A	Anorexia, dullness, death	Emaciation, splenomegaly, hepatomegaly, renomegaly, enlarged bursa	AL
99	A78/11	8/3/11	Layer	20	700	300	C	Dullness, anorexia, paralysis	Emaciation, congested liver, thickened intestines	AL
100	A79/11	9/3/11	Layer	24	6000	200	B	Weakness, dullness, paralysis, diarrhoea	Emaciation, splenomegaly, hepatomegaly with white nodular lesions	AL
101	DV19/11	11/3/11	Layer	16	600	-	-	Weakness	Hepatomegaly, grayish and swollen kidneys, nodular areas in the intestines. Lymphoblastic infiltration in the liver, spleen, kidneys	AL
102	A91/11	14/3/11	Layer	18	1500	100	A	Sudden death	Hepatomegaly with nodular lesions	AL
103	A97/11	19/3/11	Layer	16	75	30	D	-	Emaciation, nodular lesions on the liver, enteritis	AL
104	A102/11	24/3/11	Layer	20	1500	6	B	Retarded growth	Emaciation, white lesions on the liver	AL
105	A148/11	27/4/11	Swabs	-	-	-	B	-	-	AL (ELISA +VE)
106	A153/11	29/4/11	Layer	14	800	22	A	Weakness, diarrhoea	Emaciation, splenomegaly, haemorrhagic intestinal mucosa	AL
107	A154/11	29/4/11	Layer	20	600	50	A	Sudden death	Splenomegaly, nodular lesions on the liver	AL

APPENDIX 5 (CONTINUES)

108	A159/11	3/5/11	Layer	19	5000	140	A	Leg paralysis, death	Hepatomegaly with nodular lesions, haemorrhagic intestinal mucosa	AL
109	A164/11	10/5/11	Swabs	-	-	-	C	-	-	AL
110	A188/11	20/5/11	Layer	20	270	29	A	Weakness, dullness, diarrhoea, anorexia	Splenomegaly, diffuse white lesions on the liver, haemorrhagic intestinal mucosa	AL
111	A211/11	31/5/11	Layer	26	-	-	D	Weakness, leg paralysis	Thickened haemorrhagic intestinal mucosa, white nodule-like lesion on the liver	AL
112	A254/11	19/7/11	Broiler	3	300	5	F	Limb paralysis, death	-	AL (ELISA +VE)
113	A262/11	29/7/11	Layer	28	2900	1	A	Sudden death	Hepatomegaly with white lesions, renomegaly	AL
114	A265/11	2/8/11	Layer	24	250	20	D	Death	-	AL
115	A269/11	4/8/11	Layer	12	900	15	D	Drooping wings, anorexia	Hepatomegaly, splenomegaly, tumourous lesions on the kidney	AL (ELISA +VE)
116	A270/11	4/8/11	Layer	12	40	31	G	Snoring, brownish diarrhoea, death	Haemorrhagic lungs and kidneys, splenomegaly,cardiomegaly, cardiomegaly	AL (ELISA +VE)
117	A272/11	5/8/11	Layer	-	500	30	C	Weakness, drooping wings	Emaciation, hepatomegaly with white lesions, splenomegaly, renomegaly, hydropericardium	AL (ELISA +VE), Coccidiosis
118	A312/11	14/9/11	Layer	36	780	2	-	Sudden death	White spots on the liver	AL (ELISA +VE)
119	A327/11	26/9/11	Layer	-	500	50	-	-	Emaciation	AL (ELISA +VE), Coccidiosis
120	A329/11	29/9/11	Layer	12	500	25	C	Weakness, anorexia, weight loss	Splenomegaly, tumours on the liver, spleen	AL (ELISA +VE), Coccidiosis

APPENDIX 5 (CONTINUES)

121	A349/11	30/9/11	Layer	16	500	200	-	Anorexia, ruffled feathers, diarrhoea, drowsiness	-	AL (ELISA +VE)
122	A362/11	10/10/11	Layer	-	-	-	-	-	Emaciation, hepatomegaly, enteritis	AL (ELISA +VE), Coccidiosis
123	A366/11	10/10/11	Broiler	36	800	15	B	Anorexia, weakness, whitish diarrhoea	Emaciation, hepatomegaly	AL (ELISA +VE)
124	A365/11	14/10/11	Layer	-	500	25	C	Sudden death	Emaciation, hepatomegaly, haemorrhagic enteritis	AL (ELISA +VE)
125	A376/11	13/2/11	C.Layer	20	1500	100	G	Weakness, weight loss, death	-	AL (ELISA +VE)
126	DV29/11	25/4/11	Layer	-	1500	20	-	-	White nodules on visceral organs like the liver, intestines; Above tissues showed focal areas of lymphocytic infiltration.	AL
127	DV64/11	2/6/11	Layer	22	980	2	-	Weight loss	Emaciation enlarged and firm liver, spleen, kidneys.	AL
128	DV71/11	3/6/11	Layer	18	500	50	-	Dullness, weakness, diarrhoea	Emaciation, fibrin clots in the liver and pericardial sac, enlarged kidneys with pale foci.	AL
129	DV72/11	10/10/11	Layer	12	600	30	-	Dullness, weakness, drowsiness, diarrhea	Emaciation, enlarged pale liver and kidney	AL
130	DV98/11	21/10/11	Layer	24	-	-	-	Stunted growth	Emaciation, effusion in the peritoneal cavity, nodular masses in the kidneys, ovules, and lungs. Splenomegaly, hepatomegaly, enlarged bursa. Heavy lymphocytic infiltration in the liver, kidneys, lungs, and bursa.	AL

APPENDIX 5 (CONTINUES)

131	DV104/11	10/10/11	Layer	18	2500	100	-	Dullness, anorexia, weakness, diarrhoea	Emaciation, nodular masses in the proventriculus, intestinal serosa, liver. Heavy lymphocytic infiltration within the liver tissue, intestines, lungs, epicardium, and spleen.	AL, Helmithosis, Coccidiosis
132	A372/11	26/10/11	Layer	24	1000	400	B	Dullness, weakness, anorexia	Emaciation, hepatomegaly, splenomegaly	AL (ELISA +VE)
133	A377/11	2/11/11	Layer	18	500	34	B	Sudden death	Emaciation, fibrinous peritonitis, splenomegaly	AL (ELISA +VE)
134	DV113/11	2/11/11	Layer	12	500	30	-	Dullness, weakness, diarrhoea	Emaciation, enlarged pale liver	AL
135	A384/11	11/11/11	Layer	32	5000	20	E	Reduced egg production	Hepatomegaly, peritonitis	AL
136	A388/11	24/11/11	Layer	-	7000	400	G	Dullness, white diarrhoea, anorexia	Splenomegaly, tumourous lesions on the kidney	AL, Helminthosis
137	A399/11	25/11/11	Broiler	6	3000	150	A	Weakness, dullness	Hepatomegaly, splenomegaly, proventricular haemorrhages	AL (ELISA +VE), Coccidiosis
138	A400/11	25/11/11	Layer	52	300	15	B	Sudden death	Hepatomegaly	AL (ELISA +VE), Helminthosis, Coccidiosis
139	A401/11	25/11/11	Layer	16	600	200	C	Sudden death	Hepatomegaly, splenomegaly	AL (ELISA +VE)
140	DV117/11	30/11/11	Layer	18	150	6	-	Weight loss, dullness, anorexia	Emaciation, air sacculitis, nodular masses on intestinal serosa, liver.Thickened intestinal wall. Splenomegaly, swollen kidneys	AL , Coccidiosis
141	A407/11	7/12/11	Layer	22	-	-	A	Paralysis, anorexia, dullness	Emaciation, enlarged sciatic nerves, renomegaly	AL (ELISA +VE)
142	A406/11	16/11/11	Layer	44	200	10	B	Sudden death	Emaciation, tumourous growths on the liver	AL, Helminthosis
143	A410/11	16/12/11	Layer	12	600	4	D	Anorexia, death	Emaciation, hepatomegaly, splenomegaly, renomegaly	AL, Coccidiosis

APPENDIX 5 (CONTINUES)

144	DV120/11	20/12/11	Layer	32	400	3	-	Dullness, weakness	Emaciation, air sacculitis, multifocal and nodular pale lesions on the liver, kidneys, and ovaries. Splenomegaly.	AL
145	A415/11	21/12/11	Broiler	4	-	-	C	-	Hepatomegaly, splenomegaly	AL (ELISA +VE)
146	A420/11	23/12/11	Swabs	-	-	-	-	-	-	AL (ELISA +VE)
147	A02/12	6/1/12	Layer	28	200	3	B	Wing paralysis, delayed laying, diarrhoea, death	Congested oviduct and ovules	AL
148	A05/12	9/1/12	Broiler	3.5	1000	50	B	Nasal discharges, dullness, weakness	Hepatomegaly, splenomegaly, thickened intestinal mucosa	AL (ELISA +VE)
149	A17/12	24/1/12	Layer	24	500	50	B	Lameness, anorexia, reduced egg production	Emaciation, hepatomegaly, splenomegaly, diffuse focal tumours in the liver, spleen, and kidney	AL
150	A24/12	2/2/12	Layer	26	100	16	C	Dullness, paralysis	-	AL (ELISA +VE)
151	A25/12	2/2/12	Layer	28	100	12	D	Limb paralysis, dullness, anorexia	-	AL (ELISA +VE)
152	A26/12	3/2/12	Layer	-	1500	60	B	-	-	AL (ELISA +VE)
153	A40/12	10/2/12	Layer	28	500	100	B	Tumour on the skin	Emaciation, white spots on the liver, splenomegaly	AL (ELISA +VE)
154	A44/12	15/2/12	Indigenous	-	60	1	-	Inappetence	Hepatomegaly, air sacculitis	AL (ELISA +VE)
155	A45/12	16/2/12	Layer	12	384	33	B	Diarrhoea, weakness, snoring, drooping wings	Enteritis	AL (ELISA +VE)
156	DV10/12	2/4/12	Layer	-	5	1	-	Dullness, anorexia	Hepatomegaly, splenomegaly, pneumonia	AL

APPENDIX 5 (CONTINUES)

157	DV17/12	8/4/12	Layer	-	200	10	-	Sudden death	Splenomegaly with nodular cauliflower-like growths.	AL
158	DV22/12	13/4/12	Layer	18	430	6	-	Weakness, death	Emaciation, hepatomegaly, splenomegaly, renomegaly	AL
159	A74/12	16/4/12	Layer	12	185	15	-	Dullness, diarrhoea	Emaciation, hepatomegaly, haemorrhagic intestines	AL
160	A68/12	26/4/12	Layer	16	400	200	C	-	Emaciation, hepatomegaly with whitish nodules, haemorrhagic enteritis	AL (ELISA +VE), Helminthosis
161	A86/12	30/4/12	Layer	20	740	20	A	Diarrhoea, weakness, death	Hepatomegaly, splenomegaly, haemorrhagic intestinal mucosa,	AL, Helminthosis
162	A96/12	14/5/12	Layer	-	400	12	-	-	Splenomegaly, renomegaly	AL, Coccidiosis
163	DV28/12	25/5/12	Layer	17	1000	300	-	Weakness, stunted growth	Enlarged proventriculus, nodular lesions in the liver, kidney, spleen. Infiltration of mononuclear cells (lymphocytes mainly) in the kidney, liver, and heart.	AL
164	DV30/12	6/6/12	Layer	-	4	1	-	Dullness, weakness, anorexia, greenish diarrhoea	Emaciation, splenomegaly with nodular lesions, hepatomegaly, renomegaly. Infiltration of lymphocytes in the liver, kidney, spleen	AL
165	DV60/12	15/6/12	Layer	14	300	30	-	Dullness, anorexia, weakness	Splenomegaly, grey multifocal areas on the liver. Lymphocytic infiltration in the liver, spleen	AL, Helminthosis
166	DV81/12	23/6/12	Layer	28	1150	67	-	Sudden death	Emaciation, hepatomegaly with necrotic areas, splenomegaly, renomegaly	AL
167	DV85/12	29/6/12	Layer	28	400	60	-	Weight loss, anorexia, death	Emaciation, hepatomegaly, splenomegaly, enteritis	AL, Coccidiosis, Helminthosis
168	A129/12	11/6/12	Layer	22	-	-	C	Weakness	Emaciation, splenomegaly, renomegaly	AL (ELISA +VE)
169	A145/12	28/6/12	Layer	16	530	138	C	Dullness, weakness, diarrhoea, weight loss	Splenomegaly, haemorrhagic intestines	AL (ELISA +VE), Coccidiosis, Helminthosis
170	A150/12	28/6/12	-	-	30	1	-	Sudden death	Enlarged haemorrhagic bursa, renomegaly	AL (ELISA +VE), Helminthosis

APPENDIX 5 (CONTINUES)

171	A153/12	3/7/12	Layer	8	-	-	C	Weakness, anorexia	Emaciation, peritonitis	AL (ELISA +VE)
172	A154/12	6/7/12	Layer	20	300	10	C	Paralysis, weakness	Splenomegaly, renomegaly, emaciation	AL
173	A155/12	6/6/12	Layer	28	-	-	A	Dullness, greenish diarrhoea	Emaciation, renomegaly, hepatomegaly	AL (ELISA +VE), Coccidiosis
174	A156/12	10/7/12	Layer	12	500	270	C	Dullness, weight loss, diarrhoea	Emaciation, hepatomegaly, splenomegaly	AL, Coccidiosis, Colisepticaemia
175	DV31/12	11/7/12	Layer	16	320	30	-	Dullness, weakness, anorexia, diarrhoea	Nodular lesions on the liver, kidney, and heart. Enlarged intestines	AL
176	A159/12	17/7/12	Layer	18	600	60	C	Dullness, anorexia, weakness	Hepatomegaly, splenomegaly	AL
177	A165/12	19/7/12	Layer	14	700	20	C	Dullness, weakness, anorexia	Emaciation, hepatomegaly	AL (ELISA +VE)
178	A187/12	1/7/12	Swabs	-	-	-	B	-	-	AL (ELISA +VE)
179	A189/12	3/8/12	Layer	32	700	50	C	Lameness, white diarrhoea, anorexia, torticollis	Emaciation, nodular lesion on the liver	AL, Helminthosis
180	A192/12	7/8/12	Layer	24	200	30	C	Weight loss	Emaciation, tumourous and fragile liver and spleen	AL (ELISA +VE)
181	A198/12	22/8/12	Broiler	3	300	13	B	Diarrhoea, anorexia, death	Hepatomegaly, enlarged bursa, enteritis	AL (ELISA +VE), Colibacillosis
182	A200/12	27/8/12	Layer	12	1000	20	A	Sudden death	Hepatomegaly, splenomegaly, renomegaly, haemorrhagic intestinal mucosa	AL

APPENDIX 5 (CONTINUES)

183	A207/12	4/9/12	Layer	32	600	51	C	Drowsiness, diarrhoea, mouth discharges	Congestion of kidneys	AL (ELISA +VE), Helminthosis
184	A209/12	4/9/12	Layer	20	-	-	C	Lameness, blindness, diarrhoea	Hepatomegaly, renomegaly, enteritis	AL (ELISA +VE), Coccidiosis
185	A212/12	7/9/12	Layer	18	500	50	C	-	Emaciation, splenomegaly	AL (ELISA +VE)
186	A213/12	7/9/12	Layer	12	700	60	C	Lameness, greenish diarrhoea, dullness	Emaciation, hepatomegaly, heart with nodular lesions, renomegaly, nodular lesions in the intestines and proventriculus	AL (ELISA +VE), Helminthosis, Coccidiosis
187	A214/12	7/9/12	Layer	12	400	156	C	Lameness, drowsiness	Emaciation, splenomegaly	AL (ELISA +VE),Helminthosis, Coccidiosis
188	A252/12	15/11/12	Layer	-	200	5	-	Sudden death	Hepatomegaly, peritonitis	AL

Key

No.-Number

AL-Avian Leucosis

(ELISA +VE)-Enzyme linked immunosorbent assay positive

APPENDIX 6: STATISTICAL ANALYSIS OUTPUTS

Pearson's correlation results on observed clinical and pathological lesions

(Variables compared were seropositivity and observed clinical and pathological manifestations; significant if the P Value is less than 0.05 *)

Observed manifestation	P Value
Emaciation	0.1569
Nodular liver	0.6002
Nodular spleen	0.2768
Hepatomegaly	0.4957
Splenomegaly	0.2765
Cardiomegaly	0.0047 *
Renomegaly	0.0321 *
Congested spleen	0.4159
Cardiomegaly	0.0047 *
Renomegaly	0.0321 *
Congested spleen	0.4159
Congested kidney	0.8511
Congested liver	0.2768
Congested lung	0.8793
Thickened proventricular wall	0.8990
Enlarged proventriculus	0.8990
Lymphobastic infiltrations in organs	0.0062 *
Lymphocytic infliltrations	0.2071
Enteritis	0.0426 *
Enlarged bursa	0.1772
Adhession of visceral organs	0.2795
Flabby heart	0.1943
Enlarged sciatic nerves	1.0000
Anorexia	0.1377
Diarrhoea	0.2851
Unthriftiness	0.0035 *
Weakness	0.0967
Paralysis	0.8010
Dullness	0.9527
Ruffled feathers	0.8407
Skin swellings	0.1095
Cannibalism	0.5384
Drowsiness	0.3784
Torticollis	0.1291

APPENDIX 6 (CONTINUES)