ECVCP Mystery Case 2021

A case of hepatic lesion in a Scorpion Mud Turtle (*Kinosternon scorpioides*)

Contributors

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Signalment

20-year-old, female Scorpion Mud Turtle (*Kinosternon scorpioides*) in an aquarium.

Specimens

- Impression smear cytology of liver.
- Histopathology of an incisional biopsy of hepatic lesion.

History and postmortem examination

This adult Turtle was found dead with no premonitory signs. The Turtle was submitted for necropsy for post-mortem examination. On gross pathology, the Turtle was in good nutritional condition and moderately dehydrated. Gross pathological examination revealed an overall hardening of the liver and the presence of large pale white-reddish tissue. There were no other significant gross findings. Impression smears and histopathology of the liver parenchyma were performed. From fresh liver tissue samples microbiological, mycological and PCR assays were negative for bacterial, fungal, Herpesvirus and Adenovirus infection.

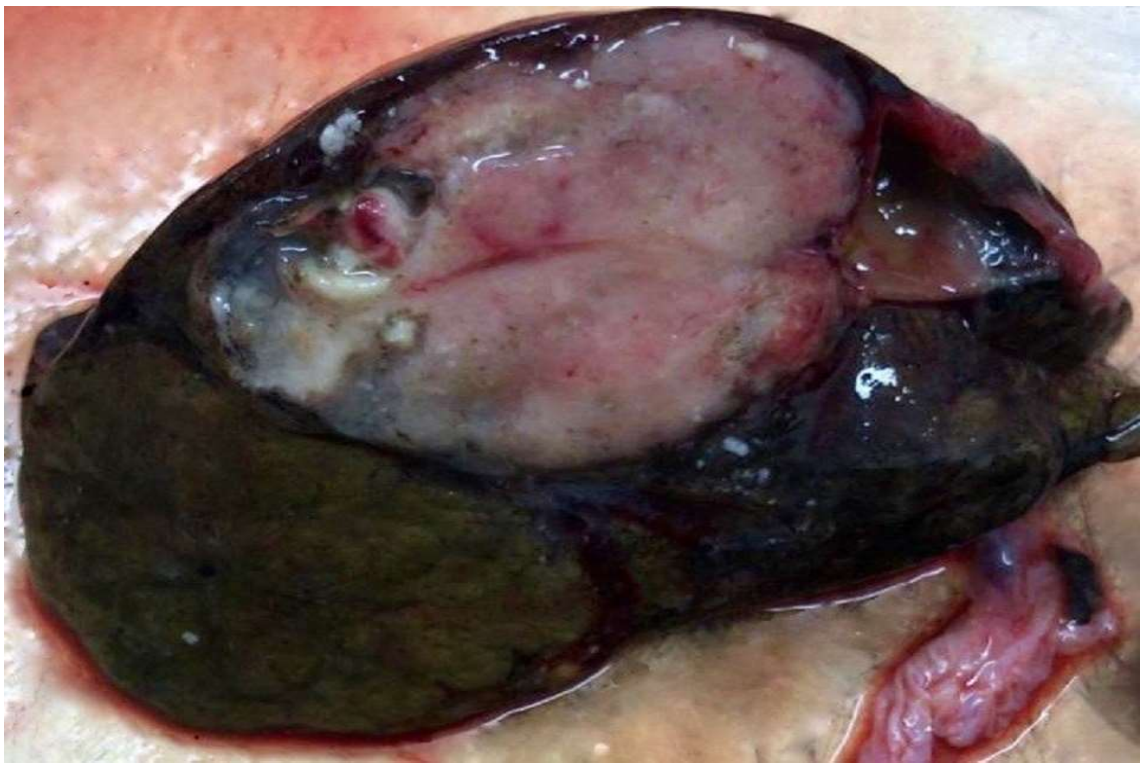

Figure 1 –Scorpion Mud Turtle (*Kinosternon scorpioides*), female, 20-year-old. Liver, gross image.

Cytological and Histological images:

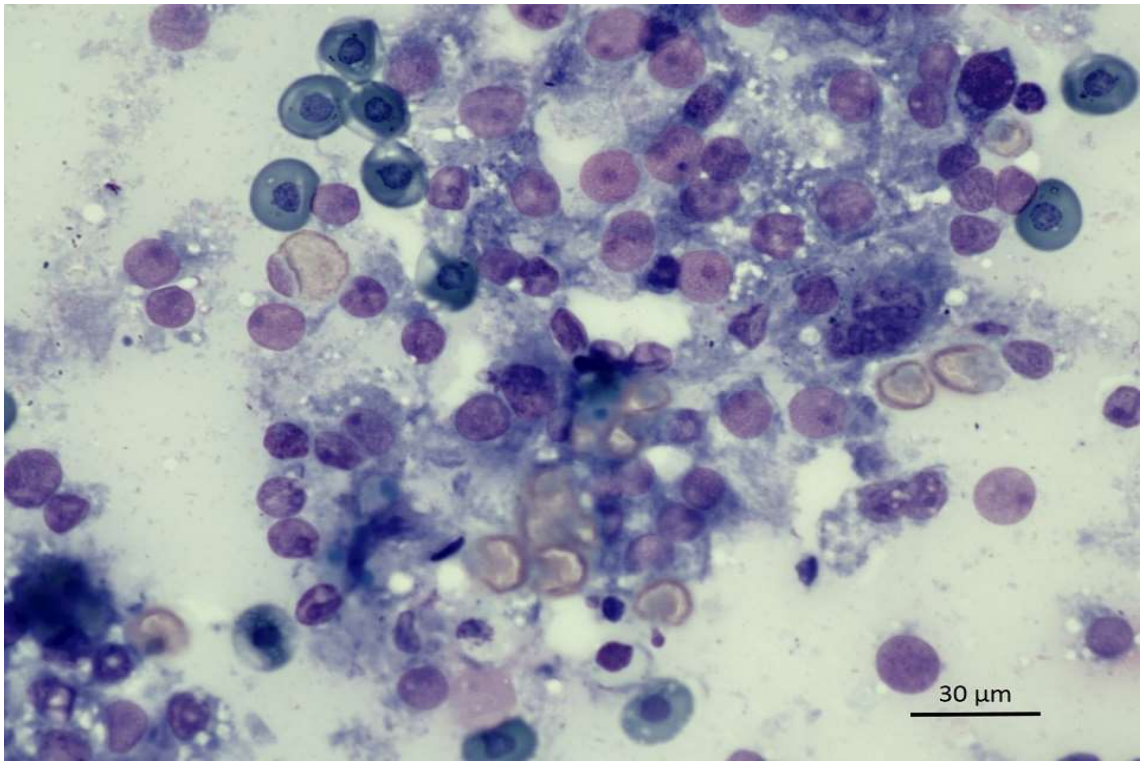

Figure 2 – Scorpion Mud Turtle (*Kinosternon scorpioides*), female, 20-year-old. Liver, impression smear, May Grünwald-Giemsa (MGG) stain, bar=30 µm.

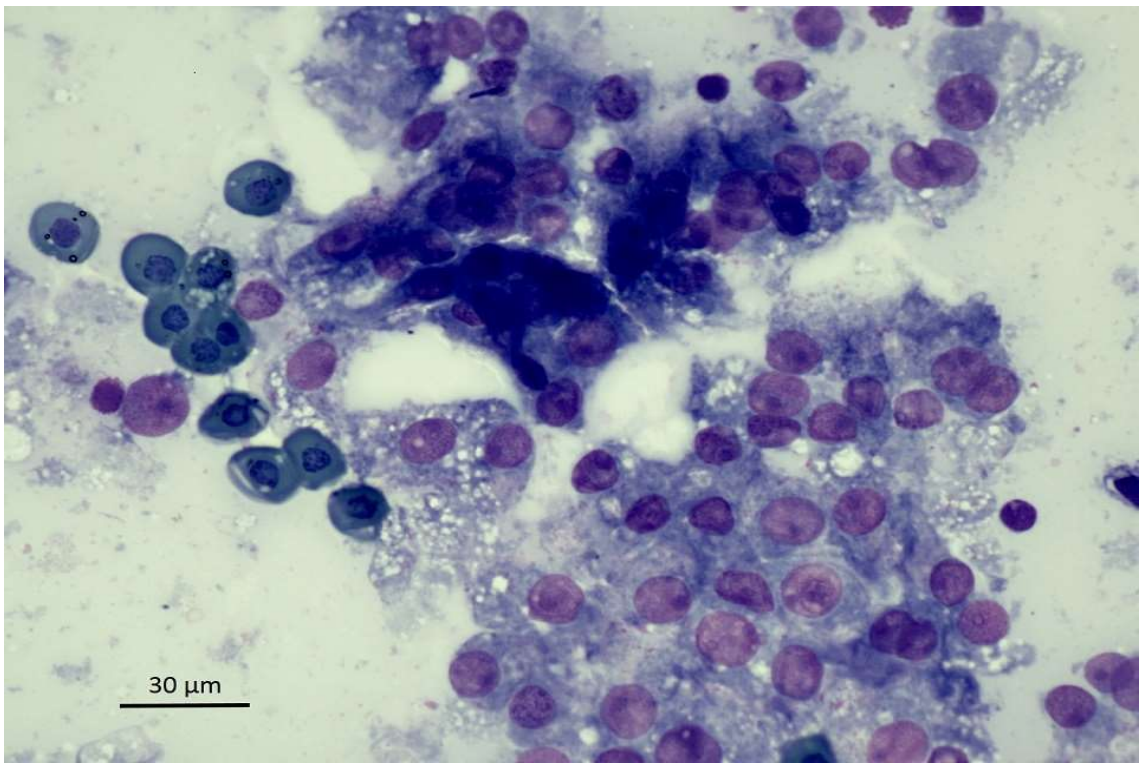

Figure 3 – Scorpion Mud Turtle (*Kinosternon scorpioides*), female 20-year-old. Liver, impression smear, MGG stain, bar=30 µm.

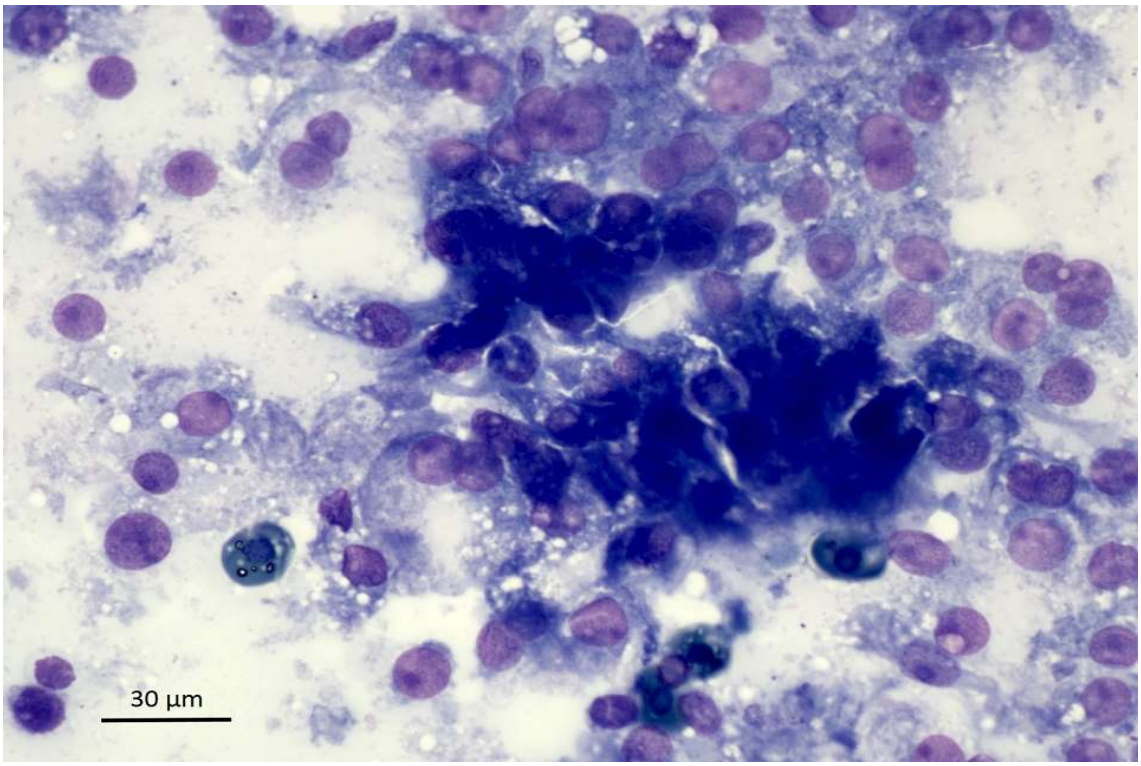

Figure 4 – Scorpion Mud Turtle (*Kinosternon scorpioides*), female, 20-year-old. Liver, impression smear, May Grünwald-Giemsa (MGG) stain, bar=30 µm.

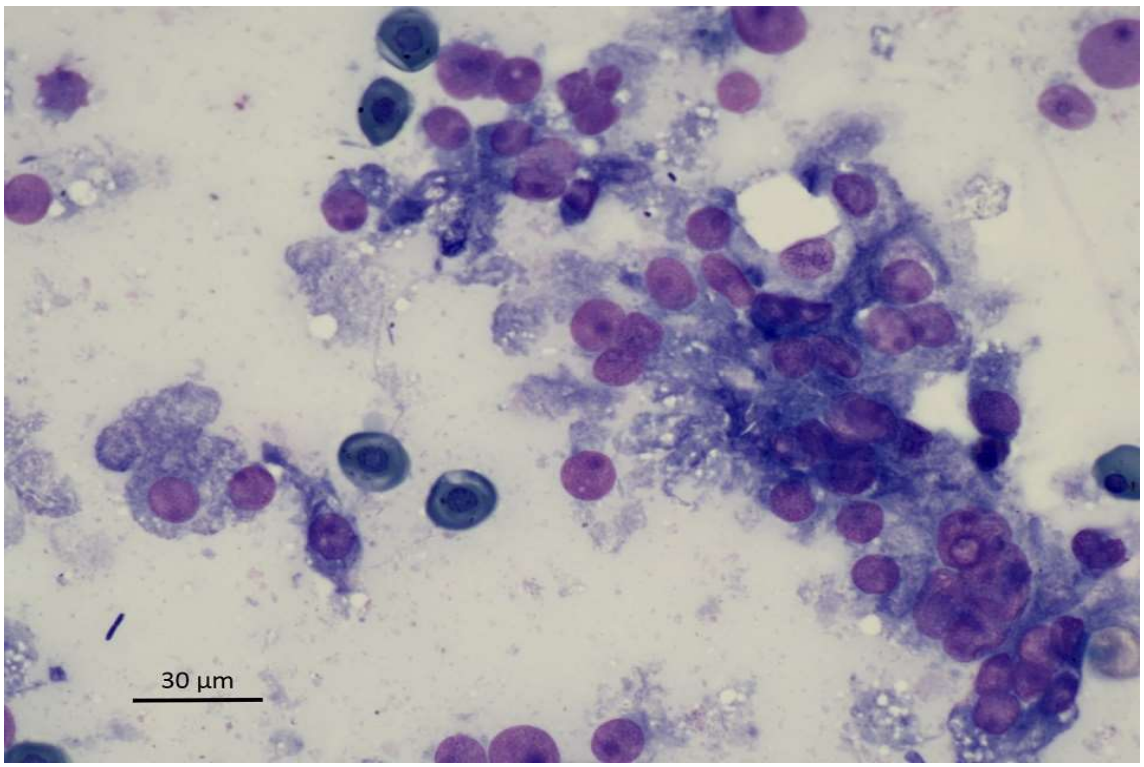

Figure 5 – Scorpion Mud Turtle (*Kinosternon scorpioides*), female, 20-year-old. Liver, impression smear. MGG stain, bar= 30 µm.

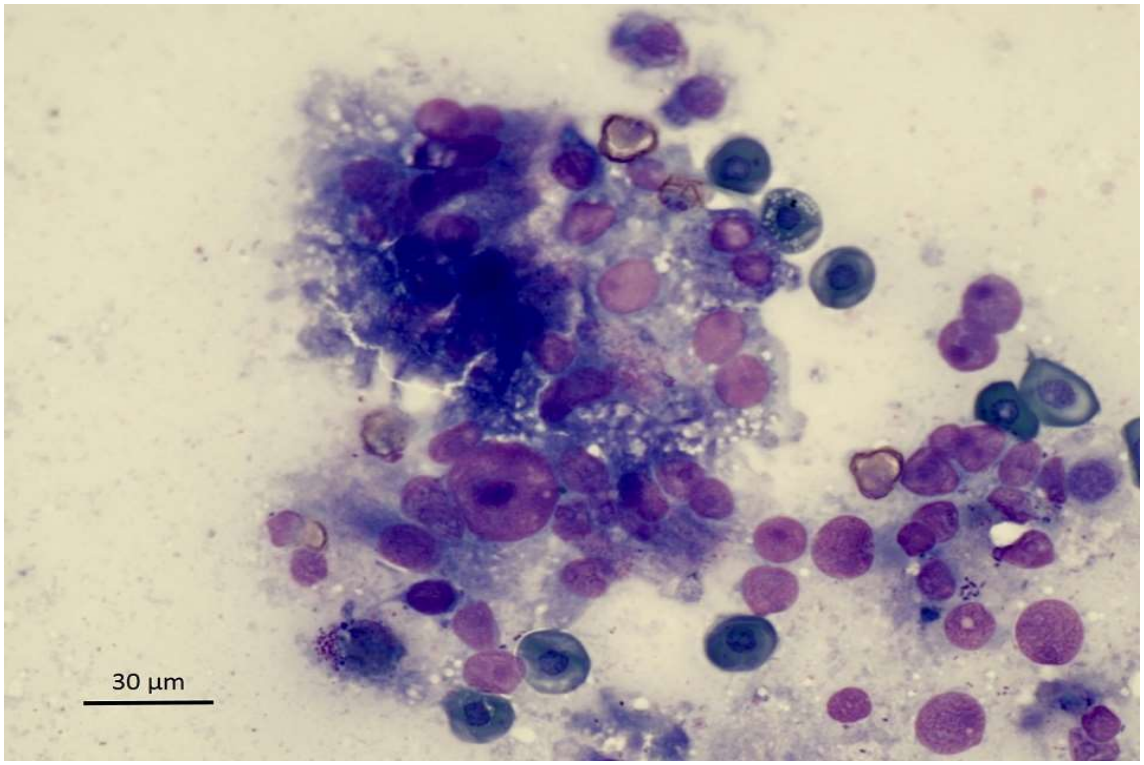

Figure 6 – Scorpion Mud Turtle (*Kinosternon scorpioides*), female, 20-year-old. Liver, impression smear. MGG stain, bar= 30 µm.

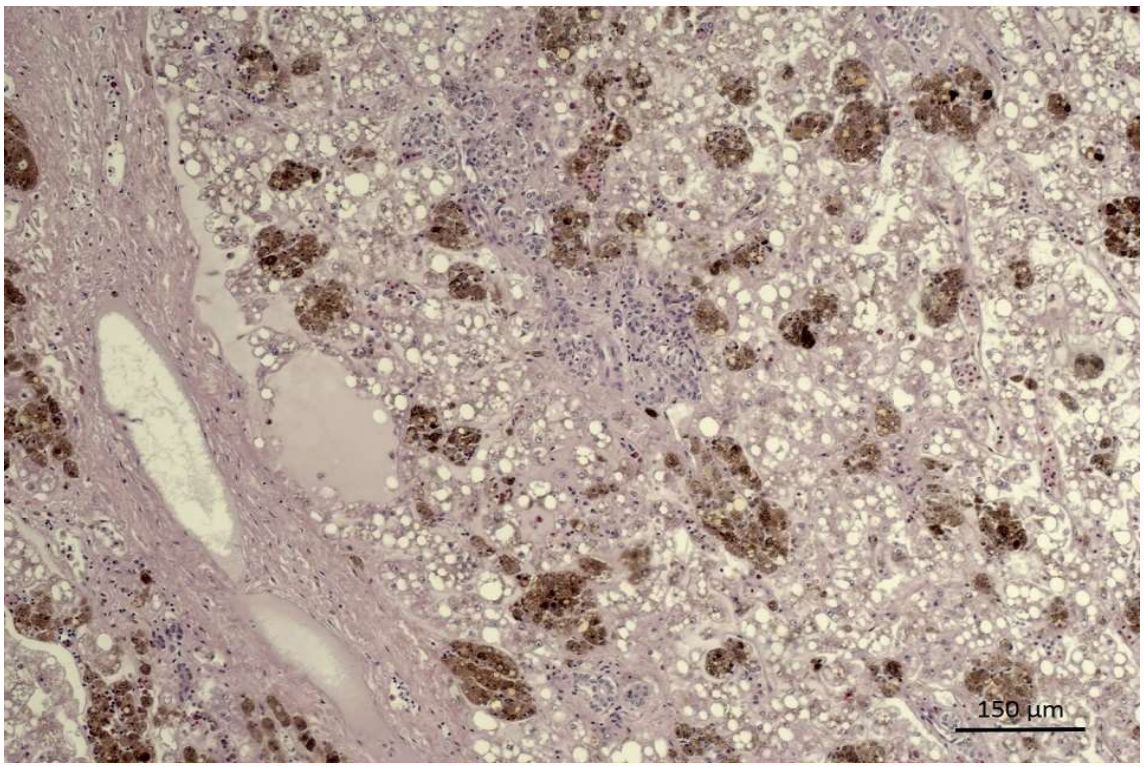

Figure 7 – Scorpion Mud Turtle (*Kinosternon scorpioides*), female, 20-year-old. Hepatic lesion, Hematoxylin and eosin (H&E) stain, bar= 150 µm.

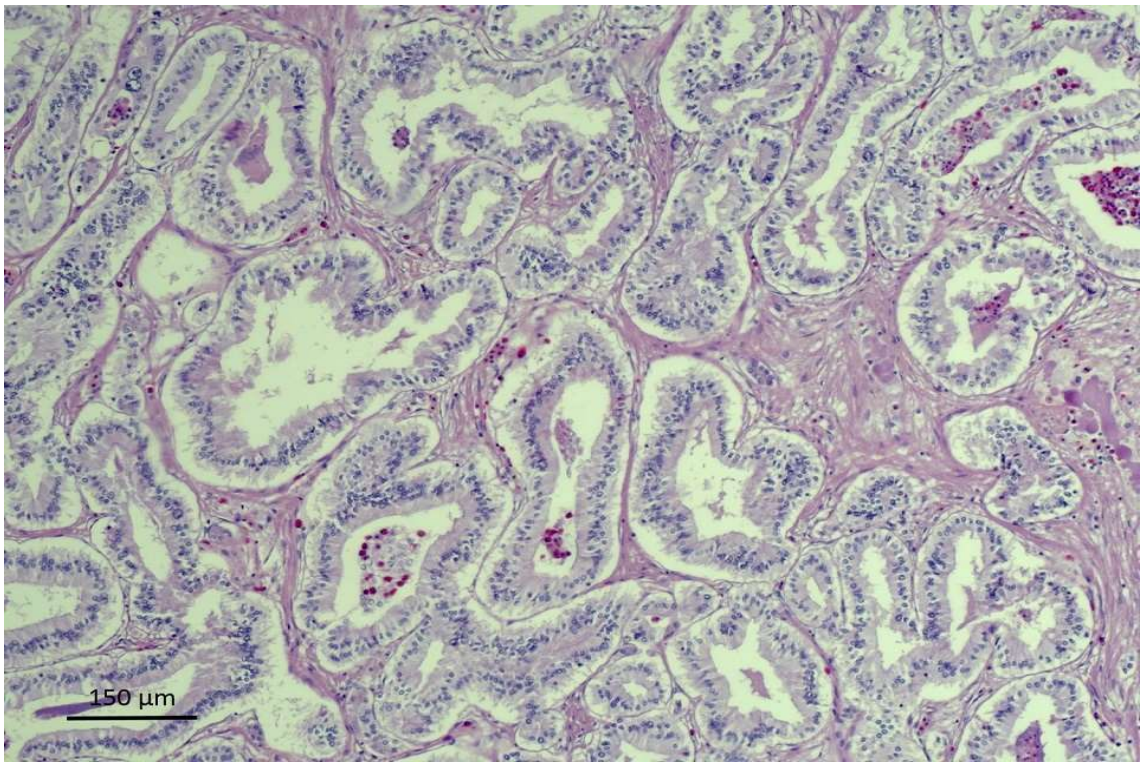

Figure 8 – Scorpion Mud Turtle (*Kinosternon scorpioides*), female, 20-year-old. Hepatic lesion, H&E stain, bar= 150 µm.

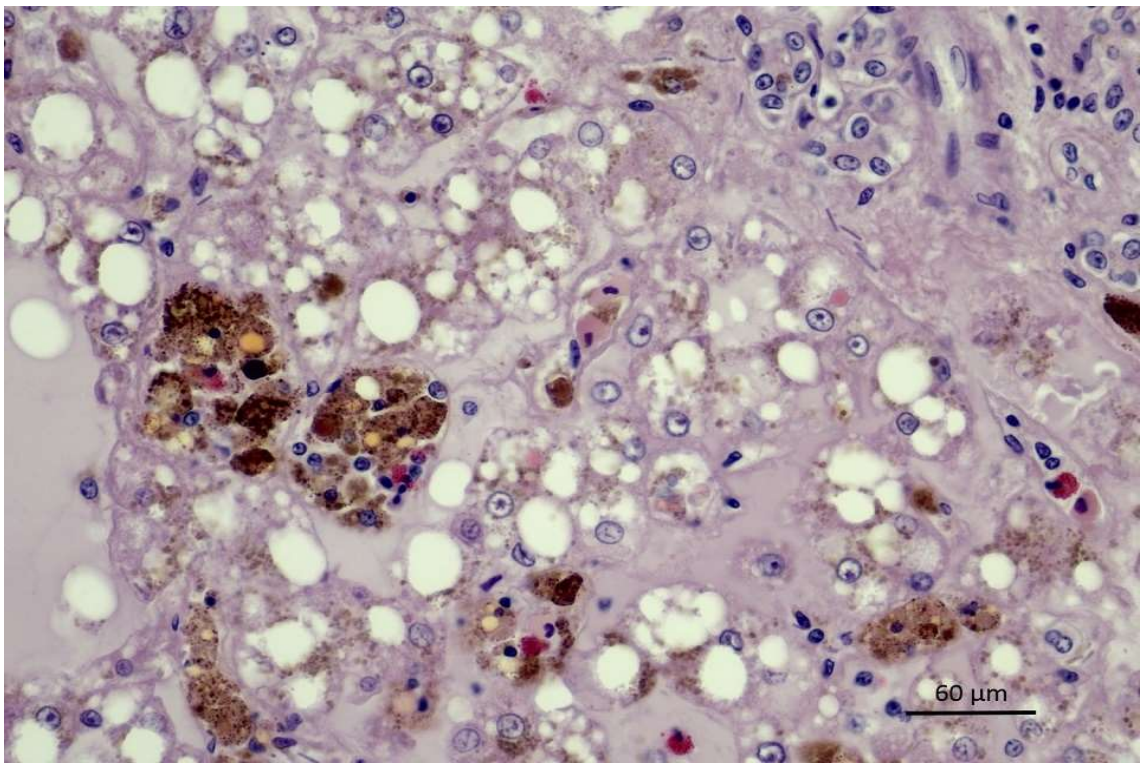

Figure 9 – Scorpion Mud Turtle (*Kinosternon scorpioides*), female, 20-year-old. Hepatic lesion, H&E stain, bar= 60 µm.

1. How could you describe the cytological and histological findings?

Cytological examination:

Hemodilution. High cellularity. Mixed population of cells with prevalence of groups of hepatocytes occasionally with indistinct cells borders, characterized by moderate/abundant amount of granular grey-bluish cytoplasm containing variable number of clear vacuoles (lipidosis), round to oval central/paracentral nucleus (15-20 µm) with reticular to fine chromatin pattern and 1-2 evident nucleoli (1-2 µm). Mild anisocytosis and anisokariosis (Figures 2, 3, 4 and 5). Many small groups of columnar cell (biliary epithelial cells) with indistinct cell borders, characterized by scant amount of granular bluish cytoplasm and basally oriented nucleus (15-20 µm) with dense granular to homogeneous chromatin and not evident nucleoli (Figures 3, 4 and 5). Moderate characters of atypia (anisocytosis and anisokariosis, macrokariosis and increased N:C ratio). No evident mitotic figures. Abundant necrotic dark blue to purple necrotic debris in the background (Figure 5). Moderate numbers of heterophils and lymphocytes (Figures 2 and 6).

Histological examination:

Histopathological examination revealed the disruption of normal liver architecture with laminar bands of fibrosis separating normal-appearing regenerative hepatocytes, occasionally with fat accumulation (lipidosis) and focal atypia (anisokariosis and prominent nucleolus) and the presence of a large neoplastic area characterized by tubular-acinar ductal structures, separated by thick fibrous stroma. The ductal structures are lined by columnar well differentiated biliary epithelial cells. The cytoplasm is pale eosinophilic with basally oriented nucleus and prominent nucleoli. The lumina of acini or ducts contain eosinophilic amorphous material (Figures 7 and 8). Many melanomacrophage aggregations (Figure 9) containing hemosiderin (Prussian Blue stain positive, Figure 10) and melanin granules (Fontana-Masson stain positive, Figure 11) hepatic necrosis and heterophilic infiltration.

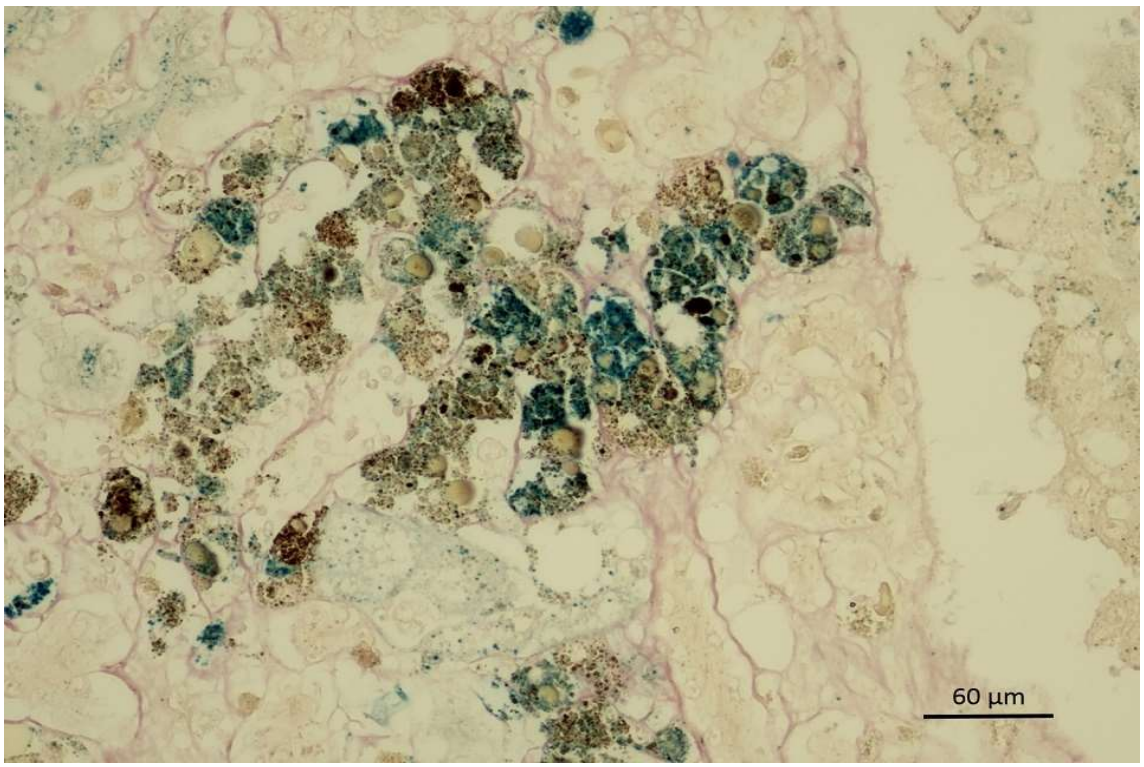

Figure 10 – Scorpion Mud Turtle (*Kinosternon scorpioides*), female, 20-year-old. Hepatic lesion, Prussian blue stain, bar= 60 µm. Presence of melanomacrophage aggregations containing Prussian Blue stain positive granules (hemosiderin).

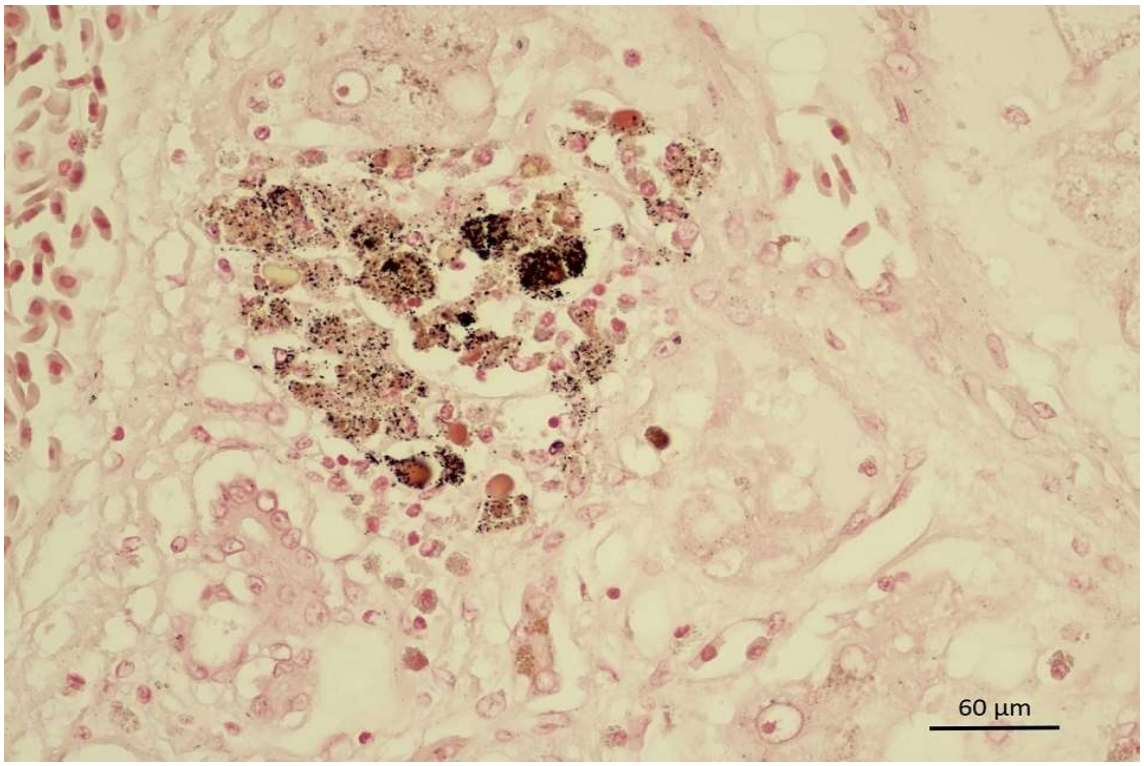

Figure 11 – Scorpion Mud Turtle (*Kinosternon scorpioides*), female, 20-year-old. Fontana Masson stain, bar= 60 µm. Presence of melanomacrophage aggregations containing Fontana-Masson stain positive granules (melanin).

2. What is your interpretation of the cytological and histological findings?

Cytological examination:
Consistent with mild to moderate heterophilic-lymphocytic hepatitis with regenerative hyperplasia of hepatocytes and biliary neoplasia.

Histological examination:
Consistent with cirrhosis and biliary adenoma "*versus*" bile duct carcinoma.

3. What are the possible differential diagnoses?
Cirrhosis and hepatobiliary neoplasia.

Discussion

Scorpion Mud Turtle (*Kinosternon scorpioides*, family *Kinosternidae*) is a medium to large-sized mud turtle with adult males in some populations generally exceeding 200 mm in carapace length. In nature, this species occurs in a variety of permanent, semipermanent and temporary aquatic habitats in Central and South America and it is primarily restricted to fresh water and/or mildly saline conditions. *Kinosternon scorpioides* is a carnivorous predator and scavenger in nature feeding on insects and their larvae, spiders, snails, worms, crabs, shrimp, fishes but also frog eggs, tadpoles, adult frogs, snake scales, bird eggshells and parts of mammals. Less frequently, this species sometimes accept plant matter such algae, fruits, nuts, seeds and flowers (Berry and Iverson, 2011).

The liver of *Kinosternon scorpioides* is located ventrally in the coelomic cavity filling almost the entire cranial portion and surrounding the pancreas, duodenum and stomach. Macroscopically, the normal liver is wide and thin with a rectangular shape and sharp borders and the parenchyma is firm and smooth. It has five lobes (two situated to the left and three to the right of the median plane) and the gall bladder is immersed in the parenchyma of the right lateral lobe (Moura et al., 2009; Moura et al., 2012). The color may range from dark-to-pale brown with black spots (which represent melanin deposits). This variation in color is dependent on lipid and glycogen deposition secondary to the contents of fat and carbohydrates in feed (Moura et al., 2009; Moura et al., 2012). Black discoloration of the parenchyma is also a common finding attributable to hypertrophy and/or hyperplasia of the melanomacrophage centers (Flint et al., 2009; Moura et al., 2009).

Melanomacrophages (MM) are normal large pigmented cells found in hematopoietic and other soft tissues of lower vertebrates and are concentrated individually or more frequently in aggregates referred as MM centers within the liver and spleen of reptiles (Christiansen et al., 1996; Johnson et al., 2005). The term “Melanomacrophages” derives from their phagocytic activity and ability to synthetize melanin. MM have diverse functions, including phagocytosis of red blood cells, foreign material, prokaryotic and eukaryotic organisms and melanin synthesis that neutralize free radicals, cations and other possible toxic agent derived from degradation of cellular material (Christiansen et al, 1996; Johnson et al., 2005). Additionally, other studies reveal that MM centers contain immunoglobulin and cytokine (granulin-1)-secreting cells (Johnson et al., 2005). The number of MM increases greatly with age as a result of a life-time of macrophage migrations to the liver and as a nonspecific response to several conditions or diseases such as emaciation, stress, chronic inflammation, and chronic bacterial infection (Christiansen et al, 1996; Flint et al., 2009). Cytologically, these cells are easily recognized by the presence of golden brown melanin pigment in the cytoplasm (Campbell TW, 2015) and with additional staining techniques (Fontana-Masson and Prussian Blue staining).

Microscopically, the liver is covered by mesothelium. Soft reticular fibers divide the parenchyma in lobules and sustain sinusoids, blood vessels and hepatocytes. Hepatocytes are arranged in tubules with laminar strings made up by two cells, as in other vertebrates. However, its structural organization differs from the mammals with these strings surrounded by twisted sinusoidal capillaries. In cross section, they resemble acini containing approximately two to five hepatocytes adjacent a probable central biliary canaliculus deposits. The hepatocytes are polyhedral in shape and variable in size with abundant frequently highly vacuolated eosinophilic cytoplasm (Hematoxylin and eosin stain) strongly PAS positive and round/oval central nucleus, shifted toward the edge. The gall bladder is composed of simple columnar epithelium underlaid by a lamina propria, although some authors report the presence of a pseudostratified epithelium in Reptiles (Moura et al., 2009; Moura et al., 2012).

Hepatic disease in Chelonians are relatively common and may range from subclinical disease reflected only by elevations of liver enzymes AST, ALT (and ALP) in serum to severe liver dysfunction/failure. Jaundice, central neurological signs and renal disease may occur secondarily depending upon the degree of liver damage (Guedes and Lavalle, 2004; Kido et al., 2017). In the majority of cases, liver disease is revealed on post mortem examination. The main hepatic lesions reported in Turtles include vacuolar hepatic degeneration (Hepatic hydropic degeneration/glycogenosis), hepatic lipidosis, acute/chronic hepatitis and/or hepatic necrosis (Guedes and Lavalle, 2004; Orós J et al., 2005; Flint et al., 2009, Campbell TW, 2015; Kido et al., 2017).

Vacuolar hepatic degeneration

Hepatic hydropic degeneration is an acute non-specific potentially reversible change characterized by an intracytoplasmic accumulation of water in hepatocytes due to the incapacity of the cells to maintain the ionic and fluid homeostasis. Several cause can lead to this injury such as inflammation/infection, heart disease or circulatory disturbances that can lead to passive hepatic chronic congestion, toxins (eg. Cadmium, Copper, Lead, Mercury, Pesticides) (Orós J et al., 2005; Flint et al., 2009; Huo et al, 2017; Huo et al, 2020). As in other species, Hepatic glycogenosis is due to the accumulation of intracytoplasmic glycogen and may be secondary to nutritional imbalance and metabolic abnormalities but it is more frequently influenced by the diet. In fact, glycogen is an important energy reserve for Turtles in several situations such as for sexual activity during the reproductive season and during hibernation in species of cold climates (Moura et al., 2009). Macro- and microscopically these changes are indistinguishable. On gross examination, the liver is enlarged, pale and soft. Microscopically, the hepatocytes are enlarged with a clear cytoplasm due to a lacey vacuolation or rarefaction and indistinct shape and limits. Histologically, arrangements of hepatic cords and hepatic sinusoids are not neat (Moura et al., 2009; Moura et al., 2012; Huo et al, 2017).

Hepatic lipidosis

Hepatic lipidosis is a relatively common lesion but poorly understood in Reptiles (Guedes and Lavalle, 2004; Orós J et al., 2005; Flint et al., 2009; Zhong et al., 2020). Hepatic lipidosis is characterized by an increased intracytoplasmic lipid deposition and can occur because of or after a number of insults including cachexia, high fat diet, toxins, nutritional imbalance and metabolic abnormalities (Guedes and Lavalle, 2004; Flint et al., 2009; Zhong et al., 2020). Macroscopically the liver may be pale yellow to light tan, swollen and highly friable (as in other species) (Guedes and Lavalle, 2004; Flint et al., 2009). On microscopic examination, hepatocytes are swollen and filled with many small or large clear cytoplasmic vacuoles, which represent the lipid that has dissolved during the staining process, and nuclei are frequently displaced to one side of the cell (Guedes and Lavalle, 2004; Campbell TW, 2015; Flint et al., 2009). Hepatic lipidosis may be macro- or microvesicular.

Acute/chronic Hepatitis

Inflammatory lesions are frequently noted in the liver of Turtles and are usually an expression of a septicemic status or systemic involvement (Orós J et al., 2005; Flint et al., 2009). Several pathogens may be involved including bacteria (eg. Escherichia coli, Proteus spp., Staphylococcus spp, Salmonella spp., Moraxella spp.), virus (eg. Adenovirus, Herpevirus), yeasts or fungi (eg. Fusarium spp.), parasites (eg. Spirorchiid eggs, Amoebiasis, larval Nematodes) (Orós J et al., 2005; Flint et al., 2009; Campbell TW, 2015; Kido et al., 2017). As in other species, hepatitis is characterized by the presence of numerous leukocytes in the cytological specimens. Based on the type and quantity of inflammatory cells, hepatitis may be further classified as heterophilic, macrophagic or mixed (Campbell TW, 2015). In Turtles, chronic granulomatous hepatitis are the most commonly observed, frequently associated with necrosis (Orós J et al., 2005; Campbell TW, 2015; Kido et al., 2017). On gross examination, yellowish-whitish lesions are scattered randomly throughout the liver (Orós J et al., 2005; Kido et al., 2017). Cytological specimens are characterized by a necrotic center surrounded by a mixed population of inflammatory cells with prevalence of vacuolated macrophages, many multinucleated giant cells and a variable number of lymphocytes, plasma cells and heterophils (Orós J et al., 2005; Campbell TW, 2015; Kido et al., 2017). In chronic lesions, fibrosis may also occur as a reparative response because of the increasing activity of fibroblasts. Cytologically, specimens are characterized by the

presence of reactive spindle cells (fibrocytes) associated with a pink amorphous collagenous stroma (extracellular matrix).

Bacteria and fungi can be found phagocytized within heterophils (which may be degenerated) and/or macrophages in addition to those that may be present extracellularly and parasites may be recognized in cytological specimens (Orós J et al., 2005; Campbell TW, 2015; Kido et al., 2017). In literature, some authors report intranuclear eosinophilic inclusions in hepatocytes of Turtles and Tortoises due to Adenovirus and Herpesvirus infection (Jacobson ER et al., 1982; Rivera S et al., 2009; Kido et al., 2017). Isolation of these pathogens using microbiological, mycotic culture and PCR are always required for a definitive diagnosis.

Hepatic necrosis

As said previously, hepatic necrosis is usually associated with hepatitis but also can be a consequence of a liver injury (especially due to toxins such as Cadmium) (Huo et al, 2017; Huo et al, 2020). Microscopically, an abundant basophilic debris in the background characterizes cytological specimens and hepatocytes appear "fuzzy" with indistinct cell outlines and definition (Orós J et al., 2005). Fibrosis may also occur as a reparative response.

Hepatic cirrhosis

The term "cirrhosis" is controversial and consensus regarding the essential features is difficult to obtain. By one definition, cirrhosis is a diffuse process characterized by the conversion of normal liver architecture into structurally abnormal regenerative nodules of hepatocytes surrounded by fibrous septa with large deposition of extracellular matrix in the perisinusoidal space and vascular disorders that often integrate both central veins and portal tracts. Cirrhosis is usually the terminal stage of several pathogenic processes characterized by necrosis or apoptosis of hepatocytes and active inflammation with chronic fibrosis (Jubb, Kennedy and Palmer's, 2016). To the author's knowledge, the literature reports only a case of hepatic cirrhosis in a red-foot tortoise (*Geochelone carbonaria*) (Guedes and Lavalle, 2004).

Hepatic neoplasia

Hepatic neoplasia may occur as a primary neoplasm of the hepatocytes or bile duct epithelium, or as a secondary neoplasia such as metastatic disease. Hepatomas and nodular regeneration cannot be differentiated based on cytology alone because both of these conditions exhibit hepatocytes that appear normal or with mild anisocytosis and anisokaryosis and hepatocellular carcinomas are often well differentiated. Neoplasia originating from the biliary epithelium (bile duct neoplasia) can also be difficult to identify because of the exfoliation of normal-appearing bile duct epithelial cells (Campbell TW, 2015).

Conclusions

As in other species, the etiology of cirrhosis is multifactorial in Turtles and the cirrhotic liver may contains a wide spectrum of lesions. In this case, in the liver were present benign and premalignant lesions. Cytological features of hepatocyte dysplasia may be mistaken for hepatocarcinoma.

The literature reports a low incidence of neoplasia in the Chelonians and to the author's knowledge, there are only two case reports of hepatic neoplasia: a biliary cystadenoma in a Piedmont Terrapin (*Pseudemys concinna*) and a multicentric lymphoblastic lymphoma in a Sea Turtle (Orós J et al., 2005; Sykes 4th JM and Trupkiewicz JG, 2006).

Bibliography

- Berry JF and Iverson JB. *Kinosternon scorpioides* (Linnaeus 1766) – Scorpion Mud Turtle. In: Conservation Biology of Freshwater Turtles and Tortoises: A Compilation Project of the IUCN/SSC Tortoise and Freshwater Turtle Specialist Group. Chelonian Research Monographs 5. Rhodin AGJ, Pritchard PCH, Van Dijk PP, Saumure RA, Buhlmann KA, Iverson JB, Mittermeier RA. Pp. 063.1-063.15, 2011
- Campbell TW. Exotic Animal Hematology and Cytology. 4° edition. Ames, Iowa: Wiley Blackwell, 2015
- Christiansen JL et al. Melanomacrophage aggregations and their age relationships in the yellow mud turtle, *Kinosternon flavescens* (Kinosternidae). Pigment Cell Res, 9(4): 185-90, 1996
- Flint M et al. Postmortem diagnostic investigation of disease in free-ranging marine turtle populations: a review of common pathologic findings and protocols. J Vet Diagn Invest, 21(6):733-59, 2009
- Guedes RMC and Lavalle GE. Hepatic cirrhosis in a red-foot tortoise (*Geochelone carbonaria*). Communication Bras Med Vet Zootec, 56(1): 123-125, 2004
- Huo J et al. Cadmium induces histopathological injuries and ultrastructural changes in the liver of freshwater turtle (*Chinemys reevesii*). Chemosphere, 186: 459-465, 2017
- Huo J et al. Effects of cadmium on the activities of ALT and AST as well as the content of TP in plasma of freshwater turtle *Mauremys reevesii*. Environ Sci Pollut Res Int, 27(15): 18025-18028, 2020
- Jacobson ER et al. Herpesvirus-like infection in map turtles. J Am Vet Med Assoc, 181(11): 1322-1324, 1982
- Johnson JC et al. Characterization of testudine melanomacrophage linear, membrane extension processes--cablepodia--by phase and atomic force microscopy. In Vitro Cell Dev Biol Anim, 41(7):225-31, 2005
- Jubb, Kennedy and Palmer's. Pathology of Domestic Animals. Volume 2. 6°edition Elsevier Saunders, St.Louis, Missouri, 2016
- Kido N et al. Hematological and pathological features of massive hepatic necrosis in two radiated tortoises (*Astrochelys radiata*). J Vet Med Sci, 79(1): 166-170, 2017
- Moura LR et al. Morphological aspects of the liver of the freshwater turtle *Phrynops geoffroanus* Schweigger, 1812 (Testudines, Chelidae). Braz J Morphol Sci, 26: 129-134, 2009
- Moura LR et al. Morphological aspects of the liver of the *Podocnemis expansa* (Testudines, podocnemididae). Journal of Morphological Sciences, 29(3): 159-166 2012
- Orós J et al. Diseases and causes of mortality among sea turtles stranded in the Canary Islands, Spain (1998-2001). Dis Aquat Organ, 63(1): 13-24, 2005
- Rivera S et al. Systemic adenovirus infection in Sulawesi tortoise (Indoestudo forsteni) caused by a novel siadenovirous. J Vet Diagn Invest, 21(4): 415-426, 2009
- Sykes 4th JM and Trupkiewicz JG. Reptile neoplasia at the Philadelphia Zoological Garden, 1901-2002. J Zoo Wildl Med, 37(1): 11-19, 2006
- Zhong Y et al. Effects of high fat diet on lipid accumulation, oxidative stress and autophagy in the liver of Chinese softshell turtle (*Pelodiscus sinensis*). Comp Biochem Physiol B Biochem Mol Biol, 240: 110331, 2020