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Technical Note

Pathological Aspects of a Subclinical Marek's Disease Case in Free-Range Chickens

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ABSTRACT

The present report aims at describing the occurrence of Marek's Disease (MD) in a free range poultry farm based on macroscopic and microscopic lesions. For this purpose, seven free-range chickens were evaluated by gross and microscopic examination. Tumor lesions were observed in the liver and ovary, in addition of enlarged kidney and spleen, skin thickening and whitish lesions in streak form in the breast muscle. Fragments of the affected organs were collected and placed in 10% formalin for processing by the usual histopathology techniques, and stained with hematoxylin-eosin. Under microscopic evaluation, we observed pleomorphic populations of neoplastic lymphoreticular cells with pyknotic nuclei in the same organs, all compatible with MD. The macroscopic and microscopic lesions presented in this study were consistent with MD, meaning that the MD virus is present in the rearing environment of the studied chicken farm.

INTRODUCTION

The health control of poultry reared free-range systems is less strict than that of commercial flocks, increasing the risk of disease outbreak. Fossum *et al.* (2009) reported significant differences in the health of poultry reared under different husbandry systems. The authors detected more health problems in poultry kept in alternative systems as compared to conventional commercial establishments, with a high prevalence of bacterial diseases. Among viral diseases, Marek's disease (MD) was diagnosed only in flocks in alternative systems.

MD is caused by an alpha-herpesvirus, whose virulence is associated with its ability to induce lymphoproliferative lesions. These lesions are characterized by enlargement of the peripheral nerves due lymphocytic infiltration and by the presence of lymphomas in various visceral organs and tissues, which may be present even in vaccinated birds (Buscaglia *et al.*, 2004; Witter *et al.*, 2005). MD can be manifested in the affected chickens as early mortality in the absence of gross or microscopic lesions; depression, pale crest, reduced feed intake and weight gain, ataxia, and paralysis (Buscaglia *et al.*, 2004).

The clinical expression of MD and the presence of lymphomas are influenced by the immune response, which may be influenced by genetic factors. Buscaglia *et al.* (2004) inoculated vaccinated SPF chickens with MD virus serotypes characterized as very virulent (vvMDV) and very virulent plus (vv + MDV), and detected a high incidence of the disease in susceptible chicken strains, but not in chickens genetically resistant to the MD virus. Resistant chicken strains tend to maintain the virus latent, while in susceptible chickens the infection causes lymphomas (Burgess *et al.*, 2001; Kaiser *et al.*, 2003).

Bird age at the time of virus exposure may also influence the disease expression. Chickens of all ages are susceptible to infection, but the frequency of lymphomas is lower in older birds, especially in more resistant strains (Witter *et al.*, 1973).

At necropsy, MD gross lesions are characterized by diffuse enlargement of the liver and the spleen, presence of lymphomas in liver, kidney, ovary, proventriculus, spleen, lungs, nerves, heart, skin, and atrophy of the bursa of Fabricius and thymus. The histopathology of affected organs shows a marked cellular polymorphism, with the presence of lymphocytes, lymphoblasts, fibroblasts and infiltration of tumor cells arranged in circumscribed or diffuse form. In the liver, these lesions are accompanied by degeneration and necrosis of parenchymal liver cells, atrophy of the hepatic ducts and vacuolization (Buscaglia *et al.*, 2004; Witter *et al.*, 2005). Necrosis and destruction of lymphoid cells are described in the thymus and bursa of Fabricius (Buscaglia *et al.*, 2004; Fodor *et al.*, 2011). Lymphomas can be found in the absence of any nerve injury or clinical signs, and therefore it may go unnoticed during rearing or even at processing. Gross lesions may also be dubious, and do not confirm the presence of MD, demanding the microscopic examination of the lesions for their proper characterization (Vieira-Pinto *et al.*, 2003), as well as for the differential diagnosis with other neoplasms or diseases causing enlargement of the peripheral nerves (Schat & Nair, 2008).

The present report aims at describing the occurrence of MD in a free-range chicken farm, characterized by the absence of clinical manifestations and diagnosed by the evaluation of gross and microscopic lesions.

MATERIAL AND METHODS

The poultry farm studied is dedicated to rearing broilers and laying hens under an extensive system. It is located in the municipality of Carapebus, state of Rio de Janeiro, Brazil. The farm stock is of 10,000 birds, divided into six layer flocks and five broiler flocks. It is not near other poultry farms, commercial or not.

The affected birds had been vaccinated against MD at the first day of age, and were approximately 50 weeks of age. No obvious clinical signs of MD were observed, but multiple whitish nodules were detected in the viscera during processing. Based on these findings, seven birds were selected, euthanized by atlanto-occipital dislocation, and necropsied. Fragments of affected organs were placed in recipients containing 10% formalin for processing by the usual histopathology techniques, according to Luna (1968), and slides were stained by hematoxylin-eosin and examined under an optical microscope (Bioval L200A, Brazil).

RESULTS AND DISCUSSION

All seven necropsied chickens presented tumor lesions in the liver. Splenomegaly, spleen discoloration, enlarged kidneys, tumors in atrophic ovaries and focal whitish thickening of the skin were also observed. In one chicken, lesions in the form of streaks were observed on the breast muscle, which gave to the organ a "boiled" appearance (Figure 1). Gross lesions, despite being very characteristic of MD, were also submitted to histopathological examination for MD confirmation and exclusion of diseases with similar characteristics, such as avian leucosis (Schat & Nair, 2008).

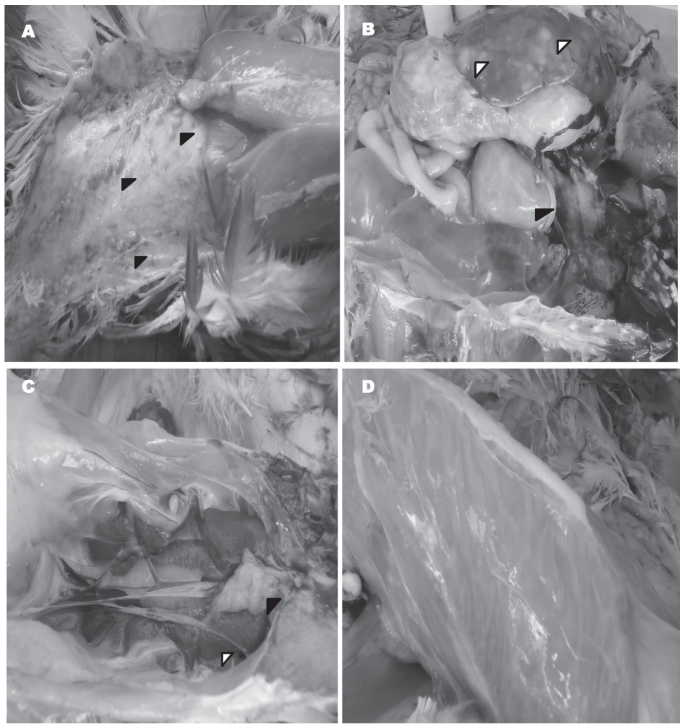

Figure 1 – Gross lesions consistent with Marek's Disease in chickens. A. Multiple cutaneous nodules of varying diameters with whitish aspect (black arrows). B. Splenomegaly with difuse discoloration of the parenchyma (black arrow) and nodular formations in the liver (white arrows). C. Nodular and diffuse formations in immature ovary (black arrow), and diffuse enlargement of the kidneys (white arrow). D. Whitish strips on the breast muscle.

Under the histopathology, spleen and liver presented neoplastic cells and, at higher magnification, pleomorphic populations of neoplastic lymphoreticular cells with pyknotic nuclei (Figure 2) were observed. The gross and microscopic lesions described in this figure are compatible with MD and similar to those described

by Buscaglia *et al.* (2004) and Fodor *et al.* (2011) in studies with chickens infected with the MD virus. Vieira-Pinto *et al.* (2003) suggests that histopathology, even in the presence of conclusive gross lesions, should be use to confirm the diagnosis of MD.

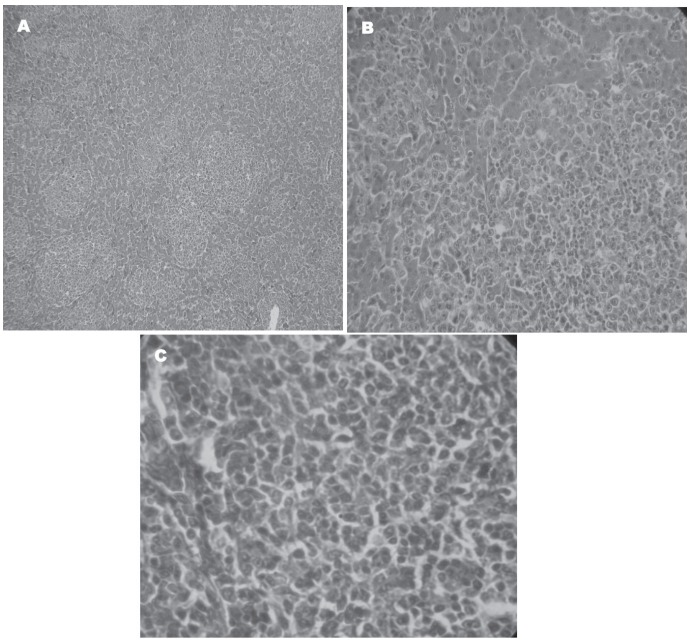

Figure 2 – Microscopic lesions consistent with Marek's disease in chickens. A. Liver. Multiple nests of oncocyts with heterogeneous aspect. HE 10x. B. Liver. Higher magnification of one oncocyt nest. Heterogeneous aspect of lymphoreticular organ invasion with hepatocyte dissociation, HE 40x. C. Spleen. Lymphoreticular infiltrate of neoplastic cells. HE100x.

The occurrence of MD, even in vaccinated birds, may be due to poor biosecurity conditions, which are frequently observed in free-range facilities (Fossum *et al.*, 2009). The presence of a highly pathogenic MD virus strain or chick vaccination failure, as well as chicken strains with low genetic resistance to MD , are also considered risk factors (Burgess *et al.*, 2001; Kaiser *et al.*, 2003;. Buscaglia *et al.*, 2004). Vaccination against MD may delay the onset of illness (Buscaglia *et al.*, 2004), which could explain the occurrence of MD in the 50-week-old birds in the present study. Injuries caused by the MD virus progress slowly (Witter *et al.*, 1973), which supports the low mortality rates observed in the studied farm.

The gross and microscopic lesions detected in this study are consistent with MD, indicating that the MD virus is present in the rearing environment of the studied farm. Effective vaccination, changing the current genetic strains, and improving biosecurity measures are required to eradicate the virus from the farm. We emphasize the purchase of replacement birds, because these are necessarily vaccinated against MD in the hatchery. The absence of nearby commercial flocks ensures that the presence of the disease does not constitute a risk to poultry production in the state of Rio de Janeiro, Brazil.

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