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AVIAN VIRAL NEOPLASTIC DISEASES: THE PATHOLOGIST'S PERSPECTIVE

BOLILE NEOPLAZICE VIRALE AVIARE: PERSPECTIVA PATOLOGULUI

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ABSTRACT

Avian neoplastic diseases are of great economic importance, considering the estimated value lost due to mortality and treatment costs. Marek's disease, avian leukosis, and reticuloendotheliosis are the major neoplastic diseases of poultry. Systematic Diagnostic techniques start with and include clinical examination, histopathology, cytology, serology such as agar gel immunodiffusion (AGID), enzyme-linked immunosorbent assay (ELISA), virus neutralisation (VN), immunohistochemistry, in situ hybridization, electron microscopy, cytogenetic, computer tomographic imaging, and molecular methods such as polymerase chain reaction (PCR). These methods have been invaluable in the diagnosis of avian neoplastic diseases. In conclusion, this review threw a searchlight on the most recent available literature on the aetiology, pathogenesis, macroscopic and microscopic pathology, hematopathology, cytopathologyand diagnosis of these economically important tumours.

Keywords: tumours, birds, diagnostic methods

REZUMAT

Bolile neoplazice aviare au o mare importanță economică având în vedere estimările de valoare pierdute din cauza mortalității și a costurilor de tratament. Boala Marek, leucoza aviară si reticuloendotelioza sunt bolile neoplazice majore ale păsărilor. Tehnicile de diagnostic sistematic includ examenul clinic, histopatologia, citologia, serologia, cum ar fi imunodifuzia cu gel de agar (AGID), testul imunoenzimatic (ELISA), neutralizarea virusului (VN), imunohistochimia, hibridizarea *in situ*, microscopia electronică, citogenetica, imagistica computer-tomografică și metode moleculare, cum ar fi reacția în lanț a polimerazei (PCR). Aceste metode au fost de neprețuit în diagnosticul bolilor neoplazice aviare. În concluzie, această recenzie oferă noi informații privind etiologia,patogeneza,patologia macroscopică și microscopică, hematopatologia, citopatologia și diagnosticul acestor tumori importante din punct de vedere economic.

Cuvinte cheie: tumori, păsări, metode de diagnostic

Neoplastic diseases of poultry fall into two categories: those with an infectious aetiology and those that are non-infectious. Those diseases in the former category are of greater economic importance because the viruses that cause them are widely prevalent in commercial stock, and the mesenchymal neoplasms that they cause affect relatively young birds. These infections and diseases can be enzootic or epizootic. Neoplasms of a non-infectious ethology occur mostly in birds older than the usual lifespan of commercial birds (28). The economic importance of neoplastic diseases and the viruses that cause them is important in poultry for several reasons. The presence of the virus or the neoplasm causes economic loss from mortality and depressed performance. For Marek's disease (MD), additional costs arise from the development, production and use of vaccines for disease control, and for avian leukosis, from the implementation of virus eradication programmes,particularly by primary breeding companies (28). Three main classes of viruses cause neoplasms in poultry, namely Marek's disease virus (MDV), a herpesvirus,avian leukosis virus (ALV), which is a retrovirus, and reticuloendotheliosis virus (REV), also a retrovirus (28).

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MAREK'S DISEASE

MDV infects chickens and causes one of the most frequent cancers in animals (5). MD, characterised by rapid-onset lymphoid tumours, immunosuppression, and paralysis, is one of the most widespread and economically important diseases of poultry worldwide (24). It has been reported in all poultry-rearing countries (10, 23) and a 2004 estimate suggested that it

causes annual losses of up to US$2 billion to the poultry industry. MD is a lymphoproliferative and neuropathic disease of domestic chickens and, less commonly, turkeys and quails (28). MDV is a highly oncogenic alpha herpesvirus that causes various clinical symptoms, including fatal lymphomas in chickens (6). MD is among the diseases with the highest economic impact on modern poultry production worldwide, and current data from the World Organisation for Animal Health (WOAH) estimates that about half of the world's countries have reported cases of MDV infection. As global requirements increase, so does our dependence on intensive poultry production facilities (28, 9, 5).

Aetiology

The aetiology is a herpesvirus, one of the most common oncogenic viruses of the avian species. This DNA-containing virus produces T-cell lymphomas in chickens and is the cause of severe economic loss worldwide. Several MDV pathotypes have been characterised over the years based on morbidity and mortality rates. These pathotypes can be distinguished into three subfamilies based on biological and genomic similarities: GaHV-2 (RB-1B, Md5, and CVI988/RISPENS), GaHV-3 (SB-1), and MeHV-1 (HVT; FC-126) (9). Currently, four pathotypes have been identified, which include mild (m) MDV, virulent (v) MDV, very virulent (vv) MDV, and very virulent plus (vv+) MDV strains. The MDV serotypes 2 and 3 are not pathogenic and are used as candidates to produce vaccines against MD (33). Similarly, a MDV-1 strain known as MDV Rispens CVI998 is the most effective vaccine strain available and can be used alone or in combination with HVT.

Pathogenesis

MDV infection starts with the inhalation of dust containing the infectious virus. Initial virus replication was previously detected in macrophages and B cells in the lung of infected animals (4). Phagocytic cells, such as macrophages, are thought to transport the virus to regional lymphatic tissues, the bursa of Fabricius, and the spleen, where other immune cells become infected (5). MDV can establish latency in infected T cells. Latently infected T cells transport the virus to the skin and feather follicle epithelia (FFE), where cell free MDV is generated. In addition, MDV can transform latently infected T cells, resulting in deadly lymphomas [10]. This virus targets lymphocytes, the principal cells of the immune system. B-lymphocytes are first targeted by the virus in a lytic infection. Following this, cytolytic infection occurs in the activated T-lymphocytes that are involved in cell-mediated immune responses. These early cytolytic events result in atrophic changes in the bursa of Fabricius and thymus, leading to severe debilitation of the immune system and marked immunosuppression. The cell-associated viraemia that develops during this period is believed to be the route by which the virus spreads throughout the body, including the feather follicle epithelium, the only site where a fully productive infection occurs which allows shedding of the virus into the environment. After the early cytolytic phase, the infection switches to a latent phase in the infected T cells, and the regressive changes in the lymphoid organs start resolving, largely restoring the architecture of these lymphoid organs. Following this, some of the latently infected T cells become targets for neoplastic transformation, resulting in lymphomatous lesions in various visceral organs. Due to the complex nature of the pathogenesis with varying periods of latency, the incubation period of MD from the point of infection to the onset of clinical disease can vary from a few weeks to several months (28). MDV results in the transformation of CD4+ T cells. The natural route of infection is defined by the inhalation of airborne cell-free virus particles within the contaminated dust and dander, shed from the infected host and produced in the terminally differentiated feather follicle epithelium, into a naïve respiratory track.

There is no transmission from chicken to egg (vertical transmission), but the birds are usually infected in the early stages after hatching (horizontal transmission) (9). MDV infection and its global presence could also be a product of natural reservoirs located in backyards and migratory birds (9).

Clinical signs

Signs associated with MD vary according to the specific syndrome. Chickens with MD lymphoma or fowl paralysis syndromes may exhibit signs, but few are specific to MD. In general, signs related to peripheral nerve dysfunction are those associated with asymmetric progressive paresis, spastic or flaccid, ataxia, torticollis, and nervous tics, eventually culminating in complete paralysis due to an enlargement of peripheral nerves. Almost any nerve in the body may be involved unilaterally or bilaterally. An uneven gait, lameness, favouring of one leg over the other, and paralysis of one leg or both wings also occur. Other signs seen include diarrhoea and a drop in egg production. Other signs of clinical MD are characterized by depression, death, stunting, lethargy, and mortality (19).

Gross, histopathological, and immunohistochemical findings

These lesions are characterized by enlargement of the peripheral nerves due to lymphocytic infiltration and by the presence of lymphomas in various visceral organs and tissues (Fig. 1 a), which may be present even in vaccinated birds. Tumor lesions were observed in the liver and ovary, in addition to enlarged kidney and spleen, skin thickening, and whitish lesions in streak form in the breast muscle (2). At necropsy, MD gross lesions are characterized by diffuse enlargement of the liver and the spleen, the presence of multiple polyorganic lymphomas (in the liver, kidney, ovary, proventriculus, spleen, lungs, heart, skin, and also nerves), sciatic nerve enlargement, and atrophy of the bursa of Fabricius and thymus (8). The histopathology

of affected organs shows a marked cellular polymorphism, with the presence of lymphocytes, lymphoblasts (Fig. 1 b), fibroblasts, and infiltration of tumour cells arranged in circumscribed or diffuse form. In the brain, there is endotheliosis, mononuclear perivascular cuffing, vasculitis, vacuolization, and an increased number of neutrophils. In the liver, the previous lesions are accompanied by degeneration and necrosis of parenchymal liver cells, atrophy of the hepatic ducts, and vacuolization. Necrosis and destruction of lymphoid cells can be found in the thymus and bursa of Fabricius (15), along with pleomorphic populations of neoplastic lymphoreticular cells with pyknotic nuclei (2). Non-encapsulated circumscribed large nodules with pleomorphic population of cells, mainly lymphoplasmacytic and mixed neutrophilic polymorphonuclear cells, can be extensively dispersed in the organism of some chickens (3). A massive infiltration of the sciatic nerve can also be detected (44). IHC findings in cases of MD showed mild to moderate immunopositivity in tumour cells and in the cytoplasm of the affected parenchymatous cells of the liver, kidney, spleen, and bursa of Fabricius (Fig. 1 c, d). In the sciatic nerve, intense immunopositivity was observed in the cytoplasm of plasma and MD cells in the lymphoproliferative areas. Evident immunostaining was observed in the medullar lymphoid cells of intrafollicular regions in the bursa of Fabricius (29, 36, 42).

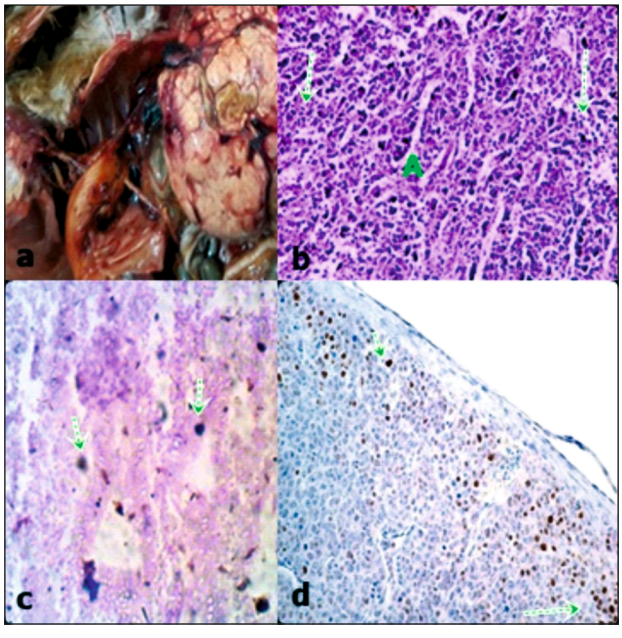

Fig. 1. a: Tumourous masses on the visceral organs (liver, kidney, and spleen), with enlargement of the pro-ventriculus and intestines; **b**: Glomerular and renal tubular coagulative necrosis (A) with periglomerular polymorphonuclear (heterophils) and cellular infiltration (thin arrows) X100 H&E; **c**: Immunopositive mo-derate immunopositively staining for CD3 X400; **d**: Photomicrograph of neoplastic liver showing characteristic intranuclear labelling of the tumour cells (arrows). Labelled with Meq (MDV1) x40.

Cytopathological findings

The cytological examination of touch imprints from the affected tissues revealed a pleomorphic cell population of small to large lymphocytes, lymphoblasts (Fig. 2), plasma cells, and macrophages (19, 25). The proportion of cell types varies with the stage of disease and the virulence of the virus. The most aggressive lymphomas may contain high numbers of lymphoblasts (22).

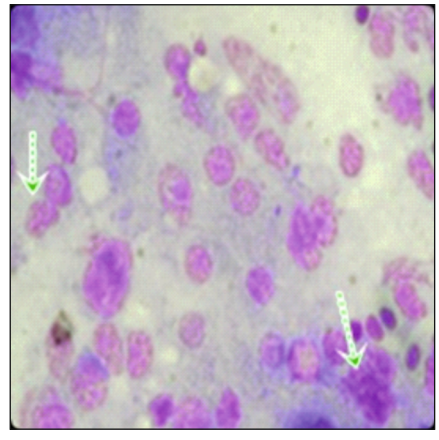

Fig. 2. Touch imprint. Liver. Marek's disease. Presence of a pleomorphic lymphoid cell population with some in mitosis (arrows), x1000 (22).

Haematological and serum biochemical findings

The importance of this finding is not clear because the haematocrit values are not routinely used as a MD parameter. Some authors reported extravascular haemolytic anaemia after infection with vvMDV strains or anaemia as a consequence of bone marrow aplasia, in cases contaminated with CIAV (34). The number of blood lymphocytes may vary during the infection cycle: T cells may be elevated while B cells are decreased. There could also be monocytosis.

Diagnosis

Rapid and reliable diagnosis of MD is primordial. Good laboratory diagnostic practices should be routinely followed upon the first suspicion of an outbreak. Clinical signs should be observed, and then a laboratory diagnosis of MD, which involves the isolation, identification, and characterization of MDV, must be followed. The diagnosis of Marek's infection in a flock can be detected by isolating the virus from the infected tissues. Materials commonly used for the isolation of the virus are buffy coat cells from heparinised blood samples, or suspensions of lymphoma and spleen cells. As MDV is highly cell-associated, the suspensions must contain viable cells. These cell suspensions are inoculated into monolayer cultures of chick kidney cells or duck embryo fibroblasts (28). The methods often chosen for the detection of virus in tissues include in situ hybridisation using MDV-specific nucleic acid probes, PCR (Fig. 3), electron microscopy, immunofluorescence, and immunohistochemical methods u-

sing polyclonal and monoclonal antibodies. Immunoreactivity for Meq mAB has been used for the definitive diagnosis of MD and differentiate it from lymphoid leukosis (22).

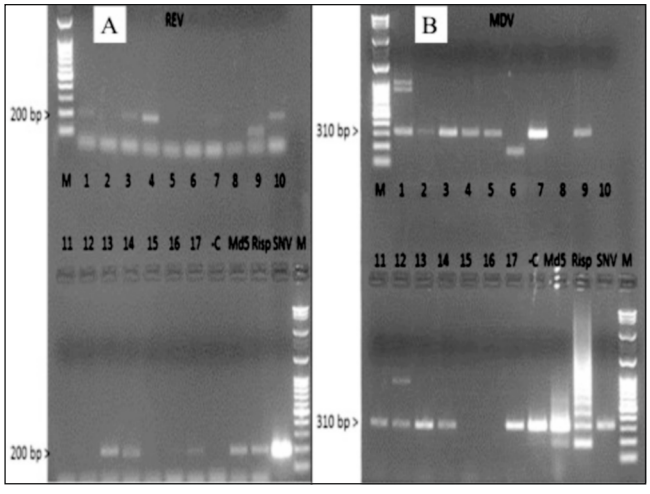

Fig. 3. PCR amplification of 310 bp of the gB gene of MDV1

Serological tests can check the presence of antibodies to MDV in birds at approximately four weeks of age. Although no serological test has been indicated for the detection of MDV-specific antibodies, the agar gel immunodiffusion (AGID) test is usually used for this purpose. Other serological tests, such as the indirect immunofluorescence test, enzyme-linked immunosorbent assay (ELISA), and virus neutralisation (VN), have been described but are used mostly for research purposes rather than for routine diagnosis (28). Imaging mass spectrometry (IMS), which allows sensitive visualization of MDV-induced lymphoma with a specific mass profile and precise differentiation from the surrounding tissue, and proteome analysis by quantitative mass spectrometry based on stable isotope labelling, have been used in the diagnosis of MD with great success (26).

AVIAN RETICULOENDOTHELIOSIS

Avian reticuloendotheliosis (RE) is a malignant disease of poultry, most frequently affecting chickens and turkeys but also waterfowl such as ducks and geese, as well as other bird species (13, 21, 28). The clinical course of RE may be like other neoplastic diseases, including MD, lymphoid leukosis (LL), or avian leucosis (41). It causes atrophy of immune organs and severe immunosuppression, in addition to other symptoms, which include avian dwarfing syndrome and chronic tumours in lymphoid and other tissues (7, 17).

Aetiology

The RE virus in chickens induces a variety of deleterious effects, including tumours and increased mortalities, causing considerable losses. The viruses of the REV group are enveloped and single-stranded, are currently classified as gammaretroviruses, and consist of pathogenic retroviruses that share structural, morphological, and antigenic similarities to mammalian type C retroviruses (41). The genomic structure of REV consists of a group-specific antigen (gag), protease (pro), polymerase (pol), and envelope (env) regions flanked by long-terminal repeats (LTRs) (32).

Pathogenesis

RE viral strains produce three distinct clinical groups of manifestations: runting, rapid-course neoplastic disease, and chronic neoplastic disease (38). Runting is usually seen 4–10 weeks after administration of contaminated vaccines to one-day-old chicks. The chronic neoplastic disease has been induced experimentally in chickens, turkeys, and ducks, while acute neoplasia, which occurs after a latency of 6–8 weeks, has also been seen in chickens, turkeys, ducks, and quail. This tumour in chickens involves T cells and may be confused with MD. RE exerts an immunosuppressive influence on the host immune system through the abrogation of T or B lymphocytes and endotheliocyte function (13). This immunosuppression is associated with the apoptosis of lymphocytes in lymphoid tissues, especially in the thymus (40). It is important to highlight that REV has a high tropism for the proventriculus, kidney, liver, lymphoid tissues, pancreas, lymphosarcoma cells, and blood vessels (40).

Clinical signs

The only specific clinical observation may be abnormal feathering of affected birds called "nakanuke syndrome" (41). Due to its main influence on the host immune system, the disease causes dysfunction of the spleen and atrophy of the bursa of Fabricius and thymus (28, 41). The most common clinical diseases induced by REV include runting-stunting disease syndrome (Fig. 4 A), characterized by growth retardation and poor feathering with acute reticular cell neoplasia and chronic B-cell and T-cell lymphomas (11,32,38).

Clinical signs in turkeys are mainly sleepiness, weakness, anorexia, diarrhoea, and reduced egg production. The pullets showed severe emaciation (Fig. 4 B), loss of back feathers, anorexia, and diarrhoea.

Gross, histopathological, and immunohistochemical findings

Necropsy of the dead turkeys and pullets showed neoplastic nodules or grey foci in the internal organs, especially the liver, intestines, and spleen. Histopathological sections of the organs highlighted a proliferation of reticular cells, with parenchymal necrosis and focal lymphocytic infiltration. Markedly emaciated carcasses (Fig. 4 A, B), unusual tumour formation in the head region, and congested and enlarged organs with whitish nodular infiltrations (mainly liver, spleen, proventriculus, and gizzard) (Fig. 4 C) were commonly observed in submitted chickens. In addition, neoplastic nodules in the intestine, pancreas and mesentery could be detected (11). There could also be present

atrophies of the bursa of Fabricius and thymus (28, 41). Other noteworthy lesions can be ulcers in the proventriculus, enteritis, or spleen and liver necrosis (41). Histopathological findings include multiple nodules consisting of homogeneous immature lymphocytes, hypercellularity, clusters of lipoblasts of varying sizes, intercellular transudate, dilated blood vessels, and the aggregation of inflammatory cells surrounding the blood vessels. Some areas were more cellular than others, these cells were mainly fibroblasts, separated from each other by intercellular transudate and focal localized aggregation of inflammatory cells such as lympho-reticular cells, lymphocytes, and histiocytes. The aggregation of inflammatory cells surrounded the congested blood vessels, resulting in an aneurysm. The dilated blood vessels sometimes contain transudate and inflammatory cells. Mitotic figures can also be observed (11, 39).

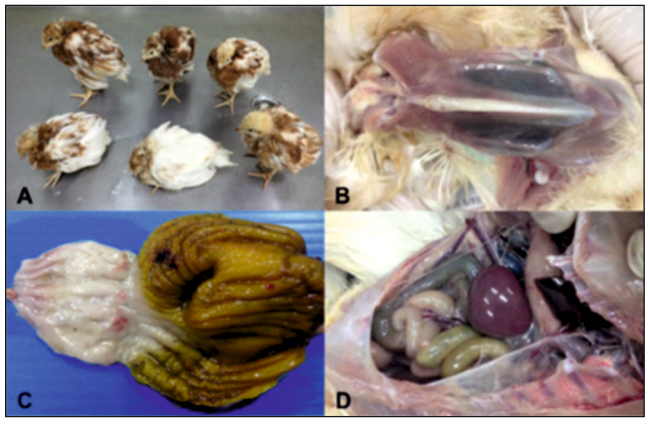

Fig. 4. A: Runting-stunting symptom. Gross lesions; **B**: Lesions of REV-infected chickens showing runting-stunting symptom included emaciation; **C**: Proventriculitis with enlargement and haemorrhagic proventricular glands; **D**: Occasional spleen enlargement (38)

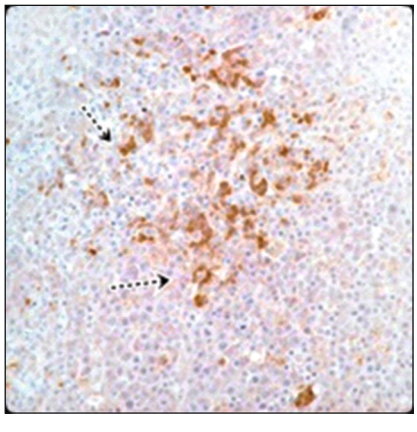

Fig. 5. Cytoplasmic labelling of the tumour cells in the liver (arrows). Labelled with 11B118 (REV) (31)

Typical pathognomonic lesions of REV are severe congestion of the central and portal veins, as well as hepatic sinusoids, associated with hepatocyte degeneration and necrosis. A massive number of pleomorphic lymphoreticular cells may replace the hepatic parenchyma. A splenomegaly (Fig. 4 D) and oedema in the wall of follicular blood vessels with diffuse reticular cell infiltration may also appear (38, 39, 12).

According to immunohistochemical findings observed in cases with Avian RE, a strong positive staining was observed in both the epithelium and musculature of the proventriculus, indicating a viral tropism for the proventriculus. The bursa of Fabricius also showed lymphoid neoplasia with positive immunostaining (32). Intracytoplasmic labelling in the liver has also been noted (Fig. 5) (31).

Cytopathological findings

Cytopathological findings are the basis of plaque assays, but the methods have not been widely used due to inconsistency in cytopathology (24). Subtle cytopathic changes, such as syncytia, may be induced in avian fibroblasts due to the replication of REV, but degenerative changes are more commonly seen. Apoptosis could be caused by the accumulation of unintegrated viral linear DNA in infected cells. Cells that can prevent early superinfection have few copies of unintegrated viral DNA and survive. The acute cell death phase lasts 2–10 days post-infection and is followed by a chronic infection state without cytopathological observations but with continued viral replication.

Haematological and serum biochemical findings

There is leukocytopenia, increased numbers of heterophils, and lymphocytosis. Basophils, monocytes, and eosinophils decreased. There were no significant differences in the serum biochemical values of total serum proteins, albumin, and alpha-, beta-, or gamma -globulins. However, there are contradictory reports of hypoproteinaemia, hyperglycaemia, and elevated levels of triglycerides, along with leucocytosis, granulocytosis and monocytosis, reported by Wang et al. (2012) (40).

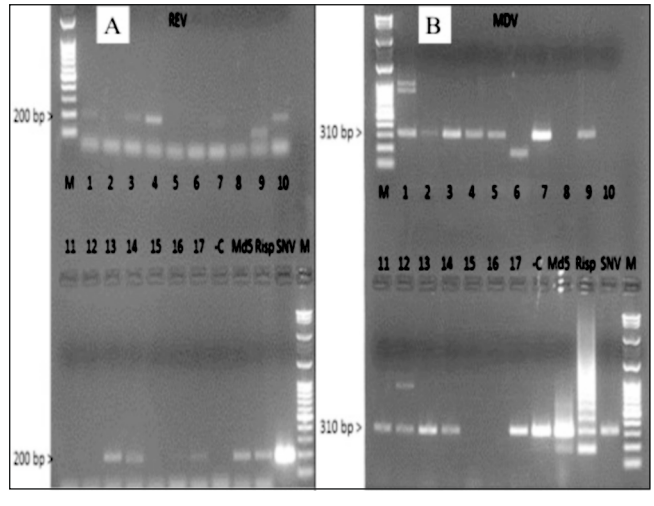

Fig. 6. PCR amplification of 200 bp of the SNV LTR of REV (32)

Diagnosis

ELISA was a successful tool for the detection of avian RE (11). A microplate indirect ELISA for antibodies against REV was consistently more sensitive than indirect immunofluorescent-antibody tests and suitable for screening chicken flocks (11). PCR (Fig.6), in situ hybridization and immunohistochemistry assays proved to be efficient for the detection of several REV strains in Japanese quail embryos during active infection (32).

AVIAN LEUKOSIS

The term leukosis encompasses several different leukaemia-like proliferative diseases of the haemopoietic system caused by the Avian Leukosis Virus (ALV) (20, 25). The components of the haemopoietic system that may undergo neoplastic change include the lymphopoietic (lymphocytic) system, the erythropoietic (red cell) system, and the myelopoietic (myelocytic) system. Therefore, there are three forms of leucosis, namely lymphoid leukosis, erythroid leucosis, and myeloid leukosis (28, 30, 44). Myeloid leukosis has become particularly prevalent in broiler breeders and less frequently in broilers, in many countries since 1996, currently being caused by the recently recognised subgroup J ALV (28).

Aetiology

ALV, a member of the avian oncornavirus or oncovirus group, *Alpharetrovirus* genus of the family *Retroviridae*, causes various diseases associated with tumour formation and decreased fertility (14, 39, 37). ALV is composed of a single positive-stranded RNA dimer. The viral genome is approximately 7.8 kb in length and contains three main coding genes: *gag*, *pol,* and *env*. ALV are usually classified into 11 subgroups, including subgroups A, B, C, D, E, F, G, H, I, J and K. The members of subgroups A, B, C, D, J, and K are exogenous viruses, that were found in naturally infected flocks (37, 27).

Pathogenesis

In susceptible chickens infected at an early age, a continuous transformation and proliferation of the stromal cells from the cortex of the bursa of Fabricius are installed. Up to the age of 6 to 8-weeks, the whole follicles can be filled with transformed lymphoblasts. Close to sexual maturity(18 to 20 weeks),the neoplastic B-cells, expressing IgM and other B-cell markers that originate in the bursa follicles, subsequently burst into the blood vessels and metastasize to other organs (28). Lymphoid leukosis has been the most common form of leukosis. It occurs in chickens at approximately four months of age (28). Erythroid leukosis (erythroblastosis) is uncommon, usually sporadic, occurring principally in adult chickens, although occasionally it can be observed in young birds (from 5 weeks old) (27, 28). Myeloid leukosis occurs in two overlapping forms: myeloblastic myeloid leukosis (myeloblastosis) and myelocytic myeloid leukosis (myelocytomatosis).

Clinical signs

The disease is often characterized by inappetence, anorexia, weakness, and diarrhoea, leading to emaciation and dehydration. Bleeding can often occur in feather follicles and claws, leading to poor feathering, paleness and anaemic combs and wattle, swollen phalanges, decreased egg production, paralysis, and death (1, 43).

Gross and histopathological findings

Generally, at the necropsy of the affected dead birds, hepatomegaly and splenomegaly with diffused neoplastic nodules or grey foci (Fig. 7 a, b) were seen. Also, a diffuse infiltration in the myocardium, lungs, and visceral organs such as the liver, spleen, heart, kidney, and ovaries may be observed. Other authors reported lung congestion and many yellowish/white tumours on the visceral surface of the sternum (1, 43). The histological sections of the liver revealed diffuse and focal infiltration of uniform-sized lymphoid cells and lymphoblasts, predominantly or sometimes pleomorphic lymphoblastoid cells, resulting in atrophy of hepatocytes due to compression and replacement. Individualisation of hepatocytes with a variable degree of degenerative changes and necrosis were described (1, 30, 43). In the spleen, a depletion of lymphocytes in germinal centres and clear nodule formation by neoplastic lymphoid cells are the common microscopic findings. Some mitotic figures and an absence of any modifications or lymphocytic infiltration in peripheral nerves may be identified. Also, in some cases, extensive proliferation and infiltration of homogenous lymphoblastic cells can be present in various organs with different degrees of degeneration (Fig. 7 c, d) (30). In the heart, a diffuse infiltration of neoplastic lymphoid cells among the myofibrils can be seen. Others mentioned a moderate fibrous tissue proliferation of the proventricular submucosa, while in the ovary, an invasion of normal tissue by a uniform lymphoblast population may be detected (30).

The gross pathological changes in lymphoid leukosis are the significant enlargement of the liver with miliary, diffuse, or nodular tumour foci and often nodular tumours in the bursa of Fabricius. Microscopically, the lesions consist of coalescing foci of extravascular uniformly immature lymphoid cells (lymphoblasts) (28).

In erythroid leucosis, the liver and spleen, and sometimes the kidneys, are moderately and diffusely enlarged, with a bright cherry-red colour. The white raised foci throughout the spleen and kidney were fragile. The bone marrow was bright red and liquid. Diffuse, multifocal white raised foci were observed on the lung surface (28). The disease is an intravascular erythroblastic leukaemia. Microscopically, the liver shows intrasinusoidal accumulations of rather uniform, round, erythroblasts. The spleen shows accumulations of erythroblasts in the red pulp, and the bone marrow shows enlarged haemopoietic sinusoids filled with erythroblasts. An erythroblastic leukaemia is visible (28). In myeloid leukosis, the liver and spleen are greatly enlarged and the liver frequently has a yellowish-grey granular ("Morocco leather")appearance.

The bone marrow is firm and reddish grey. Microscopically, the liver shows intravascular, sinusoidal accumulations of immature myeloid cells (myeloblasts and promyelocytes), and extravascular accumulations and perivascular cuffing. The red pulp of the spleen is infiltrated by myeloid tumour cells, as are the extra sinusoidal myelopoietic areas in the bone marrow. A marked myeloid cell leukaemia is observed (28).

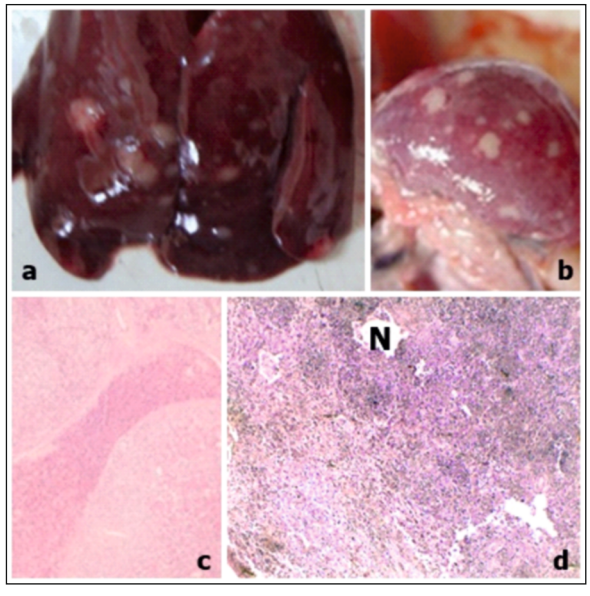

Fig. 7. a, **b**: Hepatomegaly and splenomegaly with multifocal areas of while nodulation; **c**: Spleen displayed multifocal areas of coagulative necrosis and cells infiltration (which were mainly lymphoblasts, then minority populations of heterophils, lymphocytes, and macrophages). X40 H&E; **d**: Coagulative necrosis of the hepatocytes and Kupffer cells (N) X40.

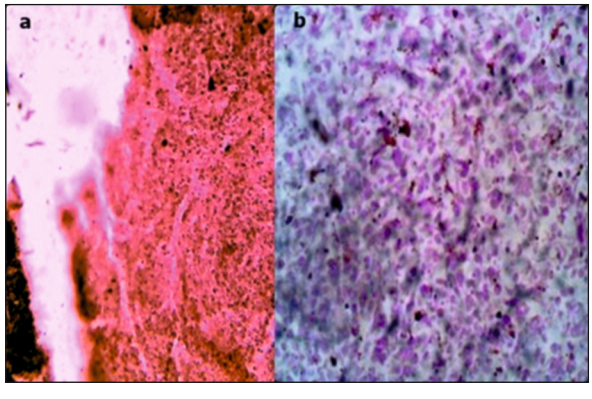

Fig. 8. **a**, **b**: Photomicrographs showing immunopositivity of the neoplastic cells for P53 in the spleen of a layer chicken X40. Moderate immunopositivity for Cd3 in the liver of a layer chicken X400.

IHC analysis using a technique detecting gp85 viral glycoprotein was used with all organs examined containing a detectable antigen. An intense immunostaining was found in the adrenal gland, heart, kidney, spleen (Fig. 8), and proventriculus. The viral antigen was most dominant in the heart, making it a possible cause of cardiomyopathy. Although recent investigations failed to demonstrate specific viral staining in bone marrow from infected chickens, we were able to show moderate staining in myelocytic precursor cells in bone marrow (18).

Haematological and serum biochemical findings

Macrocytic normochromic type of anaemia, as well as erythrocyte abnormalities such as anisocytosis, poikilocytosis, basophilic stippling, and an increased number of polymorphic erythroblasts and binucleated erythroblasts, can be remarked. Leucocytosis due to heterophily and lymphocytosis has been reported in lymphoid leukosis (16). Increased activities of ALT, AST, and ALP, as well as markedly increased serum levels of creatinine and uric acid, may be identified (16).

Cytological findings

The cytological examination of impression smears taken from tumorous growths of affected tissues revealed lymphoid cells with a thin rim of finely granular cytoplasm,a clear vesicular nucleus, and a marked degree of uniformity in size and appearance of lymphoid neoplastic cells (Fig. 9) (22, 30). In cases complicated by erythroblastosis, there were polymorphic erythroblasts with hypercellularity, basophilic cytoplasm. In the bone marrow smears, the number of erythroblasts was significantly increased. However, the cytoplasmic, nucleic features of the bone marrow erythroblasts were like those of the blood erythroblasts.

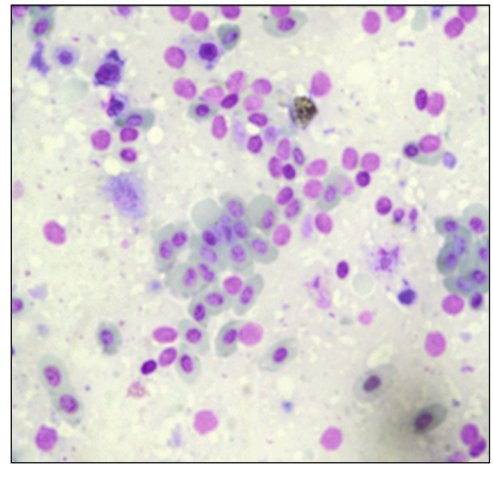

Fig. 9. Touch imprint. Liver. Lymphoid leukosis. Presence of a uniform lymphoid cell population, x1000 (22)

CONCLUSIONS

In conclusion, avian neoplastic diseases (MD, RE, and lymphoid leukosis) are caused by oncogenic viruses. They are important poultry diseases that cause significant economic loss in the industry due to the associated morbidity and mortality. Although the incubation period, the pathogenesis, the clinical manifes-

tations, the organs and tissues affected, the gross aspect, and the histopathological lesions of the diseases may vary, they are all characterized by tumour formation. They primarily affect the reticuloendothelial system and manifest similar clinical signs and gross pathological lesions. Diagnosis and differentiation of the diseases can be achieved with the aid of histopathology, cytology, clinical pathology, immunohistochemistry, serology, molecular diagnosis, electron microscopy, and in situ hybridization. In that situation, this review highlighted the aetiology and associated pathology of these neoplastic diseases, helping the veterinarians in their current practice.

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