Cardiac thrombus in a Burmese python (*Python molurus bivitattus*)

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ABSTRACT - A ten-year-old male Burmese python (*Python molurus bivitattus*) was evaluated for coelomic distension in the second third of the body in the area around the heart. On radiographic examination an increase in soft tissue opacity was seen superimposed over the cardiac silhouette. Ultrasonographic examination revealed a cystic mass in the heart. The snake died after the examination. Necropsy revealed severe distension in cardiac region with coelomic membrane adhesion to the pericardium, which was thickened and filled with a massive blood clot. The heart was increased in size with a mass inside the right atrium. Histological presentation was consistent with septic cardiovascular thrombus with signs of recanalization, diffuse necrosis and acute diffuse inflammatory reaction. Gram staining technique revealed the presence of Gram negative bacteria. The exact cause of the thrombus and the entry site for bacteria could not be determined, but chronic stomatitis that was present for more than seven years in this patient was suggested as a possible origin. Nevertheless, there are only a few reports of cardiac diseases in snakes and generally with scant information concerning the aetiology. In this work, besides reporting details of the case itself possible causes for this cardiac disease are discussed.
Key words: Heart disease, thrombus, septic, snake, stomatitis

INTRODUCTION

Cardiovascular system disease in reptiles is infrequently diagnosed. However, both primary and secondary heart diseases have been described including congenital disease, myocarditis, heart disease associated with nutrition, neoplasms and aneurysms, arteritis, and endocarditis caused by bacteria, parasites or viruses (e.g. Jacobson & Kollias, 1986; Rishniw & Carmel, 1999; Jensen & Wang, 2009; Stumpel et al., 2012). To the author's knowledge, this is the first case report demonstrating diagnostic imaging features and histopathological results of a cardiac thrombus in a Burmese python (*Python molurus bivitattus*).

CASE REPORT

A ten-year-old male Burmese python (*P. m. bivitattus*) from Curitiba´s municipal zoo was presented to the Universidade Federal do Paraná´s Wild and Exotic Animal Service for clinical examination of a coelomic distension in the second third of the body, most precisely in the area around the heart, which was noticed two weeks previously (Fig. 1). The animal weighed 8.25 kg and was dehydrated, lethargic, thin and weak. The snake had been anorexic for three months and had been treated with subcutaneous gentamicin injections for pneumonia and topical riphamicin (Rifocina Spray, Laboratório Sanofi, São Paulo, SP) on oral lesions for chronic stomatitis, diagnosed seven years before. The animal was housed at the Curitiba´s Zoo facility for seven years but originally, the snake came from animal trafficking having being held for an unknown period in a triage center for wildlife (Centro de Triagem de Animais Selvagens - CETAS). Therefore, history prior the time that the animal was housed at CETAS was not available.

Figure 1. A male Burmese python (*P. m. bivitattus*) with coelomic distension in the second third of the body. The animal´s head is to the left.

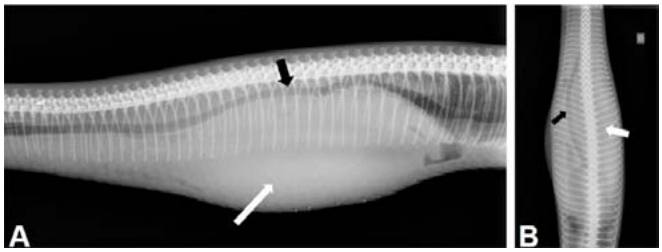

Figure 2. Radiographs of the cranial third of the male Burmese python (*P. m. bivitattus*) body. a) Lateral projection radiography showing an increase in soft tissue opacity superimposed over the cardiac silhouette (white arrow) and dorsal displacement of the trachea (black arrow). Cranial end is to the left. b) Ventrodorsal projection. Note again the soft tissue opacity over cardiac silhouette (white arrow) and lateral displacement of the trachea (black arrow).

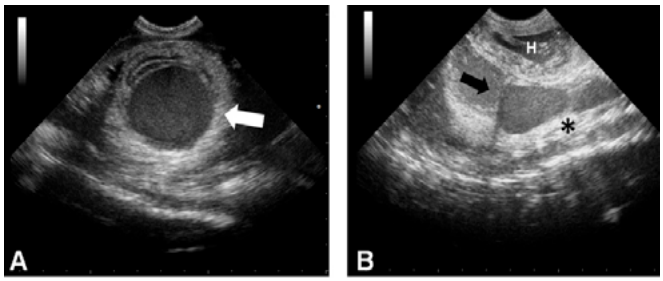

Figure 3. Ultrasonograms of a male Burmese python (*P. m. bivitattus*). a) Multiple layers mass filled with heterogeneous fluid (white arrow) inside the right atrium. b) The heart (H) was ventrally displaced. Note thickening of pericardium (asterisk) and the pericardial effusion (black arrow).

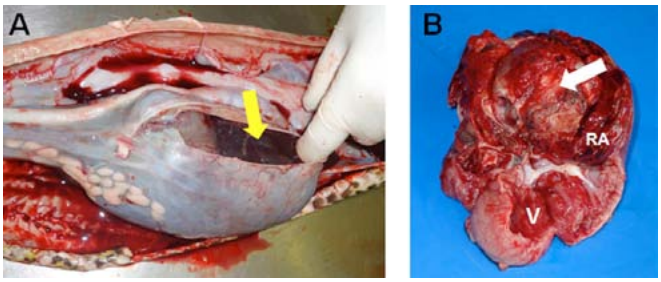

Figure 4. Postmortem examination pictures of a male Burmese python (*P. m. bivitattus*). a) Note the severe distension in cardiac region with coelomic membrane adhesion to the pericardium. The pericardium was thickened and with a blood clot inside (yellow arrow). b) The mass was observed inside the right atrium measuring 51 x 40 x 35 mm, with reddish coloration fibrous appearance and with a cystic cavity (white arrow). RA, wall of right atrium; V, ventricle.

For radiographic examination, lateral and ventrodorsal projections were performed using X-ray intensifying screens (Agfa Healthcare CR-30-X, DS5302, Mortsel, Belgium) with a tabletop technique of 55 kVp and 100 mAs. Radiographs of the cranial third of the body revealed an increase of soft tissue opacity superimposed over the cardiac silhouette, making it lose details and outline, with a dorsal-lateral displacement of the trachea (observed in lateral and ventrodorsal projections, respectively) (Fig. 2).

Ultrasonographic examination was carried out using an ultrasound system (MyLab 30 - Esaote, Genova, Italy) equipped with a 9-3 MHz micro-convex probe. This revealed a rounded structure measuring 44 vs 50 mm, with multiple layers (Fig. 3a) attached to the heart filled with heterogeneous free fluid (Fig. 3b). Pericardial thickening with large quantity of turbulent effusion was observed. At this point, differential diagnosis included a cardiac thrombus, left aortic arch aneurysm and a neoplastic mass.

The animal died immediately after the ultrasonographic examination. Necropsy revealed severe distension in the cardiac region with coelomic membrane adhesion to the pericardium, which presented as thickened with a very large blood clot present (Fig. 4a). The heart seemed to be increased in size (102 x 67 x 55 mm) with a significant dilation of the right atrium. Inside the right atrium, attached to its wall a mass measuring 51 x 40 x 35 mm was present, with reddish discoloration, firm consistency and gristly appearance. After cutting this mass a cystic cavity was seen (Fig. 4b). Mucous membranes in the oral cavity were erythematous with petechiae, plaques with caseous material appearing along the dental arcade, characterizing gingivitis, glossitis and palatitis. The liver was slightly yellow. No other major gross findings in other organs were noticed. The heart and liver tissues were collected and fixed in 10% neutral buffered formalin solution. After the heart tissue was fixed the wall of the cystic cavity mass was seen to be formed by a cluster of overlapping layers loosely bonded (Fig. 5). Tissue samples were routinely processed, paraffin embedded, sectioned at 5 mm, and stained with hematoxylin and eosin.

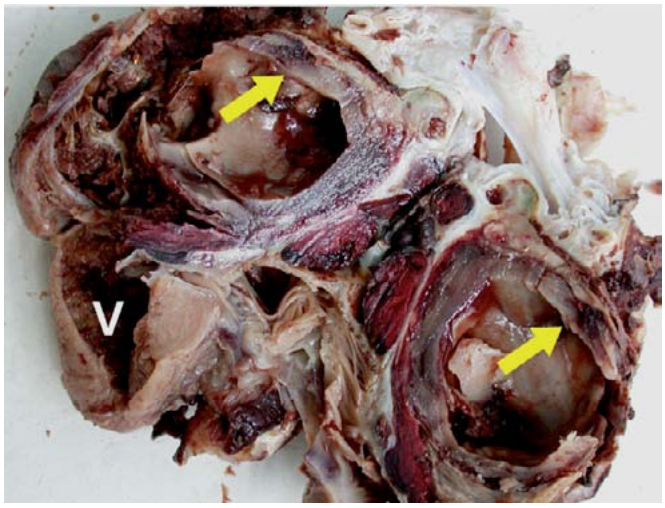

Figure 5. The heart of a male Burmese python (*P. m. bivitattus*) fixed in 10% neutral buffered formalin solution. Observe the wall of the cystic cavity formed by a cluster of overlapping layers loosely bonded (yellows arrows). (V) ventricle.

Histologically, the heart tissue was hemorrhagic and edematous, with relatively severe diffuse infiltration by lymphocytes, plasma cells, macrophages and heterophils. Muscle fibres were hyalinised but without signs of necrosis. An extensive mixed (of fibrin and erythrocytes) thrombus with the formation of sharp lines of Zahn and signs of recanalization (formation of new channels) occasionally seen, especially near the wall (Fig. 6a). Several regions exhibited deep tissue necrosis of coagulation with the presence of colonies of bacteria (bacilli) (Fig. 6b). There was no evidence or signs of neoplasia. In general, the histological presentation of this heart was consistent with a septic cardiovascular thrombus with signs of recanalization, disseminate necrosis and acute diffuse inflammatory reaction. In the liver tissue histologic changes included cytoplasmatic lipid vacuolation of hepatocytes, characterizing hepatic lipidosis. No other significant microscopic findings were seen in this animal. Gram staining technique was carried out in the thrombus tissue and showed Gram negative bacteria.

DISCUSSION

Although a great body of knowledge has been accumulated regarding the function of the reptile heart, its clinical implications in veterinary medicine have received limited attention. Literature related to the physiologic and evolutionary importance of the reptile heart is available, but there is little

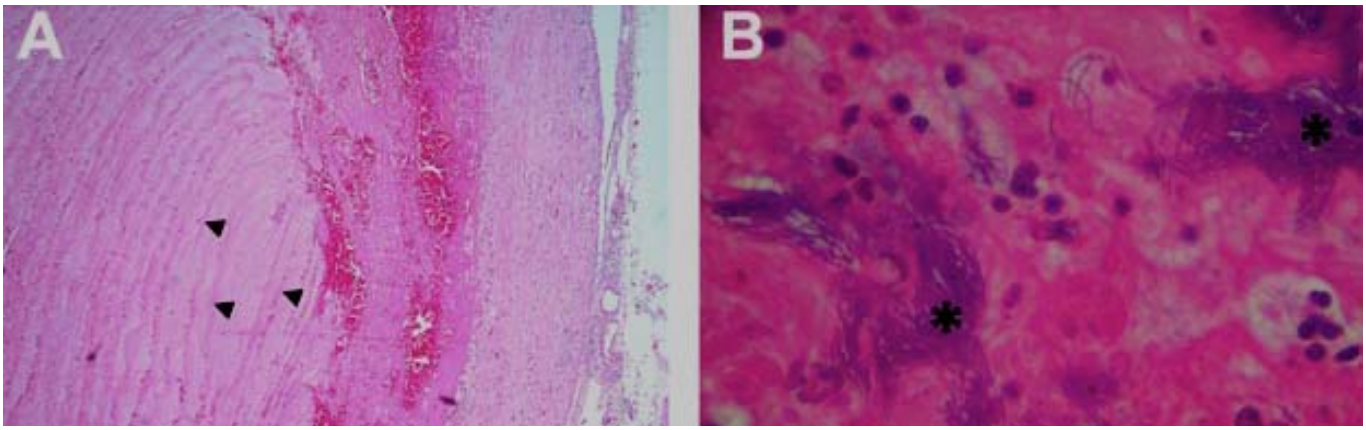

Figure 6. Photomicrographies of the intracardiac thrombus in a male Burmese python (*P. m. bivitattus*). a) Well organized, probably old blood clot evidenced by Zahn lines (arrow head) (HE 100x). b) Colonization by bacillary bacteria (asterisks) (HE 1000x).

clinical research about reptile cardiology (White, 1976). It is important to be aware of the normal anatomy and physiology of reptiles prior to diagnostic imaging or postmortem examinations. A detailed description of the snake cardiac anatomy and physiology has been described and reviewed by various authors and can be consulted for better understanding (Farrell et al., 1998; Girling & Hynes, 2004; Murray, 2006; Wyneken, 2009; Jensen et al., 2010).

A possible explanation for the presence of this contaminated thrombus would be a Gram negative septic embolus dislodged from the blood supply of the mouth as the snake had a long standing clinical history of chronic stomatitis. This septic embolus may have attached to the right atrium and initiated the process. A large number of different bacteria may be found in the oral cavity of affected reptiles, especially snakes (Fonseca, 2009). Draper et al. (1981), studying the microbiota from oral cavities of healthy and sick snakes, found Gram positive bacteria in healthy animals, whereas the sick ones presented, most frequently, Gram negative bacteria. Thus, the bacteria found in the intracardiac thrombus that have been classified as Gram negative, may have had oral origin. In cases where stomatitis is chronic and necrosis and ulceration are deep, a blood clot loaded with bacteria can be released into the bloodstream, resulting in septicemia (Jho, 2011). Despite the information about snake oral flora, investigations concerning the stomatitis influence in systemic diseases in several reptile species are scarce. One of our limitations was the lack of bacterial culture to identify which bacteria were responsible for the stomatitis and the microorganisms in the thrombus. Due to this limitation, it was not possible to confirm the origin of these bacteria.

Chronic stomatitis generates sense of pain and reduces the feeding capacity of the animal, leading to stress due starvation and weakness. Pythons have a low metabolism and capable of slowing down their metabolism for several months between each feed in order to conserve energy (O'Malley, 2005). In this case, the animal was anorexic for three months and was not cachectic, supporting O'Malley's proposition. In addition to cardiac abnormalities, we could also observe hepatic lipidosis. It may have occurred because the rapid weight loss resulted in secondary disease processes such hepatic lipidosis (Mitchell, 2009). This problem is seen most often in patients that have been starved, especially if they are obese (Donoghue, 2006).

An additional explanation for the formation of a cardiac thrombus could be blood collection by cardiocentesis. In this case report, we do not have a historical report of this technique during the seven years that the animal lived at the zoo or CETAS. Cardiocentesis is the most commonly used blood collection technique in snakes because it allows collections of large sample volumes, it carries a relatively low risk of lymphatic contamination and it can be used on snakes ranging from small neonates to large constrictors (Hernandez-Divers, 2006). Several researchers suggest that it is possible to collect blood safely by cardiocentesis in snakes, but care should be taken with asepsis and there should be few consecutive attempts (McFadden et al., 2011; Nardini et al., 2013). However, some authors suggest that risk of cardiac complications as a consequence of cardiocentesis exists, but it is poorly described. Some reports describe a dilated ventricle, lesions in the pericardium and myocardium, tissue adhesion and cardiac tamponade (Isaza et al., 2004; Selleri & Di Girolamo, 2012). The type of thrombus and observation of bacteria give us indications that blood collection by cardiocentesis may have been poorly performed.

The bacteria found in the thrombus, detected by histopatology were Gram negative. Usually, bacteria found in the body surface of most reptiles have been associated with unusual subtypes of *Salmonella* sp., a Gram negative bacterium (Mitchell, 2006). This finding could reinforce the fact that it is possible that cardiocentesis can introduce bacteria into a reptile's heart, if the technique is not carried out correctly, for instance with antiseptic care, restraint and right positioning. There are other safer techniques to collect blood samples, such as using the ventral coccygeal vein. The use of a poor technique requiring several attempts to collect blood samples has been suggested as a cause of trauma to the heart and associated structures.

Jacobson et al. (1991) reported a septic thrombus resulting in congestive heart failure, also in a Burmese python. This is the only report previously published in the literature about intracardiac thrombus in a snake. Similar to our report, the dilatation and the thrombus in the snake's heart were in the right atrium. In Jacobson's case, they found granulomas and urates deposited in the kidney, spleen and myocardium and small intestine congestion. In our case report, no other organs

had lesions, despite vacuolar degeneration in hepatocytes, a sign of lipidosis, found in both cases. *Salmonella arizona* and *Corynebacterium* sp. were isolated from the thrombus in Jacobson's case report, confirming hematogenous infection. In both cases it was not possible to affirm the source of infection, but the two reports provide important information on complementary tests for diagnosis.

This description reported an intracardiac thrombus in a snake and although the origin has not been clarified, possible causes were discussed. For several years the hypothesis that oral problems induce cardiac problems has been raised (Basilio, 2004), therefore, this would be one of many reasons to maintain appropriate oral health. Thus complete and frequent clinical examination of reptiles in order to rule out oral infections such as stomatitis is indispensable due to its systemic implications. Considering the other possible cause suggested, we concluded that antiseptic care as well as positioning and technical experience during blood collection are essential, especially in cardiocentesis.

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