Animal Health Research Institute Assuit Regional Laboratory.

CLINICAL AND PATHOLOGICAL STUDY ON MAREK'S DISEASE IN BROILER CHICKS IN UPPER EGYPT

(With 12 Figures)

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دراسه اكلينيكيه وباثونوجية على مرض المارك في دجاج التسمين في صعيد مصر

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فى أنتاء القيام بالفحوصات الإكلينيكية والتشريحية والميكروسكوبية لعينات من بداري التسمين خلال الفترة من يناير ٢٠٠١ الى أكتوبر ٢٠٠٢ فى محافظات المنيا وأسيوط وسوهاج وقنا والتى كانت تعانى من أعراض تنفسية فى أعمار ٣٥ ــ ٤٥ يوم لوحظ أن بعض العينات أظهرت تضخم واضح فى الكبد والطحال وزيادة فى سمك جدار المعدة الغدية مما يوحى باحتمال الإصابة بمرض المارك. وقد تراوحت نسب النافق الإجمالي فى هذه المزارع بين ٤ ـ ١٠٠ ، كانت جميع الطيور التي تم جمع العينات منها و فحصها لم يسبق تحصينها ضد مرض المارك بآي لقاح. ولقد اثبت الفحص الهستوباتولوجى وجود آفات مرضية لمرض المارك تتمثل فى وجود تجمعات سرطانية للخلايا الليمفاوية للأعضاء الداخلية ووجود تغيرات باثولوجية مميزة لمرض المارك. وبذلك توصى الدراسة بتطبيق الإجراءات الوقائية اللازمة للحد من انتشار هذا المرض.

SUMMARY

During post-mortum examination of broilers from private farms in El-Minia, Assuit, Sohag and Kena Governorates suffered from respiratory signs. The birds were 35-45 days old, average mortality all over the cycle was 4-10%. Examination revealed in addition to respiratory signs, lesions, enlargement of liver, spleen and thickened proventricular wall in some samples. The birds subjected to examination were not vaccinated against Marek's disease. Histopathological examination proved that the samples were positive for Marek's disease and indicate presence of

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lymphocytic nodules and diffuse polymorphic lymphocytic infiltration in liver, spleen, proventriular and intestine. This study recommended control programs for Marek's disease.

Key words: Marek's disease in broiler chicks.

INTRODUCTION

Marek's disease is the most common lymphoproliferative disease of domestic chicken and caused by cell associated herps virus (Schat, 1987). Lymphproliferative neoplasms in MD occur in various organs and tissue including viscera, skin, peripheral nerves, gonads, iris and musculature (Calnek and Wirtter, 1997).

Gimeno et al. (1999), Witter et al. (1999) and Emara et al. (2001) described that this syndrome was characterized by acute onset of neurologic signs including flaccid paralysis of neck and limbs. Heier et al. (1999) noticed the susceptibility to Marek's disease during the period from 16 to 32 weeks of age and they found that the commulative mortality was 8.2%.

Infection with Marek's disease virus can be divided into three sequential phases, early cytolytic infection, latent infection and secondary cytolytic infection with immunosuppression, and subsequent tumour development (Venugopel and Payne, 1995 and Baigent and davison, 1999). The severity of clinical Marek's disease is highly correlated with the number of copes of the viral genome present in lymphoid cell (Burnstead *et al.*, 1997 and Lee *et al.*, 1999).

Marek's disease constitutes a serious economic threat to poultry industry because of heavy annual losses (Purchase *et al.*, 1972). It is more serious in developing countries than in developed countries (Biggs *et al.*, 1982).

Recently in Egypt, Amin *et al.* (2001) reported that Marek's disease virus as single infection was the commonest cause of neoplasms among vaccinated broiler and commercial layer flocks. The Marek's virus, was demonstrated in blood sample from affected hens by Sheble *et al.* (1973).

Thus, the aim of this study is to investigate Marek's disease in broiler chicks in El-Minia, Assuit, Sohag and Kena farms. Histopathological examination with illustration were carried out and control programs for the infection were recommended.

MATERIALS and METHODS

During the period from January to October 2002, several commercial broiler private farms (5000–10000 chickens each) in El-Minia, Assiut, Sohag and Kana Governorates were examined clinically and grossly. 144 live and freshly dead broilers at 35–45 days old showing respiratory signs and/or gross lesions were collected. Tissue specimens for histopathological examination were collected from grossly affected liver, spleen, proventriculus and intestines.

Specimens were immersed in 10% neutral buffered formalin saline solution for 8 hrs and then transferred to 70% ethanol. The tissue specimens were dehydrated in graded ethanol solutions, embedded in a low melting point paraffin wax, sectioned at 5 um, mounted on glass slides and routinely stained with hematoxylin and eosin (H&E).

RESULTS

Case History:

The birds subjected to examination were not vaccinated against Marek's disease.

Clinical Signs:

The examined birds revealed varying degrees of respiratory tract infection. General signs were retarded growth, paralysis of one or more extremities. Some birds showed incoordination in movements, ruffled feathers, sever depression, anorexia and diarrhea, while others died without extensive clinical signs. The average mortality allover was 4 – 10%.

Post-mortum Examination:

On dissection, both dead and slaughtered birds revealed lesions of respiratory tract in the form of air sacculitis and pneumonia. Pericarditis and perihepatitis were also observed. Some chicks revealed hepatomegaly and spleenomegaly. The most obvious gross lesion was thickened wall of the gastrointestinal tract especially proventriculus.

Histopathological findings:

All the examined liver sections showed various degenerative changes. Among the examined cases such changes differ in intensity but were diffuse and constant in all cases. Partial loss of hepatic cords was seen in some sections. The inflammatory reaction was represented by congestion, inflammatory edema and lymphocytic segregation in sinusoids (Fig. 1 and 2) scattered hepato cellular necrosis was seen in the examined sections associated with mononuclear lymphocytic infiltration (Fig. 3). Variable size of lymphocytic nodules were present, the

lymphocyte appeared small and darkly stain, The nodules were infiltrated with pleomorphic lymphocytes (Fig. 4). Some cases showed diffuse lymphocytic segregation (Fig. 5).

In the vascular ramifications, degenerative changes in their walls were promintent in association with endothelial degeneration and perivascular infiltration by smooth densely stained lymphocytes (Fig. 6).

In the intact hepatocycte, the nuclei were mostly altered. They appeared as distinct eosinophilic bodies varying in number, size and shape. Single or several inclusions were scattered throughout the karyoplasm. They were round, angular, crescentic. Eosinophilic material occupied entire nucleus. Generally the nucleolus had central position but margination was also seen (Fig. 7 a,b, c and d)

Lesions in proventriclus and intestines were mainly hyperplastic thickening in the proventricular and intestinal mucosa (Fig. 8).

The proventricular and intestinal glands were infiltrated by polymorphic lymphocytes, heterophils and macrophage cells (Fig. 9).

Microscopic lesions in the spleen were characterized by changes in the white pulp which showed depletion of lymphoid element (Fig. 10). Immature lymphocyte were seen demarcating of red pulp (Fig. 11). Small - sized lymphocytic nodules consisting of small darkly stained lymphocytes and diffuse lymphocytic infiltrations around the capillary sinuses of splenic parenchyma with immature lymphocyte were observed (Fig. 12).

DISCUSSION

In the present investigation Marek's disease could be diagnosed from broiler flocks in upper Egypt. Several sporadic cases, or outbreak were previously reported in broiler chicks (Cho *et al.*, 1997 and Islam *et al.*, 2001).

Marek's disease diagnosis based on clinical signs and necropsy findings and the histopathological lesions onfirmed the disease. The results are nearly similar to these reported by Sharma (1985) and Silva and Witter (1996).

Marek's disease may accompany other diseased conditions of broilers especially respiratory tract infection. The disease is responsible for sever economic losses due to mortality starting as early as 35 days of age and the average mortality allover the cycle was 4-10 %. Witter (1997) reported that the age susceptibility was 6-7 weeks of age. Heier *et al.* (2000) observed that the effect of Marek's disease on the total commutative mortality in period from 16-32 weeks of age in white

leghorn flocks was 12%. Witter et al. (1999) and Emara et al. (2001) reported the acute onset of neurologic signs including flaccid paralysis of neck and limbs with an indirect effect on body weight and early mortality.

The gross pathological finding revealed enlargement of visceral organs and the presence of thickness of the wall of gastrointestinal tract, liver, spleen, kidneys and ovary. These observation were similarly described by Ekpergigin *et al.* (1982).

The histopathological change include degenerative changes, hepatocellular necrosis associated with mononuclear lymphocytic infiltration in addition to eosinophilic intranuclear inclusions. Powell *et al.* (1986) and Burgess (2001) demonstrated lymphoproferative lesions which were see microscopically into non lymphoid organs.

Cho et al. (1999) and Gimeno (1999) observed necrotizing lymphomas associated with prominent vasculitis and increase in cellularity of the neutropils. Hepatocytic intranuclear inclusion bodies supported the diagnosis of Marek's disease.

Histopathology of proventricular and intestinal gland were infiltrated by polymorphic lymphocytes which may be decreasing the surface of digestion thus it is lead to weight losses.

The histopathological lesions of spleen were characterized by depletion of the white pulp, and change in lymphocyte distribution with formation of lymphocytic nodules. Similar results were recorded by Baigent and davison (1999).

Conclusion

Marek's disease may accompany other infections in broilers causing immunosuppession, retardation of growth and share in mortality.

Marek's disease can be prevented and controlled by Marek's disease vaccination applied subcutaneousty or intramuscularly in chicks at 1 day of age (St Hill and sharma, 2000) or by in ovo vaccination of embryonated eggs (Islam et al., 2001). The success in controlling of Marek's disease other than vaccine include: quality of the vaccine, husbandry, environment, good ventilation, hygiene and sanitation in hatchery, prevent high density of birds which provides ample opportunity for airborne transmission from older housed flocks to newly housed chicks. Producers should reduce challenge and control other immunosuppressive diseases. Breeders should improve genetic resistance in their strains. Hatcheries will have to improve administration of vaccines. If all components of the industry work together, Marek's disease can be controlled.

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