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Hematological and histopathological evaluation of wildlife green turtles (*Chelonia mydas*) with and without fibropapilloma from the north coast of São Paulo State, Brazil[1]

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ABSTRACT.- Zwarg T., Rossi S., Sanches T.C., Cesar M.O., Werneck M.R. & Matushima E.R. 2014. **Hematological and histopathological evaluation of wildlife green turtles (*Chelonia mydas*) with and without fibropapilloma from the north coast of São Paulo State, Brazil.** *Pesquisa Veterinária Brasileira 34(7):682-688.* Departamento de Patologia, Faculdade de Medicina Veterinária e Zootecnia, Universidade de São Paulo, Avenida Prof. Dr. Orlando Marques de Paiva 87, Cidade Universitária, São Paulo, SP 05508-900, Brazil. E-mail: ticianamzsd@yahoo.com.br

Blood profiles were determined in 47 juvenile green turtles, *Chelonia mydas*, from São Paulo northern coast, Brazil. Twenty-nine were affected by fibropapillomas and 18 were tumor free. Complete gross and histopathologic examinations of the fibropapillo were performed in 21 green turtles. Biometrical data, size, location and amount of tumors were recorded. The papillomas varied in morphology, location, size, color and texture. We found hyperplastic stroma, rich in blood vessels and connective tissue with increase in thickness of the dermis. The tumors w0ere classified as papillomas or fibropapillomas according to their epithelial and/or stromal proliferation. The lowest Mean Corpuscular Hemoglobin (HCM) values were observed in affected turtles.

INDEX TERMS: Green turtle, *Chelonia mydas*, blood profile, histopathology, fibropapilloma.

RESUMO.- [**Avaliação hematológica e histopatológica de tartarugas verdes de vida livre (*Chelonia mydas*) com e sem fibropapilomas do litoral norte do Estado de São Paulo.**] Realizou-se hemograma de 47 tartarugas verdes, *Chelonia mydas*, provenientes de uma população de vida livre do litoral do estado de São Paulo, Brasil. Dessas, 29 apresentavam fibropapilomas e 18 não apresentavam formação tumoral. Fez-se avaliação macroscópica e histopatológica dos tumores de 21 tartarugas verdes com fibropapilomatose. Foram coletados dados biométricos dos animais, avaliação de tamanho, localização e quantidade dos tumores. As formações papilomatosas apresentaram morfologia, localização, tamanho, coloração e textura variados. Observou-se um estroma hiperplásico, rico em vasos sanguíneos e grande quantidade de tecido conjuntivo, resultando em um espessamento da derme. As formações foram classificadas como papilomas e/ou fibropapilomas, dependendo da proliferação epitelial e/ou de estroma, respectivamente. Os parâmetros hematológicos apresentaram variação, em função do acometimento tumoral, somente para Hemoglobina Corpuscular Média (HCM), sendo observados valores menores em animais com fibropapilomas.

TERMOS DE INDEXAÇÃO: Tartaruga verde, *Chelonia mydas*, perfil hematológico, histopatologia, fibropapiloma.

INTRODUCTION

The green turtle, *Chelonia mydas*, feeds and nests in Brazilian coastal areas and is classified as endangered species according to IUCN and vulnerable in Brazil (Martins & Molina 2008, Almeida 2011).

The fibropapillomatosis (FP) is a debilitating illness that can provoke the death and reaches mainly sea turtles of the *Chelonia mydas* species, representing an important

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threat for its conservation. Fibropapillomas are benign tumors that can achieve 30cm of diameter (Lackovich et al. 1999) and can involve the tegument of the fins, eyes, base of the tail, oral regions, cervical, inguinal, axillary, carapace and cloacal region (Jacobson et al. 1989, Herbst 1994).

The disease is characterized by the presence of internal and external tumors (Herbst 1994). The lesions caused by fibropapillomas can interfere with the hydrodynamics and locomotion of these animals, compromising the feeding (Adnyana et al. 1997). It can be debilitating and fatal (Aguirre et al. 1998). The affected turtle may have barnacles in the carapace and plastron, and may also presents disturbances of fluctuation, cachexia, anemia, hypoproteinemia, electrolyte imbalance (hypocalcemia, hyponatremia and hypocloremia) and elevation of liver enzymes (Norton, et al. 1990). Due to its high prevalence in immature animals, may represent in a long term a large impact on survival of endangered species or at risk (Greenblatt et al, 2005).

Before 1982, there was no register of FP in turtles captured in Florida and since then the number of cases has grown considerably (Jacobson et al. 1989). In Brazil, the first report occurred in 1986 on the coast of Espírito Santo State and the prevalence was 15.41% according to data from 2000 to 2005. There is no report in nesting areas: Fernando de Noronha/PE, Ilha da Trindade/ES and Atol das Rocas/RN (Baptistotte 2007). The first studies with the purpose of elucidate the etiology of tumors in green sea turtles caught in Brazilian areas were made by Matushima in 1995-1996, 1998-1999 and 2003. In these studies, samples were collected from tumor tissue for histopathological examination, immunohistochemistry, ultrastructural and blood for hematological and biochemical analysis (Matushima et al. 1999, 2001, Matushima 2003). After, studies were made for blood profile examination and analysis of leukocytes activity, colleting blood samples from turtles with and without tumors (Rossi 2007, 2014).

Currently, it is estimated that over 50% of the population of green turtles in the world are affected by the disease (Balazs 1991, Herbst 1994). Some records indicate that over 50% of animals caught in the region of Florida have the disease (Brill et al. 1995); according Ehrhart (1991) and Lackovich et al. (1999), this variation can be from 0 to 72.5%. Some data from the base of Ubatuba/SP indicate the increase in prevalence from 0 to 24% in the period 1986 to 1998. In the area of Vitória, Espírito Santo, the number of cases has already reached 40% in 2000 (Baptistotte et al. 2001).

Studies detected the presence of C-FP-HV (Chelonid fibropapilloma-associated herpesvirus) in samples of fibropapillomas (Lackovich et al. 1999, Ene et al. 2005, Work et al. 2009). However, interference by human industrial and agricultural activities in areas close to beaches, bays and lakes can contribute to the increase of the disease (Balazs 1991, Adnyana et al. 1997). The influence of environment on the establishment of the disease is a significant event and has been observed in several studies, as conducted in Florida (Ehrhart 1991) and in Hawaii (Balazs 1991). Pollutants can promote immunosuppression or induce the latent virus to infection (Herbst and Klein 1995). Studies in Hawaii indicated relation between high frequency of affected animals and high nitrogen concentration associated with eutrophication and presence of algae (Van Houtan et al. 2010). The turtles are very important in the ecological balance of marine ecosystem. This way, its protection is essential for the preservation of seas and coast areas (Frazier 1999).

This study aimed to evaluate the hematological profile of sea turtles with and without fibropapillomas and the histopathological features of tumors in affected animals.

MATERIALS AND METHODS

Study area and capture of the turtles

The green turtles were captured in various beaches in Ubatuba, located in the North Coast of São Paulo State (Latitude 23°26´S, Longitude 45°05´W). This region was selected due to the constant presence of young green turtles that frequent the coastal area to feed. Occasionally, some were from other regions as Guaruja (São Paulo) (Latitude 23°59'S, Longitude 46°15'W), São Sebastião (São Paulo) (Latitude 23°45'S, Longitude 45°24'W) and Ilha Bela (São Paulo) (Latitude 23°46'S, Longitude 45°21'W).

The turtles were incidentally caught in fishing nets or redeemed from the beach stranding and delivered to the Projeto Tamar-Ibama, Ubatuba/São Paulo Base. Only one specimen was seized by Ibama in captivity in the town of Parati (Rio de Janeiro) (Latitude 23°13'04'' S, Longitude 44°42'47'' W). After capture, animals were kept in captivity until they are ready for release again. The cases requiring the maintenance in captivity included animals showing fibropapillomas, disturbances of movement, body mass below average, the presence of ectoparasites or other indications which prevent the immediate return of the animal habitat.

Biometrics

Biometric data were collected as body mass (BM), curved carapace length and width (CCL and CCW, respectively) and curved plastron length and width (CPL and CPW, respectively) of each animal.

The fibropapillomas were measured using a plastic caliper with a diameter measured in centimeters. There was also the total count of fibropapillomas distributed in the body of each animal. The papillomatous formations were classified according their morphology, location, color and texture. As for texture, the tumors could be divided into verrucous and fibromatous.

It wasn't possible to determine the sex of the animals, as all the turtles were considered young, showed no external sexual dimorphism.

Hematology and histopathology

Blood samples were collected by puncture of the cervical venous sinus (Fig.1) in 47 animals to perform the complete blood count.

The blood samples obtained were placed in tubes containing sodium heparin BD® and were kept under refrigeration until processing laboratory at the Faculdade de Medicina Veterinária e Zootecnia da Universidade de São Paulo (FMVZ/USP). An aliquot was immediately separated without anticoagulant after sampling for preparation of blood extensions. The time between collection and processing did not exceed 24 hours.

Blood samples were tested as follows: Hematocrit by the technique for determination of microhematocrit, using a microcentrifuge. Total counts of red blood cell (RBC) and white blood cell (WBC) were performed in a Neubauer chamber, using Natt & Herrick (1952) diluent. Differential leukocyte count was car-

Fig.1. Blood collection by puncture of the cervical venous sinus of green turtle. Projeto Tamar-Ibama, Ubatuba/SP, 2007.

ried through the blood smears made at the time of collection of blood samples, stained with May-Grünwald-Giemsa technique (Rosenfeld 1947); the reading was held in the optical microscope objective lens of 100X. The technique that was used to count Thrombocytes was performed by counting the number of these cells in 1000 erythrocytes counted. Hemoblobin assay was determined by commercial Labtest® kit, after centrifugation of the solution to remove the free red cell nuclei following red cell lysis. Plasma proteins, using a refractometer and the erythrocyte indices (mean cell volume - MCV; mean corpuscular hemoglobin - MCH, and mean corpuscular hemoglobin concentration - MCHC) were calculated from the total count of erythrocytes, hematocrit and hemoglobin, according to Campbell (1996).

Cutaneous formations were collected from 21 specimens, with tumors of smaller size chosen as preferred to surgical excision depending on the increased possibility of being detected a possible viral agent. The asepsis was made with iodized alcohol, and then the application of xylocaine as anesthetic for superficial incision. The excised tumors were immediately fixed in a solution of formalin 10% for achievement of histopathological examination. The slides were prepared in the Histology Laboratory of FMVZ / USP, Department of Pathology (VPT), and stained with Hematoxylin--Eosin (HE).

Statistical analysis

Through statistical program (*Minitab*, by "Graphical Summary", "2 variances", "2-sample t" and "Mann-Whitney" tests), all following parameters were expressed in average and standard deviation (SD): Hematocrit (%), Hemoglobin (g/dL), Red blood cell-RBC (10^5/mm^3), MCV (fL), MCH (pg), MCHC (g/dL), White blood cells-WBC (10^3/mm^3), Plasma proteins (g/dL), Heterophils (/mm^3), Eosinophils (/mm^3), Lymphocytes (/mm^3), Monocytes (/mm^3), Basophils (/mm^3) and Thrombocytes (/mm^3).

The average values of hematological variables in groups of animals with and without fibropapillomas were compared, and submitted to statistical tests "Graphical Summary", "2 variance", "2-sample t" and "Mann-Whitney." The test "Graphical Summary" verified the normal distribution of data ($p>0.05$). In this situation, data were also submitted to the test "2-sample t", in which average were evaluated for their equality (the means were considered equal with $p>0.05$ and the average different in $p<0.05$). The test "2 variance" was conducted to evaluate the difference between the two groups, assuming that the variances were equal. Data were considered statistically identical with $p>0.05$, with significantly different data in which $p<0.05$. The non-parametric test of Mann-Whitney was realized for data that did not have normal distribution, where were considered statistically different data with $p<0.05$.

RESULTS

Biometrics

Of the 47 animals collected, 29 were affected by fibropapillomatosis and 18 had no skin tumors. Data on biometrics of animals and the tumors and their classification scores are given in Table 1 to 3.

Table 1. Biometric values of green turtles (*Chelonia mydas*) with fibropapilloma, at the coast of São Paulo, Brazil, 2006 (n=29)

	CCL (cm)	CCW (cm)	CPL (cm)	CPW (cm)	BM (kg)
Average	44.49	40.50	36.14	32.28	10.86
SD	9.39	8.91	7.98	6.60	7.72

CCL = curved carapace length, CCW = curved carapace width, CPL = curved plastron length, CPW = curved plastron width, BM = body mass.

Table 2. Biometric values of green turtles (*Chelonia mydas*) without fibropapilloma, in the coast of São Paulo, Brazil, 2006 (n=18)

	CCL (cm)	CCW (cm)	CPL (cm)	CPW (cm)	BM (kg)
Average	37.97	34.62	29.58	27.04	6.53
SD	4.76	4.82	2.20	2.95	3.21

CCL = curved carapace length, CCW = curved carapace width, CPL = curved plastron length, CPW = curved plastron width, BM = body mass.

Table 3. Values obtained through the biometrics of cutaneous formations of green turtles (*Chelonia mydas*), affected by fibropapillomatosis - Brazil, 2006 (n=27)

Turtles	Number of fibropapillomas (FP)	Classification by size			
		≤ 1 cm	1 cm < FP ≤ 5 cm	5 cm < FP ≤ 10 cm	10 cm < FP ≤ 15 cm
T1	12	12	-	-	-
T2	63	15	45	3	-
T6	84	36	46	2	-
T9	23	5	15	3	-
T10	16	8	8	-	-
T11	85	82	3	-	-
T14	5	4	1	-	-
T15	33	6	24	3	-
T16	66	9	56	1	-
T18	13	-	13	-	-
T19	38	5	32	1	-
T20	39	7	30	2	-
T21	106	60	44	2	-
T22	34	13	19	2	-
T23	30	10	20	-	-
T24	16	3	13	-	-
T29	4	3	1	-	-
T30	37	4	33	-	-
T31	17	9	8	-	-
T34	10	1	9	-	-
T39	57	16	40	-	1
T40	129	101	28	-	-
T41	36	16	20	-	-
T42	84	24	60	-	-
T43	70	19	48	2	1
T45	2	1	1	-	-
T46	78	43	35	-	-

Study of fibropapillomas

Macroscopic description. The papillomatous formations observed in studied animals exhibited morphology, location, color and texture variety. The color ranged from white, through clear pink, dark pink, green, gray and even a little back (Fig.2). Some formations, according to the high vascularization of the stroma and trauma suffered by animals, were ulcerated, with slight bleeding. In a few cases, were found trematodes eggs associated with fibropapillomas. The fibropapillomas were located in the axillary, groin, neck, base of tail, front and back fins, around the eyeballs, cornea, between the cornea shields along the oral cavity and the region pericloacal (Fig.3). With respect to size, tumors had between 0.2cm and 13.0cm in diameter with formations larger than 10.0cm in diameter observed in only two animals. The amount ranged from 4 to 129 tumors in a single turtle.

Microscopic description. The verrucous formation presents, in the microscopic examination, a large amount of wooded papillary projections, supported by an abundant fibrovascular base. The formations of fibromatous aspect contained mainly fibrous connective tissue throughout the stroma, with relatively smooth surface. In general, we find melanophores or melanomacrophages - cells that receive passively the transferred pigment by the melanocyte or that phagocytized the pigment - in the dermis of the cuts analyzed in variable amount, depending on the degree of pigmentation of the tumor.

In the 21 subjects studied, there was a hyperplastic stroma, rich in blood vessels and large amount of connective tissue, resulting in an increase in thickness of the dermis. Compared with normal epidermis, which has four to seven cell layers, the tumors showed great variation in cell proliferation, which may be minimal or extensive (more than 30 layers of cells) (Fig.4). There was also orthokeratosis in formation from keratinized tissues, in which the stratum corneum is made thicker than normal skin. In many cases, when the hyperkeratosis was pronounced and combined with a papillary hyperplasia, cysts are formed to include varying sizes and quantities. In the basal layer, there was vacuolization of the cytoplasm of cells, with necrosis. Degenerative changes that resulted in the separation layer of the dermis and epidermis were seen in some cases. When this space became large, the epidermis above could present necrosis and ulceration.

In the stratum corneum of the formations, there were vacuolated cells, often related to processes underlying the basal layer. The degenerative changes of the upper layers of the stratum corneum, however, were not always related to the basal layer. These changes included since vacuolization as described above until foci of extensive vacuole degeneration. In advanced cases of degeneration and acantholysis, the agglomeration of many granulocytic cells formed a pustule, which could progress and become an ulcer. The erosions and ulcers were extensively infiltrated with granulocytes and covered with cellular debris and proteinaceous material.

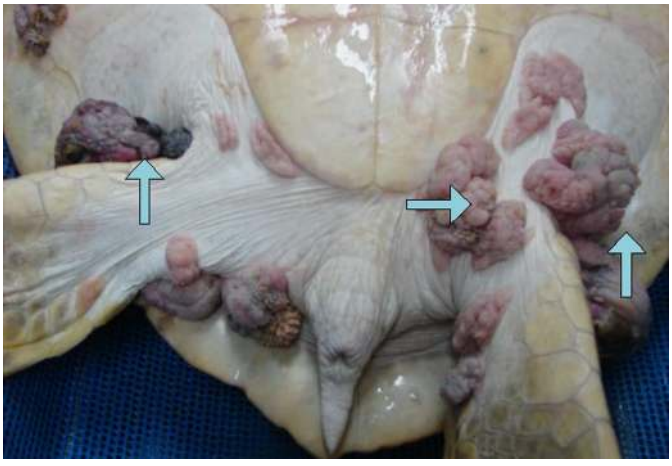

Fig.2. Multi and medium fibropapillomas with gray to pink color and appearance papillary verrucous in the inguinal region and pericloacal in *Chelonia mydas* (blue arrows). Projeto Tamar-Ibama, Ubatuba/SP, 2007.

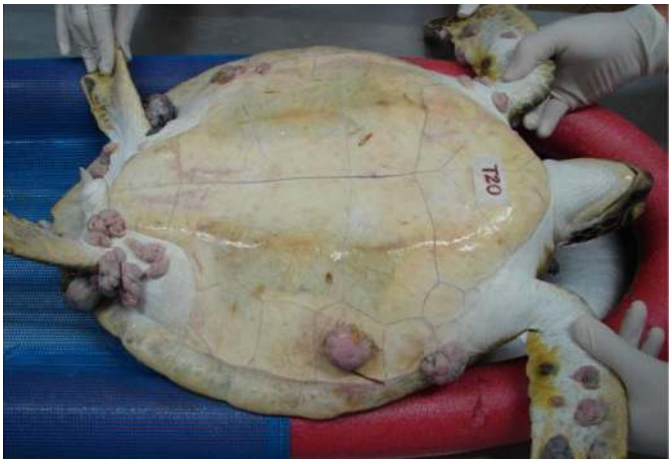

Fig.3. *Chelonia mydas* with multiple fibropapillomas in varies locations. Projeto Tamar-Ibama, Ubatuba/SP, 2007.

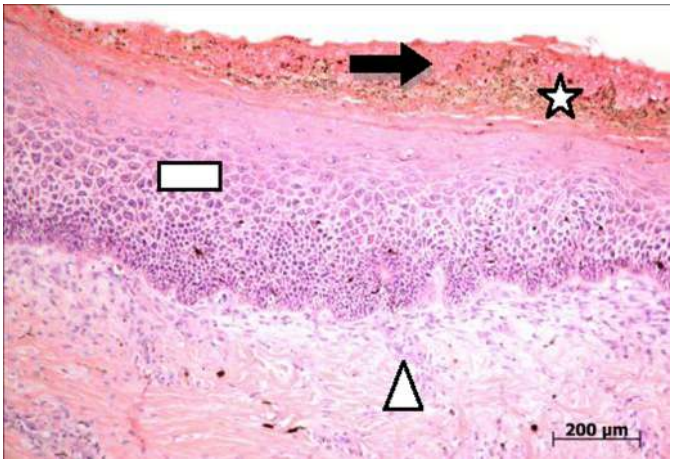

Fig.4. Histopathological section of fibropapilloma, green turtle, HE, obj.10x. There is a great proliferation of cells in the epidermis (rectangle). The *stratum corneum* is thickened, there is an area of ulceration and accumulation of dead cells (brown color - blue arrow). Below this point is a slight detachment from the corneal layer and the underlying (star). There is also proliferation of the dermis (triangle), with extensive distribution of loose connective tissue, well irrigated by vessels of different sizes.

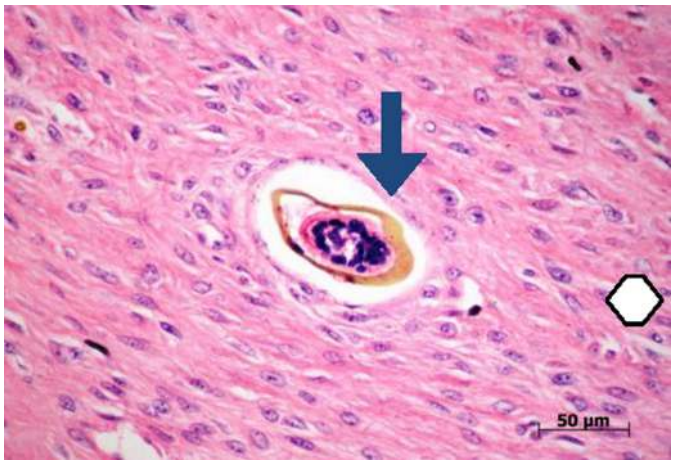

Fig.5. Histopathological section of fibropapilloma, green turtle, HE, obj.40x. Egg of Spirorchiidae in a blood vessel (blue arrow). Around, many fibroblasts form the dermal (hexagone).

In several fragments, the structures were covered by a thin membrane of brown color and containing amorphous material in it, suggestible eggs of trematodes of the Spirorchiidae family (Fig.5).

By microscopic observations associated with macroscopic, the analyzed formations were classified as a tumor of epithelial origin and benign in nature, in other words, papillomas and/or fibropapillomas, depending on the epithelial proliferation and/or stromal, respectively.

Blood profile

The statistical analysis of blood showed a significant difference ($p < 0.05$) between the two groups only for MCH, and the animals with fibropapillomatosis had the lowest average for this parameter. The results are shown in Table 4. No basophils were found in blood films during the differential counting of blood cells in both groups.

Table 4. Hematological profile of green turtles (*Chelonia mydas*) with and without fibropapillomas, in the coast of São Paulo, Brazil, 2006 (n = 47)

	Without fibropapilloma (n=18)	With fibropapilloma (n=29)
Hematocrit (%)	27.67±8.07	24.31±7.56
Hemoglobin (g/dL)	8.15±2.45	6.88±2.22
Red blood count (RBC - 105/mm3)	3.91±1,62	4.41±1.78
Mean cell volume (MCV - fL)	71.84±34.52	60.84±19.71
Mean corpuscular hemoglobin (MCH - pg)	23.45±11.07	17.12±5.62
Mean corpuscular hemoglobin concentration (MCHC - g/dL)	29.72±3.82	28.39±3.52
White blood count (WBC - 103/mm3)	6.59±3.87	6.38±3.78
Heterophils (/mm3)	5128.33±3402.37	4996.79±3169.96
Eosinophils (/mm3)	105.0±108.67	211.43±246.07
Lymphocytes (/mm3)	789.44±550.18	938.75±744.39
Monocytes (/mm3)	339.33±267.5	424.46±369.81
Thrombocytes (103/mm3)	14.87±8.60	15.82±7.71
Plasma proteins (g/dL)	4.63±1.24	5.18±0.91

DISCUSSION

The sea turtles studied were young, since Ubatuba is a region rich in algae. Young and adult green turtles are basically herbivore; the sexually mature animals habit the northeast areas of the country, where reproduce. Ubatuba is not a typical reproductive place for these animals because of the characteristics of the beaches, acting as a feeding base. Animals with fibropapillomas showed a slightly higher average of CCL that animals not affected by fibropapillomatosis (37.97cm and 44.49cm respectively). This result is consistent with the hypothesis of Adnyana et al. (1997), that older animals (and therefore larger) are more affected because they have more time to develop the disease. The same result was founded by others authors (Limpus & Miller 1990), in Australia, (Murakawa et al. 2000), in Hawaii, (Work & Balaz 1999a) and Foley et al. (2005), in USA.

In histopathological evaluation, the characteristics observed were similar to those described in previous works (Jacobson et al. 1989, Herbst et al. 1999, Work et al. 2004, Greenblatt et al. 2005). Regarding the number of tumors, it appears that animals are usually affected by multiple formations. Only one animal (turtle number 45) had two tumors. Work et al. (2004) also founded that the turtles presents multiple formations, with one individual presenting 120 tumors. Regarding size, we founded that the majority of formation (44.31%) was very small, less than 1 cm in diameter. This may be explained by the age of these animals. Because they are young, the turtles have such formations recently, and probably not yet occurred its development and progression. Work and Balaz (1999) founded the same results in Hawaii, where the turtles were more affected by small formations (less than 1cm).

Trematodes have already been suggested as a possible etiologic agent of green turtle fibropapillomatosis. However, the absence of spirorchid ova in experimentally induced fibropapillomas suggests that these are incidental findings in spontaneous tumors (Herbst et al 1999). The intense cardiovascular infection by trematodes debilitates the host and could reduce its defense against fibropapillomatosis (Adnyana et al. 1997, Aguirre 1998). The trematodes in this study suggest that turtles from the Brazilian coast may also be suffering this factor of immunosuppression.

Macroscopic studies were compatible with the existing on fibropapillomatosis (Jacobson et al. 1989, Herbst et al. 1999, Greenblatt et al. 2005). Solitary or multiple tumors were found in all regions of the body, including lines of suture adjacent to the dermal shields. The formations are described by other authors in the same way in the turtles included in this work (Jacobson et al. 1989, Norton et al. 1990, Aguirre et al. 1994, 1998, 2002, Brooks et al. 1994, Adnyana et al. 1997, Herbst et al. 1999, Work et al. 2004).

It is important to emphasize that there are many factors that can affect the values of the hematological profile and consequently the cellular function of sea turtles, such as anatomic site of venipuncture; age of the turtles; mechanism of thermoregulation, sex (McArthur et al. 2004). However, the interference of these factors was not evaluated in this work.

The analysis of hematological values obtained shows that, with respect to the erythrocyte series, the animals affected by fibropapillomatosis have lower values of hematocrit, hemoglobin, MCV, MCH and MCHC when compared with those who do not have the disease. These parameters define a framework of microcytic and hypochromic anemia. However, only the differences between the values of MCH were statistically significant in comparison of animals affected or not by fibropapillomatosis. An anemia is referred to as being microcytic when erythrocytes are small than normal; and hypochromic when cells contain a less--than-normal hemoglobin concentration (Thrall 2004). Microcytic and hypochromic anemia almost result from iron deficiency. The lower value of MCH shows a lack of hemoglobin in animals affected by the disease, which may be deficiency of oxygen to the tissues, resulting in lower activity. The papillomas could prejudice the animal's food intake, predisposing the affected turtles to a nutritional deficiency.

Studies with green turtles affected by fibropapillomatosis indicated that these animals showed lymphopenia, eosinopenia, basophilopenia, heterophilia, monocytosis and hypoproteinemia (Norton et al. 1990, Cray et al. 2001). Similar results were obtained for Work & Balazs (1999b) in the region of Hawaii, which observed lymphopenia, eosinopenia and heterophil progressive increase as the severity of the disease. The number of basophiles showed little variation and monocytes were found in greater numbers in animals most affected by fibropapillomatosis. The monocytosis may be attributed to chronic inflammation caused by the severity of the tumor. The heterophilia and lymphopenia characterize a typical white blood cell count of stress. In addition, affected animals may have non-regenerative anemia can also be caused by infection with hemoparasitas, including *Haemogregarina* sp. (Adnyana et al. 1997, Work & Balazs 1999a).

In relation to leukocyte number, it was not observed white blood characteristic of stress in animals with fibropapillomatosis (heterophilia with lymphopenia, and monocytosis eosinopenia variable) reported in the literature. The studied animals were young and the majority of formation was very small, less than 1cm in diameter. These small formations could not induce an intense and acute inflammatory response, as do the great and ulcerated ones, and so the heterophilia was not seen. Chronic inflammation leads to monocytosis, with increasing tumor size (Thrall 2004), what probably did not occur here, since the animals were young and presented early formations.

CONCLUSIONS

Macroscopic and microscopic features of tumor formation were very similar to those described in other papers. The tissue sections showed a tumor of epithelial origin and benign character.

The blood of turtles affected by fibropapillomatosis showed microcytic hypochromic anemia. With respect to white blood cell count, there was no typical leukocyte profile of the animals with fibropapillomatosis. Thus, for the animals of this study, the white blood cells did not contribute to the differentiation of animals with or without the disease.

The fibropapillomatosis remains poorly elucidated despite numerous studies worldwide. This situation is worrying, since this disease is considered one of the causes of population reduction of *Chelonia mydas*. Young turtles affected often do not survive to adult age and therefore no longer contribute to the perpetuation of the species.

The growing environmental imbalance is responsible for a major loss of habitat and this is the main cause of extinction of species. Diseases possibly related to pollution, such as the fibropapillomatosis, provide a more relevant factor for the reduction of animal population and consequently worsening the imbalance in the ecosystem.

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REFERENCES

Adnyana W., Ladds P.W. & Blair D. 1997. Observations of fibropapillomatosis in green turtles (*Chelonia mydas*) in Indonesia. Aust. Vet. J. 75(10):737-742.

Almeida P.A., Santos A.J.B., Thomé J.C.A., Belini C., Baptistotte C., Marcovaldi M.A., Santos A.S., Lopez M. 2011. Avaliação do estado de conservação da tartaruga marinha *Chelonia mydas* (Linnaeus, 1758) no Brasil. Revta Biodiv. Brasil 1(1):12-19. Disponível em <http://www.icmbio.gov.br/revistaeletronica/index.php/BioBR/article/view/87>. Acesso em: 3 set. 2013.

Aguirre A.A., Balaz G.H., Zimmerman B. & Spraker T.R. 1994. Evaluation of Hawaiian green turtles (*Chelonia mydas*) for potential pathogens associated with fibropapillomas. J. Wildl. Dis. 30(1):8-15.

Aguirre A.A., Spraker T.R., Balaz G.H. & Zimmerman B. 1998. Spirorchidiasis and fibropapillomatosis in green turtles from Hawaiian Islands. J. Wildl. Dis. 34(1):91-98.

Aguirre A.A., Balaz G.H., Spraker T.R., Murakawa S.K.K. & Zimmerman B. 2002. Pathology of oropharyngeal fibropapillomatosis in green turtles *Chelonia mydas*. J. Aquatic Anim. Health 14:298-304.

Balazs G.H. 1991. Current status of fibropapillomas in the Hawaiian green turtle, *Chelonia mydas*, p.47-57. In: Balazs G.H. & Pooley S.G. (Eds), Research Plan for Marine Turtle Fibropapilloma. U.S. Department of Commerce, National Oceanographic and Atmospheric Administration, National Marine Fisheries Service. NOAA-TM-NMFS-SWFSC 156.

Baptistotte C., Scalfone J.T., Gallo B.M.G., Santos A.S., Castilhos J.C., Lima E.H.S.M., Bellini C. & Barata P.C.R. 2001. Prevalence of sea turtle fibropapillomatosis in Brazil. Proceedings of the Twenty-first Annual Symposium on Sea Turtle Biology and Conservation. National Oceanographic and Atmospheric Administration, NOAA Tech. Memo. NMFS-SEFSC, Pennsylvania U.S. Dept Commerce, Philadelphia, p.24-28.

Baptistotte C. 2007. Caracterização espacial e temporal da fibropapilomatose em tartarugas marinhas da costa brasileira. Tese de Doutorado em Ecologia Aplicada, Escola Superior de Agricultura "Luiz de Queiroz", Centro de Energia Nuclear na Agricultura, Universidade de São Paulo, São Paulo. 63p.

Brill R.W., Balazs G.H., Holland K.N., Chang R.K.C., Sullivan S. & George J.C. 1995. Daily movements, habitat use, and submergence intervals of normal and tumor-bearing juvenile green turtles (*Chelonia mydas*) within a foraging area in the Hawaiian Islands. J. Exp. Marine Biol. Ecol. 185:203-218.

Brooks D.E., Ginn P.E., Miller T.R., Bramson L. & Jacobson E.R. 1994. Ocular fibropapillomas of green turtles (*Chelonia mydas*). Vet. Pathol. 31:335-339.

Campbell T.W. 1996. Clinical pathology, p.248-257. In: Mader D.R. (Ed.), Reptile Medicine and Surgery. W.B. Saunders, Philadelphia, PA.

Cray C., Varella R., Bossart G.D. & Lutz P. 2001. Altered in vitro immune responses in green turtles (*Chelonia mydas*) with fibropapillomatosis. J. Zoo Wildl. Med. 32 (4):436-440.

Ehrhart L.M. 1991. Fibropapillomas in green turtles of the Indian River lagoon, Florida: distribution over time and area. National Oceanographic and Atmospheric Administration (NOAA-TM-NMFS-SWFSC) 156:59-61.

Ene A., Su M., Lemaire S., Rose C., Schaff S., Moretti R., Lenz J. & Herbst L.H. 2005. Distribution of chelonid fibropapillomatosis associated herpesvirus variants in Florida: molecular genetic evidence for infection of turtles following recruitment to neritic developmental habitats. J. Wildl. Dis. 41 (3):489-497.

Foley A.M., Schroeder B.A., Redlow A.E., Fick-Child K.J. & Teas W.G. 2005. Fibropapillomatosis in stranded green turtles (*Chelonia mydas*) from the eastern United States (1980–98): trends and associations with environmental factors. J. Wildl. Dis. 41(1):29-41.

Frazier J.G. 1999. Conserving sea turtles and other natural resources. Proc. 13th Annual Symposium on Sea Turtle Biology and Conservation, Georgia, p.23-27.

Greenblatt R.J., Work T.M., Dutton P., Sutton C.A., Spraker T.R., Casey R.N., Diez C.E., Parker D., Leger J.S., Balazs G.H. & Casey J.W. 2005. Geographic variations in marine turtle fibropapillomatosis. J. Zoo Wildl. Med. 36(3):527-530.

Herbst L.H. 1994. Fibropapillomatosis of marine turtles. Annu. Vet. Fisheries Dis. 4:389-425.

Herbst L.H., Jacobson E.R., Klein P.A., Balaz G.H., Moretti R., Brown T. & Sundberg J.P. 1999. Comparative pathology and pathogenesis of spontaneous and experimentally induced fibropapillomas of green turtles (*Chelonia mydas*). Vet. Pathol. 36:551-564.

Herbst L.H. & Klein P.A. 1995. Green turtle fibropapillomatosis: challenges to assessing the role of environmental cofators. Environ. Health Persp., Washington, 103(4):27-30.

Jacobson E.R., Mansell J.L., Sundberg J.P., Hajjar L., Reichmann M.M., Ehrhart L.M., Walsh M. & Murru F. 1989. Cutaneous fibropapillomas of green turtles (*Chelonia mydas*). J. Comp. Pathol. 101:39-52.

Lackovich J.K., Brown D.R., Homer B.L., Garber R.L., Mader D.R., Moretti R.H., Patterson A.D., Herbst L.H., Oros J., Jacobson E.R., Curry S.S. & Klein P.A. 1999. Association of herpesvirus with fibropapillomatosis of the green turtle *Chelonia mydas* and the loggerhead turtle *Caretta caretta* in Florida. Dis. Aquat. Organ. 37:89-97.

Limpus C.J. & Miller J.D. 1990. The occurrence of cutaneous fibropapillomas in marine turtles in Queensland. Proc. Australian Marine Turtle Conservation Workshop, Gold Coast, Australia (R. James, compiler. Queensland Department of Environment and Heritage and Australian Nature Conservation Agency, Brisbane, p.186-188.

Martins M. & Molina F.B. 2008. Panorama geral dos répteis ameaçados do Brasil, p.327-373. In: Machado A.B.M., Drummond G.M.& Paglia A.P. (Eds), Livro vermelho da Fauna Brasileira Ameaçada de Extinção. Ministério do Meio Ambiente, Brasília.

Murakawa S.K.K., Balazs G.H., Ellis D.M., Hau S. & Eames S.M. 2000. Trends in fibropapillomatosis among green turtles stranded in the Hawaiian Islands, 1982–98. Proc. 19th Annual Symposium on Sea Turtle Conservation and Biology, South Padre Island, Texas. U.S. Dept Commerce NOAA Tech. Memo. NMFS-SEFSC 443, p.239-241.

Matushima E.R. 2003. Fibropapilomas em tartarugas marinhas: aspectos histológicos, imuno-histoquímicos e ultra-estruturais. Tese de Livre-Docente em Patologia, Faculdade de Medicina Veterinária e Zootecnia, USP, São Paulo. 111p.

Matushima E.R., Longatto-Filho A., Di Loretto C., Kanamura C.T., Gallo B. & Baptistotte C. 1999. Cutaneous papillomas of green turtles: a morphological and immunohistochemical study in Brazilian specimens. Proc. 19th Annual Symposium on Sea Turtle Conservation and Biology, South Padre Island, Texas. U.S. Dept Commerce NOAA Tech. Memo. NMFS-SEFSC 443, p.237-239.

Matushima E.R., Longatto-Filho A., Di Loretto C., Kanamura C.T., Ramos M.C.C., Sinhorini I.L. & Gallo B. 2001. Cutaneous papillomas of green turtles: a morphological, ultrastructural and immunohistochemical study in Brazilian specimens. Braz. J. Vet. Res. Anim. Sci. 38(2):51-54.

McArthur S., Wilkinson R. & Meyer J. 2004. Medicine and Surgery of Tortoises and Turtles. Blackwell Publishing, Ames, Iowa.

Natt M.P. & Herrick G.A. 1952. A new blood diluent for counting erythrocytes and leukocytes of the chicken. Poultry Sci. 31:735-738.

Norton T.M., Jacobson E.R. & Sundberg J.P. 1990. Cutaneous fibropapillomas and renal myxofibroma in a green turtle, *Chelonia mydas*. J. Wildl. Dis. 26(2):265-270.

Rosenfeld G. 1947. Método rápido de coloração de esfregaços de sangue: noções práticas sobre corantes pancromáticos e estudo de diversos fatores. Mem. Inst. Butantan 20:315-328.

Rossi S. 2007. Estudo do impacto da fibropapilomatose em *Chelonia mydas* Linnaeus, 1758 (Testudines, Cheloniidae). Dissertação de Mestrado em Ciências, Faculdade de Medicina Veterinária e Zootecnia, Universidade de São Paulo, São Paulo. 104p.

Rossi S. 2014. Avaliação da função celular de leucócitos por citometria de fluxo e a influência de bifenilos policlorados no desenvolvimento da fibropapilomatose em *Chelonia mydas* (Linnaeus, 1758). Tese de Doutorado em Ecologia Aplicada, Escola Superior de Agricultura Luiz de Queiroz, Universidade de São Paulo, São Paulo. 167p.

Thrall M.A. 2004. Veterinary Hematology and Clinical Chemistry. Lippincott Williams and Wilkins, Baltimore, Maryland.

Work T.M. & Balazs G.H. 1999a. Quantification of tumor severity and hematology in green turtles afflicted with fibropapillomatosis in the Hawaiian Islands. 19th Annual Sea Turtle Symposium, South Padre Island, Texas.

Work T.M. & Balazs G.H. 1999b. Relating tumor score to hematology in green turtles with fibropapillomatosis in Hawaii. J. Wildl. Dis. 35(4):804-807.

Work T.M., Balazs G.H., Rameyer R.A. & Morris R.A. 2004. Retrospective pathology survey of green turtles *Chelonia mydas* with fibropapillomatosis in the Hawaiian Islands, 1993-2003. Dis. Aquat. Org. 62:163-176.

Work T.M., Dagenais J., Balazs G.H., Schumacher J., Lewis T.D., Leong J.C., Casey R.N. & Casey J.W. 2009. In vitro biology of fibropapilloma-associated turtle herpesvirus and host cells in Hawaiian green turtles (*Chelonia mydas*). J. Gen. Virol. 90:1943-1950.