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CASE REPORT
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Amebiasis in a Backyard Red-Foot Tortoise (*Chelonoidis carbonaria*)

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ABSTRACT

***Background*:** Amebiasis is a parasitic infection caused by obligate or facultative amoeboid protozoans, as well as free-living forms. The genus *Entamoeba* includes both pathogenic and commensal species that can affect humans and animals. *Entamoeba histolytica* is the most important species associated with intestinal and extraintestinal infections in humans, while *Entamoeba invadens* is considered the most common and serious pathogen to many reptile species, including lizards, snakes and crocodilians. The aim of this manuscript is to report a case of amebiasis in a backyard red-foot tortoise in northeastern Brazil.

***Case*:** A 10-month-old male red-foot tortoise (*Chelonoidis carbonaria*) was presented at the Animal Pathology Laboratory of the Veterinary Hospital of Federal University of Campina Grande for necropsy with a 1-week history of anorexia, apathy, and reluctance to move. According to the owner, the animal suffered from heat stress in the backyard, where it was housed with another male red-foot tortoise. At *post-mortem* examination, there were approximately 1 mL of yellowish viscous transudate in the coelomic cavity. The liver was large, with rounded edges and multifocal to coalescing yellowish areas in the subcapsular surface. When cut, the parenchyma was more friable and yellowish. At the opening of the small intestine, the mucosa was thickened, reddened, and contained many variably sized, dark red ulcers with depressed and hemorrhagic centers. Histopathology of the liver reveals diffuse macro and microvacuolar degeneration of the hepatocyte cytoplasm, often displacing the nucleus peripherally (fatty degeneration). There were extensive and multifocal areas of necrosis characterized by shrunken, hypereosinophilic and pyknotic hepatocytes. Amebic trophozoites were seen through the areas of necrosis and degeneration and the morphological features were suggestive of the genus *Entamoeba*. In the portal triads and slightly extending to the sinusoidal spaces, there is a moderate inflammatory infiltrate of macrophages, lymphocytes, plasma cells and rare heterophils. There were amebic trophozoites and thrombi in hepatic vessels, and mild intracanalicular cholestasis. The small intestine contained areas of transmural necrosis and ulceration associated with inflammatory infiltrate of macrophages, lymphocytes and plasma cells. The ulcers were covered by a thick fibrinonecrotic exudate mixed with a varying number of heterophils and macrophages. The submucosa contained hemorrhage and edema. Similar amebic trophozoites were found within the mucosa and submucosa, and also detected in the lumens of blood vessels at the submucosa. The amebic trophozoites, seen in the liver and intestine, were intensely Periodic acid–Schiff positive.

***Discussion*:** The diagnosis of amebiasis was based on the epidemiological, clinical and anatomopathological findings. Amebiasis is a well-recognized disease that usually is diagnosed *post-mortem* in numerous species of reptiles. Unfortunately, there are no scientific articles describing these cases in Brazil. In reptiles, the major pathogenic specie is *Entamoeba invadens*, while several other species are considered non-pathogenic, such as *E. barreti, E. insolita, E. terrapinae, E. ctenosaurae,* and *E. knowlesi*, among others. Although cultivation of *E. invadens* was not undertaken, the anatomopathological findings and the morphological appearance of the agent is highly suggestive of infection with this organism. In conclusion, amebiasis is a severe infectious disease that can affect young red-footed tortoises under adverse environmental conditions. Clinical signs are nonspecific and may be difficult to identify. The diagnosis is usually made post-mortem by anatomopathological findings and the morphological appearance of the agent.

Keywords: tortoise disease, enterohepatitis, trophozoites, *Entamoeba.*

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INTRODUCTION

Amebiasis is a parasitic infection caused by obligate or facultative amoeboid protozoans, as well as free-living forms [8]. The genus *Entamoeba* includes both pathogenic and commensal species that can affect humans and animals. *Entamoeba histolytica* is the most important species associated with intestinal and extraintestinal infections in humans [7], while *Entamoeba invadens* is considered the most common and serious pathogen to many reptile species, including lizards, snakes and crocodilians [16, 17].

Entamoeba invadens has a direct life cycle with no intermediate host. The biological host is thought to be herbivorous chelonians, in which a symbiotic relationship without any pathogenicity may be observed [14]. There are two stages: the cyst, which is the quiescent infective stage; and the trophozoite, which represents the active form. Infection occurs by the ingestion of contaminated food or water containing the cystic form [1]. Trophozoites reside in the intestinal lumen and may invade the intestinal wall and occasionally spreads to other organs, mainly the liver [12].

Although early reports suggested that chelonians are usually asymptomatic carriers, there is now substantial evidence that *E. invadens* is pathogenic in tortoises and turtles [17]. Episodes characterized by high mortality attributed to *E. invadens* are reported in red-footed tortoises (*Chelonoidis carbonaria*) [11] and leopard tortoises (*Geochelone pardalis*) [17], while similar cases with detection of *Entamoeba* spp. were reported in gopher tortoises (*Gopherus polyphemus*), yellow-footed tortoises (*Geochelone denticulate*), African spurred tortoises (*Geochelone sulcate*), loggerhead flat-backed spider tortoises (*Pyxis planicauda*), musk turtles (*Sternotherus minor*), wood turtles (*Clemmys insculpta*), green sea turtles (*Chelonia mydas*) and loggerhead sea turtles (*Caretta caretta*) [1]. Therefore, the aim of this manuscript is to report a case of amebiasis in a backyard red-foot tortoise in northeastern Brazil.

CASE

A 10-month-old male red-foot tortoise (*Chelonoidis carbonaria*) was presented at the Animal Pathology Laboratory of the Federal University of Campina Grande for necropsy with a 1-week history of anorexia, apathy, and reluctance to move. According to the owner, the animal suffered from heat stress in the backyard, where it was housed with another male red-foot tortoise. The diet offered to the animal was cucumber, kale, cilantro, tomato, banana, boiled chicken eggs, and water *ad libitum*.

At *post-mortem* examination, there were approximately 1 mL of yellowish viscous transudate in the coelomic cavity (Figure 1A). The liver was large, with rounded edges and multifocal to coalescing yellowish areas in the subcapsular surface (Figure 1B). When cut, the parenchyma was more friable and yellowish (Figure 1C). Fragments of the liver floated when placed in water or 10% neutral-buffered formalin. At the opening of the small intestine, the mucosa was thickened, reddened, and contained many variably sized, dark red ulcers with depressed and hemorrhagic centers (Figure 1D).

Samples of the organs of the coelomic cavity, skin and central nervous system were fixed in 10% neutral-buffered formalin, processed routinely, embedded in paraffin wax, cut at 3 μm sections, and stained by hematoxylin and eosin (HE) and Periodic acid–Schiff (PAS).

Histopathology of the liver reveals diffuse macro and microvacuolar degeneration of the hepatocyte cytoplasm, often displacing the nucleus peripherally (fatty degeneration). There were extensive and multifocal areas of necrosis characterized by shrunken, hypereosinophilic and pyknotic hepatocytes. Amebic

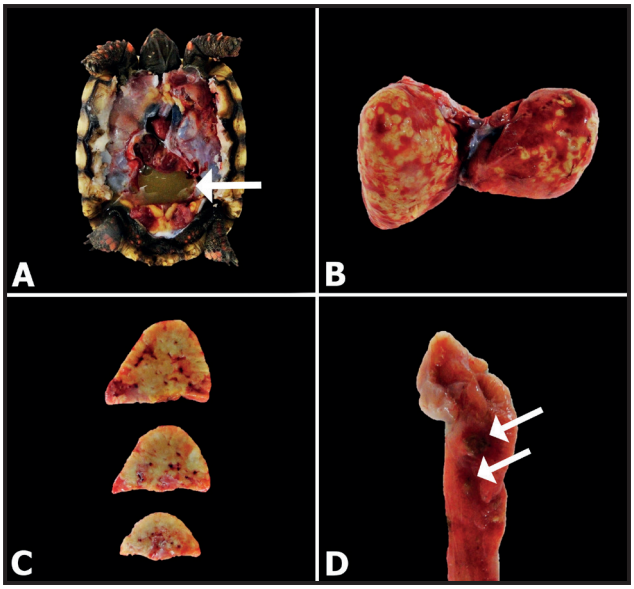

Figure 1. Amebiasis in a red-foot tortoise. A- Coelomic cavity. Yellowish viscous transudate (arrow). B- Liver enlarged with multifocal to coalescing yellowish areas in the subcapsular surface. C- Liver. Serial transverse sections showing the parenchyma yellowish. D- Small intestine, duodenum. Reddish mucosal surface with multifocal dark red ulcers with depressed and hemorrhagic centers (arrow).

trophozoites were seen through the areas of necrosis and degeneration and were characterized by round-to--ovoid organisms with eosinophilic wall, vacuolated cytoplasm, single round-to-ovoid nucleus, ranging in size from 15-20 µm (Figure 2A), morphological features suggestive of the genus *Entamoeba*. In the portal triads and slightly extending to the sinusoidal spaces, there is a moderate inflammatory infiltrate of macrophages, lymphocytes, plasma cells and rare heterophils. There were amebic trophozoites and thrombi in hepatic vessels, and mild intracanalicular cholestasis.

The small intestine contained areas of transmural necrosis and ulceration associated with inflammatory infiltrate of macrophages, lymphocytes and plasma cells. The ulcers were covered by a thick fibrinonecrotic exudate mixed with a varying number of heterophils and macrophages (Figure 2B). The submucosa contained hemorrhage and edema. Similar amebic trophozoites were found within the mucosa and submucosa, and also detected in the lumens of blood vessels at the submucosa (Figure 2C).

The amebic trophozoites, seen in the liver and intestine, were intensely PAS-positive (Figure 2D). No changes were seen in other tissues.

DISCUSSION

The diagnosis of amebiasis was based on the epidemiological, clinical and anatomopathological findings. Amebiasis is a well-recognized disease that usually is diagnosed *post-mortem* in numerous species of reptiles [1]. Unfortunately, there are no scientific articles describing these cases in Brazil.

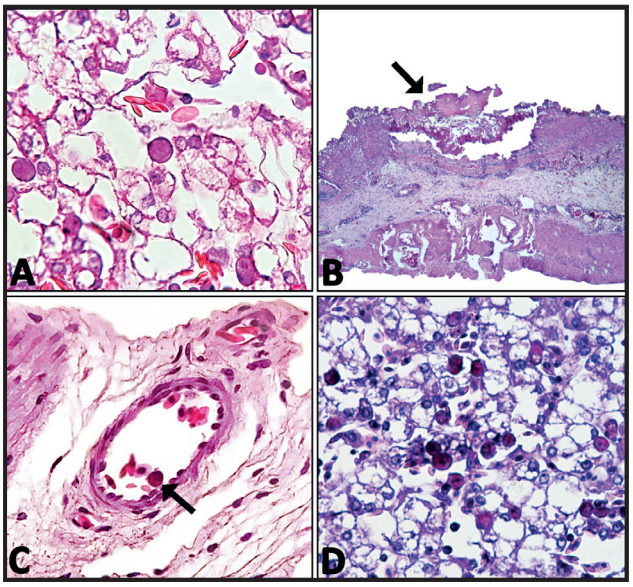

Figure 2. Amebiasis in a red-foot tortoise. A- Liver. Amebic trophozoite [HE; Obj.40x]. B- Small intestine, duodenum. Focal area of ulceration covered by a thick fibrinonecrotic exudate (arrow) [HE; Obj.5x]. C- Duodenum, submucosa. Amebic trophozoite in the lumen of blood vessel (arrow) [HE; Obj.40x]. D- Liver. Numerous amebic trophozoites within necrotic area [PAS; Obj.40x].

The genus of *Entamoeba* has adapted to live as parasite or commensal in digestive tract of human and other mammals, amphibian, brides, fishes, reptiles, and some invertebrate animals [10]. In reptiles, the major pathogenic specie is *E. invadens* [15], while several other species are considered non-pathogenic, such as *E. barreti, E. insolita, E. terrapinae, E. ctenosaurae,* and *E. knowlesi*, among others [2]. Although cultivation of *E. invadens* was not undertaken, the anatomopathological findings and the morphological appearance of the agent is highly suggestive of infection with this organism. However, this does not exclude the possibility of an equally pathogenic and morphologically similar reptilian *Entamoeba* [9].

Epidemics associated with *Entamoeba invadens* have been reported in red-footed tortoises [11], flat-shelled spider tortoise [16] and leopard tortoises [17]. In the previously reported cases, the affected animals were either suffering from weather changes or recently imported after long shipping, suggesting that pathogenicity of *E. invadens* in chelonians is largely correlated to the environmental conditions. Moreover, incomplete development of the immune system in juvenile tortoises may play a significant role in the pathogenesis of amebiasis [9].

Clinical signs in tortoises with amoebic enteritis include anorexia, lethargy, diarrhea and death, sometimes after a prolonged clinical course [11,15,16]. However, due to the behavior of chelonians, many owners may miss the early signs of the disease.

Hepatic disease in chelonians may range from subclinical disease reflected only by elevations of liver enzymes in the blood to life-threatening liver failure [5]. Although no serum biochemistry evaluation has been performed, liver enzymes are known markers of hepatocellular damage and cholestasis. Definitive identification of liver damage is often reached on biopsy, and in chelonians it is invasive, through the inguinal fossa, often by videolaparoscopy [6].

In the present study morphological evidence of amebic invasion of mucosa and blood vessels in the duodenum strongly suggested that the intestine was the primary site of infection with further spread to the liver via the portal system. It is known that the ingested

mature cysts develop into trophozoites in the intestine, invade the mucosa of the gastrointestinal tract and the small blood vessels of the submucosa, and then gain access to the superior mesentery and portal system to reach liver [11,12].

Gross lesions may include thickening, necrosis and ulceration of the small or large intestine [11,15,16]. Necrotic hepatitis is a common finding in tortoises and other reptiles with systemic amebiasis [11,16].

Definitive diagnosis of amebiasis is histological, with the identification of extracellular parasites embedded in the intestinal mucosa and/or in the hepatic sinusoids [1]. In case of systemic amebiasis, it is possible to find the parasites in other organs such as kidneys, lungs and ovaries. [1,9]. Currently, immunohistochemistry and polymerase chain reaction (PCR) are available to provide accurate identification of *Entamoeba* species [2,13].

The differential diagnosis should include hepatic lipidosis. The gross appearance of a liver affected with hepatic lipidosis is a pale yellow to light tan, swollen, highly friable organ [3,4]. In severe cases, the parenchyma is less dense, and portions will float in water or fixative [3,4], as was seen in this case. However, the liver is usually diffusely affected, and in this case the fatty degeneration was multifocal to coalescent and interposed by multifocal areas of necrosis.

In conclusion, amebiasis is a severe infectious disease that can affect young red-footed tortoises under adverse environmental conditions. Clinical signs are nonspecific and may be difficult to identify. The diagnosis is usually made *post-mortem* by anatomopathological findings and the morphological appearance of the agent.

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