

Full Article

Investigation on Incidence of Marek's Disease in Broiler Flocks of some Regions in Tehran Province, Iran

Hablolvarid^{*}, M.H.

Department of Pathology, Razi vaccine and serum research institute, Karaj, Iran

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ABSTRACT

Marek's disease (MD) is a common lymphoproliferative disease of chickens, usually characterized by mononuclear cellular infiltration in different organs. The disease is caused by a herpes virus and is transmissible. MD has been a common, important problem for poultry industry worldwide, as well as, in our country, Iran. The aim of present study was to have an estimation of the incidence of MD in broiler flocks of some major regions of chicken rearing in Tehran province (Savojbolagh, Karaj, Shahriar and Varamin). This was implemented by 35 times visiting of some poultry slaughterhouses and thoroughly inspection of chicken's carcasses and histopathological examination of various tissues and organs of suspected and normal slaughtered chickens from 80 broiler flocks, that was reared in Tehran province. Gross and microscopic examinations of chickens, in four mentioned regions, showed that 24 out of 80 flocks (30%) had been infected to different forms of the disease. This result indicated that MD has a high incidence in broiler flocks of Tehran province. The incidence of cutaneous, visceral and mixed cutaneous and visceral forms in these regions (four regions) was determined as 16.2%, 3.8% and 10%, respectively. Moreover, no case of nervous and ocular forms was seen in this study. The result of the current study gives a hint for the importance and losses behind the high incidence of the MD in broiler flocks of Tehran province, Iran. Further detailed study on MD in broiler flocks and on the effectiveness of available MD vaccines in reducing the incidence and losses of the disease is recommended.

Keywords: Marek's disease, Incidence, Chicken, Histopathology, Iran

INTRODUCTION*

MD is an important and worldwide lymphoproliferative disease of chickens caused by Marek's disease virus (MDV), an alphaherpesvirus. The disease generally characterized by mononuclear cellular

infiltrates in peripheral nerves and various other organs and tissues together with iris and skin. Before the use of vaccines, MD was a serious economic threat to the poultry industry causing up to 60% mortality in layer flocks and 10% condemnation in broiler (Witter & Schat 2003, Witter *et al* 2005). But, despite widespread use of vaccination programs, MD continues to be a serious threat to poultry production. Purchase (1985) estimated that mortality and condemnation losses due

* Author for correspondence. Email: hablolvarid@yahoo.com

to MD totaled about \$12 million in the United States in 1984 when added with economic loss from the cost of vaccine and application and reduced egg production, however, the total was about \$169 million in the United States and \$943 million worldwide. These data, clearly, shows the economic significance of the MD, as well as, the needs for more investigation on different aspect of that, in order to reduce associated losses. Presently, nearly all commercial egg-type chickens are vaccinated against MD, and this has reduced losses to less than 5% in most countries. Broiler flocks, which are vaccinated in some but not all countries, may experience mortality of 0.1-0.5% and condemnations of 0.2% or more (Purches 1985, Cho *et al* 1997, Christensen 1988). However, increasing the virulence of the MDV can be resulted in increasing of the disease (Sung 2002). Some authors do not recommend vaccination for broiler chickens in the first days of life (Biggs *et al* 1965) because the disease would not appear until the age of 42-45 days, when they are slaughtered. However, the belief of other authors is that only the morpho-clinical manifestations are not obvious to remark until that age, but the microscopic lesions are present and they have negative effects on growth and development of broiler chickens (Duguma *et al* 2005, Fodor *et al* 2009, Witter and Schat 2003). The aim of present study was to have an estimation of incidence of the disease, among the broiler flocks of some regions in Tehran province, based on thoroughly inspection of chicken's carcasses in slaughterhouses and histopathological examination of tissues and organs of suspected and grossly normal, randomly sampled, chickens.

MATERIALS AND METHODS

This study conducted as a research project, supported by Razi vaccine and serum research institute, from Oct 2005 to Nov 2007.

Geographic area of the study and statistical method.

In this study the Tehran province was regarded as ten geographic regions; Damavand, Firouz-kouh, Shemiranat, Tehran, Shahriar, Varamin, Karaj,

Eslamshahr, Shahriar and Savojbolagh. Then, based on the number of the broiler flocks, in each region, and 30% estimation of prevalence of the disease, $P=0.3$, the number of flocks witch must be sampled was determined (Quota sampling). For example in Karaj and Savojbolagh regions chickens from 25 and 16 flocks must be thoroughly inspected, in pre and post mortem examination of carcasses, and sampled. Every flock with, even, a positive MD chicken regarded as a positive MD flock.

Pre-slaughter examination of flocks. Pre-slaughter examination of the chickens, presented to different poultry slaughterhouses, was performed by close observation of the chickens. All the data related to each flock like as: the name of farm owner, place of rearing, number of the halls and population of flocks, age and average weight of chickens at slaughter, appearance and general healthy condition as well as any abnormal sign/disease of chickens was written and kept in an answer sheet, which was prepared for this purpose.

Post-slaughter examination and sampling: All the chicken's carcasses in the slaughter line had been inspected thoroughly and any abnormal condition was recorded, meantime, if there were any suspected chickens to MD samples of different organs was collected. However, in completely normal flocks, there were no abnormal grossly signs of disease, samples of different organs consisted of mainly skin, liver, spleen, sciatic nerve, proventriculus and bursa of fabricius were collected. This was performed by random collection of the mentioned organs on the beginning, middle and end of slaughter line.

Histopathological examination: Samples of different organs were kept in 10% formalin until fixation. Then tissues were routinely processed to paraffin blocks, sectioned at $5\mu\text{m}$, deparaffinized, stained with H&E and finally examined by a light microscope.

RESULTS

In the present study by 35 periods of referring to different poultry slaughterhouses and settling at

slaughter line, totally, samples from 80 broiler flocks, from Savojbolgh, Karaj, Shahriar and Varamin regions of Tehran province were collected and histopathologic examination was performed. The age of chickens in all the studied flocks was equal/less than 8 weeks. The results of this study showed that in 24 out of 80 flocks (30%) Marek's disease was present. Accordingly, the rate of the disease in Savojbolgh, Karaj, Shahriar and Varamin regions was determined as; 38.2%, 16.1%, 22.2% and 66.7% respectively (Table 1). No cases of ocular and nervous forms were found in the current study. The rate of cutaneous, visceral and mixed cutaneous and visceral forms, in four mentioned regions, was determined as; 16.2%, 3.8% and 10% respectively (Table. 2).

Table 1. Number and percentage of MD infected flocks in different regions of Tehran province.

Regions	Number of examined flocks *	Number and percentage of MD infected flocks	
		No	%
Savojbolagh	34/16	13	38.2
Karaj	31/25	5	16.1
Shahriar	9/16	2	22.2
Varamin	6/24	4	66.7
Total	80/100	24	30

*Number of the examined flocks to the total number of that was determined in statistical method.

The highest and lowest rate of cutaneous form was determined in Varamin and Karaj regions with 66.7% and 6.5%, correspondingly (Figures 1, 2). The highest and lowest rate of visceral form was determined in Savojbolagh and Karaj regions with 5.9% and 3.2%, respectively (Figures 3, 4 and 5). No cases of this form were found in Shahriar and Varamin areas. However, the highest and lowest rate of mixed cutaneous and visceral forms was seen in Savojbolagh and Karaj regions with 17.6% and 6.5%, respectively. Like as the visceral form, no cases of mixed form were found in Shahriar and Varamin regions.

Table 2. Number and percentage of various forms of MD in four region of Tehran province

Regions	Total number of investigated flocks	Number and percentage of various forms of MD					
		Skin form		Visceral form		Mixed visceral and skin forms	
		No	%	No	%	No	%
Savojbolagh	34	5	14.7	2	5.9	6	17.6
Karaj	31	2	6.5	1	3.2	2	6.5
Shahriar	9	2	22.2	0	0	0	0
Varamin	6	4	66.7	0	0	0	0
Total	80	13	16.2	3	3.8	8	10

Gross lesions in visceral organs and skin were mostly of nodular and a little diffuse types. In histopathologic examination high cellular polymorphism: small to large lymphocytes, lymphoblast, plasma cell and macrophages, were noted. Moreover, slight mitosis figures were seen in tumoral infiltration foci (Figure 5).



Figure 1. Marek's disease Neoplastic lesions of Marek's disease in the leg.

The lesions in the skin were characterized by infiltration of pleomorphic lymphoid cells in the dermis and around the feather follicles. Lesions in the bursa of Fabricius showed atrophy of follicles. Based on the

criteria: age of animals, alteration induced in skin and atrophy of the bursa of fabricius, as well as, microscopic examinations of tumor development in other organs, and the number of affected organs, all the infected flocks diagnosed as acute MD. In histopathologic examination of randomly selected organs of grossly normal chickens no positive MD case was found.

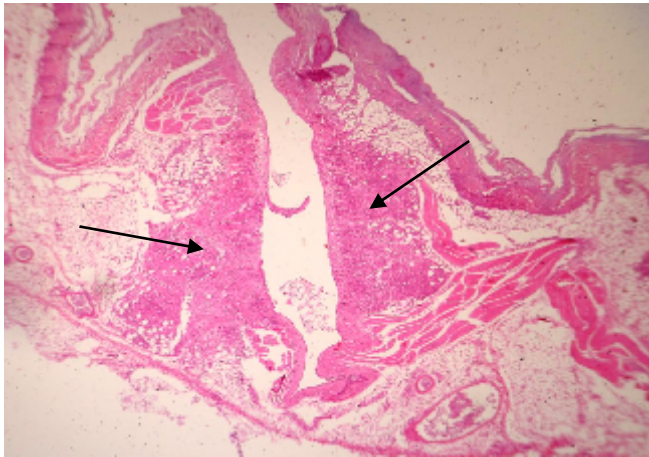


Figure 2. Marek's disease, skin- Lymphomatous infiltration around feather follicle (arrows) (H&E × 100).

DISCUSSION

Although it had been decided to do the present study in all, ten, regions of the Tehran province but, unlikely, there was no flock, presented for slaughter, from other regions except the four mentioned regions during the study.



Figure 3. Marek's disease, spleen- Multiple lymphomas in the spleens.

Nevertheless, from the results of current study evidence is emerging to suggest that the incidence of MD in broiler flocks of Tehran province, at least in the four regions, is high (30%).

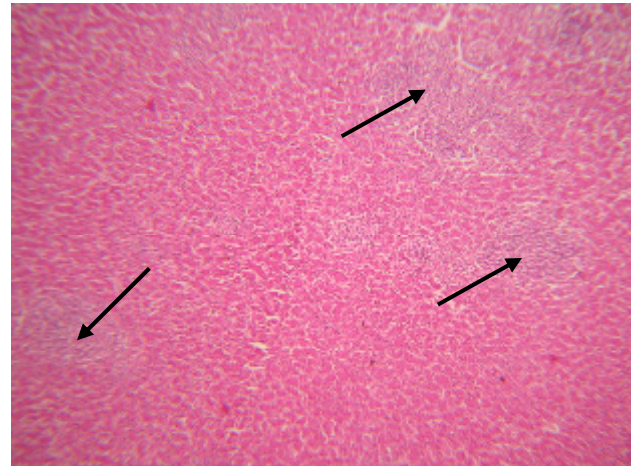


Figure 4. Marek's disease, liver- Distribution of lymphomatous lesions in the liver (arrows) (H&E × 40).

The information elicited from Savojbolagh and Karaj regions are highly reliable because the number of studied flocks is more than that was determined in statistical method, 34 vs. 25 and 31 vs. 16 flocks, respectively.

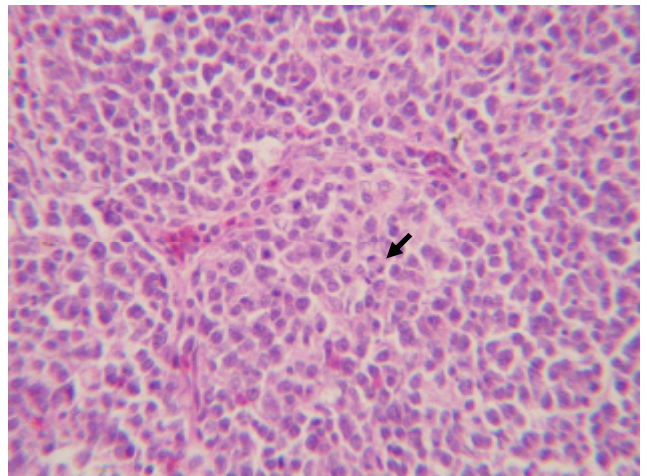


Figure 5. Marek's disease, spleen- Pleomorphic lymphomatous infiltrate in the spleen. A mitotic figure is seen (arrow head). (H&E × 400).

Whereas, the data emerging from Shahriar and Varamin regions are not sufficient for such a conclusion. Literature review on the incidence, significance and losses due to MD in broiler flocks, of Iran, showed that there are slight researches and

information. Tafti et al (1993) carried out a pathological study of MD in two broiler and eight layer chicken flocks, having natural outbreaks. One of broiler flock (57 days old) that was diagnosed as classical MD showed microscopic lesions mostly in sciatic nerve, skin, liver, spleen and proventriculus respectively. The other flock (42 days old) showed microscopic lesions mostly in the liver and proventriculus, spleen and kidney, skin, sciatic nerve, heart respectively, which was diagnosed as acute MD. In another study conducted by Farhoodi et al (2007_{a,b}) samples of five broiler flocks collected from slaughterhouses of Khorasan province was examined by histopathologic study of various organs and tissues and PCR for detection of serotype-1 MD virus in spleen. They declared that since MDV could be detected in the most of studied flocks it seems that the virus might play an important role in the development of immunosuppression syndrome among Iranian broiler chicken flocks. Ghorayshi et al (2005) in a study for detection of MDV in broiler chickens, of Markazi province, by applying histopathology and Nested-PCR techniques reported that all the samples which was diagnosed as MD in microscopic examination, were positive in PCR. Briefly, it seems that the MD is a common disease of broiler flocks of Iran and, therefore, may play a major role in death, weight losses, and condemnation of carcasses and complication of other diseases like as infection with non-highly pathogenic influenza viruses, associated with immunosuppressive property of MDV (Witter & Schat 2003). It should be noticed that the data elicited from this study had been obtained from gross and microscopic examination of the chickens carcasses. But, are these methods sufficient for such a conclusion? Although, there was a long term conventional guidelines for the pathologic diagnosis of MD (Siccardi & Burmester 1970), diagnosis of the clinical MD disease remains difficult in practice because no really pathognomonic gross lesion exists for MD. The study of Beyer et al (1980) on reliability of herd diagnosis of Marek's disease and leukosis of chickens on the basis of pathologico-

anatomic findings revealed that pathologico-anatomic findings, without histological testing, was sufficiently reliable, when undertaken as a part of herd diagnosis. On the other hand MDVs, avian leukosis virus (ALVs), and reticulo-endotheliosis virus (REVs) are prevalent in commercial poultry, often resulting in simultaneous infections complicating diagnostic efforts that depend on virological methods (Davidson and Borenstein 1999). At present time there is no accepted diagnostic standard for MD. However, Witter & Schat (2003) suggested a series of steps for diagnosis of the disease. There are some another techniques like as quantification of MDV in peripheral blood lymphocytes during early infection as a potential tool for monitoring MD in broiler flocks (Fakhrul Islam *et al* 2006) Meanwhile, gross and microscopic examinations of chicken's organs are useful techniques for diagnosis of MD, especially, when applied concurrently by virological criteria.

The result of the present study gives a hint for the importance and losses behind the high incidence of the MD in broiler flocks of Tehran province, Iran. Further detailed study on MD in broiler flocks and on the effectiveness of available MD vaccine in reducing the incidence of disease is recommended.

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