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Research Article

PATHOMORPHOLOGICAL AND IMMUNOHISTOCHEMICAL STUDIES ON MYELOCYTOMATOSIS IN LAYER CHICKEN

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ABSTRACT

The investigation included thorough postmortem examination of 40 poultry carcasses brought to the Department of Veterinary Pathology, LUVAS, Hisar during the period of six months (from August 2018 to January 2019). The cases were suspected of neoplastic conditions on the basis of abnormal enlargement or nodular growths in visceral organs and later on confirmed by histopathological examination. Maximum cases of neoplastic conditions were observed in layers. Histomorphologically, out of 40 neoplastic disease suspected cases, one case revealed the characteristic infiltration of myelocytes in visceral organs as liver, spleen, kidneys, intestines, lungs suggestive of myelocytomatosis. Immunohistochemical studies using CD79 alpha and CD3 differentiation markers were conducted on neoplastic cells for confirmation of the disease. Neoplastic myeloid cells did not reveal any reactivity for CD3 or CD79 alpha markers confirming that these cells are not of T or B lymphoid cell origin. Case of myelocytomatosis disease was further confirmed as J subgroup of Avian leukosis by conventional PCR.

Keywords: Avian leukosis, CD79 alpha, CD3, Myelocytomatosis, Neoplastic conditions, PCR

Avian leukosis, caused by avian leukosis viruses which belong to the family Retroviridae is prevalent worldwide (Fadly and Payne, 2003; Chauhan and Sushovan, 2010). Avian leukosis virus subgroup J (ALV-J) is one of ten subgroups, among six subgroups viz., A-J that were isolated from chickens and turkey (Payne, 1992). Lymphomas in poultry are common disease conditions which have economic importance due to associated mortality and condemnations of the carcassess. Myelocytomatosis is a tumour condition caused by avian leukosis virus subtype J which involves an extravascular proliferation of cells of the granulocytic series particularly the myelocytes. Subgroup J avian leukosis virus (ALV-J) was first isolated in 1989 from meat-type chickens. Diseases associated with ALV-J have caused enormous economic losses in chicken worldwide (Fadly and Smith, 1999). ALV-J has been recognized and reported in most parts of the world.

The incubation period of about 14 weeks makes it more of a problem in breeders and commercial layers (Fadly, 2010). The clinical infected hosts of ALV-J are characterized as high mortality, delayed growth and development of a variety of tumors including myelocytomas, hemangiomas, nephromas and erythroblastosis (Arshad *et al.*, 1997). In the present study, we report the diagnostic investigation of myelocytomatosis in an organized poultry farm diagnosed on the basis of gross lesions, histopathological examination, immunohistochemical differentiation and molecular techniques.

MATERIALS AND METHODS

The present study was carried out on 40 poultry carcasses suspected for neoplastic conditions on the basis of abnormal enlargement or nodular growths in visceral organs which were brought for necropsy examination to the Department of Veterinary Pathology, LUVAS, Hisar from August, 2018 to January, 2019. The detailed post mortem examination was conducted and the details regarding identification of all the carcasses viz. species, age, sex etc. were recorded.

Pathological studies: All poultry carcasses suspected for neoplastic conditions (40 cases) were examined critically for gross pathological alterations in various internal organs mainly liver. Representative tissue pieces from organs, which revealed lesions particularly heart, liver, spleen, proventriculus, intestine, ovary, lung, kidney, brain and bursa of Fabricius were collected in 10 per cent buffered formalin for histopathological examination. The fixed tissues were washed in running tap water, dehydrated in graded ethyl alcohol, cleared in benzene and embedded in paraffin wax (melting point 60-62 ºC). Paraffin sections were cut at the thickness of 4-5µ and stained with routine haematoxylin and eosin stain using Lilly Mayer's haematoxylin and 2 percent water soluble eosin (Luna, 1968). Tumours were diagnosed and classified on the basis of characteristic gross and microscopic lesions.

Immunohistochemical studies: Immunohistochemical staining using primary antibodies for B and T cell differentiation markers i.e. CD79 alpha (anti-chicken Invitrogen; CAT No. MA5-13212) and CD3 (anti-chicken Invitrogen CAT No. MA5-28699) was done in formalin fixed tumour tumour as per standard protocol. Antigen retrieval was performed by microwave irradiation in a Coplin jar containing 0.01M citric buffer, pH 6.0, for 10×2

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Table 1

Monoclonal antibodies and antigen retrieval for immunohistochemistry for differentiation of MD/LL

Sr.No.	Name of antibody (Catalog No.)	Company	Clone	Raised in species	Immunoglobulinclass	Dilution used	Antigen Retrieval (HIER)
1.	CD 79 alpha (MA5-13212)	Invitrogen	HM47/A9	Mouse	IgG	1:10	Citrate buffer pH 6.0
2.	CD 3 (MA5-28699)	Invitrogen	CT-3	Mouse	IgG	1:10	Citrate buffer pH 6.0

minutes. Optimal concentration of each primary antibody (Table 1) was determined by making serial dilution of 1:10, 1:20, 1:50 1:100 and 1:200, in PBS containing 1% bovine serum albumin (BSA) and subjecting them to standard immunohistochemical protocol. The negative controls were treated with diluent only (1% BSA in PBS) without primary antibody. The reaction was developed by using 3-Amino-9-ethyl-carbazole (AEC; Sigma Chemicals, USA) as staining substrate which gave brick red (reddish brown) colour. Counterstaining of the sections was done by using Mayer's hematoxylin (Sigma, MHS-16) for 1-2 minutes and mounted in aqueous mounting medium CC/Mount™ (Sigma Chemicals, USA). Immunohistochemical staining was accessed as negative and positive groups. Brick red or brown red colour in membrane and cytoplasm of tumour cells was taken as positive.

Molecular studies: Tumour tissue samples were collected from liver of all the 40 carcasses and stored at -20 ºC for molecular studies. Conventional Polymerase chain reaction (PCR) was carried out on all the 40 carcasses. Total genomic RNA extraction was done from the 400 mg liver tissue by Trizol reagent method. RNA quantity was determined using A260 values in biophotometer and the purity was judged using A260/280 ratio >1.8-2.0. The quality of total RNA was checked by electrophoresis in 1.5% agarose gel electrophoresis in tris-acetate-EDTA buffer, pH 8.3. cDNA synthesis was done from mRNA extracted using primers provided in the commercial kit for cDNA synthesis (Thermo Scientific Revert Aid First Strand cDNA Synthesis kit CAT no. K1621). Conventional PCR was carried out to identify the proviral gp85 for ALV-J by employing specific primer (F-5^{1}-GGATGAGGTG ACTAA_GAAG-3^{1}, R-5^{1}-CGAACC AAAGGTAACA CACG-3^{1}) as described by Pham *et al.*, 1999 using standard protocol with 54 ºC annealing temperature yielding product of size 545 bp.

RESULTS AND DISCUSSION

All the cases were present in adult birds which were more than 18 weeks age affecting layers, broilers and dual purpose birds. Maximum cases were found affecting the layers. Out of total 40 cases suspected for neoplastic conditions, one case in a layer revealed the lesions of myelocytomatosis. Grossly there was presence of variable sized tumour like nodular lesions in various visceral organs such as liver, spleen, kidney, and lung. Liver was extensively enlarged and appeared as cherry red in colour containing discrete tumour foci (Fig. 1). Microscopically there was proliferation of well differentiated myelocytes with large, eccentrically placed basophilic vesicular nucleus in different visceral organs such as liver (Fig. 2,3), spleen, kidney (Fig. 4) and lung. The cytoplasms of the cells were packed with acidophilic granules which were spherical in shape. No lesions were observed in heart, brain, nerves, bursa of Fabricius. Immunohistochemical studies on neoplastic myeloid cells did not reveal any immunoreactivity to both CD79 alpha and CD3 marker (Fig. 5, 6). This indicates that the neoplastic myelocytes were not of T or B lymphoid cell origin. Out of 40 tumour samples collected from liver tissue, one sample that was showing lesions of myelocytomatosis was positive for ALV-J serogroup, while all other samples were negative for avian lymphoid leukosis virus serogroup J. This confirmed the presence of ALV-J serogroup myelocytomatosis.

The present study demonstrated the occurrence of ALV-J in a commercial layer. Depending on the organ involved, the tumourous lesions were variable. In the liver and spleen, infiltration and proliferation of myeloid cells were associated with extensive damage in the parenchymatous tissues. The gross and histopathological findings in this case are similar to the findings of other researchers (Pejovic *et al.*, 2007; Jayalakshmi and Selvaraju, 2016). The ALV-J mainly induces myelocytomatosis because of their tropism to the cells of the myeloid rather than the lymphoid lineage (Payne and Fadly, 2003). Compression of resident cell population by the proliferative myelocytes in the liver and the ovary may lead to hepatic necrosis and ovarian atrophy which cause mortality and the decrease in egg production. ALV infections lead to economic losses due to high tumour forming potential and involvement of various visceral organs (Venugopal, 1999).

Immunohistochemically, no reactivity to CD79 alpha and CD3 was found in neoplastic cells. It confirms

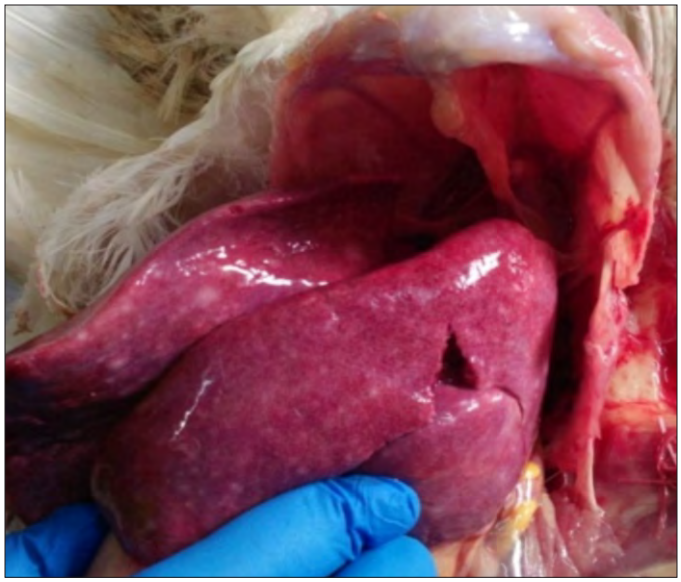

Fig. 1. Enlarged liver with cherry red colour discolartion and containing focal to coalescing whitish tumourous foci (Myelocytomatosis)

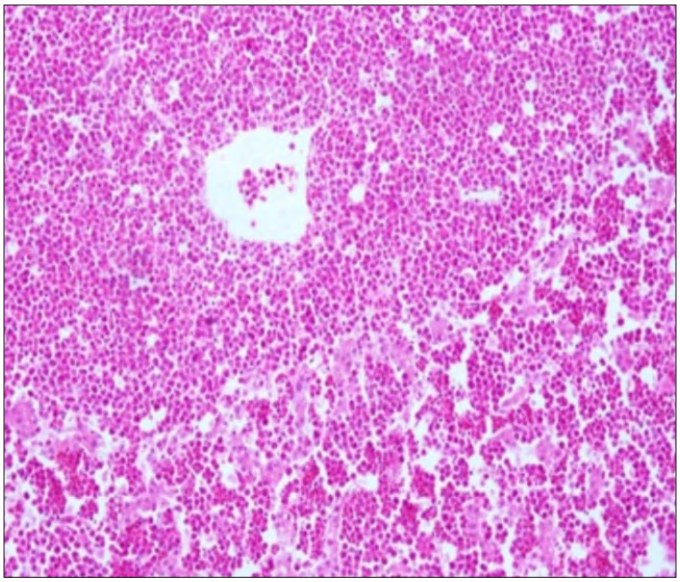

Fig. 2. Liver section showing proliferation of myelocytes (arrow) replacing hepatic parenchyma (H&E stain, 200X)

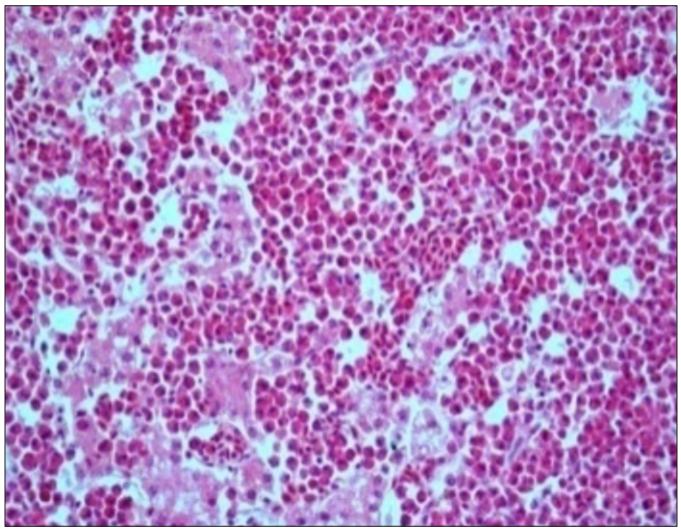

Fig. 3. Higher magnification showing diffuse proliferation of myelocytes (arrow) replacing hepatic parenchyma (H&E stain, 400X)

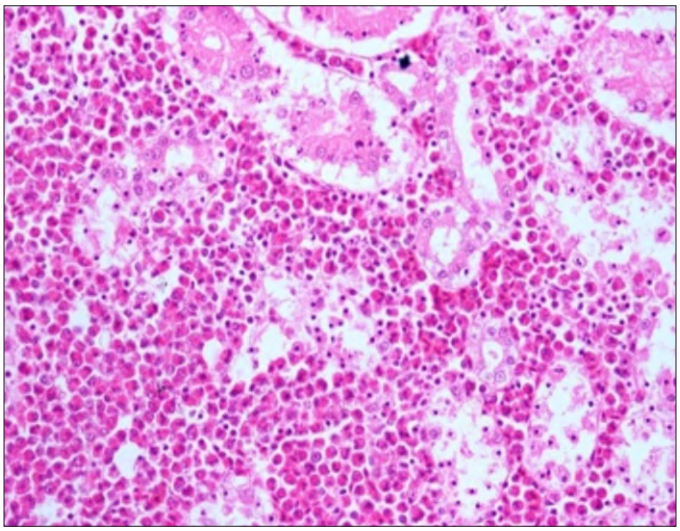

Fig. 4. Kidney showing neoplastic myelocytes (arrow) in the interstitium (H&E stain; 200X)

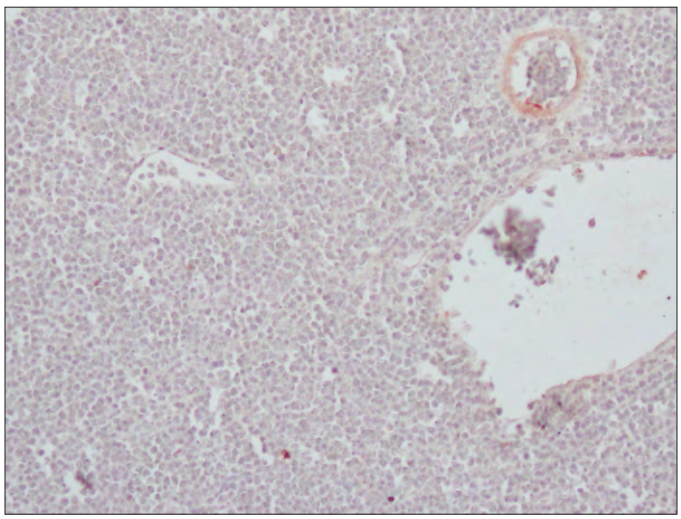

Fig. 5. Myelocytomatosis case showing no immunoreactivity to CD79 alpha marker in the neoplastic cells (IHC stain 200X)

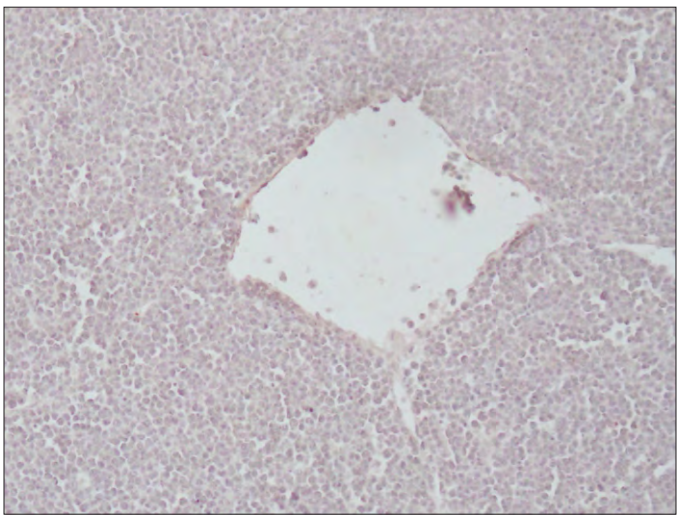

Fig. 6. Myelocytomatosis case showing no immunoreactivity to CD3marker in the neoplastic cells (IHC stain 200X)

that the neoplastic cells were negative for lymphoid origin cell markers. This indicates that that the cells were not of lymphoid origin viral disease conditions such as Marek's disease, other serogroup of ALV or Reticuloendotheliosis. CD molecules are cell surface markers which are very useful for the identification and characterization of leukocytes and the different subpopulations of leukocytes. So such studies are helpful in identifying the origin of cells among the neoplastic cells.

Further ALV-J serogroup was confirmed by PCR technique. Myeloid leukosis broadly involves both myeloblastic myeloid leukosis (myeloblastosis) and myelocytic myeloid leukosis (myelocytomatosis). The disease has become particularly prevalent in broiler breeders infected with subgroup J of ALV in many countries. Over the past decade, many myeloid tumor cases induced by ALV have been reported, especially involving ALV-J (Cheng *et al.*, 2010). At first, it was only found in white meat-type breeders. Later, it was discovered that there were a growing number of AL cases in layers and local meat-type chickens. The ALV-J have changed rapidly, which results in a change in host range, first infecting meat-type chickens and layer flocks, then detected in wild ducks in China. Molecular characterization of ALV-J in wild ducks in China identified amino acids substitutions when compared with the prototype strain HPRS-103. These substitutions might be related with changes in the host range and pathogenicity of ALV-J (Zeng *et al.*, 2014).

Avian retroviruses can be transmitted vertically from dams to offspring; embryos and tissue cultures prepared from such embryos may harbour such viruses (Fadly and Payne, 2003). Therefore, embryos or cells prepared from infected embryos could serve as a source of retrovirus contamination of poultry and of other vaccines produced from such ingredients. Source of these ALV infections to poultry might be due to the contaminated MD vaccines. Mohamed *et al.* (2010) revealed presence of ALV-A in 5 samples out of 13 (38.46%) commercial MD vaccines, while ALV-J was detected in 2 cases out of 13 commercial MD vaccines. An effective treatment or vaccine against these ALVs is not currently available. As a result, the control of ALV infections mainly depends upon early detection and limitation of the virus carriers to prevent the vertical and horizontal transmission. Meconium for chicks and fecal matter contain high concentrations of ALV and are an important source of virus for horizontal transmission to the birds. The horizontal transmission that occurs through close contact with infected birds is a major challenge for the eradication programs since the spread of infection can rise quickly during the first few weeks of infection (Venugopal, 1999). Continuous monitoring and elimination of infected birds will help in breaking is spreading life cycle of the virus and lead to ALV eradication.

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