Histopathological Perspectives of Multiple Organs in a RedFooted Tortoise (Chelonoidis carbonaria) with Suspected Metabolic Bone Disease: A Case Report

by Hani Plumeriastuti

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Case Report

Histopathological Perspectives of Multiple Organs in a Red-Footed Tortoise (*Chelonoidis carbonaria*) with Suspected Metabolic Bone Disease: A Case Report

Hani Plumeriastuti[1,*], Annise Proboningrat[1], Djoko Legowo[1], Bilqisthi Ari Putra[1], Gracia Angelina Hendarti[2], Agung Budianto Achmad[3]

ABSTRACT

Introduction: Exotic pet lovers' interest in keeping tortoises is increasing all over the world, including Indonesia. However, this trend cannot be separated from the potential emergence of various health problems in tortoises. One of the problems that often affects tortoises is metabolic bone disease. Metabolic bone disease (MBD) is a disorder related to the mechanisms of vitamin D and calcium metabolism, which generally occurs in reptiles, especially Chelonia and Lizards. Case Presentation: A 3-month-old red-footed tortoise, which was clinically suspected to have a MBD, was necropsied as an effort to support the provisional diagnosis through histopathological evaluation. The purpose of this examination was to analyze the impact of the disease on various organs microscopically in patients with suspected MBD. The results showed a decrease in the number of trabeculae and hematopoietic cells in the metatarsal bones; moderate myonecrotic changes and atrophy in the skeletal muscle; inflammation of the perineuron; acute tubular necrosis and mild edema of the renal cortex; congestion and an increase in the number of melanomacrophages in the liver; as well as epicarditis and myocarditis in the heart. Conclusion: Several forms of the histopathological changes seem to indicate a pathophysiological relationship between the suspected metabolic bone disease and the multiple organs examined.
Key words: Hematoxylin-eosin, MBD, Tortoise, Septicemia.

Hani Plumeriastuti[1,*], Annise Proboningrat[1], Djoko Legowo[1], Bilqisthi Ari Putra[1], Gracia Angelina Hendarti[2], Agung Budianto Achmad[3]

[1]*Division of Veterinary Pathology, Faculty of Veterinary Medicine, Universitas Airlangga, Surabaya, INDONESIA.*

[2]*Division of Veterinary Anatomy, Faculty of Veterinary Medicine, Universitas Airlangga, Surabaya, INDONESIA.*

[3]*Department of Health, Faculty of Vocational Studies, Universitas Airlangga, Surabaya, INDONESIA.*

Correspondence

Hani Plumeriastuti

Division of Veterinary Pathology, Faculty of Veterinary Medicine, Universitas Airlangga, Surabaya, INDONESIA.

E-mail: hani-p@fkh.unair.ac.id

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INTRODUCTION

In the last decade, the trend of domesticating tortoises as pets in urban families is increasing and popular around the world.[1] In Indonesia, the sulcata tortoise is one of the most popular tortoise pets because it is easy to find this captive breed in the reptile pet market.[2] Another tortoise that is frequently kept as a traditional pet in houses is the red-footed tortoise.[3] Their ease of care, low cost of ownership, and amazing coloration make them highly sought after by novice tortoise keepers.

The red-footed tortoise (*Chelonoidis carbonaria*) are members of the Anapsida subclass, Chelonia order, Cryptodira suborder, Testudines family, and Chelonoidis genus.[4] This species native to South America and can be found from Panama to Paraguay, as well as parts of Bolivia, Brazil, Colombia, Ecuador, and Peru.[5] *C. carbonaria* is a diurnal and terrestrial animal with a compact body and strong cylindrical limbs, ideal to support its heavy carapace and walk in rough terrain.[4,5] They are opportunistic omnivores in general, and their diet is heavily influenced by the seasonal availability of food.[6] Their main food sources are leaves, grasses, flowers, fruits, carcasses, and other food found on the ground.[4]

The increasing trend of keeping tortoises among exotic animal enthusiasts also has the potential to lead to many health problems.[1] *C. carbonaria* is the most common testudines kept as a pet in South America, which accounts for a large proportion of wildlife patients seen in veterinary practices.[4] In Indonesia, according to Raharjo *et al.*,[2] a study on the prevalence of disease in exotic pet patients at a clinic in Yogyakarta, Indonesia, during January-August 2020 showed that turtles and tortoises had the highest cases of 71.7%, compared to snakes (16.5%), iguanas (6.2%), lizards (4.1%), crocodiles (1%), and geckos (0.5%).

Management of nutrition, health, housing, and an inappropriate environment are predisposing factors to serious health issues in tortoises if not anticipated and treated immediately. Some of the health issues that tortoises in captivity can face include respiratory ailments caused by bacterial or viral infection,[7-10] gastro-intestinal disease caused by parasite or viral infection,[8,11,12] and nutritional and metabolic disorders.[13-15]

Metabolic bone disease (MBD) is one of the metabolic disorders commonly seen in captive reptiles, particularly in Chelonia (turtles, tortoises, and terrapins) and lizards, occasionally in snakes.[16] In veterinary medicine, MBD refers to a group of pathological conditions that affect the integrity and function of multiple bones.[17] They are most generally caused by genetic, dietary, and/or hormonal disorders that impact bone growth and remodeling, typically through changes in calcium/phosphorus metabolism. MBD has traditionally been broken down as fibrous osteodystrophy, osteoporosis, and rickets/osteomalacia; however, many cases are difficult to specifically classify, particularly those caused by nutritional deficiencies, because multiple conditions may coexist. Therefore, cases reported in the literature should be scrutinized carefully, and

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confirmation by histopathological evaluation should be regarded as more definitive.[18] The aim of this study was to report the occurrence of suspected MBD in red-footed tortoise (*C. carbonaria*) and describe the histopathological findings in several organs associated with the disease. This study also tried to describe the relationship between the suspected MBD and the histopathological changes that occur in several observed organs.

CASE PRESENTATION

History

A 3-month-old dead red-footed tortoise (*Chelonoidis carbonarius*) weighing approximately 50 grams with a carapace length of 6 cm (Figure 1A-B) was sent to our laboratory. According to the information from the sender, the tortoise had clinical symptoms of inappetence, anorexia, an abnormal gait, and weakness. Previously, it had a history of shipping that was too long, about seven days, to finally die. Another tortoise, similar in age and weight, maintained together, had similar complaints.

Post-mortem findings

The post-mortem investigation that had been performed showed that the carapace and plastron were tender. No abnormal conditions such as swelling, trauma, or injuries were found on the results of other external examinations. On internal examination after necropsy, no significant macroscopic changes were found in the organs, such as inflammation, bleeding, enlargement or reduction in organ size, changes in color and consistency, and accumulation of fluid in the celomic cavity. Only one colon enlargement was found. Some organs, such as the kidney, liver, heart, hind leg musculature, and metatarsals, were collected for tissue processing. Unfortunately, the intestine tissue could not be collected for histopathological examination (Figure 1C).

Histopathological findings

On the results of the microscopical examination of tortoise metatarsal bones that had been stained with hematoxylin and eosin, there was a decrease in the number of trabecular bones in the epiphysis and hematopoietic cell loss (Figure 2A). Some sections also showed the presence of hyaline cartilage in the middle of the mature trabecular bone matrix (Figure 2B). In skeletal muscles, it was observed that many cells of the skeletal muscles were necrotic and some were atrophic (Figure 3A). Inflammatory cell infiltration also occurs between the striated muscle fibers accompanied by edema (Figure 4B). Peripheral nerve cells also underwent lysis and inflammation (Figure 3C).

On microscopic examination of the kidney, edema appeared in two of the four lobes of the kidney (Figure 4A). The appearance of massive acute tubular necrosis was also clearly seen, characterized by convoluted proximal tubules that mostly underwent cell lysis and nuclear pyknosis (Figure 4B). Mild to moderate congestion was observed in the liver sinusoids (Figure 5A). An increase in the number of melanomacrophages has also occurred, and some have formed melanomacrophage centers (MMC). Additionally, there are few eosinophilic granular cells (EGCs) and some infiltrating lymphocytes that aggregate to form lymphoid follicles (Figure 5B). Furthermore, microscopic observation also showed that the tortoise heart experienced epicarditis, myocarditis, and endocarditis, indicated by fairly massive lymphocytic infiltration in the epicardium, myocardium, and lumen of the endocardium, respectively (Figure 6A-B).

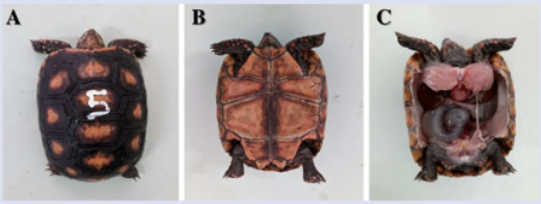

Figure 1: Gross examination of the tortoise body suspected of having MBD. A: The dorsal view. B: The ventral view. C: Gross appearance of the internal organs.

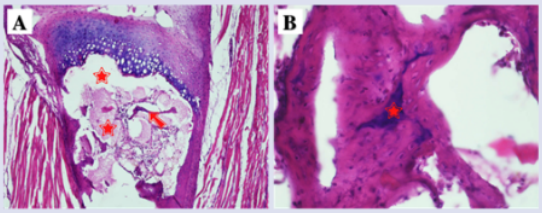

Figure 2: Histopathological appearance of the metatarsal bone of tortoise with suspected MBD (hematoxylin-eosin staining). A: metatarsal epiphyses with fewer trabeculae (arrow) and loss of hematopoietic cell (arrowhead) (100× magnification). B: hyaline cartilage (star) within the trabecular matrix (400× magnification).

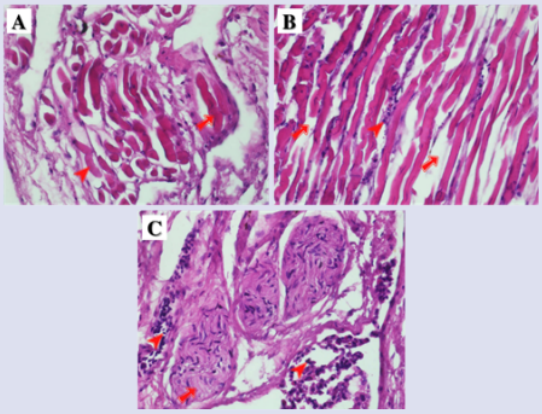

Figure 3: Histopathological appearance of tortoise skeletal muscles with suspected MBD (hematoxylin-eosin staining, 400× magnification). A: striated muscles with atrophic (arrowhead) and necrotic (arrow). B: edema (arrow) and infiltration of inflammatory cells (arrowhead) between muscle fibers. C: peripheral nerve cell lysis (arrow) and inflammatory cell (arrowhead) infiltration.

DISCUSSION

Based on clinical symptoms and the reported history, it was assumed that the tortoise that had been necropsied had MBD. One of the strong supporting reasons is hypocalcemia-induced vitamin D deficiency, which is related, first, to lack of UV light (due to long shipping journeys) and proper temperature, and/or inadequate and balanced nutritional intake. Inadequate exposure to UVB rays causes the epidermal cells of animal skin to be unable to produce vitamin D3 (cholecalciferol) which is the result of the conversion of pre-vitamin D and its precursor, pro-vitamin D (7-dehydrocholesterol). Deficiency of vitamin D3 in the blood circulation causes the liver to lack its capacity to produce calcidiol or 25-(OH)-vitamin D3 as the main storage form of vitamin D3. This continues to cause kidneys to fail to hydroxylate calcidiol to produce 1,25-(OH)2-vitamin D3 or calcitriol, which plays a vital role in the regulation of calcium and phosphorus balance.[19]

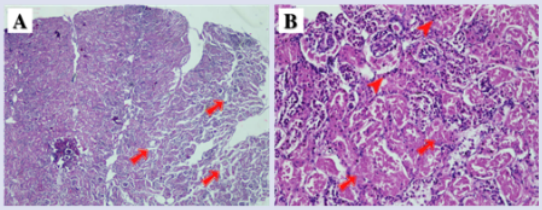

Figure 4: Histopathological appearance of the renal cortex of tortoise with suspected MBD (hematoxylin-eosin staining). A: edema (arrow) of the two right lobes of the kidney (40× maginification). B: pyknotic (arrowhead) and karyolitic (arrow) of convoluted tubule cells (200× magnification).

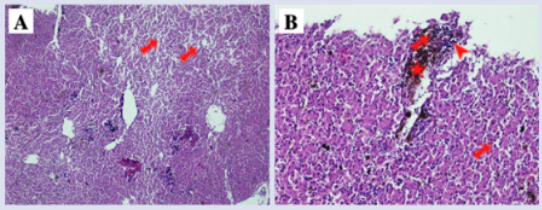

Figure 5: Histopathological appearance of the liver parenchyma of tortoise with suspected MBD (hematoxylin-eosin staining). A: some areas of the liver are congested (arrow) (40× maginification). B: infiltration of lymphocytes (arrowhead), eosinophilic granular cells (arrow), and melanomacrophage centers (star) (200× magnification).

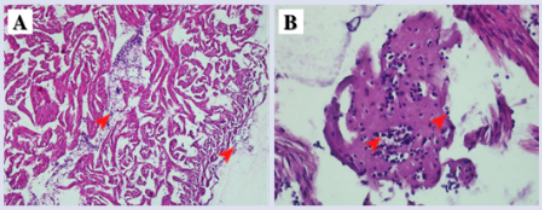

Figure 6: Histopathological appearance of the tortoise heart with suspected MBD (hematoxylin-eosin staining). A: infiltration of lymphocytic cells (arrowhead) in the epicardium and myocardium (100× maginification). B: infiltration of lymphocytic cells (arrowhead) in the lumen of the endocardium (400× magnification).

The endocrine hormone calcitriol is known to increase intestinal absorption of dietary calcium and phosphate, stimulate the storage of calcium and phosphate in the kidneys, and, together with parathyroid hormone (PTH), has a direct effect on bone by regulating calcium mobilization from bone. Lack of this hormone can cause disturbances in bone growth and development, as well as in maintaining mature bone tissue.[20]

Hypocalcemia due to hypovitaminosis D is usually compensated by increased secretion of PTH from hyperplastic parathyroid glands and subsequently hyperparathyroidism, leading to resorption of calcium from bone.[21] Unfortunately, this study did not observe the histopathological features of the parathyroids, so the hyperparathyroidism in this case could not be confirmed.

Another possibility that can occur is low calcitriol so that the body cannot limit the occurrence of osteoclastogenesis and trigger bone resorption by osteoclasts, resulting in osteopenia.[22,23] This may explain the loss of the large amount of trabecular bone in the metatarsal tortoise epiphyses that we observed. The loss of trabecular continuity leads to a reduction in the ability of the trabecular to withstand stress;[23] therefore, the tortoise appears to have an abnormal gait.

In this study, it was also found that there is cartilage within the trabecular matrix. This may be related to disturbances in endochondral ossification during the development of the young tortoise. Ostechondrosis is a disorder of chondrocyte maturation that results in delayed cartilage mineralization. In addition to calcitriol, 24,25-(OH)2-vitamin D3 produced by calcidiol hydroxylation in the proximal renal tubule also plays an important role in cartilage cell differentiation and matrix mineralization. This imbalance in plasma concentrations between vitamin D metabolites appears to be related to the disposition of osteochondrosis during the growth period of the animal.[24]

Under normal conditions, bone marrow in newborns and very young animals is mainly composed of active hematopoietic tissue and has relatively few fats.[23] This study found that the bone marrow within the epiphyseal metatarsal of the tortoise was hypocellular with a significantly reduced number of hemopoietic cells. It is still unclear how the pathophysiological relationship with the suspected MBD occurs. In another case, Turnbull *et al.*[25] also reported bone marrow hypocellularity in hypothermic sea turtles. Bone marrow hypoplasia is commonly found in animals and humans with aplastic pancytopenia, a rare condition in which all hematopoietic lines in the bone marrow are aplastic or severely hypoplasiated, resulting in bone marrow failure. The cause is usually chemical agents that are cytotoxic to hematopoietic cells, or mutations or perturbations in hematopoietic cells and their environment caused by infectious agents.[23]

In this study, indications of infection and sepsis were also found, possibly due to microbial flora, in the tortoise suspected of having MBD. The association between decreased bone mineral density (BMD) and the risk of infection and sepsis has recently been reported. Previous studies have shown that BMD is a prognostic factor for infections and sepsis in human patients. Schulze-Hagen *et al.*[26] found that low BMD was closely related to high mortality rates in intensive care units, while patients with pulmonary infections had the lowest BMD. A recent study even demonstrated that low BMD is not only a potential predictor for patients with infections and sepsis, but also a new risk factor for infections and sepsis.

Sepsis occurs after bacterial infections, leading to severe sepsis and septic shocks characterized by low blood pressure, ischemic, failure of multiple organs, and death.[27] In this case, it was observed that there was inflammation of the liver, heart, muscles, and peripheral nerves, as well as renal tubular necrosis that can lead to acute renal failure. The decrease in the number and function of osteoblasts, associated with altered expression of IL-7 and lipocalin-2, may have a negative impact on body's immunity and thus increase sensitivity to infections. Vitamin D may also explain the connection between BMD and infections and sepsis.[28] However, much remains to be done to confirm the factors and analyze the association between bone metabolism disorders and sepsis.

CONCLUSION

In conclusion, this study reports that the young tortoise necropsied had a number of pathological conditions that led to suspicion of MBD. Inflammation of multiple organs due to sepsis that we found also seems to have a pathophysiological relationship with this disorder of bone metabolism. Further study is warranted to reach a convincing confirmation by further laboratory diagnostics (blood calcium, parathyroid hormone, etc.) and investigate the relationship between MBD and the risk of infection and sepsis in animals.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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