Pathological study of lesion caused by *Serpinema microcephalus* (Nematoda: Camallanidae) in *Mauremys caspica caspica* from north of Iran

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Abstract *Serpinema microcephalus* is nematoda from family Camallanidae, which have a lot of pathogenesis species that harbored via many animals like fishes, reptiles and amphibians. Epidemiological study in different regions and histopathological examination of intestinal parasitic infections caused by *S. microcephalus* are prerequisite to develop appropriate control strategies. This study aimed to describe the lesions caused by *S. microcephalus* from Caspian turtles of north of Iran. Thirty-four adult turtles were collected from road accidents in Behshahr, northeastern city of Mazandaran province. The turtles were examined in the laboratory and parasite samples were collected from the small intestine. After clarification by lactophenol and staining, the parasites were identified as *S. microcephalus* (Nematoda: Camallanidae). For histopathological examination, tissue samples were fixed and stained with haematoxylin and eosin. Microscopic diagnoses in small intestine included hyperaemia, eosinophilic enteritis, mucosal glands hyperplasia, mucosal ulceration, intestinal exudation and fibroma in some cases. In pancreas tissue the lesions consisted of mild inflammation, fibrosis and edema. Considering the occurrence of intestinal parasitic infections (29.41 %) among Caspian turtles in this study, identification and control of the disease of the turtles are recommended.

Keywords *Serpinema microcephalus* · Camallanidae · Intestinal lesions · Caspian turtles

Introduction

Like to other wild vertebrates, reptiles are commonly infected by a wide range of nematodes, serving either as their definitive or intermediate hosts. There have been reports and papers described the apparent diseases caused by nematodes in reptilian. More than 1,000 species of nematodes have been reported in amphibians and reptiles (Baker 1984). Also a large number of aquarium and marine fish are affected by species of Camallanidae, such a type in fire-mouth cichlid fish (*Thorichthys meeki*) (Hoffman 1999) *Camallanus anabantinis* climbing perch fish (*Anabas testudineus*) (Nimai 1999). *S. microcephalus* is nematoda from family Camallanidae, which have a lot of pathogenesis species that harbored via many animals like fishes, reptiles and amphibians. *S. microcephalus* however found in the small intestine, also in pancreas seen with injuries such as necrosis and tissue destruction (Hidalgo-Vila et al. 2011). In this study we characterized pathologic changes induced by *S. microcephalus* in the gut and pancreas of Caspian turtle (*Mauremys caspica*) from north on Iran. The nematodes were recorded at the National Museum of Parasitology, Czech Republic, with registration number N-133.

Materials and methods

From July 2011 through September 2012, 34 adult turtles were collected in Behshahr, northeastern city of Mazandaran province (36.6923°N, 53.5526°E) from road accidents in dikes and dams beside roads. The turtles were transferred to the Laboratories of Veterinary Diagnostic Medicine at the

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Fig. 1 Epithelial hyperplasia (green arrow), hyperaemia (black arrows), edema (blue arrows), mucosal ulceration (yellow arrows), intestinal exudation (white arrows) and diffuse infiltration of

inflammatory cells [eosinophil (red arrows), lymphocyte] in mucosa and submucosa of small bowel of the Caspian turtles infected by *Camallanus*. H&E staining $\times 40$ magnification. (Color figure online)

Islamic Azad University-Babol branch for histopathological examination. After necropsy, the digestive tract was carefully removed. The esophagus, stomach, small and large intestines were examined for parasite. The parasites were found in small intestine killed by placing in combination of 70 % ethanol and 5 % glycerin then clarified by lactophenol and stained. Samples used for histopathology were fixed in 10 % buffered formalin, embedded in wax paraffin and sectioned at 5 μ m. Sections were stained with Hematoxylin and Eosin, mounted on glass slides, and examined by bright-field microscopy.

Results

From 34 turtle samples (21 female and 13 male), 10 samples (7 female and 3 male) were infected to parasite (Prevalence

29.4 1 %). In the present study, small intestine necropsy showed that the turtles were infected to *S. microcephalus*. Total number of 27 *S. microcephalus* (11 male and 16 female) were removed from small intestine of turtles.

The pathological study of small intestine revealed inflammatory cells infiltration, edema, hyperaemia and intestinal wall thickening. The early lesions were characterized by infiltrate of eosinophils and lymphocytes. Waste products of the parasites were chemotactic for eosinophils. Microscopically, eosinophilic inflammation as eosinophilic enteritis was prominent. Also, hyperplasia of the mucosal glands, mucosal ulceration and intestinal exudation were observed (Fig. 1).

The small masses with firm consistency were observed in the part of the intestine in two cases. Microscopically,

Fig. 2 a Mucosal edema (blue arrow), intestinal exudation (white arrow) and diffuse infiltration of inflammatory cells [eosinophil (red arrow), lymphocyte] in small intestine. H&E, $\times 40$. **b** Intestinal

fibroma. Spindle-shaped cells (black arrow) in a collagenous stroma in a whorl-like pattern. H&E, $\times 40$. (Color figure online)

Fig. 3 Increased connective tissue in intralobular, interlobular space of pancreas (black arrows), edema (blue arrow) and mild infiltration of eosinophils (red arrow) and lymphocytes. H&E, $\times 40$. (Color figure online)

these masses in the mucosal layer covered by intestinal epithelium. Lengthened spindle-shaped fibrocytes and fibroblasts with a whorl-like pattern were present among the disarranged connective tissue fibers. Inflammatory reaction was present in the tissue sections by diffuse infiltration of eosinophil and lymphocytes. Considering, the masses were not encapsulated without signs of malignancy, they were diagnosed as fibroma. We can conclude fibroma occur because of parasitic chronic inflammation in this study (Fig. 2).

Microscopic examination of pancreatic sections revealed chronic mild inflammation characterized by fibrosis, edema and mild infiltration of eosinophils and lymphocytes in four of ten infected turtles. The histopathological changes in pancreas occur because of the parasite transmission to the pancreatic tissue (Fig. 3).

Discussion

With the author's information, this is the first reported cases of eosinophilic enteritis caused by *S. microcephalus* in Caspian turtles (*M. caspica*). In a pathologic study of *S. microcephalus* in red-eared slider turtles (*Trachemys scriptaelegans*) by (Hidalgo-Vila et al. 2011) pancreatic sections revealed inflammation, fibroblasts surrounding the portions containing parasites, vascular neoformation with some lymphocytes, eosinophil granulocytes, tissue destruction of pancreas in three of the nineteen turtles. The main pancreatic lesions detected were granulomatous inflammation composed of macrophages arranged in several layers (Hidalgo-Vila et al. 2011). Rigby et al. 2008 reported two species of this family of nematode Camallanidae, *Camallanus nithoggi* and *Camallanus chelonius* in

turtles from Queensland, Australia (Hidalgo-Vila et al. 2011). According to the study in USA, *Serpinema trispinosum* Leidy, 1852 were found on helminthes in North American turtles (Platt 2008). Moreover larva form of *S. trispinosum* existed in *Thamnophis melanogaster*, the Garter Snakes in four localities from Mesa Central of Mexico (Jime'nez-Ruiz et al. 2002). *Camallanus tuckeri* was described from specimens found in the intestine of the side-necked turtles *Emydura australis* that collected from several localities in the Kimberley region of Western Australia (Kuzmin et al. 2009). Considering the turtles are a threat to the health of native fauna as a source of infection and disease, identification and control of the disease of the turtles are recommended.

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