



Comprehensive pathological studies on Marek's disease in HVT-1 vaccinated chickens flocks

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ABSTRACT

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Present study was conducted on poultry carcasses from HVT-1 vaccinated flocks received in the Department of Veterinary Pathology, LUVAS, Hisar for disease investigation. Pathological diagnosis revealed MD lesions in 9.7% cases, while Marek's disease and lymphoid leucosis (MD+LL) mixed type lesions were observed in 1.5% cases mostly affecting more than 12-24 wks age groups of layer, broiler and dual purpose breeds. MDV was also confirmed in the tumour tissues by employing Real Time PCR techniques using Meq specific primer, while ALV infection was confirmed by conventional PCR. Grossly in MD affected chicken nodular growths of different sizes was evident which were white to pale yellow in colour and were observed in organs like lungs, heart, liver, kidney, spleen, proventriculus and ovaries. However, brain, bursa of Fabricius, nerves and feather follicles did not reveal any enlargement or nodular growth. Infiltration and proliferation of neoplastic pleomorphic lymphoid cells were observed on histopathological examination of liver, spleen, lungs, intestine, kidney, ovary and proventriculus. Mixed infection (MD+LL) cases revealed islands of uniform neoplastic lymphoblasts cells in the parenchyma along with above lesions. Mean histopathological lesion score revealed severe involvement of liver, spleen and lungs. Overall mean percent histopathological lesion score was found maximum in dual purpose chicken i.e. 52.50% followed by layer (46.87%) and broiler (42.15%) breed. It may be concluded that MDV infection is still a problem in vaccinated layer and broiler breeder flocks in Haryana. The majority of neoplastic disease cases were detected in layer flocks with involvement of the liver, spleen, and lungs in the acute/visceral type of Marek's disease; but the overall severity of lesions was more in dual purpose breed.

Keywords: Chicken, Marek's disease, Lymphoid leucosis, Pathology, Lesion score

INTRODUCTION

Three main avian oncogenic viruses which cause neoplastic disorders in poultry causing huge economic losses are: Marek's disease virus (MDV), a highly contagious herpesvirus, and retroviruses such as Avian Leukosis Virus (ALV) subgroups A through J and reticulo-endotheliosis virus (REV). Furthermore multiple oncogenic virus infections also exist and have also been reported in 25% of commercial chicken and turkey flocks (Davidson, 2009). Marek's disease (MD) is caused by a highly contagious, cell-associated, oncogenic herpesvirus causing proliferative lesions of lymphoid cell population which is found mostly affecting the chickens, with less involvement of turkeys and quails (Calnek and Witter, 1997). MD is widely distributed and presents a significant risk to the chicken industry due to rising reports of vaccination failures and the advent of more virulent pathotypes.. Every year, about 40 million Indian rupees are lost due to MD outbreaks in vaccinated Indian poultry flocks. The disease is recognized in mainly two different clinical forms, the classical or neural form, where the birds exhibit paresis or paralysis affecting their legs, wings, and occasionally their neck. The disease's acute form is more virulent, causing lymphomatous lesions in

multiple organs and a significant mortality rate in flocks that are afflicted. Globally, MDV and ALVs are common, and new strains that emerge in specific areas have the potential to cross international borders and compromise national disease control initiatives (Payne and Venugopal, 2000). Therefore such studies are important to ascertain the cases of viral oncogenic diseases in particular regions so that their pathology can be determined for better effective preventive strategies.

MATERIALS AND METHODS

The analysis was done on poultry carcasses suspected of having neoplastic diseases and were presented to the Department of Veterinary Pathology, LUVAS, Hisar, for postmortem evaluation between August 2018 and January 2019. Following a thorough post-mortem examination, all carcasses were characterized using information such as species, age, sex, and other characteristics, as per the information provided in the requisition form.

Pathological studies

Every carcass was thoroughly inspected for obvious abnormal changes in different internal organs. For histopathological analysis, representative tissue samples from organs exhibiting neoplastic lesions were gathered in 10% buffered formalin. After thorough washing with

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running tap water, the preserved tissues were dehydrated in graded ethyl alcohol, clarified in benzene, and embedded in paraffin wax, which has a melting point of 60 to 62 degrees Celsius. Standard haematoxylin and eosin staining method was applied to paraffin sections using Lilly Mayer's haematoxylin and 2 percent water-soluble eosin (Luna, 1968). On the basis of distinctive microscopic and gross features, tumors were identified and categorized. MDV was confirmed by employing Real Time PCR techniques using Meq specific primer, while ALV infection was confirmed by conventional PCR in tissue samples collected for molecular diagnosis. The following formula, with minor adjustments, was used to determine the percent mean histopathological lesion score in accordance with Witter's (1982) methodology.

$$\begin{aligned} \text{Percent mean histopathological lesion score (HLS) of organs} &= \frac{\text{Mean HLS of an organ}}{4 \text{ (Maximum HLS of an organ)}} \times 100 \\ \text{Overall percent mean HLS Irrespective of various organs} &= \frac{\text{Sum of percent mean HLS of organs}}{8 \text{ (Total number of organs)}} \times 100 \end{aligned}$$

RESULTS AND DISCUSSION

Total mortality observed in vaccinated chickens due to neoplastic conditions was 12.15%, out of which MD was confirmed in 9.7% cases and mixed infection of MD and LL was seen in 1.5% cases mostly affecting more than 12-24 wks age groups of layers, broiler and dual purpose breeds. Similar to our findings regarding incidence of neoplastic disease conditions, Crespo and Senties-Cue (2015) also reported that Marek's disease (17.7%) was the most common disease conditions affecting backyard poultry. In agreement to our findings, Sani et al. (2017) also found high prevalence of neoplastic diseases in poultry that was 7.58% with overall prevalence of Marek's disease (MD) as 6.25% while that of avian leucosis (AL) as 1.07%. However, due to immunizations failures and occasional losses, other studies found that Marek's disease posed a significant economic danger to the poultry industry, accounting for up to 60% of all commercial layer deaths and 10% of broiler condemnations. The outbreak of classical form of Marek's disease was reported in a chicken 8 farms (24.2%), by Bhattacharya and Rajkhowa (2022) with a morbidity rate ranging from 9.3 to 32% and a mortality rate ranging from 2 to 18% in chickens aged 16-18 weeks. It has long been known that chickens can develop genetic resistance to MD, and that the genetic makeup of the flock affects how an MDV infection turns out (Payne and Venugopal, 2000). Additionally, research shows that female are more likely than male to get tumor disorders, confirming that sex influences the outcome of infection

because female are often more prone to developing tumors (Payne and Venugopal, 2000). This might be the reason for significant number of cases observed in layers in the present study.

Grossly various visceral organs, including the liver, spleen, kidney, heart, proventriculus, ovary, and lungs, had tumour like nodules which ranged in size and colour as white to pale yellow. Almost in all the cases liver and spleen both were found to be affected showing hepatomegaly and splenomegaly along with the presence of tumorous foci. The liver showed signs of congestion, enlargement, and friability, with distinct grayish white tumorous nodules that were dispersed and varied in size (Fig.1) destroying the parenchyma. In mixed infection (MD+LL) cases liver was extensively enlarged extending beyond the keel bone with presence of tumour foci or nodules (Fig. 2). The spleen was typically three to four times larger than usual and had a widespread white or grayish staining. (Fig.3). Lungs also showed similar type of nodular lesions with tumorous masses. Ovaries were severely affected in few cases showing grayish to whitish multiple nodular growths and marked enlargement. Tumour nodule in intestine was extended upto serosal surface in some cases (Fig.4). Kidneys appeared severely enlarged with white to grayish tumorous foci. Proventricular glands revealed congestion with nodular enlargements. The nodular lesions appeared soft and pliable, even with gray or cream-white in colouration with or without areas of necrosis. However, brain, bursa of Fabricius and feather follicles did not reveal any enlargement or nodular growth.

Present study revealed that neoplastic lesions were observed most commonly in the liver and spleen followed by kidney, heart, intestine and ovary. Similar lesions without involvement of nerves were also documented by Fujimoto *et al.* (1971). Lymphomatous visceral tumours in MD have been reported by many authors (Kurade *et al.*, 2001; Narang *et al.*, 2003; Kamaldeep *et al.*, 2007). However, the examined birds did not revealed involvement of the organs such as bursa of Fabricius, skin, sciatic nerves or eyes.

Microscopically, MD affected liver sections showed varying degrees of pleomorphic neoplastic lymphoid cell growth and infiltration, including lymphoblasts, reticulum cells, and small, medium, and large lymphocytes, compressing and changing the organ's natural structure (Fig. 5). In addition to neoplastic changes variable degrees of degenerative and necrotic changes in hepatocytes with infiltration of heterophils was observed. In cases of severe infiltration, the normal architecture was destroyed and fibrosis was present. Hepatocytes revealed fatty vacuolar degeneration along with the presence of giant cells and megakaryocytes. In mixed infection (MD+LL) cases along with above lesions there was presence of uniform neoplastic lymphoblasts

cells altering the hepatic parenchyma (Fig. 6) at places. Organ-wise the overall mean percent lesion score in liver was 67.76 % with maximum in dual purpose chicken (70.75%) followed by layers (69%) and broilers (60%).

Histopathological examination of spleen sections revealed proliferation and infiltration of pleomorphic neoplastic lymphoid cells leading to loss of normal architecture of splenic tissue (Fig. 7) along with proliferation of reticuloendothelial cells and fibrous connective tissue. The overall mean percent lesion score in spleen was 73.68% with maximum in dual purpose breed (83.25%) followed by layers (72%) and broilers (71.25%). MD specific microscopic lesions as described above were also observed in the lungs with proliferation of pleomorphic lymphoid cells in parabronchi and atrial spaces (Fig. 8) obliterating the air spaces. The overall mean percent lesion score in lungs was 57.23% with maximum in broilers (64.25%) followed by dual purpose breed (62.5%) and layers (54%).

Heart sections revealed only mild lesions with presence of pleomorphic tumour cells and mild degeneration of myocardial fibers along with congestion and hemorrhage. No lesions were observed in heart in mixed infection cases. The overall mean percent lesion score in heart was only 15.78% with maximum in dual purpose chicken (20.75%) followed by layers (16%) and broilers (10%). In kidney degeneration of tubules along with the proliferative pleomorphic lymphoid infiltrations were observed in interstitial tissue along with congestion and hemorrhages. Hyaline degenerative changes in the glomerular capillaries and severe fibrosis were noticed in the interstitium in few cases. Mixed infection (MD+LL) cases revealed presence of uniform neoplastic lymphoblasts cells in the interstitium causing atrophy of tubules with presence of hyaline casts in lumen (Fig. 9). The overall mean percent lesion score in kidney was 45.39% with maximum in broilers (46.25%) followed by dual purpose breed (45.75%) and layers (45%).

Proventriculus revealed thickening of proventricular mucosa due to severe proliferation of pleomorphic lymphoid cells in mucosa, sub mucosa and glandular tissue with atrophy of glands along with congestion and hemorrhage. Lesions were not observed in mixed infection proventriculus. The overall mean percent lesion score in proventriculus was 35.52% with maximum in layers (38%) followed by broilers (32%) and dual-purpose breed (29%). Pleomorphic lymphoid cells infiltration was observed in intestinal mucosal and sub mucosal layers causing severe atrophy of mucosal glands. In a few cases tumour lesions was found extending up to serosal layer along with severe fibrosis (Fig. 10). Proliferation of uniformly shaped lymphoblasts cells was also noticed at places in mixed infections was noticed. The overall mean percent lesion score in intestine was 48.68% with maximum in dual-purpose breed (54%)

followed by layers (49%) and broilers (42.75%). The ovarian lesions that were found were diffuse proliferation of variable sized neoplastic lymphoid cells in the stroma causing atrophy of the follicles along with congestion and hemorrhage (Fig. 11). The overall mean percent lesion score in ovary was 31.57% with maximum in dual purpose breed (54%) followed by layers (32%) and broilers (10.75%).

Neoplastic lesions were not observed in any case in the brain, bursa of Fabricius, thymus and skin tissue/feather follicles. The cellular component of tumours is identical in all affected visceral organs and in the same time it was similar to the pathological picture reported in high virulence MDVs (Narang *et al.*, 2003; Kamaldeep *et al.*, 2007; Pejovic *et al.*, 2007; Tian *et al.*, 2011; Gong *et al.*, 2013; Jayalakshmi *et al.*, 2016). Reddy *et al.* (2022) studied the incidence of Marek's disease among the vaccinated chicken flocks and reported incidence of 10.24% of MD with histological lesions of infiltration of the pleomorphic lymphoid cells in the liver, heart, lung, kidney, ovary, spleen, thymus, pancreas, proventriculus, intestine, skin, skeletal muscle and sciatic nerve.

Overall mean percent histopathological lesion score

Histopathological organ lesion score in different organs affected with neoplastic disease conditions in chickens is described in Table 1 and Fig. 12. The overall mean percent histopathological lesion score is described in Fig. 13. Lesion score was found maximum in dual purpose chicken i.e. 52.5% followed by layers (46.87%) and broilers (42.15%). Mean histopathological lesion score in neoplastic disease affected organs revealed severe involvement of liver, spleen and lungs. Review of literature did not reveal any previous study depicting the lesion scoring in MD tumours. The results of present study clearly indicate the main target organs for lymphoma development were liver and spleen with more severity in dual purpose breed.

Histopathological findings in five cases suggested a mixed infection with Marek's disease and lymphoid leucosis. Multiple infections in the poultry with about 25% incidence have also been reported by earlier researchers (Davidson, 2009; Singh *et al.*, 2017; Gowthaman, *et al.*, 2023). Main viral infection associated in mixed infections with MD is Avian Leucosis Virus (ALV). Although ALV subgroup J (ALV-J) targets myeloid lineage cells and causes late-onset myelocytomatosis (Venugopal *et al.*, 2000), subgroups Ato E of this virus, which are members of the Alpharetrovirus genus, are typically linked to lymphoid leukosis, with formation of tumors mostly affecting the bursa and internal body organs (Fadly and Venugopal, 2008). Except for ALV-J, exogenous ALVs transform B-lymphocytes to produce B cell lymphomas which develops initially in the bursa of Fabricius and thereafter spreads to other visceral organs (Ewert and deBoer,

Table 1: Histopathological organ lesion score in Marek's disease affected chickens

Species	Histopathological organ lesion score								Overall mean
	Liver	Spleen	Heart	Proven- tricus	Kidney	Intestine	Ovary	Lungs	Percent histopath- ological score
Broiler									
Mean HP score	2.4	2.85	0.4	1.28	1.85	1.71	0.43	2.57	42.15
Mean % HP score	60.00	71.25	10.00	32.00	46.25	42.75	10.75	64.25	
Layer									
Mean HP score	2.76	2.88	0.64	1.52	1.8	1.96	1.28	2.16	46.87
Mean % HP score	69.00	72.00	16	38.00	45.00	49.00	32.00	54.00	
Dual purpose									
Mean HP score	2.83	3.33	0.83	1.16	1.83	2.16	2.16	2.5	52.5
Mean % HP score	70.75	83.25	20.75	29.00	45.75	54.00	54.00	62.5	

**Fig.1:** Enlarged liver with raised grayish white tumorous nodules (Marek's disease)**Fig.2:** Enlarged liver extending up to keel bone along with the presence of diffuse white tumorous patches (Marek's disease and Lymphoid leukosis mixed infection)

1988).

Another oncogenic virus Reticuloendothelial virus (REV) has also been reported in poultry which can lead to transformation of immature B and T lymphocytes, causing lymphomas of B and T cell origin affecting both

**Fig.3:** Nodular tumorous enlargement of spleen with presence of diffuse grayish white tumorous foci (Marek's disease)**Fig.4:** Intestine showing multiple tumorous nodules. Inset showing cut surface of intestine depicting tumorous nodule on mucosal surface (Marek's disease)

chickens and turkeys (Fadly *et al.*, 2008). The syndromes caused by REV, of Gammaretrovirus genus, are distinct from those caused by the ALVs (Davidson, 2009). In the present study although molecular diagnosis for REV was not attempted, histopathologically there was no evidence

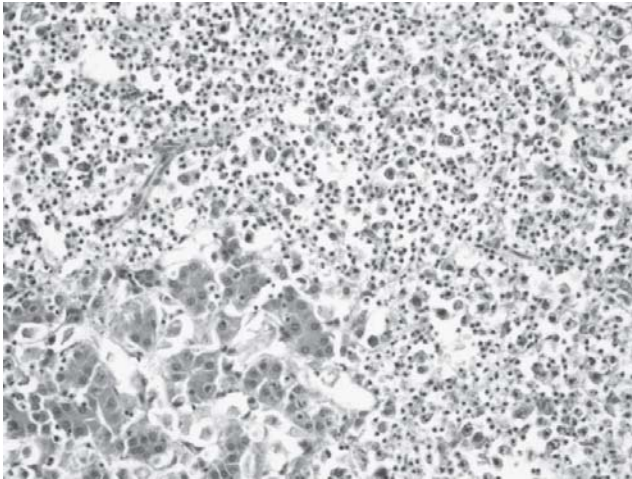


Fig.5: Liver showing neoplastic pleomorphic lymphocytes, lymphoblasts in in parenchyma (Marek's disease, H& E stain; 400X)

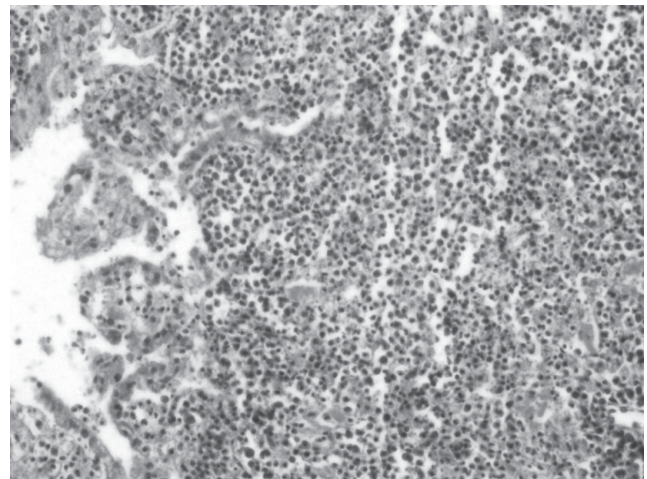


Fig.8: Lung showing diffuse presence of pleomorphic neoplastic lymphoid cells (arrow) in the parenchyma (Marek's disease, H& E stain; 200X)

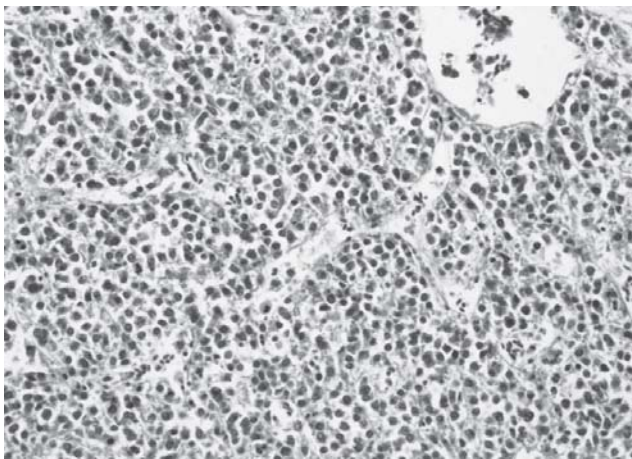


Fig.6: Liver showing presence of neoplastic lymphoblasts destroying the parenchyma (Marek's disease + Lymphoid leucosis, H& E stain; 400X)

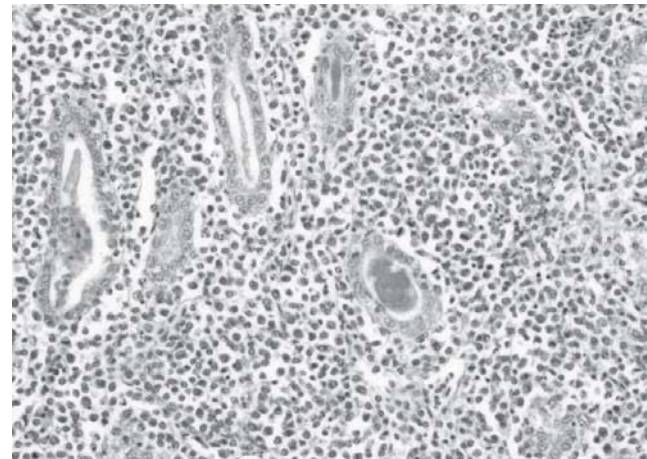


Fig. 9: Kidney showing the diffuse presence of neoplastic lymphocytes and lymphoblast cells in interstitium along with the presence of hyaline cast inside of tubules. (Marek's disease + Lymphoid leucosis, H& E stain; 200X)

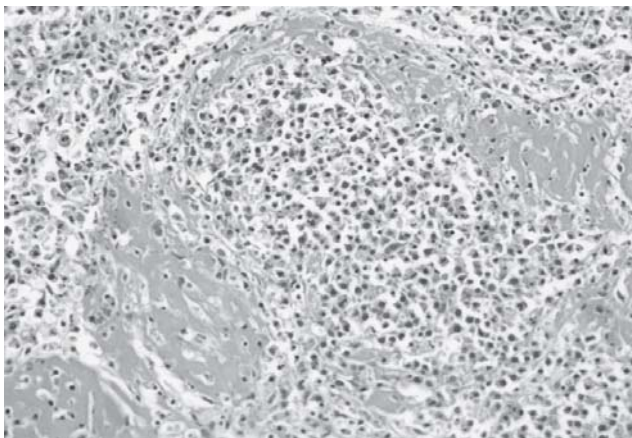


Fig.7: Spleen showing diffuse presence of pleomorphic neoplastic lymphocytes destroying the splenic architecture (Marek's disease, H& E stain; 400X)

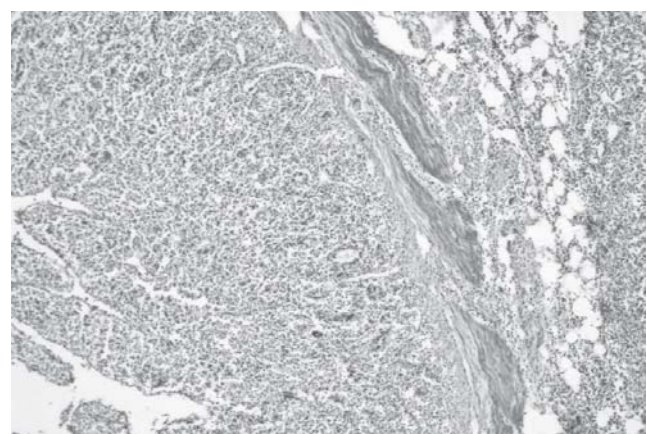


Fig. 10: Thickened intestine with diffuse presence of pleomorphic lymphoid cells in mucosa along with tumorous infiltrates in serosal layer (Marek's disease, H& E stain; 100X)

of reticular cells infiltration and proliferation in any case in visceral organs. Literature also reports that in domestic poultry and other avian species, REV can cause immunosuppression, runting disease syndrome, acute reticular cell neoplasia, and chronic neoplasia of

lymphoid tissues and other organs (Crespo *et al.*, 2002). Schat and Nair in 2008 reported that the most prevalent lymphoid proliferative disease affecting hens is Marek's disease, which alters T-lymphocytes and causes tumor

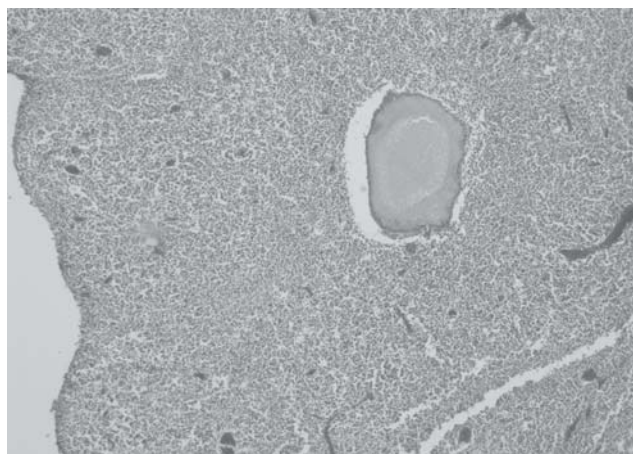


Fig. 11: Ovary tissue showing pleomorphic lymphoid cell destroying ovarian stroma along with congested blood vessels (Marek's disease, H&E stain; 100X)

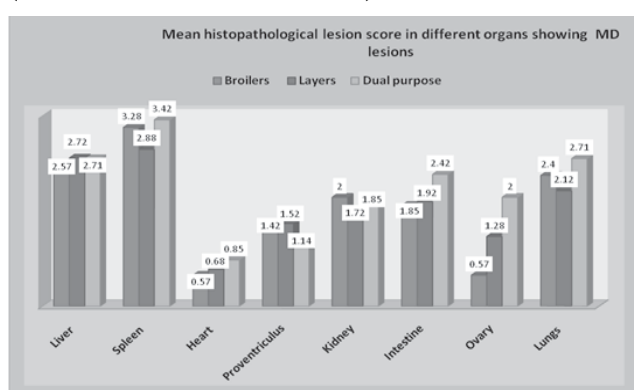


Fig. 12: Mean histopathological organ lesion score in Marek's disease affected chickens

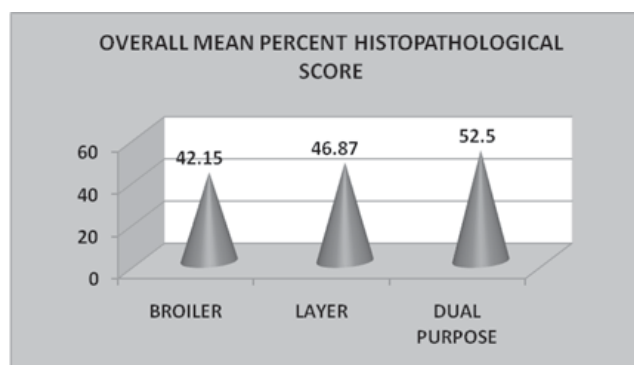


Fig. 13: Overall mean percent histopathological lesion score in Marek's disease affected chicken

development and immunosuppression. It is believed that the integration of the viral genome into the host's chromosomal DNA is linked to the transformation of afflicted cells that causes lymphomas in MD. There have been several reports of MD cases in other nations, including China, in recent years (Tian *et al.*, 2011), Egypt (Hassanin *et al.*, 2013), India (Raja *et al.*, 2008) and Japan (Murata *et al.*, 2013). It is also observed that despite being properly vaccinated with CVI988 or (CVI988 + HVT) at one day of age and subjected to strict management in the poultry flocks, chickens still exhibit symptoms of MD. Emergence of hyper virulent strains (Schumacher *et al.*,

2002; Burgess *et al.*, 2004; Gong *et al.*, 2013), and amplification in the virulence of field viruses (Witter, 1997; Gong *et al.*, 2013; Hassanin *et al.*, 2013) are suggested to be the reason for vaccine failure and change in pathology of MD (Witter, 1997; Kamal deep *et al.*, 2007; Gong *et al.*, 2013). Therefore the current investigation was conducted to obtain information on the tumour disease problems in poultry flocks in Haryana which may be caused by viral agents through the pathological alteration of this reemerging disease and characterize the field isolates on the basis of histopathological diagnosis and to identify the causes of the tumour disease in chickens and the causes of this re-emergence.

It may be concluded from the present work that MDV infection is still a problem in layer and broiler breeder flocks in Haryana even in vaccinated flocks. Layer flocks were the primary target of Marek's disease cases, which primarily affected the liver, spleen, and lungs and compared to the conventional or neurological forms of Marek's disease, the visceral type was far more common.

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