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CASE REPORT

The Dedifferentiate Tumor of the Bursa of Fabricius and the Intestinal Mucosa Associated Lymphoid Tissue in Quail (*Coturnix coturnix*): Case Report

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A B S T R A C T

After examination of 150 female quails, four of them showed the dedifferentiate tumor of the bursa of Fabricius. To the periphery, it consists of the dedifferentiate hemangiosarcoma and liposarcoma, the predominant area being constituted from lymph nodes of variable sizes. Among the lymphocytes rows the extensions of the reticular cells appeared positive for MHC II antibody. The lymphocytes showed the negativity to p53. The Bcl-2 marker manifested a limited positive reaction only in certain follicles. IgA and IgM markers showed the relatively low level of positivity in lymph nodes. Only the dense cords of the pleomorphic melanocytes manifested the positivity to Bcl-2. At the junctions between ventriculus and duodenum; ileum and cecum, especially in serosa, there were identified lymphocytes positive to MHC II, Bcl-2 and p53 markers surrounded by clusters of lipoid cells located inside the intestine wall. These structures were metastases of the dedifferentiate tumor of the bursa of Fabricius.

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INTRODUCTION

Bursa of Fabricius is the main lymphoid organ in birds and has a specific role in differentiating of the B lymphocytes. It achieves the maximum development at the age of 5 weeks (100.23±6.47 mg), then decrease quickly from 5 to 9 weeks (63.21±3.9 mg) and decrease slowly till 36 weeks when bursal folicles are almost completely degraded (He *et al.*, 2015). The most common diseases affecting bursa of Fabricius are: Marek's disease, lymphoid leukosis and mieloid leukosis.

The dedifferentiate liposarcoma is a biphasic liposarcoma with a high-grade pleomorphic sarcoma that can appear as a primary tumor or secondary as a late-stage complication of a tumor. Recently the definition was extended in order to include tumors with low-grade dedifferentiation and also suggests that low-grade dedifferentiation represents a precursor lesion of high-grade dedifferentiation (Henricks *et al.*, 1997; Yoon *et al.*, 2010). To our knowledge, this is the first report of the dedifferentiate tumor in quail.

MATERIALS AND METHODS

The examination was carried out on 150 female quails reared in university experimental unit which were sacrificed at the age of 32 weeks. Four of them had the bursa of Fabricius about 26-28mm length, burgundy-black color. The rest of internal organs did not show any visible changes in color or size. From the 4 birds, bursa of Fabricius were taken samples from the esophageal-proventricular, proventricular-ventricular, ventriculo-duodenum junctions, cecal tonsils and cecal sacs and fixed with Bouin solution. Embedded in paraffin, section of 4 µm were stained by H&E and immuno-histochemistry methods for antibodies p53 (Vector Laboratories, Burlingame, USA), Bcl-2 (BD Biosciences, San Jose, USA) IgA, IgM (Leica Biosystems, Eisfield, Germany) and MHC II (1:100) (Southern Biotech, Birmingham, USA).

RESULTS

The examination of modified bursa of Fabricius revealed tumors constituted from three distinct zones (Fig. 1). At the periphery, a zone of the dense, modified connective tissue, with very large vessels filled with round-oval cells, some of them with nucleus rich in dispersed chromatin and others with central or eccentric hyperchromatic nucleus, lesions resembling a hemangiosarcoma. The cells were negative to Bcl-2, MHC II and

p53 antibody. Among the ectasiated and distorted blood vessels were identified areas of lipoid cells and lipoblast which represent 15% of the cell population. No mitotic activity in lipoid cells was registered.

The transition from adipose tissue to the lymphoid zone consist from masses of pleomorphic cells, round, polygonal or spindle shape, histiocytes and melanocytes. The cells from this area were positive to p53, Bcl-2 and MHC II antibodies. The invasive component was represented by small groups of tumor cells located into ectasiated vessels lacking endothelial cells. The lining cell from the vessels appeared to be at the origin of the tumor cells (Fig. 2). The cells of these zones are similar to plasma cells and are negative to p53, Bcl-2 and MHC II markers (Fig. 2).

The lymphoid area consists of small lymphocytes with reduced cytoplasm and prevalent nucleus, arranged as cords. Chromatin is finely dotted and the nucleoli are less visible. The cells from this area have high mitotic rate and the apparent aspects of artifacts, in accordance with the high rate of proliferation of these neoplasma. Also, there are areas of necrosis due to intense proliferation of tumor cells. Between the cords of lymphocytes were observed extensions of the reticular cells positive to MHC II (Fig. 1). P53 protein was limited expressed at the periphery of the tumor. Bcl-2 positive cells were restricted to certain follicles (Fig. 2). IgA and M presented a relatively low positivity. There were identified dense cords of mature and immature melanocytes, pleomorphic, some of them non-melanic in HE coloration and Bcl-2 positive. The mature melanocytes were intensely brown-black colored.

At the junction areas of the digestive tract segments, MHC II, Bcl-2 and p53-positive cells were observed mainly in the serosa, forming groups associated to the lipoid cells which cross the muscular layer, reaching the mucosa. The invasive tumor cells associated with the lipoid tissue divide the muscle from the muscular tunic. At the junction from ventriculum and duodenum, in the lamina propria were observed numerous cells similar to plasma cells which did not show positivity to IgA and IgM (Table 1). The reactivity of cells from cecal bags to all the immunohistochemistry markers was reduced but the cells from mucosa of the small intestine manifested a high positivity of the IgA and lower one for IgM (Table 1).

The tumors of the lymphoid tissues associated to the intestinal mucosa consist of small lymphocytes of intermediate type having a dentate nucleus and large lymphoblasts. The intestinal tumor foci replace the normal structures of intestinal mucosa (intestinal glands and epithelium cells).

DISCUSSION

Dedifferentiated liposarcoma, traditionally defined as "nonlipogenic sarcoma" resulted from a well differentiated liposarcoma which confers metastatic potential. More than 90% of dedifferentiated liposarcomas occur *de novo,* while less than 10% happen as a relapse. The dedifferentiation component consists of pleomorphic cells, not adipocytes, having a metastatic potential. They have a local recurrence rate of 41% and about 17% metastatic (Thway *et al.*, 2016). Dedifferentiated liposarcoma which occurs outside the mediastinum, retroperitoneum or groin are extremely rare; being diagnosed radiological and histological (Yu *et al.*, 2005; Yoon *et al*., 2010). The mutations in p53 are rarely seen in the dedifferentiate liposarcoma, but are frequent in pleomorphic liposarcoma.

Barretina *et al.* (2010) have showed that 16.7% of pleomorphic liposarcomas have the mutations in p53. Similarly, Ghadimi *et al.* (2011) reported the p53 mutations at a rate of 60% in pleiomorphic liposarcoma. Neoplastic hystiocytes in histiocytoma express MHC class II molecules (Fernandez *et al.*, 2005). MHC-II antigens have been often demonstrated in tumors with lymphocyte infiltration. They were detected predominantly in the cytoplasm of tumor cells and to a lesser extent in the cell membranes.

The differential diagnosis at quail was made with other specific diseases affecting bursa of Fabricius, as Marek's disease, lymphoid leucosis and mieloid leucosis (Payne and Venugopal, 2000; Matthyssens *et al.*, 2015). Marek's disease (MD) is a lympho-proliferative disease, MD virus causing numerous and diverse clinical manifestations, paralysis, skin lesions, atrophy of the thymus and bursa of Fabricius, immunosuppression and high rate of mortality in chickens. It has been rarely reported in turkeys and quails.

Fig. 1: The dedifferentiated tumor in bursa of Fabricius in quail. Expression of p53 (IHC for p53, bar=200 µm).

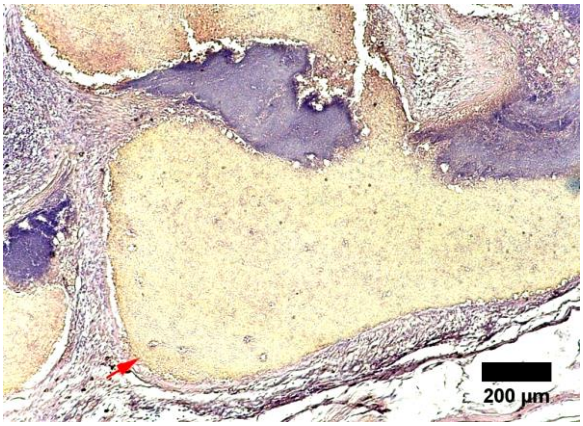

Fig. 2: Detail of hemangiosarcoma of bursa of Fabricius. The lymphocytes are located into vessels lacked of endothelial cells, showing expression of Bcl-2 (IHC for Bcl-2, bar=200 µm)

Table 1: The expression of immunohistochemistry reactions in different cell types

Organ	Cell type/location	P53	MHC II	IgA	IgM	Bcl-2
	Haemangiosarcoma cells	-	-	-	-	-
	Histiocytes	++	++	-	-	++
Bursa of	Melanocytes	-	-	-	-	+++
Fabricius	Limph nodes	-	-	-	-	-
	Reticular cells	++	+++	-	-	-
	Lymphocytes	-		+	+	-
	Serosa	+++	++	-	-	+++
	Musculosa	++	+++	-	-	+++
Digestive	Mucosa	-	-	-	-	-
tract	Plasmocytes	-	-	-	-	-
	Lymphocytes	++	-	-	-	-
	Lymphoblast	+	+++	+	+	+++

+++ overexpression; ++ medium expression; + low expression; - lack of expression.

Lymphoid leucosis appears in chickens starting with the age of four months. Miliary, diffuse or nodular extravascular tumor foci, in liver, bursa of Fabricius, spleen and other organs are observed. Microscopic tumor foci are made up of medium-sized B lymphocytes, immature (lymphoblasts) expressing immunoglobulin M (IgM) (Payne and Venugopal, 2000).

Myeloid leucosis, caused by subgroup J ALV, shows features of both myeloid and myeloblastic forms. The disease occurs in adults and occasional hybrids for meat, youth and broilers. Myelocitomas are present on the sternum, vertebrae, ribs, jaw, trachea and sometimes at eyes level. Liver, spleen, ovaries and other organs are magnified by tumor infiltration. Microscopically, the tumor cells are the well differentiated myelocytes; being possible to be an immature myeloids, sometimes at the same sick bird (Payne and Venugopal, 2000).

A variety of solid tumors can be caused by avian leucosis virus (ALV), including fibrosarcoma, chondroma, hemangioma, histiocytic sarcoma, mesothelioma, myxoma, nephroblastoma, osteoma, proliferative bone disorder, osteopetrosis, all of them characterized by the thickening of the long bones. These tumors can occur alone or can accompany the leucosis (Yu *et al*., 2005).

Reticuloendotheliosis virus (REV) can induce two types of lymphoid neoplasma in chickens. Bursa of Fabricius lymphomas can occur after a long period of time. Bursa and other organs lesions are difficult to distinguish from the same of lymphoid leucosis induced by ALV. The tumor is caused by activating the oncogene myc in the B lymphocytes. Chronic lymphomas are occurring after natural REV infection at ducks, geese, pheasants, quails and turkeys. It was not established whether these lymphomas originate from B or T cells (Payne and Venugopal, 2000).

Conclusions: The dedifferentiated tumor of bursa of Fabricius in quail is a complex disorder, consisting in hemangiosarcoma and dedifferentiation liposarcoma with positive cells to Bcl-2 and p53 markers. In our study the tumors in bursa of Fabricius metastasized to the intestine, especially at the junction areas of the digestive tract segments. MHC II, Bcl-2, P53 positive lymphocytes were observed particularly in the serous membrane, forming groups with lipoid cells.

Authors contribution: CS carried out histopathological and immunohistochemistry. MS, CS and CS performed necropsy, prepared samples and interpreted results. VF and GS critically analyzed the results and edited the manuscript. All authors approved final version of the manuscript.

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