CEVA HANDBOOK
OF POULTRY DISEASES

4 MAREK'S DISEASE

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DEFINITION

Marek's Disease (MD) is a lympho-proliferative disease affecting chickens *(Gallus domesticus)* caused by a herpesvirus, and which is characterized with neoplastic lesions affecting the visceral organs, skeletal muscles and skin, and also causing inflammatory lesions of the peripheral nerves.

HISTORY AND SYNONYMS

The history of MD begins with the report of Jozsef Marekof the Department of Royal Hungarian Veterinary School in Budapest, illustrating 4 cases of paresis in roosters (1907). In this first report, the disease is described as polyneuritis. Over the ensuing century of research, the disease has also received the names fowl paralysis, neuro-lymphomatosis, neuritis, neuritis granulomatosa infectiosa, uveitis, grey eye etc. Between 1962–1970, the etiology, route of transmission and nature of the lesions were still much debated, until the Marek's disease virus (MDV) was isolated (Churchill & Biggs, 1967) and identified as a herpesvirus (Biggs et al, 1968) and the first successful vaccine against the disease was developed (Churchill et al., 1969).

The acute form of the disease, characterized by multiple tumours in internal organs, skeletal muscles and skin, was observed during the 1950s, causing losses from death and culling at slaughter in broiler chickens, before the introduction of vaccination against MD (Benton & Cover, 1957).

An explanation for the highly contagious nature of MD was provided by the discovery that the cell-free viruses were distributed through the epithelia of feather follicles (Calnek et al., 1970).

Naturally avirulent antigenically related strains were discovered in turkeys (Witter et al., 1970) and chickens (Biggs & Milne, 1972; Cho & Kenzy, 1972) ensuring a protective immunity. An important contribution was the use of turkey herpesvirus (HVT) in the production of vaccines against MD (Okazaki et al., 1970).

By means of genetic analysis, MDV and HVT were reclassified as alphaherpesviruses (Buckmaster et al., 1988). Cantelo et al. (1994) were the first to report the existence of a recombinant MDV expressing a heterologous gene. Also, the integration of a reticuloendothelial virus (REV) in the DNA of MDV – the first report for retroviral integration in a herpesvirus – was acknowledged (Isfort et al., 1992).

CHARACTERISTICS AND NOMENCLATURE OF THE PATHOGEN

MDV is an alphaherpesvirus also known as Gallidherpesvirus 2 (GaHV-2). All MDV serotypes belong to the Alphaherpesvirinae subfamily, genus Mardivirus. Three MDV serotypes are known: serotype 1 (GaHV-2); serotype 2 (GaHV-3) and serotype 3 (*Meleagrid herpesvirus 1*), (Shahzad et al, 2007; Schat & Nair, 2008). Pathogenic MDV serotype 1 strains isolated in chickens are divided into four classes: mildly virulent (m)MDV, virulent (v)MDV, very virulent (vv) MDV and very virulent plus (vv+) MDV. Serotype 2 MDV covers non-pathogenic, strains which are isolated from clinically healthy chickens. Natural infection with such strains protects against MD (Biggs & Milne, 1972). Serotype 3 is an avirulent HVT isolated from healthy turkeys (Kawamura et al., 1969; Witter et al., 1970). The etiological agent GaHV-2 is cell-associated in tumours and in all organs except for feather follicles, where the infectious virions spread from into the body (Purchase, 1976; Payne, 1979).

The morphology of viral particles is typical for herpesviruses–hexagonal nucleocapsids measuring 85–100 nm, and enveloped particles with a diameter of about 150–160 nm. The genomes of the three serotypes are very similar, and consist of double-stranded DNA.

The structure of viral DNA in infected cells depends on the virus-cell interaction. Linear viral DNA may be detected in the nucleus of cells having undergone viral replication (Cebrian et al., 1982). The replication of the three MDV serotypes is a typical trait of other cell-associated herpesviruses. Sites of predilection for this virus include the cells of the medullary part of bursa of Fabricius' follicles, especially the epithelial cells of the renal tubules and the cornified feather follicle epithelium. The replication of complete viruses occurs only in feather follicle epithelium, where it is found in enveloped form (Calnek & Hitchner, 1969). In the other tissue (spleen, liver, gonads), the virus is in an immature form – without envelope (Obreshkov & Enchev, 1972).

Oncogenic (virulent) properties of serotype 1 only vary largely depending on the pathogenic potential of strains. The HPRS 16, 18, 19 and 20 strains provoke the acute form of MD (Purchase & Biggs, 1967). The strains HPRS–B14 and HPRS 17 induce classical MD when

inoculated to chickens (Biggs & Payne, 1963, 1967; Purchase & Biggs, 1967). The mature (enveloped) virus is highly resistant. At room temperature it can survive in feather follicles for 8 months, and in the litter and dust of farms for 1–4 months.

The MDV without envelope has a low resistance, and can be inactivated after exposure to a temperature of 56 °C for 30 min at a pH below 5.5 or over 8.4 (Obreshkov & Enchev, 1972).

EPIDEMIOLOGY

MD is prevalent worldwide, and all flocks are exposed to the etiological agent. Under natural conditions MD affects mainly chickens, but susceptibility has also been reported in turkeys, pheasants, quails etc. Other avian species such as pigeons, ducks, partridges, peacocks etc. are probably resistant (Baxendale, 1969; Grewal & Singh, 1976; Kenzy & Cho, 1969; Powell &Rennie, 1984). MD is mainly observed in birds aged 8–9 weeks, while among egg-laying birds most cases were observed between 16–20 and 24–30 weeks of age.

One-day-old chicks are most at risk, with susceptibility remaining relatively high until the 30th day of age, after which it decreases progressively with age. In both clinical forms of the disease – acute and chronic (classical), female birds are more affected than males.

The primary sources of infection are diseased birds and virus carriers which shed the MDV, since the period of incubation lasts for 16-24 months. MDV has been detected in the blood of birds more than 2 years after remission from the disease, and during that time these carriers pose a potential infection hazard. The shedding of virus in the environment by infected birds occurs mainly through desquamation of the keratinized feather follicle epithelium (Calnek et al., 1970). The mature MDV preserves its infectious properties in keratinocytes, predominantly in dust accumulated around the fans, heating devices and windows of the housing units birds are kept in, spread by ventilation systems from unit to unit.

MORTALITY AND MORBIDITY RATES

Prior to the introduction of vaccines against MD, losses in affected flocks varied from a few birds to 25–30%, with some cases of up to 60% mortality in broiler populations. Broiler chickens culled at the slaughterhouse for MD are reported to account for around 1% on average, but sometimes exceed 10%.

After the introduction of vaccinations against MD, mortality was reduced to less than 5% (Purchace, 1985).

MAREK DISEASE - RELATED SYNDROMES

Lymphodegenerative syndromes

The replication of MDV in the thymus and the bursa of Fabricius may induce transient cytolytic alterations in these organs, followed by atrophy (Shat & Nair, 2008).

Thymic atrophy is characterized by lymphocyte deficiency in both the cortex and the medulla.

Vascular syndromes

Atherosclerotic changes associated with MDV -induced atherosclerosis are observed in coronary, gastric and mesenteric arteries (Fabricant et al., 1978; Minick et al., 1979).

Transient paralysis

MD-related cases of flaccid paralysis result from vasculitis and vasogenic brain oedema that passes away after 2–3 days (Swayne et al., 1989).

IMMUNOSUPPRESSION

MDV infection is immunosuppressive and alters the host's susceptibility to other pathogens. The weakened immune response is due to the cytolytic infection of lymphocytes (Schat et al., 1978). Tumour cells may also exhibit suppressor activity (Bumstead & Payne, 1987).

CLINICAL SIGNS AND PATHOLOGY

Clinical and morphological forms of MD can be divided into acute forms, characterized mainly by visceral lymphomas and predominantly affecting younger birds, and chronic (or classical) forms, characterized by nervous lesions (fowl paralysis and ocular lymphomatosis), more common among older birds.

Acute (visceral) form

This forms of MD is characterized by diffuse or nodular lymphomatous lesions in the viscera (liver, spleen, heart, kidneys, lungs, gonads, proventriculus, pancreas etc.), skeletal muscles and skin.

The aerogenous route of infection is the most significant, and remains a risk long after birds are removed from the farm. Virus shedding from carriers may also occur via oral or faecal discharge (Witter et al., 1968). Multi-age poultry farms are at great risk of MD.

The spread of MDV is horizontal, through either direct or indirect contact between diseased and healthy birds, via feed, water, equipment, personnel etc., but particularly by aerogenic transmission. Darkling beetles (Alphitobiusdiaperinus) can act as passive virus carriers (Eidson et al., 1966; Beasley & Lancaster, 1971). Vertical transmission of MDV is not encountered. By external contamination, eggshells may induce horizontal transmission, but it is unlikely considering the short survival time of the virus at temperature and humidity maintained in incubators (Calnek & Hitchner, 1973). MD consists of several distinct pathological syndromes (Calnek, 2001). The most common and also most important is the lymphoproliferative syndrome. MD lymphoma is most commonly observed, but fowl paralysis, ocular and skin lesions are additional clinical signs with lymphoproliferative attributes. Cases of transient paralysis are caused by non-neoplastic pathological alterations, mainly vasogenic brain oedema (Gimeno et al., 1999). Subclinical syndromes may also exist, but they are difficult to define.

The incubation period has been determined in experimental conditions (Calnek, 2001). Mononuclear cell proliferation in nerves and other organs can appear after 2 weeks, but clinical signs and gross lesions are not observed until the 3rd–4th week after inoculation. After natural infection, the incubation period can last 2–3 months or more.

Fig.1

Multifold enlarged liver with a mottled marbled appearance, markedly stretched capsule and rounded edges due to diffuse lymphomatous infiltration.

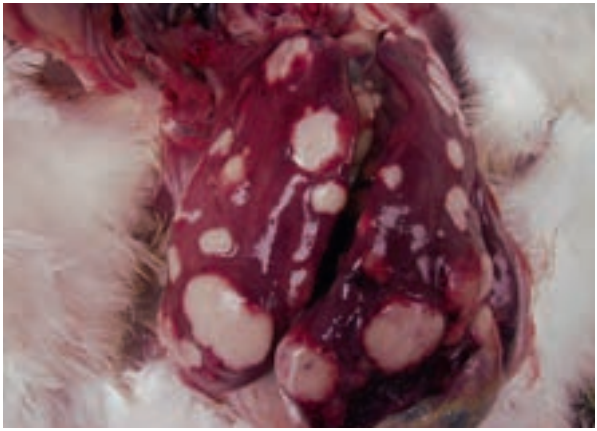

Fig.2

Multiple fat-like nodular lymphomas of various sizes, from a few millimetres to several centimetres.

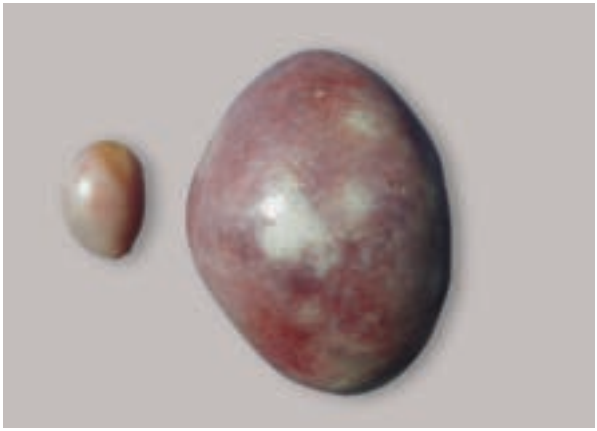

Fig.3

The spleen is enlarged by an order of between 1 and 10 due to diffuse, nodular or mixed-type neoplastic growths; left – normal appearance.

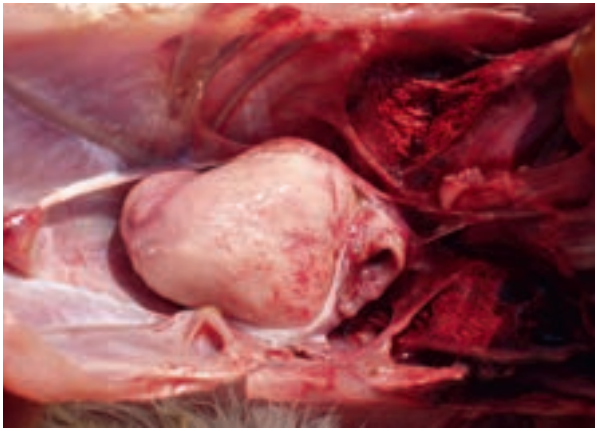

Fig.4

Neoplastic cardiac lesions also consist of nodular or diffuse fat-like masses giving the heart an amorphous tumour-like appearance.

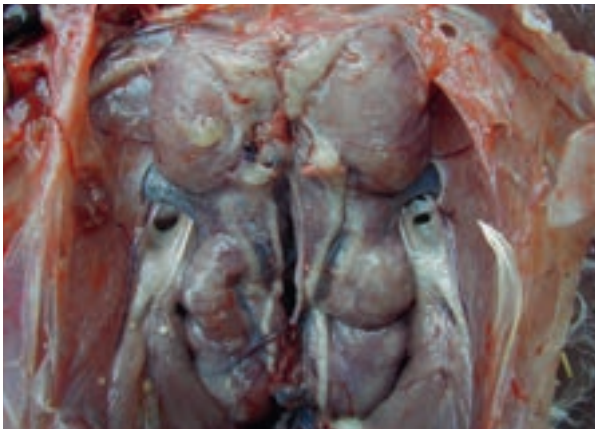

Fig.5

The kidneys are enlarged uni- or bilaterally consequently to diffuse, nodular ormixed lymphomatous growths.

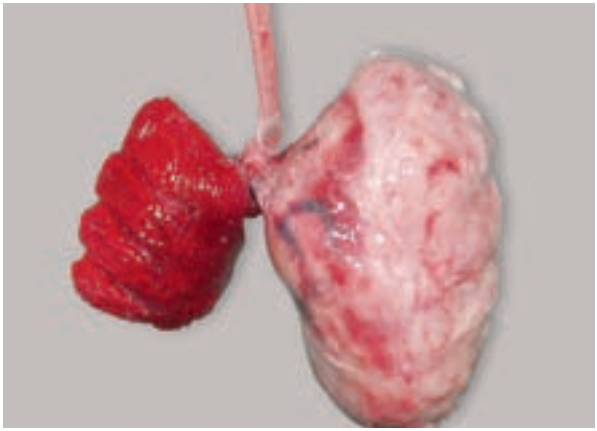

Fig.6

The lungs are also affected uni- or bilaterally by diffuse or nodular neoplastic growths. At a later stage, pneumonic foci may emerge among the neoplastic tissue. The combination of both types of lesions results in respiratory failure, which may prove fatal.

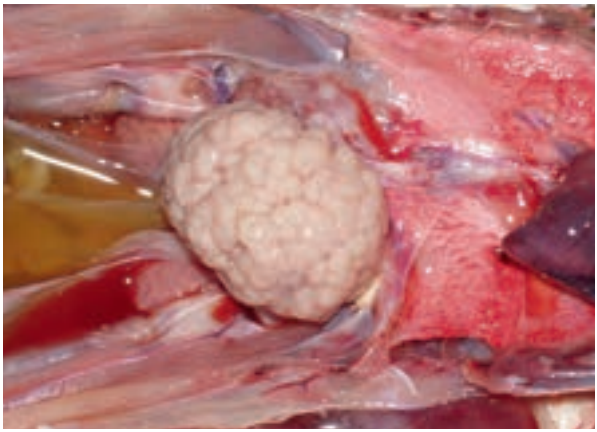

Fig.7

A specific feature of neoplastic transformation of the ovary is that it is always almost diffuse. When the entire organ is affected, it develops a characteristic cauliflower-like appearance.

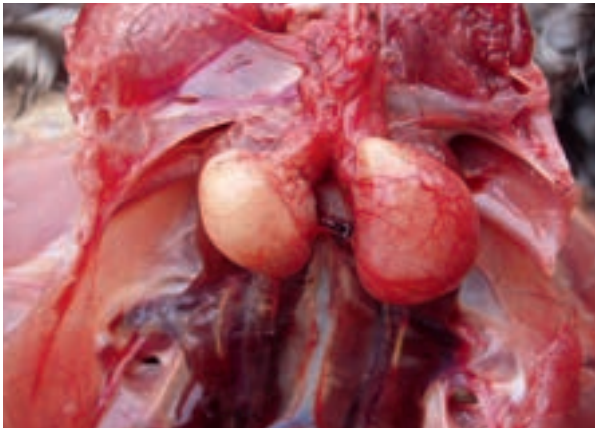

Fig.8

When one of the testes is diffusely affected, it can swell up to double normal size, resulting in a marked asymmetry when compared to the intact testis. The alterations in gonads are believed to be a valuable finding in diagnosing acute MD.

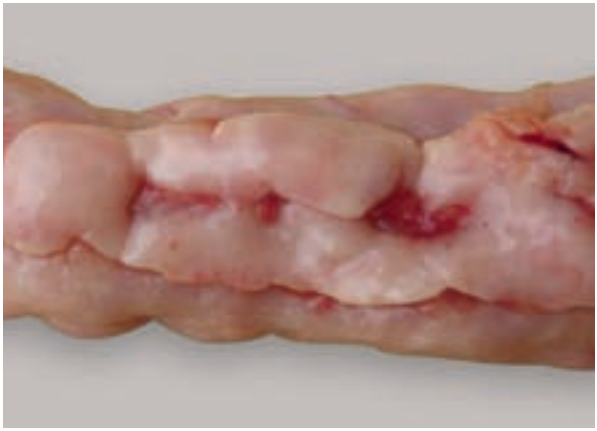

Fig.9

Neoplastic changes of the pancreas are less common. They may be diffuse or nodular. They often provoke adhesions between intestinal loops and the mesentery.

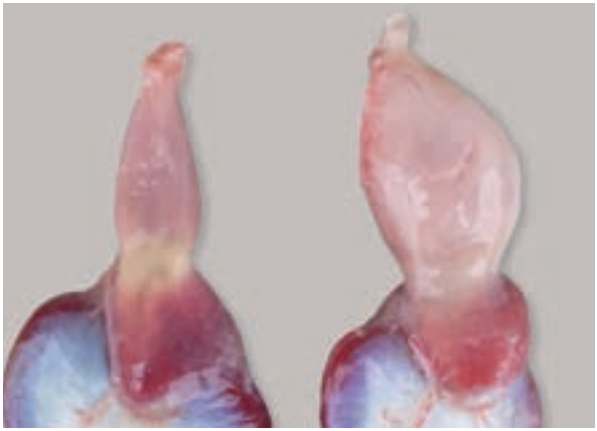

Fig.10

Another MD-specific finding is the multifold enlarged flask-shaped proventriculus, induced by diffuse neoplastic growths.

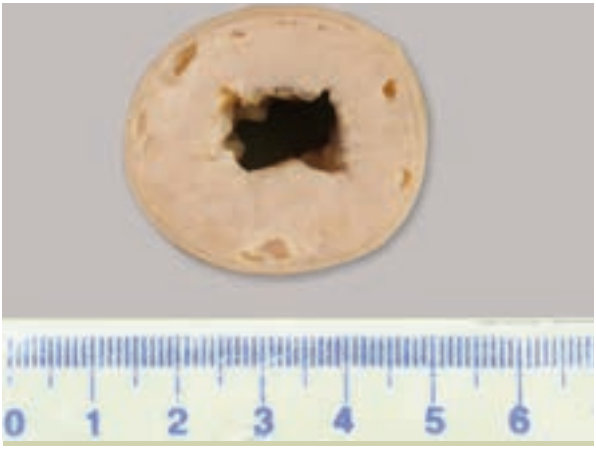

Fig.11

The wall of the proventriculus is heavily thickened by lymphoproliferative alterations, resulting in lumen narrowing and obstruction.

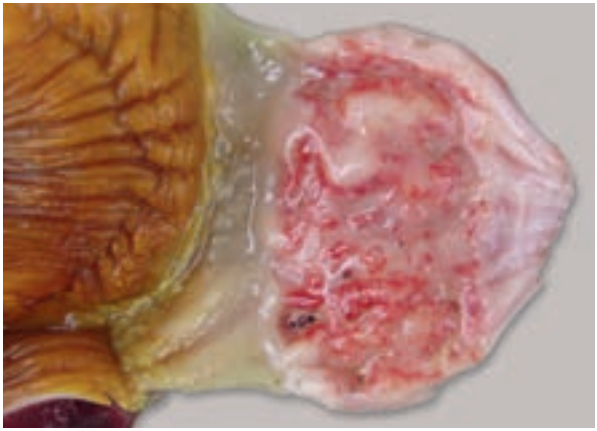

Fig.12

Haemorrhages, erosions or ulcerations are after found in the mucous coating of the proventriculus. The changes are similar to those observed in ND, but the gastric wall is neoplastically thickened.

Fig.13

Skeletal muscle lymphomas are most frequently observed in thoracic and femoral muscles. They appear as single or multiple grey-whitish nodules, prominating on the surface or perceptible deep within muscles.

Chronic (classical) form. Occurs as nervous (fowl paralysis) or ocular (ocular lymphomatosis) forms.

Fig.14

The nervous form is clinically manifested with leg paralysis.

Fig.15

Affected birds often acquire a specific for MD posture, with one leg extended forward and the other – backward (split position).

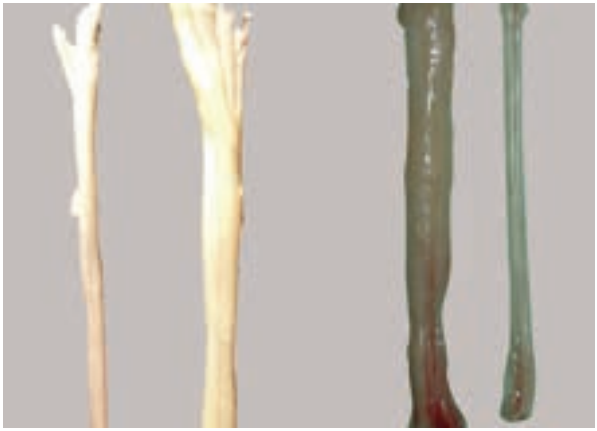

Fig.16

Almost all peripheral nerves are altered. The sciatic nerves are the most commonly examined, revealing mainly bilateral, diffuse nerve thickening to a variable degree.

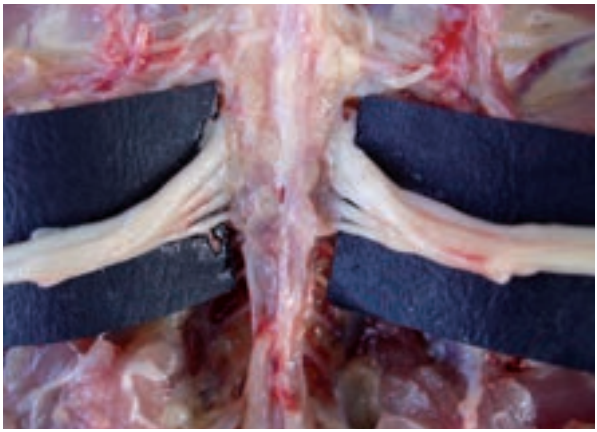

Fig.17

Enlargement of sciatic nerves is most common at the site where they exit the lumbosacral plexus. These alterations may also be associated with atrophy of the thigh and drumstick muscles.

Fig.18

The ocular form is characterized by depigmentation of the iris, deformation of the pupil, and sometimes corneal opacity and blindness.

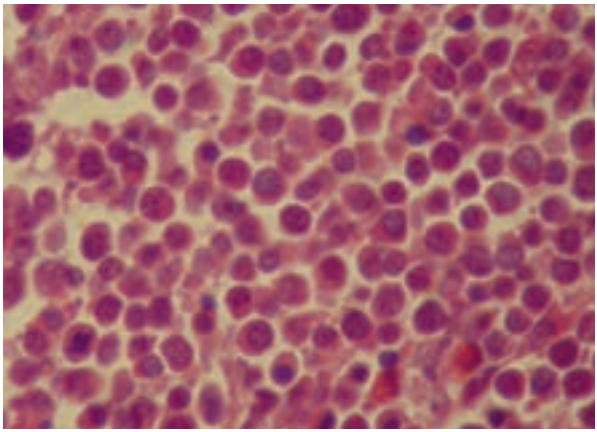

Fig.19

Histologically, pleomorphic proliferations of lymphoid cells are observed in affected internal organs, nerves or the eyes. Liver lesions are mainly focal and consist of lymphoblasts, small to large plasma cells and macrophages.

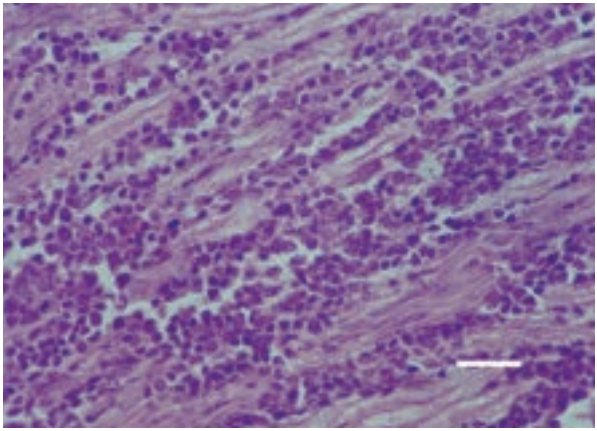

Fig.20

Peripheral nerve. A-type lesion (neoplastic type), marked lymphoid cell proliferation, absence of oedema. H/E, Bar = 25 µm.

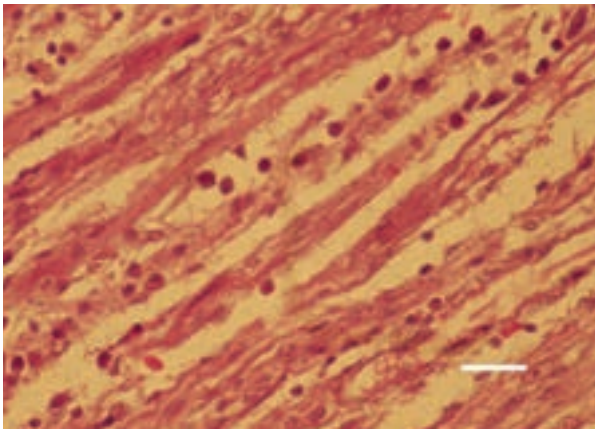

Fig.21

Peripheral nerve, B-type lesion (mostly inflammatory type). Interneuritic inflammatory oedema and slight to moderate proliferation of lymphocytes and plasmatic cells, rarely lymphoblasts. The mild version of B-type lesions is called C-type. H/E, Bar = 25 µm.

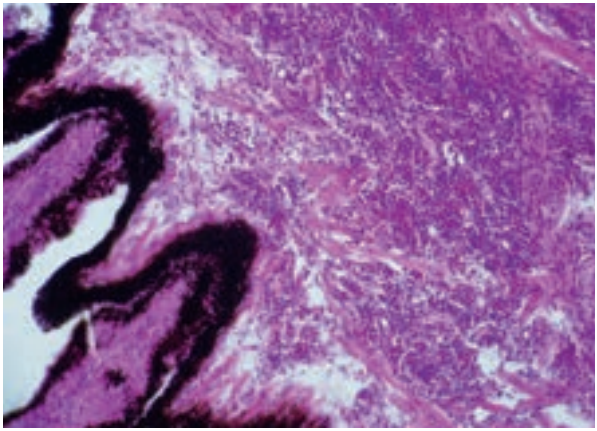

Fig.22

Lymphoid cell proliferations in the iris and ciliary muscles in the ocular form of Marek's disease. H/E, Bar = 50 µm.

Fig.23

Transient paralyses. They are observed in chickens and hens, especially those that have not been vaccinated against MD. Most cases exhibit the classical form manifested with flaccid paralysis of the neck and legs for 1–4 days followed by full recovery. The syndrome is differentiated from the nervous MD form on the basis of its transient nature and the occurrence of flaccid, non-spastic paralysis.

DIAGNOSIS OF MAREK'S DISEASE

Diagnosis is based upon epidemio–logical and clinical data, gross lesion, histological, histochemical, cytological and virological examinations.

The clinical diagnosis of MD is difficult for several reasons: gross visceral lesions are difficult to distinguish from those of lymphoid leukosis, a co-infection with other viruses inducing neoplasms in the commercial poultry industry (ALVs; REVs) is also possible (Davidson & Borenstein, 1999); there is no pathognomonic gross lesion specific to MD. Nevertheless, the following features should be taken into consideration:

a) gross lesions such as neoplastic lymphomatouslesions in the visceral organs; enlarged peripheral nerves; depigmentation of the iris.

b) detection of pleomorphic cell preparations (lymphoblasts, small or large lymphocytes, plasma cells and macrophages) in tissue sections stained with haematoxylin/eosin. Finding such signs in the peripheral nerves is particularly telling. Methyl green pyronin-stained imprint preparations are also essential to accurate diagnosis.

c) the diagnosis is confirmed by molecular techniques– PCR for detection of viral DNA in tumour samples; in situ hybridization etc.

MD should be first differentiated from lymphoid leukosis.

From the point of view of differential diagnosis, the following features deserve a special emphasis:

Lymphoid leukosis

- Usually not seen in birds younger than 14 weeks;
- Fatalities occur mostly between 24 and 40 weeks
- Distinct nodular tumours;
- Tumours in the bursa of Fabricius.

Marek's disease

Can also be observed after the age of 4 weeks;

- Peak mortality observed between the 10th and the 20th week, sometimes continues after the 20th week;
- Paralysis;
- "Grey eye";
- In some birds, the bursa of Fabricius is atrophied, in others: neoplastic.

PREVENTION

Bearing in mind that maternal immunity does not play a role in the protection against MD and that immunity in one-day-old chickens appears 2–3 weeks after vaccination, the most important biosecurity issue is to limit the exposure to MDV immediately after hatching.

With this in mind, spatial isolation between sectors housing growing and adult birds should be maintained in poultry farms. The different age groups should be reared separately with emphasis on the protection of hatchlings from MDV exposure during the first 30 days of life. With regard to production technology, the all-in/all-out principle should be adhered to strictly. The cleansing, washing and disinfection of facilities, equipment and transportation vehicles should follow a strict biosecurity procedure. The removal and decontamination of litter is subject to the same procedures.

VACCINATION

Three classes of viral vaccines are capable of protecting birds from MD:

- attenuated serotype 1 MDV – cell-associated vaccines;
- HVT – can be separated as a cell-free virus for preparation of freeze-dried vaccines;
- naturally avirulent serotype 2 MDV isolates – cell-associated vaccines.

MD vaccines can be used solely or in combination, and ensure over 90% protection. HVT, mainly the strain FC126, provides excellent results, but in case of failure bivalent vaccines can be used. Most of the bivalent vaccines in general use contain a combination of HVT strains and serotype 2 MDV strains (Purchase, 1976) , or a combination of HVT and serotype 1 strains.

With regard to the importance of early immunity, MD vaccines are applied prior to (in ovo) or at the day of hatching subcutaneously or intramuscularly. Revaccination at the age of 7–12 days is sometimes practiced in Europe.

Because of its large genome, its safety in chickens and its genetic stability, HVT is also often selected as vector to carry foreign gene(s) in the growing recombinant technology.

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