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# Pancreatitis Associated with the Helminth *Serpinema microcephalus* (Nematoda: Camallanidae) in Exotic Red-Eared Slider Turtles (*Trachemys scripta elegans*)

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ABSTRACT: Pancreatitis associated with the helminth *Serpinema microcephalus* was found in three of 19 free-ranging red-eared slider turtles (*Trachemys scripta elegans*) captured between March 2003 and September 2004 in southern Spain. Microscopic changes were associated with parasite migrations and were characterized by central areas of necrosis surrounded by leukocytes and resulted in destruction of exocrine tissue. The blood profile of one of the three female turtles revealed eosinophilia and hyperglycemia, common in helminth infections and pancreatic disorders respectively. These are the first reported cases of pancreatitis caused by the nematode *S. microcephalus* in the exotic and newly colonized host *T. s. elegans*.

*Key words:* Helminth, invasive exotic turtles, pancreatitis, reptiles, *Serpinema microcephalus*, *Trachemys scripta elegans*.

Pancreatic lesions caused by parasites commonly have been described in humans and in several other vertebrate species (McClure and Chandler, 1982; Popp and Schuster, 1989; Sandouk et al., 1997; Shad and Lee, 2001; Boruncinska and Frasca, 2002). However, only a few such cases have been reported for reptiles, including a red-bellied watersnake (*Nerodia erythrogaster*) and a pond turtle (unspecified species), both parasitized by helminths (Frye, 1991), and a radiated tortoise (*Geochelone radiata*) parasitized by a coccidian (Jacobson et al., 1994). Microscopic evidence of pancreatic helminthiasis can be detected from cross-sections of pancreas tissue with incorporated helminths or their eggs (Frye, 1991). Substantial inflammatory response in the pancreas usually occurs when helminths are accompanied by pathogenic microorganisms or are migrating, inducing concomitant pancreatitis (Frye, 1991).

The red-eared slider turtle (*Trachemys scripta elegans*) is an invasive species commonly traded worldwide as pets (Telecky, 2001). Free-ranging individuals are widely distributed in freshwater ecosystems around the world (Newberry, 1984; Luiselli et al., 1997; Chen and Lue, 1998; Cady et al., 2004; Feldman, 2007; Perry et al., 2007). In southern Spain, successful reproduction of this exotic species has resulted in the establishment of populations in the wild, where these exotic turtles coexist with native aquatic turtles of two threatened species, the Mediterranean pond turtle *Mauremys leprosa* and the European pond turtle *Emys orbicularis* (Pérez-Santigosa et al., 2006; 2008).

A study carried out during an eradication project and designed to estimate the impact of red-eared sliders on the native turtles assessed the turtles' health using hematologic, histologic, microbiologic, and parasitologic analyses (Hidalgo-Vila, 2006; Hidalgo-Vila et al., 2007; 2008; 2009). This study revealed that >70% of the exotic turtles had hepatic, renal, enteric, pulmonary, and pancreatic lesions due to various pathogenic microorganisms (Hidalgo-Vila, 2006; Hidalgo-Vila et al., 2008). In contrast, no evidence of disease was observed

in native turtle species despite the presence of similar potentially pathogenic microorganisms (Hidalgo-Vila, 2006; Hidalgo-Vila et al., 2008).

This study describes three cases of parasitic pancreatitis in free-ranging exotic red-eared sliders. Pancreatitis was caused by the nematode *Serpinema microcephalus* (Dujardin, 1845), an indirect life-cycle camallanid parasite characterized by a cephalic structure with ridges in the buccal valves (Baker, 1979). This parasite occurs only in turtles from the western Palearctic region (López-Neyra, 1947; Baker, 1979; Lluch et al., 1987; Kirin, 2001; Roca et al., 2005; Hidalgo-Vila et al., 2009). In the native habitat of red-eared sliders, the Nearctic region, these turtles are commonly parasitized by *Serpinema trispinosus* (Leidy, 1852) (Rosen and Marquardt, 1978; Esch et al., 1979). However, in the colonized areas in the south of Spain, this exotic turtle is parasitized by *S. microcephalus*, which appears to occupy a similar niche to *S. trispinosus* in its native range (Hidalgo-Vila et al., 2009).

From March 2003 through September 2004, 19 adult female free-ranging exotic red-eared sliders were captured and removed from the established population of El Portil Pond, Huelva Province, southwestern Spain (37°14′N, 7°02′W). All captured individuals were necropsied after being humanely euthanized by intraperitoneal injection of sodium thiopental (Tiobarbital, Braun Medical). Liver, digestive tract, lungs, kidneys, heart, and pancreas were removed and examined macroscopically. Digestive tracts were opened and contents were washed with physiologic saline solution and examined for helminths under a magnifying glass. Liver, lungs, kidneys, heart, and pancreas were carefully dissected and examined as above. Recovered nematodes were isolated and preserved in 70% ethanol and temporally mounted in Amman lactophenol for identification. Tissue samples used for histopathology were fixed in 10% buffered formalin, embedded in wax paraffin, and sectioned at 3 μm. Sections were mounted on glass slides, stained with hematoxylin–eosin, and examined by bright-field microscopy.

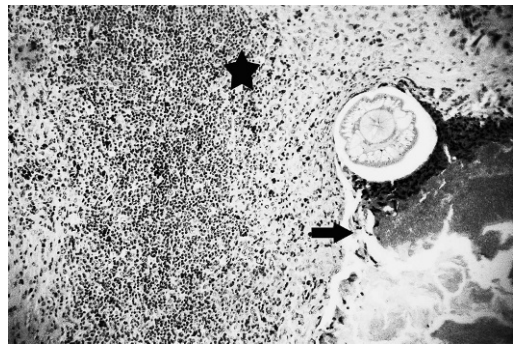

FIGURE 1. Section of nematode in the pancreas of a red-eared slider turtle (*Trachemys scripta elegans*). Note the severe granulomatous inflammatory response (arrow) due to parasite migration (star). Hematoxylin–eosin stain (400×).

Parasitologic analyses revealed nematodes in the digestive tract of the 19 turtles analyzed as well as in the pancreas of three of the 19 females. Microscopic examination of pancreatic sections revealed inflammation of the pancreas in three of the 19 individuals coinciding with the presence of nematodes in the lumen of the pancreatic ducts. According to Baker (1979), and on the basis of the identification of morphologic characteristics, some of the nematodes removed from the digestive tracts and all the nematodes found in the pancreas were identified as *S. microcephalus* (Hidalgo-Vila et al., 2009; Fig. 1). The main pancreatic lesions detected were granulomas—some of them visible macroscopically. Several nodules of 3-mm diameter with a hard consistency were included in the pancreatic parenchyma, deforming it and giving it a whitish appearance. Histologically, changes were observed in the pancreas, consisting of tissue destruction and inflammation, consequent to the transit of the parasite in the pancreatic tissue (Fig. 1). There was also granulomatous inflammation composed of macrophages arranged in several layers

and fibroblasts surrounding the portions containing parasites (Fig. 1). In these areas there was also vascular neoformation with some lymphocytes around the parasites and eosinophil granulocytes. No lesions caused by parasites were detected in the remaining organs.

As is observed in higher vertebrates, pancreatitis in reptiles is often accompanied by intense inflammatory reactions and autodigestion of the pancreas, a process whereby pancreatic enzymes destroy its own tissue, leading to inflammation (Frye, 1991). In these turtles, inflammatory responses are commonly characterized by local to diffuse infiltrations of mononuclear inflammatory leukocytes and heterophils into the lobules of the pancreas (Jacobson, 2007).

Although there was a high prevalence of *S. microcephalus* found in red-eared sliders (93.8%) in this locality (Hidalgo-Vila et al., 2009), abundance and intensity of infection were not particularly strong, being similar to the values recorded in the coexisting native Mediterranean pond turtle (Hidalgo-Vila et al., 2009). Similar prevalence, abundances, and intensities of infection by *S. trispinosum* were found in red-eared sliders and yellow-bellied sliders (*Trachemys scripta scripta*) in their native habitats of the Nearctic region (Rosen and Marquardt, 1978; Esch et al., 1979). However, no cases of pancreatitis have been reported there, even though parasites reached a much higher abundance per host (Rosen and Marquardt, 1978; Esch et al., 1979).

Additionally, hematology and blood chemistry analyses were carried out in one of the three turtles with pancreatitis in which pulmonary fibrosis, interstitial nephritis, and hepatic lipidosis were diagnosed by histopathology (Hidalgo-Vila, 2006). Hematocrit or packed cell volume was measured using a microhematocrit centrifuge technique. Red blood cell (RBC) count, white blood cell (WBC) count, and differential WBC count were determined using the methodology of Campbell (1996). Serum chemistry values were analyzed using an automated chemical analyzer (Modular Analytics, Roche Diagnostics, Basel, Switzerland).

TABLE 1. Hematology and blood chemistry in a red-eared slider turtle (*Trachemys scripta elegans*) with parasitic pancreatitis, and reference values for the species (*Trachemys scripta*).

| Parameter | Affected animal | Reference values |
|---|---|---|
| Hematocrit (%) | 19.79 | 12–26[a] |
| RBC[b] ($\times 10^6$/μl) | 0.42 | 0.37–0.78[a] |
| WBC ($\times 10^3$/μl) | 4.95 | 9.7[a] |
| Heterophils (%) | 34 | 34[a] |
| Lymphocytes (%) | 24 | 39.50[a] |
| Monocytes (%) | 2 | 1[a] |
| Eosinophils (%) | 40 | 9.4[c] |
| Basophils (%) | 0 | 1.50[a] |
| Total protein (g/dl) | 3.55 | 3.60[c] |
| Calcium (mmol/L) | 3.64 | 2.8[c] |
| Phosphorus (mmol/L) | 1.68 | 1.1[c] |
| Sodium (mEq/L) | 144.10 | 121[c] |
| Potassium (mEq/L) | 4.36 | 4.1[c] |
| Uric acid (mmol/L) | 0.10 | 0.059[c] |
| Glucose (mmol/L) | 13.66 | 3.8[c] |
| CK (IU/L) | 995 | 1,952[d] |
| AST (IU/L) | 201 | 53–83[a] |
| LDH (IU/L) | 1,304 | 213–591[a] |

[a] Stein, 1996.

[b] RBC = red blood cell; WBC = white blood cell; CK = creatine kinase; AST = aspartate aminotransferase; LDH = lactate dehydrogenase.

[c] Dessauer, 1970.

[d] ISIS, 2002.

Analyses revealed eosinophilia, lymphoid depletion, and the presence of reactive lymphocytes with phagocytosed particles, vacuolization, and an increase of the nucleus size. Increased glucose, uric acid, calcium, aspartate aminotransferase (AST), and lactate dehydrogenase (LDH) values were also measured (Table 1).

Eosinophilia is commonly detected with helminth infections (Rosskopf, 2000) and hyperglycemia is usually associated with pancreatic disorders (Stahl, 2003). Other irregular values including leukopoenia, AST, LDH, and uric acid were interpreted as due to concomitant pathologies diagnosed. Leukopoenia is commonly associated with serious infectious diseases in reptiles (Rosskopf, 2000). High levels of

AST and LDH are common with hepatic damage and increased blood levels of uric acid are explained by abnormal renal function (Divers, 2000). The high circulating levels of calcium would be related to the reproductive stage of this female individual (Campbell, 1996).

To our knowledge, these are the first reported cases of pancreatitis caused by *S. microcephalus* in the newly colonized host, the red-eared slider. Our results provide evidence that the host–parasite relationship gives rise to damage in the exotic turtles, causing unusual cases of pancreatitis. This could be due to the poor condition of these exotic turtles. In spite of their apparent health, this free-ranging exotic population had a generalized state of immunosuppression (Hidalgo-Vila, 2006), possibly due to an inability to adapt to the newly colonized areas where they are vulnerable to pathogenic organisms and diseases. In addition to the potential impact caused by competition with native turtles for basking places, nest sites, and food (Cadi and Joly, 2003, 2004), exotic turtles of this population are a source of infection and disease, and thus pose an additional threat to the health of native fauna, for which control and regulation of the exotic pet trade are recommended.

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