# SOME EPIDEMIOLOGICAL ASPECTS AND ECONOMIC IMPACT OF MAREK'S DISEASE IN SOME PARTS OF NIGERIA

**BY**

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**BY**

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**DVM/M.Sc. (Moscow Veterinary Academy) 1993**
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**A DISSERTATION SUBMITTED TO THE SCHOOL OF POSTGRADUATE STUDIES IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF THE DEGREE OF DOCTOR OF PHILOSOPHY IN VETERINARY MEDICINE**

**DEPARTMENT OF VETERINARY MEDICINE, FACULTY OF VETERINARY MEDICINE, AHMADU BELLO UNIVERSITY ZARIA, NIGERIA**

**FEBRUARY 2014**

## DECLARATION

**I declare that the work presented in this dissertation is original and conducted under the supervision of Professors P.A. Abdu, N.D.G. Ibrahim and A. J. Nok in the Faculty of Veterinary Medicine and Department of Biochemistry of Ahmadu Bello University, Zaria. The works of other investigators referred to in this study are duly acknowledged. No part of this dissertation has previously been submitted for a degree or diploma at any University.**

**Luka Daba, JWANDER**

**Name of Student** **Signature** **Date**

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## CERTIFICATION

**This Dissertation entitled 'SOME EPIDEMIOLOGICAL ASPECTS AND ECONOMIC IMPACT OF MAREK'S DISEASE IN SOME PARTS OF NIGERIA' by Luka Daba JWANDER meets the regulations governing the award of the degree of Ph.D of Ahmadu Bello University and is approved for its contribution to knowledge and literary presentation.**

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## DEDICATION

**I dedicate this work to the glory of God the father of our Lord and Saviour Jesus Christ who in spite of all odds saw me through. To the entire members of my family especially to the memory of my late Dad, Mr. Daba (Mut-bah) Bulus Jwander who taught us that God rewards hard work. To my aged Mother Mrs. Na'omi Bulus Jwander and my beloved wife Longtong Luka Jwander and my daughter Favour Cinkinan Na'Omi (Jr) who believe so much in my success as such gave themselves in prayers for me and by no means the least, Professor P. A. Abdu who meant me well in life.**

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**ABSTRACT**

**There has recently been an increase in reports of Marek's disease (MD) in commercial chickens in Nigeria. A retrospective study carried out gave 60.6% overall prevalence for MD ($p< 0.05$) from 2000-2009 out of viral cases in selected towns in Nigeria. Chickens older than 17 weeks had the highest prevalence of 85.5% ($p <0.0001$). MD prevalence in wet and dry seasons were 63.6% and 55.4%, respectively (OR 1.4 $p < 0.0003$). Questionnaire on MD was carried out at Awka involving 360 veterinary surgeons with retrieval rate of 45.28 % (163/360) to test their knowledge on MD. Seventy five per cent veterinarians put frequency of MD occurrence compared to other diseases between 5-10% and 25% put it at 20-30%. Mortality due to MD was 10-50%; drop in egg production between 10-40%. Paralysis (64%) ranked the highest sign followed by emaciation (47%) as the most common signs of MD. Liver (71%) and spleen (47%) were the most affected organs. Nodular lymphomas (60%) and diffused enlargement (39%) were the common lesions seen. Herpes turkey virus (HVT) vaccine (39%) was regularly administered. 68% of veterinarians diagnosed MD based on signs and gross lesions, while 12% on signs, gross and microscopic lesions. A questionnaire on MD was administered to 200 poultry famers across Nigeria to test their knowledge. Seventy nine per cent of the poultry farmers were aware of MD and 87.9% vaccinated against it. Intensively managed flocks experienced MD most (OR 29.6, $p < 0.0001$). Eighty five per cent of the farmers reported MD outbreak from chicks obtained from the same source. Farms rearing chicks of different ages with adults suffered MD more (OR 8.2, $p< 0.0009$). For a five year seroprevalence for MD in poultry in northern part of Nigeria was conducted**

**from 2005-2009. A total of 2,452 sera were evaluated using agar gel precipitation test and MD prevalence was 65.95% ($p < 0.0001$). Prevalence by bird type was 88.4% for layers, 65.8% pullets, and 29.8% cockerels. The overall MD antibody prevalence in 151 avian species using indirect enzyme linked immunosorbent assay was 62.91% with 68.52% in vaccinated commercial chickens, 76.47% in indigenous unvaccinated chickens, 41.67% in turkeys, 25% in quails while ducks tested negative. Marek's disease virus (MDV) antigens were detected in different organs (lungs, liver, kidneys, spleen and skin) of avian species using polymerase chain reaction. Prospective study was carried out to compare gross and microscopic lesions of MDV in experimentally challenged chickens with field outbreaks. Neoplastic lymphomas in most organs were either nodular, diffused or a mixture. The most affected organs were the liver and spleen. Histopathological findings of the field and MDV experimentally challenged chickens were comparable. Financial analysis of 31 farms with MD outbreak showed an average loss per bird to be ₦9, 120.61 ± 268.64, egg production loss per farm was ₦15, 114,465.63 and total loss of 31 farms was ₦95, 811,486:00 ($766,491:89USD). The cost benefit analysis showed that, for every ₦1 invested, a farmer was losing 27 kobo due to MD, while for the farms not experiencing MD, for every ₦1 invested, a farmer made a return of ₦5.00. Marek's disease was high and more prevalent in both immature and mature chickens (71.4-85.5%). Turkey herpes virus was the most common vaccine used. Occurrence of MD compared to other poultry diseases was 20%. Estimated reduction in eggs production due to MD was up to 40%. Marek's disease occurred more frequently in intensively managed farms and birds of different ages. Neoplastic lymphoprolipheration was observed in both organs of naturally**

**affected or experimentally infected chickens with MD. Marek's disease viral antigen was detected in organs of birds that suffered from the disease. Marek's disease was found to cause significant financial losses to the poultry industry in Nigeria. The financial implication for farmers whose flocks suffered MD outbreaks showed that they were running at a loss. Farmers should routinely vaccinate chicks with highly potent MD vaccines. Veterinarians need more knowledge on MD. Farmers should minimize the number of flocks with different ages in the same farm. Commercial poultry should not to be raised with indigenous chickens and be prevented from contact with quails and cattle egrets. All stakeholders should cooperate in the control of MD in Nigeria.**

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## LIST OF ABBREVIATIONS AND ACRONYMS

| | |
|---|---|
| **α** | **alpha** |
| **A** | **Antigen (glycoprotein designated C (gC) Soluble A antigen and cell-bound B antigen are known as gC and gB respectively** |
| **ABU** | **Ahmadu Bello University** |
| **ADOL** | **Avian Disease and Oncology Laboratory** |
| **ADOL-USDA** | **Avian Disease and Oncology Laboratory-United States Agricultural Department** |
| **ALV** | **Avian Leukosis Virus** |
| **ALSV** | **Avian Leukosis Sarcoma Viruses** |
| **AGPT** | **Agar Gel Precipitation Test** |
| **BAC** | **Bacterial Artificial Chromosome** |
| **BAC strain** | **Clone of very virulent Md11 (178532bp)** |
| **BAC strain** | **Clone of Marek's disease attenuated vaccine virus CV1988 (178311bp)** |
| **B-cell** | **Lymphomas** |
| **BCtP** | **Biological Critical Thresh Point** |
| **BCR** | **Cost Benefit analysis (Total Revenue/Total Variable Cost)** |
| **B-locus** | **linked to genetic resistance of chickens against MD** |
| **bp** | **base pairs** |
| **BSA** | **Bovine Serum Albumen** |
| **C12/130** | **A hypervirulent strain of Marek's Disease Virus** |
| **CD** | **Cluster of differentiation (of cellular antigenic marker)** |
| **$CD4^+$ T-cells** | **Cells associated with Marek's Disease Virus latency, although $CD8^+$ T-cells and B cells can be latently infected** |

| | |
|---|---|
| **$CD30^+$** | **A second antigen detected by Marek's Disease Antibodies in $CD4^+$ T-cell and Marek's Disease Virus** |
| **CEF** | **Chicken Embryo Fibroblast** |
| **cELISA** | **Competitive Enzyme Linked Immunosorbent Assay** |
| **CIAV** | **Chicken Infectious Anaemia Virus** |
| **CTL** | **Cytotoxic T lymphocytes** |
| **CFT** | **Complement Fixation Test** |
| **CI** | **Confidence Interval** |
| ***C-myc*** | **Oncogene in a B-lymphocyte (is activated in the induction of lymphoid leukosis).** |
| ***C-erbB*** | **Gene in an erythroid cell (by slowly transforming ALV gives rise to erythroid leukosis erythroblastosis)** |
| **CV1988** | **Rispens Marek's Disease Vaccines Serotype 1** |
| **DO** | **Day old** |
| **%** | **Percentage** |
| **FFE** | **Feather Follicle Epithelium** |
| **$U_L$** | **Unique Long** |
| **$U_S$** | **Unique Short** |
| **T-cell** | **Lymphomas** |
| **$T_{RL}$** | **Terminal Repeat Long** |
| **HPRS-16** | **Houghton Poultry Research Station -16 MDV Positive Standard Antigen and Antibody used** |
| **$H_2SO_4$** | **Sulphoric Acid to stop reaction in ELISA** |
| **HSV** | **Heamorrhagic Septicemia Virus** |

| | |
|---|---|
| **HVT** | **Herpes Turkey Virus Vaccines Serotype 3** |
| **ECWA Vet** | **Evangelical Church Winning All Veterinary Clinic** |
| **EL** | **Erythroid Leukosis.** |
| **EDTA** | **Ethylene Diamine Tetra-Acetic Acid** |
| **ED** | **Embryonic Day** |
| **FFE** | **Feather Follicle Epithelium** |
| **Fc126 `** | **Vaccine Serotype 3 (HVT)** |
| **FCT** | **Federal Capital Territory** |
| **FPSR** | **False Positive Serological Reactions** |
| **FOA** | **Food and Agricultural Organization** |
| **FPSR** | **False Positive Serological Reactions** |
| **g** | **Glycoproteins (g) of gB, gC, gD, gH, gI, gE, gL and gM** |
| **gB** | **GlycoproteinB** |
| **gD** | **Gene of Marek's Disease Virus** |
| **G+C** | **Guanine plus cytosine (G+C) ratio is different for the 3 serotypes and ranges from 43.9-53.6% in serotype 1 and 2, respectively, and 47.6% for HVT** |
| **G-HRP** | **Protein G-Horse Radish Peroxidase** |
| **GM** | **Gross Margin (Total Revenue – Total Variable Cost)** |
| **GS** | **Glycine Saline** |
| **GIS** | **Geographic Information System** |
| **Gs** | **Group-Specific Antigen** |
| **gp85** | **Envelope of the Marek's disease virion (contains a glycoprotein encoded by the *env*- gene which determine the subgroup specificity of the virus)** |

| | |
|---|---|
| ***gag*** | **Gene which is common to all viruses of the group and important in certain diagnostic tests** |
| ***gag, pol and env genes*** | **which form the Marek's disease virion.** |
| **IBD** | **Infectious Bursal Disease** |
| **ICFU** | **International Complement Fixation Units** |
| **ICTV** | **International Committee on Taxonomy of Viruses** |
| **ICTVdB** | **International Committee on Taxonomy of Viruses Data Base** |
| **ICP4, 0, 22, 27** | **Infected Cell Proteins 4, 0, 22, 27** |
| **iELISA** | **Indirect Enzyme Linked Immunosorbent Assay** |
| **IE** | **Immediate Early Genes** |
| **IgM** | **Immunoglobulin M (the tumour cells in LL have morphology of large lymphocytes or lymphoblasts, they have B-cell markers and carry surface IgM).** |
| **IFN** | **Gamma Interferon Test** |
| **$I_{RL}$** | **Internal Repeat Long** |
| **$I_{RS}$** | **Internal Repeat Short** |
| **KDa** | **Kilodalton** |
| **LATs** | **Latency-Associated Transcripts** |
| **LGAs** | **Local Government Areas** |
| **LPS** | **Lipopolysacharide** |
| **LTRs** | **Long Terminal Repeat (RNA copies in the virion are flanked by sequences of nucleotides of LTRs which act as promoters controlling transcription of proviral DNA to viral RNA)** |
| **LL** | **Lymphoid Leucosis (caused by the ALSV)** |
| **MAB** | **Monoclonal Antibody** |

| | |
|---|---|
| **Mab** | **Marek's Disease Antibody** |
| **MD** | **Marek's Disease** |
| **MDV** | **Marek's Disease Virus** |
| **MATSA** | **Marek's Disease Tumour Associated Surface Antigen** |
| **MATSA and CD30 suspensions** | **Can be used to enrich the transformed cells in tumours cells** |
| **MDCC** | **Marek's Disease Virus Transformed Chicken Cell-line** |
| **(MSB-1)** | |
| **mRNA** | **Messenger Reubonucleic acid** |
| **MHC** | **Major histocompatibility complex** |
| **MHC** | **genetic resistance was linked to the B-locus or major histocompatibility complex** |
| **ML** | **Myeloid leukosis (morocco leather like appearance)** |
| **₦** | **Naira (Nigerian note)** |
| **NVRI** | **National Veterinary Research Institute Vom, Plateau State, Nigeria** |
| **NDV** | **Newcastle Disease Virus** |
| **$NDV_L$** | **Newcastle Disease Virus La sota** |
| **NO** | **Nitric Oxide (presence in cell cultures reduces virus replication)** |
| **OD** | **Optical Density** |
| **OR** | **Odd Ratio** |
| **OIE** | **Office International des Epizooties** |
| **OPD** | **O-phenylenediamine Dihydrochloride Substrate** |
| **POL** | **Point of lay** |
| **ORF** | **Open Reading Frame (in a DNA sequence)** |

| | |
|---|---|
| **ORF2** | **Open Reading Frame 2** |
| **OU2** | **Cell Line 2** |
| **P27** | **Structural Protein 27** |
| **PBS** | **Phosphate Buffered Saline** |
| **PCR** | **Polymerase Chain Reaction** |
| **PP14** | **Phosphoprotein14** |
| **PP38** | **Phosphoprotein38** |
| **PI** | **Post Inoculation** |
| **qPCR** | **Quantitative Polymerase Chain Reaction** |
| **QT35** | **Quail's Cell Line 35** |
| **RR** | **Relative Risk** |
| **RRI** | **Rate of Return on Investment** |
| **REV** | **Reticuloendotheliosis Virus** |
| **R-LORF1** | **Right Long Open Region Flank 1** |
| **RK-1** | **A vv+ strain of Marek's Disease Virus** |
| **RT-PCR** | **Real Time Polymerase Chain Reaction** |
| **rCh** | **Recombinant Chicken** |
| **rChIFN-α** | **Alpha Recombinant Chicken Interferon** |
| **rChIFN-γ** | **Gama Recombinant Chicken Interferon** |
| **rFPV** | **Recombinant Fowl Pox Virus Vaccines** |
| **R2/23** | **Attenuated Serotype 1 MDV strain** |
| **REV** | **Reticuloendotheliosis Virus** |
| **RIM =** | **Resource inventory and management report** |

**RNA** Ribonucleic Acid (Dependent DNA polymerase reverse transcriptase and envelop glycoprotein: genetic makeup associated with slow cell transformation and tumour development over several months)

**SPF** Specific Pathogen Free

**SPGA** Sucrose-Phosphate-Glutamate-Albumin

**Subgroups** A, B, C, D, E and J Subgroups F, G, H and I: Allocated to endogenous ALVs occurring in pheasants, partridges and quails

**Subgroup E** Classified as endogenous leukosis virus (from within) integrated as proviral DNA sequences in the genome of germ-line and somatic cells of normal chickens and are transmitted genetically in both sexes.

**Subgroup E** Unlike other subgroups (subgroup E leukosis viruses have little or no oncogenicity for chickens)

**SORF1** Short Open Reading Frame 1

**SORF2** Short Open Reading Frame 2

**SB-1** Strain of chickens in B house on the poultry farm of the first clone MDV vaccine

**SNPs** Single Nucleotide Polymorphisms (which loosely partition between attenuate and non attenuated strains)

**$T_{RS}$** Terminal Repeat Short

**LRT** Long Terminal Repeats

**LLV** Lymphoid Leukosis Virus

**ICP** Intracellular Protein

**SORF** Short Open Reading Frame

***Taq*** **DNA polymerase A heat-stable DNA polymerase from** ***Thermus aquaticus.***

**TR** Total Revenue

**TVC** Total Variable Cost

**US$** United State Dollar

| | |
|---|---|
| **V-erbB gene** | **Viral Oncogen of Transforming ALVs (can cause erythroid leukosis)** |
| **vMDV (GA)** | **Virulent Marek's Disease Virus** |
| **vvMDV(Md5)** | **Very Virulent Marek's Disease Virus Strain** |
| **vv+MDV(584A)** | **Very Virulent Plus Marek's Disease Virus Strain** |
| **VLA–UK** | **Veterinary Laboratory Agency United Kingdom** |
| **VN** | **Virus Neutralizing Antibodies** |
| **WHO** | **World Health Organization** |
| **X** | **Multiply by** |

# CHAPTER ONE

# INTRODUCTION

## 1.1 Background of the Study

Animal protein and its products like amino acids are still grossly inadequate in meeting the daily human demands especially in view of sustained increase in population despite the advancement in technology (FAO,1985). In an attempt to augment the animal protein supply for human consumption, exotic birds were introduced into Nigeria (Ikpi and Akinwumi, 1979). It is obvious that there are different types of livestock that are profitably produced in Nigeria and the world at large. Agriculturalists and nutritionists generally agree that developing the poultry industry is the fastest means of bridging the prevailing protein deficiency gap, in Nigeria (Ikpi and Akinwumi, 1979; FAO, 1985).

Also worthy to mention is that due to its proliferation, and quick returns, the poultry industry has become an industry with a variety of business interests such as egg production, meat production, hatchery, poultry drugs and equipment business (Oluyemi *et al*., 1979).

Poultry production, especially chicken is rapidly increasing in Nigeria and other parts of the world, (FAO, 1995). Approximately 800 million fowls are found in Africa (Kidane-Mariam, 2001). This is due to fowls adaptation to most areas of the world with low economic value per unit, rapid growth and short generation interval. Another advantage of poultry production is that, there is no religious bias against meat and eggs (Stenhouse, 2004). The poultry population in Nigeria was estimated at 150 million (RIM Report, 1993). However, poultry diseases like leucosis and especially Marek's disease continued to rise with the modernization and intensification of poultry production and are among the major

setbacks to the continuous increase and expansion of the poultry industry worldwide (Coles, 1953; Blaxland, 1956; Gurdev and Balwant, 1975).

In Nigeria, Marek's disease (MD) was first reported by Davis and Hill (1960). Davis and Hill (1962) reported on the occurrence of a transmissible fibromyxosarcoma in a Rhode Island Red pullet at Ibadan and a 5.7% incidence of leucosis mainly in exotic breeds and a few cases (4.4%) of visceral and ocular lymphomatosis in local fowls. These have remained the only documented reasonable coverage on the neoplastic disease of poultry in Nigeria.

There is a dearth of information on the incidence of avian neoplasm in Nigeria as stated by Woode and Campbell (1972) when reporting on the study of a limited number of MD materials from Nigeria. Between 1960 and 1970 of the reported poultry diseases, MD is one of the leading problems of commercial poultry in Nigeria (Adene and Hill, 1977; Nawathe *et al*., 1978; Adene, 1983; Komolafe *et al.*, 1987; Adene and Akpavie, 1997; Akpavie, 2005; Abdu, 2007; Okwor and Eze, 2008; Olabode *et al.*, 2009).

Marek's disease virus (MDV) is a DNA cell-associated herpesvirus with lymphotropic properties similar to those of gammaherpesviruses (Schat, 1985). However, its molecular structure and genomic organization are similar to alphaherpesviruses (Lee *et al*., 1978a; Buckmaster *et al.*, 1988; Tulman *et al*, 2000). McGeoch *et al*. (2000) proposed to place all MDV serotypes in a separate subgroup, a3, of the *alphaherpesvirinae*. With regards to classification by the International Committee on Taxonomy of Viruses (ICTV), all MDV serotypes are grouped together in the genus *Mardivirus* (ICTVdB, 2006) within the

subfamily *Alphaherpesvirinae*. Members of the genus *ardivirus*, described as belonging to the 3 serotypes, are grouped as the 3 species *viz. Gallid herpesvirus* 2 (serotype 1), *Gallid herpesvirus* 3 (serotype 2) and *Meleagrid herpesvirus* 1 (serotype 3). On the basis of their virulence, serotype 1 strains are further divided into pathotypes, which are often referred to as mild (m) MDV, virulent (v) MDV, very virulent (vv) MDV, and very virulent plus (vv+) MDV) strains (Witter, 1997; Witter *et al*., 2005).

Replication of the 3 serotypes is typical of other cell-associated herpesviruses (Kato and Hirai, 1985; Ross, 1985; Schat, 1985; Biggs, 2004; Osterrieder and Vautherot, 2004). For initial infection of cultures or chickens by cell-free virus, enveloped virions bind to cellular receptors probably by gB in combination with other glycoproteins. Heparan sulfate, a member of the glycosaminoglycans, has been identified as one of the cellular receptor molecules (Lee *et al*., 2001). In cell cultures, viral penetration occurs within 1 hour after attachment, which is enhanced for serotype 1 by chelators such as ethylenediaminetetra acetic acid (EDTA) (Adldinger and Calnek, 1972).

Subsequent spread of infection to other cells occurs by direct contact with infected cells and virus transfer probably is accomplished through formation of intracellular bridges (Kaleta and Neuman, 1977). This is presumed to be the principal mode of virus spread after initial infection *in vitro* and *in vivo*. Recent data demonstrate the role of the Us3-encoded kinase in the morphogenesis and cell-to-cell spread of virion through the effect of stress on the fibre breakdown and polymerization of actin (Schumacher *et al*., 2005). The glycoproteins, gE, gI, and gM play a role in the transfer of virus from the infected to

uninfected cells (Schumacher *et al*., 2001; Tischer *et al.,* 2002). Replication rates vary with serotype, passage level of the virus strain, cell type and temperature of incubation (Schumacher *et al*., 2001).

The spread of virus *in vivo* from cell to cell will require intimate contact between infected and uninfected cells, which are most often lymphocytes. Epithelial cells can also be involved in this process (Tischer *et al.,* 2002). Three general types of virus-cell interactions are recognized: productive, latent and transforming. The use of an appropriate stabilizer such as sucrose-phosphate-glutamate-albumin SPGA will improve the yield of cell- free virus (Calnek *et al*., 1970a).

The principal methods to identify the presence of MD infection are isolation of the virus, demonstration of viral DNA or antigens in tissues and detection of antibodies (OIE, 2004).

Clinicopathological diagnosis is based on characteristic signs and gross lesions (Zelnik, 2004). The applications of different diagnostic procedures are widely applied for MD (OIE, 2004; Zelnik, 2004).

Marek's disease virus (MDV) infection mainly occurs in domestic chickens and is ubiquitous among poultry populations throughout the world (Venugopal *et al*., 2008). The infection of other species is rare, but occasionally the disease occurs in turkeys and quails (Venugopal *et al*., 2008). Marek's disease has also been reported in migratory white-fronted geese in Japan (Venugopal *et al*., 2008). Prevention is mainly by vaccination with

different MDV strains, biosecurity, selection for genetic resistance and good management practices (Venugopal *et al*., 2008).

## 1.2 Statement of the Research Problem

Viral diseases in domestic animals and fowls are of major threat to the economy of all nations (Payne, 1985). Veterinary measures used to curb the appearance of epizootics in animals are of major importance (Payne, 1985). The impacts of infectious diseases of poultry in selected African countries were given more attention. Among others, poultry diseases and lack of vaccinating commercial and local birds against diseases in Nigeria have adversely affected the productivity of poultry population which lead to inadequate animal protein for human consumption (Majiyagbe and Lamorde, 1997; Abdu, 2007). Adene (1975) carried some studies on the type of neoplasms of poultry in Nigeria in the southern part. A general overview of MD of poultry in Nigeria a current option and future development in its control was conducted by Adene and Akpavie (1997) and Akpavie (2004).

Marek's disease was first described by Jozsef Marek in Hungary in 1907 followed by numerous authors in many countries of the world (Jozsef, 1907). In the nineteen fifties, MD was responsible for enormous loss in the poultry industry (Nair *et al*., 2008). Mortality of up to 80% were recorded in some flocks. Economic losses from MD were attributed to both the mortality (death caused by the development of tumours in the visceral organs) and morbidity (paralysis resulting from the infiltration of nerves with lymphocytes) in the flock. At the end of the sixties, vaccines for MD were developed (Jordan, 1990; Morgan *et al*., 1991; Fred and Venugopal, 2004; Chauham and Roy, 2007; Nair *et al*., 2008; Fadly, 2008).

The World Veterinary Poultry Association International in its congress meeting in Germany June, 2001 re-emphasized the new global spread of MD and that it has taken a new dimension defying all modern prevention with very virulent pathotypes. The disease poses economic problem in terms of carcass condemnation, research and vaccine for prevention (Fred and Venugopal, 2004). Marek's disease vaccines are not produced in Nigeria making MD control more difficult by not vaccinating the birds when due and it requires hard foreign currency to import the MD vaccines for the rapid growing poultry industry. There is apparently no record quantifying the economic impact of the disease in Nigeria. Adene (1975) had reported MD with an incidence of 39.5% out of 280 specimens from suspected cases in local and commercial poultry flocks. However, severe clinical outbreaks of MD in commercial turkey flocks with mortality from tumours reaching 40-80% between 8-17 weeks of age were reported in France, Israel, the Netherlands and Germany (Fred and Venugopal, 2004). From the above observations, the partial or total lack of vaccinating local and commercial birds in Nigeria is a phenomenon that ensures the spread of MD in poultry flocks.

### 1.3 Justification of the Study

Marek's disease is a worldwide problem and has led to poultry trade restriction internationally by FAO of which Nigeria is currently listed among the countries with MD outbreaks in the world (Fred and Venugopal, 2004). Marek's disease is an economically important avian neoplastic disease posing a major set back in the poultry industry in Nigeria with no ongoing research work (Jwander, 2005).

Marek's disease is one of the few aetiologically well defined viral neoplasms of animals and it is the only vaccinally controllable neoplasm, for which an array of vaccines have been produced during the past two decades. Despite the advances in the study and control of MD worldwide, many aspects of the disease problem still remain unresolved with emerging new complications and challenges in matters relating to the aetiology (strain), pathogenesis and immunogenesis of the disease (Adene and Akpavie, 2004).

As recommended standard practice worldwide, Marek's disease vaccines must be given to chicks hatched at day old and it is not produced in Nigeria. This makes it difficult for poultry farmers to have access to MD vaccines. The general awareness on MD as a disease of universal economic importance has raised some anxiety on the need to institute official control programme in Nigeria (Adene, 1997). There are still problems and laboratory constraints in the diagnosis of MD in Nigeria (Adene, 1997**).**

Field veterinarians are faced with inadequate history due to poor record keeping and low level of awareness of the disease by poultry farmers (Jwander, 2005).

There is need for detailed studies on Marek's disease to determine the clinicopathological manifestations of the disease and also an indepth knowledge of the economic impact of MD in poultry farms is needed. In monetary terms, the economic importance of neoplastic diseases have been documented and quantified in many parts of the world but yet to be carried out in Nigeria.

### 1.4 Aim of the Study

This study was aimed at generating knowledge on the epidemiology, awareness and economic impact of MD in Nigeria.

## 1.5 Objectives of the Study

The objectives of the study were to:

1. determine the prevalence of MD from records of some veterinary clinics and hospitals in Nigeria from 2000-2009.
2. determine the level of MD awareness of some veterinarians and farmers in some states in Nigeria.
3. determine the seroprevalence of MDV infection in some states in Nigeria.
4. compare the gross and microscopic lesions of MD in naturally infected and birds experimentally challenged with Marek's diseasese virus.
5. detect Marek's disease virus
6. determine the direct financial loss due to MD in farms with outbreak.

## 1.6 Research Questions

1. Is Marek's disease outbreak on the increase since it was first recorded in Nigeria?.
2. Is Marek's disease popular among veterinarians and poultry farmers in `Nigeria?.
3. Can MDV be detected serologically in chickens in some part of Nigeria?.
4. Is MD field outbreak in chickens from Nigeria similar with the MDV experimentally challenged both grossly and histopathologically?.
5. Can MDV antigen from naturally infected birds be detected?.
6. Does Marek's disease cause any financial loss to the poultry industry in Nigeria?.

# CHAPTER TWO

# LITERATURE REVIEW

## 2.1 Definition of Neoplasm

The word neoplasm is derived from the Greek words 'neo' meaning 'new' and –plasm means 'cell'. Thus neoplasm is defined according to Baillieres Comprehensive Veterinary Dictionary (Blood and Virginia, 1988) as: any new and abnormal growth, specifically one which cell multiplication is uncontrolled and progressive. The word neoplasm is interchangeably used with 'tumour' and 'cancer'. Neoplasm could be benign, malignant or transmissible.

Cancer is any malignant, cellular tumour. The term cancer encompasses a group of neoplastic diseases in which there is a transformation of normal body cells into malignant ones. This probably involves some changes in the genetic material of the cells, deoxyribonucleic acid (DNA), perhaps as a result of faulty repair of damage to the cell caused by carcinogenic agents or ionizing radiation (Blood and Virginia, 1988). The altered cells pass on inappropriate genetic information to their progeny cells and begin to proliferate in an abnormal and destructive manner. Normally, the cells of which body tissues are made are regularly replaced by new growth, which stops when the cells are replaced; new cells form to repair tissues damaged and stops forming when healing is complete. Why they stop forming is unknown, but clearly the body in its normal processes regulates cell growth (Blood and Virginia, 1988).

In cancer, cell growth is unregulated. As the cancer cells continue to proliferate, the mass of abnormal tissue that they form enlarges, ulcerates and begins to shed cells that spread the disease locally or to distant sites. This migration is called *metastasis*. Some cells invade

neigbouring tissues, destroying and displacing normal cells and taking their place. Others may enter the blood and lymphatic vessels and are carried along in the fluid to other parts of the body. Another way in which malignancy can be spread is by entering a body cavity by diffusion and coming in contact with a healthy organ (Blood and Virginia, 1988).

Benign neoplasms grow slowly, expand without metastasis and usually do not reoccur. On the other hand, malignant neoplasms have characteristics of anaplasia, invasiveness, and metastasis while transmissible neoplasms are capable of being transmitted between individuals. Transmissible neoplasms include bovine viral leukosis, avian leukosis, rous sarcoma complex, MD, canine transmissible veneral tumour, squamous cell carcinoma of cattle and canine viral papillomatosis (Blood and Virginia, 1988).

In poultry, neoplasms could be of known or unknown origin. Tumours of unknown origin in poultry include a wide variety of begnin and malignant neoplasms derived from muscle, epithelial and nerve tissues e.g. adenocarcinoma of ovary, neuromas, hepatocellular tumours, etc. Most neoplasms of known origin in poultry are virus-induced and are transmissible, e.g MD (Charlton *et al*., 2006).

## 2.2 Definition and Synonyms of Marek's Disease

Marek's disease is a common lymphoproliferative disease of chickens, usually characterized by mononuclear cellular infiltrates in peripheral nerves and various other organs and tissues including iris and skin. The disease is caused by a herpesvirus, is

transmissible, and can he distinguished aetiologically from other lymphoid neoplasms of birds (Schat and Nair, 2008).

The old terminology of MD was confusing because early authors used a variety of names /to designate the lymphoproliferative and neurological aspects of the disease. Also, the differentiation between inflammatory and neoplastic aspects originally were confused, probably because the virus strains were less virulent and induced few if any lymphomas. The seminal description by Jozsef Marek (1907) identified the disease as polyneuritis. Other common synonyms included neuritis, neurolymphomatosis gallinarum and range paralysis. Jungherr *et al.* (1941) proposed that the term lymohomatosis be subdivided into visceral, neural and ocular forms. This nomenclature was widely applied for more than 20 years to all lymphoproliferative diseases (also called leukosis of chickens) including MD, but masked the aetiological distinctions between MD and lymphoid leukosis (LL). In retrospect, neural and ocular lymphomatosis were probably MD; whereas visceral lymphomatosis included both MD and LL (Schat and Nair, 2008). The term acute leukosis or acute MD was used in the 1960s to designate more virulent manifestations of MD characterized by visceral lymphomas. Lymphomatous lesions in the skin, which were common in broilers, were sometimes termed skin leukosis. Biggs (1961; 2004) proposed the term MD to distinguish the condition clearly from aetiologically different lymphoproliferative diseases.

Marek's disease has also been subdivided into acute and classical forms, where the latter term designates forms of the disease prevalent prior to the 1950 (Biggs, 2004). Marek's

disease virus can also induce other clinically distinct disease syndromes such as transient paralysis, early mortality syndrome, cytolytic infection, atherosclerosis and persistent neurological disease (Schat and Nair, 2008).

### 2.2.1 Historical account of Marek's disease

The seminal report by Jozsef Marek, published in 1907 of paresis in 4 roosters is the first account of the disease that now bears this author's name. Outbreaks were reported as early as 1914 in the United States and subsequently, the disease was recognized in the Netherlands, Great Britain and many other countries (Schat and Nair, 2008). The detailed description by Pappenheimer *et al.* (1926) clarified the lymphoproliferative nature of the lesions in peripheral nerves and spinal ganglia and established, as part of the syndrome, lymphoid neoplasms in the ovary and other visceral organs (Schat and Nair, 2008).

Many early attempts at transmission were equivocal or unsuccessful due to the lack of susceptible chickens and adequate bio-containment and to the failure to recognize the cell-associated nature of the causative agent (Schat and Nair, 2008). However, experiments initiated by Hutt and Cole in 1935 established the role of genetic resistance in the disease and ultimately provided susceptible chicken lines for transmission studies (Hutt *et al.*, 1941; Hutt and Cole, 1957).

Marek's disease gradually increased in severity. Pappenheimer *et al.* (1926) reported mortality from fowl paralysis reaching 20% in one pullet flock as early as 1922. A marked increase in mortality in commercial chickens occurred between 1925 and 1937, at least half

of which was due to fowl paralysis and other neoplasms (Winton, 1966). Industry concerns prompted the appropriation of federal funds to construct a new laboratory in East Lansing, Michigan dedicated to this disease. Its initial projects, launched in 1939, were directed toward the genetics and pathology of fowl paralysis in chickens (Schat and Nair, 2008).

An acute form of the disease in broiler chickens, characterized by tumours in multiple viscera, muscles and skin, was first recognized in the mid-1950s (Benton and Cover, 1957). By the early 1960s, it was a major cause of condemnation of broiler carcasses in the United States (Benton *et al.,* 1962). The incidence continued to increase during the 1960s and by 1970 about 1.5% of all broilers in the United States were discarded at processing due to neoplastic lesions (Schat and Nair, 2008). Mortality in pullets and layers reached as high as 30-60%, and the poultry industry was facing an economic crisis. The definitive transmission of MD, established in the early 1960s (Sevoian *et al*., 1962; Biggs *et al*., 1968), confirmed the infectious nature of the disease and provided an experimental system for its study. The avid cell association of the agent made identification of the agent elusive (Biggs and Payne, 1967).

Isolation of a herpesvirus in cell cultures inoculated with cells from diseased chickens was reported in 1967 by two independent laboratories (Churchill and Biggs, 1967; Nazerian *et al.,* 1968; Solomon *et al*., 1968). This virus was confirmed as the aetiological agent of MD through association studies and ultimately, by transmission with cell-free virus obtained from feather follicles (Biggs *et al*., 1968; Witter *et al*., 1969; Calnek *et al*., 1970a).

The MDV became attenuated upon serial passage in chicken kidney cultures and the attenuated virus when inoculated into newly hatched chicks, provided protection against subsequent challenge with virulent strains. Naturally, avirulent, antigenically related strains were found in turkeys and chickens and provided protective immunity (Witter, 1970; Biggs and Milne, 1972). Thus, in the span of a very few highly productive years, the causative agent was identified and vaccination was established as an effective control strategy (Schat and Nair, 2008).

Meanwhile, Calnek and Hitchner (1969), Calnek *et al*. (1970a) found that the virus was shed in a cell-free, fully infectious form from the feather follicle providing an explanation for the highly contagious nature of the disease. The virus was relatively stable in the poultry house environment and respiratory exposure to dust or dander appeared to be the mechanism of natural transmission (Beasley *et al.,* 1970). The following years continued the stream of new findings about MD. Important contributions prior to 1985 include the development of lymphoblastoid cell lines from MD tumours (Akiyama *et al*., 1973).

Characterization of the T-cell nature of the tumour cell was reported (Hudson and Payne, 1973), identification of cytolytic and immunosuppressive phases of the disease (Payne *et al.*,1976), linkage of transient paralysis with MDV infection (Kenzy *et al.*,1973), isolation of the CV1988 (Rispens *et al.*,1972) and SB-1 (Schat and Calnek,1978) strains, which proved to be successful vaccines, and identification of 3 viral serotypes based on antigenic characteristics (Lee and Witter, 1983; von Bulow and Biggs, 1975a). Genetic resistance

was linked to the B-locus or major histocompatibility complex (MHC) (Hansen *et a*l., 1967; Briles *et al*., 1977).

The stages of infection from respiratory tract to cytolytic infection of B cells, to activation, latent infection and ultimately transformation of T cells were elucidated (Calnek *et al*., 1981a; Shek *et al.,* 1982; Calnek, 1986). Synergism among vaccine viruses was identified and contributed to the development of bivalent vaccines (Schat *et al.,* 1982; Witter, 1982; Calnek *et al.*, 1983).

These and other important findings that laid the basis for our current understanding of MD are detailed in several reviews (Biggs, 2001; Biggs, 2004). Advancements of knowledge on MD during the 1960s, leading to effective vaccines, were presented during the 5$^{th}$ International Symposium on Marek's disease in 1996, and relevant information is available in the proceedings, the videotape "Legacy of the 1960s" and the historical archives of the American Association of Avian Pathologists (Schat and Nair, 2008).

### 2.2.2 Economic significance of Marek's disease

According to Purchase (1985), prior to the use of vaccines, MD constituted a serious economic threat to the poultry industry causing up to 60% mortality in layers and 10% condemnations in broilers, this is because vaccines are not 100% effective, sporadic losses still occur, but they are no longer as serious problem. Purchase (1985) estimated that mortality and condemnation losses due to MD totaled about $12 million in the United States in 1984. When combined with economic loss from the costs of vaccine and

application and reduced egg production, however, the total was about $169 million in the United States and $943 million worldwide. Morrow and Fehler (2004) quoted in 2004 that the worldwide annual losses due to MD is in the range of US$ 1 to 2 billion, but they indicated that these figures are impossible to verify. The disease remains a major concern for the poultry industry due to the unpredictability of outbreaks and the possibility that vaccines may ultimately fail as a consequence of the evolution and more virulent strains of MD virus (Schat and Nair, 2008).

### 2.2.3 Public health significance of Marek's disease

Purchase and Witter (1986) have reviewed the literatures related to MD and human health concerns, particularly human cancer. They cited numerous reports of virologic, pathologic, serologic and epidemiologic studies that support a conclusion that no aetiologic relationship exists between MDV or any of the MD vaccine viruses and human cancer (Schat and Nair, 2008). The public health significance of MD has attracted little attention. A role for MDV in the aetiology of multiple sclerosis of humans has been suggested (MacGregor and Latiwonk, 1992; MacGregor and Latiwonk, 1993; MeHatters and Scham, 1995) but was later refuted (Hennig *et al.*, 1998). In 2006, a similar claim was made for turkey herpes virus (HVT) or HVT-like viruses (Bougiouklis, 2006) lacking any supportive evidence for this speculation. The presence of DNA sequences of the gD gene of MDV was described in sera from humans with and with out exposure to poultry (Laurent *et al*., 2001; Laurent *et al*., 2004), but genes unique to MDV were not analyzed in these studies. However, Hennig *et al*. (2003) were unable to detect any MDV sequences in 300 human

plasma samples by quantitative real-time PCR. At this time (2003), no compelling evidence suggests that MDV infects humans or adversely affects human health (Hennig *et al.*, 2003).

### 2.2.4 Scientific significance of Marek's disease

Marek's disease has provided a fertile environment for contributions to veterinary medicine, basic science and comparative oncology (Schat and Nair, 2008). The disease itself is uncommonly complex, featuring an interplay of neoplasia and inflammation expressed as several distinct clinical syndromes, each modified in important ways by host genetic influence (Schat and Nair, 2008). Marek's disease virus is an alphaherpesvirus with ly mphotropic properties of gammaherpesviruses, is highly cell-associated but readily transmitted and its virulence varies and evolves. It has two unique sister viruses, both nononcogenic, that naturally infect chickens and turkeys (Schat and Nair, 2008). Infection induces complex immune responses usually resulting in high levels of protection. Vaccination for MD constitutes an outstanding example of successful disease control in veterinary medicine. Moreover, MD vaccines are the first effective vaccines against cancer in any species (Schat and Nair, 2008).

### 2.2.5 Aetiology of Marek's disease

#### *2.2.5.1 Classification of Marek's disease virus*

Marek's disease virus is a cell-associated herpesvirus with lymphotropic properties similar to those of gammaherpesviruses (Schat, 1985). However, its molecular structure and genomic organization are similar to alphaherpesviruses (Buckmaster *et al*., 1988; Lee *et al*., 2000; Tulman *et al*., 2000). McGeoch *et al.* (2000) proposed to place all MDV serotypes in a separate sub-group, a3, of the *alphaherpesvirinae*. By the classification of the

International Committee on Taxonomy of Viruses (ICTV), all MDV serotypes are grouped together in the genus *Mardivirus* (ICTVdB, 2006) within the subfamily *Alphaherpesvirinae.* Members of the genus *Mardivirus,* described as belonging to the 3 serotypes are now grouped as the 3 species viz. Gallid herpesvirus 2 (serotype 1), Gallied herpesvirus 3 (serotype 2) and Meleagrid herpervirus 1 (serotype 3). Serotype 1 MDV is the prototype virus for this group of avian viruses and except where otherwise indicated, MDV refers to serotype 1 virus (Schat and Nair, 2008). On the basis of their virulence, serotype 1 strains are further divided into pathotypes, which are often referred to as mild (m) MDV, virulent (v) MDV, very virulent (vv) MDV and very virulent plus (vv+) MDV strains (Witter, 1997; Witter *et al*., 2005).

Two additional groups of nononcogenic herpesviruses isolated from chickens (Biggs and Milne, 1972) and turkeys (Kawamura *et al*., 1969 and Witter, 1970), respectively, are considered part of the MDV group and are included in this literature. The serotypic classification for MDV and HVT strains (vonBulow and Biggs, 1975b), is originally based on the complete sequence for serotype 2 HPRS-24 and serotype 3 FC126 virus strains (Afonso *et al.*, 2001; Izumiya *et al.*, 2001; Kingham *et al*., 2001).

*2.2.5.2 Morphology of Marek's disease virus*

The morphology and morphogenesis of MDV have been reviewed by Kato and Hirai (1985) and Schat (1985). In general, viral particles are typical of those described for other herpesviruses. Scattered virions are commonly seen in the nucleus and more rarely in the cytoplasm or extracellular spaces. Hexagonal nucleocapsids 85-100 nm in diameter and enveloped particles 150-160 nm in diameter may be seen in thin sections of infected cell

cultures. Enveloped virus particles appearing as irregular amorphous structures and measuring 273-400 nm can be observed in negatively stained preparations of lysed feather follicle epithelium (FFE) (Calnek *et al*., 1970a). Thin-section preparations of the FFE revealed large numbers of cytoplasmic enveloped herpesvirus particles in keratinizing cells. The morphology of MD virions in cell cultures and FFE could be seen under high powered electron microscope (Schat and Nair, 2008).

The morphology of serotype 2 and 3 strains resembles that of MDV serotype 1. In thin sections, however, nucleocapsids of turkey herpes virus (HVT) commonly show a unique crossed appearance (Nazerian *et al.,* 1970). The morphology of serotype 2 MDV has not been studied in detail, but typical herpesvirus particles have been visualized (Schat and Calnek, 1978).

### 2.2.6 Chemical composition of Marek's disease virus

#### *2.2.6.1 Physical properties of Marek's disease virus*

The complete sequence for the 3 serotypes (Lee *et al.*, 2000; Tulman *et al*., 2000; Afonso *et al*, 2001; Izumiya *et al*., 2001; Kingham *et al.*, 2001) confirmed that the genomes are very similar consisting of linear, double-stranded DNA molecules of approximately 160-180 kb with a buoyant density in neutral CsCl of 1.706 g/mL for serotype 1 (Lee *et al*., 1971; Ross, 1985). The guanine plus cytosine (G+C) ratio is different for the 3 serotypes and ranges from 43.9-53.6% in serotype 1 and 2, respectively, and 47.6% for HVT (Izumiya *et al*., 2001; Silva *et al*., 2001). It is difficult to separate viral DNA from host cell DNA because its density is close to that of chicken DNA. Several methods have been described to obtain

viral DNA (Ross, 1985). Pulse-field electrophoresis is probably the best way to obtain pure infectious viral DNA (Isfort *et al.,* 1990; Wilson and Coussens, 1991). However, the cloning of the complete MDV genome as bacterial artificial chromosomes (BAC) has greatly facilitated the production of MDV DNA (Schumacher *et al*., 2000; Zelnik, 2003). Infectious BAC clones of the least four different MDV strains of MDV into chicken embryo fibroblasts (CEF) (Reddy *et al.,* 2001) has allowed rapid manipulation of MDV DNA by site-directed mutagenesis and the generation of recombinant viruses to gain insights into gene functions (Lupiani *et al.*, 2004 ; Silva *et al.,* 2004).

*2.2.6.2 Structural organization of Marek's disease virus*

The genomic structure (figure 1) of the 3 serotypes is typical for alphaherpesviruses as previously suggested by Lee *et al*. (2000) with a unique long ($U_L$) and a unique short ($U_S$) sequences are flanked by sets of inverted repeat sequence: the terminal repeat long ($T_{RL}$), Internal repeat long ($I_{RL}$), internal repeat short ($I_{RS}$), and terminal repeat short ($T_{RS}$) respectively (Cebrian *et al*., 1982). Alpha (α)-like sequences typical for alphaherpesviruses are located at the terminal ends of the $T_{RL}$ and $I_{RL}$ and between the $I_{RL}$ and $I_{RS}$ regions (Afonso *et al,* 2001; Izumiya *et al*., 2001; Kishi *et al*., 1991; Tulman *et al*., 2000), which can be variable in length (Reilly and Silva, 1993). These α-like sequences are believed to be important for the cleavage and packaging of viral DNA into virions (Schat and Nair, 2008).

The complete genome sequences of four serotype 1 MDV strains are Md5 (177874 base pairs, GenBank Accession number AF147806), GA (174077 base pairs, GenBank

Accession number AF147806), BAC clone of Md11 (178532 base pairs, GenBank Accession number AY510475), and BAC clone of CV1988 (178311) base pairs, GenBank Accession number DQ530348), (Silva *et al*., 2001). The genome structure and the sequences of these strains are very similar and the differences in the lengths are mostly caused by the changes in the numbers of the direct repeats in the repeat regions of the genome (Schat and Nair, 2008). Comparison between the vMDV GA and vvMDV Md5 strains shows very few structural differences between serotype 1 strains (Silva *et al*., 2001). The $U_L$ regions of GA and Md5 are very similar in length and organization, but the $U_S$ sequences of GA is longer than in Md5 (Schat and Nair, 2008). The differences in the $U_S$ and the flanking repeats result in the present of 1 copy of small ORF2 (SORF2) in GA and a second SORF2-like gene in Md5. In addition SORF1 in Md5 is completely located in the repeats, but has a frame shift resulting in a truncated SORF 1. The importance of these differences is not clear (Schat and Nair, 2008). Additional minor differences between strains can be expected. CV1988 BAC contains 14 copies of the 132-bp repeat, the expansion of which was once thought to be associated with viral attenuation (Silva *et al*., 2004; Silva and Gimeno, 2007). Kaplan *et al*. (2001) reported differences in 2 of the 3 promore regions for ICP4 between the GA. RB-1B and CV1988 strains. In vitro assays suggest

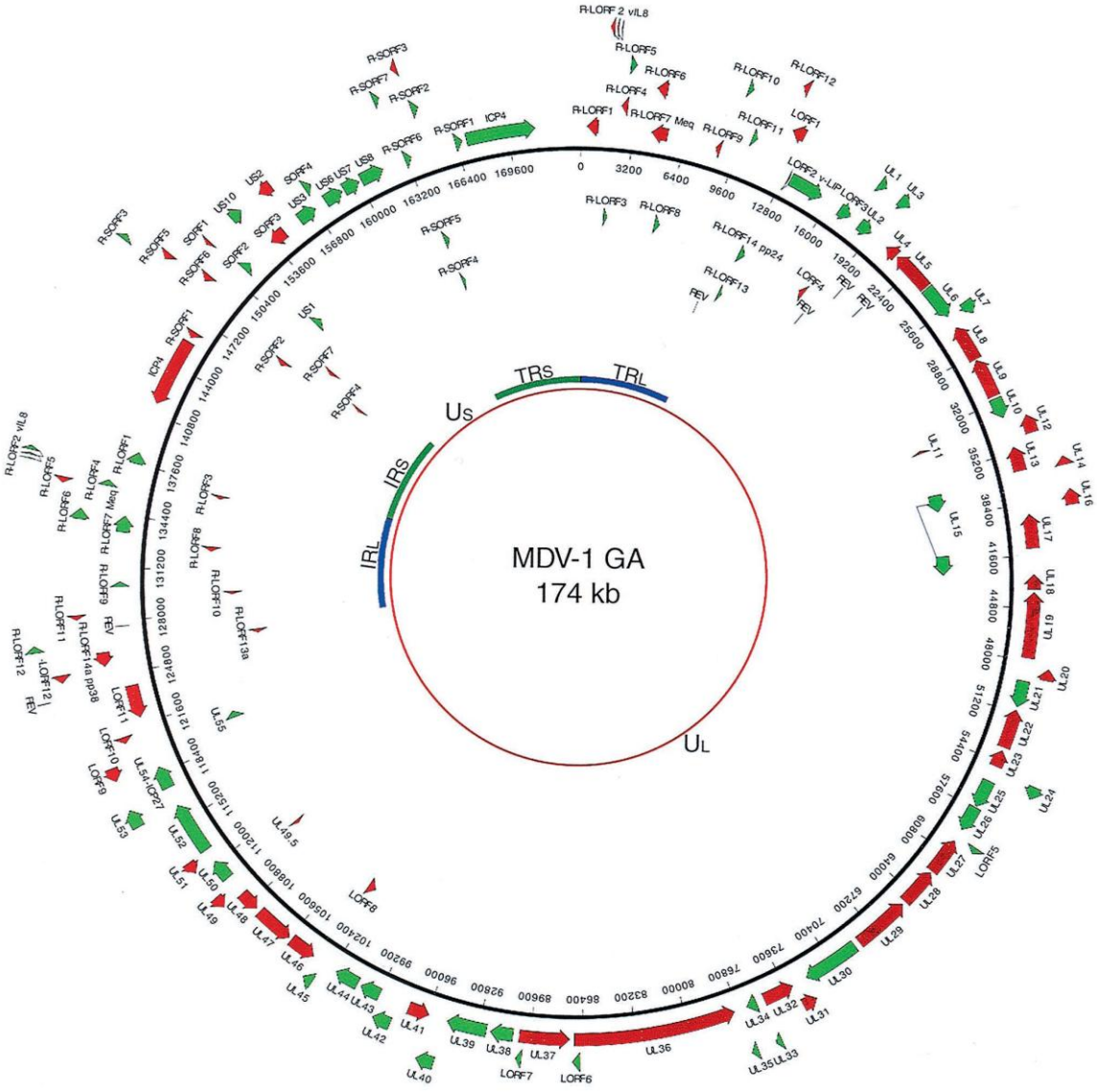



**Figure 1. Genetic organization of GA strain of Marek disease virus (Source Lee *et al.*, 2000)**

that these differences may influence the level of transcription of ICP4. Comprehensive analysis of the sequence of the $T_{RL}/I_{RL}$ regions in the genomes of 13 strains of varying virulence has identified several single nucleotide polymorphisms (SNPs) which loosely partition between attenuated and non attenuated strains (Spatz and Silva, 2007).

The complete genome sequences of serotype 2 strain HPRS 24 (GenBank Accession number NC_002577) and the serotype 3 HVT strain Fc 126 (GenBank Accession numbers AF291866, AF282130 have also been determined) (Afonso *et al*., 2001; Izumiya *et al*., 2001; Kingham et *al*., 2001). Inspection of the sequences of the 3 serotypes confirms that the genomes are collinear as had been suggested based on cross-hybridization studies with cloned fragments of the 3 serotypes (Igarashi *et al*., 1987). The three serotypes differ substantially in their restriction endonuclease digestion patterns (Hirai *et al*., 1979; Gibbs *et al*., 1983; Ross *et al*., 1983; Silva and Barnett, 1991).

*2.2.6.3 Marek's disease viral dereubonucleic acid structure in infected cells*

The structure of viral DNA in infected cells is dependent on the virus-cell interaction. Linear viral DNA can be found in nuclei of cells undergoing virus replication (Cebrian *et al*., 1982). It is still unknown how viral DNA is maintained in latently infected, nontransformed cells (Ross, 1985). The status of DNA in transformed cells has been difficult to determine in part because a variable percentage of the transformed cells may undergo viral replication at any point in time, in which case linear DNA have been done using established cell lines, which may have undergone additional selection and initial integrated and episomal DNA (Kaschka-Dierich *et al*., 1979; Rhiza and Bauer,1984).

The association of viral DNA with chromosomes by density gradient centrifugation and in situ hybridization support the idea that integration may occur. Delecluse *et al.* (1993) showed that viral DNA can be intergrated into primary tumour cells and tumour cell lines. Integration into primary lymphoma cells occurred randomly at multiple sites. In cell lines, integration occurred at 2-12 sites, which were characteristic for the individual cell lines (Schat and Nair, 2008). Integration sites were preferentially located at the telomeres of large- and mid-size chromosomes or in minichromosomes. It is noted that the terminal ends of the MDV genome contains host cell telomere-like sequences which are thought to assist in the preferential integration of the viral DNA near to the telomeres of the host cellular DNA in latently infected cells (Kish *et al*., 1991; Delecluse and Hammerschmidst, 1993).

*2.2.6.4 Marek's disease virus structural changes by recombination and mutation*

Spontaneous recombination among the 3 MDV serotypes under field conditions is probable a very rare occurrence, despite the fact that chickens vaccinated with all 3 serotypes frequently are super infected with virulent MDV with concomitant infections in the same tissue and cells have been reported (Cho, 1977; Nii *et al.*, 1989). Only 1 case of a recombination between 2 serotypes has been reported when a virus was isolated from the MSB1-41C cell line with serotype 1 and 2 sequences. Cloning of the junction fragment showed that this was indeed a recombinant virus (Hirai *et al*., 1990). Serotype 1 strains quickly develop altered biologic characteristics upon serial passage *in vitro,* such as loss of oncogenicity-reduced expression of the so-called A antigen (glycoprotein C (gC) and decreased replication *in vivo* indicating that spontaneous mutations may have occurred (Churchill *et al*., 1969a; Schat *et al*., 1985). The gradual evolution of pathotypes toward

greater virulence and the changes in biologic properties of MDV during *in vivo* backpassage further support the mutability of MDVs (Witter *et al*., 1987).

These biological changes are accompanied by several molecular changes, although it is not clear which molecular change correlates with a specific biological change. An expansion was found within the BamHI D and H fragments that are commonly associated with cell culture passage and attenuation in serotype 1 strains (Hirai *et al*., 1981a; Fukuchi *et al*., 1985; Silva and Witter, 1985). This expansion was caused by a tandem amplification of direct 132 bp repeat (Maotani *et al*., 1986; Hoofvan *et al*., 1990; Ross *et al*., 1993). Further changes have also been described including the deletion of 400 bp in the BamHI A fragment of CV1988 CLONE c (988C) and 988C/R6 and a deletion of 200 bp in the BamH1 L fragment of the vvMDV strain Md11 (Hoofvan *et al*., 1990; Wilson and Cousens, 1991). Additional changes have been reported for CV1988 in the meq gene and the ICP4 promoter/ enhancer region of CV1988 (Lee *et al*., 2000; Kaplan *et al*., 2001). It is not clear whether these differences are a consequence of cell culture passage or reflect strain differences (Schat and Nair, 2008).

Serial passage of serotype 2 and serotype 3 strains can also lead to biological and structural changes. The biological changes are often associated with loss of *in vivo* replication and protective immunity (Yachida *et al*., 1986; Witter and Offenbecker, 1997; Witter, 2001). Other changes have also been reported such as the release of large quantities of cell-free HVT or the development of resistance to inhibition by phosphonoacetate (Lee *et al*., 1978; Yachida *et al*., 1986). Structural DNA changes have been described using restriction

endonuclease patterns and total size estimates based on pulsed field electrophoresis experiments (Hirai *et al.,* 1989; Silva and Barnett, 1991; Wilson and Cousens, 1991).

In *vitro* co-cultivation of MDV or HVT with avian leukosis virus (ALV) or reticuloendotheliosis virus (REV) resulted in spontaneous insertion of long terminal repeats (LRT) of the retroviral provirus in the MDV genome (Isfort *et al*., 1994; Jones *et al.*, 1996), but full-length, infectious provirus occasionally can intergrate (Isfort *et al*., 1994). Integration of endogenous ALV sequences into MDV has also been reported (Sakaguchi *et al*., 1997). The integration occurs often at the border regions of the repeats and unique sequences of MDV and in the gD gene (Brunosvkis and Kung, 1996). Davidson and Borenshtain (2001), Davidson and Borenshtain (2001) and Davidson *et al*. (2002) reported the *in vivo* integration of retrovirus sequences into MDV in commercial poultry. The relevance of the integration of retrovirus for the pathogenesis of MD is not clear, although LTR insertions can cause increased transcription of MDV genes (Jones *et al*., 1996).

*2.2.6.5 Marek's disease viral genes and proteins*

Over the last 20 years (1993), a number of individual genes of MDV-1 have been identified and sequenced and the proteins have been characterized (Ross, 1999; Witter and Schat, 2003). Comprehensive reviews based on the complete sequences for the 3 serotypes recently have been published including the lists of ORFs and their putative products (Izumiya *et al*., 2001; Lupiani *et al*., 2001; Silva *et al*., 2001; Osterrieder and Vautherot, 2004). The location of the ORFs indicates the number of ORFs with homologues of heamorrhagic septicemia virus (HSV) and equine herpesvirus 1 and 4, and the genome organization is similar to these two alphaherpesviruses (Lupiani *et al*., 2001). The MDV

genes are grouped into two general categories: genes with homologues in alphaherpesviruses and genes unique for MDV. Only the genes that are important for the pathogenesis and immune responses are reviewed briefly (Schat and Nair, 2008).

#### *2.2.6.6 Genes with homologues in alphaherpesviruses*

Marek's disease virus broad category of homologue in alphaherpesviruses genes can be divided into immediate early (IE), early and late genes, which are with few exceptions important for virus replication (Anderson *et al*., 1992).

#### *2.2.6.7 Immediate early genes and early genes have homology to heamorrhagic septicemia virus*

The immediate early (IE) genes are important transcriptional regulators. Four IE genes have been identified: intracellular protein (ICP) 4, ICP0, ICP22, and ICP27 (Schat and Nair, 2008). Anderson *et al*. (1992) identified ICP4 as a 4245 bp ORF, but sequence data indicated the presence of an open reading frame (ORF) of 6969 bp. This is in agreement with the finding that two functional promoter/enhancer regions are located upstream of the larger ORF and that the putative promoter/enhancer region for the short ORF was nonfunctional in *in vitro* assays (Kaplan *et al*., 2001). Proof that ICP4 protein is a transactivator was provided by transfection of the MD cell line (MDCC) MSB-1 with the short form of ICP4, showing increased transcription of the PP38 and pp24 genes and the endogenous ICP4 gene (Pratt *et al*., 1994; Endoh *et al*., 1995). The ICP4 protein coded for by UL48 and present in the tegument of MDV particles (Boussaha *et al*., 1996). However, Kaplan *et al.* (2001) were unable to confirm this observation and the requirement for VP16 in a cell-associated virus may not be absolute.

The role of ICP22 (US1) protein in MDV replication is poorly understood (Brunovskis and Velicer, 1995). Deletion mutants lacking ICP22 is not essential for *in vivo* infection, oncogenesis, and reisolation of virus (Parcells *et al*., 1994; Parcells *et al*., 1995). The MDV ICP27 phosphoprotein) localizes in the nucleus, can transactivate pp38 and pp14 independently of ICP4, and represses the early thymidine kinase gene (Ren *et al*., 1994; Ren *et al*., 1996). Although the precise functions of the MDV ICP27 have not been demonstrated, reports on the role of HSV ICP27 in the mRNA export pathway (Koffa *et al*., 2001) and NFkβ activation (Pratt *et al*., 1994) suggest important roles for this protein in MD biology. Intracellular protein (ICP) 0, (R-LORF1) has been identified as an ORF in the TRL and IRL and a recent study using proteomic approaches has demonstrated that the ICP0 gene product is expressed in MDV-infected CEF (Liu *et al*., 2006). However, it is unknown if the protein has similar functions as ICP0 in HSV (Schat and Nair, 2008).

The early genes with homology to HSV-1 have been identified. The functions of these genes are expected to be similar to those in other alphaherpesviruses (Lupiani *et al*., 2001). The MDV genes MDV 008 and MDV 073 encode the related phosphoproteins pp24 and pp38 respectively (Tulman *et al*., 2000).

#### *2.2.6.8 Late genes of Marek's disease virus*

The late gene products include the nucleocapsid proteins, the tegument proteins, including VP16, and the glycoproteins (Lupiani *et al.,* 2001). The glycoproteins (gB, gC, gD, gH, gI, gE, gL, and gM) are presumed to be important for infection of cells, transfer of virus from cell to cell and immune responses. Churchill *et al*. (1969b) identified 2 glycoproteins by

AGP tests: the soluble A antigen and the cell-bound B antigen, which are now known as gC and gB, respectively (Churchill *et al*., 1969b).

gB, encoded by UL27, consists of a complex of 3 glycoproteins with molecular weights of 100, 60, and 49 kD (gp100, gp 60, and gp 49) (Pepose *et al*.,1981; 1992; Ikuta *et al*., 1984; Silva and Lee, 1984; Isfort *et al*., 1986a; Chen and Velicer, 1992). gB is important for cell attachment and penetration based on the production of gB-specific virus-neutralizing (VN) antibodies (Ikuta *et al*.,1984; Isfort *et al*., 1986a; Nazerian *et al*., 1992; Ross *et al*., 1993; Ono *et al*., 1996). Deletion of gB from MDV prevented the cell to cell spread demonstrating the essential nature of this protein for MDV replication as is the case with all other herpesviruses (Pereira, 1994; Schumacher *et al*., 2000)

The UL44 gene encodes gC, a 57-65 kD glycoprotein identified in some early references as gA, which is extensively synthesized in productively infected cells and is expressed on the cell surface and cytoplasm. In addition, gC is actively secreted by infected cells and is one of the major antigens to which the chicken immune system mounts a substantial serological response (Ikuta *et al*., 1985; Isfort *et al*., 1986b; Coussens and Velicer, 1988). The production of gC in cell culture decreases with attenuation probably due to reduced transcription of UL44 (Churchil *et al*., 1969a; Ikuta *et al*., 1983). Recent data suggest that the reduction of MDV replication due to over expression of gC in cultured cells is possibly caused by the secreted forms of gC (Tischer *et al*., 2005). A gC deletion mutant had an attenuated phenotype with a decrease in infectivity, horizontal transmission and oncogenicity. However, a revertant virus needs to be generated to confirm that the

attenuation was caused by deletion of gC (Morgan *et al*., 1996). Recent *in vivo* studies using a gC-negative mutant of RB-1B showed that although the viral load in the peripheral blood was not affected, it was compromised in establishing latency and inducing tumours suggesting a major role for gC in MD pathogenesis (Osterrieder *et al*., 2006).

The importance of gD, coded by US6, is poorly understood. It is expressed poorly or not at all in vitro probably as a consequence of no or limited transcription (Ono *et al*., 1996; Tan *et al*., 2001). Limited expression of gD compared to pp38 and gB has been described in FFE may be needed for the production of gD. A gD deletion mutant constructed in the oncogenic RB-1B strain was fully oncogenic and capable of horizontal transmission indicating that gD is nonessential (Anderson *et al*., 1998). Single nucleotide polymorphism (SNP) analysis in the gD gene was used in a retrospective molecular epidemiological study to determine the variation among MDV strains (Laurent *et al*., 2004).

The functions of the other glycoproteins have not been studied in detail. The gI and gE proteins interact with each other based on immunoprecipitation assays (Tan *et al*., 2001). It is not clear whether the gE/gI complex functions as Fc receptors as described for other alphaherpesviruses. Mutants constructed in a bacterial artificial chromosome (BAC) clone carrying deletions in the gM, gI, or gE gene indicate that the encoded glycoproteins are essential for virus replication because the deletion mutants are unable to transfer infectivity from infected to uninfected cells (Schumacher *et al*., 2001; Tischer *et al*., 2002).

### 2.2.7 Marek's disease virus replication

Replication of the 3 serotypes is typical of other cell-associated herpesviruses and has been reviewed extensively (Kato and Hirai, 1985; Ross, 1985; Schat, 1985; Biggs, 2004; Osterrieder and Vauther *et al*., 2004). For initial infection of cultures or chickens by cell-free virus, enveloped virions bind to *cellular receptors probably by gB* perhaps in combination with other *glycoproteins*. Heparan sulfate, a member of the *glycosaminoglycan*s, has been identified as one of the cellular receptor molecules (Lee *et al*., 2001). In cell cultures, viral penetration occurs within 1 hour after attachment, which is enhanced for serotype 1 by chelators such as ethylenediaminete-traacetic acid (EDTA) (Adldinger and Calnek, 1972). Subsequent spread of infected cells and virus transfer probably is accomplished through formation of intracellular bridges (Kaleta and Neumann, 1977). This is presumed to be the principal mode of virus spread after initial infection *in vitro* and *in vivo*. Recent data demonstrate the role of the $U_S3$-encoded knase in the morphogenesis as well as cell-to-cell spread of virions through the effect of stress on fibre breakdown and polymerization of actin (Schumacher *et al*., 2005). The glycoproteins gE, gI and gM play a role in the transfer of virus from infected to uninfected cells (Schumacher *et al*., 2005; Tischer *et al*., 2002). Replication rates vary with serotype, passage level of the virus strain, cell type and temperature of incubation (Schumacher *et al*., 2005).

The spread of virus in vivo from cell to cell will require intimate contact between infected and uninfected cells, which are most often lymphocytes, although epithelial cells also can be involved in this process. The precise interaction between these cells remains one of the important unsolved issues (Schumacher *et al*., 2005).

### *2.2.7.1 Marek's disease virus-cell interactions*

Three general types of virus-cell interactions are recognized; productive, latent and transforming.

### *2.2.7.2 Marek's disease virus productive infection*

During productive infection, replication of viral DNA occurs; proteins are synthesized and in some cases, virus particles are produced (Schat and Nair, 2008).. The number of genome copies per cell can increase 100-fold and exceed 1,200 in the case of HVT (Kaschka Dierich, 1979). Two types of productive infection exist. Fully productive infection in the FFE of chickens results in development of large numbers of enveloped, full infectious virus by disruption of cells (Schat and Nair, 2008). The use of an appropriate stabilizer such as sucrose-phosphate-glutamate-albumin (SPGA) will improve the yield of cell-free virus (Calnek *et al*., 1970a). A variant strain of HVT that releases large quantities of cell-free virus into the medium of infected cell cultures has been described. This strain is replication-defective in chickens (Yachida *et al*., 1986). In all susceptible cells, productive infection leads to intranuclear inclusion body formation and lysis of the cell. A gene for the viral host shut-off protein has been identified, UL41 that is probably responsible for the initiation of the lytic process (Lupiani *et al*., 2001). Lytic infection *in vivo* can cause frank necrobiotic lesion formation. Because of this, productive infection has been termed cytolytic and the terms are used synonymously (Calnek, 1986). In productively infected fibroblasts, most of the MDV genome that produced infection with virulent and attenuated serotype 1 strains have been described (Bradley *et al*., 1989; Ross *et al*., 1993) and are mostly associated with a study employing a mass soectrometry-based proteomic approach

has further confirmed the expression of the majority of MDV-encoded proteins during cytolytic infection in CEF (Liu *et al*., 2006).

Productive infection in cell cultures is influenced by several factors. Inhibitors of viral DNA polymerase and thymidine kinase will inhibit virus replication in productively infected cell cultures but do not influence the growth of lymphoblastoid cell lines (Ross, 1985; Schat, 1985). The presence of nitric oxide (NO) in cell cultures reduces virus replication in a dose-dependent manner (Djeraba *et al*., 2000; Xing and Schat, 2000). *In vivo* infection can change transcription regulation of cellular genes in infected and in neighbouring cells. Microarrays have been used to determine which genes may be up-or down-regulated by infection with MDV (Morgan *et al*., 2001). For example, MHC class I and II genes and two IFN-response elements were upregulated. The importance of these results needs to be further examined. Hunt *et al*. (2001) reported that productive-restrictive MDV replication down regulated expression of MHC class I expression in OU2 cells and in lymphoblastoid cell lines. Upregulation of MHC genes actually occur in noninfected cells probably through IFN (Kent *et al*., 2001; Levy *et al*., 2001). This is certainly compatible with the up regulation of IFN-response genes. IFN, probably IFN-α, may be produced at least by some MDV and HVT strains (Hong and Sevoian, 1971; Kaleta and Bankowski, 1972).

#### *2.2.7.3 Marek's disease latent infection*

Latent herpesvirus infection has been defined as the presence of viral DNA in the absence of viral transcripts and proteins, although latency-associated transcripts (LATs) have been

described for many herpesviruses (Schat and Nair, 2008). This definition is appropriate for the non transforming serotype 2 and 3 strains. For serotype 1 strains, the distinction between latency and transformation is often problematic (Schat and Nair, 2008). In both cases, the viral genome is present, but no information is available on differences in transcriptional regulation between latently infected and transformed cells, because it is impossible to separate latently infected, nontransformed cells from noninfected cells. As a consequence, studies on latency have often been done in MD transformed cell lines (Schat and Nair, 2008).

Marek's disease virus latency is mostly associated with $CD4^+$ T cells, although $CD8^+$ T cells and B cells can be latently infected (Calnek *et al*., 1984; Lee *et al*., 1999). Fewer than 5 copies of the viral genome are present in latently infected cells (Ross, 1985). *In vitro* latent infections have been described in REV-transformed (Pratt *et al*., 1992), OU2 (Abujoub and Coussens, 1997) and QT35 cell lines (Yamaguchi *et al*., 2000). The MDV genome can be reactivated from latently infected cells and tumour cells by inoculation of susceptible chickens, co-cultivation with permissive cells, and in vitro cultivation of latently infected lymphocytes (Calnek *et al*., 1981b). The latter approach can be used to estimate the number of latently infected cells by enumeration of antigen-positive cells at 0 hour and 48 hours in culture (Calnek *et al*., 1981). The presence of latent MD infections can also be detected by polymerase chain reaction (PCR) assays as in diagnosis. However, the PCR assay needs to be combined with real time (RT-PCR), demonstrating the absence of early and late transcripts to ensure that the amplified DNA is obtained from latently and not from the few productive-restrictive infected cells that commonly occur in populations

of latently infected lymphocytes. The up regulations of infected cell proteins (ICP4) transcripts and down regulation of latency-associated transcripts (LATs) have been associated with reactivation of early and late genes in latently infected cells (Abujoub and Coussens, 1997; Yamaguchi *et al*., 2000).

At least 2 cytokines produced by concanavalin A-stimulated spleen cell cultures can help maintain latency in cultured lymphocytes (Buscaglia and Calnek, 1988). Subsequently, 2 recombinant chicken (rCh) cytokines, rChIFN-α and rChIFN-γ, have been shown to suppress production of immediate early genes (IE), early, and late viral antigens in latently infected lymphocytes (Volpini *et al*., 1995; Kaplan *et al*., 2001). Curiously, IFN-α was more effective in the suppression of viral genes in later stages of latency than during early stages (Volpini *et al.*, 1995). In *vivo* selective reactivation of latently infected cells can be induced by treatment with cyclosporine of betamethazone, but not by infection with immunosuppressive viruses, such as infectious bursal disease (IBD) virus (IBDV) or reticuloendotheliosis virus (REV) (Buscaglia and Calnek, 1988). Chicken infectious anemia virus (CIAV) may influence latency in MD based on its influence on cytokine production and cytotoxic T lymphocytes (CTL) (Schat, 2003)

#### *2.2.7.4 Marek's disease transforming infection*

Transforming infections occur only in cells infected with serotype 1 MDV (Schat and Nair, 2008). Selection of transformed cells from the background of immunologically committed and non committed cells would facilitate comparative studies on transformed cells in tumours and tumour cell lines (Payne and Roszkowski, 1972). The search for specific surface markers associated with tumours has yielded two potential antigens. An

MD tumour-associated surface antigen (MATSA) was detected on cells from MD lymphomas and lymphoblastoid cell lines but not on the surface of productively infected cells (Powell *et al*., 1974; Witter *et al.*, 1975). Marek's disease tumour-associated surface antigen was also detected on lymphocytes from chickens vaccinated with HVT or serotype 2 strains of MDV with subsequent studies revealing MATSA to be present on activated T cells from uninfected chickens (Schat and Calnek, 1978; Kitamoto *et al*., 1979; Powell and Rennie, 1980; McColl *et al*., 1987). A second antigen, CD30, detected by Marek's disease antibody (MAb) AV37, has been associated with MD-transformed $CD4^+$ T cells and MDV-infected cells during the cytolytic phase. However, this antigen can also be detected on B cells and REV-transformed cells (Burges and Davison, 1996; Ross *et al*., 1997). Studies confirm that $CD30^{hi}$ expression is characteristic of MD lymphomas suggesting that CD30 is a component of a critical intracellular signaling pathway perturbed in neoplastic transformation (Burges *et al*., 2004). Both MATSA and CD30 can be used to enrich the transformed cells in tumours cells suspensions. Rose *et al*. (1997) purified cells from lymphomas using $CD4^+$ and AV37 MAbs and found that meq and SAR transcripts were abundant in these populations. In contrast, PP 38 and VP16 transcripts were not detected. Most other studies have used lymphoblastoid cell lines to identify transcripts (Schat and Nair, 2008). Antigens normally are not detected in lymphomas or lymphoblastoid cell lines by FA tests with convalescent serum, except for occasional cells that probably have converted to a productive infection and by definition, are no longer transformed. Some transformed lymphocytes can be induced to produce viral antigens by treatment with IUdR or by culture at suboptimal temperatures (Akiyama and Kato, 1974; Duncan and Nazerian, 1977; Calnek *et al*., 1978; Calnek *et al*., 1981; Lee, 1993).

## 2.3 Viral Replication of Other Marek's Disease Virus Serotypes

In most reports, replication of MDV serotype 2 and HVT are similar to serotype 1. Because HVT and serotype 2 MDV are nononcogenic no cell lines have been developed equivalent to those derived from MD lymphomas and transforming infections have not been recognized (Witter *et al*., 1970; Schat and Calnek, 1978).

Latent infections have been demonstrated in chickens infected with serotype 2 and 3 viruses (Shek *et al*., 1982). The phenotype (s) of cells latently infected with serotype 2 and 3 strains have not been elucidated. The observation of latent infection of SB-1 in ALV-transformed B cell lines suggests that B cells are likely target cells for latent infection with serotype 2 strains (Fyan *et al*., 1993). Occasionally, HVT can be present in MDV-transformed T cell lines, probably representing a latent infection (Calnek *et al*., 1978; Hirai *et al*., 1981b). Hammand *et al.* (2001) reported the presence of HVT transcripts encoded in the $TR_L$ and $IR_L$ in the thymus and spleen, but not the bursa of Fabricius, in the absence of gB expression. Interestingly, these transcripts were also detected in peripheral nerves and feather-associated tissues (Schat and Nair, 2008).

### 2.3.1 stock production and stability of Marek's disease virus

Productively infected cell cultures are a common source of cell-associated virus stocks for all 3 viral serotypes and for cell-free HVT stocks (Schat and Nair, 2008). Techniques for the production and cryopreservation of cell-free and cell-associated virus stocks have been described (Churchil, 1985). Cell-associated stocks of MDV or HVT are routinely stored at -196°C. The infectivity of such stocks however, is directly related to viability of the cells contained in these preparations and depends also on optimal freezing and thawing

techniques. Under ideal conditions, the half-life of diluted, cell-associated virus stocks or vaccines should be at least 2-6 hours (Thornton, 1985).

Cell-free serotypes 1 and 2 virus stocks are best obtained from feather follicle epithelium (FFE) for low-passage virus or infected cell cultures (high-passage virus). Small quantities of low-passage virus can be obtained from infected cell cultures by lysing cells in SPA (Calnek *et al*., 1970b). The production of cell-free HVT is best achieved by lysing heavily infected cell cultures. Cell-free MDV and HVT can be stored at -70°C or lyophilized (Calnek *et al*., 1970c). The potency of both cell-associated and cell-free vaccines can be affected adversely by storage temperature, reconstitution technique, choice of diluents and holding time and temperature after reconstitution (Halvorson and Mitchel, 1979; Pattison, 1985).

### 2.3.2 Susceptibility of Marek's disease virus to chemical and physical agents

The stability of cell-associated MDV serotype 1 and 2 strains is completely depending on the viability of the cells. Any treatment affecting cell viability will impact directly the infectivity of virus stocks. Cell-free MDV obtained from the skin of infected chickens was inactivated when treated for 10 mins at pH 3 or 11 and stored for 2 weeks at 4°C, 4 days at 25°C, 18 hours at 37°C, 30 mins at 56°C or 10 mins at 60°C (Calnek and Adldinger, 1971). Dander, litter and feathers from infected chickens are infectious and presumably contain cell-free virus from the FFE bound to cellular debris. The infectivity of such materials was retained for 4-8 months at room temperature (Hlozanek *et al.,* 1973; Witter *et al*., 1968) and for at least 10 years at 4°C (Calnek, 1979). Virus infectivity was inactivated by a variety of common chemical disinfectants within a 10-minute treatment period (Calnek and

Hitchner, 1973; Hlozanek *et al*., 1977). Survival of virus in litter may be affected adversely by increased humidity (Witter *et al*., 1968).

### 2.3.3 Classification of Marek's disease virus serotypes

Von Bulow and Biggs (1975a; b) classified the MDV herpesvirus group into 3 distinct virus groups that correlated with biologic properties. Type-specific monoclonal antibodies usually are used to determine virus serotype (Ikuta *et al*., 1982; Lee *et al*., 1983). Although distinguishable by serologic tests, the 3 serotypes also share many common antigens. Thus, sera against a serotype usually will react with antigens of other serotypes, although somewhat less vigorously than with homologous antigens (Von Bulow and Biggs, 1975b). A number of biological characteristics are associated with viral serotypes (Biggs and Milne, 1972; Schat, 1985). Low-passage serotype 1 viruses grow best in duck embryo fibroblast (DEF) or chicken kidney cell (CKC) cultures, grow slowly and produce small plaques (Schat and Nair, 2008). Serotype 2 viruses grow best in chicken embryo fibroblasts (CEF), grow slowly and produce medium plaques with some large syncythia. HVT grows best in CEF, grows rapidly and produces large plaques. More infectious virus can be extracted from HVT-infected cells than from cells infected with serotype 1 or 2 viruses (Schat and Nair, 2008).

### 2.3.4 Epidemiology of Marek's disease

*2.3.4.1 Marek's disease* present itself in many forms

Marek's disease consists of several distinct pathologic syndromes of which differences are among the types of MDV induced syndromes typically seen in layer, breeder, broiler and

those induced in the laboratory. Lymphoproliferative syndromes are most frequently associated with MD and have the most practical importance. Marek's disease lymphoma is probably the most common (Erf *et al.,* 2001). However, fowl paralysis, persistent neurological disease, skin leukosis and ocular lesions are additional clinical manifestations that have lymphoproliferative components. Some of the lymphoproliferative syndromes may also have degenerative components. Several additional clinical syndromes characterized solely by degenerative and inflammatory lesions, often with accompanying immunosuppression are induced by MDV infection in the laboratory (Schat and Nair, 2008). Non neoplastic brain pathology, mainly vasogenic oedema is responsible for transient paralysis (Gimeno *et al.,* 1999). Under laboratory conditions, young chicks inoculated with tumour cells may develop localized or diffused transplantable tumours (Theis *et al.,* 1974; Stephens *et al.,* 1980).

Some of the syndromes induced under laboratory conditions are said to be rare or non existent in the field, probably because most commercial chickens are hatched with passive MD antibodies and receive MD vaccines at or before they are hatched. Subclinical MD syndromes may also occur but are more difficult to define (Schat and Nair, 2008). Purchase *et al*. (1972) found that vaccinated flocks produced more eggs than non vaccinated flocks, indicating that MDV may depress productivity in otherwise normal appearing non vaccinated chickens.

*2.3.4.2 Incidence and distribution of Marek's disease*

Marek's disease exists in poultry-producing countries throughout the world (Schat and Nair, 2008) probably every flock of chickens raised in areas where poultry is prevalent becomes infected and many experience some losses (Schat and Nair, 2008). Reporting systems vary, however, it is difficult to determine the true incidence of MD. The incidence of MD infection is surely much higher than the MD incidence causing the disease. Even in susceptible chickens, MD infection does not always induce clinical disease and in genetically resistant or vaccinated chickens, infection may rarely cause overt disease (Schat and Nair, 2008).

In the United States, the incidence of MD losses can be illustrated through the use of broiler condemnation data, collected consistently with maximum frequencies around April and minimum frequencies around August, perhaps reflecting the effects of temperature stress and limited ventilation during winter months (Purchase, 1985). Regional differences are striking, analysis of the devastating 1960s outbreak showed that the initial losses occurred in the Delmarva and northeastern states, but the disease spread quickly to other regions of United States (Witter, 1997).

Historically and to the present time, condemnations in Delaware have been consistently lower than mean values for the United States (Witter, 1997). Long and short-term trends due to the introduction of vaccines can also be evaluated through condemnation data. Since 1971, when HVT vaccine was introduced through 2000, yearly condemnations have decreased by 79-fold, 169-fold, and 958-fold in Delaware, the United States and Georgia, respectively (Santin *et al.*, 2006). Up turns in the early 1980s and the early 1990s were both

successfully resolved by the introduction of more effective vaccines. However, during the early part of 2005 outbreaks occurred in the Delmarva region with condemnations of almost 10% in broilers vaccinated with bivalent and trivalent vaccines. Similar trends have been experienced in layer and breeder flocks (Santin *et al.,* 2006).

Sporadic outbreaks of MD occur on individual farms or regions in the United States, several reports lend credence to the implication of exceptionally virulent MDV isolates in vaccine failures (Eidson *et al.,* 1978; Witter *et al.,* 1980; Schat *et al.,* 1982; Powell and Lombardini, 1986). It is usually difficult to associate increased virulence of MDV isolates with regional fluctuations in MD frequency, but layer outbreaks in northwestern Ohio in 1995 yielded several isolates of unusual virulence (Witter, 1997; Calnek *et al.,* 1998).

### 2. 3 .5 Natural and experimental hosts of Marek's disease

Chickens are by far the most important natural host for MD, but quails, turkeys and pheasants are also susceptible to viral infection and disease. Virtually all chickens including game fowl, native breeds and jungle fowl are susceptible to MDV infection and tumour development. (Amos *et al.,* 1981). Most other avian species including ducks, sparrows, partridges, pigeons and peafowls are probably refractory although MDV-inoculated ducks developed antibodies (Amos *et al.,* 1981). Mammals, including several species of primates are also refractory to experimental inoculation. Mice inoculated with tumour cells from chickens from field cases of MD developed a high rate of neoplasms, but the aetiological role of MDV in these tumours is unsubstantiated (Amos *et al.,* 1981).

#### *2.3.5.1 Quails*

In Japanese quails, natural outbreaks of MD in commercial flocks are relatively common. Affected birds develop lymphomas in various visceral organs, but peripheral nerves are rarely affected. Mortality can reach 10-20%, but deaths occur relatively late. Marek's disease virus occasionally can be isolated from blood, although titres can be low or detected by PCR assays. Transmission of MDV can occur from chickens to quails and from quails to chickens by experimental inoculation or contact exposure to MDV strains of both chicken and quail origin (Schat and Nair, 2008). The experimental disease appears similar to that of natural outbreaks. Many commercial quail flocks are vaccinated with HVT. Kobayashi *et al*. (1986) considered vaccination to be protective. However, Paul and Pradhan (1991) reported that quails were poorly protected by HVT against challenge at 10 days of age with an MDV strain of quail origin. Bobwhite quails are also susceptible to MD, but perhaps less so than Japanese quail (Powell and Rennie, 1984).

#### *2.3.5.2 Turkeys*

In turkeys, MD-like tumours have been occasionally reported, but causal associations with MDV usually have been lacking and natural outbreaks have been rare at least until the late 1990s (Elmubarak *et al.,* 1982; Nazerian *et al.,* 1982). However, turkeys are susceptible to experimental infection with MDV with mortality around 22-70% at 8-19 weeks of age. Enlarged peripheral nerves were rare or absent in studies conducted. Characteristics of the viral infections were similar to those in chickens but generally are more subdued (Schat and Nair, 2008). Virus could be re-isolated from peripheral blood lymphocytes and antigen detected in lymphoid tissues and lungs of inoculated turkeys but less frequently and at

lower concentrations than in chickens. Immunosuppression was also noted in turkeys that developed tumours (El-Mubarak *et al.,* 1982; Nazerian *et al.,* 1982). Interestingly, vaccination with HVT provided no protection against MDV challenge. Both B-cell and T cell lines have been developed from turkey lymphomas induced by MDV inoculation, suggesting that both cell types are susceptible to transformation (El-Mubarak *et al.,* 1982; Nazerian *et al.,* 1982).

Severe clinical outbreaks of MD in commercial turkey flocks were reported in France, Israel, Germany and Scotland, mortality from tumours reached 40-80% between 8-17 weeks of age (Coudert *et al.,* 1997; Voelckel *et al.,* 1999; Davidson *et al.,* 2002; Pennycott and Venugopal, 2002). In some of these cases, the affected turkey flocks were raised in proximity to broilers (Coudert *et al.,* 1997). The lesions were similar to those produced experimentally, although paralysis was noted in some birds and peripheral nerves were occasionally infiltrated with lymphocytes (Coudert *et al.,* 1997). Serotype 1 MDV was detected by virus isolation or PCR (Davidson and Borenstein, 1999). Vaccination of turkeys with CV1988 appears to offer protection. Marek's disease has not yet shown the ability to become endemic within commercial turkey populations (Coudert *et al.,* 1997).

2.3.5.3 *Marek's disease in other avian species*

Pheasants and related species like the black Francolin may also be susceptible as indicated by occasional reports of typical lymphomas and nerve lesions. Challenge of the common pheasant (*Phasianus colchicus*) with virulent MDV induced paralysis, visceral lymphomas and precipitating antibodies within 75-85 days post infection (PI). Unlike quails and

turkeys, pheasants appear to be more susceptible to neurological lesions of MD (Murata *et al.,* 2005). However, the disease has not been well characterized in these species (Schat and Nair, 2008). Marek's disease was reported in white-fronted geese (*Anser albiforms*) in Japan. Subsequent analysis showed high prevalence of MDV genome in the feather tips of these geese (Murata *et al.,* 2005; Schat and Nair, 2008).

### 2.4 Transmission, *C*arriers and Vectors of Marek's Disease

Marek's disease virus is transmitted readily by direct or indirect contact between chickens, apparently by the airborne route (Biggs, 1985). Epithelial cells in the keratinizing layer of the feather follicle replicate fully infectious virus and these cells serve as a source of contamination to the environment and to other chickens. Virus associated with feathers and dander is infectious and contaminated poultry house dust remains infectious for at least 4 to 8 months in shaded places at 20-25°C and for years at 4°C (Calnek, 1986). The virus is known to survive for 4 months in droppings and litter and up to 1 year in dander and feather follicles (Chauhan and Roy, 2007).

Under commercial field conditions, young chickens are most commonly exposed to MDV by inhalation of infective particles with residual dust and dander in the growing house or by the introduction of these materials by aerosols (from adjacent chicken houses), fomites, or personnel (Chauhan and Roy, 2007). After the virus is introduced into a chicken flock, regardless of vaccination status, infection spreads quickly from bird to bird. Virus excretion begins about 2 weeks after inoculation or exposure, with maximal shedding occurring between the third and fifth week (Chauhan and Roy, 2007). Once infected, chickens appear

to shed virus indefinitely (Chauhan and Roy, 2007). Quantitative (q) PCR assays have been established to measure virus load in FFE and dust for MDV serotype 1 including the vaccine strain CV1988, serotype 2 and serotype 3. Preliminary data confirm the earlier observations that maximal shedding occurs early after the presence of virus in the FFE (Kenzy and Biggs, 1969; Witter *et al.,* 1971; Chauhan and Roy, 2007).

Darkling beetles (*Alphitobius diaperinus*) were shown to passively carry the virus, but free-living litter mites, mosquitoes, and coccidial oocysts could not be associated with transmission (Chauhan and Roy, 2007). Vertical transmission of MDV does not occur (Solomon *et al*., 1970; Rispens *et al.,* 1972; Solomon and Witter, 1973). Transmission from dam to progeny as the result of external egg contamination is also unlikely because of poor virus survival at temperature and humidity levels used during incubation (Calnek and Hitchner, 1973).

Experimental transmission is accomplished by innoculation of day-old, genetically susceptible chicks with blood, tumour suspensions or cell-free virus by virtually any parenteral route. Exposure by direct or indirect contact with infected chickens is also effective. The virus can be transmitted by intratracheal instillation or inhalation exposure using cell-free virus preparations. Cellular inocula contain few, infectious virions and are unlikely to cause infection except by parenteral inoculation (Calnek and Hitchner, 1973; Chauhan and Roy, 2007; Schat and Nair, 2008).

## 2.5. Incubation Period of Marek's Disease

The incubation period for experimentally induced MD is well established. Mononuclear infiltrations may be found in nerves and other organs after about 2 weeks. Clinical signs and gross lesions, however, generally do not appear until between the third and fourth weeks (Schat and Nair, 2008). The incubation periods can be short for several non lympphomatous syndromes associated with MDV infection. Cytolytic infections occur at 3-6 days post inoculation (PI) and are followed by degenerative of the thymus and bursa of Fabricius within 6-8 days PI. The clinical expression of both acute and classical forms of transient paralysis usually occurs from 8-18 days PI. Field cases of transient paralysis are seen mainly between 6-12 weeks of age, probably reflecting MDV exposure 8-10 days prior to the onset of symptoms. Development of atherosclerosis requires 3-7 months. Induction of tumours within 10-14 days after inoculation of cellular material is suggestive of transplantation response of local lesions in the wing web visible in 3-4 days PI with allogenetic MDV-infected Chicken kidney cell (CKC) (Payne and Rennie, 1973; Witter *et al.,* 1999; Calnek, 2001; Schat and Nair, 2008).

Under field conditions, MD outbreaks sometimes occur in unvaccinated layer chickens as young as 3-4 weeks. Most of the serious cases begin after 8-9 weeks but also sometimes commence well after the onset of egg production. Marek's disease outbreaks in commercial, vaccinated chickens have been termed as early and late breaks, referring to the apparent failure of vaccines to provide protection. The late breaks are especially troublesome and have been known to occur late in the laying cycle or even after chickens have been induced to molt and the second laying cycle has commenced. It is uncertain

whether late onset of disease is caused by early (old) or late (recent) infection. Witter and Gimeno (2006) infected birds between 18 and 102 weeks of age and found that nonvaccinated birds developed MD within 68 days PI when challenged with highly virulent strains. In contrast, vaccinated birds of similar ages did not develop MD after challenge. The authors suggested that late breaks were not likely the result of recent infections alone and that additional factors are needed to cause the late breaks (Nicholis, 1984; Kreager, 1997; Witter, 2001; Witter and Gimeno, 2006).

## 2.6 Pathogenesis of Marek's Disease

The pathogenesis of MD is complex, with infection occurring through the respiratory route from the inhalation of poultry house dust contaminated with the virus. After an early cytolytic infection mainly in B lymphocytes in the bursa, spleen and thymus at 3-5 days post-infection, the virus infects the activated T lymphocytes, mainly of the $CD4^+$ phenotype. The infection in the T lymphocytes becomes latent at 6-7 days post-infection and the virus is spread throughout the body by infected lymphocytes, which persist as a cell-associated viraemia. A secondary cytolytic infection occurs in the feather follicle epithelium from about 10 days after infection, from where infectious-cell-free virus is produced and shed into the environment in feather debris and dander. The latently infected T lymphocytes are subsequently transformed, leading to the development of lymphomatous lesions in the visceral organs. The main target cells for transformation in natural infections are the $CD4^+$ T cells, although the virus also has the potential to transform CD8+ T cells (Payne and Rennie, 1973; Shek *et al*., 1983; Payne, 2004; Chauhan and Roy, 2007; Nair *et al*., 2008).

## 2.7. Clinical Signs Associated With Marek's Disease

Clinical signs associated with MD vary according to the specific syndrome. Chickens with MD lymphoma or fowl paralysis syndromes may exhibit signs, but few are specific to MD in general, signs related to peripheral nerve dysfunction are those associated with asymmetric progressive paresis and later, complete spastic paralysis of one or more of the extremities (Gimeno *et al.,* 1999). Involvement of the vagus nerve can result in paralysis and dilation of the crop and gasping. Because locomotory disturbances are easily recognized, incoordination or tilted gait may be the first observed sign (Gimeno *et al.,* 1999). A particularly characteristic clinical presentation is a bird with one leg stretched forward and the other back as a result of unilateral paresis or paralysis of the leg (Gimeno *et al.,* 2001).. However, chickens with MD lymphomas may exhibit few signs and instead, become depressed and comatosed prior to death (Gimeno *et al.,* 2001).. Other chickens may appear clinically normal and still have extensive neoplastic involvement when euthanized (Gimeno *et al.,* 2001). Nonspecific signs such as weight loss, paleness, anorexia and diarrhoea may be observed, especially in birds in which the course is prolonged. Under commercial conditions, death often results from starvation and dehydration because of the inability to reach food and water or, in many cases, from trampling by pen mates. Some birds develop nervous torticollis 18-26 days after virus exposure, often after recovery from classical transient paralysis. This syndrome has been termed persistent neurological disease and can be induced by paralysis. However, the central nervous system (CNS) signs are difficult to distinguish from those associated with MD nerve lesions (Gimeno *et al.,* 1999; Gimeno *et al.,* 2001).

Birds with ocular involvement may show evidence of blindness, which can be unilateral or bilateral, although recognition of clinical blindness requires careful observation. Affected eyes gradually lose their ability to accommodate to light intensity (Ficken *et al*., 1991).

Early mortality syndrome results in high mortality 8-16 days PI of young chickens with virulent MDV strains (Ficken *et al*., 1991). Chickens become depressed and comatose prior to death, which occurs within 48 hours of the onset of signs. Some affected chickens may also exhibit flaccid neck paralysis prior to death. Chickens undergoing acute cytolytic infection at 3-6 days PI may be depressed but rarely die during this period, although some may die later from early mortality syndrome. Immunosuppressed chickens may succumb to auxillary infections, but some chickens die 20-40 days PI with few signs (Witter *et al.*, 1999)

A transient paralysis syndrome has been described in field flocks and is associated with MDV infection. However, it has been observed infrequently in the field since vaccination for MD has become widespread. Two forms of MD exist, classical and acute. In the classical form, affected chickens display varying degrees of ataxia and flaccid paralysis of the neck or limbs beginning 8-12 days after exposure to the virus by inoculation or contact. Signs typically last 1-2 days followed by a rapid and complete recovery, although recovered chickens may succumb a few weeks later with MD lymphomas. The acute (fatal) form results in death within 24-72 hours following the onset of paralytic signs (Witter *et al.*, 1999).

## 2.8 Morbidity and Mortality Due to Marek's Disease

The incidence of MD is quite variable in commercial flocks. Although a few birds that develop signs apparently recover from the clinical disease (Biggs and Payne, 1967; Burgess *et al*., 2004). The recovery is rarely permanent; chickens that develop clinical disease usually die. Prior to the use of vaccines, losses due to MD in affected flocks were estimated to range from a few birds to 25 or 30% and occasionally as high as 60%. In broilers, MD condemnations averaged 1.0% in 1970 and could reach 10% or higher in individual flocks. Nearly all commercial egg-type chickens are vaccinated against MD, and this has reduced losses to less than 5% in most countries (Purchase, 1985). The average condemnation rate for MD in the United States has decreased dramatically, falling to less than 0.001% in most regions in 2002 (Santin *et al*., 2006). However, spikes in condemnations can occur as was noted in Delmarva in 2005 (Santin *et al*., 2006). Some flocks experience significant disease outbreaks despite vaccination. After the disease appears, mortality builds gradually and generally persists for 4-10 weeks. Outbreaks occur in isolated flocks or occasionally in several flocks in a region or in succeeding flocks on a farm (Schat and Nair, 2008). An interesting, albeit fortunate, tendency has developed for regional outbreaks to abate spontaneously. The reasons for the occurrence and cessation of regional outbreaks are poorly understood (Schat and Nair, 2008).

Response rates or mortality approaching 100% for lymphomas, early mortality syndrome, acute cytolytic infection, or transcient paralysis can be achieved following inoculation or exposure to MDV. It is believed that the response frequency is influenced by many factors as indicated in 2.8, laboratory experiments can be designed to produce a wide range of specific clinical and pathologic responses (Karel *et al*., 2008).

## 2.9 Factors that Influence Mortality and Lesions Due to Marek's Disease

### 2.9.1 Marek's disease virus strain

The virulence of MDV strains varies widely and appears to have increased over time. Compared to the milder forms of the disease, which caused mainly peripheral nerve lesions, the more virulent MDV pathotypes frequently induce higher mortality and more visceral lymphomas and have the tendency to more frequently break through genetic host resistance or immunity induced by vaccination (Witter, 1997; Schat and Nair, 2008). In some outbreaks, a high incidence of blindness and ocular lesions appeared to be related to only certain virus strains. However, in other trials, many contemporary MDV strains appeared to induce ocular lesions. The extent of disease induced by a given strain depends in part on the genetic constitution of the host (Witter *et al.,* 1980; Schat *et al.*, 1981; Ficken *et al.,* 1991; Spencer *et al.,* 1992; Witter, 1997; Schat and Nair, 2008).

### 2.9.2 Dose and route of exposure of Marek's disease virus

Dosage may influence disease frequency under natural conditions. Although, the MD response in genetically susceptible birds given virulent virus was found to be maximal even when a limiting dilution of virus was inoculated, route of exposure probably functions in the same manner because less efficient routes may effectively decrease the dose to the bird (Smith and Calnek, 1974; Schat and Nair, 2008).

### 2.9.3 Host gender response to Marek's disease

Biggs cited several studies in which it was observed that females birds died earlier and experienced higher losses than males. With the genetic strain, it was more pronounced with susceptible strains of chickens and was most apparent with viruses of higher virulence. In practice, the influence of gender appears variable and is probably less important than other factors (Biggs, 1973; Schat and Nair, 2008).

### 2.9.4 Maternal antibody

Chubb and Churchill (1969) reported that maternal antibody reduces and delays MD mortality and virtually all other manifestations of the disease, probably by limiting the spread of virus in tissues during the first few days post exposure (Payne and Rennie, 1973; Calnek,1992). Transient paralysis can be reproduced consistently only in chickens lacking maternal antibodies (Kenzy *et al*., 1973). The early mortality syndrome is reduced markedly in chickens with maternal antibodies (Witter *et al*., 1980). Breeder stocks are vaccinated uniformly and exposed to virulent MDV and therefore, virtually all chickens are hatched with maternal antibodies. Specific pathogen free (SPF) flocks are a source of antibody-free chicks for laboratory studies. Thus, unlike some other virus infections, passive antibodies do not provide a sterilizing immunity and antibody-positive chickens can be infected successfully and vaccinated, with reduced responses (Schat and Nair, 2008).

### 2.9.5 Host genetics and age at exposure to Marek's disease

Genetic factors and age at initial exposure are important determinants of MD susceptibility (Bacon *et al*., 2001a; Bumstead and Kaufman, 2004). Genetic resistance correlated with the development of virus neutralizing antibody and retention of cell-mediated immune functions (Calnek, 1972; Sharma and Stone, 1972; Schat and Calnek. 1978a; Lee *et al*., 1981). This resulted from a sparing of immune competence in the case of resistant birds, rather than from an inherent difference between strains (Smith and Calnek, 1973; Higgins and Calnek, 1975). Newly hatch chicks and older chickens are both susceptible to infection and cytolytic infection, but in older chickens, cytolytic infections are resolved more rapidly

and virus load is somewhat lower (Buscaglia *et al*., 1988; Davison *et al*., 1998; Calnek, 2001).

The frequency of lymphomas is variable and often markedly reduced in older chickens compared to newly hatched chicks, especially in genetically resistant lines (Calnek and Hitchner, 1973; Witter *et al*., 1973; Anderson *et al*., 1992). However, nonvaccinated, SPF, older chickens may develop high rates of lymphomas and transient paralysis following challenge with vv and vv+ strains (Rosenberger *et al*., 1997; Witter and Gimeno, 2006). Age-related resistance can be abrogated by neonatal thymectomy suggesting that other immunosuppressive stress factors may increase the susceptibility of older chickens to disease at least under some conditions. Lesion regression has been identified as the basis for age-related resistance (Sharma *et al*., 1973).

### 2.9.6 Prior to infection with Marek's disease

Before the use of vaccines, naturally occurring infections with serotype 2 MDV often provided some protection against later exposure to oncogenic strains (Jackson *et al*., 1976). Mild strains of serotype 1 MDV also can induce protective immune responses (Smith and Calnek, 1974). However, the influence of natural infections with avirulent serotype 1 MDV's on disease response in vaccinated chickens probably is minimal and at present, such infections are not likely to have much clinical importance (Schat and Nair, 2008).

### 2.9.7 Effects of environmental and stress factors in spread of Mareks disease

Various environmental factors and intercurrent infections appear to affect the incidence of MD, probably through interference with host immune responses. Gross (1972) observed increased incidence of MD among chickens selected for high concentrations of plasma corticosterone or subjected to a high degree of social stress. The administration of corticosteroids to latently infected chickens precipitated the appearance of clinical MD and the feeding of corticosteroid inhibitors tended to increase resistance to MD (Colmano and Gross, 1971; Powell and Davison, 1986)). Restricted feed intake delayed and reduced incidence of MD (Han and Smyth, 1972) whereas high-protein diets or the selection of chickens for fast growth rate were associated with increased susceptibility to the disease (Han and Smyth, 1972**).**

Effects of intercurrent infections with other avian pathogens and MD have been extensively studied (Biggs *et al*., 1968; Abbassi *et al*., 1999). Because MDV infection may depress host immune responses in its own right, concurrent infections are often exacerbated, examples include coccidiosis and cryptosporidiosis (Biggs *et al*., 1968; Abbassi *et al*., 1999). However, when the concurrent infection is itself immunosuppressive, the resulting immunosuppression usually will exacerbate both disease processes, as in the case of IBDV, REV and CIAV (Witter *et al*., 1979; Jeurissen and de Boer, 1993). Unfortunately, MDV stocks occasionally have become contaminated with other viruses, especially when propagated in chickens (Schat and Nair, 2008). Problems with CIAV contamination invariably interfere with the evaluation of MDV stocks for relative virulence (Miles *et al*., 2001).

## 2.10 Pathology Due to Marek's Disease

### 2.10.1 Gross pathology

Pathologic changes in MD consist mainly of nerve lesions and visceral lymphomas (Payne, 2004). Enlarged peripheral nerves are a frequent finding in affected birds. Macroscopic changes are not seen in the brain, but gross enlargements can be found in spinal ganglia. Lesion distributions appear to be similar for naturally occurring and experimental disease (Schat and Nair, 2008). Goodchild (1969) found that many nerves and plexi were commonly affected, but the celiac plexus was most commonly involved. Usually, plexi of the sciatic and brachial nerves are more enlarged than the respective trunks. Witter (1995) as cited by Schat and Nair (2008) has found the cervical vagus to be of particular diagnostic importance of MD.

#### *2.10.1.1 Nerves involvement due to Marek's disease*

Severely affected peripheral nerves may show loss of cross striations, gray or yellow discolouration and sometimes an oedematous appearance. Localized or diffuse enlargement causes the affected portion to be 2-3 times normal size, in some cases much more. However, minimal enlargements may be important indicators of disease in experimental infections. Because lesions are often slight or unilateral, it is helpful to examine opposite nerves and in experiments, to compare with age-matched normal controls to detect changes. Careful examination of the various nerve ramifications may be necessary to expose gross lesions in some birds, because enlargements can vary in both presence and degree from one portion of an affected nerve to another (Schat and Nair, 2008).

*2.10.1.2 Visceral organs involvement due to Marek's disease*

Lymphomas may occur in one or more of a variety of organs and tissues. Lymphomatous lesions can be found in the gonad (especially the ovary), lungs, heart, mesentery, kidneys, liver, spleen, bursa, thymus, adrenal glands, pancreas, proventriculus, intestine, iris, skeletal muscles and skin (Witter, 1997). Probably no tissue or organ is without occasional involvement. Both the genetic strain of chicken and the virus strain can influence the organ distribution of lesions. Visceral lymphomas are common in more virulent forms of the disease (Witter, 1997). Visceral tumours can occur in the absence of gross nerve lesions, especially in certain strains of chickens. Marek's disease lymphomas in most viscera appear as diffuse enlargements, sometimes to several times the normal size, and a diffuse white or grayish discoloration is often present. Alternatively, lymphomas may occur as focal nodular growths of varying sizes. Nodules are white or gray in colour and firm, and the cut surface is smooth. Necrosis is rare but may occur in the centre of rapidly growing lesions (Schat and Nair, 2008).

Diffuse infiltration of the liver causes loss of normal lobule architecture and often gives the surface a coarse granular appearance. Nodular tumours may also be seen in the liver. Lesions in the immature ovary are observed as small to large grayish translucent areas. With large tumours, the normal foliated appearance of the ovary is obliterated. Mature ovaries may retain function, even though some follicles are timorous (Schat and Nair, 2008). Marked involvement of MD is indicated by cauliflower-like. The proventriculus becomes thickened and firm as a result of focal leukotic areas within and between the glands, which may be seen through the serosal surface or if involvement is diffused and is detected by palpation (Schat and Nair, 2008). Affected areas are pale from diffuse

infiltration or have single or multiple nodular tumours in the myocardium, or pinpoint foci may be seen in the epicardium. Involvement of the lungs and proventriculus may be indicated by increased firmness of the organ upon palpation. Muscle lesions may be present in both superficial and deep layers and are most common in the pectoral muscles. Gross changes vary from tiny whitish streaks to nodular tumours in visceral organs (Schat and Nair, 2008).

*2.10.1.3 Integument involvement due to Marek's disease*

Skin lesions, probably the most important cause of condemnation in broiler chickens, usually are associated with feather follicles. The nodular lesions may involve few scattered follicles or they may be numerous and coalesce. The distinct whitish nodules especially evident in dressed carcasses may become scab-like with brownish crust formation in extreme cases. Lapen and Kenzy (1972) found that lesions in certain feather tracts are more frequent than in others; the highest incidences were in external and internal crural and dorsal cervical tracts. Erythematous involvement of the shank integument is seen, especially in virulent forms of the disease in broiler chickens and is commonly known as Alabama red leg. Swelling of the comb or wattles may indicate lymphoma growth in underlying tissues (Schat and Nair, 2008).

*2.10.1.4 Eyes involvement due to Marek's disease*

Gross ocular changes include loss of pigmentation in the iris (gray eye) and irregularity of the pupil, both results in mononuclear infiltration of the iris. Ficken *et al.* (1991) described cases in which conjunctivitis occasionally with multifocal haemorrhages and corneal

oedema were observed. Nearly all field isolates induced ocular lesions in nonvaccinated or HVT-vaccinated chickens with ocular occurance rates ranging from 5-100% (Witter, 1997; Schat and Nair, 2008).

*2.10.1.5 Other syndromes of Marek's disease*

Gross lesions are associated with at least some of the other syndromes that are associated with MDV infection. The lymphodegenerative syndromes, related to intense cytolytic infections of lymphoid organs, usually are characterized by severe atrophy of the bursa of Fabricius and thymus. The cytolytic infection is first evident 3-6 days after infections but in some cases persists and is more obvious at 8-14 days after infection (Witter *et al.*, 1980; Venugopal and Payne, 1996; Calnek *et al*., 1998). After inoculation with highly virulent field strains, some chickens may die at 20-50 days without gross lesions except severe bursal and thymic atrophy (Witter, 1997). Some chickens also develop a transient splenomegaly within 4-12 days after inoculation (Calnek, 1979).

The splenomegaly is a nonneoplastic response to viral replication because it is induced by both virulent and avirulent serotype 1 strains as well as serotype 2 and 3 strains (Schat and Nair, 2008). Vascular syndromes are manifested principally by occlusive atherosclerosis (Fabricant *et al*., 1978). Susceptible P-line chickens inoculated with the CU2 isolate of MDV developed grossly visible fatty atheromatous lesions in large coronary arteries, aortas, major aortic branches and other arteries. Lymphoid tumour transplants and local lesions are experimental syndromes characterized by nodular growths at the site of inoculation, although some transplantable tumours metastasized readily to the liver and

spleen, causing diffuse enlargements (Stephens *et al*., 1980). The gross appearance of transplants varies with the transplant, the host chicken and the route of inoculation (Schat and Nair, 2008).

### 2.10.2 Histopathologic changes due to Marek's disease

Histopathologic changes associated with MD lymphoproliferative lesions have been described by numerous workers who are in general agreement about the types of histologic lesions and the cells involved (Payne and Roszkowski, 1972; Payne *et al*., 1976).

#### *2.10.2.1 Nerves lesions in Marek's disease*

Two main types of lymphoproliferative lesions are recognized in periphera nerves. One type is considered neoplastic, consisting of masses of pleomorphic lymphocytes; in some cases, demyelination and Schwann cell proliferation are associated with this lesion. The second type is essentially inflammatory and is characterized by diffuse, light-to-moderate infiltration by small lymphocytes and plasma cells, usually with oedema, and sometimes, with demyelination and Schwann cell proliferation. A few macrophages may be found. Payne and Biggs (1967) referred to these nerves lesions as type A and B, respectively and noted that the 2 types may be observed on different nerves of the same bird or even in different areas of the same nerve. Lawn and Payne (1979) observed cellular infiltrations as early as 5 days PI, which gradually increased in intensity until 3 weeks when severe proliferative (type A) lesions were seen in the absence of paralysis or demyelination. Coincident with initial neurologic signs seen at 4 weeks PI, areas of widespread demyelination could be found within the proliferative lesions. Characteristic inflammatory

type B (oedema, sparse infiltrations) appeared. Payne *et al*. (1976) have reviewed the sequence of events in detail on characteristic changes in nerves due to Marek's disease.

*2.10.2.2 Brain lesions in Marek's disease*

Initial reports identified mild perivascular cuffing usually accompanied by gliosis but without primary demyelination as the principal CNS lesion in MD (Payne *et al*., 1976). Wight (1962) found that the CNS of affected birds was often histologically normal or with only minimal lesions. Following experimental inoculation with less virulent MDV strains, lesions could be seen as early as 7-10 days PI and were of moderate severity (Sevoian and Chamberlains, 1964; Payne and Rennie, 1973). However, lesions induced by vvMDV strains appeared earlier than 7 days and were more extensive (Gimeno *et al*., 2001).

The initial lesions also described under CNS Syndromes involve vascular elements; endotheliosis occurs at 6 days PI and is followed at 8-10 days PI by a moderate to severe infiltration of lymphocytes and marcrophages around blood vessels and scattered throughout the neuropil (Gimeno *et al*., 2001). The vasculitis and oedema disappear and may be followed by lymphoproliferative infiltrations of large lymphocytes and glial cells. These lesions tend to persist and are associated with persistent neurological disease. Severe lymphoblastic infiltration, often accompanied by extensive vacuolated areas that may correspond with secondary demyelination, has been shown to occur 4 weeks after inoculation with a highly virulent MDV (Gimeno *et al*., 2001). Infection with the C12/130 (a hypervirulent strain) and RK-1 (a vv+ strain) showed infiltration with monocytes in addition to T cells in the perivascular cuffins. These monocytes were expressing pp38 after

infection with C12/130 but not after infection with RK-1 (Barrow *et al.,* 2003; Njaa *et al*., 2004). However, in RK-1 infected birds many of the monocytes were in mitosis and apoptotic cells were often observed first in the white matter and later in the gray matter. Cho *et al*. (1998) reported severe necrotizing and nonnecrotizing lesions in the brains of chickens up to 10 weeks PI with vvMDV. Thus, as in nerves, brain lesions are both inflammatory and lymphoproliferative. In contrast with nerves, however, the inflammatory lesions are induced first (Gimeno *et al*., 1999). Severe lymphocytes infiltration is seen in the cerebellum **(**Gimeno *et al*., 1999**).**

*2.10.2.3 Marek's disease lesions in visceral organs*

Lymphomatous lesions in visceral organs are more uniformly proliferative in nature than those in nerves. Cellular composition is similar to that of the proliferative lesions described for nerves, consisting of diffusely proliferating small-to-medium lymphoblasts, and activated and primitive reticulum cells (Purchase and Biggs, 1967). Plasma cells are rarely present (Purchase and Biggs, 1967). Macrophages are also present in the tumour mass, especially in slow-growing tumours, perhaps reflecting the host immune response. The cellular composition of tumours is similar from one organ to another, even though the gross pattern of involvement may vary. Pradhan *et al.* (1988) found immune complexes in the kidneys, leading to glomerulopathy, in MDV-infected chickens, suspecting that these lesions might be one of the major causes of death in MD (Schat and Nair, 2008).

*2.10.2.4 Marek's disease lesions in the integument*

Lesions in the skin appear largely inflammatory but may also be lymphomatous. They usually are localized around infected feather follicles. In addition, compact aggregates of proliferating cells, often perivascular and a few plasma cells and histiocytes may be seen in

the dermis (Helmboldt *et al*., 1973; Payne and Biggs, 1967). With small lesions, the architectural integrity of the skin is maintained, but massive proliferative lesions may cause disruption of the epidermis, and resultant ulcer. Moriguchi *et al*. (1989) described both inflammatory and lymphoproliferative lesions in the feather pulp;with the latter closely related to the incidence of MD. Feather pulp lesions may be useful for antimortem diagnosis of MD. Lymphoproliferative nodules often surround feather follicles that contain MDV viral antigens and inclusions in the FFE (Calnek *et al*., 1970a; Cho *et al*., 1997).

*2.10.2.5 Marek's disease lesions in the eyes*

The most constant change in the eye is mononuclear infiltration of the iris, but infiltrates may also be found in eye muscles, especially in rectus lateralis and ciliaris (Jungherr and Hughees, 1965). Granular or amorphous material is sometimes present in the anterior chamber. Others but more rarely, observed lesions involve the cornea (near Schlemm's canal), bulbar conjunctiva, pectin and optic nerve. The unusually severe ocular lesions described by Ficken *et al.* (1991) include uveal changes with increased aqueous humour protein and vascular engorgement and mild hyperemia to severe swelling of the iris as well as severe inflammatory changes and oedema of the cornea, including intranuclear inclusion bodies. Sevoian and Chamberlain (1962) and Smith *et al*. (1974) reproduced ocular lesions experimentally. They latter reported that infiltration of proliferating lymphoreticular cells in optic and ciliary nerves and uvea were followed by similar infiltrations throughout the eye. Dukes and Pettit (1983) found cataracts present in seven of 18 spontaneous cases of ocular MD (Schat and Nair, 2008).

*2.10.2.6 Marek's disease lesions in the blood*

Blood leukocyte counts may be elevated largely because of increased number of large lymphocytes and lymphoblasts (Patterson *et al*., 1969). Payne *et al*. (1974) identified the

majority of leukemic cells as T cells. The leukemic response is not consistent and may be absent or only a mild leukocytosis may be present (Sevoian and Chamberlain, 1964; Jungherr and Hughes, 1965). Infection with the hypervirulent C12/130 strain caused significant increase in the absolute number of blood monocytes around 8 days PI. The B cells, $CD4^+$ and $CD8^+$ T cells decreased during the early cytolytic infection followed by an increase between 8 and 10 days PI, but these changes were also seen for T cells after infection with HPRS-16 (Barrow *et al*., 2003). Bone marrow changes in MD have variously been reported to include multiple tumour nodules, aplasia but the MDV strains was later found to be contaminated with CIAV or changes were not observed (Sevoian and Chamberlain, 1964; Powell and Rennie, 1984; Schat, 2003). Gilka and Spencer (1995) described an extravascular haemolytic anemia in MDV-inoculated chickens characterized by reduced packed cell volumes. The importance of this finding is not clear because haematocrit values are not routinely used as a MD parameter (Schat and Nair, 2008).

### 2.11 Differential Diagnosis of Marek's Disease

Lymphoid leukosis is a clonal, bursal lymphoma induced by ALV and under some conditions, by REV in chickens older than 16 weeks of age. Chickens usually have gross tumours in the bursa of Fabricius and tumour cells are uniform, blast-like, and pyroninophilic and express B-cell markers and IgM (Table 2.1). Also, the tumour cells have clonal insertions of proviral DNA near the c-myc gene. Nerve enlargements, runting, and nonbursal T-cell lymphomas can be induced by REV but, thus far, have only been observed under experimental conditions or where chickens have been inoculated with contaminated vaccines (Cooper *et al*., 1991). Lymphocytes obtained from REV-induced nerve lesions or tumours are not known to express pp*38* or m*eq*. Cells from nonbursal REV lymphomas are negative for MHC class II and predominantly stain for $CD8^+$ antigen

(Cooper *et al*., 1991). Exclusion of ALVs or REVs, where possible, through negative PCR, histochemical assays on tumours or antibody tests may provide strong support for a diagnosis of MD when other MD-related criteria are positive (Cooper *et al*., 1991).

Peripheral neuropathy is a neurological disease of uncertain aetiology that causes paralysis and nerve enlargement in a low proportion of commercial chickens 6-12 weeks of age and has been described in commercial and SPF chicken flocks (Biggs *et al*., 1982; Witter and Bacon, l995). Affected chickens lack visceral lymphomas; the nerve lesions are uniformly B-type and MDV is rarely, if ever, demonstrated. Other diseases that may present confusing gross lesions or paralytic signs are myelocytomatosis (myeloid leukosis), myelobastosis, erythroblastosis, carcinoma of the ovary, various other nonviral neoplasms, riboflavin deficiency, tuberculosis, histomoniasis, genetic gray eye, Newcastle disease, avian encephalomyelitis and joint infections or injuries (Schat and Nair, 2008).

Myeloid leukosis is a common tumour in broiler breeder flocks that superficially resembles MD but can be differentiated histologically. The tumor cells are myeloid in nature and lack T cell and MD viral markers (Schat and Nair, 2008). Multicentric histiocytosis is a disease of broiler chickens characterized by enlarged mottled spleens and lesions in other viscera composed mainly of histiocytes (Hafner *et al*., 1996). No lympbocytes were identified and MD was not demonstrated in tumours, although an association with subgroup J ALV has been suggested (Arshad *et al*., 1997).

**Table 2.1: Differential diagnosis of Marek's disease.**

| Feature | Marek's disease | Lymphoid leukosis | Reticuloendothelisosis |
|---|---|---|---|
| Age | Any age 3 weeks or older | Not under 14 weeks | Not under 16 weeks |
| Signs | Frequently paralysis | Non specific | Non specific |
| Incidence | Frequently above 5% in unvaccinated flocks. Rare in vaccinated flocks | Rarely above 5% | Rare |
| Macroscopic lesions | | | |
| Neural involvement | Frequent | Absent | Infrequent |
| Bursa of Fabricius | Diffuse, enlargement atrophy | Nodular tumours | Nodular tumours |
| Tumours on skin, muscles, proventriculus grey eye | May be present | Usually absent | Absent |
| Microscopic lesions: | | | |
| Neural involvement | Yes | No | Infrequent |
| Liver tumours | Often perivascular | Focal or diffuse | Focal |
| Spleen | Diffuse | Often focal | Focal or diffuse |
| Bursa of Fabricius | Interfolliculartumour/ arophy of follicles | Intrafollicular tumour | Intrafollicular tumour |
| Central nervous system | Yes | No | No |
| Lymphoid proliferation in skin and feather follicles | Yes | No | No |
| Cytology of tumours | Pleomorphic lymphoid cells, small, medium and large lymphocytes and reticulum cells. Rarely can be only lymphoblasts. | Lymphoblasts | Lymphoblasts |
| Category of neoplatic lymphoid cell involved | T-cell lymphocyte | B-cell lymphocyte | B-cell lymphocyte |

Source: OIE, 2010

## 2.12 Diagnosis of Marek's Disease

Despite long-established guidelines for the pathologic diagnosis of MD, diagnosis of the clinical disease remains difficult in practice for several reasons. First, no truly pathognomonic gross lesion exists for MD (Siccardi and Burmester, 1970). Gross lesions of MD can resemble those of other neoplasms and unrelated (Table 2.1 and Figure 2) conditions characterized by tumours in visceral organs or grossly enlarged nerves. Finally, MDVs, ALVs and REVs are widespread in commercial poultry, often resulting in simultaneous infections, complicating diagnostic efforts that depend on virological methods (Davidson and Borenstein, 1999). The absence of a generally accepted confirmatory test makes even properly performed pathologic diagnoses subject to controversy. No currently accepted diagnostic standard exists for MD. However, the following model was suggested (Schat and Nair, 2008). Diagnosis of MD must primarily be addressed by consideration of the characteristics of the proliferating cell populations that constitute the disease. Other disease-specific criteria, such as epidemiological factors, are also valuable. Virological criteria are less valuable but can help to establish the presence of the causative agent or more importantly, the absence of certain other agents. The process commences with the acquisition of a flock history and a sufficient number (5-10) of representative sick and dead chickens showing the lesions of the disease and proceeds as a series of steps such as clinical data and gross pathology, histology, cytology and histochemistry of tumour cells and virology due to MD etc are required (Schat and Nair, 2008).

Techniques for diagnosis of infection with MDV are different (Table 2.1) from those needed for differential diagnosis of the disease. The MDV is ubiquitous, but the MD is not.

The principal methods to identify the presence of infection are isolation of the virus, demonstration of viral DNA or antigens in tissues and detection of antibody. The applications of different diagnostic procedures have been reviewed (OIE. 2004; Payne, 2004; Zelnik, 2004; Chauhan and Roy, 2007; Nair *et al*., 2008).

### 2.12.1 Marek's disease virus isolation from chicken

Marek's disease virus can be isolated as early as 1 or 2 days PI or 5 days after contact exposure (Adldinger and Calnek, 1973) and throughout the life of the chicken. Intact viable cells are the preferred inoculum because, in most cases, infectivity is avidly cell-associated although cell-free preparations from skin, dander or feather tips of infected chickens may contain the virus (Calnek *et al*., 1970a). Inocula may consist of blood lymphocytes, heparinized whole blood, splenocytes or tumour cells. The MDV can often be recovered from infected cell suspensions following storage for 24 h at 4°C, thus facilitating transport of samples (Witter and Lee, 1984; Witter and Schat, 2003).

### 2.12.2 Cell culture techniques for the isolation of Marek's disease virus

Probably, the most widely used method for primary isolation of MDV is inoculation of susceptible tissue cultures with blood lymphocytes or single-cell suspensions from lymphoid tissues of infected chickens. Chicken kidneys cell and DEF cultures are preferred substrates for primary isolation of serotype 1 MDV; whereas CEF normally are used for isolation of viruses of serotypes 2 and 3 as well as for attenuated serotype 1 vaccine strains. Although CEF are less as permissive for growth of low passage serotype 1 virus, some contemporary isolates appear to grow well in CEF, even on primary isolation (Churchill,

1968; Witter, 1995; Schat, 2005). Cultures are inoculated with 1-2 x $10^6$ cells, although some inhibition of viral plaque formation may be encountered with doses greater than 8 x $10^6$ cells for some viruses (Calnek *et al*., 1982). After 24-48 h, the inoculum is washed off and the culture is maintained under liquid or agar medium, usually without subculture Calnek *et al*., 1982; Wakenell and Sharma, 2008).

Development of typical plaques in inoculated cultures within 3-12 days and the absence of such changes in comparable uninoculated (or sham inoculated) control cultures are evidence for isolation of MDV. The plaques induced by serotype 1, 2 and 3 viruses can be distinguished, with practice, by morphologic criteria, but immunofluorescent staining with serotype-specific monoclonal antibodies provides a more accurate differentiation (Witter, 1983; Schat, 1985). Optimal time for observation of plaques varies with the cell substrate and serotype of the virus. Marek's disease virus also has been isolated by direct culture of kidney cells from infected chickens or by inoculation of normal kidney cultures with trypsinized kidney cells from infected chickens (Witter *et al*., 1969).

### 2.12.3 Identification of Marek's disease virus isolate

Marek's disease virus serotype 1 isolates should be free of contaminating MD vaccine strains. It normally is useful for the isolate to be plaque purified or cloned at the early possible passage. Serotype identity and purity can be confirmed using staining techniques with serotype-specific monoclonal antibodies (Lee *et al*., 1983). Pathotyping of serotype 1 MDVs, although not routine, may be accomplished by comparison of pathogenicity with that of prototype strains by inoculation of nonvaccinated chickens as well as chickens vaccinated with HVT or bivalent vaccines (Witter, 1997; Witter *et al.*, 2005). Freedom

from extraneous viruses is also critical, because contamination with passage viruses may alter the apparent pathogenicity of the isolate (von Bulow *et al*., 1983; Jeurissen and de Boer, 1993; Miles *et al*., 2001). Propagation of MDV isolates for up to 6 passages in CEFs or CKC cultures appears to exclude contaminants such as CIAV and permits preparation of seed and working stocks, which can be more easily standardized and titrated (Yuasa, 1983). Although the possibility of attenuation during cell culture propagation must be considered, effects on pathogenicity are difficult to detect until after 20 cell-culture passages. To preserve virulence, some workers have preferred to propagate serotype 1 viruses in vivo, preparing stocks of cryopreserved spleen or buffy coat cells from infected chickens (Schat and Nair, 2008).

### 2.12.4 Marek's disease virus assay and titration

Viruses of serotypes 1, 2 and 3 can be assayed by *in vitro* techniques similar to those described for virus isolation. Methods differ for different serotypes, but most rely on plaque induction in susceptible cell cultures. Enumeration is done as soon as plaques become mature (time varies with isolate), because secondary plaques may occur when cultures are maintained with liquid medium. Agar overlay methods have been described but are not commonly used, probably because plaque formation is often delayed under agar and because secondary plaque formation under liquid medium has not been considered a significant problem (Spencer and Calnek, 1970). Procedures for titration of vaccine viruses have been reviewed and are not fundamentally different from those for pathogenic isolations (Spencer and Calnek, 1970; Thornton, 1985; Wakenell and Sharma, 2008).

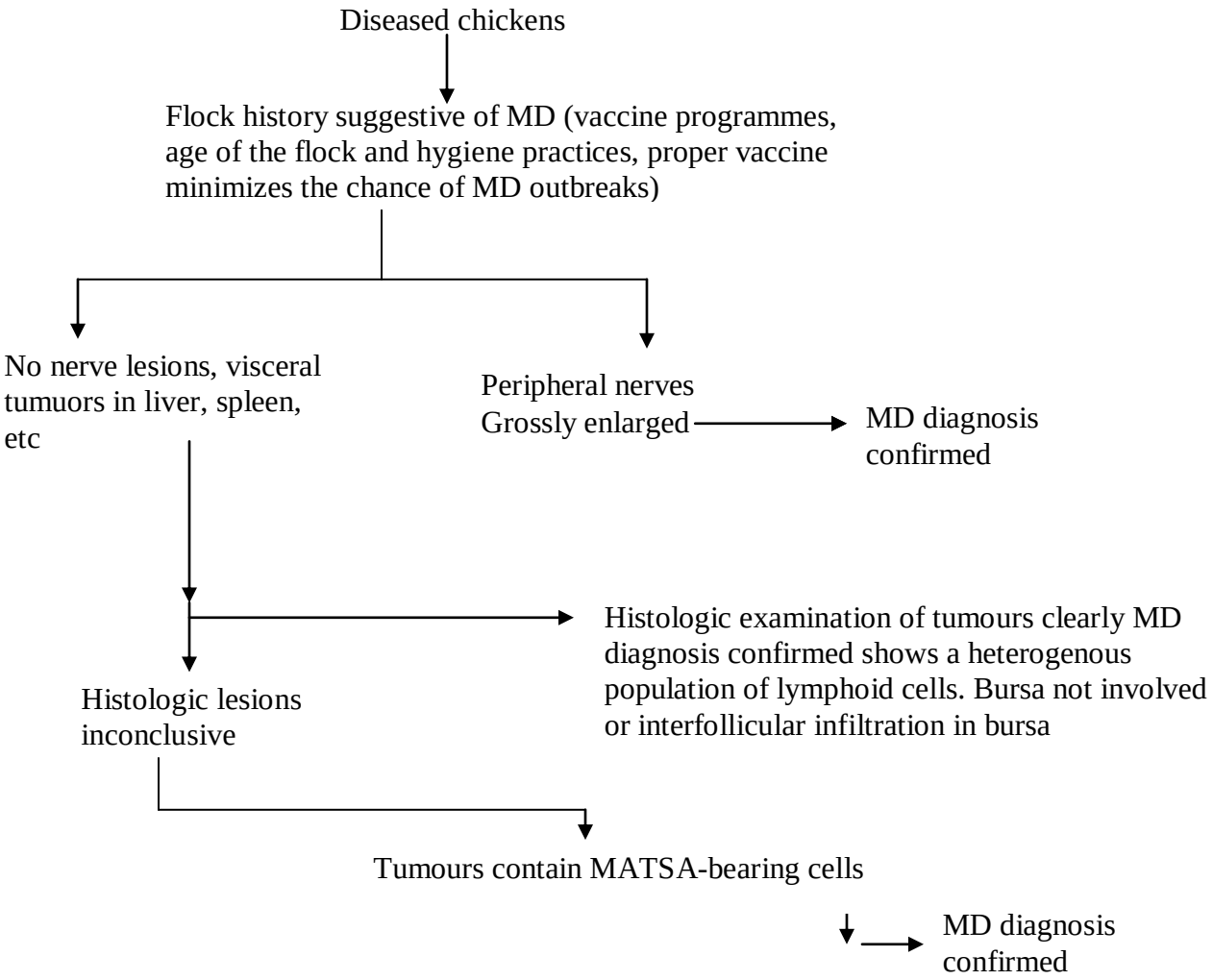



**Figure 2: Scheme of field diagnosis of Marek's disease.**

If suspected chickens lack characteristics gross or histologic lesions, the diagnosis can be confirmed by demonstrating the presence of MD tumour associated surface antigen (MATSA) on tumour cells which is not found on any tumour cells (Source: Payne, 1985)

### 2.12.5 Marek's disease viral markers in tissues

It is often desirable to detect the presence of viral infection in chickens without isolating the virus in culture. Such infection markers also have value for the identification of putative MDV isolates in cell cultures (Wakenell and Sharma, 2008; Schat and Nair, 2008).

### 2.12.6 Marek's disease viral antigen detection

Marek's antibodies (MAb) prepared against type-common and type-specific epitopes of all 3 MDV serotypes (Lee *et al*., 1983) are now used in preference to polyclonal antibodies can be detected in feather tips and FFE, cytolytically infected lymphoid tissues, brain or infected cell cultures with appropriate antibodies by fluorescent antibody tests, immunohistochemistry, agar gel precipitin tests and enzyme-linked immunosorbent assays (Spencer and Calnek,1970; Haider and Lapen-Kenzy, 1970; Cauchy, 1974; Lesnik *et al*., 1978; Davidson *et al*., 1988; Scholten, 1990). Antigen-positive cells are relativeely rare in lymphomas and latently infected tissues, although pp 38 and Meq have been identified frequently in the brain). In MD lymphomas, positive cells occasionally are observed and Meq expression may be relatively common (Naito *et al.*, 1986; Ross *et al*., 1997; Gimeno *et al.*, 2001).

### 2.12.7 Polymerase chain reaction for Marek's disease virus

The availability of the nucleotide sequences of different genes from a large number of viruses including the complete genome sequence of the 3 serotypes of MDV allow the use of PCR-based methods for specific detection of MDV. Primers designed to amplify 132 bp sequences specific to MDVs of serotype 1 have been described (Becker *et al*., 1992; Silva, 1992; Zhu *et al*., 1992). Polymerase chain reaction assays using these primers can discriminate between attenuated and wild-type strains and may detect viral DNA in

lymphomas (Davidson *et al*., 1995; Silva, 1997). A wide variety of other primer sequences have also been used. However, PCR may not always be sensitive enough to detect latent infection due to the lower frequency of positive cells and the lower number of viral genomes per cell. qPCR assays using various primer sequences recently have been used to assay viral load in tissues from infected chickens and are becoming essential tools for diagnosis and epidemiological studies of MD (Bumstead *et al*., 1997; Burgess and Davison, 1999; Reddy *et al*., 2000; Islam *et al.*, 2004; Baigent *et al*., 2006).

The qPCR assays have been adapted for the detection of related MD vaccine strains and PCR assays have been used to differentially detect MDV and HVT in both blood lymphocytes and feather tips of field chickens. Because clinical samples may contain inhibitors that can reduce DNA polymerase activity and produce false-negative results, an internal control is useful to establish the absence of such inhibitors. More recently, qPCR assays were carried out to study different aspects of MD biology including replication kinetics of MDV in feathers and lymphoid tissues, correlation between protection and vaccine load, the association between replication rates of viral genomes and virulence and the impact of specific gene deletions on replication. The qPCR tests for the absolute quantitation of serotype 2 and serotype 3 MDV have also been developed (Bumstead *et al*., 1997; Handberg *et al*., 2001; Yunis *et al*., 2004; Baigent *et al* ., 2006; Brown *et al* ., 2006; Islam *et al* ., 2006; Renz *et al* ., 2006).

*2.12.7.1 Marek's disease virus deoxyribonucleic acid probes*

Methods using DNA-DNA dot-blot hybridization with DNA probess for the detection of MDV DNA in feather tip extracts have been described (Davidson *et al*., 1998). Furthermore, localization of virus-infected cells has been accomplished by *in situ* hybridization for both MDV and HVT (Ross *et al*., 1981; Endoh *et al*., 1996; Holland *et al*., 1996).

*2.12.7.2 Electron Microscopy*

Herpesvirus particles can be detected by electron microscopy in the FFE and in productively infected cells *in vitro* (Calnek *et al*., 1970a).

*2.12.7.3 Marek's disease antibody detection*

Tests for identifying the presence of specific antibodies in chicken sera are useful in studies of viral pathogenesis and for monitoring SPF flocks. A number of procedures including agar gel precipitin, fluorescent antibody, enzyme-linked immunosorbent assays and virus-neutralization tests are in common use (Sharma, 1998; Zelnik *et al*., 2004). Commercial reagents are available only for the agar gel precipitin test, which is the least sensitive test when compared with fluorescent antibody and ELISA but is adequate to detect serologic responses in infected or vaccinated chicken flocks. None of these tests, agar gel precipitin, fluorescent antibody, enzyme-linked immunosorbent assays and virus-neutralization however, can differentiate antibodies of the 3 serotypes. The biologic significance of antibodies detected by different methods may vary (Calnek, 1972).

*2.12.7.4 Clinical signs and gross pathology*

Although enlarged peripheral nerves and visceral lymphomas are common in MD and one or both are invariably present, neither lesion occurs consistently nor pathognomonic. Thus, other criteria, such as age and lesion distribution, must be considered in the postmortem diagnosis of MD. Chickens may be diagnosed provisionally as MD if at least one of the following conditions is met:

1. leukotic enlargement of peripheral nerves
2. lymphoid tumours in various tissues (liver, heart, gonads, skin muscles and proventriculus) in birds under 16 weeks of age or
3. visceral lymphoid tumours in birds 16 weeks or older that lack neoplastic of the bursa of Fabricius or
4. iris discolouration and pupil irregularity.

Proper examination of the bursa is particularly important and requires incision of the organ with close inspection of the epithelial surface. However, diagnoses based on gross pathologic criteria are not definitive and additional steps such as clinical data and gross pathology, histology, cytology and histochemistry of tumour cells and virology due to MD etc are required (Chauhan and Roy, 2007; Schat and Nair, 2008).

*2.12.7.5 Histology, cytology and histochemistry of tumour cells*

Tissues fixed in formalin or fresh-frozen are used to prepare paraffin cryostat sections. Impression smears of tumours may also be used. Essential diagnostic features may be seen in routine histologic sections stained with hematoxylin and eosin or touch preparations stained with methyl green pyronin or Shorr's stain (Siccardi and Burmester, 1970). Cellular

and viral antigens can be demonstrated in frozen or in some cases, paraffin sections by immunohistochemistry. A mixed population of small to larger lymphocytes, lymphoblasts, plasma cells and macrophages are typically found in MD tumours and nerve lesions (Payne and Biggs, 1967). The proportion of cell types varies with the stage of the disease and virulence of virus and the most aggressive lymphomas may contain high numbers of lymphoblasts. Marek's disease tumours are a mixed population and most cells express MHC class 11 antigen and T-cell markers, especially $CD4^+$ (although $CD8^+$ cells may also be present) (Schat *et al*., 1991). IgM is present on less than 5% of cells. $CD30^+$ is also commonly found in MD tumour cells (Ross *et al*., 1997). Marek's tumour surface antigen (MATSA) (Witter *et al*., 1975) can be demonstrated with polyclonal or monoclonal antibodies on 5-40% of Marek's disease tumour cells but is also present on activated T cells (Lee *et al*., 1983; McColl *et al*., 1987). Tumour lesions tentatively identified as MD in predominantly express $CD4^+$/$CD8^+$ markers and where expression of IgM or B-cell makers is limited or absent, may be diangnosed provisionally as MD. Demonstration of $CD30^+$ or MATSA may potentially be useful, but more data are needed (Schat and Nair, 2008).

*2.12.7.6 Virology of Marek's disease virus*

For tumours that satisfy MD criteria listed in steps 2.12.7.4 and 2.12.7.5 or for typical tumours, the association of MDV with the tumour cell is a useful confirmatory procedure. Viral antigens such as pp38 or meq may be detected in tumour cells by immunohistochemisty or fluorescent antibody tests but because neither antigen is expressed in all cells or in all tumours, negative results are less helpful than positive results (Naito *et al*., 1986; Ross *et al*., 1997). Also, very small number of positive cells may reflect the

presence of inflammatory T cells incidentally infected with MDV, which are unassociated with the primary tumour. In situ hybridization is another technique to localize MDV genome in tumour cells (Ross *et al*., 1997). Polymerase chain reaction assays may also detect MDV DNA in tumours, especially in tissues with high concentrations of tumour cells (e.g tumour nodules). Polymerase chain reaction assays on other tissues and virus isolation from buffy coat or spleen cells demonstrate the virus in the bird but do little to associate the virus with the tumour cells. Similar information is provided by antibody tests. There may be a quantitative association between virus load and MD tumours. Low levels of virus or viral DNA may be detected in lymphocytes from non tumour-bearing chickens, but most tumour- bearing chickens have high viremic titers and are usually PCR positive (Witter *et al*., 1971; Davidson *et al*., 1995; Silva and Witter, 1996).

Therefore, the possibility that infection criteria in excess of some arbitrary threshold levels, as yet undetermined, would better correlate with lymphomatous lesions could be explored. Thus, the demonstration of high quantities of virus or viral antigen in tumour cells at the exclusion of other relevant tumour viruses should be sufficient, along with other criteria such as clinical data and gross pathology, histology, cytology and histochemistry of tumour cells and virology due to MD etc are required to establish a diagnosis of MD (Schat and Nair, 2008).

#### *2.12.7.7 Pathotyping of Marek's disease virus strains*

The concept of MDV pathotyping has arisen from the recognition of the existence of strains that are associated with increased virulence, which show correlation with breaking

of vaccinal immunity in the field (Witter *et al*., 2005). Methods to differentiate between classical strains of MDV and the more virulent pathotypes have been examined. However, the ADOL (Avian Disease and Oncology Laboratory) method of pathotyping, based on the induction of lymphoproliferative lesions in chickens vaccinated with different vaccination regimes, has been the most widely used. This method was used to characterize more than 45 isolates into distinct v MD, vvMDV, or vv+MDV pathotype group (Witter, 1997). Even though ADOL method stipulates the use of line 15x7 chickens for pathotyping, experiments with other lines of birds also have given similar results (Buscaglia *et al*., 2004; Witter *et al*., 2005).

### 2.13 Control of Marek's Disease

The development of successful vaccines for control of MD was a significant achievement (Churchill *et al*., 1969b; Okazaki *et al*., 1970; Rispens *et al*., 1972). Vaccination represents, for now and the foreseeable future, the central strategy for the prevention and control of MD. Genetic resistance and biosecurity, however, are critical adjuncts to vaccination. No effective practical treatment exists for the disease in individual chickens or infected flocks (Schat and Nair, 2008). An integrated strategy to prevent early infections, to slow the acquisition of virulence of field strains and to provide superior immune responses seems most likely to succeed (Witter, 1997).

### 2.13.1 Vaccination against Marek's disease

*2.13.1.1 Types of Marek's disease vaccine*

Several different types of MD vaccines are in common use, both individually and in various combinations (Schat and Nair, 2008). The most widely used products are low pathogenic serotype 1 MDV, naturally avirulent HVT and serotype 2 viruses (Okazaki *et al*., 1970; Rispens *et al*., 1972; Schat and Calnek, 1978; Witter *et al*., 1987). The serotype 2 strains usually are combined with HVT to take advantage of the synergistic activity documented between serotypes 2 and 3 (Schat *et al*., 1982; Witter, 1982).

All vaccines types are protective but to varying degree (Witter, 1970). Turkey herpes virus (HVT), mainly strain FC126 continues to be extensively used because it is effective and economical to produce and combine well with other products (Witter, 1970). Although both cell-free and cell-associated forms of HVT are available, the latter has been most widely used because it is more effective than cell-free virus in the presence of maternal antibodies (Witter and Burmester, 1977). Bivalent vaccines based on combinations of HVT and either SB-1 (Schat and Calnek, 1978) or 301B/1 (Witter, 1987) strains of serotype 2 MDV were introduced in the mid 1980s, The CVI988 strain used in The Netherlands and other countries since the early 1970s, was introduced to the United States in the early 1990s (Rispens *et al*., 1972). Another attenuated serotype 1 strain, R2/23 was also introduced in the 1990s (Witter, 1991). Serotype 1 and 2 vaccines are available only as cell-associated products (Chauhan and Roy, 2007; Schat and Nair, 2008).

Research continues to be directed toward the development of improved vaccines through recombinant DNA approaches, but with limited practical success (Ross, 1998). Recombinant fowl poxvirus, HVT and MDV vaccines expressing various MDV genes have shown some protective efficacy (Ross *et al*., 1993; Nazerian *et al*., 1996; Hirai and Sakaguchi, 2001; Lee *et al*., 2005). The rFPV vaccines were effective to some degree in chickens positive for maternal antibodies to MDV, but were not tested in chickens with maternal antibodies to FPV (Lee *et al*., 2003).

Incorporation of cytokines in vaccines in recombinant vaccines may improve vaccine efficacy as suggested by the inclusion of the gene for avian myelomonocytic growth factor in FPV (Djeraba *et al*., 2002). However, the lack of protection with rFPV vaccines expressing avian influenza genes against avian influenza in birds with antibodies to FPV (or with mutations in the biological critical thresh point (CtBP-interaction**)** domain of the *meq* (were protective against challenge with vv or vv+ MDV (Swayne *et al*., 2000; Brown *et al*., 2006). In a limited experiment, DNA vaccination using pBAC20, derived from attenuated MDV, provided limited protection against challenge (Tischer *et al*., 2002).

Efforts to derive more efficacious serotype 1 vaccines through classical virological approaches (Witter, 2001) or retroviral insertion (Witter, 1997) yielded several promising candidates. However, these strains did not protect better than the best commercial CV1988 vaccines (Witter and Kreager, 2004) and also questioned if the efficacy of MD vaccines is limited by some types of biological threshold. The emergence of increasingly virulent viral strains, coupled with an apparent reduction of vaccine efficacy during the past 20 years

(1993), has prompted justifiable concern. This suggests that vaccination by itself does not provide a complete control programme and is not the ultimate solution for MD. Strict biosecurity procedures to reduce early exposure and the presence of genetic resistance are essential adjuncts to a successful vaccination programme (Witter and Kreager, 2004; Schat and Nair, 2008).

*2.13.1.2 Marek's disease vaccine administration*

Marek's disease vaccines are administered to chicks at or before hatch because early immunity is essential. Both cell-associated and cell-free vaccines are given by subcutaneous or intramuscular inoculation at a dose usually in excess of 2,000 plaque-forming units per chick. Vaccines are also effective when administered to embryos at embryonic day (ED) 18th (Sharma and Burmester, 1982). *In ovo* vaccination is now performed by automated technology and is widely used for vaccination of commercial broiler chickens, mainly because of reduced labour costs and greater precision of vaccine administration (Johnston *et al*., 1997). Deposition of the vaccine by the amniotic or intraembryonic route is essential for optimal protection (Wakenell *et al*., 2002). Proper handling of vaccine during thawing and reconstitution is crucial to ensure that adequate doses are administered (Halvorson and Mitchel, 1979; Jackson, 1999).

*2.13.1.3 Marek's disease vaccination strategies*

Efficacy data available that compare certain group of MD vaccines are either bivalent or polyvalent (Witter, 1985; Schat, 1997; Witter, 2001). Bivalent serotype 2+3 vaccines and R2/23 (attenuated Serotype 1 MDV strain) are clearly more effective than HVT (Witter,

1992). The most effective vaccine, however, appears to be the original CV1988 vaccine, a result consistent with earlier reports from Europe (Witter, 1992; Velitz and Landgraf, 1986). Efficacy ranking of vaccines, however, have not always been reproducible when performed in different laboratories and should be interpreted with caution. Marek's disease vaccines as a class are unusually effective, often achieving greater than 90% protection under commercial conditions (Witter, 2001).

However, attention is often focused on flocks in which MD losses are perceived to be excessive (Zanella, 1982; Kreager, 1997; Jackson, 1998). Causes for such vaccine failures are difficult to ascertain by retrospective analysis (although early exposure and the emergence of new MDV strains with increased virulence may be important (Witter, 2001). The propensity of MDVs to evolve to greater virulence is critical to the strategic use of vaccines for MD control (Witter, 1997). Vaccination itself no doubt contributes to this virulence increase, which, in turn, tends to make earlier vaccines obsolete (Witter, 1997). Kreager (1998) has noted that the useful life of an MD vaccine has been about 10 years under current management conditions. Although this is perhaps an overstatement, the implication is serious. Since CV1988 was introduced in the United States, some evidence already suggests that contemporary strains have increased their virulence in CV1988-vaccinated chickens (Witter, 2001).

The choice of a vaccination programme is an important consideration for MD control. A rational approach is to use the least effective product required to control the disease on a particular farm at a particular time (Witter, 1997). In practice, HVT alone provides

adequate protection to many broiler flocks under normal conditions (Witter, 1997). Broiler flocks, especially during winter months and layer/breeder flocks often require bivalent (serotype 2+3) vaccines rather than HVT alone (Witter, 1997). Where these vaccines are insufficient, the CV1988 strain is used. Mixtures of CVI988 with HVT or both serotype 2+3 vaccines are also used, although little evidence exists for synergistic activity of CV1988 with other vaccine serotypes (Witter *et al*., 1995).

*2.13.1.4 Factors affecting Marek's disease vaccines efficacy*

Vaccines typically are given at doses of 2,000-6,000 plaque forming units per chick, but these may be significantly reduced in broilers. Higher doses (Eidson *et al*., 1978; Witter, 1983) or revaccination (Ball and Lyman, 1977) offered little improvement. Revaccination at 7-12 days continues to be popular in Europe and occasionally is used in the United States, but the effectiveness of this procedure has not been validated in laboratory studies (Witter, 1983). Maternal antibodies reduce the effectiveness of cell-associated vaccines but do not abrogate the protective effect (Calnek and Smith, 1972). Vaccination of breeders with serotype 1 or 2 virus leaves progeny more responsive to vaccination with HVT (King *et al.*, 1981).

The shorter the interval is between vaccination and exposure to the virulent field virus, the poorer the level of protection (Okazaki *et al.*, 1970). Early exposure is undoubtedly one of the most important causes of excessive MD in vaccinated flocks because field exposure usually occurs very soon after placement of chickens (Witter *et al*., 1970) and because at least 7 days is required to establish solid immunity after vaccination (Basarab and Hall,

1976). The vaccine strain of virus also has a major influence on vaccine efficacy. Immunity induced by weaker vaccines such as HVT may be excellent against low virulence challenge but can be completely overwhelmed by early challenges with highly virulent strains (Witter, 1997). Although high virulence strains are commonly invoked to explain field outbreaks of disease, many alternate causes should be considered. The strain of chicken is also an important determinant of vaccine efficacy. Schat *et al.* (1982) found that HVT vaccine in genetically resistant chickens resulted in a stronger immunity than did the bivalent (HVT + SB-1) vaccine in susceptible chickens.

Stress appears to interfer with the maintenance of vaccinal immunity. In chickens properly vaccinated at hatch and well protected following challenge, Powell and Davidson (1986) induced MD lesions and mortality by immunosuppressive treatment at 10 weeks of age. The possibility that immunosuppressive stress may play an important role in MD outbreaks in vaccinated flocks, especially those that occur after the onset of egg production, deserves consideration (Witter, 2001). Infectious bursa of Fabricius disease virus (IBDV) and chicken infectious anaemia virus (CIAV) have been reported to interfere with the induction of vaccinal immunity, although very specific conditions sometimes required REV and Reoviruses (Witter *et al.,* 1979; Rosenberger, 1983; Sharma, 1984; Otaki *et al.*, 1988a; Otaki *et al.*, 1988b; Yuasa and Imai, 1988).

*2.13.1.5 Use of genetic resistant birds in Marek's disease control*

The well-known variation in susceptibility of different lines of chickens to MD challenge is determined by genetic factors and provides a unique opportunity to consider genetic

approaches to the control of MD (Cole, 1985). The importance of genetic resistance in the control of MD was reported several decades ago and was the first method used to control the disease (Cole, 1968). There are two groups of genes in the chicken that influence MD resistance: major histocompatibility complex (MHC) genes and non-MHC genes. The relevance of MHC genes in MD resistance has been amply demonstrated and is well characterized (Bacon *et al.,* 2001a). Particularly remarkable is the influence that the MHC haplotype has on the efficacy of the response to MD vaccines and how this influence depends on the serotype of the vaccine used (Bacon and Witter, 1992; 1994a; 1994b).

Theoretically, the most appropriate vaccine could be selected based on the predominant MHC haplotype of a particular poultry strain. The non-MHC genes involved in MD resistance have not been well studied. Indeed, poultry breeders have included resistance to MD in selection programmes for many years. However, genetic resistance can be overcome by challenge with highly virulent MDVs and is best applied in concert with vaccination and biosecurity to achieve optimal control (Bacon *et al*., 2001a; Bumstead and Kaufman, 2004).

#### *2.13.1.6 Biosecurity in farms against Marek's disease*

There are two critical points that any biosecurity plan should include; reducing the initial levels of MDV on a farm by preventing the entry of MDV into a building and avoiding contamination of the environment by preventing MDV from leaving the infected building (Schat and Nair, 2008). Measures to reduce initial levels of MDV by practicing all-in-all-

out systems, single-age buildings, etc. have been widely stressed but are not always followed. Measures to avoid contamination of the environment are not always well recognized and there are two issues that deserve more attention; air management and the disposal of chicken carcasses and manure. Biofilters, usually designed to remove odours of air emanating from livestock facilities, could be used to decrease the level of MDV contamination in the effluent air from poultry farms. Carcasses are usually burned or buried, but disposal of manure does not normally receive adequate attention. Composting has been shown to be a satisfactory alternative procedure to discarding both carcasses and manure (Spencer, 2001; Chauhan and Roy, 2007; Schat and Nair, 2008).

## CHAPTER THREE

## RETROSPECTIVE STUDIES OF MAREK'S DISEASE DIAGNOSED IN VETERINARY CLINICS IN SOME PARTS OF NIGERIA FROM 2000-2009

### 3.1 Abstract

A retrospective study of MD outbreak diagnosed from 2000 to 2009 by Veterinary Clinics and Hospitals at Zaria, Kaduna State (north west), Ibadan, Oyo State (south west), Nsukka, Enugu State (south east) and Bukuru, Plateau State (north central) Nigeria was conducted. Out of the 1,970 viral diseases recorded, the over all prevalence for Marek's disease was 60.6% in the studied States. The prevalence of MD was significantly higher ($p < 0.05$) among flocks aged 17 weeks and above (85.5%) compared to those aged 9 – 18 weeks (71.4%), 5 - 8 weeks (25.3%) and 1-4 weeks (14.7%)**.** On age of birds**,** a total of 319 outbreaks in broilers with mean age of 7.8 weeks (95% CI: 6.7 – 8.9), 77 cockerels at 15.0 weeks, 952 pullets at 12.9 weeks and 602 layers at 29.9 weeks. Layers had an MD prevalence of 86.7%, followed by pullets with 62.1%, turkey had 40.0% and broilers had 17.2%. An MD prevalence of 63.6% was recorded during the rainy season while 55.4% was recorded during dry season. It was observed that the risk factors for MD using odds ratio analysis to test for association on age, type, region of the country where farm is located and season, showed statistically significant associations. Flocks aged 18 weeks and above were 34.3 times more at risk of having MD compared to 1–5 weeks. High risk of MD were also observed in flock aged 9–18 weeks (OR 14.5; 95%) and 5–8 weeks (OR 2.0; 95% CI: 1.3–3.0) compared to 1–5 weeks. On the type and species of birds compared to broilers, layers had higher chances of having MD (OR 31.3; 95%) followed by pullets (OR 7.9; 95%) and turkeys (OR 3.2; 95%). On locations, the chances of having MD were significantly higher in the south (OR 7.8; 95%) compared to the north and higher during the rainy season (OR 1.4; 95%) compared to dry. The outbreaks of MD decreased in 2006

(OR 0.7; 95) and 2007 (OR 0.7; 95%) compared to 2000 but increased in 2008 (OR 1.5; 95%) and 2009 (OR 2.0; 95%). The prevalence of MD was high and increased over the years and it has become endemic in Nigeria. Due to frequent outbreaks of MD, the importance of diagnostic surveillance of the disease and good management practices by farmers should be encouraged.

## 3.2 Introduction

Marek's disease, a lymphoproliferative neoplasm of domestic fowl is caused by a herpes virus and was a major disease problem and source of economic losses in poultry until the early 1970's with the discovery of the turkey herpes virus lyophilized vaccine for MD. This was followed with progress in the control of the disease (Okazaki *et al*., 1970; Purchase and Schultz, 1985). In Nigeria, routine MD vaccination has not been fully adopted especially in local chickens and some commercial breeds probably due to economic problem or difficulty in obtaining the imported vaccines (Jwander, 2005). Adene (1983) reported that the poultry industry appeared to have benefitted from the spill-over effects of the vaccination programmes and practices in the poultry exporting countries. The pathological similarity between MD and avian Leucosis (AL) which is another economically important neoplasm of poultry; a diagnostic surveillance for both diseases is important (Adene, 1975; Adene and Akpavie, 2004). Based on available literature, nationwide studies on MD are yet to be conducted in Nigeria. To determine the prevalence of MD from records of some veterinary centres in Nigeria from 2000-2009 which is the aim and objectives of this study. Few studies with reported outbreak of MD in different parts of the country showed low prevalence (Fatumbi and Adene, 1986; Adene and Akpavie, 2004; Akpavie, 2005; Okwor and Eze, 2008; Olabode *et al*., 2009). Wakawa *et*

*al.* (2012), Musa *et al*. (2013) in Zaria, Nigeria reported overall prevalence of MD to be 8.3% and 4.9% respectively while Salih *et al*. (2013) reported the overall prevalence of MDV to be 49.5% in six provinces in southern Iraq**.**

## 3.3 Materials and Methods

### 3.3.1 Study area

Selection of the study area in Nigeria was based on the likely sources of reported MD outbreaks, due to long time presence of Veterinary Teaching Hospitals, Clinics and Diagnostic centres in strategic geographical locations of the country. Mokola Veterinary Centre and University of Ibadan Veterinary Teaching Hospital (latitude 0.7$^{o}$C 22'N and longitude 0.3$^{o}$C 58'E), Ibadan in Oyo State. Nsukka Private Veterinary Clinics and University of Nigeria Nsukka Veterinary Teaching Hospital (latitude 0.6$^{o}$C 52'N and longitude 0.7$^{o}$C 24'E) Enugu State. Evangelical Church Winning All (ECWA) Veterinary Clinic Bukuru (latitude 10$^{o}$C 0'N and longitude 9$^{o}$C 30'E) in Plateau State and Veterinary Teaching Hospital (latitude 11$^{o}$C 12'N and longitude 0.7$^{o}$C 37'E) Ahmadu Bello University Zaria in Kaduna State, (Ekong *et al.,* 2012). The Veterinary Clinics and Hospitals were selected because they served as the referral points; represented the country and the population of poultry farmers (figure 3).

### 3.3.2 Data collection

Data collection were carried as follows: a prepared structured form was used in data collection. The information obtained from the records involved reporting of outbreaks, age

and type of birds, dry and raining season, region of country of north west, north central, south east and south west as reported by Ekong *et al*. (2012).

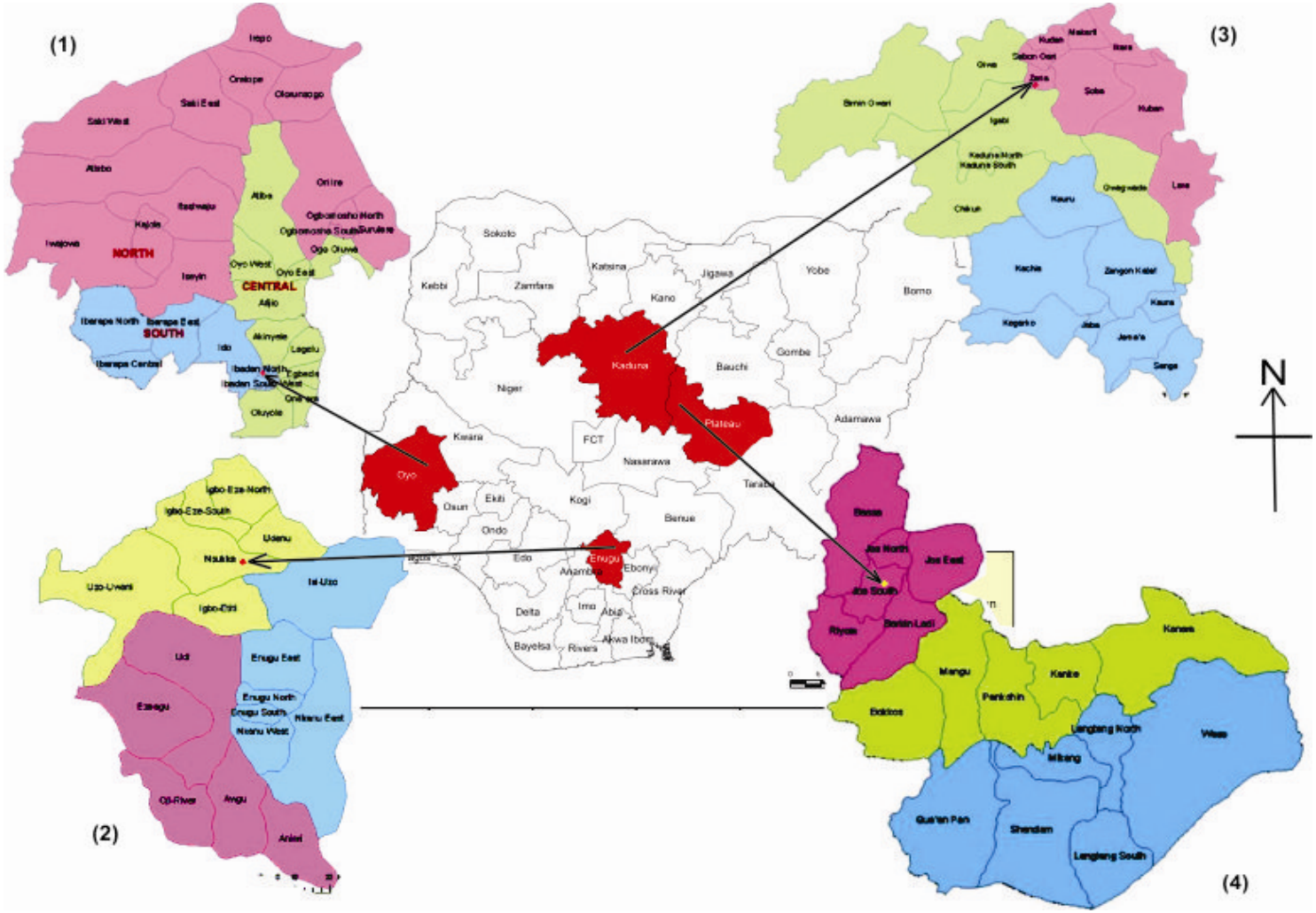



**Figure 3: Map of Nigeria showing States with LGAs with Veterinary Hospitals and Clinics as points of data collection on Marek's disease.(1) Ibadan Norrth in Oyo State, (2) Nsukka in Enugu State, (3) Zaria in Kaduna State and (4) Jos South in Plateau State.**

All cases for which a diagnosis of MD were made by postmortem examination and by histopathology within the study period were considered, controls included other viral diseases of poultry diagnosed within the same study period (2000-2009) for which MD was excluded.

### 3.3.3 Data analysis

Data collected were entered into excel spread sheet and were further transferred into MedCal version 11.2. Analysis was carried to determine the odd ratio and relative risk among variables such as, year, age, type, season, region and zone.

## 3.4 Results

### 3.4.1 Year

Out of the 1,970 poultry viral disease cases recorded, 1,193 (60.6% at 95%) were positive for Marek's disease. The cases of MD decreased in 2006 (OR 0.7) and in 2007 (OR 0.7 at 95%) compared to 2000 but increased in 2008 (OR 1.5 at 95%) and 2009 (OR 2.0 at 95% ) (Table 3:1).

### 3.4.2 Age

The prevalence of MD was highest among flocks aged 18 weeks and above (85.5%) compared to those aged 9 – 17 weeks (71.4%) and 5 - 8 weeks (25.3%), with the least in those aged 1-4 weeks (14.7%). Flocks aged 18 weeks and above were 34.3 (95% CI: 22.5–52.1) times more likely at risk of suffering from MD compared to age 1 – 5 weeks, followed by age 9 – 17 weeks with odd ratio 14.5 (95%) and ages 6 – 8 weeks with OR 2.0 (95% CI: 1.3–3.0) compared to 1 – 5 weeks (Table 3.2).

**Table 3.1: Yearly distribution of Marek's disease diagnosed between 2000-2009 in some veterinary diagnostic centres in Nigeria.**

| Year | MDx (Pr %) | TVDx (Pr %) | Odds Ratio | 95% C.I | *p* value |
|---|---|---|---|---|---|
| 2000 | 120 (60.0) | 80 (40.0) | 1 | RF | |
| 2001 | 75 (46.3) | 87 (53.7) | 0.6 | 0.4 - 0.9 | 0.0095 |
| 2002 | 20 (76.9) | 14 (23.1) | 35.4 | 2.1 - 589.3 | 0.0129 |
| 2003 | 106 (66.3) | 54 (33.7) | 1.3 | 0.9 - 2.0 | 0.2233 |
| 2004 | 39 (97.5) | 1 (2.5) | 26.0 | 3.5 - 193.1 | 0.0014 |
| 2005 | 42 (95.5) | 2 (4.5) | 14.0 | 3.3 - 59.5 | 0.0003 |
| 2006 | 274 (49.6) | 278 (50.4) | 0.7 | 0.5 - 0.9 | 0.0122 |
| 2007 | 106 (50.0) | 106 (50.0) | 0.7 | 0.5 - 1.0 | 0.0419 |
| 2008 | 324 (69.5) | 142 (30.5) | 1.5 | 1.1 - 2.2 | 0.0171 |
| 2009 | 81 (75.0) | 27 (25.0) | 2.0 | 1.2 – 3.4 | 0.0089 |

MDx – Marek's Disease Diagnosed
TVDx – Total Viral Diseases Diagnosed
Pr – Prevalence of Marek's Disease
CI – Confidence Interval
RF – Reference Group

**Table 3.2: Age frequency distribution of Marek's disease in poultry diagnosed between 2000 and 2009 in some veterinary diagnostic centres in Nigeria.**

| Age group (in weeks) | MDP (Pr %) | TVD (Pr %) | Odds Ratio | 95% C.I | *p* value |
|---|---|---|---|---|---|
| 1 – 4 | 35 (14.7) | 203 (85.3) | 1.0 | RF | |
| 5 – 8 | 93 (25.3) | 275 (74.7) | 2.0 | 1.3 - 3.0 | 0.0021 |
| 9 – 17 | 490 (71.4) | 196 (28.6) | 14.5 | 9.8 – 21.5 | <0.0001 |
| > 17 | 561 (85.5) | 95 (14.5) | 34.3 | 22.5 – 52.1 | <0.0001 |

MDP-Marek's disease positive cases
TVD-Total viral diagnosed cases
Pr %– Prevalence of Marek's disease in percentage
CI – Confidence interval
RF- Reference group

### 3.4.3 Type of birds

In all, 319 broilers with a mean age of 7.8 weeks (at 95%), 77 cockerels with mean age 15.0 weeks (at 95%), 952 pullets, mean age 12.9 weeks (at 95%) and 602 layers, mean age 29.9 weeks (95%) were involved in the study. Layers showed a higher prevalence of MD (86.7%), followed by pullets with 62.1%. Compared to broilers, layers had the highest odds of Marek's disease (OR 31.3; 95%CI) followed by pullets (OR 7.9; 95% CI) and turkeys (OR 3.2; 95% CI) (Table 3.3).

### 3.4.4 Season

Rainy (wet) season recorded 63.6% cases of Marek's disease while, 55.4% was recorded during the dry. The chances of MD being diagnosed was higher during the rainy season (OR 1.4; 95% CI) compared to dry (Table 3.4).

### 3.4.5 Region

The odds of having MD infection were significantly higher in the southern part 150/164 (91.5%)with odd ratio 7.8 ( 95% CI: $p< 0.00001$) compared to the northern part 1043/1806 (57.8%) of the country (Table: 3.5).

### 3.4.6 Zone

The odds of having MD were significantly high in the south west (Oyo state) followed by north west (Kaduna state) when compared to north central (Plateau state) 129/130 (99.2%) (OR 113; 95% CI: 15.8-810.3 $p < 0.0001$) and Kaduna 179/187 (95.7%) (OR 19.6; 95% CI, $p < 0.0001$) respectively (Table: 3.5).

**Table 3.3: Distribution of Marek's disease by types of bird between 2000 and 2009 in some veterinary diagnostic centres in Nigeria.**

| Type of bird | MDx (Pr%) | TVDx (Pr%) | Odds ratio | 95% C.I | *p* value |
|---|---|---|---|---|---|
| Broiler | 55 (17.2) | 264 (82.8) | 1.0 | RF | |
| Cockerel | 18 (23.4) | 59 (76.6) | 1.5 | 0.8 – 2.7 | 0.2146 |
| Pullet | 591 (62.1) | 361 (37.9) | 7.9 | 5.7 - 10.8 | <0.0001 |
| Turkey | 4 (40.0) | 6 (60.0) | 3.2 | 0.9 - 11.7 | 0.079 |
| Layer | 522 (86.7) | 80 (13.3) | 31.3 | 21.6 – 45.5 | <0.0001 |

MDx – Marek's disease diagnosed
TVDx – Total viral diseases diagnosed
Pr %– Prevalence of Marek's disease
CI – Confidence interval
RF- Reference group

**Table 3.4: Seasonal distribution of Marek's disease diagnosed between 2000 and 2009 in some veterinary diagnostic centres in Nigeria**

| Season | MDx (Pr %) | TVDx (Pr %) | Odds ratio | 95% C.I | *p* value |
|---|---|---|---|---|---|
| Wet-rainy | 787 (63.6) | 450 (36.4) | 1.4 | 1.2 - 1.7 | 0.0003 |
| Dry | 406 (55.4) | 327 (54.6) | 1 | RF | |

MDx – Marek's Disease Diagnosed
TVDx – Total Viral Diseases Diagnosed
Pr% – Prevalence of Marek's Disease
CI – Confidence Interval
Wet Season (April-September)
Dry (October-March)
RF- Reference Group

**Table 3.5: Regional and zonal distribution of Marek's disease diagnosed between 2000 and 2009 in some veterinary diagnostic centres in Nigeria**

| Variable | MDx (Pr %) | TVDx (Pr%) | Odds Ratio | 95% C.I | *p* value |
|---|---|---|---|---|---|
| Region | | | | | |
| South | 150 (91.5) | 14 (8.5) | 7.8 | 4.5 - 13.7 | <0.0001 |
| North | 1043 (57.8) | 763 (42.2) | 1 | RF | |
| Zone | | | | | |
| North west | 179 (95.7) | 8 (4.3) | 19.6 | 9.6 - 40.1 | <0.0001 |
| South east | 21 (61.8) | 13 (40.2) | 1.4 | 0.7 - 2.9 | 0.33 |
| South west | 129 (99.2) | 1 (0.8) | 113 | 15.8 - 810.3 | <0.0001 |
| North central | 863 (53.3) | 756 (52.7) | 1 | RF | |

MDx – Marek's Disease Diagnosed
TVD – Total Viral Diseases Diagnosed
Pr %– Prevalence of Marek's Disease
CI – Confidence Interval
RF- Reference Group

### 3.5 Discussion

Based on available records, this is the first, current and highest MD prevalence (60.6%) reporteed in Nigeria which increased from 2000 to 2009 with noticeable peaks in 2002, 2004 and 2005. Olabode *et al.* (2009) in a ten year prevalence study of MD (1998 to 2007) carried out in Ilorin, Kwara State of Nigeria reported a prevalence of 41.77%. They also reported that the peak of MD infection was in 1998. In addition, a ten-year prevalence study of MD by Fatunmi and Adene (1986) in Nigeria between 1972 and 1984 reported two noticeable peaks of MD in 1977 and 1983 similar to this work. The differences within the years could be attributed to adequate use of MD vaccines, good management and biosecurity which reduced the disease outbreak. While the years which recorded high outbreak could be due to financial economic problems for example, lack of available MD vaccines as importation is the only source of vaccines, poor management practices and could be due to virulent MDV strains as against the vaccinal strains used as reported by OIE (2000) and Witter (2001).

On the age distribution and type of birds diagnosed with MD, birds at the point of lay and above experienced MD more. This could be due to stress as a result of management practices as repoted by Proudfoot and Aitken (1969). Infection occurs through the respiratory route from the inhalation of poultry house dust contaminated with the MD virus. Birds at point of lay and above easily fly around thereby facilitate the spread of MD from the infected ones from the dusted house. Since infection occurs in the feather follicle epithelium where infectious-cell-free virus is produced and shed into the environment in feather debris and dander The prevalence of MD in adult layers with mean age 29 weeks could also be due to late vaccine breaks as a result of wild MDV strain. While early MD

outbreaks in young birds could be due to MD vaccine failure as reported by Witter and Schat (2003). Marek's disease was more in layers and Pullets and this supports the report of Owoade and Oni (2008) that MD causes economic impact during onset of egg production in laying birds. Apart from chickens as the main host of MD, turkeys were diagnosed with MD could be due to keeping them in close contact with chickens.

The seasonal distribution of the MD indicated high occurrence in the wet season (April-September) and this could be due to intercurrent infections like coccidiosis and cryptosporidiosis and other avian pathogens as reported by Biggs *et al.* (1968) and Abbassi *et al*. (1999) as a result of wet litre, poor ventilation with constant closure of windoors. There are more hatcheries in the south west and high human movement of people could be one of the reasons for high MD risk factor in both the zone and region. Handberg *et al*. (2001) reported continuous out break of MD in infected areas as well as Okwor and Eze (2008) reported outbreaks and persistence of MD in batches of birds reared in a poultry farm located in populated area in Nsukka, South Eastern Nigeria which could be due to contaminated environment.

The diagnoses of MD across the country were based on the clinical signs, gross and histopathology which need to be supported by modern techniques as reported by Nair *et al*. (2008) and OIE, (2010). However, more specific methods as ELISA, PCR for surveillance of the prevalence of MDV as reported by Handberg *et al*. (2001) would be desirable.

Owoade and Oni (2008) used molecular detection of Marek's disease virus which was highly specific in some poultry flocks in the southern region and western zone of Nigeria

which ruled out the possibility of other neoplastic diseases in the diagnosis of MD, while Salih *et al*. (2013) conducted similar study using PCR on MD in six provinces in southern Iraq.

### 3.6 Conclusions

Year prevalence differs within the years from 2000 to 2009 for MD. Birds aged above 17 weeks had the highest prevalence rate of MD and are more at risk of suffering from MD. Layers had the highest prevalence of MD and are more at risk of having MD. Season has an effect on the occurrence of MD and the risk of suffering from the disease. The sourth-western zone and region of the country had highest risk of Marek's disease.

### 3.7 Recommendations

1. There should be proper diagnostic records keeping in veterinary centres.
2. The best practice against MD is by keeping birds of the same age at a time .
3. Good biosecurity against MD is needed in poultry producing areas.
4. In diagnosis of MD, Veterinarians should include modern techniques such as ELISA and PCR, etc.

### 3.8 Limitations

Records of some clinics and hospitals were not available hence, analysis by months was not possible. Retrospective study was based on Marek's disease cases over the total viral cases instead of all poultry diseases recorded. There was no differentiation of MD and other tumours by some diagnostic centres.

# CHAPTER FOUR

## VETERINARIANS EXPERIENCES AND PRACTICES AND POULTRY FARMERS' AWARENESS AND BIOSECURITY PRACTICES ON MAREK'S DISEASE IN NIGERIA

### 4.1 Abstract

As one of the diseases that have a worldwide economic impact, a structured questionnaire study on MD in Nigeria was carried out, involving 360 veterinarians during their 46th annual conference held on the 19th to 23rd October, 2009 at Awka in Anambra State. Out of 360 questionnaires served, 45.28% (163/360) were retrieved for evaluation, 27 (7.5%) unanswered while, 170 (47.22%) questionnaires could not be accounted for. Of those responding, 60% (97/163) were clinicians, 12% (19/163) veterinary input providers, 25% (40/163) academicians and 3% (7/163) diagnosticians (work in the laboratory). Of the 134 veterinarians that responded on regular diagnosis of MD, 75% (99/134) put the frequency of diagnosed MD when compared to other poultry diseases between 5 and 10%, while 25% (35/134) put it between 20 and 30%. On the type of birds frequently attended to, 58% (95/163) attended to layers and on the age at which MD is diagnosed, 8% (13/163) diagnosed MD in birds less than 4 weeks, 75% (123/163) in birds between 4 and 16 weeks while 17% (27/163) diagnosed MD in birds 17 weeks old and above. About 33% (54/163) of veterinarians put mortality due to MD between 10 and 50% in a flock while 84% (137/163) put drop in egg production due to MD between 10 and 40%. Paralysis was ranked highest as the clinical manifestation of MD by 64% (105/163) of veterinarians. Liver was scored highest 71% (115/163) as the organ most frequently affected by MD, then spleen 47% (77/163), nerve 33% (54/163). On the type of MD vaccine most regularly used, 39% (64/163) of veterinarians used HVT, 26% (43/163) used Rispens/CVI988 and 9%

(15/163) used SB126 vaccines. 68% (112/163) of veterinarians confirmed the diagnosis of MD based on clinical signs and gross lesions while, 12% (20/163) diagnosed on clinical signs, gross and microscopic lesions. The relationship between MD and factors responsible for the endemicity and outbreak were also investigated through a structured questionnaire to determine the level of awareness among poultry farmers in Nigeria from 2005 to 2009. A total of 200 questionnaires were distributed to poultry farmers from Enugu, Oyo, Plateau, Nasarawa, Kaduna, Kano and Jigawa states and the retrieval rate was 75% (150/200). Seventy nine per cent of farmers (119/150) have heard about MD and 87.9% (132/150) vaccinated their birds against it. A comparison between the chance of having MD in intensive and semi-intensive management systems with extensive was highly significant (OR. 29.6 and 5.0, $p < 0.0001$). There was no significant difference for farmer's gender, closure of poultry farm due to MD, proximity of $10 \geq 100$ metres to neighbouring farms and source of water used. Veterinarians scored MD high as one of the diseases that is having economic effect on the poultry industry and needed specific laboratory for diagnosis. Some farmers had no knowledge of the disease and did not protect their birds by vaccinating against MD. Both veterinarians and poultry farmers need more information on MD.

## 4. 2 Introduction

Records from veterinary hospitals and clinics in Nigeria indicated that there have been regular report of clinical cases of MD (personal observation Jwander, 2005). Marek's disease cause neurological acute infiltration of the central nervous system and nerves resulting in floppy broiler syndrome and transient paralysis, long-standing paralysis of legs and wings, eye lesions; tumours in heart, ovaries, testes, muscles, lungs and feather follicles

(Baigent and Davison, 2004). Morbidity is 10-50% and mortality cause up to 100% (Anon, 2004). Mortality in an affected flock typically continues at a moderate or high rate for a few weeks. In late Marek's disease, mortality varies as the virulence of the MDV strains differ and mortality due to MD can extend to 40 weeks of age (Ananymous, 2004). Affected birds are more susceptible to parasitic and bacterial diseases **(**Witter *et al.*, 2005**)**.. The route of infection is usually respiratory and MD is highly contagious being spread by infective feather follicle, dander and fomites **(**Witter *et al.*, 2005**).** The virus survives at room temperature for a long time when cell associated and is resistant to quaternary ammonium and phenol (Paul, 2010). Gross lesions commonly seen are; grey-white foci of neoplastic tissue in liver, spleen, kidneys, lungs, gonads, heart, and skeletal muscle, thickening of nerve trunks and loss of nerve striation (OIE, 2010). Microscopically, lymphoid infiltration is polymorphic. Diagnosis of MD based on, history, clinical signs, distribution of lesions, age affected, histopathology are differentiated from lymphoid leukosis, botulism, deficiency of thiamine, Ca/Phosphorus/Vitamin D, especially at the start of lay **(**Witter *et al*, 2005).

Prevention of MD is by good management practices such as hygienic environment, keeping of birds of the same source and age by *all-in* and *all-out* production to break any disease reservoir, using birds that are resistant to MD, vaccinating chicks generally with 1500 PFU of HVT at day old (but increasingly by *in*-ovo inoculating the embryonated eggs), use of polyvalent vaccines like SB1 Sero-type 2 and Rispen's (Paul, 2010; Witter *et al.*, 2005). This study was aimed at testing the level of experiences and practices of veterinarians and poultry farmers' awareness and biosecurity on Marek's disease.

## 4. 3 Materials and Methods

### 4.3.1 Veterinarians

A structured questionnaire (Appendix 1) on MD in Nigeria was distributed to veterinarians from different parts of the Nigeria during the 46th Annual conference of the Nigerian Veterinary Medical Association held on the 19th to 23rd October, 2009 at Awka, Anambra State. The questionnaire was administered through direct contact to veterinarians.

### 4.3.2 Poultry farmers

Study area was selected based on the presence of a major Veterinary service provider (veterinary drugs, feeds and equipment), Veterinary Teaching Hospitals and Clinics consulting on poultry health and rural poultry production in different states (Appendix 2). According to Adene and Oguntade (2006), the study areas have high poultry population with 3,723,326 in Enugu, 4,828,898 Jigawa, 2,821,093 Kaduna, 3,852,135 Kano, 584,551 Nasarawa 3,113,368 Oyo and 3,799,580 Plateau state (Figure 4). Structured questionnaire was designed, pretested and adjustments made accordingly. Questionnaire gathered information on farmer's identification, awareness, biosecurity practices, effects of Marek's disease on poultry production. Farmers were selected based on reference of the veterinarians consulting poultry farms within the study areas. The questionnaires were administered by practicing Veterinarians.

### 4.3.3 Data analysis

**T**he data that was entered in excel spread sheet was transferred to MedCal data base and analysed into descriptive statistics and odd ratio (OR). The data were reduced into tables of positive response (yes or no) grouped into different variables of association using odd ratio

at 95% confidence interval (CI) to determine the significance of the association between the variables with regards to Marek's disease.

## 4. 4 Results

### 4.4.1 Veterinarians response on Marek's disease

Of the three hundred and sixty (360) questionnaires served, 45.28% (163/360) were retrieved for evaluation. Of the responding Veterinarians, 60% (97/163) were clinicians practicing in clinics and hospitals, 25% (40/163) academicians, 12% (19/163) veterinary input providers and 3% (7/163) diagnosticians in research institutes (Tables 4.1).

Out of the 134 veterinarians that responded on the frequency (how often) of diagnosis of MD cases visa vis other viral poultry diseases, 75% (99/134) indicated the frequency of MD diagnosed compared to other poultry diseases to be between 5 and 10%, while 25% (35/134) of veterinarians indicated the frequency between 20 and 30% (Table 4.2).

On the type of birds frequently attended to, 58% (95/163) veterinarians attended to more layers, 27% (44/163) to broilers, 6% (9/163) to parent stock, 3% (5/163) to mixed type of birds (different types of birds reared) and about 6% (9/163) to local birds. As regards the age at which MD is diagnosed, 8% (13/163) of the veterinarians diagnosed MD in birds

**Table 4.1: Occupation of veterinarians served with questionnaire on Marek's disease.**

| Occupation | Frequency (%) |
|---|---|
| Clinicians | |
| Yes | 118 (72.4) |
| No | 45 (27.6) |
| Total | 163 (100.0) |
| Veterinary input providers | |
| Yes | 19 (11.7) |
| No | 144 (88.3) |
| Total | 163 (100.0) |
| Veterinary academicians | |
| Yes | 40 (24.5) |
| No | 123 (75.5) |
| Total | 163 (100.0) |

Yes = belong to the occupation
No = do not belong to the occupation
Clinicians = veterinarians working in the clinics and hospital
Veterinary input provider = veterinarians providing feeds, drugs and equipment services
Veterinary academician = veterinary lecturers

**Table 4.2: Percentile comparison of Marek's disease with other avian diseases by Veterinarians.**

| Frequency of Marek's disease diagnosed vis avis other poultry diseases | Frequency (%) |
|---|---|
| 5% | 53 (39.6) |
| 20% | 19 (14.2) |
| 30% | 16 (11.9) |
| Total | 134 (100.0) |

Frequency (%) = the number of veterinarians that responded in percentage

less than 4 weeks of age, 75% (123/163) diagnosed MD in birds between 4-16 weeks of age, while 17% (27/163) diagnosed MD in birds above 16 weeks old. 33% (54/163) veterinarians reported that mortality due to MD is between 10 and 50% of the flock. Also, 84% (137/163) of veterinarians indicated the drop in egg production due to MD to be between 10 and 40%.

Paralysis was indicated as the highest clinical manifestation of MD by 64% (105/163) veterinarians, followed by emaciation by 8% (13/163), stunted growth, 12% (20/163) and pale comb and wattles by 16% (26/163). Regarding the organ most frequently affected by MD, the liver was the highest according to 39% (64/163) of the respondent veterinarians, then the spleen, 27% (44/163) and nerves 13% (21/163) followed by the kidneys, gonads, lungs, heart and skin and were ranked 9% (15/163), 4% (7/163), 3% (5/163), 3% (5/163) and 2% (3/163) respectively (Tables 4.3). Nodular lesions were mostly seen at postmortem by 60% (98/163) of the veterinarians followed by diffused lesions by 39% (64/163). On the types of MD vaccine most regularly used, 39% (64/163) veterinarians used HVT, 26% (43/163) used Rispens/CVI988 vaccines and 9% (15/163) used SB126 vaccines (Table 4.4). 26% (42/163) Veterinarians did not respond on the use of MD vaccines as a means of disease control.

**Table 4.3: Organs frequently affected by Marek's disease as indicated by veterinarians.**

| Organ | Frequency (%) |
|---|---|
| Liver | |
| Yes | 115 (70.6) |
| No | 48 (29.4) |
| Total | 163 (100.0) |
| Spleen organ | |
| Yes | 77 (47.2) |
| No | 86 (52.8) |
| Total | 163 (100.0) |
| Nerves organ | |
| Yes | 54 (33.1) |
| No | 109 (66.9) |
| Total | 163 (100.0) |

Yes = frequently affected by Marek's disease
No = not frequently affected by Marek's disease

**Table 4.4: Types of Marek's disease vaccine frequently used by veterinarians.**

| Vaccine used | Frequency (%) |
|---|---|
| Rispens/CV 1988 | |
| Yes | 43 (26.4) |
| No | 120 (73.6) |
| Total | 163 (100.0) |

Yes = vaccine frequently used
No = vaccine not frequently used

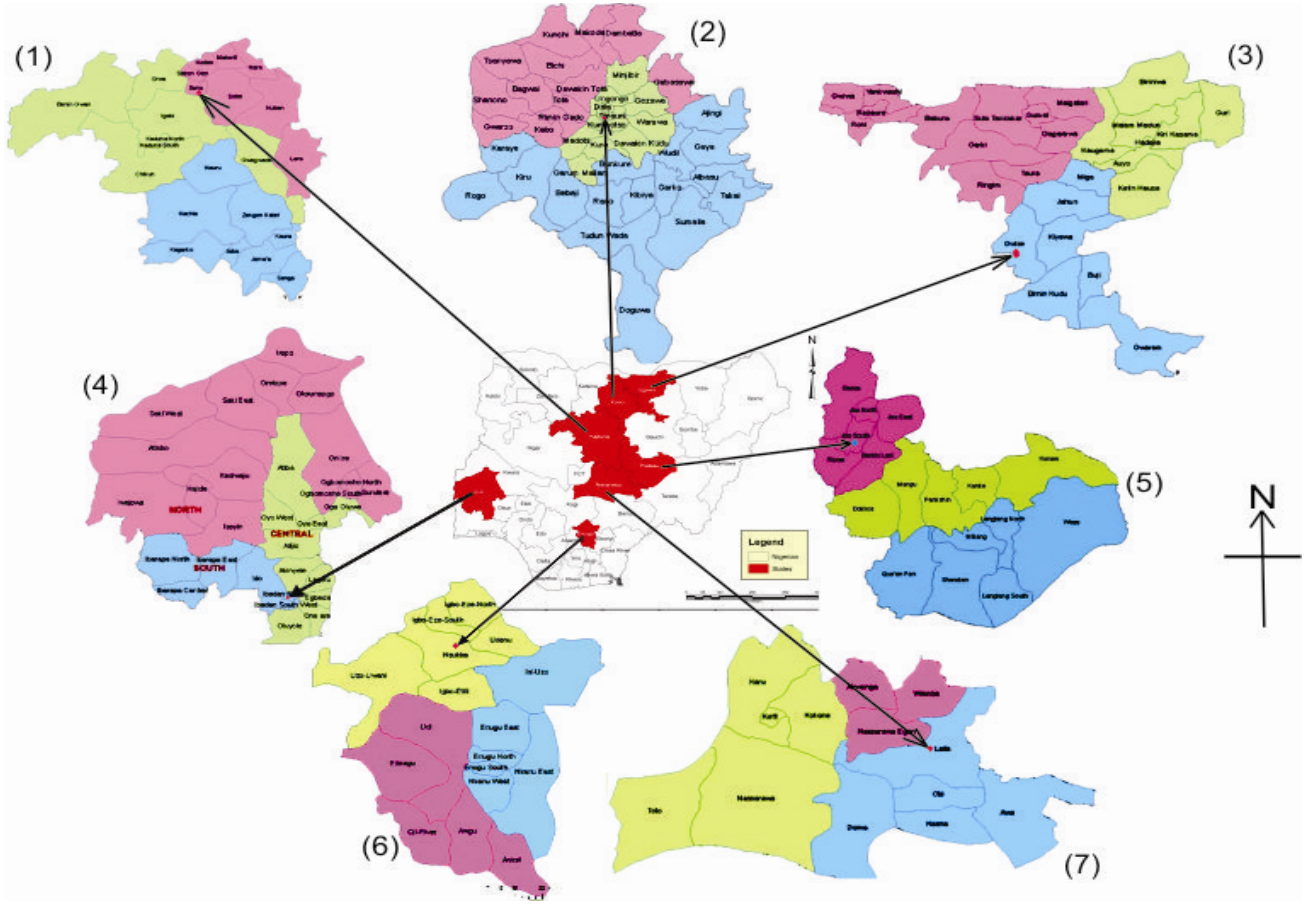



**Figure 4: Map of Nigeria showing States in red colour wih LGA locations (1) Zaria in Kaduna State, (2) Tarauni in Kano State, (3) Dutse in Jigawa State, (4) Ibadan North in Oyo State, (5) Jos South in Plateau State, (6) Nsukka in Enugu State, (7) Lafia in Nasarawa State testing Poultry farmers' level of awareness on Marek's disease.**

**Table 4.5: Some factors associated with Marek's disease in Nigeria.**

| Variables associated with darek's Disease | Positive Response (%) | Negative response (%) | Odd ratio (95% C.I) | p value | Overall p value |
|---|---|---|---|---|---|
| States | | | | | |
| Enugu | 11 (91.7) | 1 (8.3) | 22.0 (2.3-2.12) | 0.0076 | |
| Kaduna | 14 (58.3) | 10 (41.7) | 2.8 (0.8-10.0) | 0.1127 | |
| Kano | 4 (44.4) | 5 (64.6) | 1.6 (0.3-8.3) | 0.5743 | |
| Nasarawa | 3 (33.3) | 6 (76.7) | 1 (0.2-5.5) | 1.0000 | |
| Oyo | 16 (94.1) | 1 (5.9) | 32.0 (3.4-302.2) | 0.0025 | |
| Plateau | 13 (65.0) | 7 (45.0) | 3.7 (1.0-14.2) | 0.0556 | 0.1300 |
| Jigawa | 6 (33.3) | 12 (76.3) | 1 | RF | |
| Farmers' gender | | | | | |
| Female | 23 (65.7) | 12 (34.3) | 1.3 (0.6-3.1) | 0.5335 | 0.5335 |
| Male | 41 (59.4) | 28 (40.6) | 1 | RF | |
| Occupation | | | | | |
| Farmer | 18 (54.6) | 15 (45.4) | 1.1(0,3-3.5) | 0.9141 | |
| Business | 12 (75.0) | 4 (25.0) | 2.7(0.6-11.7) | 0.1937 | |
| Civil servant | 20 (60.6) | 13 (39.4) | 1.4(0.4-4.5) | 0.6034 | 0.6770 |
| Others | 9 (52.9) | 8 (47.1) | 1 | RF | |

RF = Reference group

**Table 4.6: Management and practices associated with the spread of Marek's disease in poultry farms in Nigeria.**

| Management practices | Positive response (%) | Negative response (%) | Odd ratio (95% C.I) | p value | Overall p value |
|---|---|---|---|---|---|
| Management system | | | | | |
| Intensive | 55 (80.9) | 13 (19.1) | 29.6 (7.7 - 114.5) | <0.00001 | |
| semi-intensive | 5 (41.7) | 7 (58.3) | 5.0 (0.9 - 26.5) | 0.0585 | <0.0001 |
| Extensive | 3 (12.5) | 21 (87.5) | 1 | | |
| Source of birds | | | | | |
| Commercial | 52 (89.7) | 4 (10.3) | 39.0 (11.5 - 132.6) | <0.00001 | |
| Mixed (different types) | 2 (66.7) | 1 (33.3) | 9.0 (0.7 - 116.2) | 0.0923 | <0.0001 |
| Local | 6 (18.2) | 27 (81.8) | 1 | RF | |
| Source of water | | | | | |
| Pump | 13 (52.0) | 12 (48.0) | 0.8 (0.3 - 2.1) | 0.6057 | |
| Well/Pump | 12 (92.3) | 1 (7.7) | 8.6 (1.0 - 71.4) | 0.0469 | |
| River | 0 (0.0) | 6 (100.0) | 0.06 (0.003 - 1.04) | 0.053 | 0.0840 |
| Well | 28 (70.0) | 12 (30.0) | 1 | RF | |
| Sources of feed | | | | | |
| Commercial | 9 (25.0) | 27 (75.0) | 0.3 (0.07-1.6) | 0.1724 | |
| Comm/self milled | 8 (100.0) | 0 (0.0) | 17.0 (0.7-391.7) | 0.0767 | 0.0440 |
| Self milled | 4 (50.0) | 4 (50.0) | 1 | RF | |
| Isolate sick birds | | | | | |
| No | 51 (67.1) | 25 (32.9) | 2.3 (1.0-5.5) | | 0.0543 |
| Yes | 14 (46.7) | 16 (53.3) | 1 | RF | |
| Dead birds disposal | | | | | |
| Burry | 10 (66.7) | 5 (33.3) | 1.3 (0.4-4.0) | | 0.7046 |
| Throwaway | 56 (61.5) | 35 (38.5) | 1 | RF | |
| Availability of Vet Services | | | | | |
| No | 43 (69.4) | 19 (30.6) | 2.4 (1.1-5.3) | 0.0357 | 0.0357 |
| Yes | 21 (48.9) | 22 (51.1) | 1 | RF | |
| Proximity to other farms | | | | | |
| 10-50 m | 21 (50.0) | 21 (50.0) | 0.4 (0.1-1.1) | 0.3889 | |
| 50-10 m | 20 (64.5) | 11 (35.5) | 0.7 (0.2-2.2) | 0.5518 | 0.0680 |
| ≥100 m | 18 (72.0) | 7 (38.0) | 1 | RF | |

Positive response = had Marek's disease outbreak
RF = reference group

**Table 4.7: Marek's disease biosecurity measures by poultry farmers in Nigeria.**

| Variable (Measures) | Positive response (%) | Negative responses (%) | Odd ratio | p value | Overall p value |
|---|---|---|---|---|---|
| Vaccinate against MD | | | | | |
| No | 51 (87.9) | 7 (12.1) | 21.9 (6.8-70.0) | <0.0001 | <0.0001 |
| Yes | 7 (25.0) | 21 (75.0) | 1 | RF | |
| Revaccination | | | | | |
| No | 13 (92.9) | 1 (7.1) | 10.5 (1.3-83.7) | 0.0269 | 0.0269 |
| Yes | 46 (55.4) | 37 (54.6) | 1 | RF | |
| Keep birds of different ages together | | | | | |
| No | 18 (40.9) | 26 (59.1) | 0.2 (0.1-0.5) | 0.0005 | <0.0001 |
| Yes | 44 (76.6) | 18 (23.4) | 1 | RF | |
| Different Farmers complained of Marek's disease in chicks bought from same source (contaminated) | | | | | |
| Yes | 33 (84.6) | 6 (15.4) | 5.5 (2.0-14.9) | 0.0009 | 0.0001 |
| No | 31 (50.0) | 31 (50.0) | 1 | RF | |
| Raise chicks separately | | | | | |
| Yes | 18 (94.7) | 1 (5.3) | 8.2 (1.1-64.9) | 0.0453 | 0.0009 |
| No | 59 (68.6) | 27 (31.4) | 1 | RF | |

Positive response = had Marek's disease outbreak
RF= reference group

**Table 4.8: Effect of Marek's disease on poultry production and poultry farmers in Nigeria.**

| Variable | Positive response (%) | Negative response (%) | Odd ratio (95% C.I) | p value | Overall p value |
|---|---|---|---|---|---|
| Types of birds | | | | | |
| Commercial | 61 (84.7) | 11 (15.3) | 27.7(9.4 - 82.2) | <0.00001 | <0.0001 |
| Local | 6 (16.7) | 30 (83.3) | 1 | | |
| Likely season of Marek's disease | | | | | |
| Wet-Rain(May-October) | 11 (78.6) | 3 (21.4) | 6.4 (1.6-26.5) | 0.0101 | |
| Hot (February-April) | 24 (82.6) | 5 (17.4) | 8.4 (2.7-26.3) | 0.0003 | 0.0001 |
| Harmattan (November – January) | 16 (36.4) | 28 (63.6) | 1 | | |
| Sold and close farm because of Marek's disease? | | | | | |
| Yes | 6 (85.7) | 1 (14.3) | 4.4 (0.5-38.2) | 0.1783 | 0.1783 |
| No | 49 (57.7) | 36 (42.3) | 1 | | |
| Heard about Marek's disease? | | | | | |
| No | 49 (79.0) | 13 (21.0) | 6.5 (2.7-15.8) | <0.0001 | <0.0001 |
| Yes | 15 (36.6) | 26 (63.4) | 1 | RF | |

Positive response = Farmers that experienced Marek's diseased
RF = Reference group

### 4.4.2 Farmers' awareness on Marek's disease

The level of awareness of poultry farmers on MD (Figure 4) was put at 79.0% (with over all p = 0.1300). Enugu (p = 0.0076), Oyo (P = 0.0025), Plateau (p = 0.0556), Kaduna (p = 0.1127), Nasarawa (p = 1.0000) by locations of experiencing MD most in descending order when compared with the odd ratio of Jigawa state (Table 4.5). On gender of poultry farmers, there was no significant difference between the risk of having MD in different gender. The risk of experiencing MD by occupation of poultry farmers was high among farmers in business and civil servants than farmers whose job is purely poultry farming (Table 4.5). On management practices, intensive and semi-intensive management systems showed high risk of MD with odd ratio of 29.6 and 5.0 with overall p < 0.00001 respectively when compared with extensive system. Commercial and mixed source of birds, well and pump water, commercial self-feed milling were at high risk of MD infection (Table 4.6). Poultry farmers that did not isolate birds and had no access to veterinary services were at high risk of MD infection. Mode of carcass disposal and close proximity between poultry farms had no significant difference in the spread of MD (Table 4.6). Not vaccinating, revaccinating birds, keeping birds of different ages and chicks bought from MD contaminated source had high risk of experiencing MD (Table 4.7). Commercial birds, hot and rainy seasons, farms that were closed due to MD experienced high risk of the disease (Table 4.8).Farmers without the prior knowledge of MD, experienced it most (Table 4.8).

## 4.5 Discussion

Out of 360 questionnaires served to Veterinarians, 163 (45.28%) were answered, 27 (7.5%) were retrived but unanswered. It could mean, they do not attached importance to MD or are not avian specialists or have lost touch with the veterinary practice and do not know much about MD. The MD diagnosed by the veterinarians is likely to be based on clinical observations (this further explained that they could still be very much in contact with the disease in the field thereby making this investigation more reliable). On the type of chickens most regularly presented for diagnosis, the veterinarians were more in contact with commercial layers, mixed birds and broilers and this could be due to rapid growth and wide distribution of commercial poultry across the country. The contact of the veterinarians with mostly commercial poultry is an indication that there is a change from the past reports on zoning of commercial and local birds to a particular region in Nigeria by Nawathe *et al*. (1978) and Adene (1980)

The changing epidemiology of MD as reported by Morrow and Fehler (2004) has complicated diagnosis: some veterinarians were reluctant to consider MD where tumours were not seen until after 16 weeks of age and MD can be confused with avian leukosis (AL) in the field. It means that birds above 16 weeks of age without nervous involvement as seen from MD could be AL or mixed infection that needs further laboratory investigation as reported by Witter and Schat (2003).

Chickens below 16 weeks of age are considered important pointer or maker towards the diagnosis of MD as AL virus is considered as slowly manifesting in chickens but, MD outbreaks in broiler breeders are more likely to start with significant number of tumours

appearing at the point of lay and that late onset of tumour in MD are now very common as reported by Morrow and Fehler (2004).

The highest mortality reported in 2007-2009 was 50% with frequency 16 (11.9%). When compared with previous reports in Nigeria (Adene and Hill, 1977; Nawathe *et al*., 1978; Okwor and Eze, 2008; Olabode *et al*., 2009), it means that the prevalence of MD is increasing and this report is the highest in Nigeria (> 60%). Egg drop in birds with MD for 2007 to 2008 was between 10-40% with the following frequency, 51 (37.2%); 38 (27.7%) and 25 (18.2%) respectively. These figures agreed with previous reports that MD has economic effect especially in laying birds (Witter and Schat, 2003; Hunt, 2009).

Turkey herpes virus (HVT) vaccine is the most regularly used for MD control in Nigeria. Unfortunately this vaccine cannot protect birds from virulent strains of MDV as reported by Witter *et al*. (1980) and Witter (2001). The lack of MD vaccines as indicated by veterinarians and the wide spread use of HVT vaccine could likely be the cause of sharp increase in MD outbreaks with high mortality. Witter (1997) reported the continuation of an evolutionary trend of MDV towards greater virulence as the cause of increased losses from MD in vaccinated flocks. The present study showed that of the 58 farmers who vaccinated against the MD, 51 (87.9%) experienced MD outbreaks.

Farmers' awareness on MD is an important factors in the disease control; from the results of this study, 36.6% of the farmers were aware of MD while 79.0% were not.. Poor management and lack of good biosecurity have adverse effect on poultry farms. Prevention

of Marek's disease requires optimal hygiene and management. Apart from the use of vaccines, hygiene is the second important measures in the fight against MD as reported by Morrow and Fehler (2004). The level of awareness of MD plays a great role in the disease spread and control. Farmers' awareness of any disease will reduce its spread.. From this study, 49% of farmers do not have access to veterinary services and had higher risk of MD outbreak.

The risk of having MD in intensive and semi-intensive management system when compared with extensive system was highly significant with OR. 29.6 and 5.0 ($p < 0\text{-}0001$) respectively. This could be that MD spread horizontally in a flock which is aided by close contact of the infected birds shedding the virus in the flock as reported by Baigent and Davison (2004).

Risk factors such as bird type, source of birds, feed, isolation of sick birds, distance between poultry farms are good indicators of getting the farm infected with MD. Farmers experiencing MD was higher during the raining season probably due to poor ventilation and the presence of other mixed infections. Farmers who kept birds of different ages together experienced MD due to reinfection from the adults to the young ones.

Farmers (84.6%) who had MD and bought chicks from the same source means that, chicks from the hatcheries or chicks vendors might have been contaminated with MD virus prior to supply. Farms (94.7%) that raised chicks separately from adult birds experienced MD

indicating that, MD is endemic in these farms with constant pollution of the environment hence separating birds by age makes no difference in already infected environment.

## 4.6 Conclusions

Most Veterinarians diagnosed MD based on the clinical signs and gross pathological alteration. Most Veterinarians used HVT vaccine to prevent MD. Most farmers were not aware about MD and not all commercial birds were vaccinated against Marek's disease.

## 4.7 Recommendations

1. There is the need to educate and update veterinarians on MD.

2. The use of polyvalent MD vaccines such as Rispens CVI1988 combined with either HVT or SB 126 is recommended.

3. There should be routine vaccination by the farmer or hatchery of day old chicks with Rispens/CVI988 vaccine stored at minus (-196$^{o}$C) to ensure viability.

# CHAPTER FIVE

# SEROPREVALENCE OF MAREK'S DISEASE IN BIRDS FROM FEDERAL CAPITAL TERRITORY, KADUNA STATE, BAUCHI STATE, PLATEAU AND NASARAWA STATES OF NIGERIA

## 5.1 Abstract

Blood samples were obtained from 1,700 commercial and 752 local chickens from 36 flocks in the FCT, Kaduna, Bauchi, Plateau and Nasarawa States of Nigeria between 2005 and 2009. The study area was chosen based on reported suspected MD outbreak that needed follow up and the presence of live bird markets and rural households. The local chickens had no records of vaccination. The commercial flocks were kept either on deep litter or battery cages. Sera obtained were tested for antibodies to MDV by agar gel precipitin test. Analysis of risk factors associated with seropositivity for MDV antibody in commercial and local chickens, univariate and multivariate logistic regression was done using stata/se 10. A factor or independent variable of interest was considered to have an influence on seropositivity of MD which was significant ($p \leq 0.05$) with overall seroprevalence of 66.0% ($p < 0.0001$). Another study surveyed for MDV antibodies from 151 sera from different avian species from the FCT, Nasarawa, Kaduna, Bauchi and Plateau states using ELISA. Ninety five samples were positive which gave the overall MDV antibody prevalence to be 62.91% (95/151). Antibody prevalence obtained were as follows: from the FCT 59.09% (13/22), Nasarawa State 40% (8/20), Kaduna State 45% (18/40), Bauchi State 60% (6/10) and Plateau State 84.75% (50/59). Out of 108 sera from vaccinated chickens, 68.52% (74/108) were positive while of the 17 sera from unvaccinated local chickens, 76.48% (13/17) tested positive for MDV antibodies and of the 12 sera from turkeys, 41.67% (5/12) were positive. Also, 3 out of the 12 sera from quails

were positive (25%), while ducks were seronegative (0/2). The detection of the MDV antibody in unvaccinated commercial chickens, local chickens, turkeys and quails was an indication that they were exposed to field MDV. This study gives the seroprevalence of MD in the study area. A nationwide screening of chickens for MDV antibody in Nigeria is needed to establish its true status.

## 5.2 Introduction

Marek's disease (MD) is a lymphomatous and neuropathic disease of domestic fowl caused by an alphaherpesvirus (OIE, 2010). It belongs to the genus *Mardivirus* (Osterrieder and Vautherot, 2004). It is considered by the Food and Agriculture Organization (FAO) and Office Internationl des Epizooties (OIE) as one of the most widespread avian neoplastic infection in the world (OIE, 2010 as cited by Pastoret (2004). Three of the MD serotypes have been identified but serotype 1 is pathogenic (Lee *et al*., 1983; OIE, 2010).

Field MD is diagnosed based on clinical signs, gross or microscopic lesions (Nair *et al*., 2008; OIE, 2010). Infection by MDV is detected by virus isolation and the demonstration of viral antibody **(**OIE, 2010**).** In chickens, MD occurs at 3–4 weeks of age or older and is most common between 12 and 30 weeks of age **(**Nair *et al*., 2008; Schat and Nair, 2008; OIE, 2010**).** Under field conditions, most chickens become infected with MDV during the first few weeks of life and then carry the infection throughout their lives, often without developing overt disease (OIE, 2010). Antibodies to MDV develop within 1–2 weeks of infection and are detected by the agar gel precipitin test, indirect fluorescent antibody test and enzyme-linked immunosorbent assay (OIE, 2010). Extensive distribution of MD susceptible and carrier avian species in Nigeria and the need for effective surveillance,

prevention and control of the disease poses significant challenges (Adene and Akpavie, 2004). The threat to poultry production caused by MD in Nigeria is likely underestimated, considering the poor reporting and diagnostic services for MD (Durojaiye and Adene, 2004). Two studies were carried out to determine the seroprevalence of MD in the FCT, Kaduna, Bauchi, Plateau and Nasarawa States in Nigeria using AGPT and indirect ELISA.

## 5.3 Materials and Methods

### 5.3.1 Study area, species and locations of birds sampled

The study area was chosen based on reported suspected MD outbreak that needed follow up and presence of live bird markets and rural households. The areas were the FCT, Plateau and Nasarawa states (North-central region); Kaduna state (North-west region); Bauchi state (North-east region). In Kaduna state, blood samples collected were from Aviation, Sabon gari, Samaru and Gadan mallam Mamman along Abuja road. Samples from Plateau state were from Jos North, Jos South, Jos East, Pankshin and Mangu. Nasarawa state samples were from Lafia, Akwanga, Nyanya and Masaka. Samples from the FCT (Abuja) were from Wuse and Karu. The samples from Bauchi state were from Toro area (Figure 5). The species of birds sampled were local chickens, commercial chickens, turkeys, quails, and ducks. All local chickens, turkeys, quails, ducks and some commercial chickens were unvaccinated against MD. Commercial chickens and quails were from poultry farms while local chickens, turkeys, ducks were from live bird markets and households. Blood was obtained from the jugular veins of commercial and domestic chickens, turkeys, quails and ducks while for some local and commercial chickens, blood was collected during slaughter.

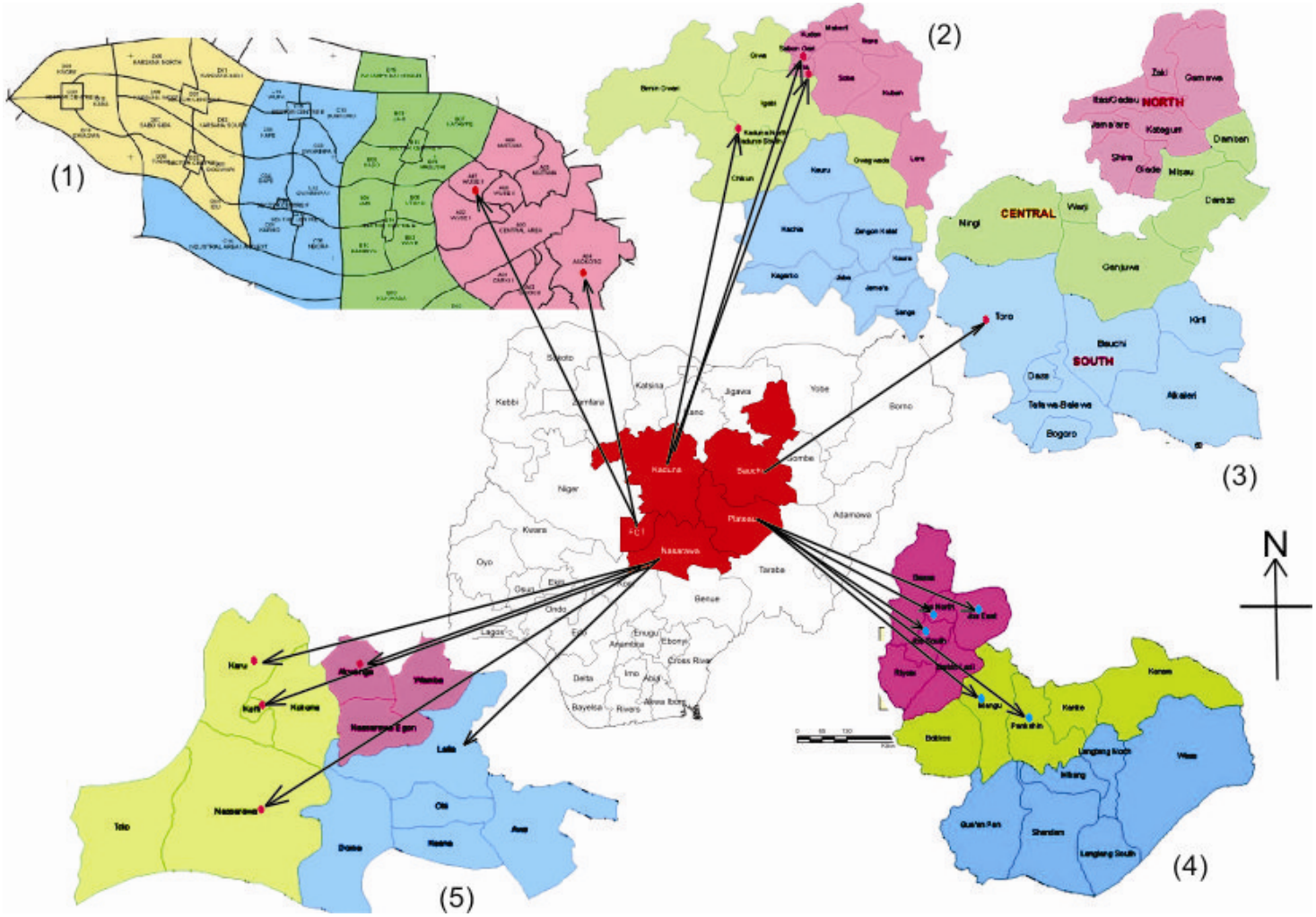



**Figure 5: Map of Nigeria showing location of States in red colour with LGAs where birds were sampled for Marek's disease antibodies. (1) Wuse and Nyanya in Asokoro district of FCT, (2) Kaduna north, Zaria and Sabo in Kaduna State, (3) Toro in Bauchi State, (4) Jos south, nort and east, Mangu and Pankshin in Plateau State, (5) Lafia, Akwanga, Keffi, Nasarawa and Karu in Nasarawa State.**

The commercial flocks were mostly kept on deep litter with few in battery cages. The source of feed was commercial or self-milled for commercial chickens and quails while local birds were extensively managed. Local birds are of different ages and sources were housed together while the commercial chickens were mostly sourced from the southern part of Nigeria.

### 5.3.2 Sample size

The sample size for the seroepidemiological survey for MD was determined by using the method of Mahajan (1997) which used the formula:

$N = Z^2pq/L^2$

where N = sample size (minimum), Z = 1.96; q = 1-p ; L = allowable error = 5%; P = prevalence rate 39.5% (Adene, 1977); N = 376 (minimum sample size). This number was multiplied by 4 to obtain 1, 469. At the end of the survey, 2,452 sera were collected and tested for antibodies against MDV.

### 5.3.3 Sampling technique

Blood samples were conveniently taken from 20 birds in a flock. A data collection form was filled to indentify location, age, source, species and type of bird, vaccination status, flock size and source of feed and birds.

### 5.3.4 Blood collection

Using 5 ml syringes, about 1.5-3 ml of blood from the jugular veins were collected. The syringes were slanted and the blood was allowed to clot at room temperature for about 3 hours. Sera were then transferred into labeled cryovials and stored in a freezer until used

for AGPT at the NVRI Vom. The labeled 151 sera were transported in a cooler packed with ice to the laboratory of the Department of Veterinary Medicine, University of Ibadan and tested using ELISA.

### 5.3.5 Preparation of agar gel

i) Put 1g of agarose, 8 g sodium chloride in flat bottom flask and added 100 ml PBS (pH 7.4). The mixture was boiled and placed in a water bath at 56$^{o}$C. ii) 15 ml of agar was dispensed into a medium sized Petri dish to form a thickness of agar gel of about 3 mm. iii) Wells were punched in the agar gel using a standard template with a centre well and 6 wells spaced at equal distance around it. The diameter of each well was approximately 5.3 mm and the wells were about 2.4 mm apart as described by OIE (2010).

### 5.3.6 Qualitative agar gel precipitin test procedure

Marek's disease virus (HPRS-16) positive **s**tandard antigen and serum lot No PA156 Batch No MP 062 Batch 11-4-84 and PA0157 Batch No MP 063 BR were purchased from Veterinary Laboratory Agencies (VLA-UK) for AGPT. The antigen and serum were diluted as recommended by the producer.

Using micropipette, 50 uL of the standard antigen was placed in the central well and the standard antiserum in alternate exterior to serve as control. Test sera were placed in the remaining wells. The Petri dishes were covered with lids and incubated in a humid jar at 37°C for 24 hours. Visible precipitin lines were considered as positive for MDV antibodies (Plate I). The results were transferred into laboratory result sheet and then, into field sample form.

### 5.3.7 Enzyme linked immunosorbent assays

The MDV antibodies were detected using ELISA protocol of Adeniran and Oyejide (1995). In preparing antigen for the ELISA, wells of an enzyme linked immunosorbent assays (ELISA) for detecting MDV antibodies were made available in 96-round bottom wells of microtitre plate coated with MD commercial vaccines as antigen in coating buffer.

### 5.3.8 Procedures for Marek's disease virus antibodies detection using indirect enzyme linked immunosorbent assays

The coating buffer used was carbonate bicarbonate and the washing buffer was PBS in 0.05% Tween 20 (pH 7.4) and the dilution buffer was PBS-Tween 20- 1% BSA. The antigen, 1,000 doses of MD HVT vaccine, was reconstituted in 1ml of distilled water and then diluted 1:50 in bicarbonate buffer and then added 50 µl to the wells and incubated at room temperature for 1 hour then 24 hours at 4°C. The microtitre plate was emptied and washed three times at 5 minutes interval with washing buffer. The standard positive, negative and test serum were diluted each 1:100 in dilution buffer and 50 µl was added to each well and then incubated at room temperature for 1 hour, wells were then emptied and washed three times at 5 minutes interval with washing buffer. Conjugate diluted at 1:2,000 in dilution buffer was added at 50 µl to each well and incubated at room temperature for 20 minutes. The plate was then emptied and washed three times at 5 minutes interval. Then, 50 µl of O-phenylenediamine dihydrochloride (OPD) substrate was added into each microtitre well and incubated at room temperature until colour develops. Reaction was stoped using 50 µl $H_2SO_4$ to the wells and the optical density was read at 492 nm using ELISA plate reader.

### 5.3.9 Statistical analysis

Statistical analysis was conducted using the MedCalc statistical software version 11.2. The outcome (dependent) variable was individual MDV seropositivity status (AGPT), coded as 0 (negative serum) or 1 (positive serum). In the analysis of risk factors associated with the seropositivity of MDV, univariate and multivariate logistic regression was done using stata/se 10 (StataCorp, 2003). A factor or independent variable of interest was considered to have an influence on seropositivity which was significant at a probability level of $p \leq 5\%$.

## 5.4 Results

### 5.4.1 Result of agar gel precipitin test

#### *5.4.1.1 Location*

Two thousand four hundred and fifty two sera, 1,840 from North-central (Plateau, Nasarawa States) and FCT; 592 from North-west (Kaduna State) and 20 from North-east (Bauchi State) were collected and examined for MDV antibodies by AGPT (Plate I). The overall seroprevalence of MD in the study areas were 66.0% (Table 5.1). The specific seroprevalence of MD in North-central, North-west and North-east was 63.8%, 72.1% and 80.0% respectively. Overall, there was a significantly higher risk in the North-west zone (RR 1.13) and North-east zone (RR 1.25) compared with North-central zone (Table 5.1).

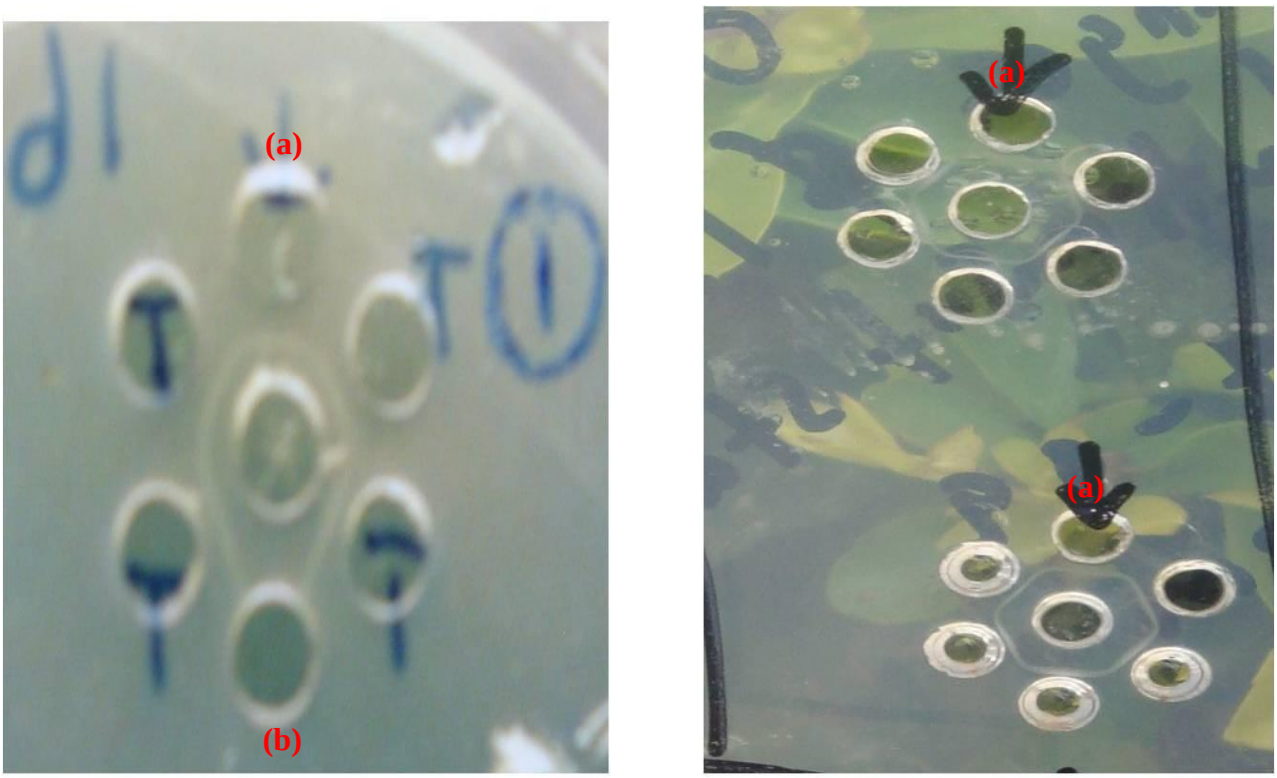



**Plate I**: **Results of A and B plates showing cross section of agar gel precipitin test of Marek's disease positive lines of diffusions.**

A and B = Precipitin lines in positive field cases.

Arrows at (a) in plates A and B pointing at the standard serum from UK.

T = test sera in the surrounding wells from field samples while negative control at (b) in plate A. The centre wells contain the standard MD antigen from Veterinary Laboratory Agencies (VLA-UK).

**Table 5.1: Seroprevalence of Marek's disease in chickens in some parts of Nigeria, 2005 - 2009.**

| | Seroprevalence of MD | | | |
|---|---|---|---|---|
| **Variable** | **Tested positive (%)** | **Tested negative (%)** | **95% CI** | ***p*- value** |
| Zones | | | | |
| Overall seroprevalence of MD | 1617 (65.9) | 835 (34.1) | 63.9 – 67.8 | <0.0001 |
| Studied zones | | | | |
| North-west (Kaduna) | 427 (72.1) | 165 (27.9) | 68.3 – 75.7 | < 0.0001 |
| North-east (Bauchi) | 16 (80.0) | 4 (20.0) | 56.3 – 94.3 | 0.0021 |
| North-central (Plateau,Nassarawa and FCT) | 1174 (63.8) | 666 (36.2) | 61.6 – 66.0 | < 0.0001 |

MD- Marek's disease CI-Confidence interval

*5.4.1.2 Type of birds*

There was no significant difference in seroprevalence of MD between commercial 66.9% and local chickens 63.7% (RR 1.05, p = 0.1249) (Table 5.2).

*5.4.1.3 Vaccination status*

The relative risk of MD among unvaccinated commercial birds (76.4%) compared with unvaccinated local birds (64.4%) was 1.19 (at 95% CI), was significant (p = 0.0062) (Table 5.3). The relative risk of MD among vaccinated commercial birds (66.1%) compared to unvaccinated commercial birds (76.4%) was 0.87 (at 95% CI), was significant (p = 0.0039) (Table 5.3). The relative risk of MD among vaccinated birds (66.1%)) compared to unvaccinated birds (68.1% 311/457) was 1.01 (at 95% CI), was not significant (p = 0.843) (Table 5.3).

Result from the univariable analysis indicated a significant higher risk of MD infection; 1.52, 1.28, 1.16, 1.18 among birds from FCT, Bauchi, Kaduna, Nasarawa respectively, compared with birds from Plateau State (Table 5.4).

*5.4.1.4 Age of birds*

Birds age below and above 18 weeks had high seroprevalence. There was no significant difference in seroprevalenvence of MD among age groups when age 5-10 weeks with 11-18 and >18 weeks were compared (p = 0.0836 and 0.6578, RR= 0.97 and 1.1.2 respectively) (Table 5.4).

**Table 5.2: Seroprevalence of Marek's disease in type of birds in some part of Nigeria, 2005 – 2009.**

| Variable | Seroprevalence of MD | | | |
|---|---|---|---|---|
| | Tested positive (%) | Tested negative (%) | 95% CI | *p*- value |
| Type of birds | | | | |
| Commercial | 1,138 (66.9) | 562 (33.1) | 64.6-69.1 | < 0.0001 |
| Local | 479 (63.7) | 273 (36.3) | 60.2-67.1 | < 0.0001 |

MD = Marek's disease
CI = Confidence interval
% = Per cent

**Table 5. 3: Univariable analysis of vaccination status among commercial and local chickens as risk factors for Marek's disease in some parts of Nigeria, 2005–2009.**

| | MD sera tested | | | | |
|---|---|---|---|---|---|
| **Variable** | **Tested positive (%)** | **Tested negative (%)** | **Relative risk** | **95% CI** | ***p* – value** |
| Overall unvaccinated flocks | | | | | |
| unvacc.commercial chickens | 107 (76.4) | 33 (23.6) | 1.2 | 1.05-1.34 | 0.0062 |
| unvacc. local chickens | 204 (64.4) | 113 (35.6) | 1 | | |
| Commercial farms | | | | | |
| Unvacc.commercial chickens | 107 (76.4) | 33 (23.6) | 1.2 | 1.05-1.28 | 0.0039 |
| Vacc.commercial chickens | 1031 (66.1) | 529 (33.9) | 1 | | |

**Table 5.4: Univariable analysis of risk factors for Marek's disease infection in chickens in some parts of Nigeria, 2005-2009.**

| | MD sera tested | | | | |
|---|---|---|---|---|---|
| **Variable** | **Tested positive (%)** | **Tested negative (%)** | **Relative risk** | **95% CI** | ***p* – value** |
| Location | | | | | |
| Abuja | 19 (95.0) | 1 (5.0) | 1.52 | 1.37-1.69 | <0.0001 |
| Bauchi | 16 (80.0) | 4 (20.0) | 1.28 | 1.03-1.60 | 0.028 |
| Kaduna | 427 (72.1) | 165 (27.9) | 1.16 | 1.09-1.23 | <0.0001 |
| Nasarawa | 132 (73.3) | 48 (26.7) | 1.18 | 1.07-1.29 | 0.0009 |
| Plateau | 1023 (62.4) | 617 (37.6) | 1 | | |
| Zone | | | | | |
| North-west (Kaduna) | 427 (72.1) | 165 (27.9) | 1.13 | 1.06-1.20 | 0.0001 |
| North-east (Bauchi) | 16 (80.0) | 4 (20.0) | 1.25 | 1.01-1.57 | 0.046 |
| North-central (Plateau, Nasarawa, FCT) | 1174 (63.8) | 666 (36.2) | 1 | | |
| Type of bird | | | | | |
| Commercial | 1138 (66.9) | 562 (33.1) | 1.05 | 0.99-1.12 | 0.1249 |
| Local | 479 (63.7) | 273 (36.3) | 1 | | |
| Flock size | | | | | |
| >5000 | 157 (87.2) | 23 (12.8) | 1.37 | 1.28-1.46 | <0.0001 |
| >1000-5000 | 322 (66.4) | 163 (33.6) | 1.04 | 0.97-1.12 | 0.259 |
| 1-1000 | 1138 (63.7) | 649 (36.3) | 1 | | |
| Age | | | | | |
| >18 weeks | 367 (67.9) | 173 (32.1) | 0.97 | 0.85-1.11 | 0.6578 |
| 11-18 weeks | 263 (78.5) | 72 (21.5) | 1.12 | 0.99-1.28 | 0.0836 |
| 1-10 weeks | 84 (70.0) | 36 (30.0) | 1 | | |
| Type of feed | | | | | |
| Self milled | 361 (75.2) | 119 (24.8) | 1.23 | 1.15-1.32 | <0.0001 |
| Commercial | 626 (61.1) | 399 (38.9) | 1 | RF | |
| Source of chicks | | | | | |
| South | 302 (75.5) | 98 (24.5) | 1.14 | 1.04-1.24 | 0.0033 |
| North | 295 (66.3) | 150 (33.7) | 1 | | |

*5.4.1.5 Source of birds*

Chicks sourced from the southern 75.5% (302/400) part of the Nigeria showed a significantly higher exposure (RR 1.14) of developing antibodies to MDV compared to those from the northern 66.3% (295/445) part of Nigeria (Table 5.4).

*5.4.1.6 Flock size*

Farms with more than 5,000 birds 87.2% (157/180) have a significant higher risk of MD (RR 1.37) (Table 5.4).

*5.4.1.7 Source of feed*

Farms that used self milled 75.2% (361/480) feed have a higher risk of (RR 1.23) of MD infection compared to farms that used commercial feeds (Table 5.4).

*5.4.2.1 Results of indirect enzyme linked imunosorbent assays*

The overall MDV antibody in avian species was 62.9% (95/151). Vaccinated commercial chickens tested positive 68.5% (74/108) for MDV antibody. Local unvaccinated chickens 76.5% (13/17) tested positive. Among other avian species, turkeys 41.7% (5/12) and quails 25.0% (3/12) were positive while, ducks 0% (0/2) sera tested negative for MDV antibody. Of the birds tested from Kaduna state, 45.0% (18/40), from Plateau state 84.8% (50/59), Nassarawa state 40.0% (8/20), Bauchi state 60.0% (6/10) and the FCT 59.0% (13/22) were positive for MDV antibodies (Table 5.5).

**Table 5.5: Seroprevalence of Marek's disease virus antibody in avian species in some parts of Nigeria using indirect enzyme linked immunosorbent assays.**

| Variable | Total sera tested | Number of sera positive | Percentage positive |
|---|---|---|---|
| **Location** | | | |
| Kaduna | 40 | 18 | 45.0 |
| Plateau | 59 | 50 | 84.8 |
| Nasarawa | 20 | 8 | 40.0 |
| Bauchi | 10 | 6 | 60.0 |
| FCT | 22 | 13 | 59.0 |
| Avian **species/vaccination status** | | | |
| Vaccinated commercial chickens | 108 | 74 | 68.5 |
| Unvaccinated local chickens | 17 | 13 | 76.5 |
| Unvaccinated turkeys | 12 | 5 | 41.7 |
| Unvaccinated quails | 12 | 3 | 25.0 |
| Unvaccinated ducks | 2 | 0 | 0.0 |

## 5.5 Discussion

Earlier seroprevalent studies in Nigeria on MD from the South West were reported by Adene (1983) who reported 16.4% and 8.3% seroprevalence for exotic and local chickens respectively. Nawathe *et al.* (1978) also reported a seroprevalence of 33.1% in local chickens, 12.0% in guinea fowls and 23.0% in improved commercial chickens in Jos, Plateau State, Nigeria, but they were unable to detect MDV antibodies in ducks and turkeys. In the present study, the seroprevalence of MD was 63.7% in local chickens and 66.0% in improved chickens which are higher than the previous findings. This could be explained based on the difference in sample locations, source, and size and the increase in the number and size of commercial farms, incidence of MD and level of MD vaccination. However, it is difficult to explain why the seroprevalence reported by Nawathe *et al*. (1978) was higher in local chickens than in improved poultry. The lack of significant difference in seroprevalence of MD by age group is an indication of equal chance of exposure to MDV or MD vaccines. Local chickens that are normally unvaccinated might have been exposed to MDV via direct contact with infected commercial poultry or indirectly via inhalation of infected dander or feather or contaminated dust or manure from commercial poultry.

Overall seroprevalence obtained by AGPT and ELISA were comparable. Hence for screening of Marek's disease virus antibody, AGPT can be used as a substitute without compromising the validity of the results as reported (OIE, 2010).

Plateau State had the highest seroprevalence for MDV and this could be due to high concentration of commercial poultry farms. It is possible that the level of MD vaccination was highest in the state.

Chickens between the ages of 11 to 18 weeks had the highest seroprevalence for MDV because they were mostly growers reaching the point of lay which agreed with similar reports that in chickens, MD occurs at 3-4 weeks of age or older and is most common between 12 and 30 weeks of age (Nair *et al*., 2008; Schat and Nair, 2008; OIE, 2010).

Chicks sourced from the South had the highest seroprevalence for MDV because of the high concentration of hatcheries and commercial poultry farms. It is possible that the chance of MDV exposure is high due to commercial poultry activities in the south.

The report by Nawathe *et al.* (1978) that Marek's disease prevalence was higher in local than in improved poultry is in agreement with the 76.5% seroprevelance obtained in this study using iELSA. Nawathe *et al*. (1978) had earlier reported MDV antibody in guinea fowls but the findings of the present work is the only evidence of the presence of antibody to MDV in turkeys and quails in Nigeria. The findings of high seroprevalence for commercial chickens for MDV could be due to MDV vaccination, genetic susceptibility or birds being housed in close contact.

That the seroprevalence for MDV in unvaccinated commercial birds was higher than in unvaccinated local chickens, this could be because of genetic susceptibility, close bird

contact and production stress which facilitate spread of infections . Vaccinated commercial birds had high seroprevalence for MDV than unvaccinated commercial birds this could due to MD vaccination. Overall, the MDV seroprevalence in vaccinated birds was higher than in unvaccinated birds due to vaccination.

Farms with flock size above 5000 birds had the highest seroprevalence for MDV this could be due to close contact since MDV spreads horizontally therefore high population density will increase the risk of exposure to MDV infection. Birds fed with self-milled feed was higher in MDV seroprevalence than the commercial feed fed because of the high chances of feed contamination and exposure of the birds to MD infection. Zelnik *et al*. (2004) reported that the continuous rise in MDV antibody response in vaccinated birds strongly suggests a recurring exposure of the immune system to antigens produced in the lytic phase of MD. Lee *et al*. (1983) reported that sera from MDV infected convalescent chickens generally have low titre and cross reactive antibodies this could account for the variations in optic densities and results in ELISA. The results showed that even after vaccination against Marek's disease, not all vaccinated commercial chickens acquired over 90% positivity as reported by Witter and Schat (2003).

Ducks tested not positive to MDV antibodies probably because they were not exposed or infected by MDV. All the locations were positive for MD indicating that the disease is widespread as already reported in the south western Nigeria (Adene, 1975; 1983). The ELISA seroprevalence of MD in 151 sera of different poultry found to be 62.9% was higher than the estimated MD seroprevalence reported in some parts of the country by

Adene (1983) and Nawathe *et al*. (1978). This study has revealed that the ELISA is a good screening test for MD in unvaccinated birds as recommended by OIE (2010).

## 5.6 Conclusions

The study gives the current status on MD in the study area. The overall MD seroprevalence using AGPT was high (66.0%). The seroprevalence of MD was highest in North East Nigeria (80%). Seroprevalence was high in both layers (66.9%) and local chickens (63.7%). The overall MD seroprevalence using ELISA was high (62.9%). Marek's disease virus antibodies were detected in commercial vaccinated chickens (68.5%) and local unvaccinated chickens (76.5%), turkeys (41.7%) and quails (25%) but not in ducks using ELISA. The sera were screned using AGPT and ELISA because ELISA is more sensitive and specific than AGPT.

## 5.7 Recommendations

1.There should be strict adherence to biosecurity practices among poultry farmers to reduce early exposure of chicks to field MDV before establishment of vaccinal immunity.

2. Futher research is needed to screen more avian species for MDV antibodies, isolate and characterize field MDV.

3. A nationwide screening of chickens for MDV antibody in Nigeria is needed to establish its true status.

**4.** Birds should analyzed according to type of of bird.

# CHAPTER SIX

# CLINICAL SIGNS, GROSS AND MICROSCOPIC LESIONS IN CHICKENS NATURALLY INFECTED AND THOSE EXPERIMENTALLY CHALLENGED WITH MAREK'S DISEASE VIRUS IN NIGERIA AND USA

## 6.1 Abstract

The history, clinicopathological manifestations and the extent of mortality due to MD in commercial poultry farms in some parts of Kaduna, Plateau, Nasarawa and Bauchi States and the FCT were evaluated. Tissues of MD affected organs from carcasses of chickens were collected between 2005 and 2009 were fixed in 10% buffered formalin, trimmed and sectioned at about 6 microns and stained with haematoxylin and eosin, made into slides for microscopic examination. The gross and histopathological lesions due to field MD were compared with chickens experimentally challenged with vvMDV in Avian Disease Oncology Diagnostic Laboratory East-Lansing, Michigan. As a control measure for MD, the type of vaccines used by the farmers were as follows: 15 farms (48.39%) out of 31 farms gave HVT and 6 Rispens (19.35%) while 2 farms (6.45%) did not vaccinate chickens. Also, there was no record of Marek's disease vaccination by 8 farms (25.8%). The management systems were deep litter which was widely practiced, 27 (87.09%) and battery cage system 4 (12.91%). Clinical manifestations of MD investigated were morbidity and mortality, paralysis, diarrhoea, somnolence, inappetence, poor development of comb and wattles, ruffled feathers and stunted growth. On presentation, there were generalized emaciation with prominent keel bones, sunken eyes, paled comb and wattles were the signs recorded. Postmortem examinations showed MD lymphomas in cutaneuos-muscular (skin leukosis), respiratory (lungs), cardiovascular (heart), digestive (liver, proventriculus, gizzards and intestine), lymphoid (thymus, spleen and bursa of Fabricius),

nervous (nerve trunks and brain), urogenital (kidneys with adrenal glands) and reproductive (testicles and ovaries) systems. The lymphomas in organs affected were either nodular (discrete, multifocal, localised, raised and greyish white about 0.2 to 10 mm in diametre), diffused (enlarged, multifocal pin point or milliary). Grossly, the most affected organ was the liver followed by the spleen. Histopathological findings of the field cases were comparable with experimentally challenged chickens. Cellular infiltrations were predominantly pleomorphic lymphocytes. This investigation clinicopathologically confirmed that MD outbreaks in investigated farms were likely to be caused by virulent strains of MDV. It is recommended that the MD viruses in Nigeria be isolated, characterized and pathogenicity studies conducted. More efficacious vaccines than HVT and biosecurity would assist in controlling MD outbreaks in Nigeria.

## 6.2 Introduction

Marek's disease is an economically important neoplastic disease of poultry (Purchase, 1985; Jwander, 2008). Marek's disease virus infection has many effects (Handberg *et al*., 2001). Marek's disease virus initially cause cytolytic infection in B-lymphocytes and the infected activated T-lymphocytes where it becomes latent (Nair *et al*., 2008). In susceptible chicken genotypes MDV transforms $CD4^+$ lymphocytes, causing visceral lymphomas or neural lesions and paralysis (Nair *et al*., 2008). Chickens may be vaccinated with either MDV-1 or HVT vaccines (OIE, 2010). Layers vaccinated against MD are not hundred per cent protected against Marek's disease (Witter and Schat, 2003). The typical signs of MD are paralysis of the legs, wings and neck, general morbidity and listlessness, weight loss, blindness (Davidson and Nair, 2005). This study was conducted to investigate field outbreak of MD in Nigeria to compare the clinical signs, gross and histospathological

lesions with those of chickens experimentally challenged at the ADOL-USDA, USA so as to serve as diagnostic control.

## 6.3 Materials and Methods

### 6.3. 1 Study area

Investigations were carried out in 31 farms in the following areas; Samaru, Zaria and Kaduna environs in Kaduna State; Bukuru, Topp, Vom, Jos North, Jos East and South, Mangu, Pankshin and its environs in Plateau State; Toro in Bauchi State; Lafia, Akwanga, Keffi, Nasarawa, Karu, Mararaba and Nasarawa State and Nyanya in the FCT (Abuja) (Figure 6).

### 6.3. 2 Outbreak of Marek's diease in Nigeria

Thirty-one outbreaks of MD in five years in 93.6% (2005 -2009) commercial flocks were reported to veterinary clinics and diagnostic centres in the study areas among which were the Poultry Unit of the Ahmadu Bello University Veterinary Teaching Hospital (ABUVTH), Zaria Kaduna; Evangelical Church Wining All (ECWA) Veterinary Clinic Bukuru and Central Diagnostic Laboratory of the National Veterinary Research Institute (NVRI), Vom both in Plateau State. The MD affected farms were visited and flock histories such as flock size, age of chickens, number dead, age of the chickens at the time of onset of the disease, MD vaccination status and egg production were obtained. The clinical observations included were morbidity and mortality, paralysis, diarrhoea, somnolence, inappetence, poor development of comb and wattles, ruffled feathers and stunted growth. On presentation, there were generalized emaciation with prominent keel

bones, sunken eyes, paled comb and wattles were the signs recorded and pathological observations included were gross lesions that might be suggestive of neoplastic growth in the visceral organs such as the liver, lungs, heart, spleen, kidneys, nerves, skin and brain (Table 6.1).

All carcasses and moribund birds submitted were routinely subjected to postmortem examination for MD as proposed by Goodchild (1969) and reviewed by Adene (1980), OIE (2008). Pieces of the liver, heart, lungs, skin, kidneys, gizzard, ovaries, sciatic nerves and the brain were collected and fixed in 10% buffered formalin. The tissues were fixed, trimmed and sectioned at about 6 microns and stained with haematoxylin and eosin, made into slides for microscopic examination.

### 6.3.3 Study of chickens experimentally challenged with very virulent strain of Marek's disease virus

With the kind permission of the Director of Avian Disease and Oncology Laboratory, United States Department of Agriculture (ADOL-USDA), East Lansing, Michigan USA, tissue samples from different organs were obtained from chickens experimentally challenged with virulent MDV strains by Dr. Dunn John. The experimentally challenged chickens used were strictly raised and kept in isolators of specific pathogen free (SPF). Regular visits were made to the isolators and carcasses were collected before the terminal stage of the experiment at about the 16th week of age. During postmortem examination, tissues from different organs collected were fixed in 10% buffered neutral formalin and.

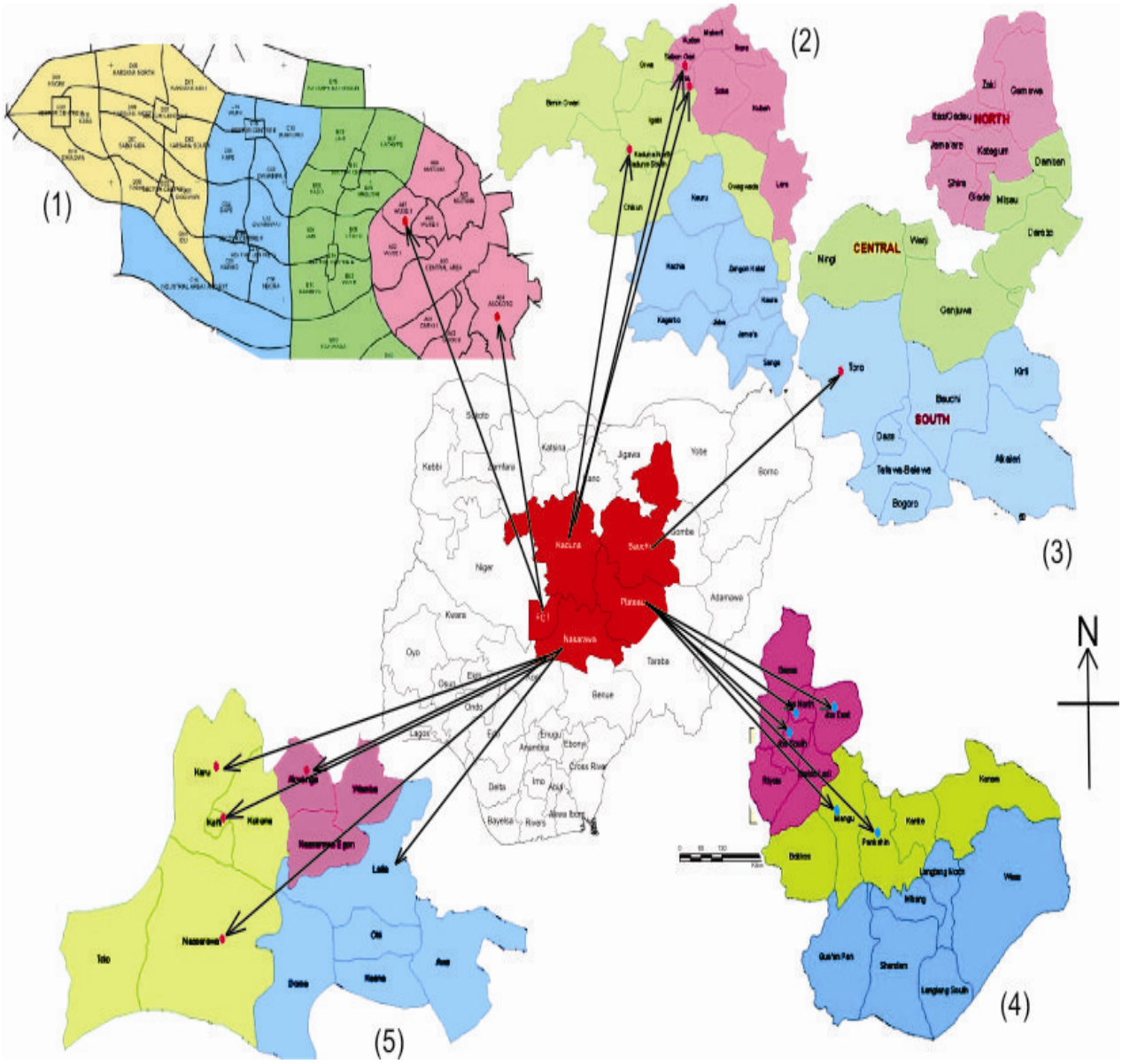



**Figure 6: Map of Nigeria showing some location of States in red colour with LGAs where Marek's disease field outbreak were investigated between 2005-2009. (1) Wuse and Nyanya in Asokoro district of FCT, (2) Kaduna north, Zaria and Sabo in Kaduna State, (3) Toro in Bauchi State, (4) Jos south, nort and east, Mangu and Pankshin in Plateau State, (5) Lafia, Akwanga, Keffi, Nasarawa and Karu in Nasarawa State. (Map of study site was designed in ArcView GIS version 3.1).**

sent to the Diagnostic Center for Population and Animal Health, Michigan State University for processing into slides for microscopic examination.

## 6.4 Results

### 6.4. 1 Results of investigation, Marek's disease outbreak and experimentally challenged

In all, 31 poultry farms were involved with suspected MD outbreaks. Deep litter system (27 farms; 87.09%) was widely practiced with very few battery cages 4 farms; 12.91%). Two of the farms in Vom were not vaccinated against MD. Vaccines mostly used against MD in farms were HVT (15; 48.39%), Rispens 6; 19.35%) and 2 (6.45%) farms were not vaccinated while, 8 (25.8%) farmers could not disclose the type of MD vaccines used.

### 6.4.2 Clinical signs of Marek's disease

Affected chickens died suddenly without premonitory signs in some flocks while in others there were loss of weight, anorexia, ataxia, pale underdeveloped comb and wattles, stunted growth, diarrhoea of different colourations, drop in egg production, eye opacity and paralysis of legs, neck and wings (Plate II, III and IV). Experimentally challenged chickens showed the following clinical signs: motality, mobidity, somnolence, ruffled feathers, loss of weight, poor growth, lack body size uniformity and underdeveloped comb and wattles.

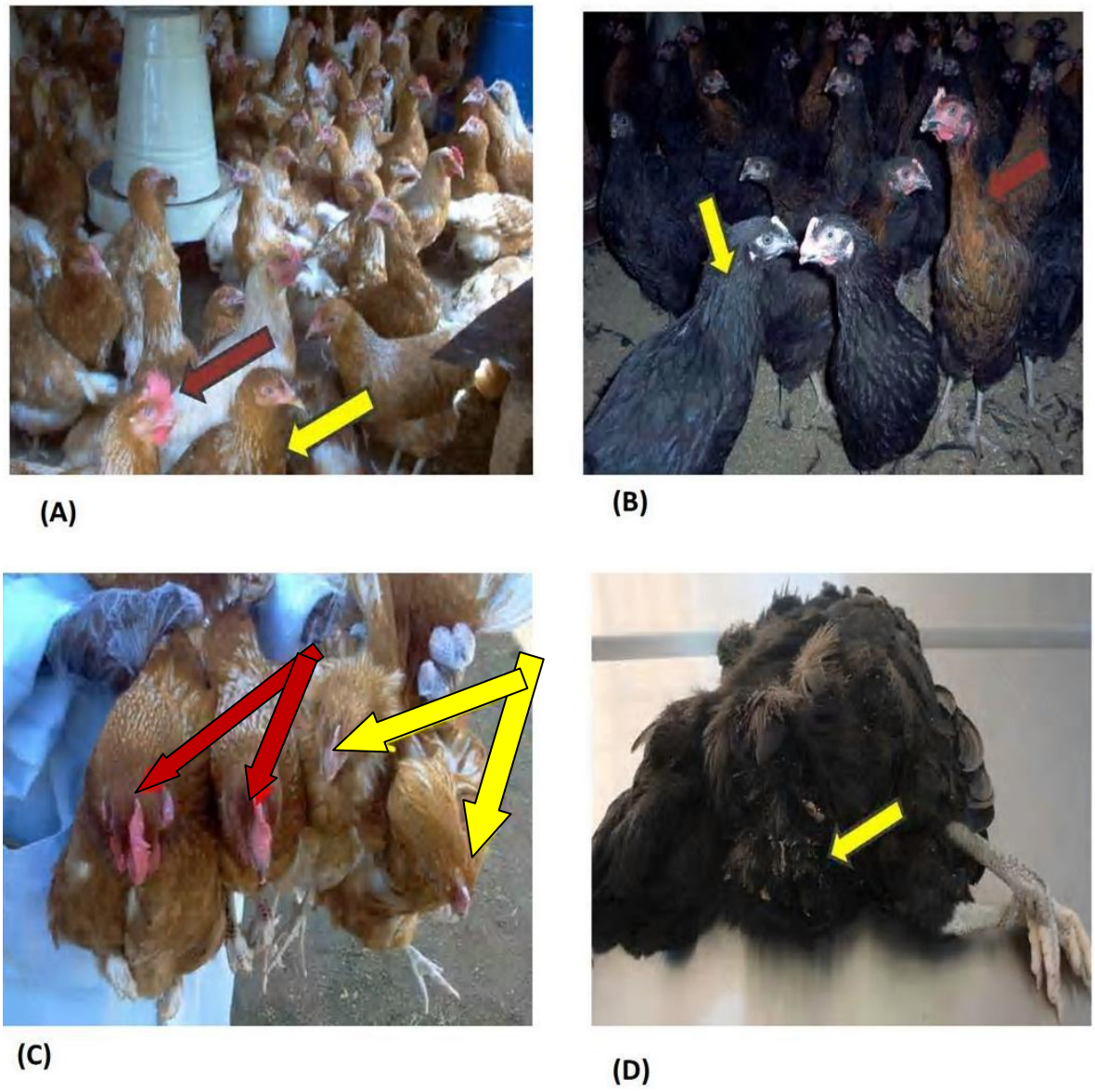



**Plate II: Field pictures of comb, wattles and matted vent showing cross section of clinical signs of different flocks of layers similar to Marek's disease**.

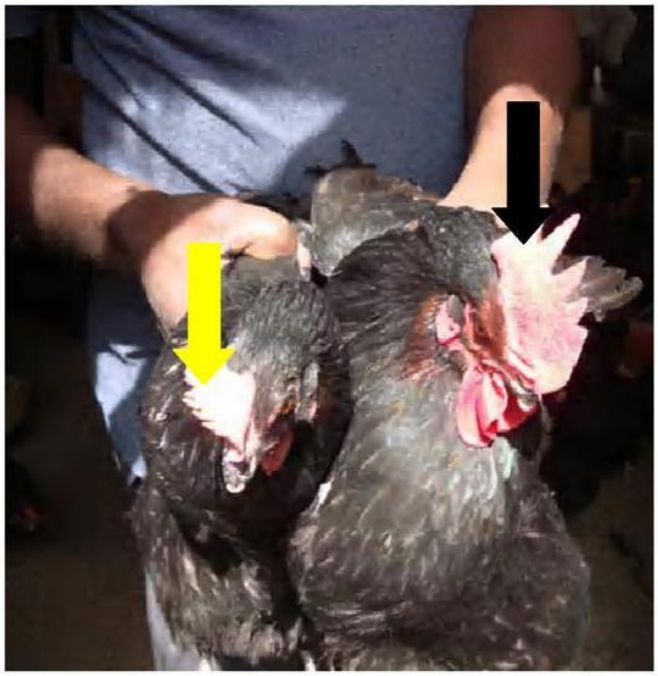
A = red arrowed chicken that is laying while, yellow arrow = chickens not in lay, B = Layers not in production, C = Poor growth, pale, undeveloped comb/wattles in layers from the same flock in yellow arrow while red arrows are layers in egg production. D = Moribund layer with matted vent.



(A)



(B)



(C)

**Plate III: Field Marek's disease further affecting organs of layers involvement.**

A = Field chicken that suffered from MD showing matted vent with greenish yellow diarrhea, B = Field chicken that suffered from MD showing ocular opacity, C = Field chicken that suffered from MD with poor comb and wattles development in yellow arrow.

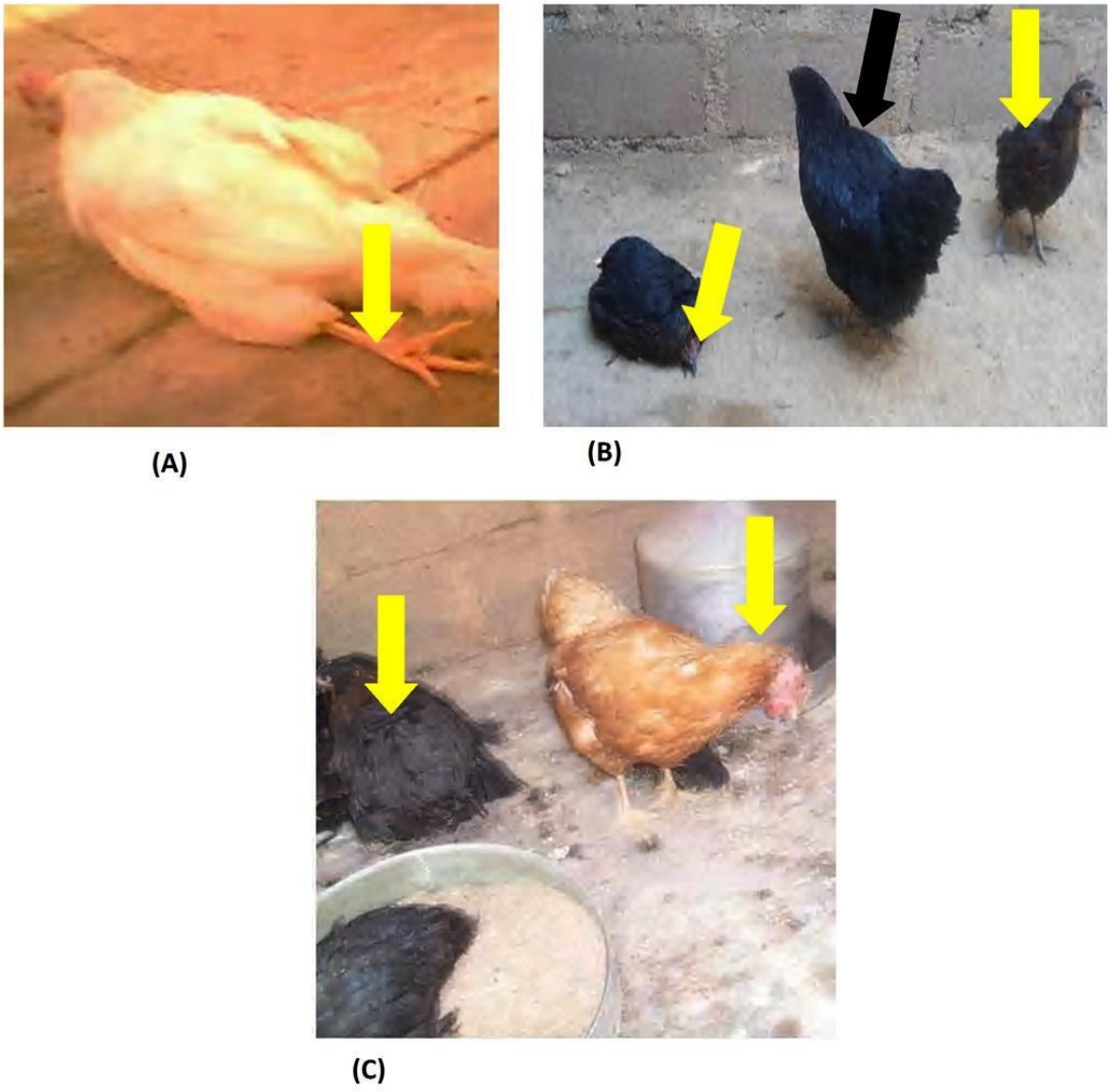



**Plate IV: Field chickens that suffered Marek's disease showing neurological syndromes and growth variations.**

A = Field cock that suffered from MD showing classical unilateral leg paralysis in an unvaccinated flock.

B = Field chickens that suffered from MD in the same flock of layers, showing variable stunted growth, twisted wings and neck (yellow arrows) with normal layer (black arrow)

C = Field chickens that suffered from MD; twisted neck in a brown layer and somnolence in black layers of the same flock.

### 6.4.3 Gross pathology

The neoplastic lesions which were diffused or focal, commonly affected one or more of parenchymatous organs. The focal lesions produced nodules of varying sizes ranging from about 0.2 mm to 10 mm in diametre. The tumours on the skin sometimes coalesce and formed tumour masses (Plates V). Nodular and diffused lesions were seen on the heart while the lungs had nodules (Plate VI). The liver had nodules but was sometimes diffusely enlarged in field and challenged (Plate VII). Nodules were present in the small intestine and proventriculus (Plate VIII).

The spleen had nodules or was diffusely enlarged (Plate IX). Thymic lobes were moderately or severely atrophied (Plate X). Testicular and adrenal gland involvement was characterized by diffuse enlargement and greyish discoloration of the organs while the ovaries were cauliflower like in the challenged and the kidneys were enlarged and grayish (Plate XI; Plate XII). The vagus nerves showed unilateral enlargement in the challenged (Plate XIII). Some carcasses showed no gross lesions in the same flocks.

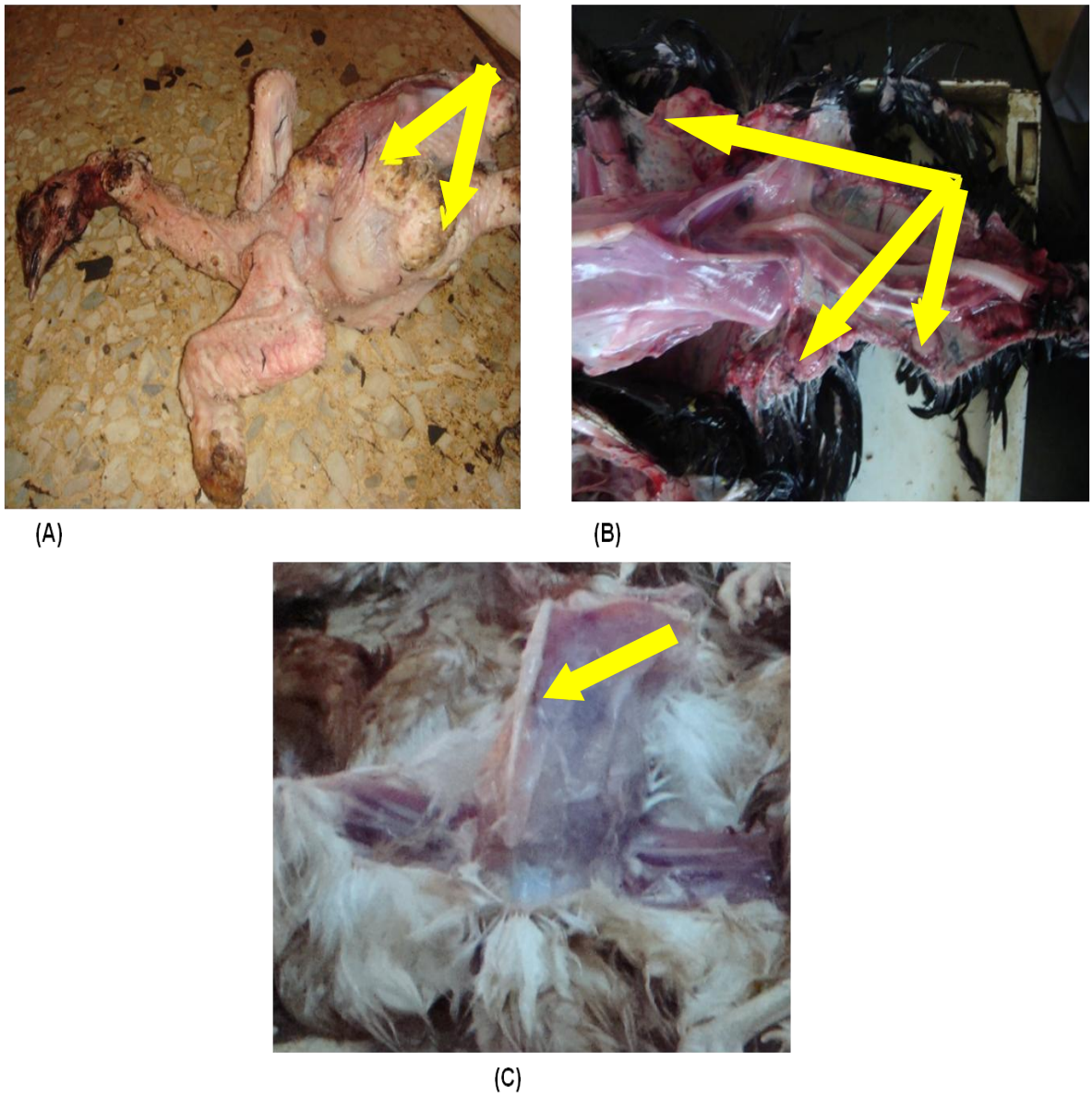
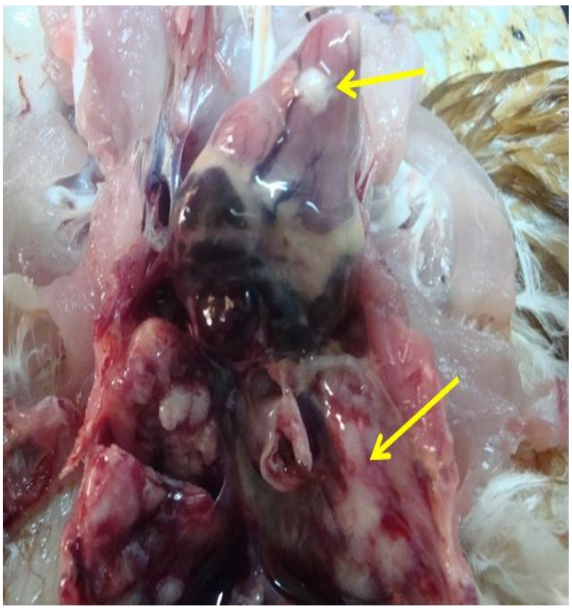


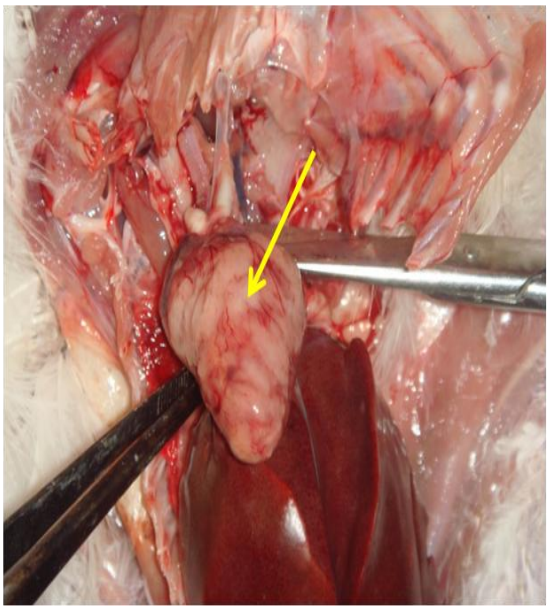

**Plate V: Field carcasses from layers that suffered from Marek's disease involving cutanous and musculoskeletal systems.**

A and B = Field carcasses from chickens that suffered from MD; feather follicles, skin and muscular tumours.

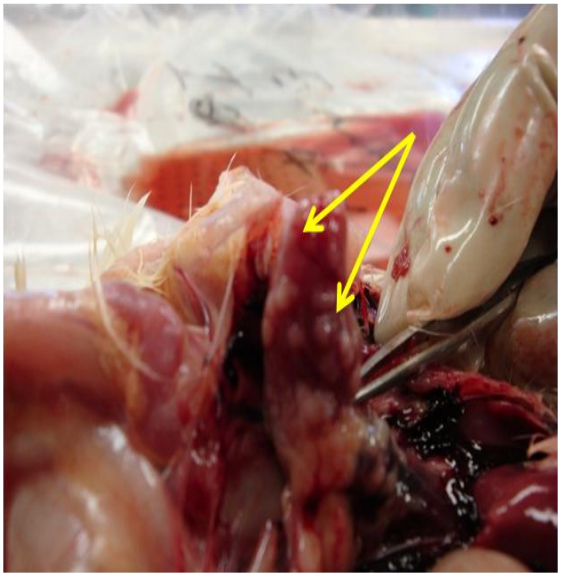
C = Field carcass from chicken infected with MD; severe loss of flesh with prominent keel bone.



(A)



(B)



(C)

**Plate VI: Carcasses with lymphomas in the heart and lungs due to field and challenged Marek's disease.**

A = Field chicken carcass with MD; lymphomas in heart (focal) and lungs (multiple foci). B and C = chicken carcasses with challenged MD; lymphomas of heart muscles (diffused and generalized in B while multifocal in C).

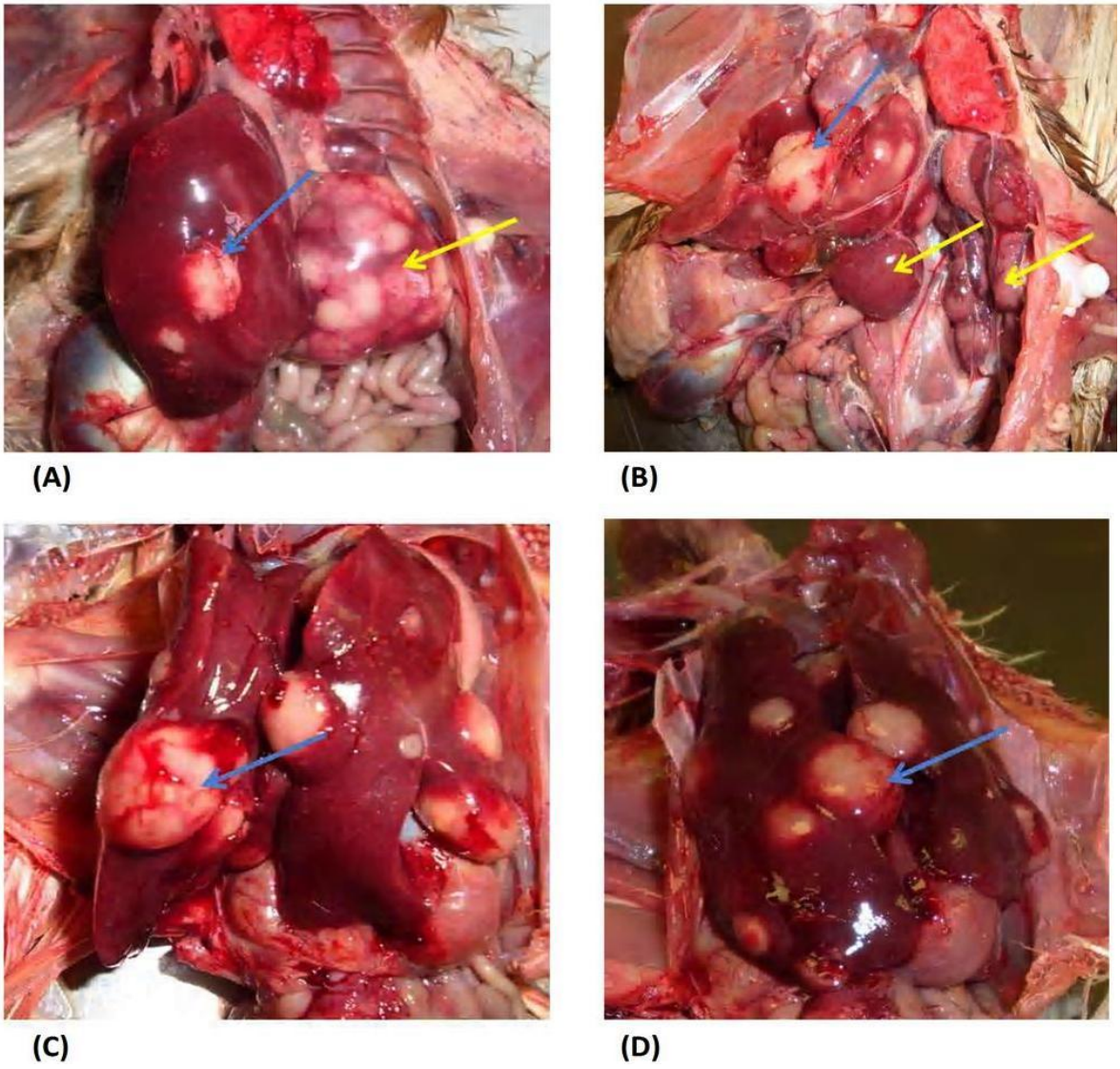



**Plate VII: Chicken carcasses with lymphomas in the liver and spleen in field and challenged Marek's disease cases.**

A and B = Field chicken carcasses with MD; lymphomas in livers (blue arrows), spleens and kidneys (yellow arrows).

C and D = Chicken carcasses with challenged MD; lymphomas in the livers showing massive raised multiple tumours.

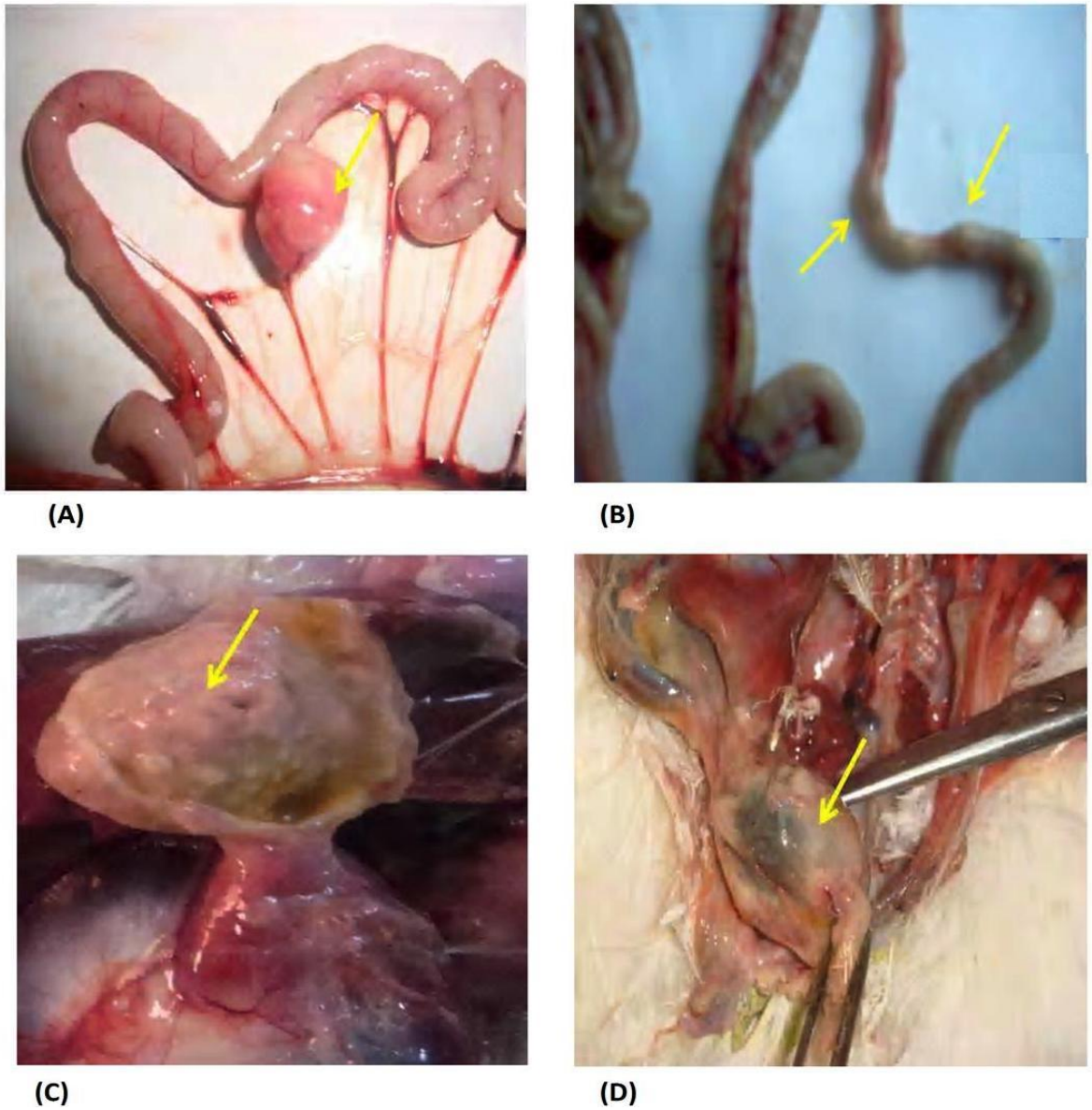



**Plate VIII: Lymphomas of the mesenteric artery, intestine and proventriculus due to field Marek's disease.**

A = from field MD; mesenteric artery with discrete firm tumour, B = from field MD; localized intestinal nodules, C and D = from field MD, proventricular nodules.

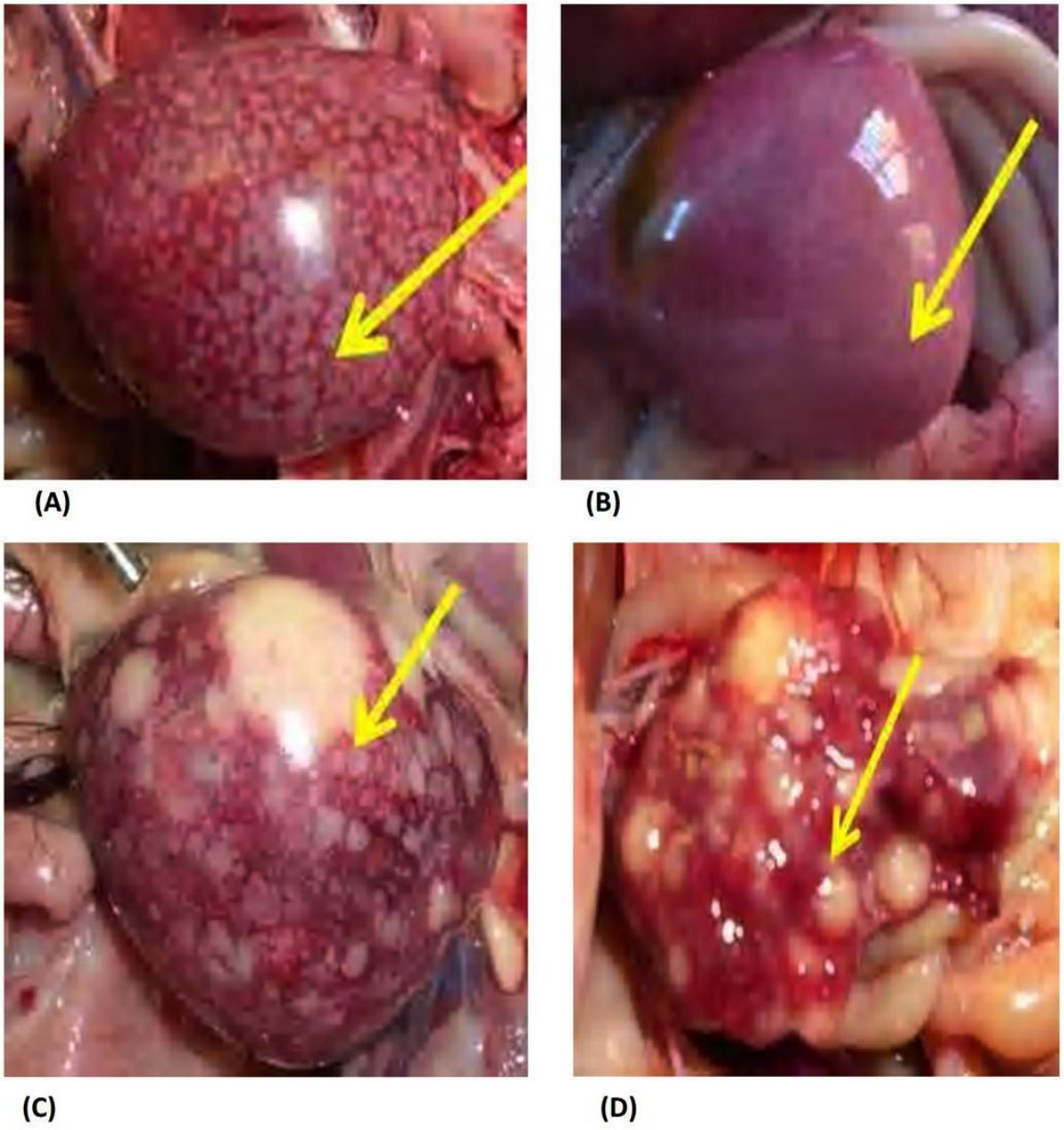



**Plate IX: Chicken carcasses with lypmhomas in the spleen due to field and challenged Marek's disease.**

A, C and D = Chicken carcasses with challenged MD; lymphomas (enlarged and wide spread milliary, diffused and nodular) in the spleens,

B = Field Chicken carcass with MD; lymphomas in the enlarged and diffused spleen.

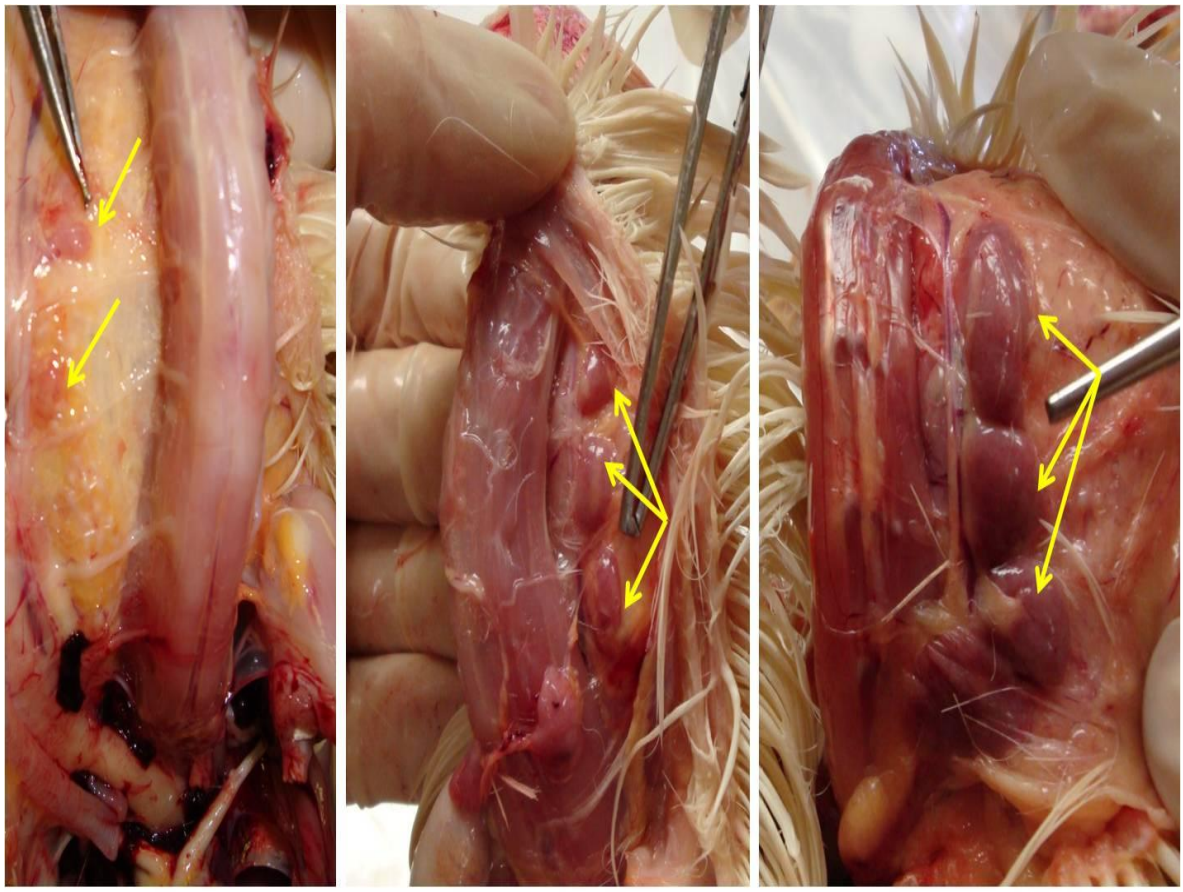



**Plate X: Atrophy of the thymic lobes in Chicken carcasses challenged with Marek's disease.**

A and B = Chicken carcasses with challenged MD; atrophy of the thymic lobules (with A severe, and B moderate) compared with control (C).

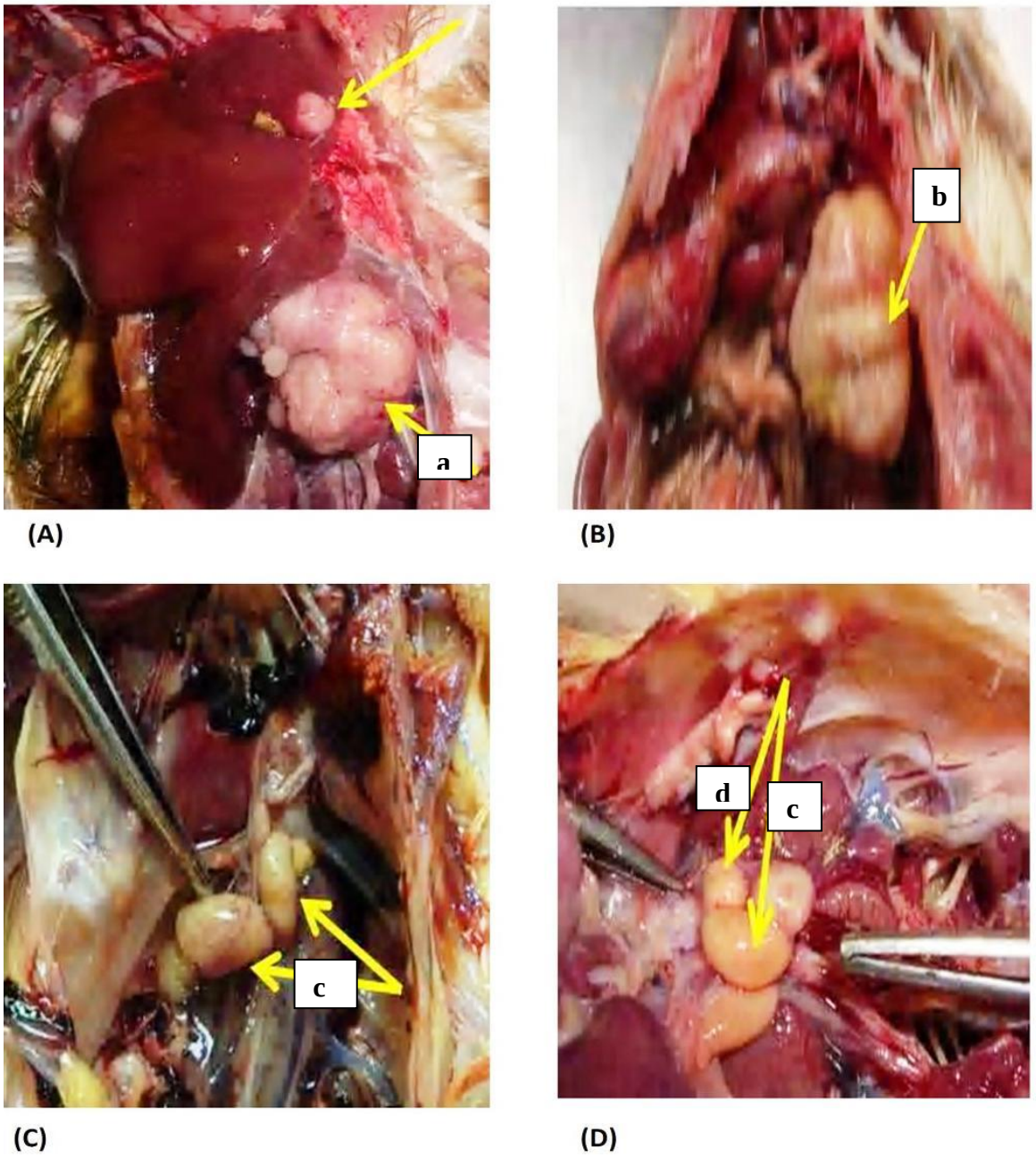
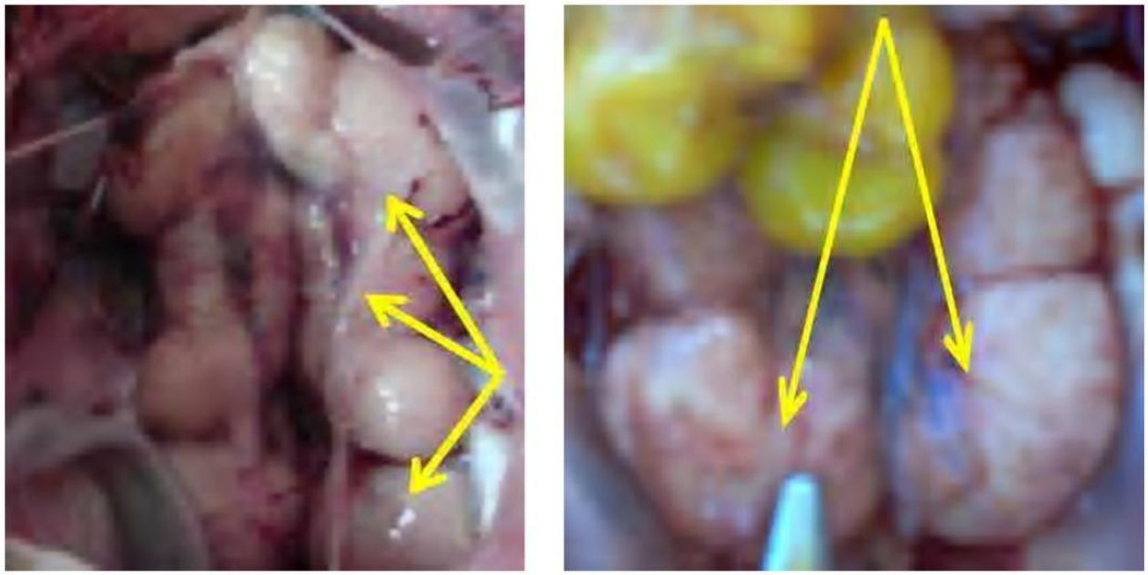



**Plate XI: Enlargement or atrophy of the ovary, testicles and adrenal glands in field and challenged due to Marek's disease .**

A = Carcas from field chicken with MD; (a) atritic ovary (cauliflower-like) with liver showing discrete tumour.

B = Chicken carcass with challenged MD; (b) ovary (cauliflower-like), C and D = challenged cockerel with MD; lymphomas of unilateral testicle (c) and adrenal gland (d).



(A)



(B)



(C)

**Plate XII: Chicken carcasses that suffered from Marek's disease showing lymphomas and diffused enlargement of the kidneys in field and challenged cases.**

A and B = Carcasses from field chickens with MD; kidneys severely enlarged

C = Carcass with MD (challenged) severely enlarged and haemorrhagic kidneys

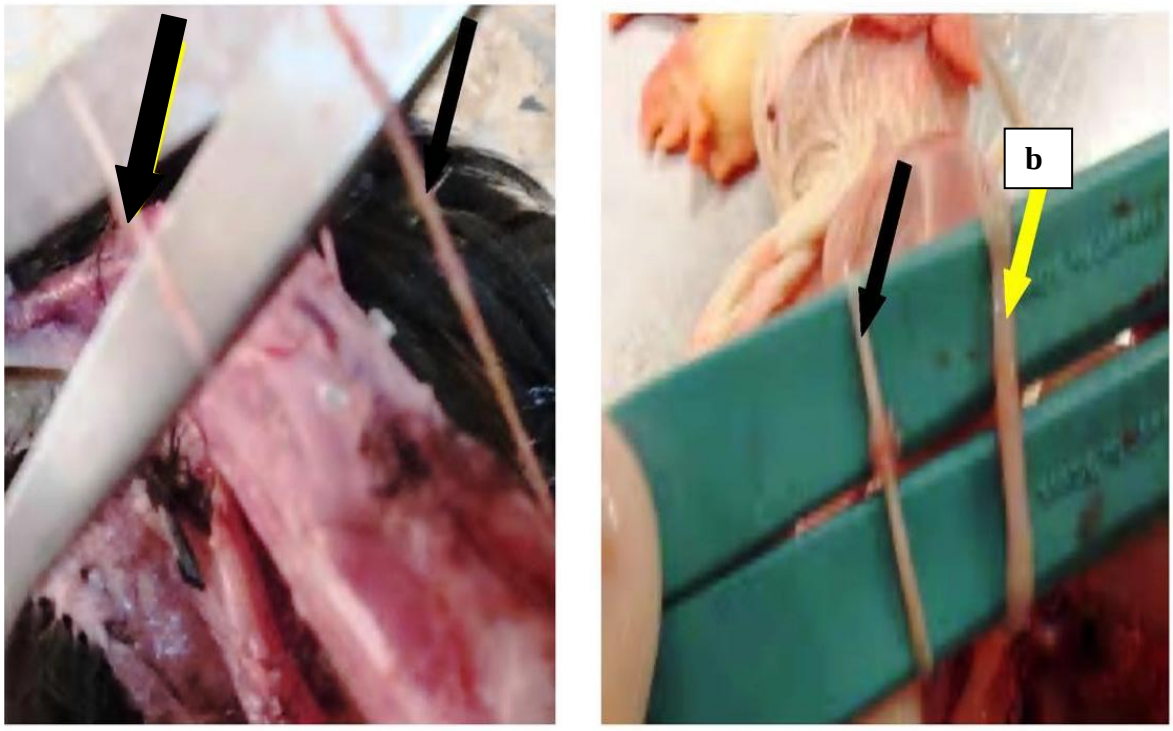



**Plate XIII: Carcasses from Field (A) and challenged (B) chickens with Marek's disease; showing enlargement of vagus nerve in yellow arrows with normal in black arrows.**

Carcass with challenged MD (B) showing unilateral enlargement of vagus nerve (b) yellow arrows), about three times the normal size (black arrow).

## Table 6.1: Summary of history and clinicopathological lesions of Marek's disease outbreak investigated in 31 farms in Nigeria.

| Farm S/N | Flock size at day old | Birds type/ sytem | Present age of birds | Age onset of MD (wks) | No. dead | Type of vaccine used | Clinical signs | Gross lsesions |
|---|---|---|---|---|---|---|---|---|
| 6 | 1500 | ly,dl | 30 | 2 | 1440 | HVT | sd,pg, wt, du,dh, | elv, espl, ekd, pov |
| 28 | 3,000 | ly,bc | 20 | 4 | 2137 | - | sd,wt,pcw,du | enlv,espl, cgkd, |
| 10 | 1800 | y,dl | 24 | 7 | 524 | HVT | sd,wt,pcw,du | espl,net,ekd |
| 1 | 1500 | ly,dl | 23 | 8 | 900 | - | wt,pcw,du,dh | nlv,cgs, cgkd,eln |
| 12 | 860 | ly,dl | 23 | 8 | 444 | HVT | wt,pcw,du | dflv,elspl,pov |
| 16 | 500 | pl,dl | 15 | 8 | 140 | Rispens | sd wt,pcw,du | enlv,espl, cgkd, |
| 22 | 550 | pl,dl | 15 | 8 | 150 | HVT | sd wt,pcw,du | enlv,espl, cgkd, |
| 27 | 2512 | pl,dl | 13 | 8 | 502 | No | sd,wt,pcw,du, | st, nlv, cgs, cgkd |
| 5 | 4000 | ly,dl | 25 | 9 | 2000 | HVT | sd,wt,pcw,du | enlv,espl, cgkd, |
| 7 | 700 | ly,dl | 25 | 9 | 212 | Rispens | wt,pcw,du,dh | enlv,espl, cgkd, |
| 11 | 24 | ck,dl | 16 | 9 | 19 | No | lp,wp,du,pcw,dh, | nlv,cgs, cgkd |
| 2 | 350 | ck,bc | 24 | 10 | 190 | - | wt,pcw,du | enlv,cgs, cgkd, |
| 4 | 1000 | ly,dl | 28 | 12 | 200 | HVT | wt,pcw,du | dflv,dfspl, cgkd,pov |
| 9 | 1500 | ly,dl | 21 | 12 | 331 | HVT | sd,wt,pcw,du | nlv,cgs, cgkd,eln |
| 17 | 600 | pl,dl | 15 | 12 | 108 | - | wt,pcw,du | elv,net |
| 3 | 1500 | ly,dl | 30 | 13 | 200 | HVT | wt,pcw,du,dh | elv, espl, ekd, pov |
| 14 | 2100 | ly,dl | 19 | 13 | 100 | Rispens | wt,pcw,du | dflv,dfspl, cgkd,pov |
| 15 | 1500 | pl,dl | 17 | 14 | 200 | HVT | sd,wt,pcw,du | nlv,cgs, cgkd |
| 21 | 1400 | ly,dl | 25 | 14 | 538 | HVT | wt,pcw,du | nlg,nht, nlv |
| 26 | 13,000 | ly,dl | 27 | 14 | 4000 | HVT | lp,wt,pcw,du | elv,cgspl,pov |
| 25 | 3,700 | ly,dl | 25 | 16 | 140 | Rispens | sd,wt,pcw,du,dh | dflv,dfspl, cgkd,pov |
| 13 | 2000 | ly,dl | 32 | 18 | 200 | Rispens | wt,pcw,du | elv, espl, ekd, pov |
| 19 | 4000 | ly,bc | 22 | 18 | 850 | - | wt,pcw,du,dh | nlv,dfspl, cgkd, |
| 20 | 1000 | ly,dl | 44 | 18 | 480 | - | wt,pcw,du | dflv,npr,elkd,pov |
| 23 | 100000 | ly,dl | 25,60 | 18 | 40,000 | HVT | sd,wt,pcw,lp,np,wp,du,dh | enlv,cgs, cgk,pov |
| 24 | 30,000 | ly,dl | 57 | 18 | 4216 | HVT | wt,pcw,du,dh | nlv,enspl,cfov |
| 30 | 5,000 | ly,dl | 36 | 18 | 2,200 | - | wt,pcw,du,dh | enlv,espl, cgk,cfov |
| 18 | 2000 | ly,dl | 32 | 19 | 400 | HVT | sd,wt,pcw,du | Nht,nlg,enlkd,pov |
| 31 | 2,520 | ly,dl | 37 | 19 | 280 | Rispens | wt,pcw,du | enlv,cgs, cgk, |
| 8 | 12,000 | ly,dl | 26 | 21 | 3740 | - | wt,pcw,du | st, nlv, cgs, cgk |
| 29 | 6,400 | ly,bc | 42 | 27 | 800 | HVT | wt,pcw,du,dh | dflv,dfspl, cgk,pov |
| Total | 208,516 | | 27.28 | Mean 13 | 67,641 | | | |

KEYS: **Bird type**; p = pullet, bl = broilers, ck = cockrel, 1y= laryers. **Management system**; dl = deep litter, bc = battery cage. **Vaccine type;** HVT = turkey herpes virus vaccine, Rispens/CV1988 vaccine, No = not vaccinated, - = indifferent about MD vaccination. **Clinical signs**; sd = sudden death, du = dullness, ob = ocular blindness, pg = poor growth, pcw = pale comb/wattles, dh = diarrhoae, wt = weight lost, lp = leg paralysis, pw = wing paralysis, np = neck paralysis. **Gross lesions**; st = skin tumour mass, cglg = congested lungs, nlg = nodular lungs, nht = nodular heart, eht = enlarge heart dfht = diffuse heart, elv = enlarged liver, cglv = congested liver, nlv = nodular liver, dflv = diffuse liver, espl = enlarge spleen, eln = enlarge nerves,cgkd = congested kidneys, cgspl = congested spleen, enlg = enlarge lungs, ekd = enlarge kidneys, dfs = diffused spleen, pov = poor ovary,cfov = cauliflowerlike overies.net = nodular intestine, npr = nodular proventriculus.

### 6.4.4 Histopathology

Massive infiltrations of neoplastic lymphoid cells occurred in the affected organs: brain (Plate XIV), heart (Plate XV), lungs (Plate XVI), liver (Plate XVII), kidneys (Plate XVIII), nerves (Plate XIX).

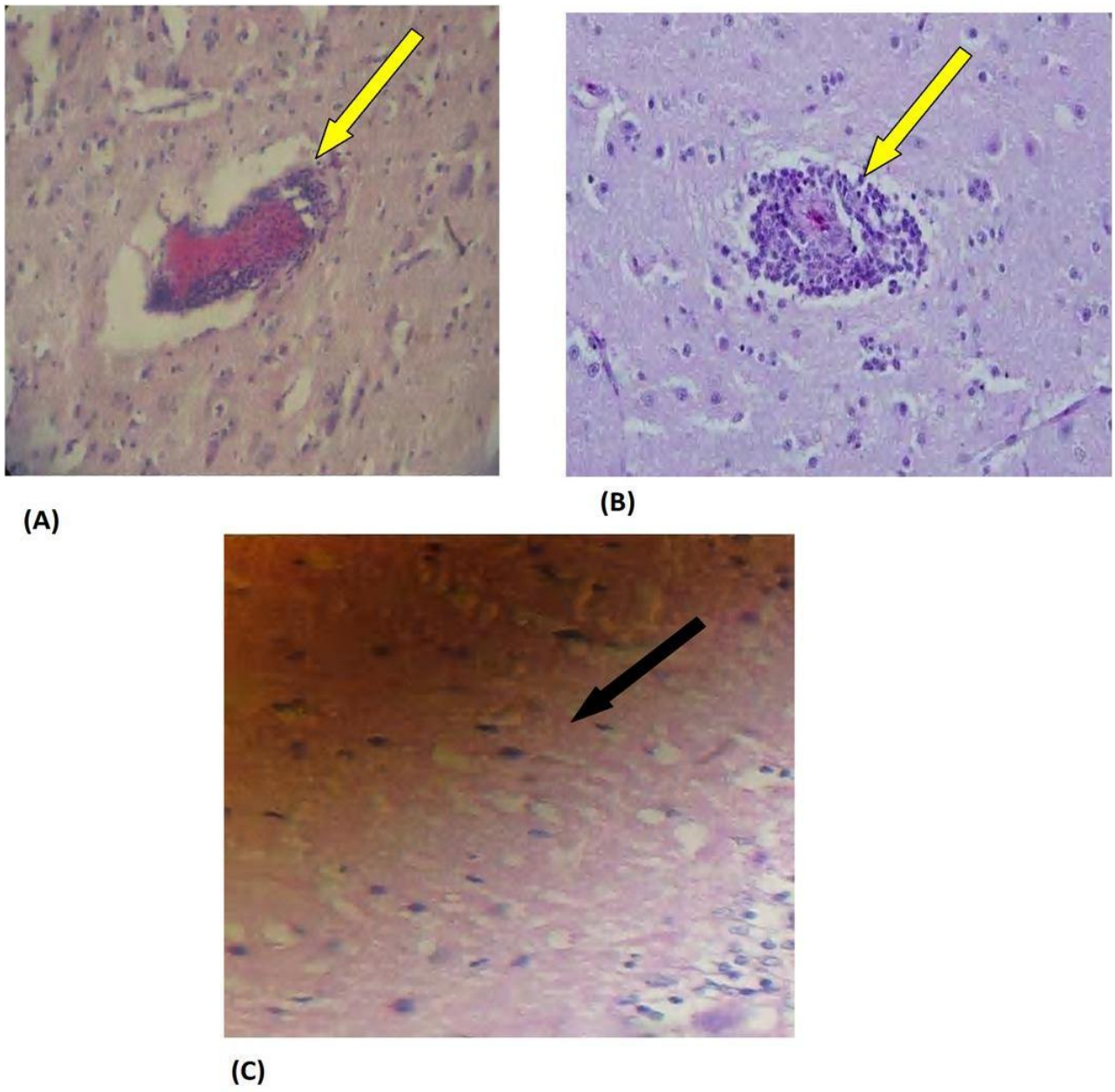
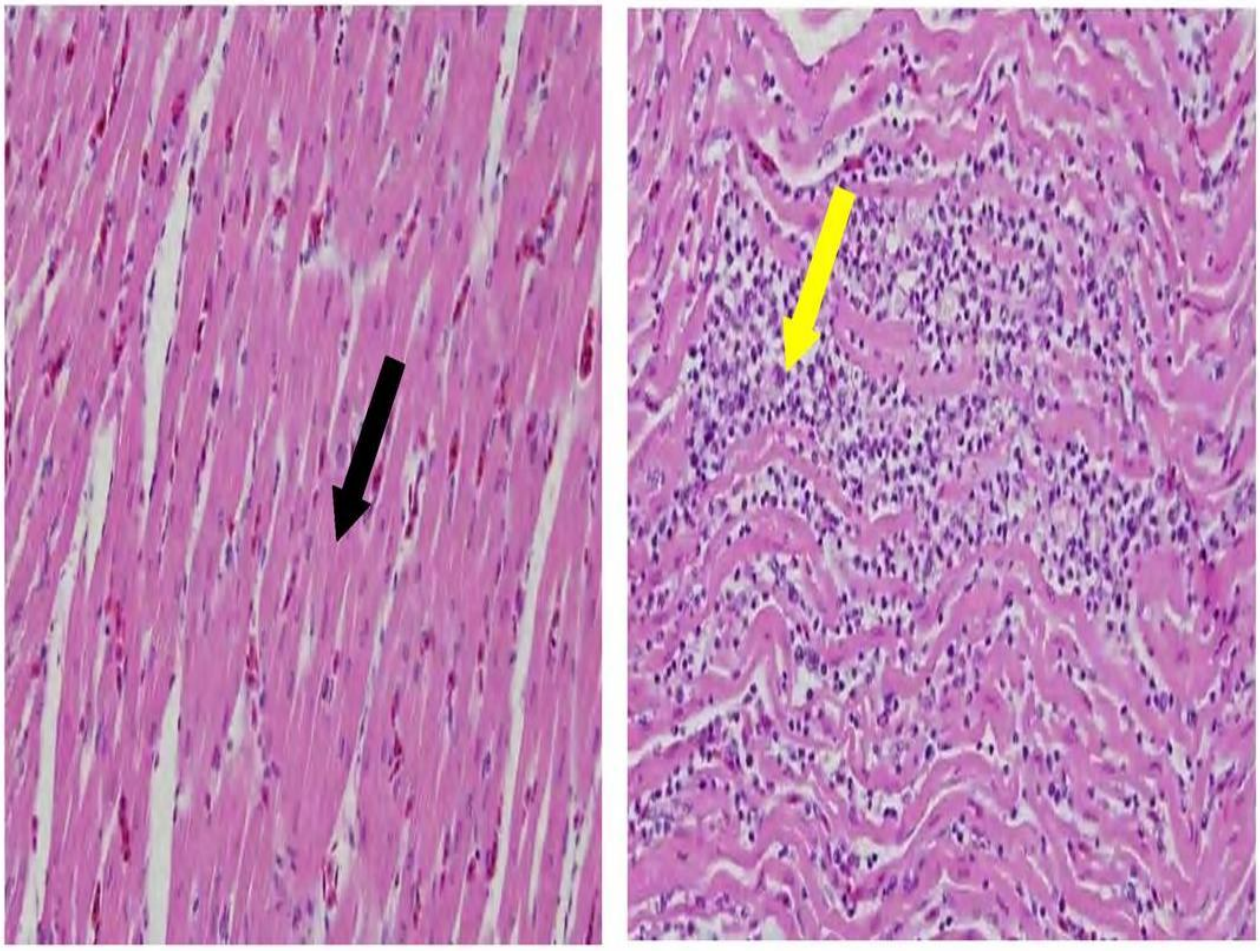



**Plate XIV: Molecular layer of chicken carcasses with cerebellum showing perivascular cuffing due to field and challenged Marek's disease virus (Haematoxylin and Eosion, X 400 MG).**

A = Carcasses from field chicken with MD; perivascular cuffing of the cerebellum (yellow arrow).

B = Carcass from MD (challenged) chicken showing the perivascular cuffing of the cerebellum (yellow arrow).

C = Chicken carcass with normal cerebellum (black arrows).



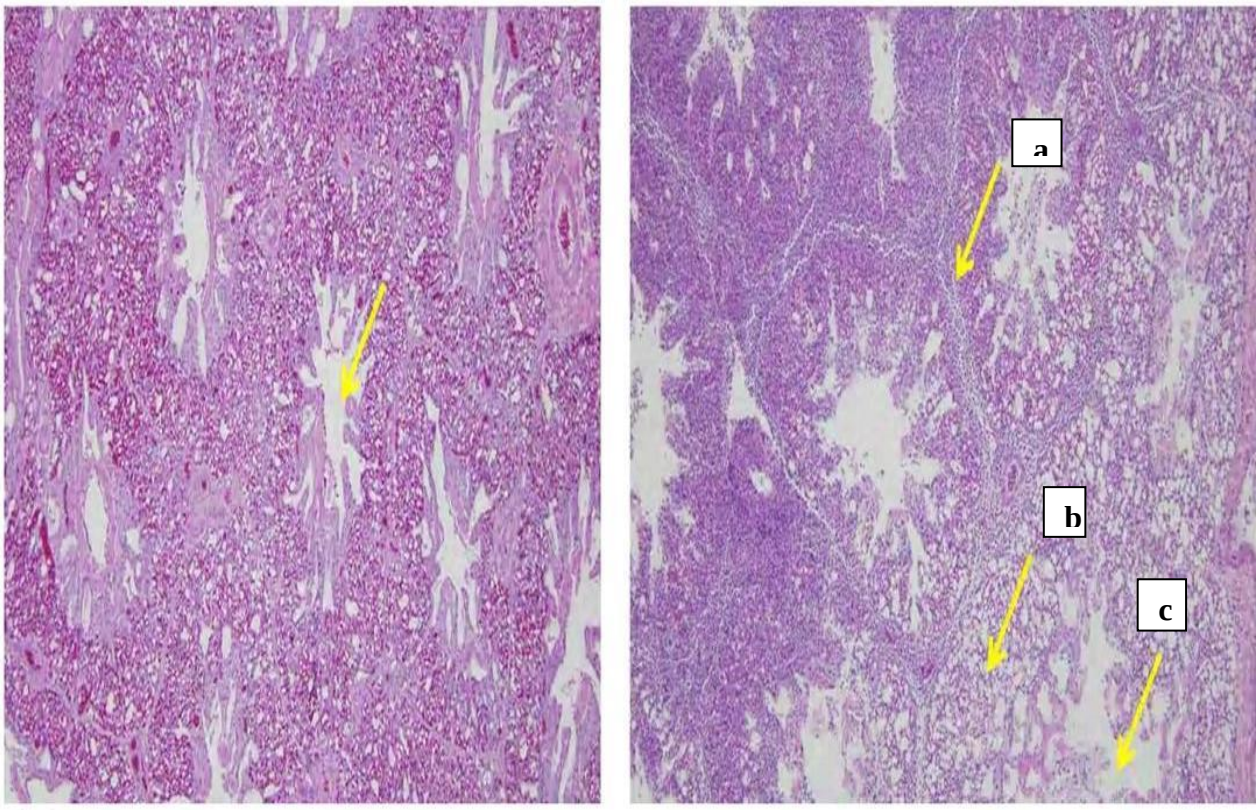
(A) Normal heart X 100

(B) Marek's disease infected heart with diffused infiltration of neoplastic lymphocytes

**Plate XV: Infected myocardium (B) from chicken carcass experimentally challenged with Marek's disease virus (Haematoxylin and Eosion X100).**

A = Normal heart of a chicken carcass with longitudinal myocardial fibre (black arrow).

B = Infected heart from challenged chicken carcass with MDV; myocardial fibre with diffused cellular infiltration yellow arrow.



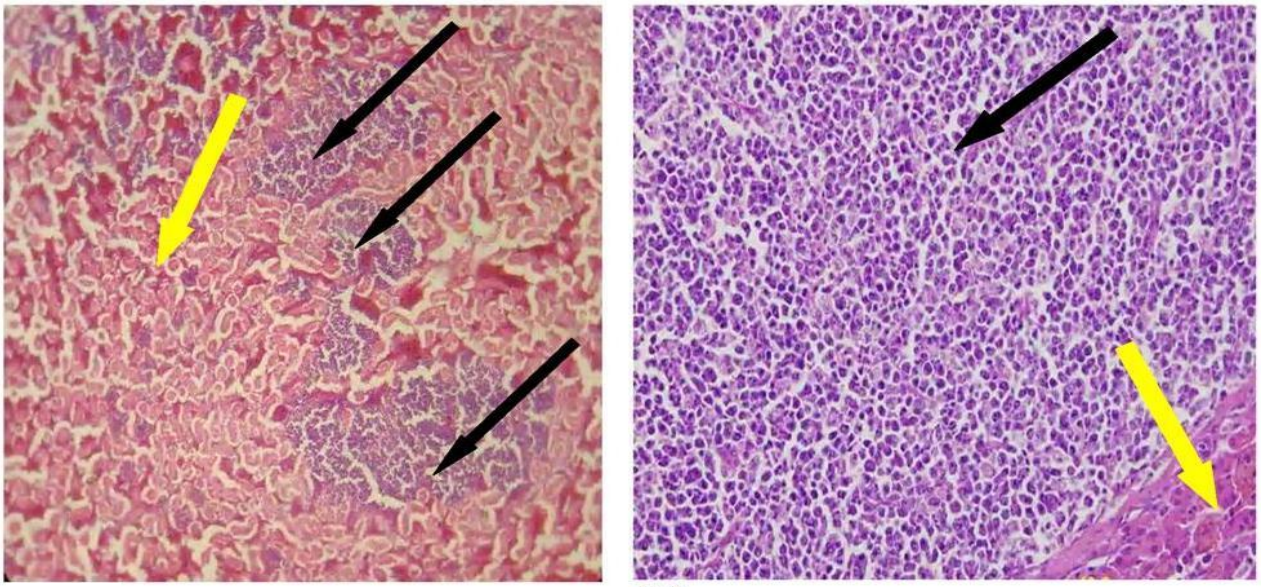

(A) Normal lung X100

(B) Lung challenged with Marek's disease virus X100

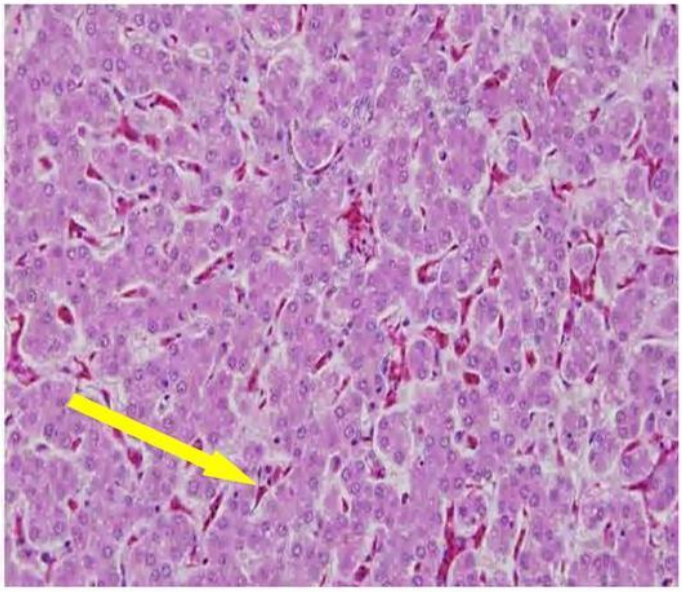
**Plate XVI: Infected lung (B) due to challenged Marek's disease virus (Haematoxylin and Eosion X100 MG).**

A = Normal lung with empty aveoli from chicken carcass.

B = Lung from chicken carcass challenged with MDV showing prominent septre (a), loss of aveoli architecture (b), filled with cellular infiltration and fluid (c).



(A) (B)



(C)

**XVII: Liver from field X50 (A) and experimentally challenged chicken (B) carcasses with Marek's disease virus showing cellular infiltration and normal liver (C) (Haematoxylin and Eosion X100 MG).**

A = Liver from field chicken carcarss infected with MDV; with necrotic foci and cellular infiltration in black arrows.

B = Liver from challenged chicken carcass with MDV showing discrete cellular infiltration in black arrow with normal in yellow arrow.

C = Chicken carcass with normal liver in yellow arrow showing hepatocytes.

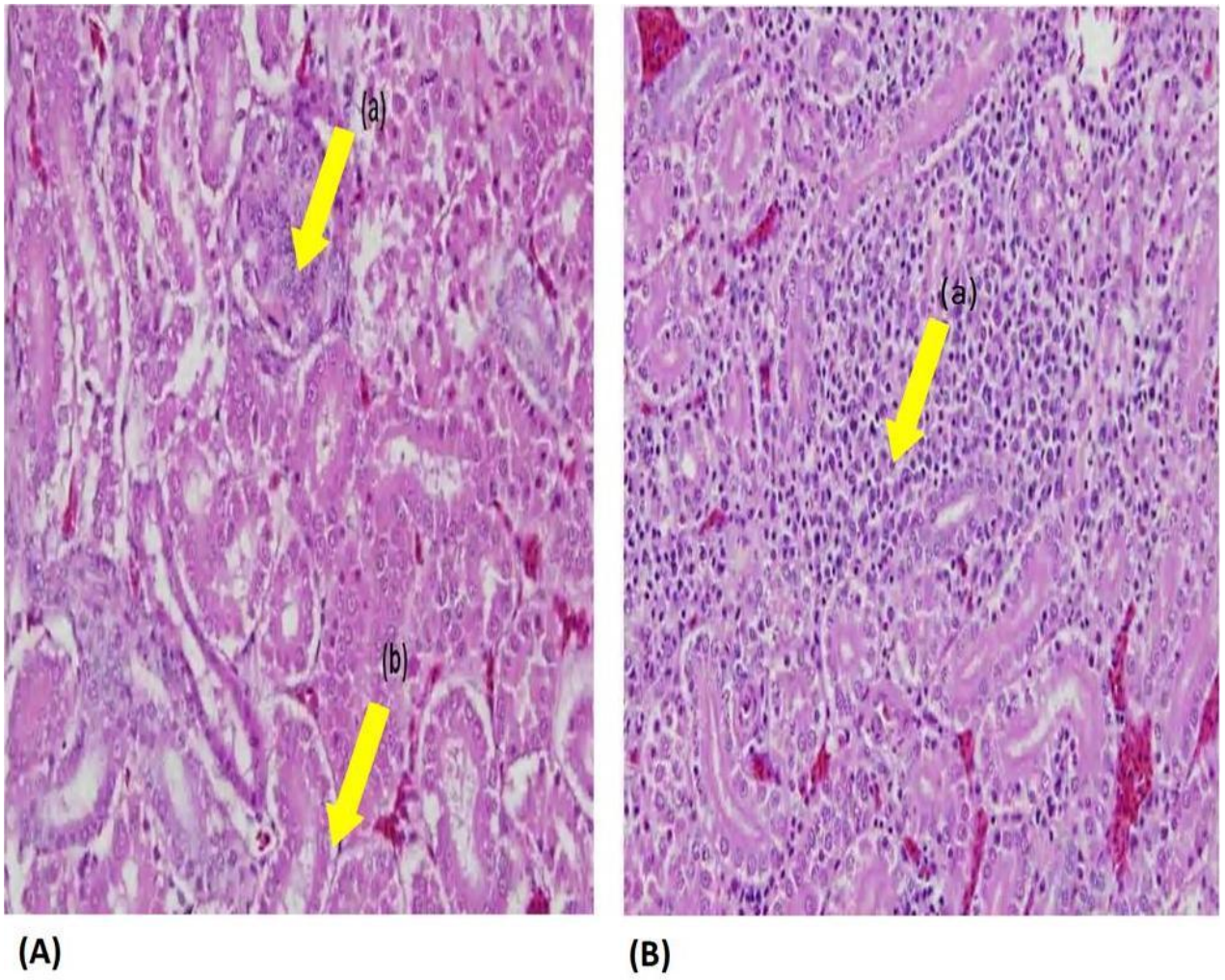



**Plate XVIII: Infected chicken carcass kidney (B) experimentally challenged with Marek's disease virus showing cellular infiltration (Haematoxylin and Eosion X 400 MG).**

(A) = Normal kidney showing the glomerilus (a) and tubule (b).

(B) = Kidney from challenged chicken carcass with MDV showing loss of kidney achitecture with severe cellular infiltration (a).

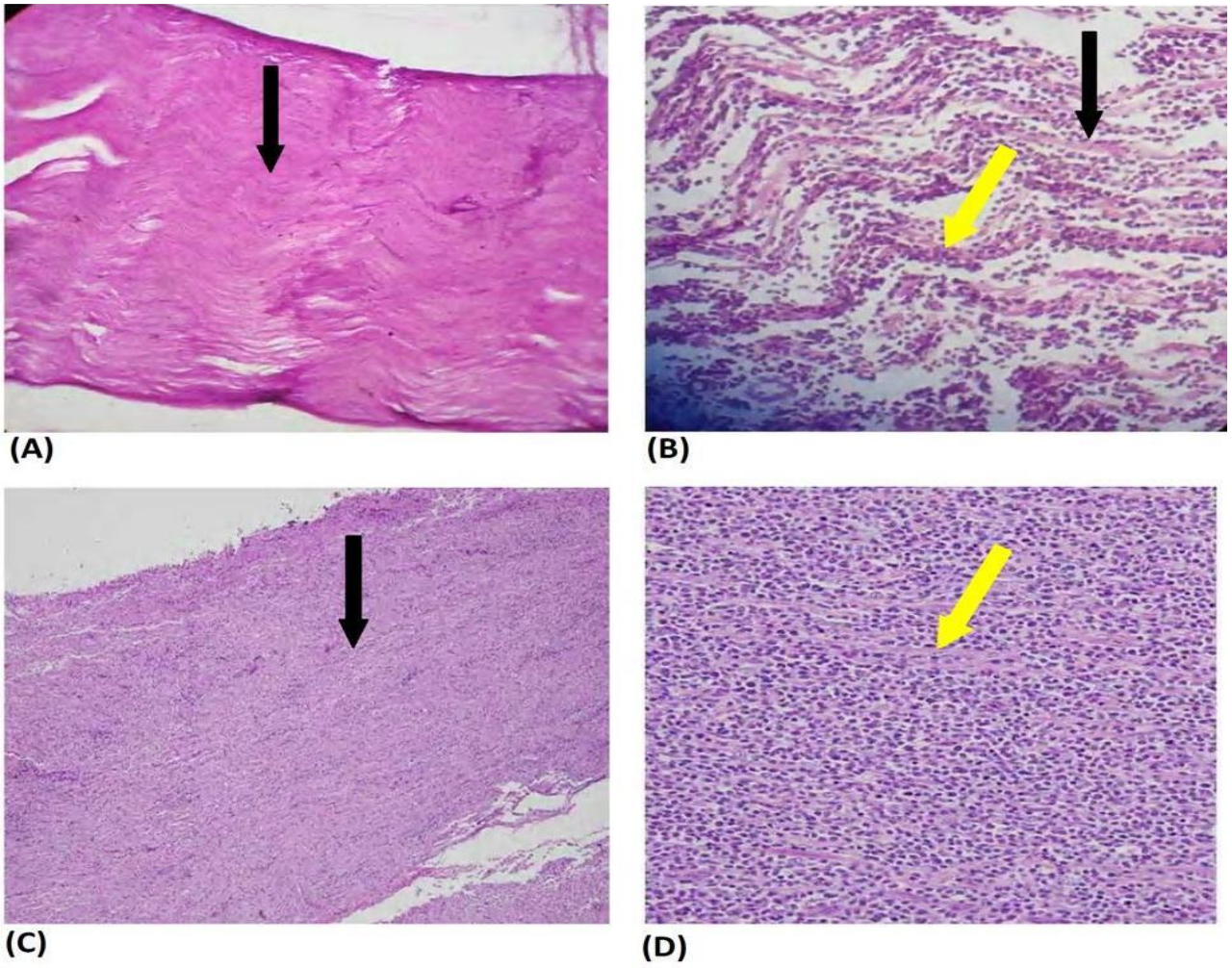



**Plate XIX: Nerve from field infected chicken carcass and chicken carcass experimentally challenged with Marek's disease virus showing sciatic nerves with cellular infiltration (Haematoxylin and Eosion X 100 MG).**

A = Normal sciatic nerve fibre black arrow.

B = Nerve from field MD infected chicken carcass showing loss in striation (a) black arrow with severe cellular infiltration and scanty nerve fibres in yellow arrow (b).

C and D =Sciatic nerve from challenged chicken carcass with MDV (lower X 50 MG) and higher (X 100MG) showing scanty nerve fibres (black and yellow arrows) due to loss in architecture and severe cellular infiltration.

## 6.5 Discussion

Deep litter management which was widely practiced by most farmers is a risk factor for MD as reported by Heier and Jarp (2000) who stated that, a higher risk of MD exist in laying birds reared on the floor than those maintained in cages probably due to MDV contaminated environment. Farms that reared chickens of different ages as was observed being practiced, had been reported to have increased risk of MD as reported by Heier and Jarp (2000). Some of the outbreaks of MD investigated may be due to poor biosecurity and management practices. This is because, the MDV survives at ambient temperature for 65 weeks when cell associated and is resistant to quaternary ammonium compound.

Bains (1979) reported sudden deaths, variable stunted growth with moderate to severe loss of weight, depression, ruffled feathers, paleness and underdeveloped combs and wattles and matted vents feathers with yellowish to greenish white diarrhea as observed in this study. Also, the neck, wings and legs paralysis seen agreed with the report of Witter (1997). Ocular involvement manifested as was seen in only one of the 31 flocks investigated suggested that the ocular form of MD is rare.

According to Venugopal and Payne (1996) and Witter (1997), the acute form of MD demonstrates lymphoma and enlargement of visceral organs like liver, lungs, heart, gonads and kidneys, which were observed in this study. The naturally infected and experimentally challenged chickens showed similar gross and histopathological lesions, which were characteristics of MD lymphomas as reported by earier workers (Bacon *et al.*, 2001a; Witter, 2001). The liver was the most affected organ followed by the spleen with lymphomas and therefore, the examination of these organs are important for the diagnosis of MD as recommended by Schat and Nair (2008) and Jwander (1993).

Not all carcasses had lymphomas and not all chickens manifested the clinical signs of MD, even the experimentally challenged chickens in this study. This agreed with the report of Bains (1979) that, a variable number of infected chickens will develop tumours and that tumour development is dependent on the individual chick's immune response, intercurrent disease, stress, maternal immunity, exposure dose, virulence of the virus and genetic resistance of the birds.

The pleomorphic cells seen especially in nerves in the present study are characteristics of MD and are used to differentiate it from avian leukosis (Jwander, 1993; Akpaive, 2005). According to Witter (1997), several MD responses including lymphomas, mortality, early mortality with bursal and thymic atrophy and frequency of visceral lymphomas or ocular lesions in nonvaccinated chickens were positively correlated with virulence. The mortality in farms investigated and the presence of lymphomas in the skin and visceral organs were similar to the findings of Witter (1997).

## 6.5 Conclusions

The clinicopathological findings of the field outbreaks of MD were similar to those of MDV experimentally challenged chickens. The management practices as observed in most of the farms greatly contributed to the field MD outbreaks in this study. The cause of the outbreak in vaccinated flocks could be "vaccines failure" or "vaccine break". The most common clinical signs were paralysis, loss of body weight, poor growth, underdeveloped comb and wattles and ocular opacity. Skin tumour and lymphomas of the visceral organs

were the common pathological lesions seen. Lymphoproliferation of neoplastic lymphocytes were seen in affected tissues.

### 6.7 Recommendations

1. The pathological lesions from the experimentally challenged can serve as control for the field diagnosis of Marek's disease cases in Nigeria.
2. Isolation of the field virus is needed to determine the MDV strains causing the farm for possible production of local MD vaccine.
3. Good biosecurity management practices by farmers are recommended to reduce the MD outbreak.
4. Early vaccination of chicks from hatcheries against MD will protect them from early exposure to MD.

# CHAPTER SEVEN

## MAREK'S DISEASE VIRUS DETECTION IN SOME SELECTED AREAS OF NIGERIA


### 7.1 Abstract

The aim of this study was to detect the occurrence of Marek's disease virus among different birds' species in Nigeria. Tissues were collected from layers, local chickens, ducks, quails and cattle egrets presented for disease diagnosis at veterinary facilities in the FCT, Plateau, Nasarawa, Bauchi and Kaduna States. Polymerase chain reaction (PCR) was applied to identify viral nucleic acid from the lungs, liver, spleen, kidneys, skin, pancreas and ovaries. The extraction and isolation of DNA from tissue homogenates was done using QIA amp viral DNA Mini Spin Protocol (2008). The PCR detected a common band of MDV from the lungs, liver, spleen, kidneys and skin of layers aged between 8 and 24 weeks at 491bp. About 34% (5/15) of the field samples were positive for MDV. Percentage positivity was 100% (1/1) for the lungs, spleen, skin and kidneys and 11.1% (1/9) for the liver. Fifty per cent of the layers (5/10) were positive while MDV antigen was not detected in local chickens, ducks, quails and cattle egrets. Layers from, Plateau, Bauchi and Kaduna States tested positive. This study confirmed the presence of MDV in layers suspected of suffering from MD. Layers should be routinely vaccinated against MD.


### 7.2 Introduction

The traditional diagnosis of MD is based on clinical signs and pathological alterations (Handberg *et al.,* 2001). The detection of viral antigen in the feather follicle epithelium by agar gel precipitation (AGPT) test has been described by Haider and Lapen-Kenzy (1970), but the sensitivity of that test is inferior to that of ELISA and DNA hybridization

(Davidson *et al*., 1986). Different MDV serotypes can be differentiated serologically using specific monoclonal antibodies (Lee *et al*., 1983). The preferred samples for virus isolation are buffy coat cells, which can be co-cultivated with susceptible primary cell cultures. Immunofluorescent) or ELISA can be used for subsequent identification of the MDV serotype (Kitamoto *et al*., 1979; Cheng *et al*., 1984). Alternatively, restriction endonuclease analysis or polymerase chain reaction (PCR) can identify the serotype (Wang *et al.*, 1993; Ross *et al*., 1997). *In-situ hybridization* has been used for detection of MDV genome in infected tissues (Endoh *et al.*, 1996; Ross *et al*., 1997), but the technique is probably too laborious for routine diagnoses (Davidson *et al*., 1995). Davidson *et al*. (1996) applied MDV serotype 1 specific PCR technique to full blood and tumour tissue samples from commercial chickens and turkeys, the majority of which had neoplastic disease. Young and Gravel (1996), Wang *et al.* (1993) and Silva and Witter (1996) applied a MDV serotype 1 specific PCR protocol to various tissues of chickens experimentally inoculated with JM/102 strain.

The advent of hybridoma technology has made it possible to produce monoclonal antbodies of high specificity (Lee *et al*., 1983) developed monoclonal antibodies with specificity for the three different serotypes of Marek's disease viruses in chickens. Monoclonal antibody T81 is type common (detects serotype 1, 2 and 3 of both MDV and HVT), antibody L78 is specific for HVT, antibody Y5 detects serotype 2 while antibody H19 detects MDV of serotype 1 (Lee *et al*., 1983).

The principal methods of identifying the presence of Marek's disease infection also include isolation of the virus and demonstration of viral DNA or antigens in tisues (Witter and

Schat, 2003). The polymerase chain reaction (PCR) can detect viral nucleic acid of MDV in productively and latently infected cells as well as in cells from lymphomas. The most commonly used primers amplify the 132 base pair repeat sequences (Becker *et al*., 1992; Silva, 1992; Zhu *et al*., 1992; Handenberg *et al*., 2001), although primers to amplify other regions of the genome may also be used (Becker *et al*., 1992; Handenberg *et al*., 2001; Gimeno *et al*., 2005). The 132 base pair repeat primers can detect virulent or attenuated serotype 1 MDV. Quantitatve PCR has been used to detect MDV in latently infected birds (Bumstead *et al.*, 1997; Reddy *et al*., 2000). Real-time PCR has been used to quantify MDV in tumours (Gimeno *et al*., 2005). Briefly, DNA was extracted from tumours using a Puragene DNA isolation kit (Gentra System, Minneapolis, MN). Samples were amplified using primers for glycoprotein B (gB). High load of MDV DNA in tumours were considered diagnostic of MDV as the causative agent (Wakenell and Sharma. 2008). Detection of the MDV serotypes in birds is yet to be given consideration in Nigeria. The purpose of this study was to extract and detect the presence of field MDV for diagnostic purpose in chicken tissues in FCT, Plateau State, Nasarawa, Bauchi and Kaduna States of Nigeria using PCR.

## 7. 3 Materials and Methods

### 7. 3. 1 Study area

The study area was made up of veterinary clinics and hospitals where suspected MD cases were reported. The veterinary establishments were located in Plateau, Kaduna, Nasarawa and Bauchi States and the FCT.

### 7. 3. 2 Tissue samples used for Marek's disease virus detection by Polymerase Chain Reaction

Tissues sampled from the liver, lungs, kidneys, spleen, skin, ovaries and proventriculus were obtained from layers with suspected MD and apparently healthy local chickens, ducks, quails and cattle egrets and prepared for MDV-DNA extraction and detection using PCR. Tissues were manually homogenized in PBS (7.4 pH) in ratio of 1 g to 2 ml respectively at NVRI, Vom in Plateau State and then transported in cooler packed with ice blocks and kept at -20$^{o}$C for onward use in University of Ibadan. Positive Rispens and HVT commercial vaccines were used as standard references.

### 7. 3. 3 Extraction Kit for Marek's disease virus DNA

The source of extraction kit required using a standard (491 base pair) was obtained from Dr. A. A. Owoade of University of Ibadan. The extraction and isolation of DNA from tissue homogenates was done using QIAamp viral DNA Mini Spin Protocol, 2008 which was obtained from Dr.A. A. Owoade of University of Ibadan.

### 7. 3. 4 Marek's disease virus DNA extraction

The general procedure for extraction was conducted as follows:

560 µl of prepared Lysis buffer AVL and aliquote pipette into sterile microcentrifuge tubes (1.5 ml) proportionate to the number of labeled samples and kept at 4$^{o}$C; votex for 15 seconds to break crystals formed (if not used directly). This was followed by the addition of 140 µl of every sample to the labeled corresponding buffer AVL above.Votex, then incubated at 25$^{o}$C room temperature for 10 minutes. After centrifuge for 10 seconds, add ethanol 560 µl (96-100%), then vortex for 15 seconds. It was then centrifuged for 20

seconds to allow drops from caps (covers) to settle using Biofuge pico Heraeus 8.0 x1, 000 rpm. Qiaspin column contains white filter 630 µl sample each into labeled corresponding tube from above, then centrifuge as above 8,000 rpm/minute. Remove the silicagel tube with filter which contains the DNA (the silicagel filter has high affinity for DNA), discard the filtrates and carefully transfer the filters into their corresponding collection tubes (2 ml size). The transfer process is repeated until all samples are finished (630 µl of the mixture). Two types of washing buffer to purify the DNA were used; Add 500 µl washing buffer -1 (tip should not touch the sample if only one tip used to dispense) then centrifuge at 8,000 rpm for a minute. A new set of collection tubes with corresponding labels were arranged on the rack and then insert the silca gel filter after the centrifugation above into the tubes.

Next, add 500 µl washing buffer-2 to further purify the DNA then centrifuge at 13,000 rpm (STD 14,000) for 3 minutes. Transfer the silica gel filter into new labeled ependorf tubes with cover. Add 60 µl elution buffer AVE to dissolve the DNA making it to pass to the bottom for harvest. Next, incubate the filtrates for a minute, then centrifuge at 8000 rpm for a minute. The purified DNA is settled at the bottom and then kept at -20$^{o}$C till ready for use. Marek's disease vaccines, Biovac (HVT- FC 126 strain) and Marek's- vac (Rispens CV1988) were similarly treated and used as positive control for serotype 3 and 1 respectively.

### 7. 3. 5 Marek's disease DNA detection

The PCR Reagents used were product from Invitrogen. The primer pair gBF 5'GTGGAAAGAGGTGACTGAAATG3'and gBR 5' AGAAATTGGAGCATGGCGA3' according to Ross *et al.* (1989) was specific to highly conserved region of the gB of the MDV gene for all serotypes was used for the PCR which produce bands at 491bp

**Master Mix Preparation**

| | | |
|---|---|---|
| PCR Buffer | X10 | |
| Magnasium chloride | 50 mM | |
| dNTP | 10 mM | |
| Primer F | 25 uM | gBF |
| (491bp expt) Primer R | 25 uM | gBR |
| Taq (platinum) enzyme | 5 µl | |

Reaction pool volume = 25µl

| Mixed Protocol | X µl x20 (17+3 samples) | |
|---|---|---|
| Denuclease $H_2O$ | 18.2 | 364 |
| 10 x PCR Buffer | 2.5 | 50 |
| $MgCl_2$ | 1.5 | 30 |
| dNTP | 0.5 | 10 |
| Primer F | 0.1 | 2 |
| Primer R | 0.1 | 2 |
| Taq (platinum) | 0.1 | 2 |
| Total | 23 µl | |

### 7. 3. 6 Running Polymerase Chain Reaction

The PCR was carried out in a total volume of 25 µl as follows:

i) Pipette out 2 µl of the extracted and purified DNA kept above into labeled corresponding PCR tubes, next add 23 µl of the Master Mix to all and mix up and down carefully using multi-channel pipette to give the final total volume of 25 µl per PCR tube.

ii) Then cover the above mixture in the PCR tubes (keep at +4$^{o}$C**)**

Thermocycler Protocol

iii) The PCR mixes and the thermal cycles used were optimized in Luxembourg. The PCR mix was vortexed and 23 µl was aliquot into each PCR tube, 2 µl of the DNA was added to the PCR mix and was vortexed and put in the thermocycler to undergo the PCR thermal procedure as follows:

**1$^{st}$** denaturation which breaks double strands of DNA to single DNA;

| | |
|---|---|
| 94 $^{o}$C for 5 minutes | Initial denaturation |

**2$^{nd}$** anealing Tempperature (primer attached to DNA) for Forward and Reverse primers

| | | |
|---|---|---|
| 94 $^{o}$C for 30 seconds | Denaturation | 35 cycles |
| 55 $^{o}$C for 30 seconds | Annealing | |
| 72 $^{o}$C for 1 minute | Extention | |
| 72 $^{o}$C for 7 minutes | Final extention | |

Electrophoresis of the PCR product

1. Gel preparation

| | | |
|---|---|---|
| Agarose | 0.6g | |
| TBE Buffer | 0.5% | 30ml |

The above were poured into a conical flask respectively then microwaved for a minute, then cool to about 60 $^{o}$C before adding 0.1µl Ethidium bromide (dye) so as not to denature it.

2. Loading dye with bromophenol blue (LD) 1 µl /well was used for each sample. Mix by adding 5 µl of the sample DNA.

3. Next, standard ladder already prepared (1kb).

4. As in 1 above, TBE buffer was poured inside the sizeable gel electrophoresis tank with combs fixed to give comb wells on solidification.

5. The 1st and last well of each lane of the gel were loaded with 5 µl of ladder or maker. Each sample (5 µl) was mixed with 1 µl of loading dye as in 2 above and was put into corresponding well using single channel micropipette without breaking up the the comb. The electrophoresis tank was then connected to the power source and then run the gel for 30 minutes at 120V. DNA migrates from cathode (-ve) to anode (+ve). The bands formed were made visible using UV light connected to a camera (photographed) and then using a connected computer reader.

### 7. 4 Results

The PCR detected Marek's disease virus DNA from the field samples from different tissues at a uniform band of 491bp (Plate XXIV and Table 7.1).

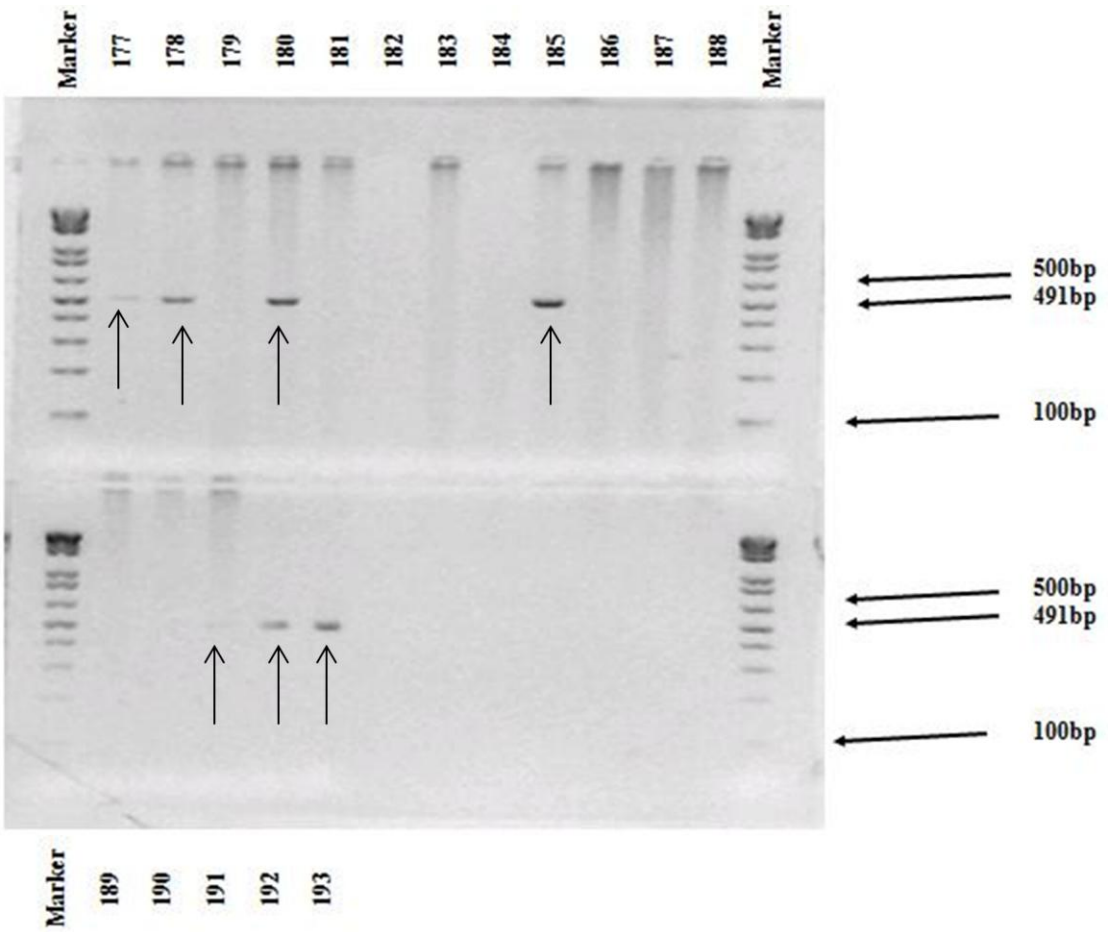



**Plate XX: Tissues showing cross section of the PCR positive bands at 491base pairs (bp).**

Lungs (177), liver (178), kidneys (180), spleen (185), skin tumour (191), HVT (192) and CVI988/Rispens (193) as controls.

**Table 7.1: Summary of positive samples for Marek's disease virus DNA in different states, age, avian species and organs.**

| SN | State | Age and species of bird | Type of Organ | PCR Results |
|---|---|---|---|---|
| 1 | Kaduna | 21 weeks layer | Lungs | Positive |
| 2 | Plateau | 12 weeks pullet | Liver | Positive |
| 3 | Bauchi | 16 weeks pullet | Ovary | Negative |
| 4 | Bauchi | Layer | Kidneys | Positive |
| 5 | Nasarawa | Layer | Liver | Negative |
| 6 | FCT | Layer | Pancreas | Negative |
| 7 | FCT | Duck | Liver | Negative |
| 8 | Plateau | Cattle egret | Liver | Negative |
| 9 | Plateau | 24 weeks layer | Spleen | Positive |
| 10 | Nasarawa | Pullet | Liver | Negative |
| 11 | Plateau | Quail | Liver | Negative |
| 12 | Plateau | Quail | Liver | Negative |
| 13 | Plateau | Local chicken | Liver | Negative |
| 14 | Kaduna | 20 weeks layer | Liver | Negative |
| 15 | Kaduna | Pullet | Skin tumour | Positive |
| 16 | | HVT (control) | HVT (control) | Positive |
| 17 | | CV1988/Rispens (control) | CV1988/Rispens | Positive |

HVT=Herpes Turkey Virus Vaccine Serotype 3
CV1988/Rispens= Marek's Disease Vaccines Serotype 1

## 7.5 Discussion

The PCR detected MDV in different tissues and species of birds in three northern states. Owoade and Oni (2008) detected MDV in feathers of apparently healthy chickens and the liver and spleen of 11-44 week old sick commercial chickens in Lagos and Oyo States. In the present study MDV was detected in the lungs, liver, kidneys, spleen and skin of 12-24 week old commercial chickens suspected of suffering from MD. The diagnosis of MD was based on combination of MDV detection by PCR and clinicopathological findings.

In this study, the PCR detected the MDV at 491 base pairs (bp) amplicon common for all the MDV serotypes and specific to the conserved region of gB MDV gene was amplified. The amplification of the gB MDV gene is necessary in diagnosis because of its importance for cell attachment and penetration (Ikuta *et al.*, 1984) as cited by Owoade and Oni (2008). The inability to use specific PCR primers that differentiate MDV serotype 1, 2 and 3 is a limitation of this study (OIE, 2010).

Marek's disease virus was detected in the lungs, liver, kidneys, spleen, and skin showing the wide distribution of the MDV in different organs. Tissues from the liver of quails, duck and Cattle egret tested negative for MDV this could be due to the few samples tested hence more samples are needed to ascertain that MD is mainly a disease of domestic chickens as reported by Davison and Nair (2004) and Schat and Nair (2008). The PCR was able to detect MDV in samples from Kaduna, Plateau and Bauchi states indicating how widely distributed the virus is in the areas thus, confirming that MDV is ubiquitous (Davison and Nair, 2004).

Cattle egrets mingle between farms particularly they feed on poultry waste and manure dumped within poultry houses could make them to expose chickens to diseases such as MD hence, there is the need for good biosecurity measures in poultry farms and hatcheries.

## 7.6 Conclusions

Marek's disease virus was detected in the lungs, liver, spleen and kidneys in chickens from Kaduna, Plateau ans Bauchi. This study confirmed the presence of MDV in layers suspected of suffering from MD.

## 7.7 Recommendations

1. Further studies are necessary to serotype the MDV across Nigeria.
2. Cattle egret and local chickens should not have access to commercial chicken flock.
3. Further research is needed to select local chickens for genetic resistance against MDV.

# CHAPTER EIGHT

## FINANCIAL LOSSES IN FARMS DUE TO MAREK'S DISEASE OUTBREAK FROM 2005 TO 2009 IN SOME STATES IN NIGERIA

### 8.1 Abstract

Following field reports of Marek's disease (MD) outbreaks in commercial birds from Kaduna, Plateau, Nasarawa, Bauchi States and FCT, economic data were collected from 2005-2009. Structured questionnaire was served to farmers in Samaru, Zaria, and Kaduna in Kaduna State, Bukuru, Topp, Vom, Jos North, Jos South, and East in Plateau State, Toro in Bauchi State, Lafia, Akwanga, Keffi, and Nasarawa in Nasarawa State and Karu, Mararaba, Nyanya, Kuje in Abuja Nigeria to determine the input and output of MD infected and uninfected farms. Investigations carried out in 31 farms with MD outbreaks showed mortality varied from flock to flock, farm to farm and that not all chickens in a flock were affected with the disease. Data showed that most farms vaccinated their birds with HVT and and few used Rispens vaccines while some farmers were not sure whether MD vaccine was given to chicks from source, while others did not vaccinate against MD as means of prevention and control. Majority of the farms raised birds on deep litter with few in battery cage system. Birds of different ages were seen in most farms.Total flock size of 31 farms at day old was 208,516 chicks. The economic losses with average loss per bird was ₦9, 120.61 ± 268.64 and total mortality was 32.4%, mean age of onset of MD was 13.0 ± 0.9 weeks old. Marek's disease case fatality was 79.5% which was highest in chicks with age frequency between 1-6 weeks old while production loss of ninety million, seven hundred and seventy six thousand four hundred and sity nine naira (₦90,776,469:00) was

highest in layers at the point of lay from ≥ 18 weeks old. Egg production was below expectation in MD farms. The mean cost benefit ratio of 31 farms without experiencing Marek's disease outbreaks was 6.07, which implies that the farm is profitable, that is for every one naira invested in the business the farmer is getting ₦5.00 in return. The mean cost benefit ratio of 31 farms that suffered Marek's disease was 0.73, which implies that the farmers were running the farms at a loss, meaning for every ₦ 1 invested the farmer is losing 27 kobo. Control and prevention of Marek's disease will reduce economic losses in poultry production by helping farmers to realize substantial financial returns from high production cost inputs, which undoubtedly will contribute towards increase in food production. However, tools for quantitative evaluation of the losses and the exact amount are relative as social indicators are difficult to asses.

## 8.2 Introduction

Marek's disease is an important and ubiquitous lymphoproliferative disease of chickens caused by Marek's disease virus (MDV), an alphaherpesvirus (Witter *et al*., 2005; Owoade and Oni, 2008). A variety of vaccines and good vaccination procedures can provide protection against Marek's disease (Biggs *et al.,* 1970; Mustaffa-Babjee and Spradbrow, 1973; Calnek *et al*., 1983; Witter and Lee, 1984; Witter *et al.,* 1984; Sharma *et al*., 1995; Lee *et al*., 1999; Ricks *et al*., 1999; Islam *et al*., 2001b); however, vaccination does not preclude infection, vaccinal and virulent viruses can co-exist together in the same host (Witter *et al*., 1984; 1987; Lee *et al*., 1999; Islam *et al*., 2001a). MDV infection can persist in resistant chickens without any clinical signs or pathological lesions (Witter *et al*., 1971; Nair, 2005).

Wittwer's (1975) report as cited by Purchase and Schulz (1985) on the economics of MD control in the United States, that improved technology through continuous research is the world's only hope of substantially increasing food production. That in the contemporary world of nowadays full of environmental disasters and economic uncertainty, food can become an instrument in international diplomacy. The control of MD losses in poultry production undoubtedly contributes to increase in food production. However, tools for the quantitative evaluation of losses and the amount are not standardized and social indicators are difficult to asses. MD costs the poultry industry nearly $1 billion annually (Purchase, 1985). Genetically resistant lines were shown to have greater vaccinal immunity and higher egg production than susceptible lines (Spencer *et al*., 1974; Gavora and Spencer, 1979). This study examines the economic impact of MD on 31 farms that experienced MD outbreak in some parts of Nigeria to determine whether they are running the farms at financial loss.

## 8.3. Materials and Methods

### 8.3.1 Study area

Visits were made to ABUVTH, Zaria Kaduna State; ECWA Veterinary Clinic Bukuru and Central Diagnostic Laboratory of National Veterinary Research Institute (NVRI), Vom, Plateau State. Records of MD were obtained and farm visits were made to suspected MD flocks.

### 8.3.2 Data collection

A structured questionnaire was administered to the farmers to obtain information on flocksize at day old, present age of chickens, onset of MD in weeks, number dead, number left, age at point of lay in weeks, eggs laid per day, MD vaccination status and cost of both MD vaccine and vaccination per chick.

### 8. 3. 3 Data analysis

Data collected were entered into Excel spread sheet for input and output computation to determine the fanicail loses due to MD. Data extraction and computation were carried out to calculate individual farm input and output among the variables such as: Farm serial number, date and location, flock size, present age of birds in weeks, onset of MD in weeks, mortality or number dead, number left, age of birds at point of lay in weeks, eggs collected per day, farm production of lost birds and MD vaccine cost with labour

### 8. 3. 4 Assumptions made based on market survey

1. Egg unit output per layer per year; 300 eggs/365 days = 0.8 eggs in a day (Smith, 2004)
2. Cost price of a 25kg bag of vital feed was ₦ 1,800.00 (Jos market survey, 2005)
3. Unit cost of feed consumed/day/layer (120g/25,000g multiply ₦1,800.00) = ₦8.64 Kobo/layer/day (Jwander, 2005)
4. Unit cost of MD vaccine with labour/chick ₦ 10.00 (ECWA, 2005)
5. Unit cost of a pullet day old chick = ₦ 190 (Jwander, 2005)
6. Unit cost of a layer ₦ 800.00 (Jwander, 2005)

7. Chickens at point of lay (POL) = 20 weeks
8. Age of a spent layer = 80 weeks
9. Unit output of a spent layer ₦ 800.00 (Jwander, 2005)
10. Unit output of an egg ₦ 25 (Jos market survey, 2005)
11. 100 birds produced 25 bags of 100 kg each of manure upto spen layer according to Titiloye (1997).
12. A unit output of 100 kg bag of manure was N1000.00 (Jwander, 2005)
13. Official exchange rate of Naira (₦) to US Dollar S was ₦ 125 = 1 USD (Jwander, 2005)
14. Total loss of a farm = cost of production (day old + feeding + medication/labour + eggs + manure + spent layers)
15. Loss per bird = Total loss over number dead

### 8.3.5 Revenue calculations

The revenue calculations were made based on the followings:

Inputs made by farmers that suffered MD (Table 8.2)

Outputs made by farmers that suffered MD (Table 8.2)

Inputs made by farmers without MD (Table 8.3)

Outputs made by farmers without MD (Table 8.3)

### 8.3.6 The financial outputs made by 31 farms

The cost benefit analysis were calculated for farms that suffered MD and for farms that did not suffered from Marek's disease.

*8.3.6.1 Calculation of cost benefit ratio analysis*

**Cost Benefit Ratio Analysis or Rate of Return on Investment (%)**

Cost benefit ratio (CBR) or Rate of return on Investment (RRI) gives the basis for the profitability that is, what will one naira invested into the business give (return on investment). So, in order to conclude whether the enterprise is profitable or not, there is the need to compute the profitability index. Profitability index is the gross revenue divided by total costs. However, a ratio of 1 is normally an indicator that the venture is at breaking position. The following profitability measures were calculated:

Rate of returns on investment or cost benefit ratio (%)

CBR= GM/TVC…… (i)

GM = Total Revenue -Total Variable Cost (TR- TV)…… (ii)

Where GM = Gross margin

TVC= Total variable cost

The ratio should be greater than 1 for the investment to be profitable.

## 8.4 Results

Day old chicks loss = ₦190 (cost per one) X number dead.

Feed loss = (No of chicks dead X cost of feed ₦25.2 X 8 week) + (No of growers dead X N29.11 X 10 week) + (No of adults dead X ₦59.51 X 12 weeks of the current age). The adult current age for example farm 1 = 30 weeks (present age) – 18 weeks (period of lay) = 12 weeks

1. Cost of Medication from day old to 8 weeks= ₦83/ chick (gendox, IBD, MD, IBD, NDVL, anticoccidiosis, FP, FT, Fc, debeaking (Jwander, 2005)

2. 9-18 weeks medication = ₦45/per grower (bronchovav, newvaxidrop vaccines, anticoccidiosiss, dewarming)
3. ₦10/layer (vitalytes/egg boosters) medication for the remaining production period
4. Medication and labour = ₦148 X No dead

Eggs = No. of dead birds X ₦131.25/week X (No of birds alive)

Manure = No. of dead birds X ₦5.49/week X (Number of weeks left to lay)

Spent layer = No. of dead birds X ₦800/ bird.

Nineteen farms (61.29%) out of 31 at the age frequency between 7-17 weeks surfered most from MD outbreaks (Table 8.1).

**Table 8. 1: The age frequency, mortality and production loss due to Marek's disease in 31 farms in Nigeria.**

| Age (weeks) | Farm frequency (%) | Total number of birds (Mean) | Number dead | %mortality | Production loss in Naira (₦) |
|---|---|---|---|---|---|
| 1 – 6 | 2 (6.45) | 4500/2 (2250) | 3577 | 79.49 | 526,140:00 |
| 7 – 17 | 19 (61.29) | 39096/19 (2058) | 10898 | 27.88 | 5,873,313:00 |
| ≥ 18 | 10 (32.26) | 164920/10 (16492) | 53166 | 32.24 | 90,776,469:00 |

%= Percentage

Total financial egg loss of 31 farms with MD = ₦453, 433, 968.8 (Table 8.2)**.** Total input and output loss of 31 farms with MD = ₦ 625, 087, 517.64 (Table 8.2). Total financial input of the 31 farms without MD = ₦ 47, 541, 648.00 (Table 8.2).

Total financial output of the 31 farms without MD = ₦166, 812, 800.00 (Table 8.3).

The mean cost benefit ratio of 31 farms that suffered Marek's disease was ₦0.73, which implies that the farmers were running the farms at loss, meaning that, for every ₦1 invested a farmer was losing 27 kobo (Table 8.5).

The mean cost benefit ratio of 31 farms without experiencing Marek's disease outbreak was ₦6.07, which implies that the farms were profitable, that is for every one naira invested in the business the farmer was getting ₦5.00 in return (Table 8.6).

**Table 8.2: Marek's disease infected 31 farms showing input cost and output loss.**

| | Input cost in Naira (₦) | | | | | Output loss in Naira (₦) | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Farm | Flock size | No. dead | Day old (₦) | Feeding (₦) | Medication/labour (₦) | Eggs (₦) | Manure (₦) | Spent layer (₦) | Total (Input, Output) (₦) | Overall loss / bird (₦) |
| 1 | 1500 | 1440 | 273600 | 1737820.8 | 184320 | 9450000 | 395280 | 1152000 | 13193020.8 | 9161.82 |
| 2 | 3000 | 2137 | 406030 | 1307245.64 | 273536 | 16828875 | 703927.8 | 1709600 | 21229214.44 | 9934.12 |
| 3 | 1800 | 524 | 99560 | 445274.24 | 67072 | 3851400 | 161098.56 | 419200 | 5043604.8 | 9625.2 |
| 4 | 1500 | 900 | 171000 | 711225 | 115200 | 6733125 | 281637 | 720000 | 8732187 | 9702.43 |
| 5 | 860 | 444 | 84360 | 350871 | 56832 | 3321675 | 138940.92 | 355200 | 4307878.92 | 9702.43 |
| 6 | 500 | 140 | 26600 | 56751.8 | 17920 | 1139250 | 47653.2 | 112000 | 1400175 | 10001.25 |
| 7 | 550 | 150 | 28500 | 60805.5 | 19200.004 | 1220625 | 51057 | 120000 | 1500187.5 | 10001.25 |
| 8 | 2512 | 502 | 95380 | 174269.3 | 64256 | 3953250 | 165358.8 | 401600 | 4854114.1 | 9669.55 |
| 9 | 4000 | 2000 | 380000 | 1818540 | 256000 | 14437500 | 603900 | 1600000 | 19095940 | 9547.97 |
| 10 | 700 | 212 | 40280 | 192765.24 | 27136 | 1530375 | 64013.4 | 169600 | 2024169.64 | 9547.97 |
| 11 | 24 | 19 | 3610 | 8255.12 | 2432 | - | 6362.91 | 15200 | 35860.03 | 1887.37 |
| 12 | 350 | 190 | 36100 | 161454.4 | 24320 | 1396500 | 58413.6 | 152000 | 1828788 | 9625.2 |
| 13 | 1000 | 200 | 38000 | 217560 | 25600 | 1365000 | 57096 | 160000 | 1863256 | 9316.28 |
| 14 | 1500 | 331 | 62890 | 222177.13 | 42368 | 2563181.25 | 107214.21 | 264800 | 3262630.59 | 9856.89 |
| 15 | 600 | 108 | 20520 | 43779.96 | 13824 | 878850 | 36761.04 | 86400 | 1080135 | 10001.25 |
| 16 | 1500 | 200 | 38000 | 241364 | 25600 | 1312500 | 54900 | 160000 | 1832364 | 9161.82 |
| 17 | 2100 | 100 | 19000 | 55221 | 12800 | 800625 | 33489 | 80000 | 1001135 | 10011.35 |
| 18 | 1500 | 200 | 38000 | 92718 | 25600 | 1653750 | 54900 | 160000 | 2024968 | 10124.84 |
| 19 | 1400 | 538 | 102220 | 489187.26 | 68864 | 3883687.5 | 162449.1 | 430400 | 5136807.86 | 9547.97 |
| 20 | 13000 | 4000 | 760000 | 4113160 | 512000 | 27825000 | 1163880 | 3200000 | 37574040 | 9393.51 |
| 21 | 3700 | 140 | 26600 | 127297.8 | 17920 | 1010625 | 42273 | 112000 | 1336715.8 | 9547.97 |
| 22 | 2000 | 200 | 38000 | 265168 | 25600 | 1260000 | 52704 | 160000 | 1801472 | 9007.36 |
| 23 | 4000 | 850 | 161500 | 621129 | 108800 | 6470625 | 270657 | 680000 | 8312711 | 9779.66 |
| 24 | 1000 | 480 | 91200 | 979180.8 | 61440 | 2268000 | 94867.2 | 384000 | 3878688 | 8080.6 |
| 25 | 100000 | 40000 | 7600000 | 41131600 | 5120000 | 278250000 | 11638800 | 32000000 | 375740400 | 9393.51 |
| 26 | 30000 | 4216 | 801040 | 11862095.44 | 539648 | 12727050 | 532354.32 | 3372800 | 29834987.76 | 7076.61 |
| 27 | 5000 | 2200 | 418000 | 3440536 | 281600 | 12705000 | 531432 | 1760000 | 19136568 | 8698.44 |
| 28 | 2000 | 400 | 76000 | 530336 | 51200 | 2520000 | 105408 | 320000 | 3602944 | 9007.36 |
| 29 | 2520 | 280 | 53200 | 454549.2 | 35840 | 1580250 | 66099.6 | 224000 | 2413938.8 | 8621.21 |
| 30 | 12000 | 3740 | 710600 | 3623237.2 | 478720 | 26507250 | 1108760.4 | 2992000 | 35420567.6 | 9470.74 |
| 31 | 6400 | 800 | 152000 | 1536752 | 102400 | 3990000 | 166896 | 640000 | 6588048 | 8235.06 |
| SUM | 208516 | 67641 | 12,851,790.00 | 77,072,326.83 | 8,658,048.00 | 453,433,968.80 | 18,958,584.06 | 54,112,800.00 | 625,087,517.64 | 282,738.99 |
| MEAN | | 2,181.97 | 414,573.87 | 2,486,204.09 | 279,291.87 | 15,114,465.63 | 611,567.23 | 1,745,574.19 | 20,164,113.47 | 9,120.61 |
| S.E.M | | 1278.33 | 242882.88 | 1350601.51 | 163626.36 | 9169449 | 371537.94 | 1022664.77 | 11996668.2 | 268.6383 |

S.E.M = Standard Error of Mean; Average loss per bird 9120.61 ± 268.64 Naira (Lower limit = 8851.97; Upper limit = 9389.25)

**Table 8.3: Input and output calculation of 31 ideal farms without suffering from Marek's disease.**

| | Input cost in naira (₦) | | | | | | | Output cost in naira (₦) | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Farm | Flock size | Day old | Chick feed (₦) | Grower feed (₦) | Layer feed (₦) | Medication/ labour (₦) | Total input (₦) | Egg (₦) | Manure (₦) | Spent layer (₦) | Total output (₦) |
| 1 | 1500 | 285000 | 302400 | 436650 | 5533920 | 342000 | 6899970 | 10237500 | 428505 | 1200000 | 11866005 |
| 2 | 3000 | 570000 | 604800 | 873300 | 11067840 | 684000 | 13799940 | 20475000 | 857010 | 2400000 | 23732010 |
| 3 | 1800 | 342000 | 362880 | 523980 | 6640704 | 410400 | 8279964 | 12285000 | 514206 | 1440000 | 14239206 |
| 4 | 1500 | 285000 | 302400 | 436650 | 5533920 | 342000 | 6899970 | 10237500 | 428505 | 1200000 | 11866005 |
| 5 | 860 | 163400 | 173376 | 250346 | 3172780.8 | 196080 | 3955982.8 | 5869500 | 245676.2 | 688000 | 6803176.2 |
| 6 | 500 | 95000 | 100800 | 145550 | 1844640 | 114000 | 2299990 | 3412500 | 142835 | 400000 | 3955335 |
| 7 | 550 | 104500 | 110880 | 160105 | 2029104 | 125400 | 2529989 | 3753750 | 157118.5 | 440000 | 4350868.5 |
| 8 | 2512 | 477280 | 506419.2 | 731243.2 | 9267471.36 | 572736 | 11555149.76 | 17144400 | 717603.04 | 2009600 | 19871603.04 |
| 9 | 4000 | 760000 | 806400 | 1164400 | 14757120 | 912000 | 18399920 | 27300000 | 1142680 | 3200000 | 31642680 |
| 10 | 700 | 133000 | 141120 | 203770 | 2582496 | 159600 | 3219986 | 4777500 | 199969 | 560000 | 5537469 |
| 11 | 24 | 4560 | 4838.4 | 6986.4 | 88542.72 | 5,472.00 | 110399.52 | 163800 | 6856.08 | 19200 | 189856.08 |
| 12 | 350 | 66500 | 70560 | 101885 | 1291248 | 79800 | 1609993 | 2388750 | 99984.5 | 280000 | 2768734.5 |
| 13 | 1000 | 190000 | 201600 | 291100 | 3689280 | 228000 | 4409980.19 | 6825000 | 285670 | 800000 | 7910670 |
| 14 | 1500 | 285000 | 302400 | 436650 | 5533920 | 342000 | 6899970 | 10237500 | 428505 | 1200000 | 11866005 |
| 15 | 600 | 114000 | 120960 | 174660 | 221568 | 136800 | 2759988 | 409500 | 171402 | 480000 | 4746402 |
| 16 | 1500 | 285000 | 302400 | 436650 | 5533920 | 342000 | 6899970 | 10237500 | 428505 | 1200000 | 11866005 |
| 17 | 2100 | 399000 | 423360 | 611310 | 7747488 | 478800 | 9659958 | 14332500 | 599907 | 1680000 | 16612407 |
| 18 | 1500 | 285000 | 302400 | 436650 | 5533920 | 342000 | 6899970 | 10237500 | 428505 | 1200000 | 11866005 |
| 19 | 1400 | 266000 | 282240 | 407540 | 5164992 | 319200 | 6439972 | 9555000 | 399938 | 1120000 | 11074938 |
| 20 | 13000 | 2470000 | 2620800 | 3784300 | 47960640 | 2964000 | 57799740 | 88725000 | 3713710 | 10400000 | 102838710 |
| 21 | 3700 | 703000 | 745920 | 1077070 | 13650336 | 843600 | 17019926 | 25252500 | 1056979 | 2960000 | 29269479 |
| 22 | 2000 | 380000 | 403200 | 582200 | 7370560 | 456000 | 9191960 | 13650000 | 571340 | 1600000 | 15821340 |
| 23 | 4000 | 760000 | 806400 | 1164400 | 14757120 | 912000 | 18399920 | 27300000 | 1142680 | 3200000 | 31642680 |
| 24 | 1000 | 190000 | 201600 | 291100 | 3689280 | 228000 | 4599980 | 6825000 | 285670 | 800000 | 7910670 |
| 25 | 100000 | 19000000 | 20160000 | 29110000 | 368928000 | 22800000 | 459998000 | 68250000 | 28567000 | 80000000 | 791067000 |
| 26 | 30000 | 5700000 | 6048000 | 8733000 | 110678400 | 6840000 | 137999400 | 204750000 | 8570100 | 24000000 | 237230100 |
| 27 | 5000 | 950000 | 1008000 | 1455500 | 18446400 | 1140000 | 22999900 | 34125000 | 1428350 | 4000000 | 39553350 |
| 28 | 2000 | 380000 | 403200 | 582200 | 5533290 | 456000 | 7354690 | 13650000 | 571340 | 1600000 | 15821340 |
| 29 | 2520 | 478800 | 508032 | 733572 | 9296985.6 | 574560 | 11591949.6 | 17199000 | 719888.4 | 2016000 | 19934888.4 |
| 30 | 12000 | 2280000 | 2419200 | 3493200 | 44271360 | 2736000 | 55199760 | 18900000 | 3428040 | 9600000 | 94928040 |
| 31 | 6400 | 1216000 | 1290240 | 1863040 | 23611392 | 1459200 | 29439872 | 43680000 | 1828288 | 5120000 | 50628288 |
| SUM | | | 39.618,040.0 | 42,036,825.60 | 60,699,007.60 | 765,207,070.50 | 47,541,648.00 | 955,126,159.90 | 671,137,950.00 | 59,566,765.70 | 166,812,800.00 |
| Mean | | | 1,356,026.63 | 1,958,032.50 | 24,691,246.40 | 1,533,601.55 | 30,810,521.29 | 23,941,490.32 | 1,921,508.57 | 5,381,058.07 | 53,206,815.02 |
| Standard Error of Mean | | | 660308.13 | 953450.87 | 12090825.32 | 746777.05 | 15065849.2 | 6938826.05 | 935665.79 | 2620270.34 | 25909429.98 |

**Table 8.4: Showing summary of input and output of normal and Marek's disease infected farms.**

| Farm | Flock size @ day old | Normal output (₦) | Normal input (₦) | Profit from normal farm (₦) | Number of birds dead (%) | Total input and output loss (₦) |
|---|---|---|---|---|---|---|
| 1 | 1500 | 11,866,005.00 | 6,899,970.00 | 4,966,035.00 | 1440 (96.0) | 13,193,020.80 |
| 2 | 3,000 | 23,732,010.00 | 13,799,940.00 | 9,932,070.00 | 2137 (71.2) | 21,229,214.44 |
| 3 | 1800 | 14,239,206.00 | 8,279,964.00 | 5,959,242.00 | 524 (29.1) | 5043604.80 |
| 4 | 1500 | 11,866,005.00 | 6,899,970.00 | 4,966,035.00 | 900 (60.0) | 8732187.00 |
| 5 | 860 | 6,803,176.20 | 3,955,982.80 | 2,847,193.40 | 444(51.6) | 4307878.92 |
| 6 | 500 | 3,955,335.00 | 2,299,990.00 | 1,655,345.00 | 140(28.0) | 1400175.00 |
| 7 | 550 | 4,350,868.50 | 2,529,989.00 | 1,820,879.50 | 150(27.3) | 1500187.50 |
| 8 | 2512 | 19,871,603.04 | 11,555,149.76 | 8,316,453.28 | 502(20.0) | 4854114.10 |
| 9 | 4000 | 31,642,680.00 | 18,399,920.00 | 13,242,760.00 | 2000(50.0) | 19095940.00 |
| 10 | 700 | 5,537,469.00 | 3,219,986.00 | 2,317,483.00 | 212(30.3) | 2024169.64 |
| 11 | 24 | 189,856.08 | 110,399.52 | 79,456.56 | 19( 79.2) | 35860.03 |
| 12 | 350 | 2,768,734.50 | 1,609,993.00 | 1,158,741.50 | 190 (54.3) | 1828788.00 |
| 13 | 1000 | 7,910,670.00 | 4,409,980.19 | 3,500,689.81 | 200(20.0) | 1863256.00 |
| 14 | 1500 | 11,866,005.00 | 6,899,970.00 | 4,966,035.00 | 331(22.7) | 3262630.59 |
| 15 | 600 | 4,746,402.00 | 2,759,988.00 | 1,986,414.00 | 108(18.0) | 1080135.00 |
| 16 | 1500 | 11,866,005.00 | 6,899,970.00 | 4,966,035.00 | 200(13.3) | 1832364.00 |
| 17 | 2100 | 16,612,407.00 | 9,659,958.00 | 6,952,449.00 | 100(4.8) | 1001135.00 |
| 18 | 1500 | 11,866,005.00 | 6,899,970.00 | 4,966,035.00 | 200(13.3) | 2024968.00 |
| 19 | 1400 | 11,074,938.00 | 6,439,972.00 | 4,634,966.00 | 538(38.4) | 5136807.86 |
| 20 | 13,000 | 102,838,710.00 | 57,799,740.00 | 45,038,970.00 | 4000(30.8) | 37574040.00 |
| 21 | 3,700 | 29,269,479.00 | 17,019,926.00 | 12,249,553.00 | 140(3.8) | 1336715.80 |
| 22 | 2000 | 15,821,340.00 | 9,191,960.00 | 14,901,380.00 | 200(10.0) | 1801472.00 |
| 23 | 4000 | 31,642,680.00 | 18,399,920.00 | 13,309,360.00 | 850(21.3) | 8312711.00 |
| 24 | 1000 | 7,910,670.00 | 4,599,980.00 | 3,310,690.00 | 480(48.0) | 3878688.00 |
| 25 | 100000 | 791,067,000.00 | 459,998,000.00 | 331,071,000.00 | 40,000(40.0) | 375740400.00 |
| 26 | 30,000 | 237,230,100.00 | 137,999,400.00 | 99,230,700.00 | 4216(14.1) | 29834987.76 |
| 27 | 5,000 | 39,553,350.00 | 22,999,900.00 | 16,553,450.00 | 2,200(44.0) | 19136568.00 |
| 28 | 2000 | 15,821,340.00 | 7,354,690 | 8,466,650.00 | 400(20.0) | 3602944.00 |
| 29 | 2,520 | 19,934,888.40 | 11,591,949.60 | 8,343,938.80 | 280(11.1) | 2413938.80 |
| 30 | 12,000 | 94,928,040.00 | 55,199,760.00 | 39,728,280.00 | 3740(31.2) | 35420567.60 |
| 31 | 6,400 | 50,628,288.00 | 29,439,872.00 | 21,188,416.00 | 800(12.5) | 6588048.00 |
| TOTAL | 208,516 | 1,649,411,266.00 | 955,126,159.90 | 694,285,106.10 | 67,641 | 625,087,517.64 |
| MEAN | 6726.3 ± 3275.3 | | | | 2181.9 ± 1278.3 | |

**Table 8.5: Cost Benefit Revenue of 31 farms that suffered Marek's disease outbreak in Nigeria.**

| Farm | Total input (₦) | Total output (₦) | Benefit (₦) | CBR (₦) |
|---|---|---|---|---|
| 1 | 6899970 | 11866005 | 4966035 | 0.719718 |
| 2 | 13799940 | 23732010 | 9932070 | 0.719718 |
| 3 | 8279964 | 14239206 | 5959242 | 0.719718 |
| 4 | 6899970 | 11866005 | 4966035 | 0.719718 |
| 5 | 3955983 | 6803176 | 2847193 | 0.719718 |
| 6 | 2299990 | 3955335 | 1655345 | 0.719718 |
| 7 | 2529989 | 4350869 | 1820880 | 0.719718 |
| 8 | 11555150 | 19871603 | 8316453 | 0.719718 |
| 9 | 18399920 | 31642680 | 13242760 | 0.719718 |
| 10 | 3219986 | 5537469 | 2317483 | 0.719718 |
| 11 | 110399.5 | 189856.1 | 79456.56 | 0.719718 |
| 12 | 1609993 | 2768735 | 1158742 | 0.719718 |
| 13 | 4409980 | 7910670 | 3500690 | 0.793811 |
| 14 | 6899970 | 11866005 | 4966035 | 0.719718 |
| 15 | 2759988 | 4746402 | 1986414 | 0.719718 |
| 16 | 6899970 | 11866005 | 4966035 | 0.719718 |
| 17 | 9659958 | 16612407 | 6952449 | 0.719718 |
| 18 | 6899970 | 11866005 | 4966035 | 0.719718 |
| 19 | 6439972 | 11074938 | 4634966 | 0.719718 |
| 20 | 57799740 | 1.03E+08 | 45038970 | 0.779224 |
| 21 | 17019926 | 29269479 | 12249553 | 0.719718 |
| 22 | 9191960 | 15821340 | 6629380 | 0.721215 |
| 23 | 18399920 | 31642680 | 13242760 | 0.719718 |
| 24 | 4599980 | 7910670 | 3310690 | 0.719718 |
| 25 | 4.6E+08 | 7.91E+08 | 3.31E+08 | 0.719718 |
| 26 | 1.38E+08 | 2.37E+08 | 99230700 | 0.719066 |
| 27 | 22999900 | 39553350 | 16553450 | 0.719718 |
| 28 | 7354690 | 15821340 | 8466650 | 1.151191 |
| 29 | 11591950 | 19934888 | 8342939 | 0.719718 |
| 30 | 55199760 | 94928040 | 39728280 | 0.719718 |
| 31 | 29439872 | 50628288 | 21188416 | 0.719718 |
| Sum | 47541648 | | | 0.737974 |
| Mean | 30810521 | | | |
| Standard error of mean | 15065849 | | | |

CBR= Cost Benefit Revenue

**Table 8.6: Cost benefit revenue of 31 farms that did not experience Marek's disease outbreak in Nigeria.**

| Farm | Input (₦) | Ouput (₦) | Profit (₦) | CBR (₦) |
|---|---|---|---|---|
| 1 | 2195741 | 10997280 | 8801539.2 | 4.00846 |
| 2 | 1986812 | 19242403 | 17255591 | 8.685066 |
| 3 | 611906.2 | 4431698.6 | 3819792.3 | 6.242447 |
| 4 | 997425 | 7734762 | 6737337 | 6.75473 |
| 5 | 492063 | 3815815.9 | 3323752.9 | 6.75473 |
| 6 | 101271.8 | 1298903.2 | 1197631.4 | 11.82591 |
| 7 | 108505.5 | 1391682 | 1283176.5 | 11.82591 |
| 8 | 333905.3 | 4520208.8 | 4186303.5 | 12.5374 |
| 9 | 2454540 | 16641400 | 14186860 | 5.779845 |
| 10 | 260181.2 | 1763988.4 | 1503807.2 | 5.779845 |
| 11 | 14297.12 | 10997280 | 8801539.2 | 4.00846 |
| 12 | 221874.4 | 1606913.6 | 1385039.2 | 6.242447 |
| 13 | 281160 | 1582096 | 1300936 | 4.627031 |
| 14 | 327435.1 | 2935195.5 | 2607760.3 | 7.964204 |
| 15 | 78123.96 | 1002011 | 923887.08 | 11.82591 |
| 16 | 304964 | 1527400 | 1222436 | 4.00846 |
| 17 | 87021 | 914114 | 827093 | 9.504522 |
| 18 | 156318 | 1868650 | 1712332 | 10.95416 |
| 19 | 660271.3 | 4476536.6 | 3816265.3 | 5.779845 |
| 20 | 5385160 | 32188880 | 26803720 | 4.97733 |
| 21 | 171817.8 | 1164898 | 993080.2 | 5.779845 |
| 22 | 328768 | 1472704 | 1143936 | 3.479463 |
| 23 | 891429 | 7421282 | 6529853 | 7.325152 |
| 24 | 1131821 | 2746867.2 | 1615046.4 | 1.426945 |
| 25 | 53851600 | 321888800 | 268037200 | 4.97733 |
| 26 | 13202783 | 16632204 | 3429420.9 | 0.25975 |
| 27 | 4140136 | 14996432 | 10856296 | 2.622208 |
| 28 | 657536 | 2945408 | 2287872 | 3.479463 |
| 29 | 543589.2 | 1870349.6 | 1326760.4 | 2.440741 |
| 30 | 4812557 | 30608010 | 25795453 | 5.36003 |
| 31 | 1791152 | 4796896 | 3005744 | 1.678107 |
| | | | | N6.094056 |

N6.0 = 6 Naira return on every N1 invested
CBR=Cost Benefit Revenue

## 8.5 Discussion

According to Purchase (1985), prior to the use of vaccines, MD constituted a serious economic threat to the poultry industry causing up to 60% mortality in layers and 10% condemnation in broilers and that is because vaccines are not 100% effective, sporadic losses still occur which agreed with the results of this study with mortality of 32.4%. The mean age of the onset of MD was 13 weeks old in this study which is similar to what was reported by Chauhan and Roy (2007**).** Purchase (1985) also estimated that mortality and condemnation losses due to MD totaled about $12 million in the United States in 1984. While results from this study showed total mortality loss of 31 farms due to MD to be ₦95, 811, 486.00 (766,491.89 USD). When combined with financial losses from the costs of vaccine and application and reduced egg production, the total was about $169 million in the United States and $943 million worldwide. In this study, financial loss of MD vaccine and labour in dead birds from 31 affected farms was estimated at ₦676, 500.00 (5,412.00 USD). Total egg loss due to MD in 31 farms was ₦453, 433, 968.80 (3,627,471.70 USD) which agreed with previous reports (Witter and Schat, 2003; Hunt, 2009), that MD has a worldwide economic effect especially in laying birds. Total profit in 31 farms without experiencing MD was ₦694, 285, 106.10 (5,554,280.8 USD).

A farmer lost ₦9,000.00 per bird on the average due to MD. For every ₦1 invested, a farmer was losing 27 kobo due to MD. For the farms without experiencing MD for every ₦1 invested, a farmer made a return of ₦6.00. The financial implication of this is that farmers that had MD were running at a loss. The decision whether to keep or dispose of

MD infected birds is easier. Farmers that obtained loan to start layer production would be unable to pay the bank interest if they kept MD infected flock up to the end of production.

According to Morrow and Fehler (2004) the worldwide annual losses due to MD was in the range of US$ 1 to 2 billion, but they indicated that these figures are impossible to verify. The disease remains a major concern to the poultry industry due to the unpredictability of outbreaks and the possibility that vaccines may ultimately fail as a consequence of the evolution and more virulent strains of MD virus (Witter, 1997; Schat and Nair, 2008) this agreed with the findings of this study where 11 farms were vaccinated against MD yet, there were outbreaks of the disease.

## 8.6 Conclusions

Mortality due to MD in this study was 32.4% which was high in any poultry management practice. Disease reporting and accurate records keeping to quantify the economic losses due to MD in Nigeria was still a major challenge. Farmers with MD affected flocks were running at financial loss.

## 8.7 Recommendations

1. General proper record keeping (financial input, output) by poultry farmers is very important.
2. Regular extension poultry farmers enlightenment campaing on selected poultry diseases like MD be encouraged.
3. Proper control programme on MD.

## 8.8 Limitations

1. Mortality of the 31 farms due to other diseases and management problems apart from MD were not considered.

2. Regular follow up visits to each farm up to the point of spent layer was not carried out to determine the final impact of MD.

3. Details of MD vaccination status of some farms were not available.

4. Standard values used were based on direct Market survey during investigations.

5. The number of poultry diseases and MD cases recorded between 2005 and 2009 by the three referral veterinary units were not included under this investigation to determine the incidence.

# CHAPTER NINE

# CONCLUSIONS AND RECOMMENDATIONS

## 9.1 Conclusions

Marek's disease prevalence was high and more frequent in immature (71.4%) and mature (85.5%) chickens age from 7-17 weeks and >18 weeks). Vaccine commonly used by veterinarians to control MD was HVT. Seventy five per cent veterinarians put the frequency of the occurance of MD compared to other poultry diseases at less than 20%. Seventy five per cent veterinarians diagnose MD in chickens between 4-16 weeks of age. Eighty four per cent veterinarians estimated reduction in eggs production due to MD at up to 40%. Paralysis was the commonest clinical sign of MD observed by veterinarians. Veterinarians observed that the most frequently affected organ by MD was the liver. The presence of nodules was the most commonly observed lesions in MD affected organs. Most poultry farmers were aware of MD and vaccinated against the disease. Most farmers reported that MD occurred more frequently in intensive farms. Farms that kept birds of different ages together had higher chance of suffering from MD. Antibodies to MD were prevalent in layers, commercial chickens and indigenous chickens. Nodular lymphomas developed mostly in the liver of layers with MD. Neoplastic lymphocytic infiltrations were observed in the organs of both MD naturally affected and experimentally infected chickens. The lungs, liver, spleen, kidneys and skin of layers suffering from MD had viral antigens. Antibodies to MDV were detected in unvaccinated indigenous chickens that had no lymphomas. Marek's disease causes highly significant financial loss to poultry.

## 9.2 Recommendations

1. Farmers should routinely vaccinate chicks with highly potent MD vaccines.

2. Veterinarians need more knowledge on MD.

3. Farmers should minimize the number of flocks with different ages in the same farm.

4. Commercial poultry should not be raised together with indigenous chickens.

5. Commercial poultry should be prevented from contact with quails and cattle egrets.

6. Marek's disease neoplastic tissues from chicken affected lungs, liver, spleen, kidneys and skin can be used for diagnosis of MD.

7. All stakeholders should cooperate in the control of MD in Nigeria.

## 9.3 Limitations

1. The financial costing was based on Jos Plateau State market's survey.

2. The affected farms were not monitored to the end of their disposal.

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## Appendix 1

**Questionnaire on Marek's disease cases in poultry among Veterinarians during the 46th Annual Conference of Nigerian Veterinary Medical Association on the 19th to 23rd October, 2009 at Awka in Anambra State.**

Dear respondent,

Kindly assist and answer the under listed questioins to the best of your knowledge on Marek's disease in birds/chickens for the past three (3) years. Rest assured that your response will be treated as confidential.

1. State …….LGA……..
2. (a) Clinician (b) Veterinary input provider (drugs etc) (c) Academician (d) Diagnostician
3. Type of birds frequently attended to; (a) Layers (b) Broilers (c) Parent Stock (d) Mixed birds (e) Local birds
4. The frequency of diagnosis of Marek's disease/Avian tumour cases visa vis other poultry diseases; (a) 5%) (b) 10% (c) 20%) (d) 30% (e) Others
5. Age of birth suspected (a) < 4 wks (b) 4-8wks (c) 8-16 (d) > 16 wks
6. Tick most frequent clinical signs for diagnosing MD; (a) Emaciation (b) Paralysis (c) Stunted growth (d) Pale comb/wattles (e) Others specify…….
7. Type of tumour usually seen at post mortem. (a) Nodular lesions (b) Diffused lessions (c) Others specify ……
8. Organs frequently involved; (a) Lungs (b) Heart (c) Liver (d) Spleen (d) Kidneys (f) Gonads
9. Drop in egg production in laying birds can be put at; (a) 10% (b) 20% (c) 30% (d) 40%
10. Type of vaccine commonly used: (a) Rispens/CV 1988 (b) HVT (c) SB-126 (d) Others……
11. Highest mortality ever reported in percentage……
12. How do you confirm your diagnosis for Marek's disease? ……..……
    ……
    ……..

Thank you.

## Appendix 2

**Questionnaire on poultry farmers' awareness on Marek's disease in Nigeria.**

Name of interviewer ----------

INTRODUCTION

Disease is one of the most poultry production constraints be it local or commercial. Therefore in order to assist poultry farmers reduce disease incidence in their flocks and increase production, we would like to know more about the major problems associated with the poultry production. The response given by the farmers to this questionnaire will be of assistance in making recommendation for improvement.

1. Personal datails Date----------

Name/Farm
District
LGA
Village/town
Male/female
Occupation

| Mangement tick "X" | | check |
|---|---|---|
| (i) | Intensive | ( ) |
| (ii) | Semi intensive | ( ) |
| (iii) | Extensive | ( ) |

2.Type and Number of chickens you have:

| Local | Number |
|---|---|
| Cock s | |
| Hens | |
| Growers | |
| Chicks | |

| Commercial | Number |
|---|---|
| Layers | |
| Broilers | |
| Parent stock | |

3. Type of chicken: Mark only x

A

| **Local:** | **check** |
|---|---|
| Type | X |
| Dwarf | |
| Naked neck | |
| Frizzled (curl feather) | |
| White | |
| Black | |
| Mottled | |
| Barred | |
| Long legged | |
| Others specify | |

| **B.** **Comercial**: | check |
|---|---|
| Broilers | |
| Layers | |
| Parent stock | |
| Colour: | |
| Brown | |
| Black white | |
| Others e.g. Variegated Black/white, Ash etc | |

4. Which of these species of birds do you have?

| Species | Number |
|---|---|
| Turkeys | |
| Pigeons | |
| Guinea fowsl | |
| Others (specify) | |

5. Source of your birds (mark correct "X")

| **A Local Birds** | "X" | check |
|---|---|---|
| Purchased from market | | |
| As gift | | |
| Inherited | | |
| Others specify | | |

**B. Comercial:** "X"

Obasanjo farm

S & D

ECWA

Zartech

Others specify

| Source of water mark "X" | Check |
|---|---|
| Well water | |
| River/stream | |
| Rain water | |
| Pump/tap | |
| Pond | |
| Borehole | |

6. What food/feed do you give your birds? Check

| **Type of food** | Response "X" |
|---|---|
| Nothing (scavenge) | |
| Millet | |
| Maize | |
| Rice/brand | |
| Maize brand | |
| Millet brand | |
| Kitchen waste | |
| Maize grit | |
| Commercial feed: ECWA | |
| Vital | |
| Feed masters | |
| Others (specify) | |

7.In your opinion what are the main causes of mortality (death in your birds?

| Test | "X" |
|---|---|
| Disease | |
| Theft | |
| Birds prey (hawks) | |
| Car accident | |
| Dogs/cats | |
| Snakes | |
| Lack of feeds | |
| Others (specify) | |

8. If diseased, do you isolate sick birds? Yes ( ) No ( )

9.What do you do with the dead birds? sell ( ) cooked ( ) throw away ( ) etc check

10.How does the bird behave when sick? loss of weight ( ) not eating well ( ) pale comb/wattles ( ) poor growth ( ) twisted neck ( ) egg drop ( ) weakness ( ) paralysis ( )

11. Do you have a veterinary clinic near by? Yes ( ) No ( )

12. Have you heard about Marek's disease (Tumour) Yes ( ) No ( )

13. How many of your birds died within six months?

14 Mortality (death) from Marek's disease (Tumour).

| | Chicks | Adult | Slaughter/PM Chicks | Adult |
|---|---|---|---|---|
| Chickens | | | | |
| Ducks | | | | |
| Turkey | | | | |
| Guinea fowl | | | | |
| Others specify | | | | |

1. In which month of the year is Marek's Disease more likely to occur? Place "X" next to appropriate month (s).

| | | | |
|---|---|---|---|
| January | April | July | October |
| February | May | August | November |
| March | June | September | December |

2. Have you ever vaccinated your local bird's agaisnt Marek's? yes( ) No ( )

17 Do you revaccinate your commercial chicks against Marek's disease? Yes ( ) No ( ) and at what age? ----------

18. Do you keep both chicks and Adult birds in the same building? Yes ( ) No ( )

19. Do you sell your sick birds? Yes ( ) No ( ) some times ( )

20. Marek's Disease in commercially affected birds: do you close the farm by selling off the birds? Yes ( ) No ( )

21. How many times have you experienced Marek's Disease on your farm? once ( ) twice ( ) never ( )

22. Have you ever had the same Marek's Disease complains at the same time with another farmer you know with the same source of chicks? Yes ( ) No ( )

23. What is the percentage loss of your birds due to Marek's Disease?
5% 10% 20% 30% 40% 50%

24. In your opinion do you consider Marek's Disease – posing a serious threat to poultry farmers in Nigeria? Yes ( ) No ( )

25. Due to Marek's Disease on your farm, have you decided to brood and raise chicks in new environment then return them to the permanent site as growers? yes ( ) Not at all ( ) the same farm ( )

26. Any other comments ?

## Appendix 3

**Views of laboratories used in the investigation of Marek's disease in the field and experimentally challenged chickens in Nigeria and USA with postmortem showing lymphomas based on the system involved (yellow arrows)**




(A) (B)

(C) (D)

A and B = Central Diagnostic Laboratory NVRI, Vom Plateau State, Nigeria C and D = Avian Disease and Oncology Laboratory, East Lansing Michigan, USA. Arrows showed samples collection from chicken carcasses.