MAREK'S DISEASE

(Commonly also k.a. **Polyneuritis**, **Fowl Paralysis**, **Range Paralysis**, and **Neural Lymphomatosis; Neurolymphomatosis; Gray Eye; Pearl Eye; Avian Reticulosis**)

Marek's disease (MD) is a lympho-proliferative and neuropathic disease of domestic fowl/chickens caused by **alpha-Herpesvirus**, a DNA virus, named as **Marek's disease virus** (MDV). *MDV belongs to the genus* **Mardivirus** *that includes three species* (*serotypes*) *designated as Gallid herpesvirus 2* (*serotype 1*), *Gallid herpesvirus 3* (*serotype 2*) and *Meleagrid herpesvirus 1 or herpesvirus of turkeys* (*HVT*) (*serotype 3*). The **ocular form** is also suppose to occur along with the **lymphopathic and neural form**.

The disease was first described by the Hungarian veterinarian **Jozsef Marek** in 1907, after whom it is named "Marek's disease". MD can occur at any time, beginning at 3–4 weeks of age or older (2-4 month age mostly affected), sometimes even well after the onset of egg production.

AETIOLOGY

Marek's disease virus (MDV) is an enveloped, linear double-stranded DNA virus, with lymphotropic properties similar to those of gamma-herpesviruses. The MDV and related herpesviruses have been classified into three serotypes. Type-specific monoclonal antibodies are used to determine virus serotypes. The **Serotype-1** comprises oncogenic strains of MDV. **Serotype-2** is a group of naturally non-pathogenic strains of MDV. **Serotype 3** is the apathogenic and antigenically related herpesvirus of turkeys (HTV). Thus, it can be seen that virulence or oncogenicity is associated only with serotype 1 viruses and these have been subdivided into three pathotypes: 1. Mildly virulent (mMDV), 2. Virulent (vMDV), and 3. Very virulent (vvMDV). Virus infectivity *in vivo* and *in vitro,* is strictly cell-associated except in the **feather follicle epithelium, where cell-free virus is produced**.

SPREAD

The virus spreads rapidly from infected to uninfected birds. The virus is present in desquamated feather follicle epithelial cells, and in oral, nasal, and tracheal secretions. Feather dander (white covering that wraps the developing feathers) is the most common culprit of transmission and feather follicle cells are the most important source of infection, and are responsible for the infectivity of dander (minute scales from feathers or skin), poultry house dust, and litter.

Direct or indirect contact between birds spreads the virus. Airborne spread of virus and infection through the respiratory tract is considered to be the most important route. The virus is **not transmitted through the egg**, and thus chicks are hatched free of infection, however eggs before hatching may be contaminated with marek's virus wherein chicks are infected at time of hatching. Once contracted, the infection persists throughout the life of the chicken, and infected 'birds continue to contaminate the environment by shedding the virus.

PATHOGENESIS

Pathogenesis of Marek's disease can be described in four phases of infection:

1. Early productive-restrictive virus infection, causing mainly degenerative (cytolytic) changes,
2. Latent infection,
3. A second-phase of cytolytic, productive-restrictive infection coinciding with permanent immunosuppression, and
4. A proliferative phase involving non-productively infected lymphoid cells which may or may not progress to lymphoma formation.

The virus enters through the respiratory tract, where it is picked up by the phagocytic cells. An acute phase of the disease follows within 3-4 days, characterized by cytolytic infection of the lymphoid system, most marked in the bursa of Fabricius, thymus, and also spleen. The

primary target cells in all these organs are B cells, although some activated T cells become infected and undergo degeneration as well. Resting T-cells are resistant to infection.

The necrotizing effects of this early infection provoke an acute inflammatory reaction with infiltration of macrophages, granulocytes, and lymphocytes. Infected birds normally recover from the acute phase of the disease and after 6-7 days the infection becomes latent.

At about 6-7 days, the infection switches to latency. This coincides with the development of immune responses.The virus is spread throughout the body by infected lymphocytes, and a persistent, cell-associated viraemia is present. A secondary cytolytic infection occurs in the feather follicle epithelium (FFE) 2-weeks after primary infection and infectious cell-free virus is produced and shed into the environment in feather debris and dander. The latent infection is persistent, and can last for the life of the bird.

Infection in genetically resistant birds does not progress beyond the second phase (latency). Susceptible birds, on the other hand, develop a second wave of cytolytic infections after the 2nd to 3rd week coinciding with permanent immunosuppression. The lymphoid organs are again involved, and foci of infection occur in tissues of epithelial origin in various organs (e.g., kidney, pancreas, adrenal gland, proventriculus etc.), and especially in the skin, where a prominent infection of the feather follicle epithelium (FFE) occurs. Infection of FFE is unique in that it is the **only known site of complete virus replication**.

Lymphoproliferative changes constitute the final response and may progress to tumour development. Transformed T cells proliferate in peripheral nerves and other tissues and organs, leading to type-A nerve infiltrations and lymphoma formation. Transformed T cells contain viral genome, but the synthesis of viral particles is severely restricted.

CLINICAL SIGNS

Marek's disease affects chickens from about 4-6 weeks of age (2-4 months). Clinical signs observed are paralysis of the legs and wings, with enlargement of peripheral nerves, although nerve involvement is sometimes not seen, especially in adult birds. Clinical disease occurs in several forms.

Classical Marek's Disease

Characterised mainly by the involvement of nerves, mortality rarely exceeds 10–15% and can occur over a few weeks or months.

Most common clinical sign is partial or **complete paralysis of the legs and wings**. The characteristic finding is enlargement of one or more peripheral nerves. The signs depend on the peripheral nerves affected. Most commonly affected and easily seen at post-mortem are the vagus, brachial and sciatic plexuses, and leads to progressive spastic paralysis (i.e., paralysis accompanied by muscular rigidity) of the wings and legs. Sometimes, when the cervical nerves are involved, there may be **torticollis (twisting of the neck);** and if the vagus and intercostals nerves are affected, **respiratory signs** may develop.

'**Gray eye'** (also called **Pearl Eye**) is discoloured iris, caused by **iridocyclitis or ocular lymphomatosis**, renders the bird unable to accommodate the iris in response to light and causes a distorted pupil – seen in older (16–18 week) birds, and may be the only presenting sign.

Acute Marek's Disease

Typical finding is widespread, visceral lymphomas (diffuse lymphomatous involvement) of the liver, gonads, spleen, kidneys, lungs, proventriculus and heart with disease incidence of 10–30%. Mortality in this form is usually much higher than in the classical form.

Sometimes lymphomas also arise in the **skin around the feather follicles** and in the **skeletal muscles**. In younger birds, **liver enlargement** is usually moderate in extent, but in adult

birds the liver may be greatly enlarged and the gross appearance identical to that seen in lymphoid leukosis, from which the disease must be differentiated. Nerve lesions are often absent in adult birds with MD.

Non-neoplastic disease involving brain pathology (neuropathy) with vasogenic oedema resulting in transient paralysis is increasingly recognised with MD with virulent virus, similar to those seen in the classical form.

Transient Paralysis

This is an uncommon encephalitic manifestation of Marek's disease in birds between 5 and 18 weeks of age. Birds suddenly develop varying degrees of paresis (partial paralysis) or paralysis of the legs, wings and neck (hurdling stance; one leg paralysed one normal). Signs usually disappear within 24-48 hours.

GROSS LESIONS

In classical Marek's disease, the characteristic lesion is enlargement of one or more peripheral nerves. Vagus and Sciatic nerves are commonly affected and may enlarge upto 2-3 times the normal thickness. The affected nerves loses its glistening white appearance , become gray-yellowish with nodular swellings, with loss of striations. Nerves commonly affected are the brachial and sciatic plexi (singular, plexus = a network of nerves), coeliac plexus, abdominal vagus, and intercostal nerves. Some paralysed birds show no visible nerve enlargement at necropsy, but characteristic nerve lesions are found microscopically.

Depending on the strain of MDV, lymphomatous lesions can occur in multiple organs such as the ovary, liver, spleen, kidneys, lungs, heart, proventriculus and skin. Tumours produced by MDV may also resemble those induced by retroviral pathogens such as avian leukosis virus and reticuloendotheliosis virus and their differentiation is important. Compared with the uniform cell populations observed in lymphoid leukosis, MD lymphomas consist of pleomorphic lymphoid cells of various types and are mostly small, soft, and grey in appearance, or rarely large, yellowish, and lobulated.

Acute Marek's disease in young birds is characterised by cerebral symptoms predominating lymphomatous lesions, that often leads to partial or complete paralysis of wings and legs. “Clutching” of toes’ is characteristic symptom and affected birds hobble or walk on hunched toes or hopp with affected legs.

In growing birds, its characterized by diffuse lymphomatous involvement, and enlargement of the liver, gonads, spleen, kidneys, lungs, proventriculus and heart. Sometimes, lymphomas also arise in the skin in association with feather follicles (known as "**skin leukosis**" in the USA), and in the muscles. In younger birds liver enlargement is moderate, but in adult birds the liver is greatly enlarged, which is similar to that in lymphoid leukosis.

Capricious appetite, stunted growth and loss of condition are other symptom noticed.

MICROSCOPIC LESIONS

The basic pathological process is the same in classical and acute Marek's disease. The disease begins as a proliferation of lymphoid cells. Proliferation is progressive in some cases, and undergoes regressive changes in others. In the classical form regressive changes are more common than in the acute form, in which the lymphoid proliferation leads to widespread tumour formation.

In the **Nervous System**, peripheral nerves are affected by proliferative, inflammatory or chronic but minor lesions, termed type A, B, and C, respectively.

Type-A lesion consists of proliferating lymphoid cells, lymphoblasts, and small, medium and large lymphocytes. Type B lesion is characterized by inter-neuritic oedema, Schwann cell proliferation, and light to moderate infiltration of mainly small lymphocytes and plasma cells. A primary cell mediated

demyelination occurs in the type A and B nerves, causing paralysis. Type C lesion consists of lightly scattered lymphocytes and plasma cells.

Type A lesion is the primary reaction, usually associated with lymphoid tumours and is seen most commonly in chickens dying early in the disease. Type B lesion follows type A, and is usually seen in chickens with disease of longer standing. Type C lesion is seen in infected birds which show no clinical signs. Lesions of the CNS include proliferative lymphoid lesions of the type-A, but inflammatory lesions characteristic of a viral encephalomyelitis are more common. The most prominent change is perivascular cuffing, but microgliosis and endotheliosis may also occur.

In **Visceral Organs**, most common lesions are lymphoid tumours. These are made up of cells similar to the type-A lesion of peripheral nerves. The malignantly transformed cell is a thymus-dependent lymphocyte (T cell), and the lymphoma consists of a mixture of malignant T cells and reactive, bursa-dependent lymphocytes (B cells), T cells, and macrophages.

On the **Skin**, there is gross enlargement of **feather follicles**, appearing as nodular elevations, making skin very rough.

In **Ocular Form** (Gray Eye; Pearl Eye), the **iris** is infiltrated with small round cells and polyblast-like cells, that is caused by **iridocyclitis or ocular lymphomatosis;** also involving choroid, retina and ocular muscles at times.

DIAGNOSIS

- Diagnosis is made on clinical signs and gross or microscopic lesions. Definitive diagnosis must be made by diagnosing the disease (tumour), not the infection alone.
- Serological tests: Viral antigens can be detected in feather tips and feather follicle epithelium, infected lymphoid tissues, or infected cell cultures with appropriate antibodies (monoclonal) by fluorescent antibody (FA) tests, immunoperoxidase tests, agar-gel precipitation (AGP) tests, and enzyme-linked immunosorbent assays (ELISA):-
  - ✓ Agar gel Immunodiffusion (AGID): There is no test suitable for certifying individual animals prior to movement, but the AGID test is employed commonly to detect antibody. A variation of agar gel immunodiffusion (AGID) test used for serology may be used to detect MDV antigen in feather tips as an indication of infection by MDV
  - ✓ The indirect fluorescent antibody test (IFAT) demonstrates the ability of a test serum to stain MDV plaques in cell cultures. These tests are group specific and more sensitive than AGID test.
  - ✓ ELISA: for detecting MDV antibodies are available
  - ✓ Virus Neutralization test:
- Polymerase chain reaction (PCR): used to confirm and differentiation of oncogenic and non-oncogenic strains of various MDV serotypes.
- Virus Isolation: Infection by MDV in a flock may be detected by isolating the virus from the tissues of infected chickens. MDV is highly cell associated, so buffy coat cells (WBCs) from heparinised blood samples, or suspensions of lymphoma cells are used as samples. Cell-free virus (MDV) can be extracted / isolated from Feather tips / Feather follicles.

Differential Diagnosis of Marek's disease from Lymphoid Leukosis (LL) and Reticuloendotheliosis is important, whose differentiating features are presented below:-

Table. Differentiating Marek's Disease from Lymphoid Leukosis

Features	Marek's Disease	Lymphoid Leukosis	Reticuloendotheliosis
AGE	Any age; 6 weeks or older (young affected)	Not under 16 weeks (older birds affected)	Not under 16 weeks (older birds affected)
SIGNS	Frequently paralysis	Non-specific	Non-specific
PARALYSIS	Frequently seen; above 5%	Rarely above 5%	Rare
MACROSCOPIC LESIONS			
Neural involvement	Frequent	Absent	Infrequent
Bursa of fabricius	Diffuse enlargement or atrophy	Nodular tumours	Nodular tumours
Tumours in skin, muscle and proventriculus, 'grey eye'	May be Present	Usually absent	Usually 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	Interfollicular tumour and/or atrophy of follicles	Intrafollicular tumour	Intrafollicular tumour
CNS	Yes	No	No
Lymphoid proliferation in skin and feather follicles	Yes	No	No
CYTOLOGY OF TUMOURS	Pleomorphic lymphoid cells, including lymphoblasts, small, medium and large lymphocytes and reticulum cells. Rarely can be only lymphoblasts	Lymphoblasts	Lymphoblasts
Category of NEOPLASTIC LYMPHOID CELL	T cell	B cell	B cell