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# Anatomopathological findings of Testudines necropsied in the Distrito Federal, Brazil[1]

Uilton G. Santos[2], Cintia R.R. Queiroz[3], Líria Q.L. Hirano[4], Maria V.B. Santos[5], Ana K.S. Cavalcante[5], Juliana T.S.A. Macêdo[3] and Pedro M.O. Pedroso[2,3]*

**ABSTRACT.-** Santos U.G., Queiroz C.R.R., Hirano L.Q.L., Santos M.V.B., Cavalcante A.K.S., Macêdo J.T.S.A. & Pedroso P.M.O. 2022. **Anatomopathological findings of necropsied Testudines in the Distrito Federal, Brazil.** *Pesquisa Veterinária Brasileira 42:e06953, 2022.* Laboratório de Patologia Veterinária, Fundação Universidade de Brasília, Campus Universitário Darcy Ribeiro, Via L4 Norte s\n, Brasília, DF 70910-970, Brazil. E-mail: pedrosovet@yahoo.com.br

In order to determine the main anatomopathological findings of Testudines necropsied in the Distrito Federal, all necropsy records performed at the "Laboratório de Patologia Veterinária" of the "Universidade de Brasília" (LPV-UnB) on Testudines during the period from January 2008 to July 2020 were reviewed. The 72 cases reviewed were grouped and classified according to species, sex, origin, season of occurrence, and diagnosis. In 69.44% of the cases the species was informed in the necropsy protocols, which included *Phrynops geoffroanus* (38%), *Trachemys dorbigni* (36%), *Chelonoidis carbonaria* (14%), *Chelonoidis denticulata* (10%) and *Podocnemis expansa* (2%). In 30.55% of the cases this parameter was not informed and were classified only as Testudines. In 41.66% of the cases the sex was informed, being female 22.22%, male 19.44%, and 58.33% were not informed. Of these animals 79.16% were from environmental agencies and 20.84% from zoos and/or guardians. In 70.83% of the animals analyzed they were directly related to the autumn and winter seasons, with June being the most frequent month (29.17%). The conclusive diagnosis was possible in 68.05% of the cases. The category of disorders caused by injurious agents (48.97%) was the most prevalent, followed by inflammatory disorders (32.65%) and nutritional and metabolic disorders (28.57%). The main diagnoses were carapace and/or plastron fracture with 30.61%, hepatic steatosis (20.40%) and pneumonia (10.22%). Most cases of carapace or plastron fracture and hepatic steatosis occurred in animals from environmental agencies.

INDEX TERMS: Anatomopathology, necropsy, Testudines, Brazil, reptiles, hepatic lipidosis, pneumonia, fracture.

**RESUMO.-** [**Achados anatomopatológicos de Testudines necropsiados no Distrito Federal, Brasil.**] Com o objetivo de determinar os principais achados anatomopatológicos de Testudines necropsiados no Distrito Federal, foram revisadas todas as fichas de necropsia realizadas no Laboratório de Patologia Veterinária da Universidade de Brasília (LPV-UnB) em Testudines, durante o período de janeiro de 2008 a julho de 2020. Os 72 casos revisados foram agrupados e classificados quanto à espécie, sexo, procedência, estação do ano de ocorrência e diagnóstico. Em 69,44% dos casos havia a espécie informada nos protocolos de necropsia, que incluíam *Phrynops geoffroanus* (38%), *Trachemys dorbigni* (36%), *Chelonoidis carbonaria* (14%), *Chelonoidis denticulata* (10%) e *Podocnemis expansa* (2%). Em 30,55% dos casos não tiveram esse parâmetro informado e foram classificados apenas como Testudines. Em 41,66% casos foi informado o sexo, sendo fêmea 22,22%, macho 19,44% e não informados 58,33%. Destes animais 79,16% eram de órgão ambiental e 20,84% de zoológicos e ou tutores. Em 70,83% dos animais analisados tiveram direta relação com as estações de outono e inverno, sendo o mês de junho o mais frequente (29,17%).

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[2] Graduate Program in Animal Sciences in the Tropics (PPGCAT), Faculdade de Medicina Veterinária e Zootecnia, Universidade Federal da Bahia (UFBA), Av. Adhemar de Barros 500, Ondina, Salvador, BA 40170-110, Brazil.

[3] Laboratório de Patologia Veterinária, Universidade de Brasília (UnB), Campus Universitário Darcy Ribeiro, Via L4 Norte s/n, Brasília, DF 70910-970, Brazil. *Corresponding author: pedrosovet@yahoo.com.br

[4] Clínica e Cirurgia de Animais Silvestres, Hospital Veterinário, Universidade de Brasília (UnB), Campus Universitário Darcy Ribeiro, Via L4 Norte s/n, Brasília, DF 70910-970, Brazil.

[5] Laboratório de Estudos em Morfofunção Animal, Prédio de Biologia "Elismar V. Adorno", Centro de Ciências Agrárias, Ambientais e Biológicas, Universidade Federal do Recôncavo da Bahia (UFRB), Rua Rui Barbosa 710, Campus Universitário, Cruz das Almas, BA 44380-000, Brazil.

O diagnóstico conclusivo foi possível em 68,05% dos casos. A categoria de distúrbios causados por agentes lesivos (48,97%) foi a mais prevalente, seguido por distúrbios inflamatórios (32,65%) e dos distúrbios nutricionais e metabólicos (28,57%). Os principais diagnósticos foram fratura de carapaça e ou plastrão com 30,61%, esteatose hepática (20,40%) e pneumonia (10,22%). A maior parte dos casos de fratura de carapaça ou plastrão e de esteatose hepática ocorreram em animais provenientes de órgão ambiental.

TERMOS DE INDEXAÇÃO: Anatomopatologia, Testudines, necropsia, Brasil, répteis, lipidose hepática, pneumonia, fratura.

## INTRODUCTION

The order Testudines (terrapins, jabutis, and sea turtles) represents a group of reptiles of the subclass Anapsida, whose specimens do not present temporal apertures (Gaffney 1984, Ferreira & Werneburg 2019) and are distinguished from other animals by presenting a unique morphology (Gaffney & Meylan 1988). These animals have the spinal column attached to the carapace, with the presence of dermal bones that surround much of the body, forming a bony framework with the carapace in the dorsal region, and the plastron ventrally. They are subdivided into two suborders, the Cryptodira and the Pleurodira (Gaffney & Meylan 1988, Guillon et al. 2012, Fritz & Havaš 2013).

Retrospective studies that sought to determine the causes of death in wild animals have been conducted over the years and are widely found in the literature (Sanches 2008, Batista et al. 2010, Vanstreels et al. 2011, Dutra et al. 2012, Sharma et al. 2014, Navas-Suárez et al. 2016, Gomes et al. 2017, Tremori et al. 2018, Fósse et al. 2020, Rocha 2020). However, there remains a shortage of retrospective studies performed in the laboratory aiming to diagnose possible diseases causing deaths in Testudines (Jacobson 1994, Orós et al. 2005, Medina et al. 2013, Assis & Caldara 2016). Thus, the objective of this work was to perform a retrospective study and verify the main anatomopathological alterations of necropsied Testudines in the routine of the "Laboratório de Patologia Veterinária" (Laboratory of Veterinary Pathology) of the "Universidade de Brasília" (LPV-UnB) in the period from January 2008 to July 2020.

## MATERIALS AND METHODS

All the necropsy records of Testudines performed at the "Laboratório de Patologia Veterinária" (Laboratory of Veterinary Pathology) of the "Universidade de Brasília" (LPV-UnB), during the period from January 2008 to July 2020, were reviewed. From the records, data were compiled, with grouping and classification as to species, sex, origin (environmental agencies, zoos, guardian), season of occurrence (autumn-winter, April to August or spring-summer, September to March). Based on necropsy results, the diagnoses were analyzed and classified into conclusive, inconclusive, and autolysed. The conclusive cases were divided into four groups: injurious agents, inflammatory diseases, nutritional and metabolic disorders. When morphological alterations were not enough to establish the cause of death, the cases were considered inconclusive or established as autolysed, due to the state of preservation of the corpse, which presented cadaveric emphysema and colliquation. When necessary, a survey of the laminar, paraffin blocks, formalin-embedded material and the photographic collection was carried out. The histology was routinely processed and stained with hematoxylin and eosin (HE).

## RESULTS

In the interval of twelve years, 72 necropsies of Testudines were accounted for. In 50 (69.44%) cases the species reported in the necropsy protocols was present, which included: Brazilian terrapin (*Phrynops geoffroanus* - 19/50, 38%), water tiger (*Trachemys dorbigni* - 18/50, 36%), jabuti-piranga (*Chelonoidis carbonaria* - 7/50, 14%), jabuti-tinga (*Chelonoidis denticulata* - 5/50, 10%), and Amazonian turtle (*Podocnemis expansa* - 1/50, 2%). In 22 (30.55%) cases this parameter was not reported, they were classified only as Testudines. In 41.66% (30/72) of the cases the sex was informed, being female 22.22% (16/72), male 19.44% (14/72) and not informed 58.33% (42/72).

Regarding the origin, 79.16% (57/72) of the Testudines submitted to necropsy were from environmental agencies and 20.84% (15/72) were from zoos and/or guardians. As for the environmental agency, the cases were from the "Centro de Triagem de Animais Silvestres" (CETAS - Wild Animal Sorting Center). This agency is responsible for receiving, screening, rehabilitating, and destining wild animals rescued and seized by law enforcement agencies and spontaneous deliveries by private individuals.

As for the season in which the cases occurred, 70.83% of the necropsies occurred in autumn-winter, with the months of July (29.17%) and August (18.05%) being the most frequent. The distribution of the number of necropsies according to the months of the year are shown in Figure 1.

Of the total cases evaluated in this study, 68.05% (49/72) had a conclusive diagnosis, in 20.83% (15/72) inconclusive and in 11.11% (8/72) were autolysed. Among the cases in which the diagnosis was classified as conclusive, disorders caused by injurious agents were the most frequent with 48.97% (24/49), followed by inflammatory disorders 32.65% (16/49) and nutritional and metabolic disorders 20.40% (10/49). The main categories of diagnoses can be seen in Figure 2.

Of the disorders caused by injurious agents the most frequent diagnosis was carapace fracture (Fig.3) and or plastron with 30.61% (15/49).

Of the inflammatory disorders pneumonia accounted for 10.22% (5/49). Three cases had as an entry point for multisystemic infection, the complete fracture of the carapace.

Among the nutritional and metabolic disorders, hepatic steatosis was the alteration found in 20.40% of the cases (10/49). The liver appeared with a diffuse pale yellowish

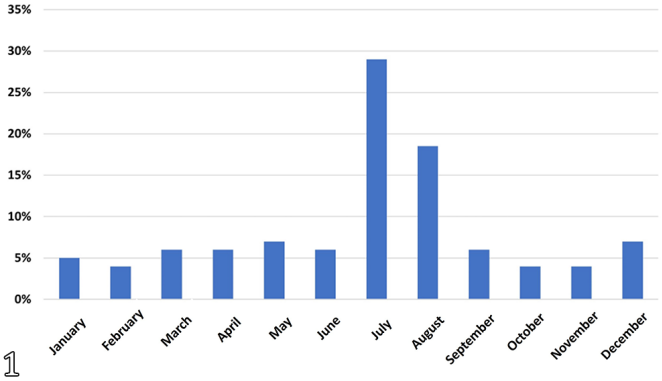


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Fig.1. Distribution of the number of necropsies performed on Testudines according to the months of the year.

coloration, with rounded edges on the diaphragmatic and celomatic surfaces (Fig.4-5). Microscopically, the hepatocytes were diffusely expanded by intracytoplasmic macro- and microvacuoles and the nucleus was displaced to the periphery (Fig.6).

Most cases of carapace or plastron fracture and hepatic steatosis occurred in animals from environmental agencies, corresponding to 80% (12/15) and 70% (7/10) respectively. The prevalence of conclusive diagnostic categories in Testudines can be seen in Table 1.

## DISCUSSION

This study provides epidemiological data and an overview of the main anatomopathological alterations observed in necropsied Testudines at the LPV-UnB from 2008 to 2020. Of the Testudines represented in this study, only three (*Phrynops geoffroanus*, *Chelonoidis carbonaria* and *Chelonoidis denticulatus*) of the five species occur naturally in the Distrito Federal and surrounding areas. For the other two species (*Trachemys dorbigni* and *Podocnemis expansa*) there is no description for the region (Costa & Bérnils 2018). *T. dorbigni* is a species with natural distribution restricted only to the southern region of Brazil, Uruguay and northern Argentina, thus in other regions of the country, including the Distrito Federal, it is described as an exotic species (Santos et al. 2020). *P. expansa*, popularly known as the Amazon turtle, has wide distribution in Colombia, Venezuela, northeastern Peru, eastern Ecuador and northern Bolivia. In Brazil the species occurs throughout the north region, reaching the limits of the states of Tocantins and Goiás (Rhodin et al. 2017, Costa & Bérnils 2018) so that for the Distrito Federal this animal came from a zoo.

Fractures of the carapace and/or plastron caused by harmful agents (mechanical energy) were the most frequent diagnoses in necropsies of Testudines. According to Barten (2006) and Melidone & Selleri (2008), traumatic alterations that caused shell fractures in chelonians were the most representative in their studies, having as consequences automobile accidents, bites by predatory animals, and falls. Orós et al. (2005) also

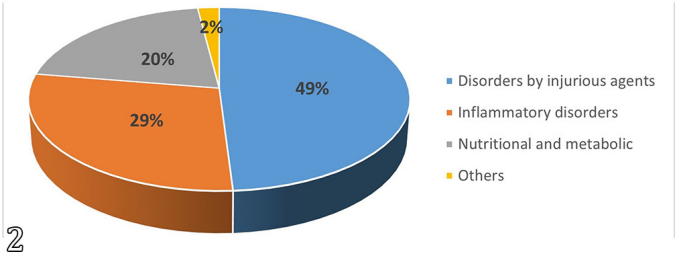


Fig.2. Percentage of different conclusive diagnoses in the Veterinary Pathology Laboratory of the "Universidade de Brasília" (LPV-UnB) from 2008 to 2020.


Fig.3. Water Tiger (*Trachemys dorbigni*). Dorsal view with carapace fracture in the cranial portion.

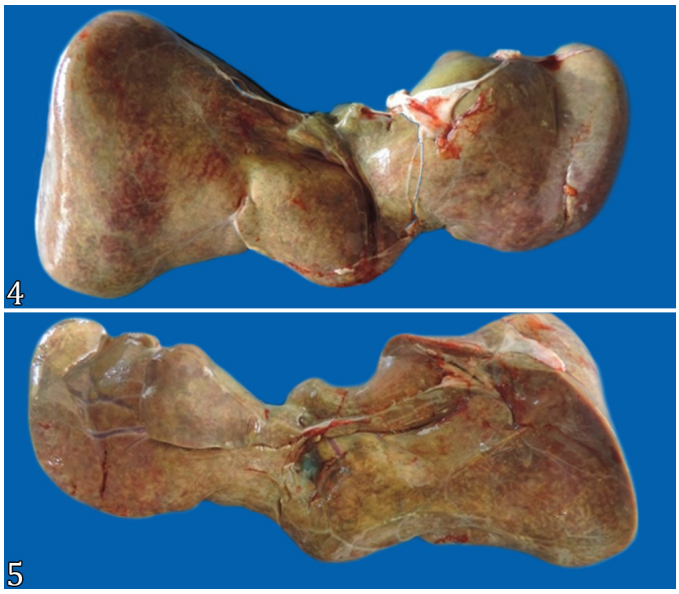


Fig.4-5. Liver of jabuti-piranga (*Chelonoidis carbonaria*) with hepatic steatosis. Note a yellowish color and accentuation of the diffuse lobular pattern and rounded edges on the (**4**) diaphragm and (**5**) coelomatic surfaces.

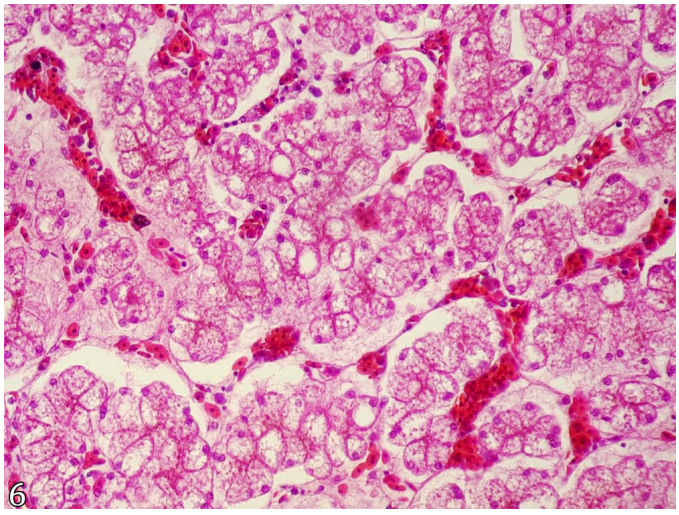


Fig.6. Liver of jabuti-piranga (*Chelonoidis carbonaria*) with hepatic steatosis. Note hepatocytes diffusely expanded by intracytoplasmic macro and micro vacuoles and a nucleus displaced to the periphery. HE, obj.20x.

reported significant consequences caused by traumas, such as lung lesions, necrotic pneumonia, and, in more severe cases, fibrinogenic cellomitis, septicemia, and death of the animal.

The shell of Testudines acts as a natural insulation mechanism that protects the internal structures preserving their components from the external environment (Barten 2006, Melidone & Selleri 2008). This barrier acts against invading microorganisms, protects against solar radiation, assists in thermoregulation, and prevents dehydration on land and excessive water entry into the body in aquatic environments. Thus, when there is a change that compromises this structure, internal organs may be exposed, allowing the entry of opportunistic bacteria that, in most cases, can cause serious multisystemic infections, compromising the survival of the animal (Orós et al. 2005, Santos et al. 2009). Which corroborates this study, in which the cases of sepsis attributed to inflammatory disorders had carapace fracture as the gateway. In a retrospective study in hospitals evaluating dermatological lesions in reptiles, it was found that cases of sepsis associated with shell trauma or dermal lesions in Testudines had significant results, highlighting the importance of management in maintaining the health of these animals (White et al. 2011).

Regarding the specimens coming from environmental agencies, the carapace/plastron fracture was more representative. However, due to insufficient data on the life history of the animal, it was not possible to prove the cause of the fractures. Nevertheless, behavioral characteristics such as slow locomotion, diurnal habits and mating needs in reproductive period, associated with a flow of vehicles close to their natural habitat (Hels & Buchwald 2001, Cunha et al. 2015), may condition the mortality of these animals by run over. Other factors are also attributed as possible causes of fracture in free-living Testudines such as predation of these animals in the wild, accidents by falling and physical trauma by boat propellers or lawn mowers (Jacobson 1994, Barten 2006, Joy et al. 2010).

Inflammatory disorders were the second category of illnesses diagnosed in Testudines, with pneumonia and septicemia being the leading cause of death. Pneumonia is a common multifactorial respiratory disease in reptiles and may be associated with environmental and nutritional management problems (Schumacher 2003). In Testudines, infectious agents may be capable of causing primary pneumonia, however, in most cases they are described as secondary agents due to management problems in captive animals, and it is very important to determine the disease as a primary or secondary (Origgi & Jacobson 2000, Silveira et al. 2014). Traumatic shell alterations can cause severe lung injury, triggering secondary infections by bacteria or other opportunistic agents (Ciccarelli et al. 2020). In addition, severe debilitation, depression, and other diseases can restrict normal breathing function in these animals. In this study three cases had as a gateway to multisystemic infection, complete carapace fracture.

Septicemia, the second most common cause of inflammatory disorders observed in the animals of this study is well described in the literature in reptiles, usually attributed to immunocompromised individuals and caused by the diverse microbiota resident in these animals. In Testudines, cases of septicemia can also occur, in acute conditions, following bacterial infection through lesions on the skin, through the oral cavity, under the action of pathogenic bacteria from the environment, or in more chronic situations, at sites where the infection was unnoticed or inadequately treated (Chitty & Raftery 2013, Morselli et al. 2016). According to Barten (2006), shell infections resulted from bite wounds, trauma or, more commonly, inadequate environmental management, can favor the occurrence of sepsis in these animals. In this survey it was observed that sepsis had as a gateway fractures of the shell or plastron, demonstrating the importance of these injuries in these animals.

Testudines are considered resilient animals and can survive for long periods without food and dehydrated (Norton 2005). These animals are ectothermic and their body temperature depends on the environmental temperature, so that several

**Table 1. Prevalence of conclusive diagnosis categories in Testudines necropsied at the LPV-UnB, from 2008 to 2020**

| Causes | *Phrynops geoffroanus* | *Trachemys dorbigni* | *Chelonoidis carbonaria* | *Chelonoidis denticulata* | Testudine | (%) |
|---|---|---|---|---|---|---|
| Disorders by injurious agents | | | | | | |
| Carapace fracture/plastron | 9 | 3 | 1 | | 2 | 30.61 |
| Piercing-cutting injury | 1 | 1 | | | 1 | 6.12 |
| Burn | | | 1 | | 2 | 6.12 |
| Sepsis with fracture | 3 | | | | | 6.12 |
| Inflammatory disorders | | | | | | |
| Pneumonia | | 1 | 1 | | 3 | 10.22 |
| Sepsis | | | | | 3 | 6.12 |
| Gastritis | 1 | | | | | 2.04 |
| Celomit | 1 | | | | | 2.04 |
| Falit | | | | | 1 | 2.04 |
| Myosit | | | | | 1 | 2.04 |
| Hepatitis | 1 | 1 | | | | 4.08 |
| Nutritional and metabolic disorders | | | | | | |
| Hepatic steatosis | 2 | 1 | 1 | 1 | 5 | 20.40 |
| Others | | | | | | |
| Sand accumulation | | | | 1 | | 2.04 |
| TOTAL | | | | | | 100 |

metabolic aspects can be affected by temperature (Dutra 2014). Nutritional disorders have been reported as the main cause of diseases, which are considered limiting to the health and survival of these animals (Paranzini et al. 2008, Marchiori 2013). Still, these disorders may have their origin in the inadequate management of food, either by excess or reduced consumption, developing in the animals some deficiency of vitamins and minerals essential for their performance and development (Frye 1991). Ten diagnoses that had nutritional and metabolic disorders as a cause were represented by hepatic steatosis. This is a metabolic condition caused by the accumulation of fat in the hepatocytes, leading to liver dysfunction, which has been frequently diagnosed in reptiles, especially in Testudines, and should be differentiated from physiological steatosis (Divers & Cooper 2000).

In this study, this alteration was responsible for the third most prevalent in Testudines, which mostly came from CETAS. In these cases, wild animals may have been rescued and/or seized by law enforcement agencies or spontaneously surrendered by people. A possible cause for hepatic steatosis in free-living Testudines was reported by Cardells (2012) in his study with *Trachemys scripta*, in which changes related to icteric or lipidic liver were more expressive in free-living reptiles than that of captive animals, where this condition can be considered of multifactorial origin (bacterial, parasitic and viral agents, metabolic disorders, prolonged fasting, hormonal changes, hibernation, reproductive cycles, inadequate temperatures, neoplasia), since these reports have been found in other reptiles, birds and mammals (Echeto & Aranjibel 1999, Mcarthur et al. 2004, Sanches 2008, Osorio & Cañas 2012, Andrade et al. 2020). Given the above, the need for further research on the etiopathogeny of diseases in these animals is evident. In captive animals, hepatic steatosis has been described as a relatively common hepatic alteration, rarely existing in isolation, and in many cases, it may come associated with other chronic conditions such as inadequate management and nutrition, high-fat diets, obesity, hyperparathyroidism, anorexia secondary to other diseases, and the action of bacterial and fungal toxins (Divers & Cooper 2000, Mcarthur et al. 2004, Divers 2019). The cases of hepatic steatosis in captive animals diagnosed in this work suggest that when empirically fed by humans, they become more susceptible to nutritional deficiencies and metabolic alterations due to inefficient nutritional management associated with inadequate environments (Flosi et al. 2001, Marchiori et al. 2015). Corroborating Otero et al. (2014) who state that inadequate nutritional management of captive animals leads to metabolic alterations that induce the occurrence of hepatic steatosis due to excess of fat acquired in the diet.

In the present study, 11.11% of the cases presented a process of autolysis that made diagnosis impossible, a fact attributed to the time of arrival of the cadaver to the laboratory, which in free-living wild animals is longer, since most of these animals are found randomly in the environment hours after death, and the inadequate conditions of preservation of the body leads to rapid tissue destruction by the action of proteolytic enzymes produced during cadaveric decomposition. These alterations may mask the real lesions and induce mistaken interpretations, which makes diagnosis difficult or impossible (Coelho 2002). Thus, it is recommended to perform the necropsy soon after the death of the animal, so that all changes are preserved and the diagnosis of the cause of death is reached.

## CONCLUSIONS

Through this study it can be concluded that the disorders caused by harmful, inflammatory and nutritional and metabolic agents were the most frequent in the necropsied Testudines.

Carapace/plastron fractures were the main pathological findings in Testudines in the LPV-UnB area, followed by hepatic steatosis, pneumonia, sepsis, fracture with sepsis, burns and sharp injuries.

This study contributes to the knowledge of the main pathological findings in Testudines in the Distrito Federal and surrounding area diagnosed in the LPV-UnB routine, highlighting the importance of further research aimed at the knowledge of diseases that affect these animals, especially free-living animals, which can be reservoirs of zoonoses in the locality. Such records can also serve as support for teaching in veterinary medicine, directing wildlife veterinarians to the diagnosis and differential diseases that affect Testudines.

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