CLINICOPATHOLOGICAL STATUS OF MAREK'S DISEASE AT DINAJPUR DISTRICT

A THESIS

BY

SONIA SARDI RINI

REGISTRATION NO: 1405088

SEMESTER: JANUARY-JUNE/2015

SESSION: 2014-2015

MASTER OF SCIENCE (M.S.)

IN

PATHOLOGY

DEPARTMENT OF PATHOLOGY AND PARASITOLOGY

HAJEE MOHAMMAD DANESH SCIENCE AND TECHNOLOGY UNIVERSITY, DINAJPUR

JUNE, 2015

Dedicated

To My

Beloved Parents

ACKNOWLEDGEMENT

All praises and compliments to the almighty Allah without whose desire I could not have completed the degree of Master of Science (MS) in Pathology. And then I express my deepest sense of gratitude, sincere appreciation and indebtedness to my respected research supervisor, Dr. Md Nazrul Islam, Associate Professor, Department of Pathology & Parasitology, Hajee Mohammad Danesh Science & Technology University, Dinajpur for his cordial and scholastic guidance, continuous encouragement, valuable suggestions, constructive criticism and affectionate feelings throughout the course of the research work and preparation of the manuscript. I also would like to thank my research co-supervisor, Dr. Md. Haydar Ali, Assistant Professor & Chairman with my heartfelt gratefulness for his sympathy, sincere cooperation, inspiration and valuable suggestions for the completion of research work. Profound respect and gratitude are expressed to my honorable teachers Prof. Dr. S. M. Harun–ur-Roshid, Dr. Md. Nazrul Islam, Dr. Mominul Islam, Dr. Md. Golam Azam (Rana), Department of Pathology and Parasitology, Hajee Mohammad Danesh Science & Technology University, Dinajpur for the contributions, encouraging suggestions and kind cooperation, they have given me throughout my academic career. I also express my cordial thanks to Prof. Dr. S. M. Harun–ur-Roshid, Dr. Md. Nazrul Islam, Dr. Mominul Islam and all other MS Students for their constant inspiration and co-operation during the entire period of my academic life in HSTU, for their kind support and guidance throughout my research work. My gratefulness also goes to all technician and office staffs of the Dept. of Pathology and Parasitology, HSTU, Dinajpur. Most importantly I wish to extend my heartfelt gratitude to my parents, sisters and brother without whom it was impossible for me to lead my life. Finally I want to offer thanks to my all relatives and well-wishers.

The Author

June 2015

ABSTRACT

The study was designed to investigate the pathological conditions of Marek's disease in the small scale commercial layer farms at different region in Dinajpur district. Chickens are the most important natural host for Marek's disease virus, a highly cell-associated but readily transmitted alphaherpes virus with lymphotropic properties of gammaherpes viruses. The objective of the present study was to investigate the pathological features of Marek's disease in the recent outbreaks in commercial poultry farms at dinajpur district. A total of 18 dead or sick birds were obtained from 6 different layer farms at dinajpur district with clinical suspicion of Marek' disease, during the period from January to June 2015. On necropsy, Grossly large or military whitish nodular lesions were found in the liver, spleen & enlargement of sciatic nerve. Histologically, lymphomatous lesions of various extent and nature were observed in different organs. In the liver and spleen, the lymphomatosis was associated with extensive damage of the parenchyma were characterized by diffuse as well as focal and nodular proliferation of lymphocytes. In the period of six month, total 61 diseased birds were affected of which 18 dead or sick chickens are examined and taken sample for pathological investigation. The present study showed that overall prevalence at Dinajpur district were 0.45% whereas 0.48%, 0.53%, 0.40% 0.17% 0.78%and 0.35% in 6 farms respectively. The mortality rate 0.25% whereas 0.20%, 0.27%, 0.25%, 0.13%, 0.44 % and 0.20% The mortality was higher in nonvaccinated than in vaccinated birds. The findings of the present study would suggest that Marek's disease can be expected its etiology, conventional and advance tools and techniques being used for its diagnosis, prevention and control strategies in poultry.

TABLE OF CONTENTS

TABLE OF CONTENTS (Contd.)

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS AND SYMBOLS

MD	- Marek’s disease
CD	- Cluster of differentiation
PCR	- Polymerase chain reaction
(GaHV-2)	- Galid herpes virus-2
GaHV-3	- Galid herpes virus-3
MDV	- Marek’s disease virus
vMDV	- virulent Marek’s disease virus
vvMDV	- Very virulent Marek’s disease virus
vv+MDV	- Very virulent plus Marek’s disease virus
ALV	- Avian leukosis virus
REV	- Reticulo-endothelial virus
CNS	- Central nervous system
RNA	- Ribonuclic acid
DNA	- Deoxy Ribonuclic acid
ELISA	- Enzyme Linked Immunosorbent Assay
AGPT	- Agar gel precipitation test
MHC	- Major histocompatibility complex
H & E	- Hemoatoxylin and Eosin
e.g	- example
no	- number
ml	- Mililiter
%	- percentage
g	- gram

CHAPTER I

INTRODUCTION

Bangladesh is one of the highly density countries of the world has a population of 160 million people. About eighty percent people of this country still live in villages and extremely poor. Poultry farms, mainly chicken farms producing meat and eggs, can be highly specialized operations. Both the government and a variety of non-governmental organizations (NGO's) are actively promoting poultry development at all levels. The Bangladesh Rural Advancement Commission (BRAC), the largest shows in its 'annual report that more than 70% of rural households are involved in poultry keeping. But they face serious constraints, as the mortality rate of poultry is said to be as high as 25%, due to a combination of improper feeding practices, ignorance of management needs and poor distribution of vaccines (Khairul Islam *et al.*, 2014). Many reports have established this, and also provided evidence that an increase in livestock production based on smallholder systems not only increase cash income but also household consumption of livestock products (LID, 1999; Kurup, 2001). The poultry sector in Bangladesh is very important for the reduction of poverty and creation of employment opportunities. The livelihoods of a substantial section depend directly on this industry. Most birds are kept in small flocks under a scavenging system with feed generally available from household waste, homestead pickings, and crop residues. Productivity of the local hens is low and losses due to diseases and predators are high. Constraints to productivity however, are not only related to disease but also to management systems, lack of supplementary feeding, predators, and inappropriate breeds (Saleque, 2001). For sustainable development of poultry sector some specific recommendations (establishment of effective data bank, formation of poultry farmers 'organization etc.) are made for consideration by the concerned stakeholders of this industry (S. K. Raha, 2013).

Marek's disease (MD) is a contagious disease of chickens characterized by development of lymphoid tumors in the viscera and lymphoid infiltration of the peripheral nerves (K. Nazerian and R.L. Witter, 1970). Birds get infected by inhalation of infected dust from the poultry houses, and following a complex life cycle, the virus is shed from the feather follicle of infected birds (Baigent & Davison, 2004). Published literature on MD outbreaks in Bangladesh is rather limited. In Bangladesh, 22.73% of chicken mortality occurs due to different type of viral diseases. And among them about 1.09% of total

mortality is due to Marek's disease and avian leukosis (Rahman *et al.,* 2003). MD is caused by serotype 1 oncogenic Marek's disease virus (MDV). MDV causes a lymphoproliferative disease that appears in visceral and nervous forms. Skin tumors and ocular lesions have been reported as well (Ficken *et al.,* 1991; Witter and Schat, 2003).

Marek's disease, is a lymphoproliferative disease of chickens and characterized by number of conditions such as lymphomatous tumors in different visceral organs, enlargement of sciatic nerve and brachial nerve due to infiltration of lymphoblasts. Birds of any age are susceptible; most commonly at above 2-5 months of age are more susceptible. Adult birds were commonly seen to the vaccination can prevent formation of tumor but generation of infectious virus is not prevented. The virus is shed in the environment as dander dust after maturation in the feather follicle epithelium (Ahmed, 1982). The most commonly affected organs and tissues are peripheral nerves, iris gonads, spleen, heart, lungs, liver and muscle. Paralysis is evident with ataxia for period of several days. Diagnosis is based on enlarged nerves, lymphoid tumors in viscera and confirmation is by demonstration of tumor – associated surface antigen on some of the individual cells by immunofluorescence. A real-time PCR method is developed, optimized and validated, to enable quantization of Marek's disease virus genomes. Vaccination is the principal method of control. Genetic resistance of chickens to the disease has been exploited in the laboratory to develop resistant breeds. Chemoprophylaxis is of little success (K. Shahzad, 2007). Because MDV is not transmitted vertically, partial control may also be achieved through bio-security procedures sufficient to delay exposure, such as placement of newly hatched chicks in thoroughly cleaned and disinfected houses that are well separated from houses with older chickens. Genetic selection and management have been used more frequently as adjuncts to vaccination rather than as primary control strategies but are critical components of an integrated control system (R. L. Witter, 1998).

Histopathologic examination of proliferative lesions is of paramount importance in diagnosing MD. Lymphomatous lesions in MD consist of small and medium size lymphocytes and blast cells. The cells are pleomorphic and vary in size (Payne, 1976). The target cells for malignant transformations are mostly of CD4+CD8- phenotype (Schat *et al.,* 1987). Besides the pathomorphological examination, histochemical staining can help in differentiating MD from lymphoid leucosis (LL) as well as from other neo-plastic diseases.

MD can be diagnosed by clinical history, necropsy and histopathology. Pathomorphological examination and molecular detection of MD by PCR technique can help in confirmatory diagnosis of MD and further studies like molecular characterization will help knowing the genetic properties because nothing is known about the genetic properties of MDVs circulating in Bangladesh. So, keeping the above in view, the study was undertaken with the following objectives:

- To study the clinical findings of Marek's disease in chickens at dinajpur district.
- To study the gross features of Marek's disease in field outbreaks.
- To evaluate the histopathological changes in liver & spleen of affected chickens.
- To know the prevalence of Marek's disease in chickens.

CHAPTER II

REVIEW OF LITERATURE

Available literature on pathology and diagnosis of Marek's disease is reviewed in this part of the thesis along with a brief overview on the history, etiology, epidemiology, clinical manifestations, pathogenesis, diagnosis and vaccination against MDV.

2.1 History

Marek's disease is a common lymphoproliferative disease of chickens affecting the peripheral nerves, other tissues and visceral organs. The name "Marek's disease" was first proposed by Biggs (1961) and was regarded as synonymous with "fowl paralysis" or "neurolymphomatosis (Fujimoto, Y. *et al.,* 1971) An association of lymphoid tumors in the peripheral nerves as well as visceral organs and used the name "neurolymphomatos is gallinarum" (Pappenheimer *et al.,* 1929). Outbreaks of MD in 1914 were reported by United States; subsequent of the disease came from The Netherlands, Great Britain, and many other countries (Biggs *et al.,* 1968). Several outbreaks of the disease in the United States reported by Kaupp (1921), whose observations dated to 1914 (Van der Walle and Winkler-Junius, 1924) indicated that the disease was also present in the Netherlands. Marek's disease was first recorded in Great Britain by Galloway in 1929. It was subsequently noted in any other countries (Biggs, 1966). Now-a-days, Marek's disease is considered to have world-wide distribution. In Bangladesh, A pathological study was conducted on the poultry disease occurring at Rajshahi region of Bangladesh during the period 4 January, 2001 to February, 2002 about 0.61% of total diagnostic cases were recorded as Marek's disease (Hossain *et al.,* 2004). Outbreaks of the disease producing unusually high mortality have been observed since at least 1949 and is now quite common (Benton and Cover, 1957; Benton *et al.,* 1962; Biggs *et al.,* 1965; Dunlop *et al.,* 1965). These are usually characterized by the occurrence of visceral lymphoid tumors, often without accompanying gross nerve enlargement. Biggs (1966) has referred to this form as "acute Marek's disease" whiles the form in which neural involvement predominates was termed "classical Marek's disease".

2.2 Etiology

Marek's disease viruses (MDVs) belong to the family Herpesviridae, the subfamily Alphaherpesviridae and the genus Mardivirus (Churchill and Biggs, 1967; Biggs *et al.,* 1968; Nazerian *et al.,* 1968; Solomon *et al.,* 1968; Witter *et al.,* 1969; Gimeno, 2004; Fauqet *et al.,* 2005). The causal agent of Marek's disease was reported to be a herpes virus following independent studies by workers in Great Britain (Churchill and Biggs, 1967) and the United States (Nazerian *et al.,* 1968; Solomon *et al.,* 1968). Within this subfamily, MDV was classified originally under the genus `Marek's disease-like virus'. Generally three species are available within this genus, Galid herpesvirus 2 (GaHV-2), Gallid herpesvirus 3 (GaHV-3) and Meleagrid herpesvirus I (MeHV-1). These species are previously classified according to the MDV type I or serotype 1, MDV type 2 or 5 serotype 2, turkey herpes virus (HVT) or serotype 3, respectively (Kingham *et al.,* 2001). MDV has a linear, double stranded DNA genome that is about 160-180 kb in size and the genome contains at least 90 open reading frames (Izumiya *et al.,* 2001; Lupiani *et al.,* 2001). All three MDV serotypes have genome structures consisting of a long unique region and a shorter unique region, each flanked by inverted repeats (Cebrian *et al.,* 1982).The genomes of all three serotypes are similarly organized (Igarashi *et al.,* 1987; Ono *et al.,* 1992). Serotype 1 has the largest genome, followed by serotype 2 then lastly serotypes 3 having the smallest genome size (Cebrian *et al.,* 1982, Hirai *et al.,* 1997). All three serotypes also differ in their restriction end onuclease digestion patterns (Witter *et al.,* 2003). The patho types described by these analyses are generally classified as mildly virulent (mMDV), virulent (vMDV), and very virulent (vvMDV) and very virulent plus (vv+MDV) (Witter *et al.,* 1997). Although these classifications describe a continuum of virulence from a field perspective, it is important that the distinctive lesions appear to be associated with the vvMDV (high incidence of visceral lesions) and vv+MDV (high incidence of stunting, neurologic lesions and rapid transmission rate) pathotypes (Rosenberger, 1997; Gimeno *et al.,* 1999). Pathotyping of virus isolates involves pathogenicity tests in vaccinated or unvaccinated chickens (Witter, 1988). No in vitro methods have yet been developed.

MDV can be found in multiple forms, such as 85-100 nm in diameter nucleocapsid or an enveloped particle 150-160 nm in diameter. MDV is also found in feather follicle epithelium as an enveloped 273-400 nm particle (Witter *et al.,* 2003).

2.3 Epidemiology

2.3.1 Source of infection

Infection persists and virus is shed from follicles along with desquamated cells. This dander can remain infective for several months in dust and litter in poultry house.

2.3.2 Age and host range

Chickens between 12 to 25 weeks of age are most commonly clinically affected. Occasionally pheasants, quail, game fowl and turkeys can be infected. Marek's disease will often affect chickens from 4 to16 weeks old up to 3 years of age, but can occur any time throughout their lifetime ***(Sarah Tilley** et al.,* 2013). Cytolytic and latent infection of lymphoid cells and oncogenic transformation of CD4+ T cells in susceptible chickens. Infection of a young susceptible chick with the alpha herpes virus is followed after 4 to 7 days by a short period of virus replication in lymphoid cells and reticulum cells in thymus, spleen and bursa of fabric us. Following an early cell associated cytolytic infection of lymphocytes a switch to latent infection occurs at about 7 days. Congenital infection does not occur and chicks are protected by maternal antibodies for first few weeks of life**]** Epidemics involve sexually immature birds' 2-5months old (Shahzad *et al.,* 2007).

2.3.3 Incubation Period

Generally, young birds age are susceptible but in most cases susceptibility is strength, number of birds seen at four weeks of age It is difficult to determine the incubation period of the disease under field conditions. It commonly appears in 3 to 4 weeks old chickens and gradually builds to a peak between 12 and 30 weeks of age (Morgan *et al.*, 2008). Chicks inoculated at 1 day of age excrete virus beginning at the 2nd or 3nd week and develop microscopic lesion as early as 2 weeks p.i. Clinical signs and gross lesions generally do not appear until between the 3nd and 4th week (Biggs and Payne, 1963, 1967; Sevoian *et al.,* 1962 and Vickers *et al.,* 1963) when exposure is by contact with inoculated birds, the latent period is delayed by a period about equal to the time required before the virus is excreted form the inoculates (Biggs and Payne,1967).). Liver, kidneys, lungs, heart, proventriclus, ovary and nerves of sciatic plexus collected from 20 chickens aged 8 to 24 weeks were examined. Lymphoproliferative enlargement in various organs of focal and diffuse character was found.

2.3.4 Transmission

Marek's disease virus is transmitted by air within the poultry house. It is in the feather dander, chicken house dust, feces and saliva. Infected birds carry the virus in their blood for life and are a source of infection for susceptible birds. Disease virus is transmitted horizontally only, and international spread in hatching eggs and day-old chicks can be prevented by appropriate hygiene precautions. Transmission of ALV and REV occurs both horizontally and vertically (through the egg), and measures to prevent international spread are more demanding(Pnye *et al.*, 2000) Epithelial cells in the keratinizing layer of the feather follicle allow replication of fully infectious virus (Calnek *et al.,* 1970), and these cells serve as a source of contamination to the environment. Virus associated with feathers and dander contaminated poultry house dust remains infectious for at least several months at 20-250 C and for years at 40 C (Calnek, 1980; Crabb *et al*., 2009). Birds get infected by inhalation of infected dust from the poultry houses, and following a complex life cycle, the virus is shed from the feather follicle of infected birds (Baigent and Davison, 2004). Direct or indirect contact between birds affects virus spread, apparently by the airborne route (Biggs, 1985). Once the virus is shed into the environment, it can remain infectious for many months (Kreager, 1998; Rodriguez *et al.,* 2007). Apparently, there is no vertical transmission of MDV (Witter and Solomon, 1972), and 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 employed for incubation (Calnek and Hitchner, 1973).

2.3.5 Morbidity and mortality

Morbidity (number affected) in unvaccinated flocks can reach 60 percent. Vaccinated flocks fare better with less than 5 percent affected. Mortality is high in affected birds reaching nearly 100 percent over a 10-week period. Pullets are more likely to be affected than cockerels.

Factors associated with the host which affect disease incidence include sex, genetic constitution, and age. Biggs and Payne (1967) and Cole (1968) observed that females experienced higher losses then males and agreed that the greater susceptibility of females was manifested in a shorter latent period. According to the former workers, the difference was apparently not due to sex hormones. Cole (1968) noticed that the difference was less pronounced with relatively resistant strains than with susceptible

strains of chickens, and Purchase and Biggs (1967) observed that the susceptibility differences between the sexes was apparent only in the case of infection with the highly virulent virus isolates. Genetic factors play an important role in determining the outcome of exposure to virus and are evident in the case of both natural and experimental infection (Spencer, 1969; Calnek and Hitchner, 1969). Panneerselvam *et al.,* (1990) reported that the percentage of mortality due to Marek's disease was higher in the younger age group (9-20 weeks) than that of older birds (above 20 weeks) and peak mortality was encountered between 16-26 weeks of age. Since immunological responsiveness is reduced with Marek's disease (Purchase *et al.,* 1968) infected chickens may fail to develop immunity to other diseases. This appears to explain the association of Marek's disease with coccidiosis (Biggs *et al.,* 1968) and could also account for apparent relationships between Marek's disease and other disease. The pathologic response was also affected by age; neural lesions predominated in the younger birds while visceral lesions were most common in the older group (Sevoian and Chamberlain, 1964). Biggs and Payne (1967) inoculated 1-day-old and 50-day-old chickens with the B-14 isolate and observed incidences in the two groups of 73% and 6%, respectively. Panda *et al.* (1983) observed higher mortality (28.15 %) due to MD at 21 to 40 weeks of age as compared to 8.86 per cent mortality at 9 to 20 weeks of age.

2.4 Clinical Signs

Marek's disease has 4 different forms including cutaneous (Skin form), neural (nerve form), ocular (eye form) and visceral (internal-organ form) (Kozdrun *et al.,* 2001). Clinical signs of MD are associated with asymmetric, progressive paresis and finally, complete paralysis of one or more of the extremities. Either one or several nerves in the body may be affected. Wing involvement is followed by drooping of the limb. If nerves of the neck muscles are affected, the head may be held down and there may be some torticollis. Characteristic attitude Of MD is that, one leg stretched forward and the other backward as a result of unilateral paresis or paralysis of the leg (Calnek and Witter, 1991). In acute forms of MD, the symptoms are more explosive and initially are characterized by a high proportion of severe depression of birds. After few days, Lymphomatous visceral tumors in MD have been reported by many authors (Purchase and Biggs, 1967; Rathore *et al.,* 1985; Narang *et al.,* 2003, Kamaldeep *et al.,* 2007).

Ability to accommodate to light intensity gradually decrease by affected eyes. If iris affects blindness is the final outcome. At the start point, pupils become irregular and at advance stages is only a small pinpoint opening (Jungherr and Hughes, 1965; Fujimoto *et al.,* 1972). Other signs which include weight loss, paleness, anorexia, and diarrhea may be observed, especially in birds in which the course is prolonged. In case of commercial farming death often results from starvation and dehydration because of inability to reach food and water or in many cases from trampling by pen mates Marek's disease has been observed infrequently since vaccination has been practiced. Varying degrees of ataxia and partial or whole body paralysis is manifested by affected birds beginning 8-12 days after virus inoculation and lasting 1-2 days (Swayne *et al.,* 1989).

2.5 Pathology

The gross and microscopic lesions in chickens infected with MDV can be greatly affected by many factors such as age, chicken strains, virus strains, and sex (Calnek and Witter, 1991). Although all strains of serotype 1 have oncogenic potential, the outcome of infection depends largely on the combination of virus strain and genetic resistance of the chicken. Among the serotype 1 MDV strains, very virulent MDV (vvMDV) strain is known to cause a higher incidence of MD lymphomas in genetically resistant chickens and higher early mortality in genetically susceptible chickens (Witter *et al.,* 1980). The Gross and microscopic lesions associated with MD are discussed below:

2.5.1 Gross lesions

Liver, kidneys, lungs, heart, proventriclus, ovary and nerves of sciatic plexus collected from 20 chickens aged 8 to 24 weeks were examined. Lymphoproliferative enlargement in various organs of focal and diffuse character was found. The affected organs were enlarged, compact and very brittle, irregular in shape, grayish-red or grayish white and of fatty consistency. Most often characteristic changes were in the spleen, liver, proventriculus and ovary that usually had a compact or ribbed, fatty cauliflower like formation, different in size (PEJOVIN *et al.,* 2007) Major gross lesions were severe emaciation, thickened proventriculus and flabby heart with loss of coronary fats (Musa I. W. *et al.,* 2013) Grossly, tumors or nodules (pin-point to 2 mm in diameter), grayish-white in color, were seen on the liver, spleen, ovary and kidney parenchyma, which were firm in consistency. Such nodular or miliary lymphoid tumors in liver, spleen, heart, kidney, proventriculus and gonads in birds suffering from acute MD without involvement of

peripheral nerves have been demonstrated by (Fujimoto *et al.,* 1972).Gross changes in visceral organs of infected chickens, enlargement of the affected organs, sometimes to several times than normal size is evident, and there is diffuse grayish discoloration. The bursa of fabricius usually atrophic, if affected may develop tumors that appear as diffuse thickening owing to interfollicular distribution of tumor cells (Purchase *et al.,* 1987).

The normal lobule architecture of liver is disappeared and often gives a coarse granular appearance on the surface due to diffuse infiltration of lymphocytes. Lesions also observed in the ovary as small to large grayish translucent areas. The normal foliated appearance of the ovary is obliterated. Mature ovaries may retain function even though some follicles are tumors. Marked involvement is indicated by a cauliflower-like appearance (Yutaka *et al.,* 1971). Gross changes vary from tiny whitish streaks to nodular tumors. Affected areas are a lusterless whitish gray or may have a definite yellow-orange color (probably associated with necrosis). Muscle lesions can also include atrophic changes of neurogenic origin when nerve trunks are severely affected (Madarame *et al.,* 1986; Wight, 1966). Benton and Cover (1957) studied acute form of MD in broiler chickens, and observed numerous tumors of varying degrees of size on visceral organs, muscle and skin. MD lymphomas in visceral organs have been reported by several workers (Kamaldeep *et al.,* 2007; Narang *et al.,* 2003 and Rathore *et al.,* 1985).

2.5.2 Histopathological lesions

Lymphomatous lesions are uniformly proliferative in nature in the visceral organs. Proliferating small to medium lymphocytes, lymphoblast and activated and primitive reticulum cells are the component of cellular composition (Payne and Biggs, 1967).specific pathologic features of eye lesions are demonstrable only by histological examination. The most constant change is mononuclear of the iris but infiltrates may also be found in the eye muscles, especially the rectus lateralis and ciliaris. Sometimes in the anterior chamber, granular or amorphous material is observed (Jungherr and Hughes 1965).

The visceral lesions were classified of Marek's disease into the following 3 categories to the histological characteristics: i) lymphoigranulomatous lesions, ii) reticulosarcoma-like or lymphosarcomalike lesions, iii) lymphoblastoid lesions (Yamamoto *et al.,* 1969).

The histopathologic changes associated with Marek's disease have been described by numerous workers who were in general agreement about the types of histological lesions and the cell types involved (Campbell, 1959; Furth, 1935 and Payne and Biggs, 1967).

For purpose of histopathological examination a total of 767 tissue samples were received from different parts of India and Bangladesh during the period from July 2006 to June 2007. Marek's disease involved the liver (34.34%), spleen (26.26%), kidneys (12.12%), ovaries (7.07%), proventriculus (8.08%), lung (4.04%), sciatic nerve (3.03%), intestine (2.02%), skin (1.01%) and mesentery (1.01%) (Balachandran *et al.,* 2009).

Skin lesions appear as inflammatory or lymphomatous in nature. They are localized around infected feather follicles. Sometimes in the dermis, massive accumulations of mononuclear cells around feather follicles, compact aggregates of proliferating cells, often perivascular and a few plasma cells and histocytes are seen (Payne and Biggs, 1967).

Chicks was injected with the CONN-A isolate of virus and noted that central nervous system lesions, while apparent from the 2nd week post inoculation, were most pronounced at 4-7 weeks when clinical manifestations were most sever. They observed mostly immature lymphocytes and only a few blast cells (Vickers *et al.,* 1963).

Proliferation and infiltration of lymphoblasts and lymphocytes in the sections of liver, spleen, kidney, sciatic nerve and ovary of the affected birds were observed on histopathological examination (Frazier, 1974; Lobago and Woldemeskel, 2004; Goyal *et al.,* 2006). Panneerselvam *et al*. (1990) reported lesions of Marek's disease in the liver, spleen, kidney, proventriculus, ovary, nerve, heart and lungs of layers. The composition of tumours is the same from one organ to another even though the gross pattern of involvement may vary. Sevoian and Chamberlain (1964) concluded that the lesions mainly consisted of proliferated cells originating from the primitive mesenchymal cells of the tunica adventitia of the arterioles, neurilemmal cells and the lining cells of the hepatic sinusoids, in descending order of incidence. In the study of Fujimoto *et al.* (1971), cell proliferation was markedly seen in the interlobular connective tissues, especially around the small blood vessels in the liver. In the spleen, proliferation was seen around the capillary sheathed arteries. In the ovary, adrenals and kidneys, etc., proliferation started around the capillary or small arterioles in various tissues and extended into the adjoining tissues. In severe cases, proliferation had become so massive

as to suggest a distinct neoplasm. Payne and Biggs (1967) described an unusual cell with very basophilic pyroninophilic and vacuolated cytoplasm and a nucleus with little or no detail. The authors called it a "Marek's disease cell" and thought it to represent a degenerative process in a blast type cell. It was frequently seen in proliferative lesions. Pejovic *et al.* (2006) found on histological examination, proliferation of small and medium size lymphocytes, lymphoblast cells, Marek's disease cells and activated reticulum cells. Tumor proliferates predominantly consisted of lymphoblasts and had all the characteristics of a lymphoma. Cho *et al.* (1999b) detected histological, visceral tumors and peripheral nerve lesions in 84% and 97% birds in the non-necrotizing category, and 88% and 100% birds in the necrotizing category. They had typical MD visceral and nerve lesions consisting of various compositions of lymphoid cells, mainly from small lymphocytes to timorous lymphoblasts. In some of the MD visceral lesions, the necrotizing blood vessels with necrotic lymphoid cells were concurrently observed with necrotizing CNS lesions in three birds. Five birds showed MD visceral and nerve lesions, but no CNS lesions. In contrast to the visceral lymphomas, lesions of the skin appear more inflammatory. In addition to the sometimes massive accumulations of mononuclear cells around the feather follicles, complete aggregates of proliferating cells, often perivascular, and a few plasma cells and histiocytes are seen in the dermis (Helmboldt *et al.,* 1963; Payne and Biggs, 1967; Moriguchi *et al.,* 1989). With small lesions the architectural integrity of skin maintained, but massive proliferative lesions may cause disruption of the epidermis resulting in an ulcer.

Lesoins in peripheral nerves consist of light to heavy mononuclear cells, sometimes associated with edema, myelin degeneration and Schawann cell proliferation. Axonal degeneration is rare. The offending cells are usually a mixture of several types including small and medium lymphocyte, plasma cells, and lymphoblast (OIE, 2004). A few macrophages may be found. Wight (1962) quoted several reports which described the essential changes in affected nerves as an infiltration of inflammatory cells which are at first peri vascular but subsequently increase in number until the nerve tissue is largely replaced by masses of cells. Wight (1962) classified the lesions into three types, two of which were essentially inflammatory or degenerative while the third one was neoplastic. Type I lesions were characterized by cellular infiltration relatively little edema. Most cells were small lymphocytes or plasma cells but there were also some lymphoblasts in cases of massive infiltrations. In Type II, edema was marked and only a few infiltrating

cells (mostly plasma cells) were present. Fibrosis was occasionally seen. Type III was declared neoplastic because of massive infiltration with lymphoblastic cells and the observation of frequent mitosis. Sometimes there were also small lymphocytes and some groups of these had germinal centers. All three types were considered histologic variants of the same condition, but it was thought that the neoplastic changes followed the inflammatory lesions. Payne and Biggs (1967) studied the pathogenesis of the experimental disease in chicks in order to examine the stages leading to advanced lesions. Chicks which had been inoculated at 1 day of age with the B-14 isolate developed microscopic changes which they categorized as A, B or C type. Type A lesions were those first observed (14-21 days post inoculation) and consisted of proliferating lymphoid cells; in some cases there was demyelinatin and Schwann cell proliferation. “Marek’s disease cells” were present. Type B lesions consisted of diffuse infiltration by plasma cells and small lymphocytes usually with edema and sometimes with demyelination and Schwann cell proliferation. They were not seen until 28 or more days post inoculation and were sometimes mixed with type lesions. A third lesion type (C) in which there was only a light infiltration by plasma cells and small lymphocytes, was observed in 4 or 6 clinically normal birds examined at 10 weeks. Thus in contradiction to Wight (1962) and others, Payne and Biggs considered the more inflammatory type changes to follow the proliferative lesions. Histopathologic changes in the brain were described by Pappenheimer *et al.* (1926) and Cho *et al.* (1999). Lesions were always focal in distribution and consisted of either compact perivascular cuffs of small densly staining lymphocytes or sub-military nodules composed of lymphocytes and paller elements. Jungherr and Hughes (1965) stated that the latter were probably of glial organ. The spinal cord had, in addition to regional infiltrations, focal accumulations in the white matter and occasionally in the central gray matter. Root ganglia were intensely infiltrated but the ganglion cells were intact. Wight (1965) found the central nervous system of affected birds often histological normal or with only minimal lesions and concluded that to be Marek’s disease of peripheral nerves. He did not find plasma cells in the brain. Vuckers *et al.* (1967) injected chicks with the CONN-A isolate of virus and noted that central nervous system lesions, while apparent from the 2nd week post inoculation, were most pronounced at 4-7 weeks when clinical manifestations were most severe. They observed mostly immature lymphocytes and only a few blast cells. In studies on transient paralysis, Swayne *et al.* (1989) described a vasculitis leading to vasogenic edema. Lesions were most consistently seen in the cerebellum. Ultra structural

changes did not include demyelination (Kornegay *et al.,* 1983; Swayne *et al.,* 1989). Jungherr and Hughes (1965) pointed out that the specific pathologic features of eye lesions are demonstrable only by histologic examination. The most constant change is mononuclear infiltration of the iris but infiltrates may also be found in the eye muscles, especially the rectus lateralis and ciliaris. Granular or amorphous material is sometimes present in the anterior chamber. Other but more rarely observed lesions involve the cornea (near Schlemm's canal), bulbar conjunctiva, pecten, and optic nerve. The iris and ciliary muscle lesions have been experimentally repeoduced by Sevoian and Chamberlain (1962) who inoculated the JM isolate directly into the anterior chamber of the eye of dayoldchicks. Changes in the Bursa of Fabricius and thymus of experimentally infected birds have been reported by Purchase and Biggs (1967) and Jakowski *et al.* (1969). In the bursa there were cortical and modularly atrophy, necrosis, cyst formation and interfollicular lymphoid infiltration. Atrophy of the thymus was sometimes severe and also involved both the cortex and medulla. In some cases there were areas of lymphoid proliferation in thymus. The bursa of Fabricius while usually atrophic when affected (Purchase and Biggs, 1967), may rarely develop tumors which appear as a diffuse thickening due to the interfollicular distribution of tumor cells. This lesion differs from the nodular tumor characteristic of lymphoid lukosis and may be easily differentiated histologically. Jakowski *et al.* (1970) observed that when chickens free of parental antibody were inoculated with MD virus at 1 day of age, necrosis and loss of architecture in the bursa of Fabricius and thymus were accompanied by a drastic reduction in packed cell volume and an aplasia of bone marrow.

2.6 Pathogenesis

Three phases are recognized 1) productive – restrictive infection. 2) Latent infection. 3) Neo-plastic transformation**.** Subclinical infection with virus shedding is common. Infection is acquired by inhalation of dander. Epithelial cells of respiratory tract are infected and contribute to cell-associated viremia involving macrophages. By sixth day there is productive infection of lymphoid cells in variety of organs including thymus, bursa of fabricius, bone marrow, spleen resulting in immune suppression (Shahzad *et al.,* 2007).

Humoral immunity, primary and secondary antibody response is decreased in the body as described by Purchase *et al*. (1968). In cellular immunity, median skin graft rejection

time in infected birds was either normal or slightly delayed and hypersensitivity to tuberculin was slightly decreased in significantly depressed MD birds. The presence of infection may also increase the susceptibility of fowl to other diseases**.** The production of cytokine mRNAs, in addition to viral DNA was quantified by quantitative reverse transcription – PCR in splenocytes during the course of Marek's disease virus infection in susceptible and resistant inbred chicken lines. MDV replicates similarly to other cell associated with Herpesviruses. At first, virus binds with the cellular receptors likely by the fuse of glycoprotein B, C and D and fuses and penetrates the target cell. The virus then uncoating with the aid of cellular enzymes which releases the viral DNA to be transported to the nucleus, the nucleus synthesized messenger RNA and then transported into the cytoplasm for translation. Then the virus enters into cells and infects other cells by direct contact possibly through formation of intracellular bridged (Kaleta., *et al.*, 1977). In addition to the virus going through exocytosis in Golgi vesicles, the release of progeny viruses are accompanied by death of the target cells (Davidson *et al.,* 2004). There are 4 basic phases of MDV pathogenesis based on the Cornell model (Calnek and Witter, 1985; Schat, 1987; Schat and Xing, 2000). These include an early cytolytic phase (2-7 dpi), a latent phase (7-10 dpi), late cytolytic and immunosuppressive phase (18 dpi) and a proliferative phase (28 dpi onward).

In the early cytolytic phase MDV is picked up by macrophages and ellipsoid associated reticular cells (EARCs) from the lungs and enters the blood stream; the cells then enter the secondary lymphoid tissues (e.g. spleen, gut-associated lymphoid tissue, cecal tonsil, Harderian gland). The virus gains entrance via the respiratory tract, where it is probably picked up by phagocytic cells. Shortly thereafter, cytolytic infection can be detected in the spleen, bursa of Fabricius, and thymus, peaking at 3-6 days. Shek *et al*. (1983) discovered that the primary target cells in all three organs are B cells, although some activated T cells become infected and undergo degeneration as well. Resting T cells are refractory to infection (Calnek *et al.,* 1985). The necrotizing effects of this early infection provoke an acute inflammatory reaction with infiltration of various cells including macrophages, granulocytes, and both immunologically committed and uncommitted lymphocytes (Payne and Roszkowski, 1973). A hyperplastic response in the spleen can follow, and at about 7 days, a transient immunosuppression may occur due to the presence of suppressor macrophages. Ultimately, there can be atrophy of the bursa and thymus. Chickens of susceptible and resistant strains of differing ages are

equally susceptible to infection (Calnek, 1973; Sharma, 1973; Witter *et al.,* 1973), and the level of infection in all birds is equally high in all cases during the early cytolytic period (Fabricant *et al.,* 1977). However, the pathogenicity of the virus strain may affect the severity of early infection. At about 6-7 days, the infection switches to latency coincident with the development of immune responses. Cell-mediated immunity has been shown to be important in the switch (Buscaglia *et al.,* 1988). Most latently infected cells are activated T cells, although B cells can also be involved (Shek *et al.,* 1983; Calnek *et al.,* 1984). The latent infection is persistent and can last for the lifetime of the bird (Witter *et al.,* 1971). Infection in genetically resistant birds often does not progress past the second phase (latency). Susceptible birds, however, develop a second wave of cytolytic infections after the 2nd or 3rd week coincident with permanent immune suppression. The lymphoid organs are again involved and localized foci of infection can be found in tissues of epithelial origin in various visceral organs (e.g., kidney, pancreas, adrenal gland, proventriculus, etc.) and especially in the skin, where a striking infection of the feather follicle epithelium occurs. The latter is unique in that it is the only known site of complete virus replication. There is focal necrosis, and inflammatory reactions develop around affected areas. The extent of infection during this phase depends on factors known to govern incidence of tumors; the most susceptible birds develop the most widespread and intense infections. The cause of inflammatory CNS lesions associated with MDV-induced transient paralysis is not clear, but it is known that the syndrome is under the control of genes of the major histocompatibility complex and that B cells are required for its induction (Schierman and Fletcher, 1980). Lymphoproliferative changes constituting the ultimate response in the disease may progress to tumor development, although regression of lesions can and commonly does occur either before or after frank lymphomas are apparent (Sharma *et al.,* 1973). Death from lymphomas may occur at any time from about 3 week onward. The composition of lymphomas is complex, consisting of a mixture of neoplastic, inflammatory, and immunologically active cells. Both T and B cells are present, although the former predominate (Hudson and Payne, 1973; Rouse *et al.,* 1973). During 2nd week after infection, there is persistent cell – associated viremia followed by proliferation.

2.7 Diagnosis

Diagnosis is made histological or by demonstration of tumor associated antigen (MATSA) on some individual cells by immunofluorescence Serum antibodies to Gallid

herpesvirus 2 may be demonstrated using virus neutralization. Primers that can distinguish attenuated and wild type strains have been developed for PCR assays**.** Feathers can be sampled readily from live birds and feather tip extracts are useful as a source of Marek's disease virus DNA for polymerase chain reaction (PCR) amplification for detection of MDV antigens by Elisa. However, compared with conventional PCR, real-time PCR is rapid, sensitive, reproducible, and has a wide dynamic range and, being a closed system requiring no post-amplification manipulation (Shahzad *et al.,* 2007). The traditional diagnosis of Marek's disease is based on the clinical signs and pathological alterations. The detection of viral antigen in the feather follicle epithelium by the agar gel precipitation test (AGPT) has been described by Haider *et al*. (1970). The different serotypes can be differentiated by the agar gel precipitation test (Lee *et al.,* 1983), but the sensitivity of that test is inferior to that of enzyme linked immunosorbent assay (ELISA) and DNA hybridization (Davidson *et al.,* 1986). The serotype can be identified by restriction endonuclease analysis (Ross *et al.,* 1983) or polymerase chain reaction (PCR) (Wang *et al.,* 1993). Zanella and Raymonds (1969) carried out AGPT for testing serum samples from chicken of various poultry farms for detecting MDV antibodies. MDV antigens HPRS-16, LCBS- 212 and LCBS-216 were used for the test. Antibodies were found in the birds from 6-7 weeks onwards and were present in almost 100 per cent birds at 17 to 18 weeks of age. Ianconescu and Samberg (1971) studied the spread of MDV infection among commercial flocks by using the AGPT. In all the flocks tested, and over 90 per cent of 12 weeks old fowls were found to have MDV antibodies. The incidence was much higher among meat breeds than layers.

Adene (1983) carried out serological survey of MDV in exotic and local chickens in Nigeria of 152 fowls of exotic commercial strains and of 108 local fowls, 16.4 and 8.3 percent respectively had positive precipitating antibody titers for MDV of 110 exotic and 105 local fowls tested for MDV feather follicle antigen, 41.8 and 12.4 per cent respectively were positive. Witter (1983) reported the presence of MDV in 63 problem flocks in USA. Antibodies were measured by indirect immunofluorescence and by AGPT. vvMDVs were isolated from 7 of 29 flocks. Immunofluorescent assay or ELISA (Cheng *et al.,* 1984) can also be used for subsequent identification of the MDV serotype. Alternatively, the serotype can be identified by restriction endonuclease analysis (Ross *et al.,* 1983) or polymerase chain reaction (PCR) (Wang *et al.,* 1993). In situ hybridization has been used for detection of MDV genome in infected tissue (Ross *et al.,* 1997).

Sung *et al*. (1997) tested feather tips collected from field broiler chicken for MDV infection both by AGPT test and PCR to compare their relative sensitivity. They found 12 out of 35 farms (34.0%) positive for MDV by PCR whereas only three farms (8.6%) were positive by AGPT. In a nationwide survey using PCR technique from DNA extracted from feather follicle of broiler birds, MDV infection was detected in 31 farms out of 80 tested in the Republic of Korea. On of T lymphoblastoid cells and a week later, death begins to occur.

2.8 Prevention and Control

Several methods have been developed to prevent the disease. The variation of the innate susceptibility to chickens is exploited in laboratory and used to develop resistant lines. Genetic resistance to MD is associated with genes within the B locus, encoding the chicken major histocompatibility complex (MHC)**.** The MHC of the chicken is composed of three classes of genes, *B-F* (class I), *B-L* (class II), and *B-G* (class IV). MHC-associated resistance to MD is mapped to the *B-F* region rather than to *B-G* region. Although the influence of the chicken classical MHC in resistance to MD is well established, the role of the recently identified, genetically independent, MHC-like region known as *Rfp-Y* is unclear. The contagious feature of the disease forces many to eradicate the disease. Chemoprophylaxis against Marek's is of little success though a substituted benzimidazole appears to partly prevent tumor development but not the replication of virus. Prevention of marek's by vaccination is possible and in United States the vaccine is cell associated virus and consists of HVT-infected live tissue culture cells preserved by diethyl sulfoxide in liquid nitrogen. MD vaccine viruses establish a persistent infection which reduces early viraemia, after subsequent exposure to pathogenic strains, and protects against tumour formation and hence mortality so infection has no economic consequences. Research on Marek's disease (MD) has accomplished a great number of success within the last 50 years, such as the development of the first most widely used anticancer vaccine around the world; the very efficient control of one of the most devastating diseases for the poultry; and the development of a technology that permits immunization of embryos against infectious poultry diseases. But in doing so the fact to be realized is that the vaccines that protect against the development of the disease do not stop the infection or transmission and are only a temporary solution that might drive the pathogen to higher virulence (Shahzad *et al.,* 2007).

CHAPTER III

MATERIALS AND METHODS

The present Studies were conducted during the time period of January to June 2015, in the Pathology laboratory of the Department of Pathology and Parasitology, Faculty of Veterinary and Animal Science, Hajee Mohammad Danesh Science and Technology University, Dinajpur. All of the cases were, after necropsy, provided histopathololological observation by already establishing methods. The detailed outline about the Materials and Methods used are given below.

3.1 Histopathology

3.1.1 Study area and period

Suspected liver samples were collected from the poultry farm in Dinajpur. The present research work was conducted between January to June, 2015.

3.1.2 Study item

Liver, spleen was subjected for histopathological examination and these samples were preserved in 10% neutral buffered formalin.

Histopathological procedure fixed tissue sections were processed, paraffin embedded, sectioned and were routinely stained with hematoxylin and eosin (H&E) as per standard procedure.

3.1.3 Laboratory preparation

All the instruments were placed in their appropriate place to conduct laboratory operation collect and accurately. Personnel's who works in the laboratory must were apron and hand gloves before laboratory work. All the surgical instruments were kept clean and also disinfect to prevent any kinds of contamination. After finishing the laboratory work all personnel put off their apron, hand gloves and wash hands before leave the laboratory, the dissecting table and the laboratory room kept clean after each postmortem operation.

3.2 Materials

3.2.1 Samples

Sources of the population in this study were different layer farms raised commercially by farmers from in Dinajpur district. From the flocks suspected with neo-plastic disease in poultry all the dead as well as sick birds were collected for furthers examination. The organs or tissue like liver spleen were submitted to the laboratory of the Department of Pathology and Parasitology, Hajee Mohammad Danesh Science and Technology University, Dinajpur for the final diagnosis.

3.2.2 Instrument and Appliances

3.2.2.1 Equipment and appliances for necropsy

- Birds (Liver, spleen)
- Scissors
- Forceps
- Gloves
- Musk
- Scalpel
- Knife
- A pair of shears,
- 10% neutral buffered formalin

3.2.2.2 Equipment and appliances

- Samples (Liver and spleen)
- 10% neutral buffered formalin
- Chloroform
- Paraffin
- Alcohol
- Tape water
- Xylene
- Hematoxylin and Eosin stain
- Distilled water
- Clean slides
- Cover slips

- Mounting media (DPX)
- Microscope

3.2.2.3 Cleaning and Sterilization of Required Glassware

Test tubes, glass tubes, glass slides, cover slips, beakers, pipettes, reagent bottles, glass bottle, spirit lamp, measuring cylinders etc. were used in this study. The conical flask, measuring cylinder, beakers, glass slides, cover slip, for slide preparation for histopathological study and staining of organisms after smear and pipettes, reagent bottle, glass tubes for different biochemical tests. New and previously used glassware were collected and dipped in 2% sodium hypochlorite solution and left there until cleaned. After overnight soaking in a household dishwashing detergent solution, the glassware were cleaned by brushing and washed thoroughly in running tap water and rinsed three times in distilled water. The cleaned glass wares were then dried on a bench at room temperature or in an oven at 50-70^{0}C.

3.2.3 Chemical and Reagents Used

10% neutral buffered formalin, Xylene, Hematoxylin and Eosin stain. PBS, Distilled water etc were used for necropsy and histopathology of collected samples.

3.2.4 Preparation of Harris' Hematoxylin Solution

Hematoxylin crystals	5.0g
Alcohol (100%)	50.0 ml
Ammonium or potassium alum	100 g
Distilled water	1000.0 ml
Mercuric oxide (red)	2.5 g

Hemoatoxylin was dissolved in alcohol and alum in water by heat. The two solutions were thoroughly mixed and boiled as rapidly as possible. After removing from heat, mercuric oxide was added to the solution slowly. The solution was reheated to a simmer until it became dark purple, and then the vessel was removed from heat and immediately plunged into a basin of cold water until it became cool. 2-4ml glacial acetic acid was added per 100 ml of solution to increase the precision of the nuclear stain. Before use, the prepared solution was filtered.

3.2.5 Preparation of Eosin Solution

3.2.5.1 1% stock alcoholic eosin

Eosin Y, water soluble	1 g
Distilled water	20 ml
95% alcohol	80 ml

Eosin was dissolved in water and then 80 ml of 95% alcohol was added.

3.2.5.2 Working eosin solution

Eosin stock solution	1part
Alcohol, 80%	3 parts

0.5ml of glacial acetic acid was added to 100 ml of working eosin solution just before use.

3.3 Methods

3.3.1 Experimental Layout

Detection of Farms

⇩

Recorded the details about the flock (Age, breed, vaccination, Morbidity, Mortality etc)

⇩

Collection of dead and sick birds from suspected flocks

⇩

Necropsy of birds

⇩

Collection of tissue or organ for histopathology

⇩

Preservation in 10% formalin

⇩

Processing of sample (trimming, processing, paraffin embedding, sectioning)

⇩

Staining with H & E

⇩

Examined under microscopic

CHAPTER IV

RESULTS

4.1 Clinical findings

Dead or sick birds were received from different farms with clinical history suggestive of Marek's disease. The main clinical history as reported by the farmers was regular mortality with emaciation. Lameness, torticollis and paralysis were also reported in some cases.

Table 1. Summary of clinical history of submitted samples

Farm No.	No. of samples	Age	Clinical history
1	1	12 weeks	Emaciated body, loss of appetite and lameness
2	2	30 weeks	Emaciated body, loss of appetite and lameness
3	3	35 weeks	Emaciated body, loss of appetite and lameness
4	4	12 weeks	Regular mortality, emaciation, lameness, neck twisting and anorexia
5	2	9 weeks	Regular mortality with emaciation and lameness
6	2	30 weeks	Regular mortality with emaciation, paralysis and twisted neck
7	2	17 weeks	Regular mortality, emaciation and paralysis
8	2	23 weeks	Regular mortality and emaciation

Figure 1: Paralyzed leg due to Marek's disease

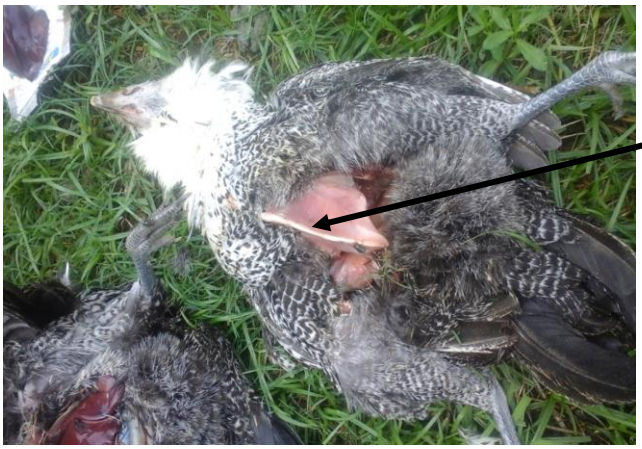

Figure 2: Emaciated keel bone

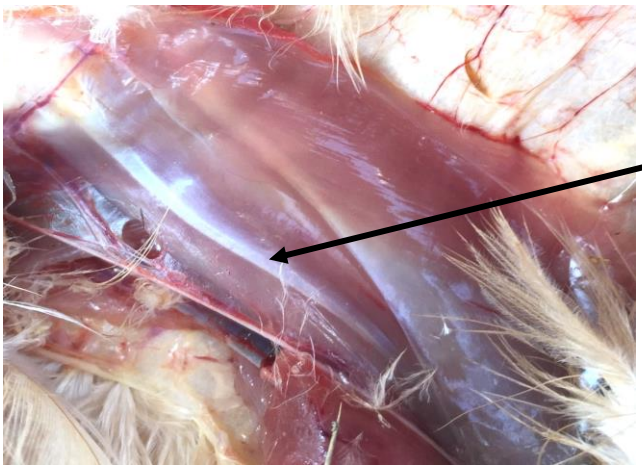

Figure 3: Emaciated carcass

4.1.1 Necropsy findings

The gross lesions and organ involvement varied from bird to bird. Diffuse enlargement or nodular lesions were found in the liver, spleen, proventriculus, heart, kidney, ovary and Intestine.

Table 2: Lesions distributed in different organs of affected birds in different farms

farm No.	No. of birds examined	No. of birds having lesions in	
		Liver	Spleen
1	1	1	1
2	2	0	0
3	3	0	0
4	4	0	0
5	2	2	2
6	2	2	0
7	2	1	1
8	2	2	2
Total	18	8	6

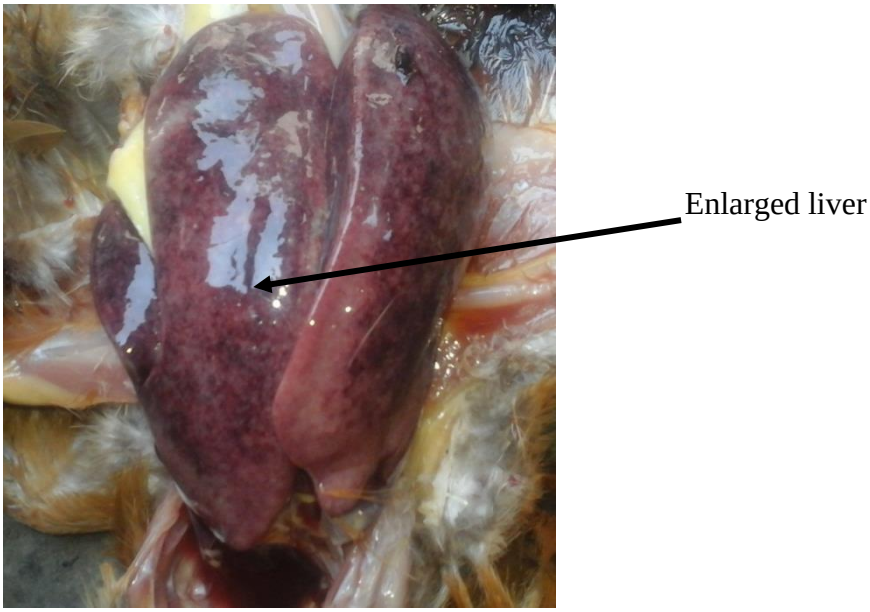

Figure 4: Enlarged liver

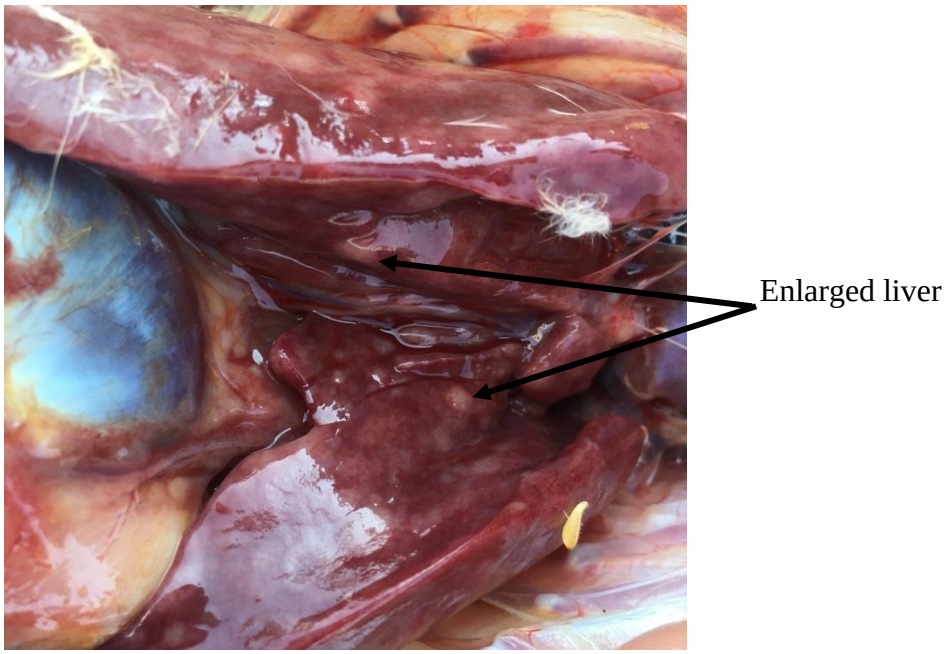

Figure 5: Multiple tumors were found in liver which were whitish in color

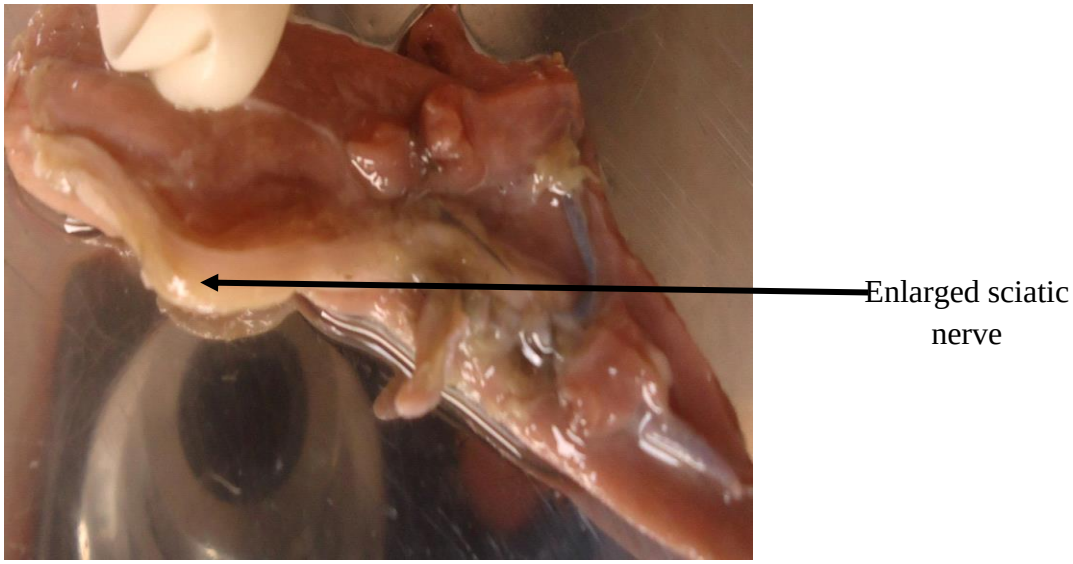

Figure 6: Enlarged sciatic nerve

4.1.2 Histopathological finding

4.1.2.1 Liver

Microscopic examinations of affected livers revealed diffuse proliferation of pleomorphic lymphocytes. Lymphocytic proliferation was so extensive that the normal architecture of hepatic lobules was largely distorted leaving only islands of hepatic cords. Lymphocytic proliferation was associated with marked haemorrhage and congestion.

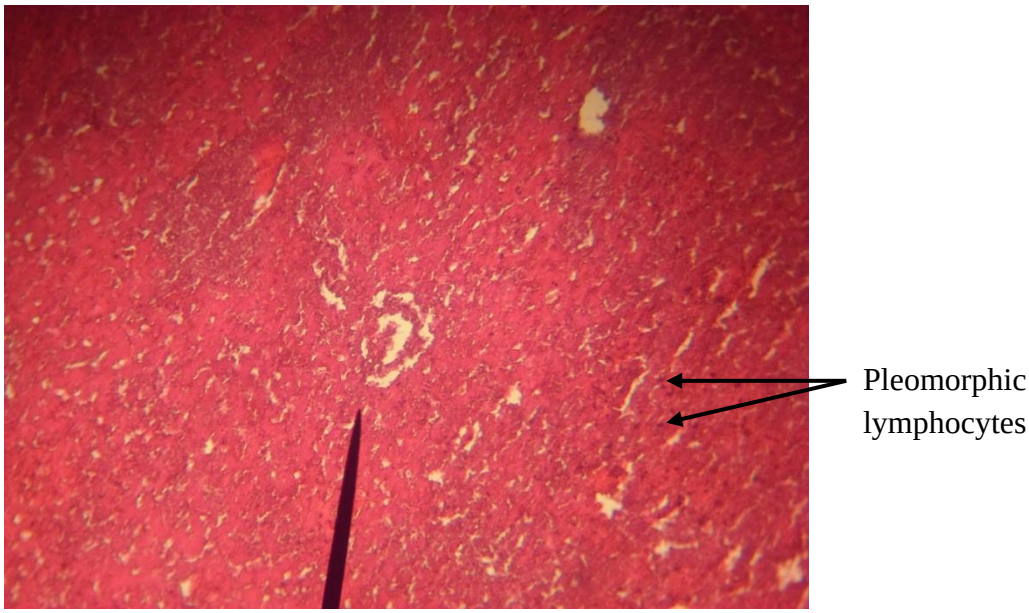

Figure 7: Proliferation of pleomorphic lymphocytes in liver

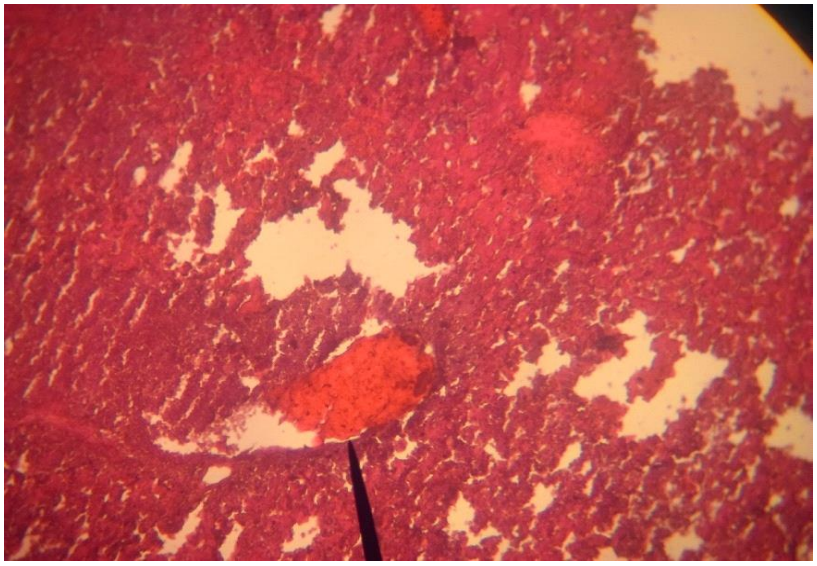

Figure 8: Lymphocytic proliferation was associated with marked haemorrhage and congestion in liver

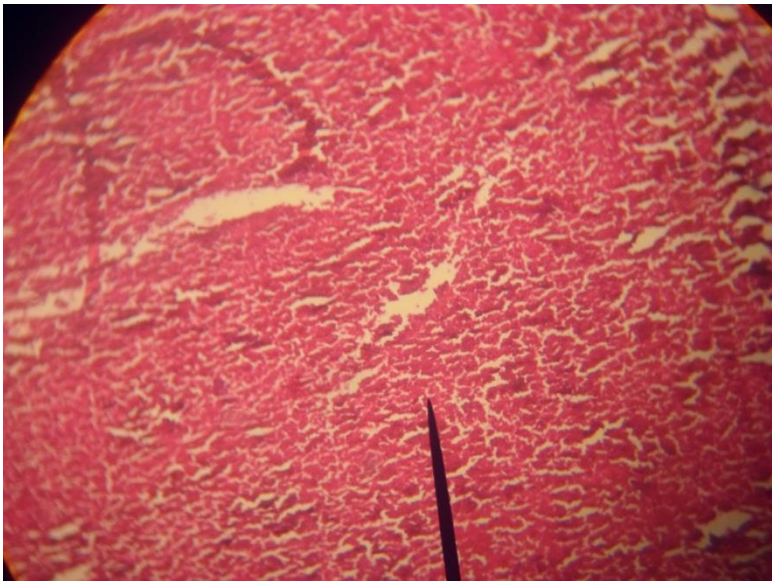

Figure 9: Normal architecture of hepatic lobules was largely distorted in liver

4.1.2.2 Spleen

Unusual pleomorphism due to proliferation of neoplastic cells was observed in the spleen.

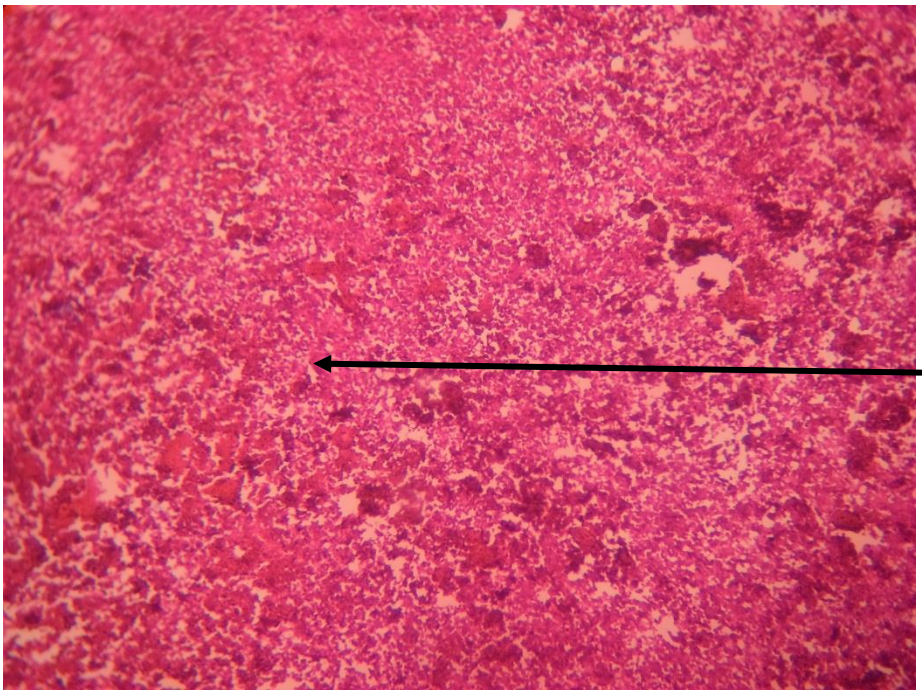

Figure 10: Pleomorphic infiltration of pleomorphic lymphocytes in spleen

Table 3: Prevalence and mortality of different farms at Dinajpur district

Farm No.	No. of total birds	No. of infected birds	Percentage of prevalence	No. of dead birds	Percentage of mortality
1	2500	12	0.48^{c}	5	0.20^{c}
2	3000	16	0.53^{b}	8	0.27b^{c}
3	2000	8	0.40^{cd}	5	0.25b^{c}
4	2300	4	0.17^{e}	3	0.13^{d}
5	1800	14	0.78^{a}	8	0.44^{a}
6	2000	7	0.35^{cd}	4	0.20^{c}
Total	13600	61	0.49	33	0.24
LSD			0.70**		0.43**
CV %			64.90		53.42
Mean ± SEM			0.45±0.05		0.25±0.03

CHAPTER V

DISCUSSION

Marek's disease (MD) is a common and important neoplastic disease of chickens. Following the introduction of HVT vaccine about 1971, losses from MD in broiler and layer chickens were dramatically reduced. Based on this early success, the poultry industry has relied on vaccination as the principal means of control. However, control can also be achieved through selection for host genes associated with resistance to tumor induction.(R.L.Witter11998). Vaccination against MD is commonly practiced in Bangladesh, even after outbreaks of MD are occasionally observed in commercial poultry farms of Vaccination against MD is commonly practiced in Bangladesh, even after outbreaks of MD are occasionally observed in commercial poultry farms of Bangladesh. The present study was undertaken to investigate the pathological condition of Marek's disease at small scale commercial layer farm in dinajpur district from January to June, 2015. Prevalence of Marek's disease at different commercial broiler farms are showing total 6 farms in dinajpur district. Total 61 diseased birds were affected of which 18 dead or sick chickens are examined and taken sample for pathological investigation. The present study showed that overall prevalence at Dinajpur district were 0.45% whereas 0.48%, 0.53%, 0.40% 0.17% 0.78%and 0.35% in 6 farms respectively. The mortality rate 0.25% whereas 0.20%, 0.27%, 0.25%, 0.13%, 0.44 % and 0.20% respectively. Biggs and Payne (1967) inoculated 1-day-old and 50-day-old chickens with the B-14 isolate and observed prevalence in the two groups of 73% and 6%, respectively. Panda *et al.* (1983) observed higher mortality (28.15 per cent) due to MD at 21 to 40 weeks of age as compared to 8.86 per cent mortality at 9 to 20 weeks of age. This result variation may be due to the geo-climatic condition, biological barriers, immunization status, social awareness and mostly on the health status of the birds. 18 dead or sick chickens observed from 6 different layer farms having clinical suspicion of MD were subjected to pathological examination. Progressive emaciation, regular mortality, leg weakness with or without paralysis were the main clinical manifestations. Similar clinical signs were described for MD by Calnek and Witter (1997), Biggs and Payne (1967) and Swayne *et al.,* 1989. Grossly visible visceral neoplastic lesions were observed most commonly in the followed by liver, spleen which appeared as enlargement of the organs with white large or miliary nodules. Similar lesions were found by Fujimoto *et al.*

(1971). Lymphomatous visceral tumors in MD have been reported by many authors (Purchase and Biggs, 1967; Ahmed, 1982; Rathore *et al.,* 1985; Narang *et al.,* 2003, Kamaldeep *et al.,* 2007).

Histopathological examinations were conducted on obtained samples. Infiltration and proliferation of pleomorphic lymphoid cells were observed on histopathological examination of liver and spleen. Depending on the organ involved the lymphomatous lesions were variable. In the liver infiltration and proliferation of lymphoid cells were associated with extensive damage in the parenchymatous tissues and haemorrhage and congestion. Changes in the spleen were characterized by unusual pleomorphism of lymphocytes. The lesions were in general consistent with those described by others (Frazier, 1974; Payne *et al.,* 1976; Lobago and Woldemeskel, 2004; Goyal *et al.,* 2006).

CHAPTER VI

CONCLUSION

It is concluded from the present study represent that after post mortem examination, enlargement of liver and spleen and sciatic nerve, Liver are multiple tumor in whitish color and spleen are dark red in gray yellow nodule of different shape are also seen. Mortality usually experienced at the age of 12-24 weeks and above. It is unusual at early age. Microscopic examinations of affected livers revealed diffuse proliferation of pleomorphic lymphocytes. Lymphocytic proliferation was so extensive that the normal architecture of hepatic lobules was largely distorted leaving only islands of hepatic cords .Lymphocytic proliferation was associated with marked haemorrhage and congestion. Unusual pleomorphism due to proliferation of neoplastic cells was observed in the spleen. Finally, it is concluded that histopathological lesions of collected samples were positive for marek's disease. Transmission of Marek's disease virus horizontally and vertically (through egg) and measures to prevent spread are more demanding. Marek's disease is controlled by virus eradication programmes mainly primary breeding level. On the basis of this study, it is assumed that although Marek's disease is a serious problem at poultry industry in Bangladesh, it possible to control under routine preventive and control measure which is essential for substantial improvement in poultry industry. Further molecular study should be carried out for isolation, characterization and development of effective vaccine.

REFERENCES

Adene, D. F. (1983). Studies on the neoplastic diseases of local and exotic chickens in and L. H. Schwarte (eds.). Disease of Poultry, 5th ed. Iowa State University Press, Ames, IA, pp.512-567.

Balachandran, C., N. Pazhanivel, S. Vairamuthu and B. Muralimanohar (2009). Marek's disease and lymphoid leucosis in chicken – A histopathological survey. Tamilnadu J. Vet. Anim. Sci., 5 :167-170.

Buscaglia, C., B. W. Calnek and K. A. Schat (1988). Effect of immunocompetence on the establishment and maintenance of latency with Marek's disease herpesvirus. J Gen. Virol., 69:1067-1077.

Biggs, PM. and Milne, BS. (1972). Biological Properties of a number of Marek's Disease Virus Isolates. In: Oncogenesis and Herpesviruses. Ed. Bggs, PM; G.de Th6 and Payne, LN. IARC scientific publication No. 2 Lyon: *International Agency for Research on Cancer*, p. 88.

Biggs, PM; Churchill, AE; Rootes, DG and Chub, RC. (1968). The etiology of Marek's disease- an oncogenic herpes-type virus. *Perspectives Virology*, 6: 211-237.

Biggs P. M. and L. N. Payne (1967). Studies on Marek's disease. I. experimental transmission. J. Nat. Cancer. Inst., 39:267-280.

Biggs PM. (1966). Avian leucosis and Marek's disease. Thirteenth World's poultry Congr. Symp. Papers, 10: 91-118.

Benton, W. J., M. S. Cover and W. C. Krauss (1962). The incidence of avian leucosis in broilers at processing. Avian Dis., 6: 430-435.

Benton, W. J. and M. S. Cover (1957). The increased incidence of visceral lymphomatosis in broiler and replacement birds. Avian Dis., 1:320-327.

Crabb, J. R., S. W. Walkden-Brown, S. Baigent, L. Smith and V. Nair (2009). Rate of natural transmission of Marek's disease virus (MDV) to susceptible chickens in the post-challenge period: effect of vaccination with HVT and association with MDV genome count in air. Poult. Res. Foundation, Sydney. pp 184-187.

Cho, K. O., K. Ohashi and M. Onuma (1999b). Electron Microscopic and Immunohistochemical Localization of Marek's Disease (MD) Herpesvirus Particles in MD Skin Lymphomas.Vet. Pathol., 36:314–320.

Calnek, B. W., and R. L. Witter. (1991). Marek's disease. In B. W. Calnek, H. J. Barnes, C. W. Beard, W M. Reid, and H. W. Yoder, Jr. (eds.). Diseases of Poultry, 9th ed. Iowa State University Press, Ames, IA, pp. 342. 385.

Calnek, B. W., and R. L. Witter. (1991). Marek's disease. In B. W. Calnek, H. J. Barnes, C. W. Beard, W M. Reid, and H. W. Yoder, Jr. (eds.). Diseases of Poultry, 9th ed. Iowa State University Press, Ames, IA, pp. 342. 385.

Calnek, B. W. and R. L. Witter (1985). Marek's disease—a model for herpesvirus oncology. Crit. Rev. Microbiol., 12:293-320.

Cheng, Y. Q., L. F. Lee, E. J. Smith and R. L. Witter (1984). An enzymelinked immunosorbent assay for the detection of antibodies to Marek's disease virus. Avian Dis., 4:900– 911.

Calnek, B. W., K. A. Schat, L. J. N. Ross, W. R. Shek and C. L. H. Chen (1984). Further characterization of Marek's disease virus-infected lymphocytes. I. In vivo infection. Intl. J. Cancer, 33:289-398.

Cebrian, J., C. Kaschkar-Deitrich, N. Berthelol and P. Shrldrick (1982). Inverted repeat nucleatide sequences in the genome of Marek's disease virus and Herpes virus of turkey. Proc. Natl. Acad. Sci., 79**:**555-558.

Calnek, B. W. (1980). Marek's disease virus and lymphoma. In F. Rapp (ed.). Oncogenic Herpesviruses. CRC Press, Boca Raton, FL, pp. 103-143.

Calnek, B. W. and S. B. Hitchner (1973). Survival and disinfection of Marek's disease virus and the effectiveness of filters in preventing airborne dissemination. Poultry Sci., 52:35-43.

Calnek, B. W., T. Ubertini and H. K. Adldinger (1970). Viral antigen, virus particles and infectivity of tissues from chickens with Marek's disease. J Nat. Cancer Inst., 45**:**341–351.

Cole, R. K. (1968). Studies on genetic resistance to Marek's disease. Avian Dis., 12: 9-28.

Churchill, A. E., and P. M. Biggs (1968). Herpes-type virus isolated in cell culture from tumors of chickens with Marek's disease. II. Studies in vivo. J. Nat. Cancer Inst., 41:951-956.

Churchill, A. E. and P. M. Biggs. (1967). Agent of Marek's diseases in tissue culture. Nat., 215:528-530.

Campbell, J. G. (1956). Leucosis and fowl paralysis compared and contrasted. Vet. Rec., 68:527-28.

Davidson, I; Nair, V and Kung, HJ. (2004). Marek's disease virus oncogenicity:molecular mechanisms. In: Marek's Disease, an Evolving Problem. Davison. F; Nair, V. (edition), Elsevier Ltd. San Diego. pp. 204.

Dunlop, W. R., S. D. Kottaridis, J. R. Gallagher, S. C. Smith and R. G. Strout (1965). The detection of acute avian leucosis as a contagious disease. Poultry Sci., 44:1537- 1540.

Fauqet, C. M., M. A. Mayo, J. Maniloff, U. esselberger, and L. A. Balls (2005). Virus Taxonomy. Eighth Report of the International Committee on Taxonomy of Viruses. London: Elsevier.

Fabricant, J., M. Ianconescu and B. W. Calnek (1977). Comparative effects of host and viral factors on early pathogenesis of Marek's disease. Infect Immunol., 16:136-144.

Frazier, J. A. (1974). Ultrastructure of lymphoid tissue from chicks infected with Marek's disease virus. J. Natl. Cancer Inst., 52:829-837.

Fujimoto, Y; Nakagawa, N;Okada, K and Matsukawa, K. (1972). Pathological studies of Marek's disease. I. The histopathology of field cases in Japan Jap. J.Vet. Res.,19:7-26.

Fujimoto, Y., N. Nakagawa, K. Okada, M. Okada and K. Matsukawa (1971). Pathological studies of Marek's disease. I. The histopathology of field cases in Japan. Jap. J. Vet. Res., 19: 7-26.

Fujimoto, Y., N. Nakagawa, K. Okada, M. Okada and K. Matsukawa (1971). Pathological studies of Marek's disease. I. The histopathology of field cases in Japan. Jap. J.Vet. Res., 19: 7-26.

Furth, J. (1935). Lymphomatosis in relation to fowl paralysis. Arch. Pathol., 20:329-428.

Goyal, D., A. Singh, N. Sood, K. Gupta and N. K. Sood (2006). Adenocarcinoma of liver with Marek's disease in poultry. Indian Vet. J., 83:562-563.

Gimeno, I. (2004). Future stragegies for controlling Marek's disease. . In: Marek's Disease: An Evolving Problem. Biology of Animal Infections. Davison, F., Nair.V (ed.). Elsevier Academic Press, San Diego pp.186-198.

Gimeno, I. (2004). Future stragegies for controlling Marek's disease. . In: Marek's Disease: An Evolving Problem. Biology of Animal Infections. Davison, F., Nair.V (ed.). Elsevier Academic Press, San Diego pp.186-198.

Gimeno I. M., R. L. Witter, H. D. Hunt, L. F. Lee, S. M. Reddy and U. Neumann (2001). Marek's Disease Virus Infection in the Brain: Virus Replication, Cellular Infiltration, and Major Histocompatibility Complex Antigen Expression, Vet Pathol., 38:491–503

Gimeno I. M., R. L. Witter and W. M. Reed (1999). Four distinct neurological syndromes in Marek's disease: effects of viral strain and pathotype. Avian Dis., 43:721–737.

Hossain MK; Ahamed, M; Kabir, H; Jalil, MA and Adhikary, GN. (2004). Poultry Diseases at Rajshahi in Bangladesh. *J. Ani. Vet. Adv.*, 3:656-658.

Hirai, K., K. Ikuta and S. Kato. (1997). Comparative Studies on Marek's Disease Virus and Herpesvirus of Turkey DNAs. J. Gen. Virol., 45:119-131.

Hudson, L. and L. N. Payne. (1973). An analysis of the T and B cells of Marek's disease lymphomas of the chicken. Nat. (New Biol.), 241:52-53.

Haider, S. A., R. F. Lapen and S. G. Kenzy (1970). Use of feathers in a gel precipitation test for Marek's Disease. Poultry Sci., 49:1654–1657.

Helmboldt, C. L. F., F. K. Wills and M. N. Frazier (1963). Field observations of the pathology of skin leucosis in Gallus gallus. Avian Dis., 7:402-411.

Igarashi. T., M. Takagashi, J. Donovan, J. Jessip, M. Smith, K. hirai, A. Tanaka and M. Nonoyama (1987). Restriction enzyme map of herpes virus of turkey DNA and its collinear relationship with Marek's disease virus DNA. J. Virol., 157:351-358.

Ianconescu, M. and Y. Samberg (1971). Etiology and immunological studies in Marek's disease. II. Incidence of Marek's disease precipitating antibodies in commercial flocks and in eggs. Avian Dis., 15 :177-186.

Jakowski, T. M., T. N. Fredrickson, T. W. Chomiak and T. E. Luginbuhl. (1970). Hematopoietic destruction on Marek's disease. Avian dis., 14:274-285

Jakowski, R. M., T. N. Frederickson, R. E. Luginbuhl and C. F. Helmboldt (1969). Early changes in bursa of Fabricius from Marek's disease. Avian dis., 13:215-222.

Jungherr, E. L. and W. F. Hughes (1965). The avian leukosis complex. In: H.E. Biester and L. H. Schwarte (eds.). Disease of Poultry, 5th ed. Iowa State University Press, Ames, IA, pp.512-567.

Kamaldeep (2007). Occurrence of Marek's Disease in Vaccinated Poultry Flocks of Haryana (India) International Journal of Poultry Sci., 6: 372-377.

Kamaldeep, P; Sharma, C and Narang, G. (2007). Occurrence of Marek's disease in Vaccinated Poultry Flocks of Haryana (India). 6:372-377.

Kozdrun W., E. Samorek-Salamonowicz and H. Czekaj (2001). Polymerase chain reaction for the differentiation of Marek's disease virus strains. Bull. Vet. Inst. Pulawy., 45:5-10.

Kornegay, J. N., E. J. Gorgacz, M. A. Parker, J. Brown, and L. W. Schierman (1983). Marek's disease virus-induced transient paralysis: Clinical and eletrophysiologic findings in susceptible and resistant lines of chickens. Am. J. Vet. Res., 44:1541-1544.

Kaleta, E. and U. Neumann (1977). Investigations on the mode of transmission of the herpesvirus of turkeys in vitro. Avian Pathol., 6:33.

Lobago, F. and M. Woldemeskel (2004). An outbreak of Marek's disease in chickens in central Ethiopia. Trop. Anim. Hlth. Prodn., 36:397-406.

Lupiani, B., L. Lee and S. Reddy (2001). Protein-coding content of the sequence of Marek's disease virus serotype 1. Curr. Top. Microbiol. Immunol., 255:159-190.

Musa I. W., Bisalla M., Mohammed B., Sa'idu L. and Abdu P. A. (2013). "Retrospective and clinical studies of Marek's disease in Zaria, Nigeria" Journal of Bacteriology Research Vol. 5(2), pp. 13-21,

Morgan, R; Anderson, A; Bernberg, E; Kamboj, S; Huang, E; Issacs, G; Green, PJ and Burnside, J. (2008). Sequence conservation and differential expression of Marek's disease virus Micro RNAs.82:12213-12220.

Muhammad K. Shahzad, Khalid Abdul Majeed, and Muhammad Younus **(**2007). Marek's Disease: A Mini-Review IJAVMS, Vol. 1: 4-8.

Moriguchi, R., M. Oshima, F. Mori, I. Umezawa and C. Itakura. (1989). Chronological change of feather pulp lesions during the course of Marek's disease virus-induced lymphoma formation in field chickens. In: S. Kato, T. horiuchi, T. Mikami, and K.Hirai (eds.). Advances in Marek's Disease Research. Japanese Association on Marek's Disease, Osaka, Japan, pp. 338-343.

Madarame, H; Fujimoto, Y and Moriguchi, R. (1986). Ultrastructural studies on muscular atrophy in Marek's disease. II. Muscular lesions in spontaneous cases. 34:51-75.

Narang, G; Jindal, N and Kumar, S. (2003). Occurrence of Marek's disease in vaccinated layer flocks in Haryana. Indian Vet. J., 80: 580-590

Nazerian, K. and B. R. Burmester (1968). Electron microscopy of a herpesvirus associated with the agent of Marek's disease in cell culture. Cancer Res., 28:2454-2462.

Nigeria with particular reference to Marek's disease. Ph. D. Thesis, University of Ibadan, Nigeria, p. 298.

Office International des- Epizooties (OIE). (2004). Marek's disease. In: Manual of Standards for Diagnostic Tests and Vaccines. OIE Terrestrial Manual. 5th Edn.,Rome.

Pejovi, N, Velhner Maja, Polak V, Aleksi Kova, Evi Sanja, Marinkovi, D And Knevi Milijana (2007). Morphological And Immunohistochemical Examination Of Tumor Cells In Marek's Disease "Acta Veterinaria European Journal of Business and Management (Beograd), Vol. 57, No. 1, 27 35.

Panneerselvam, S., N. Dorairajan, C. Balachandran and B. Murali Manohar, (1990). Incidence of Marek's disease in Namakkal, Tamil Nadu. Cheiron, 19: 143-144.

Panda, B. K., S. C. Arya, H. K. Pradhan, and D. C. Johri (1983). Marek's disease in chicken in various strains of White Leghorn. Indian J. Poult. Sci., 18: 100.

Payne, L. N. and J. Roszkowski (1973). The presence of immunologically uncommitted bursa and thymus dependent lymphoid cells in the lymphomas of Marek's disease. Avian Pathol., 1:27-34.

Purchase, HG and Oocazaki, W. (1971). Effect of Vaccination with Herpesvirus of Turkeys (HVT) on Horizontal Spread of Marek's Disease HerpesvirusAvian Dis.,31:225-233.

Purchase, H. G., R. C. Chubb and P. M. Biggs. (1968). Effect of lymphoid leukosis and Marek's disease on the immunological responsiveness of the chicken. J. Nat. Cancer inst., 40: 583-592.

Purchase, H.G. and P.M. Biggs (1967). Characterization of five isolates of Marek's disease. Res. Vet.Sci., 8:440-449.

Payne, L. N. and P. M. Biggs. (1967). Studies on Marek's disease. II. Pathogenesis. J.Nat. Cancer Inst., 39:281-302.

Pappenheimer, AM; Dunn, LC and Cone, V. (1929). A study of fowl paralysis (neurolymphomatosisgallinarum). I. clinical features and pathology. J. Exp. Med.,49: 63-86.

Pappenheimer, A. M., L. C. Dunn and V. Cone (1926). A study of fowl paralysis (neurolymphomatosis gallinarum). Storrs Agri. Expt. Sta. Bull., 143:187-290.

Rosenberger, J.K. (1997). Control of Marek's disease. Vineland Update, 52: 1-4.

Rathore, BS; Singh, R and Khera, SS. (1985). Survey of cases of poultry mortality in India: Based on postmortem examinations conducted at ten diagnostic centres. Indian J. Poult. Sci., 20: 135-139.

Ross, L. J., B. Milne and P. M. Biggs (1983). Restriction endonuclease analysis of Marek's disease virus DNA and homology between strains. J. Gen. Virol., 64:2785–2790.

Rouse, B. T., R.J. H. Wells and H. L. Warner (1973). Proportion of T and B lymphocytes in lesions of Marek's disease: Theoretical implications for pathogenesis. J. Immunol., 110:534-539.

Sarah Tilley, North Carolina State University David D. Frame, (2013). Utah State University Extension Poultry Specialist *Mark C. Bland*, Cutler Associates International Marek's Disease (MD), Range Paralysis, Gray Eye.

Schat, K. A. and Z. Xing (2000). Specific and nonspecific immune responses to Marek's disease virus. Dev. Comp. Immunol., 24:201-221

Sung, H. W., S. J. Kim, C. S. Song, Y. J. Lee, C. W. Lee, K. S. Kim and S. J. Kim (1997). Detection of pathogenic Marek's disease (MD) virus and survey on MD in broiler farms by the polymerase chain reaction. J. Vet. Sci., 39:38-44

Swayne, D. E., O. J. Fletcher and L. W. Schierman (1989). Marek's disease virus induced transient paralysis in chichens: Alterations in brain density. Demonstration of vasogenic brain oedema by an immunohistochemical method. J. Comp. Path., 101:451-462.

Schat, K. (1987). Marek's disease: a model for protection against herpesvirus-induced tumors. Cancer Surv., 6:1-5.

Shek, W. R., B. W. Calnek, K. A. Schat and C. L. H. Chen (1983). Characterization of Marek's disease virus-infected lymphocytes: Discrimination between cytolytically and latently infected cells. J. Natl. Cancer. Inst., 70:485-491.

Schierman, L. W. and O.J. Fletcher (1980). Genetic control of Marek's disease virus inducedtransient paralysis: Association with the major histocompatibility complex. In P. M. Biggs (ed.). Resistance and Immunity to Marek's Disease. Commission European Communities, Luxembourg, pp. 429-442.

Sharma, J. M., R. L. Witter, B. R. Burmester and J. C. Landon (1973). Public health implications of Marek's disease virus and herpesvirus of turkeys. Studies on human and subhuman primates. J. Natl. Cancer Inst., 51:1123-1128.

Solomon, JJ; Witter, RL; Nazerian, K and Burmester, BE. (1968). Studies on the etiology of Marek's disease. I. Propagation of the agent in cell culture Proc. Soc.Exp. Boil. Med., 127:173-177.

Sevoian, M. and D. M. Chamberlain (1964). Avian lymphomatosis. IV Pathogenesis. Avian Dis., 8:281-310.

Sevoian, M; Chamberlain, DM and Counter, FT. (1962). Avian lymphomatosis. I. experimental reproduction of the neural and visceral forms. Vet. Med., 57:500-501.

Vickers, KH; Helmoldt, CF and Luginbuhl, RE. (1963). Pathogenesis of Marek's disease (Connecticut a Isolate). *Avian Dis.*,11:531-545.

Van der Walle and E. Winkler-Junius (1924). De Neuritis-Epizootic bijkippente Barneveld in 1921. Tijdschr. Vergel. Geneesk, Enz., 10:34-50.

Witter R. L. and K. A. Schat (2003). Marek's disease, In: Saif YM editor in chief, Disease of Poultry, 11th Edition, Blackwell Publishing Co, Iowa State Press, 407-465.

Wang, R. F., J. N. Beasley, W. W. Wei, M. F. Slavik, M. C. Johnson, F. Wang, and W. C. Wei (1993). Development of PCR method specific for Marek's disease virus. Mol. and Cellr. Probes, 7:127–131.

Witter, R. L. (1988). Very virulent Marek's disease viruses: Importance and control. In Proc 18th World's Poult. Congress. World's Poultry Congress, Nagoya, Japan, pp. 92-97.

Witter, R. L. (1983). Characteristics of Marek's disease viruses isolated from vaccinated commercial chicken flocks: association of viral pathotype with lymphoma frequency: Avian Dis., 27:113-132.

Witter, R. L., J. M. Sharma, J. J. Solomon and L. R. Champion (1973). An age-related resistance of chickens to Marek's disease: Some preliminary observations. Avian Pathol., 2:43-54.

Witter, R. L., J. J. Solomon and G. H. Burgoyne (1969). Cell culture techniques for primary isolation of Marek's disease-associated herpesvirus. Avian Dis., 13:101-118.

Wight, PAL. (1966). Histopathology of the skeletal muscles in fowl paralysis (Marek's disease). Brit. Vet. J., 121:278-282.

Wight, P. A. L. (1962). The histopathology of the central nervous system in fowl paralysis. J. Comp. Pathol. Therap., 72:348-359.

Yoshida S., L. F. Lee, N. Yanagida and K. Nazerian (1994). The glycoprotein B genes of Marek's disease virus serotypes 2 & 3: Identification and expression by recombinant fowlpox viruses. J. Virol., 200:484-493.

Yamamoto, H., K. Ishino, H. Hihara, R. Ani, T. Shiroto and M. Yamazaki (1969). Proceeding of the 67th Meeting of the Japanese Society of Veterinary Science, Ibid., 31, Suppl., 125.

Zanella, A. and S. Raymonds (1969). Serological survey with immuno diffusion test in Marek's disease. Cinica. Vet. Milano., 92:424-428.