ORIGINAL ARTICLE

MACROSCOPIC AND MICROSCOPIC FINDINGS IN THE LIVER, GALLBLADDER, SPLEEN AND KIDNEYS OF CAPTIVE RETICULATED PYTHONS (*Python reticulatus,* Schneider*,* 1801) (Reptilia: Pythonidae) WITH PNEUMONIA

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ABSTRACT

The macroscopic and microscopic findings in the liver, gallbladder, spleen and kidneys of 12 reticulated pythons with pneumonia were described through gross and histopathological examinations. Gross findings in the liver observed were lesions on the surface (milliary white nodules and adhesions) and within the parenchyma of the organ. The gallbladder had a circumference of 15.71 ± 4.75 cm and length of 6.28 ± 2.01 cm. Gross lesions in the kidneys include congestion, thickened capsule and web-like adhesions. Twenty-five percent of the animals were found to have spleen that was irregularly shaped, oblate-spheroid organs with varying discolorations. Histopathological examination revealed signs of hepatitis, nephritis and nephrosis, splenitis and hyperplasia of the pseudostratified epithelium of the gallbladder. Significant histopathologic findings include presence of visceral larva migrans in the liver and kidneys, granuloma formation, presence of intracytoplasmic eosinophilic round inclusion bodies within the hepatocytes and renal tubule as well as presence of lipid vacuoles and cholesterol crystals in the splenic parenchyma. Macroscopic and microscopic findings suggest that presence of lesions in other organ systems is highly likely in snakes with pneumonia.

Keywords: gallbladder, histopathology, kidney, liver, pneumonia, reticulated python, spleen

INTRODUCTION

Pneumonia or lower respiratory tract disease is common in snakes, especially captive ones, and has a predilection to pythons and boas (Mayer *et al.,* 2009). Possible causes for pneumonia include bacterial, viral, fungal, parasitic and non-infectious agents (Mader, 2006; Mayer *et al.,* 2009). Non-infectious causes are usually associated with poor husbandry conditions such as inappropriate temperature and humidity levels within the enclosure, poor ventilation, dietary and nutrition-related issues and captive-social stress and reproduction-related stress (Mader, 2006; Ballard *et al.,* 2013). Snakes are exotherms, and thus, they derive their body temperature from the environment (Jacobson, 2007). They have a preferred optimum temperature zone (POTZ) requirement as well as

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humidity level requirement that must be maintained within their enclosure in order for their respiratory system and immune system to function normally (Mader, 2006; Ballard *et al.,* 2013; Mayer *et al.*, 2013). For reticulated pythons, their POTZ requirement ranges from 27-33°C with a humidity of 70%.

In the diagnosis of pneumonia, systemic evaluation is important since most reptiles showing signs of respiratory distress are already critically ill, they may already be in the stage where the disease has spread to other organ systems (Mader, 2006). Once diagnosed, an aggressive therapeutic regimen is usually recommended; however, prognosis for pneumonia is still dependent on the state of the animal upon presentation (Mader, 2006; Mayer *et al.*, 2013). Prognosis for wild-caught animals is usually poor, and it is unfavorable for chronic cases (Mayer *et al.*, 2013).

This study was conducted to observe macroscopic and microscopic changes in the liver, gall bladder, spleen and kidneys in reticulated pythons with pneumonia to determine if pneumonia predisposes the animal to pathological changes in other organs. The findings of this study may assist the clinician in the proper management of reticulated pythons with pneumonia.

MATERIALS AND METHODS

Twelve (12) reticulated pythons from the Biodiversity Management Bureau- Wildlife Rescue Center (BMB-WRC), Department of Environment and Natural Resources, Quezon City, Philippines were used as subjects in the study. The pythons with clinical signs of pneumonia were selected from a list of animals due for euthanasia. The animal's medical history, prior observations from their keepers, physical examination and hematologic profile were also utilized during animal selection.

An initial screening was done in the selection of the animals with pneumonia. An ocular examination was performed and the animals were inspected for the occurrence of anorexia, dehydration, poor body condition and presence of mouth lesions, respiratory sounds, foamy and/or ropey saliva, nasal exudates and caseous lesions in the mouth. Animals which showed at least three of the above-mentioned criteria were included in the study.

Manual restraint of the animal was done by an experienced handler to facilitate physical examination. The general appearance of the snake from head to tail was examined and the appearance of the skin and the musculature were noted. The presence of viscous or frothy saliva as well as hypermia of the mucous membranes, presence of caseous exudates and necrosis in the oral cavity were also noted.

Body length (cm) and tail girth (cm) of the animals were determined using a tape measure. Necropsy was performed in accordance with the suggested procedure set by Terrell *et al.* (2007). The organs were examined *in situ* before *in toto* evisceration of the organs. Deviations from normal gross appearance of the organs were recorded. The cranial and caudal borders as well as the middle portion of the liver and kidneys were collected. The gallbladder and spleen were collected whole. The organs were labelled and placed in sample bottles containing 10% formalin solution placed in zip-lock bags.

For histopathology, the tissues were stored inside sample bottles with formalin for at least three days for fixation. Afterwards, the samples were cut into 2 cm portions before each organ was individually packed with gauze moistened with 10% formalin solution. The samples were sent to Hi-Precision Diagnostics, Del Monte, Quezon City for processing, then examined histopathologically.

RESULTS

Macroscopic findings

During necropsy, the organs were examined for any presence of gross lesions and or deviations from their normal appearance. Gross measurements of the dimensions of each organ were also obtained. Upon gross examination of the liver, 17% had adhesions, 25% were observed to have milliary, white-raised pinpoint nodules on the surface of the organ (Figure 1), 25% had both adhesions and milliary white nodules while 8% of the samples had adhesions and presence of diffused raised brownish lesions that had a mottled appearance (Figure 3).

No gross lesions were apparent upon examination of the gallbladder aside from its varying sizes: 8.76 to 27.82 cm (Figure 5). Gross examination of the kidneys showed that 92% of the samples had no apparent gross lesions (Figure 6) on both the left and right kidneys, while the other 8% were congested with pale areas on both left and right kidneys as well as thickened capsule and web-like adhesions (Figure 7).

Gross examination of the spleen revealed that 75% of the animals had no apparent lesions while the remaining 25% had small, irregularly shaped oblate spheroid organs with varying changes in coloration (pale, whitish, yellow brown and brown) (Figures 8 and 9).

Microscopic findings

Histopathological examination of the liver revealed that all samples had signs of hepatitis where 42% were found to be acute while 58% were chronic. Significant histopathological findings in the liver included: 1) random multifocal areas of coagulation necrosis characterized by nuclear pyknosis, karyolysis and karyorrhexis with homogenous eosinophilic cytoplasm (Figures 2 and 6) or pale appearance of cellular structure (ghost-like) with presence of vacuolations (Figure 6), loss of cellular detail and cellular architecture but with retained basement membrane framework; 2) random multifocal areas of hydropic degeneration characterized by translucent vacuolations in the cytoplasm (Figure 2); 3) heterophilic infiltration; 4) melanomacrophage center hyperplasia characterized by increase in size and concentration of melanomacrophages present and steady decrease of brown coloration as the size increases (Figure 11); 5) hemosiderosis characterized by the presence of intracytoplasmic yellow-brown to golden-brown globules; 6) granuloma formation characterized as discrete concentrically arranged aggregates with either heterophils (heterophilic) or macrophages (histiocytic) at its center (Figures 7 and 1). Chronic granuloma is characterized as having a necrotized center, lymphoid infiltrates and peripheral fibrosis (Figure 7). Chronic hepatitis due to presence of parasitic larvae within the parenchyma of the liver was found in one animal. Parasitic larvae were found

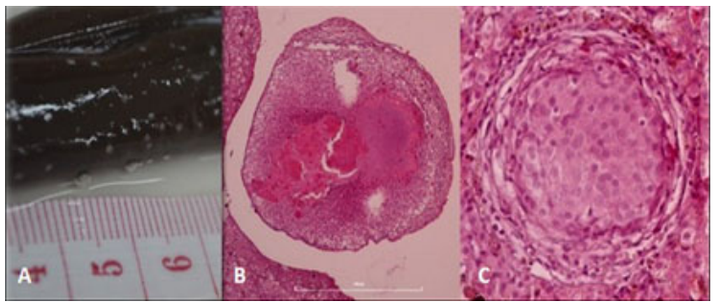

Figure 1: Liver. Grossly, there were milliary white raised nodules (A). Microscopically, there were random multifocal areas of granuloma formation that can be chronic (B) or histiocytic (C) in nature.

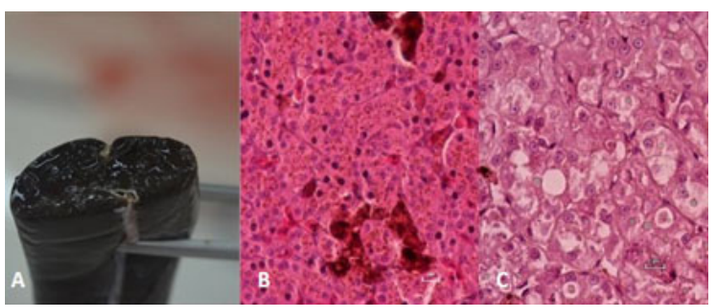

Figure 2: Liver. Grossly, the liver was turgid with fluid accumulation in the parenchyma (A). Microscopically, there was coagulation necrosis characterized by nuclear pyknosis and karyolysis and homogenous eosinophilic cytoplasm (B) and hydropic degeneration (C).

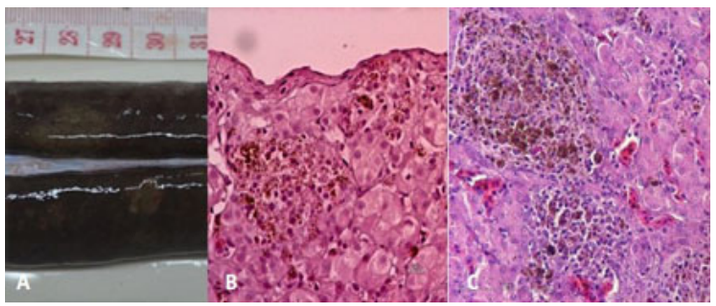

Figure 3: Liver. Grossly, the liver had multifocal raised brownish mottled lesions (A). Microscopically, there was aggregation of hyperplastic melanomacrophage centers at the periphery of the organ (B) and in the liver parenchyma (C).

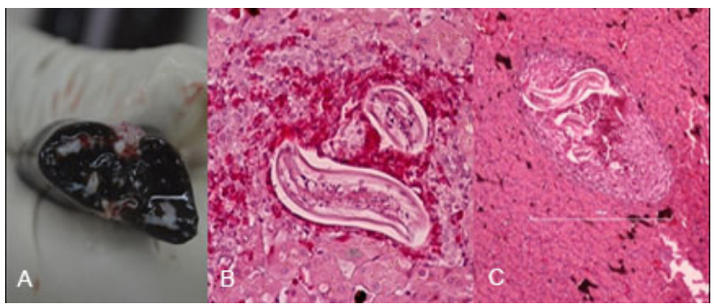

Figure 4: Liver. Grossly, the liver had multifocal areas of soft white lesions within the parenchyma (A). Microscopically, there was presence of parasitic larvae that were surrounded by heterophilic infiltrative cells (B) and presence of parasitic granuloma formation (C).

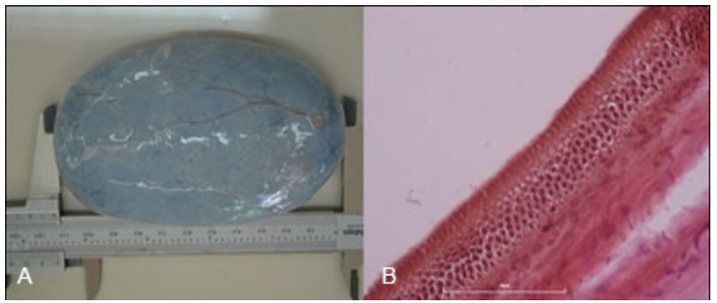

Figure 5: Gall bladder. Grossly there was enlargement (A). Microscopically, there were random areas of hyperplasia of the pseudostratified columnar cell epithelium of the gallbladder (B).

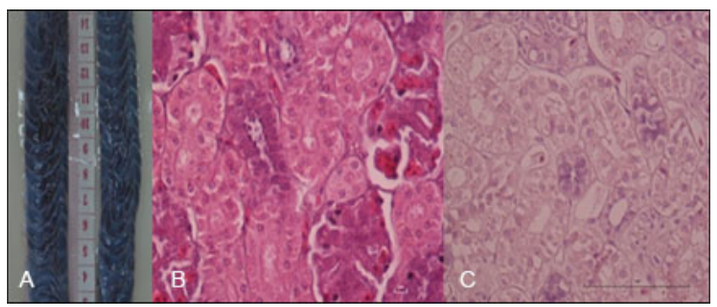

Figure 6: Kidneys. Grossly there were no significant lesions on the left and right kidneys (A). Microscopically, there was tubuloglomerular nephrosis (coagulation necrosis) characterized by nuclear pyknosis and karyolysis (B & C) with paling of the cellular structures (ghost-like) (C), loss of cellular detail and presence of vacuolations but with retained basement membrane framework (C)

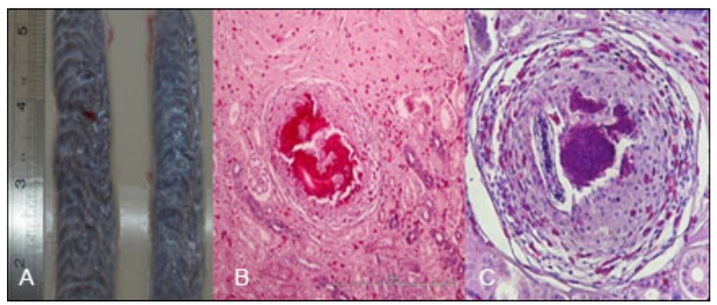

Figure 7: Kidneys. Grossly, the kidneys were pale and surrounded by thick web-like adhesions (A). Microscopically, there was tubulo-interstitial nephritis characterized by extensive coalescing areas of fibrosis and heterophilic infiltrates with intralesional chronic heterophilic granuloma (B) and parasitic granuloma formation (C).

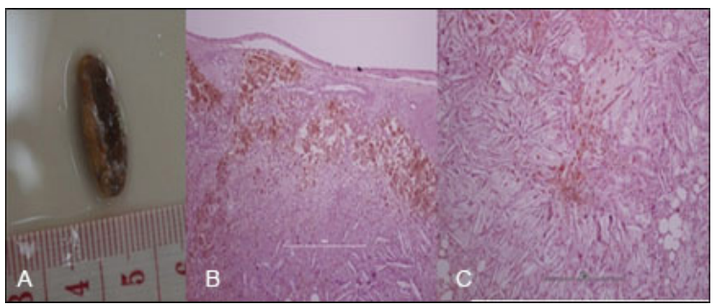

Figure 8: Spleen. Grossly, the spleen was small, ellipsoid yellow-brown in coloration (A). Microscopically, extensive melanomacrophage center aggregation on the periphery was observed (B). Extensive areas of cholesterol crystal formation characterized by their distinct thin rhomboidal appearance that looks like shards of glass and intralesional lipid vacuoles were observed in the parenchyma (C).

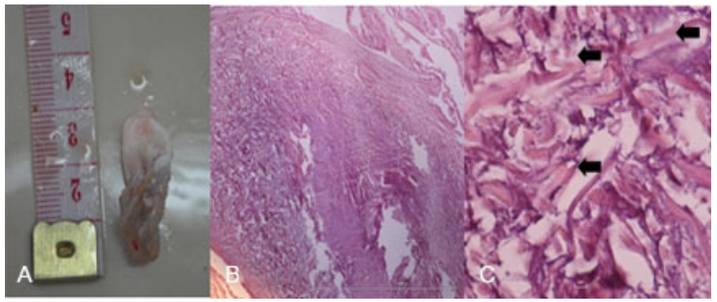

Figure 9: Spleen. Grossly, the spleen was white, firm and irregularly shaped (A). Microscopically, there was loss of 95% of normal tissue architecture (B) suspectedly due to eosinophic infiltrates (arrows) in the splenic parenchyma (C).

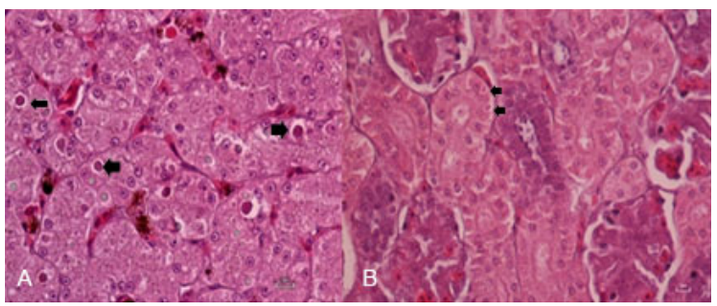

Figure 10: Intacytoplasmic eosinophilic round inclusion bodies (arrows) found in the liver (A) and kidneys (B) of captive reticulated python with pneumonia.

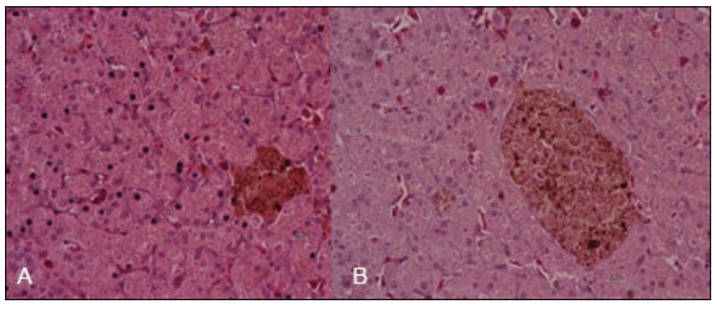

Figure 11: Liver. Normal melanomacrophage center (A) and hyperplastic melanomacrophage center (B).

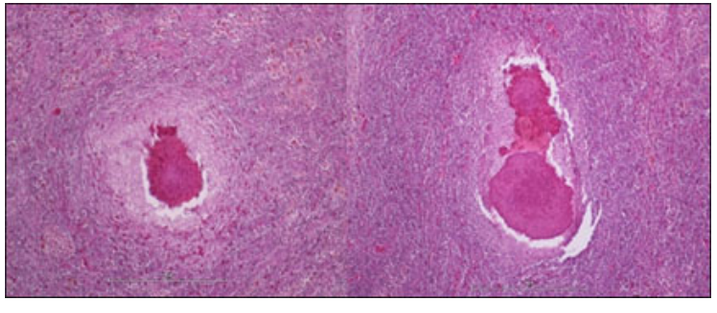

Figure 12: Spleen. Chronic granuloma formation in the splenic parenchyma.

to be surrounded by heterophilic infiltrates or within granuloma formation (Figure 4); and 7) numerous intracytoplasmic eosinophilic round inclusion bodies (Figure 10) were found on on one animal, which is highly suggestive of inclusion body disease. Histopathologic examination of the gallbladder revealed random, multifocal areas of hyperplasia of the pseudostratified columnar cell epithelium in all the animals (Figure 5).

Histopathologic examination of the kidneys showed that 50% of the animals had tubuloglomerular nephrosis while 17% had tubuloglomerular nephritis and 33% had tubulo-interstitial nephritis. Significant histopathological findings in the kidneys included: 1) random multifocal areas of coagulation necrosis and hydropic degeneration (Figure 6); 2) heterophilic infiltration; 3) melanomacrophage center hyperplasia (Figure 11); 4) hemosiderosis; 5) granuloma formation with presence of pararitic granuloma (Figure 7); and 6) numerous intracytoplasmic eosinophilic round inclusion bodies (Figure 10) were found in one animal, which is highly suggestive of inclusion body disease (8%).

Histopathologic examination of the spleen revealed that 75% of the animals had inflamed spleens while 25% had variable degrees and severe loss of parenchymal architectural integrity. Significant histopathological findings included: (1) heterophilic infiltration; 2) granuloma formation of varying chronicity and type (Figure 12); 3) varying degrees and severity of cholesterol crystal formation characterized by distinct thin rhomboidal appearance that looks like shards of glass (Figure 8); 4) lipid vacuole formation (Figure 8); 5) melanomacrophage center hyperplasia; and 6) eosinophilic infiltrating strands (Figure 9).

DISCUSSION

Histopathological examination of the liver, gallbladder, kidneys and spleen revealed various pathological changes in the organs. Hydropic degeneration is one of the most common signs of cell injury which is characterized by cell enlargement due to failure of the cell to maintain homeostasis and is usually found secondary to cellular injuries such as hypoxia, infectious (bacterial, viral, fungal) disease, mechanical and immune-mediated injuries (Myers, 2007).When causative agent is not addressed, it could lead to oncosis or oncotic necrosis, specifically coagulation necrosis (Myers, 2007; Rubin *et al.,* 2011).

Hepatitis was observed in all samples wherein there was inflammation in the parenchyma of the liver characterized by the presence of hepatocellular necrosis and presence of varying proportions of heterophilic infiltrates (Cullen, 2007). Reptilian inflammatory response differs from that of mammals. Instead of a suppurative exudate, inflammatory exudates form granulomas wherein its origin depends on which inflammatory cell comprises its center (Stacy *et al.,* 2007). Histopathological findings confirmed that 83% of liver samples exhibited varying types and chronicity of granuloma formation - one of which is parasitic in nature. Granuloma formation was not used as a marker for chronicity in this study. Instead, presence of marked increase in concentration and size of melanomacrophage centers (MMC), associated with seasonal variation, stress emaciation, chronic inflammation (Jacobson, 2007, Stacy *et al.,* 2007) was used as basis for chronicity.

Milliary, white, nodules were observed in 50% of the liver of the animals. Grossly seen nodule, confirmed to be granuloma under histopathological examination, is usually associated with either Mycobacteriosis or Chlamidophilosis (Soldati *et al.,* 2004; Rosenthal *et al.,* 2006; Jacobson, 2007) both of which are also known to be associated with pneumonia in reptiles (Mader, 2006; Mayer, 2013). Given that there has been no successful treatment protocol for reptiles, euthanasia is an option for disease management. *Chlamydia spp.* also has zoonotic potential and is associated with granulomatous lesions in snakes (Soldati *et al*., 2004). *Chlamydia,* specifically *Chlamydophila pneumonia* infection should be considered as a differential diagnosis for granulomatous lesion in reptiles and is of significant concern because of its zoonotic potential (Mayer, 2013).

The presence of intracytoplasmic eosinophilic round inclusion bodies within the hepatocytes and renal tubules was observed in one animal. This lesion is highly indicative of inclusion body disease (IBD) that is suspected to be caused by a Retrovirus-like agent (Schumacher, 2006). It is usually associated as a trigger for bacterial pneumonia in boas and pythons (Schumacher, 2006; Schmidt *et al.,* 2013).

During histopathologic examination of the liver and kidneys, parasitic larvae within the tissue parenchyma inciting extensive heterophilic infiltration and granuloma formation were observed. The adult form of the parasite appears to be round worms found within the gastrointestinal tract of the animal. Nematodes are the most frequently diagnosed endoparasite in snakes and lesions and clinical signs attributed to them are brought about by tissue damage caused by larval migration (Jacbson, 2007; Miller, 2014). Clinical signs may include obstruction, torsion, pneumonia, renal disease, muscle wasting, etc. And they also predispose the animal to secondary bacterial infection (Greiner *et al.,* 2006; Miller, 2014).

Gross examination of the gallbladder showed a distinct increase in its size compared to that of Aguisanda *et al.* (2011). This is reflected by the presence of random, multifocal areas of hyperplasia of the pseudostratified columnar cell epithelium of the gallbladders during histopathological examination. The gall bladder is where bile is concentrated and stored. Bile acids are synthesized from cholesterol from the liver. One of their functions would be in the digestion and absorption of dietary fats specifically cholesterol (Johnson *et al.,* 2012). During time of fasting, hepatic bile is diverted into gallbladder where it is stored and concentrated (Toouli, 2006; Johnson *et al.,* 2012). According to Toouli (2006), the gallbladder releases 75% of stored bile after a meal. At the end of the meal, hepatic bile is once again stored in the gallbladder and would be released again on the next meal. This may explain why gallbladder distension is marked in animals with prolonged anorexia and weight loss since at times of starvation, there is increase in fat mobilization (triglycerides) (Myers *et al.,* 2007)

Gross findings of the spleen revealed that 25% deviated in appearance from the small, reddish round structure as described by Terrel (2007). Instead, the spleen found in the study appeared to be small, irregularly shaped oblate spheroid. The coloration of the spleen varied throughout the samples from pale-whitish, yellow brown to brown.

Histopathologic examination of the spleen revealed that 58% of the animals had granulomatous inflammation. This is usually the organ's response to intracellular facultative pathogens such as *Mycobacteria* spp. (Fry, 2007). Cholesterol crystal formation, on the other hand, was found on 17% of the animals; however, this lesion is not considered significant by itself since it simply indicates the site of an old haemorrhage or area of necrosis (Myers, 2007). Lipid vacuolation was found in 17% of the animals. Lesions may be attributed to increased mobilization of triglycerides due to extended periods of starvation (Myers, 2007). Two of the animals had been found to be anorexic for 13-20 months and had lost 1.4-5.6 kg in weight.

Macroscopic and microscopic findings of some non-respiratory organs of the present study are in agreement with Mader (2006) who stated that reptiles, in this case reticulated pythons, diagnosed with pneumonia may already be suffering from a multisystemic illness wherein disease spreads and causes pathological lesions to other organ systems.

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