



## Study on subclinical and clinical Marek's disease (MD) in the broiler chickens using histopathology

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### ABSTRACT

Marek's disease (MD) is the most common lymphoproliferative diseases of chickens; During the last year the incidence of MD was investigated among slaughter houses and suspected broiler farms, located in Isfahan (Iran) as the region of highest density of poultry husbandry by histopathology, For subclinical MD investigation, in total 1550 chickens originated from slaughter houses were examined, Parallely for clinical MD investigation, 100 chickens from 10 broiler farms were examined. Based on the obtained results, about 6.81% of the liver samples (with average age of 60 days) collected from slaughter houses showed pleomorphic lymphoid cell proliferation and infiltration of mononuclear cells as subclinical MD. The microscopic results of examined broilers (average age of 57 days) with clinical signs of MD, showed pleomorphic lymphoid cell proliferation in sciatic nerves (62%), spleens (66%) and livers (71%). Now in Iran, the outbreaks of subclinical and clinical MD in broiler chickens increased but MD vaccination is not permitted so regarding to the results it will be to an obligation for hatchery MD vaccination, and/or decreasing the slaughtering age besides a good bio security of broilers farms.

**Key words:** MD, Histopathology, Clinical, Subclinical, Broiler

### INTRODUCTION

Marek's disease is one of the most common lymphoproliferative diseases of chickens which causes mononuclear infiltration to one or more of the peripheral nerves, gonads, iris, muscle, viscera, and skin (Bacon et al. 2001). MD is caused by herpes virus with three serotypes of MDV which have many common antigens and are distinguished by serologic tests (Carter et al., 2006). The first phase of MD infection is degenerative changes caused by early productive-restrictive virus infection, then latent infection, third phase is cytolytic infection associated with permanent immunosuppression, and final phase is nonproductive infected lymphoid cells, MD commonly affects pullets between 12-24 weeks of age, but can infect broilers as early as 6 weeks of age. The incubation period ranges from 3-4 weeks to several months (Okazaki et al., 1980; Otaki et al., 1988). Asymmetric progressive included paralysis of one or more of the extremities, Wing and limb drooping, and torticollis, Vagal involvement will lead to dilatation of the crop and/or gasping and sometimes "grey eye" due to iris involvement. Many birds die suddenly without symptoms. There are widespread nonspecific signs such as weight loss, paleness, anorexia, and diarrhea, (Spencer, 1984). Macroscopic lesions is visible in nerves as grayish, edematous,

two or three times the normal thickness, and loss of the normal striated white glistening appearance commonly in the brachial and sciatic plexus, celiac plexus, abdominal vagus and intercostals nerves. Nerve enlargement may not always be seen in affected birds at necropsy, although characteristic lesions may be found histologically. Also, appearance in affected viscera, are indistinguishable which are enlarged tumors with diffuse grayish discoloration with the exception of the bursa of Fabricius (Fadly and Ewer, 2008). Microscopic lesions show two main types of lesions in peripheral nerves. Type A is interpreted as neoplastic in character, consisting of masses of proliferating lymphoblastic cells. Sometimes, demyelination and proliferation of Schwann cells are seen with these lesions, (Spencer et al., 2000). Type B is inflammatory in nature and is characterized by diffuse infiltration of lymphocytes and plasma cells, edema, and sometimes demyelination and Schwann cell proliferation. Lymphomatous lesions in visceral organs are more uniformly proliferative in nature. Deposition and diffuse proliferation of small to medium lymphocytes, lymphoblasts, and primitive reticulum cells are seen (Witter and Schat, 2003). A step-wise process has been proposed for diagnosis of Marek's disease which firstly includes history, epidemiology, then clinical observations and gross necropsy, characteristics of the tumor cell, and virological characteristics (Calnek et al., 1998; Marsh et al., 1995). The aim of this study was investigation the MD outbreaks during the last year among slaughter houses and suspected broiler farms, located in Isfahan (Iran), for improving prevention strategy in broilers.

### MATERIAL AND METHODS

Isfahan province as the region of highest density of poultry husbandry in Iran and the most broiler meat production (200,000 tones / year) in Iran, used for clinical and subclinical MD investigation during the last year. In subclinical MD investigation, randomly and totally 1550 chicken livers ( $n = t^2pq/d^2$ ,  $p = 1\%$ ,  $q = 99\%$ ,  $d = 5\%$ ), originated from Isfahan slaughter houses were sampled for histopathology examination, these samples were labeled and the owner name, farm address, age of the birds and probable clinical sign of the related farms recorded in the questioner forms. Parallely in clinically MD suspected farms with some MD signs such as drooping of the limb, anorexia, torticollis, weight loss, paleness and diarrhea, totally 100 alive chickens related to 10 broiler farms were selected for histopathology sampling, the total specimens, were 100 sciatic plexuses, 100 livers and 100 spleens, which labeled and farm address, age of birds, probable clinical signs and name of owner recorded in the questioner forms. Samples washed 3 times in 10% formalin and finally floated in 10% formalin in 300 ml screwed tubes and transport to histopathology lab. The specimens has been processed and histological sections have been placed onto glass slides for hematoxylin and eosin (H&E) staining, the prepared slides were examined by microscope ( $>X40$ ) and describing the histological findings as pathology reports (Thomas et al., 1997).

### RESULTS AND DISCUSSION

Based on the simple statistic analysis of the data of questioner forms, the average age of the birds used for random sampling in Isfahan slaughter houses were about 60 days, these birds peaked up from 32 different broiler farms ready for slaughtering. In histopathology examination of the liver samples for subclinical form of MD, about 6.81% of the examined slides revealed pleomorphic lymphoid cell proliferation, deposition and diffuse proliferation of small to medium lymphocytes, lymphoblasts, and primitive reticulum cells in the various slides. (Figures. 1, 2, 3 and 4).

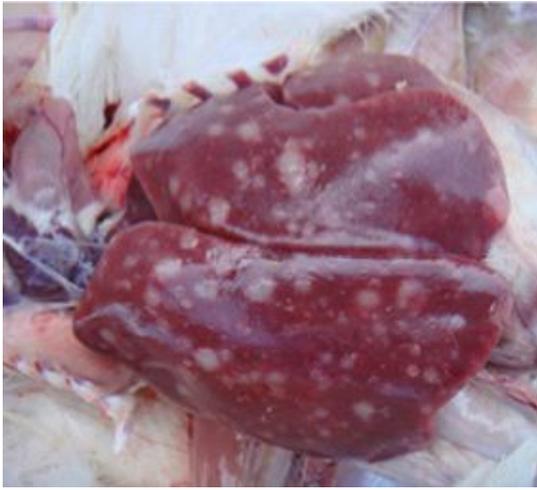


Figure.1-The tumoral gross lesions of MD in Broiler liver

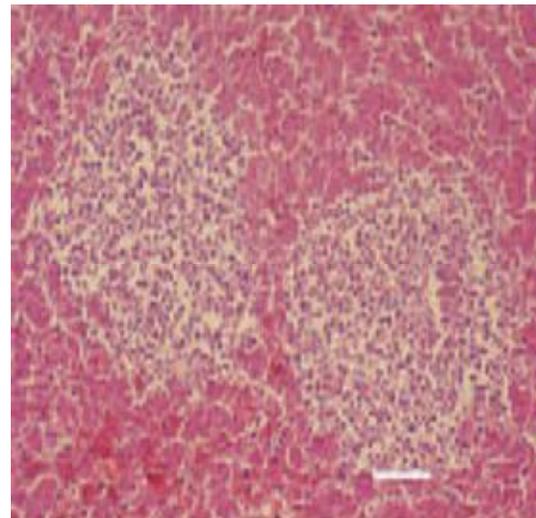
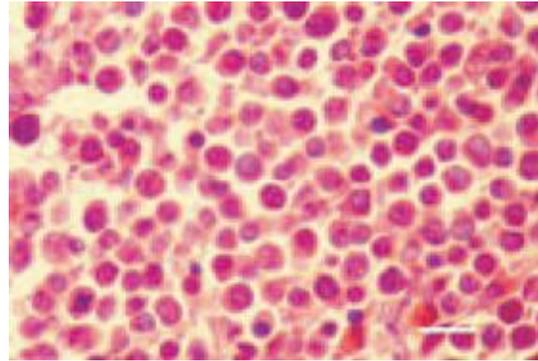
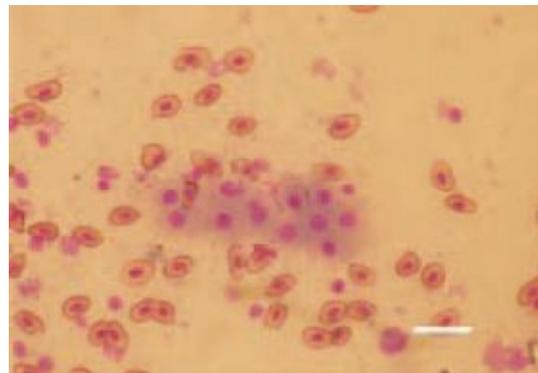


Fig. 2- Symmetrical neoplastic foci of pleomorphic cells, liver H/E, Bar = 50  $\mu$ m.



**Fig. 3-** Acute MD (visceral) form, liver, Lymphomatous lesions, mainly consisting of lymphoblasts, and small to medium-sized lymphocytes. Among the proliferate, single plasmatic and reticular cells are outlined. H/E, Bar = 10  $\mu$ m.



**Fig. 4-** Marek's disease, Broiler , touch imprint preparation of a liver cross section. Lymphoid cells of a various size: lymphoblasts and lymphocytes. Diff Quik, Bar = 10  $\mu$ m.

The investigation of clinical form of MD in the broilers averagely aged 57 days, using histopathology examination showed pleomorphic lymphoid cell proliferation in 62% of sciatic nerves, 66% of spleens and 71% of the livers .In microscopic study on the sciatic nerve the type A lesion were more than the type B. The cellular composition of tumors generally was similar between organs, but sometimes though the gross pattern of involvement may vary. (Carter et al.,2006 ,Okazaki et al.,1980). The rate of invasive proliferative lymphocytes (small to medium and mature cells, to sciatic nerves were less than livers and spleens , but the most clinical features of the MD in the flocks were peripheral dysfunction such as the limb and wing drooping, anorexia, torticollis (Witter et al.,1980 ;Bacon et al.,2001), it shows that nervous signs are more exhibit than the other signs in the MD, and few are specific to MD. (Figures. 5 and 6).



Fig.5-Neoblastic spleen in the broiler with clinical MD



Fig.6- Sciatic nerves inflammation in a broiler with clinical MD signs

MD exists in poultry-producing countries throughout the world and probably every flock of chickens could become infected. The incidence of infection is surely much higher than the incidence of disease. Even in susceptible chickens, infection does not always induce clinical disease and in genetically resistant or vaccinated chickens infection may rarely cause overt disease. (Witter et al., 1984; Witter and Schat, 2003). The subclinical MD investigated in current study with lymphomatous lesions in the livers and clinical form of MD with involvement of sciatic nerves, spleens and livers showed an unexpected frequency of MD in the broiler farms which is not desired due to its immunosuppressive effects in alive young birds and carcass condemnation of slaughtered broilers (up to 10%), meanwhile the vaccination failure and outbreaks of different infectious diseases would be resulted. (Soffer et al., 1990). MD is an age-dependent viral disease and its causative agent (MDV) is resistant to most of disinfectants and infects day-old chickens easily, so poor biosecurity and increasing the age of broilers, resulted in more subclinical and clinical MD outbreaks in current study. Now in Iran, MD vaccination is not permitted for broiler chickens so regarding to the results it will be an obligation for hatchery MD vaccination, and/or decreasing the slaughtering age besides a good biosecurity of broiler farms for improving the MD outbreaks, better immune response and decreasing slaughter condemnations.

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