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Pathological Studies on Acute Marek's Disease in Native and Crossbred Chickens of Organized Poultry Farm

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in female than male birds.

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Introduction

Marek's Disease (MD) is a lymphoproliferative and neuropathic disease of domestic chickens and less commonly of turkeys and quails, caused by a highly contagious, cell-associated, oncogenic herpes virus (Schat and Nair, 2008). Marek's Disease Virus (MDV) strains that induce disease in chicken are classified as serotype-1 under *alpha-herpesvirus*. The disease is usually characterized by mononuclear cellular infiltration in peripheral nerves and various other organs and tissues including iris and skin. Marek's disease virus spreads rapidly throughout flocks by direct and indirect contact with infected chicken, premises, litter, dust and chopped feathers. Most important is the airborne route of infection (Josipovic, 1990). This study was aimed to describe the epidemiology of Marek's disease based on gross and microscopic lesions, i.e. visceral lymphoma, in seven different native and crossbred chicken strains maintained at University farm in Anand and received for post-mortem in Pathology department over a period of one year.

Materials and Methods

The present study was carried out for a period of one year, i.e. from February 2017 to

Indian J. Vet Sci. Biotech (2018) Vol. 14 No. 2

Abstract The present study was aimed to detect the occurrence of Marek's

disease in a native and cross-bred chickens based on gross and

microscopic lesions. Seven different native as well as crossbred

strains of poultry flocks were evaluated the mortality pattern

based on farm as well as autopsy records and for the gross and

microscopic examination of the birds suspected for Marek's disease. Visceral lymphomas like lesions were recorded in liver,

spleen, proventriculus, heart, kidney and gonads. Fragments of

the affected organs were collected and placed in 10% formalin

for routine histopathology. Under microscopic examination,

pleomorphic population of lymphocytes and lymphoblasts were observed. The incidence of MD observed was from 12 to 61

weeks of age with peak mortality from 12 to 31 weeks of age

during summer in different strains. The strain wise mortality due

to MD ranged between 13.42 (random bred controls) to 36.57%

(IWN×Native, F1) with an average of 26.80 %, and it was higher

January 2018, on seven different native as well as crossbred strains, viz., Anand Specific White Leghorn (ASWLH), Bantomized White Leghorn (BWLH), Rhode Island Red (RIR), Native, RC (Random breed Control), F1 (IWN×Native) (First generation cross), TC (F1×RIR) (Terminal Cross) and Indian White Native (IWN) of poultry flocks maintained at Poultry Research Station, AAU, Anand and received for post-mortem diagnosis and suspected for Marek's disease based on gross pathological lesions, i.e. visceral lymphoma. The study comprised the epidemiological information in relation to mortality due to MD with respect to strain, age, sex, season and month wise susceptibility, gross and histopathological examination of different organs.

Epidemiological Study

The information pertaining to the strength of different native and crossbred strains, and age and sex wise as well as month wise mortality due to Marek's disease was collected based on records available at Poultry Research Station of the College. The necropsy incidence of Marek's disease from February-2017 to January-2018 was worked out based on necropsy records available at Department of Pathology.

Gross Lesions and Histopathology

Regular autopsies were conducted on the birds of different strains. Detailed post-mortem examination was carried out and gross pathological lesions observed were recorded giving special attention to visceral lymphoma. During autopsy, the tissue pieces of organs showing gross lesions of Marek's disease like liver, spleen, heart, proventriculus, kidney, gonads and sciatic nerve were collected for histopathological examination. Tissues were preserved in 10% formalin for further processing. The trimmed tissues were processed in automatic tissue processor and sections of 5 to 6 micron thickness were cut with automatic section cutting machine (Leica, Germany). The sections were stained with haematoxylin and eosin stain and histopathological lesions were observed using light microscope.

Results and Discussion

Epidemiological Studies

The present study recorded incidence of MD

between 12 to 61 weeks of age with peak mortality between 12 to 31 weeks of age during summer months of March to June 2017 in different strains.

The strain wise mortality due to MD ranged between 13.42 to 36.57 % with an average of 26.80 %. Out of total 4,498 birds of different strains, 1203 birds were died due to Marek's disease. Among the total population of different strains, the highest mortality was observed in IWN×Native (F1) (36.57%) followed by Native (32.09%), BWLH (30.08%), F1×RIR (TC) (25.29%), ASWLH (25.18%), RIR (23.64%) and RC (13.42%) [Fig. 1]. Among the birds died due to MD, the mortality pattern was of the order of Native (24.94%) followed by BWLH (18.20%), RIR (18.04%), ASWLH and F1 (14.38%), RC (6.40%), and TC (3.66%).

The age wise mortality pattern revealed that the mortality due to MD started from 12 weeks onwards and lasted till 61 weeks of age. The majority of birds (94.74%) belonged to all the strains died between the ages of 12 to 31 weeks of age, and overall age wise mortality ranged between 0.16 to 51.45 % among different age groups of all strains. The highest mortality due to MD was observed in the age group of 17-21 weeks (51.45%) followed by 22-26 weeks (21.94%), 12-16 weeks (15.54%), 27-31 weeks (5.81%), 32-36 weeks (2.41%), 42-46 weeks (1.25%), 37-41 weeks (0.74%), 47-56 weeks (0.33%) and 58-62 weeks (0.16%) of age [Fig. 2].

The month wise mortality pattern showed that majority of birds (95.50%) died during months of February to June 2017. The incidence decreased from July onwards till January in all the strains and amounted to 4.5% only. The highest mortality due to MD was observed during March (39.23%) followed by April (32.75%), May (10.88%), February (8.81%), June (3.82%), July (1.99%), September (1.16%), November (0.50%), August, October (0.33% each) and December and January (0.08% each). The month wise mortality incidence reflected the season wise incidence as 86.69 % during summer months, 9.47 % during winter months and 3.82 % during monsoon months. The incidence was high during hot months of summer during March to June.

Where the population of both male and female birds was reared together, the sex wise mortality in male and female birds was 22.97 and 26.23 % in ASWLH, 24.00 and 32.38% in BWLH and 15.58 and 17.50% in native strain, respectively.

The mortality due to MD has been reported in birds above 12 weeks of age or during peak production among layer flocks (Zhuang *et al.*, 2015; Balasubramaniam *et al.*, 2017). Present study also recorded incidence of MD between 12 to 61 weeks of age with peak mortality between 12 to 31 weeks of age during summer months (March to June) in all the strains. The significance of higher mortality during summer months in the present study could not be explained as the age of the flocks above 12 weeks coincided with months of February onwards till June.

Clinical Signs

Chickens with MD lymphoma initially exhibited non-specific clinical signs such as emaciation and anorexia almost in all the affected flocks which progressed to depression, weight loss, diarrhoea and paleness of comb and wattles. At later stage, there was marked reduction in egg production and skin around feather follicles was raised and roughened with enlargement of feather tips. The most severely affected birds died due to starvation and dehydration, with presence of visceral lymphomas in various organs including liver, spleen, proventriculus, heart, kidneys, ovary and testes.

Gross Pathological findings

The gross pathological lesions were predominantly found in various visceral organs. The gross lesions in liver and spleen varied from few large size greyish white nodules to many multiple small size nodules with little or no normal tissue visible [Fig.3, 4, 5]. The lesions in the heart were nodular growths with diffuse enlargement and single small greyish nodules commonly noticed on the ventricular muscles leading to distortion of the shape. Proventriculus revealed diffuse thickening of the wall with white nodular areas through the serosal surface and focal ulceration and inter-papillary haemorrhages on the mucosal surface [Fig. 6]. In kidneys bilateral enlargement due to lymphoma formation with nodular growths of greyish white colour involving few lobes of the kidney was observed. The gross

lesions in the ovary were whitish nodular growths and typical cauliflower-like appearance due to tumorous masses. The testes showed marked asymmetry following unilateral or bilateral enlargement.

Gross lesions in visceral organs suggestive of MD, i.e. presence of greyish white tumorous nodules or diffuse enlargement of the organs like liver, spleen, kidneys and gonads (ovary and testes) were reported from time to time by various workers (Kamaldeep et al., 2007; Abd-Ellatieff et al., 2018). Significant gross lesions of numerous lymphomas of various size and greyishwhite in colour on the surface of the visceral organs and thickening of proventricular wall suggestive of Marek's disease were observed in number of birds affected with MD. There were no gross lesions in peripheral nerves especially in brachial and sciatic nerves in any of the strains like enlargement and oedema with loss of striations.

Histopathological findings

Microscopic changes were most consistently observed in tissue sections of liver, spleen and proventriculus representative of all the strains. The lesions in the hepatic parenchyma and spleen were characterized by proliferation of pleomorphic lymphoid cells, i.e. lymphoblasts and small to large lymphocytes with perivascular lymphoid proliferation in some of the sections [Fig. 7, 8]. In proventriculus, there was extensive infiltration of lymphoid cells which has replaced and distorted the mucosal and glandular architecture [Fig. 9]. Kidney sections showed extensive infiltration and proliferation of pleomorphic lymphocytes in the interstitial connective tissue accompanied by partial to complete obliteration of renal tubules of cortex and medulla. In ovary there was infiltration and proliferation of pleomorphic lymphoid cells with focal infiltration of lymphoid cells and plasma cells in the stromal tissue, whereas in testes pleomorphic lymphoid cells proliferation was observed. In the heart lesions were characterized by focal aggregation of pleomorphic lymphoid cells among the muscle fibres resulting in separation of muscle fibres noticed in the interstitial spaces [Fig. 10].

Focal to diffuse infiltration of pleomorphic

Indian J. Vet Sci. Biotech (2018) Vol. 14 No. 2



Fig.1. Graph showing strain wise incidence



Fig.2.Graph showing age wise incidence of MD in different pure as well as cross-bred strains



Fig.3.Liver showing diffuse enlargement with large sized greyish-white masses on its surface



Fig.4. Spleen showing diffuse enlargement with greyish-white nodular growth



Fig.5. Mottling of spleen with diffuse enlargement



Fig.6.Proventriculus: focal ulceration, small to medium sized nodular growth and inter papillary haemorrhages

Indian J. Vet Sci. Biotech (2018) Vol. 14 No. 2



Fig.7. Liver section showing extensive pleomorphic perivascular lymphocytic proliferation replacing the normal parenchyma (H & $E \times 120$)



Fig.9. Proventriculus section showing diffuse infiltration of pleomorphic lymphoid cells in glandular parenchyma (H & E × 480)

cells in liver, spleen, heart and lungs leading to disruption of the architecture of these organs and infiltration of pleomorphic cells in mucosa, submucosa, muscularis and serosal layers of proventriculus, intestines and inter tubular spaces of renal parenchyma are the characteristic microscopic lesions commonly observed in Marek's disease (Sharma et al., 2017). During the present study most of the gross and microscopic lesions of lymphoma were observed in visceral organs suggestive of acute visceral form of marek's disease in native and crossbred strains reared in confinement. As the gross and microscopic lesions suggestive of MD were not evident in sciatic nerve and brain, the present outbreak of Marek's disease was acute form of Marek's disease without involvement of classical or neural form.



Fig.8. Liver section showing neoplastic lymphoid cell proliferation in the hepatic parenchyma (H & E × 1000)



Fig.10. Heart showing pleomorphic lymphoid cells proliferation in the pericardium (H & $E \times 480$)

Conclusion

The epidemio-pathological study of Marek's disease occurrence in seven native and crossbred strains of chicken revealed varying pattern of mortality based on farm as well as autopsy records with clear visceral lymphomas like lesions in vital organs like liver, spleen, proventriculus, heart, kidney and gonads. The incidence of MD varied between 12 to 61 weeks of age with peak mortality between 12 to 31 weeks of age during summer months. Highest mortality was seen in March (39.23%), in age group of 17-21 weeks (51.45%), in IWN×Native, (F1) strain (36.57 %), and in female birds.

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Indian J. Vet Sci. Biotech (2018) Vol. 14 No. 2

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Conflict of Interest

Authors have no conflict of interest.

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